International Journal of Molecular Medicine

ISSN 1107-3756
eISSN 1791-244X

VOLUME 44, SUPPLEMENT 1, 2019

Proceedings of the Abstracts of

The 24th World Congress on Advances in Oncology, and 24th International Symposium on Molecular Medicine

10-12 October, 2019, Mystras Grand Palace Resort, Sparta, Greece

International Journal of Molecular Medicine

ISSN 1107-3756
eISSN 1791-244X

Fax: +30 210 725 2922; Tel: +30 210 722 7809
e-mail: contact@spandidos-publications.com
Web-site: www.spandidos-publications.com

Submission of manuscripts
Online: www.spandidos-publications.com

By courier service/overnight mail: Professor D.A. Spandidos,
Editorial Office: 10, Vriaxidos Street, Athens 116 35, Greece

Aims and scope: *International Journal of Molecular Medicine* is a journal providing rapid publication in the field of molecular and clinical research on the mechanisms of diseases related to man, including biology, biochemistry, physiology, immunology, pharmacology, pathology, gene therapy, genetics, virology, hematology, endocrinology, microbiology, oncology, epidemiology, neurosciences, infectious diseases, molecular pathogenesis, human genomics, molecular cardiology, molecular surgery, molecular psychology. Studies *in vitro* or *in vivo* on experimental model systems relevant to the mechanisms of disease are also considered. All manuscripts submitted to the *International Journal of Molecular Medicine* are subject to review.

Publication data: *International Journal of Molecular Medicine* is published monthly.

Annual subscription rates: Print and online subscription rates available at contact@spandidos-publications.com

Editorial board:
The Editorial Board of the *International Journal of Molecular Medicine* consists of experts in their field who are members of The Editorial Academy of the *International Journal of Molecular Medicine*.

Editorial Academy of the International Journal of Molecular Medicine:
To qualify as a member of The Editorial Academy each editor must have 1000 citations of his work as first author or over 2000 in total or over 100 publications in refereed journals and to have contributed significantly to molecular medicine. To apply to become a member of The Editorial Academy you should submit your CV to the Editorial Office.

Bibliographic services: Articles in the *International Journal of Molecular Medicine* are listed in bibliographic services including Current Contents/Life Sciences, Index Medicus, MEDLINE, PubMed Central, Science Citation Index, Biochemistry & Biophysics Citation Index, Science Citation Index Expanded (SciSearch), ISI Web of Science, Chemical Abstracts, BIOSIS and Index Copernicus.

International Journal of Molecular Medicine

ISSN 1107-3756
eISSN 1791-244X

Editorial Board

Organized by the *International Journal of Oncology, Oncology Reports, International Journal of Molecular Medicine, Molecular Medicine Reports, Experimental and Therapeutic Medicine, Oncology Letters, Molecular and Clinical Oncology, Biomedical Reports and World Academy of Sciences Journal*

Welcome to Greece

Dear Colleagues,

On behalf of Spandidos Publications, it is my great pleasure to welcome you to Sparta for the 24th World Congress on Advances in Oncology and the 24th International Symposium on Molecular Medicine. This conference will allow the communication of the latest advances in the fields of oncology and molecular medicine by internationally renowned scientists. It will further facilitate the active collaboration between different research groups worldwide.

We are proud to report that the past year has built on the success of previous years for Spandidos Publications as we continue to grow, with record numbers of papers published in our nine journals. The newly released Impact Factors (2018 Journal Citation Reports® Edition) revealed further increases for several of our journals, indicating that the papers we publish are continuing to be more highly read and cited.

The scientific program for 2019 comprises lectures, short oral presentations and poster presentations, the best of which will be recognized with Certificates of Achievement at our traditional, end of Congress Award Dinner to be held on Saturday the 12th of October. In addition, we have organized six workshops. The first, pre-congress workshop, entitled 'SNP genotyping and rare mutation detection in cancer with molecular methods', is co-organized with TATAA Biocenter, Sweden, and will take place on the 8th and 9th of October. This workshop, which is the fifth in a series of annual pre-congress workshops, will focus on methods for nucleic acid analysis in cancer covering SNP genotyping, rare mutation analysis, methylation, copy number analysis, gene expression analysis, splice variants, microRNA and exosome analysis. Second, third and fourth of the workshops, entitled 'Workshop on Tumor Immunology, Microenvironment and Therapy', 'Workshop on Polyamines' and 'Workshop on Inflammation: The Cornerstone of Chronic Disease' will take place on the 11th of October and the fifth and sixth, entitled '5th Workshop on Paediatric Virology' and 'Workshop on Immuno-Dermatology/Oncology: Updates in Skin Cancer Therapies', on the 12th of October.

In addition, while you are here in Sparta, we hope that you will be able to explore some of the cultural and historic landmarks of Greece. This year, our Congress tours will include excursions to Monemvasia and Mani-Diros Caves. Monemvasia or the 'Gibraltar of the East' as it is also known, is a castle-town with a 15 centuries-old history, where you can enjoy a fantastic trip into a labyrinth of old paths winding till the hilltop, spectacular churches and gorgeous remains of fortifications. Mani-Diros Caves, one of the most beautiful lake caves in the world, is a large underground network of caves full of impressively shaped rock formations. The combination of colors, the wild beauty of the scenery and the turquoise waters of the bay will make this experience unforgettable. This could be an excellent opportunity to reunite with old acquaintances and make new ones, whilst exploring the magnificent natural environment and historical monuments.

We thank you for contributing to the success of this Congress and wish you a very pleasant stay in Greece.

Demetrios A. Spandidos

101

PKC-ι and PKC-ζ are heavily responsible for upregulating epithelial-mesenchymal transition (EMT) and activating Vimentin to facilitate cellular motility in prostate cancer cell lines

Wishrawana S. Ratnayake, Christopher Apostolatos, Sloan Breedy, Robert Hill, Clare Dennison, Mildred Acevedo-Duncan
Department of Chemistry, University of South Florida, Tampa, FL, USA

Prostate carcinoma is the most common type of cancer among males in terms of the number of new cases reported each year. Metastasis is responsible for >90% of prostate cancer-related deaths. Therefore, the understanding of the cellular mechanisms behind prostate cancer metastasis is crucial. The expression of Vimentin is a hallmark of metastatic mesenchymal prostate cancer cells, which is initiated by EMT. Our previous studies showed that atypical protein kinase C- iota (PKC-ι) and zeta (PKC-ζ) inhibition attenuated the activation of the NF-κB pathway by diminishing NF-κB nuclei translocation. The present study demonstrated that the siRNA knockdown of PKC-ι and PKC-ζ downregulated Snail1, PRRX1 and Vimentin, while upregulating E-cadherin and thereby diminishing EMT. *In vitro* migration and invasion assays for PC-3 and DU-145 prostate cancer cell lines demonstrated a significant reduction in cellular migration and invasion in the PKC-ι and PKC-ζ knockdown samples. Immunoprecipitation experiments suggested the direct association of Vimentin with PKC-ι and PKC-ζ separately. Laser-stimulated confocal immunofluorescence and immuno-gold transmission electron microscopic techniques were used to further confirm the relationship of Vimentin with aPKCs. qPCR was used to analyze the mRNA levels of targeted markers to further validate the transcriptional downregulation of Vimentin, which was observed in western blot analysis upon aPKC *si*RNA knockdown. Overall, the results revealed a stronger relationship between PKC-ι and Vimentin over PKC-ζ with Vimentin. Microscopic results also showed PKC-ι concentrated along the cell membrane together with Vimentin in addition to the abundant distribution throughout the cell. In addition, our results suggested that both aPKCs target multiple activation sites (S33, S39 and S56) on Vimentin, thereby playing a crucial role in the regulation of Vimentin dynamics, which is essential for increased prostate cancer cell motility. We used a novel PKC-ι specific inhibitor (ICA-1S) to conduct *in vivo* experiments on murine models. Excised tumors were analyzed for pathways observed in *in vitro* experiments. Immunohistochemical and western blot analysis of the tumor samples confirmed the relationship of aPKCs with Vimentin. In addition, these samples will be analyzed for the miRNA expression upon PKC-ι inhibition. Overall, the results suggest that both aPKCs are essential for the upregulation of EMT and for the activation of Vimentin to facilitate the metastasis of prostate cancer cells. Finally, the results suggest that PKC-ι and PKC-ζ can be effectively targeted using specific inhibitors to develop targeted therapeutics for metastatic prostate carcinoma.

102

Effective antitumor immunity against murine gliomas using dendritic cells transduced with hTERTC27 recombinant adenovirus

Hanxian Gong[1], Lei He[2], Ying Peng[2]
[1]Nanhai Hospital Affiliated to Southern Medical University, Foshan, Guangdong, P.R. China; [2]Sun Yixian Memorial Hospital of Sun Yat-sen University, Guangzhou, Guangdong, P.R. China

hTERTC27, a 27-kDa hTERT C-terminal polypeptide has been demonstrated to cause hTERT-positive HeLa cell apoptosis and inhibit the growth of mouse melanoma. hTERTC27 has been associated with telomere dysfunction, the regulation of gene-regulated apoptosis, the cell cycle and the activation of natural killer (NK) cells, although its mechanisms of action are not yet fully understood. Herein, we report that dendritic cells (DCs) transduced with hTERTC27 can increase T-cell proliferation, and augment the concentration of interleukin-2 (IL-2) and interferon-γ (IFN-γ) in the supernatants of T cells. The T cells co-cultured with rAd-C27 DCs produced 75.54±5.32 pg/ml of IL-2 and 61.35±2.33 pg/ml of IFN-γ, which were higher than those in rAd-EGFP DCs and the normal control DCs groups. The cytotoxic activity of rAd-C27 DCs was 50.38±2.95% at a 40:1 effector/ target ratio (E/T), while no obvious lysis by rAd-EGFP DCs or DCs was detected, even at the highest E/T ratio (29.53±1.49%, 27.53±2.71%). It was demonstrated that the cytotoxic T lymphocytes (CTL) against glioma cells were mainly induced by hTERTC27 peptides. To further evaluate whether intratumoral injections with rAd-C27 DCs influence the induction of tumor-specific T cell responses, tumor-bearing mice were immunized twice. The cytotoxicity in the mice elicited by rAd-C27 DCs was much higher than the other groups at the E/T ratios of 5:1, 10:1 and to 40:1. On Day 21 following tumor implantation, four mice from each group were euthanized to obtain brain tissues and compare the tumor volume. As a result, the average tumor sizes in rAd-C27 DCs group were 10.53±1.24 mm^3, which was significantly smaller than other groups (P<0.001). The mice administered Ad-C27 DCs exhibited a significantly prolonged survival compared with rAd-EGFP DCs or DCs. These data suggest that hTERTC27 gene-transduced DCs can efficiently enhance immunity against gliomas *in vitro* and *in vivo*.

Key words: dendritic cells, cytotoxic T lymphocytes, immunotherapy, hTERTC27

103

The SWI/SNF complex subunit genes and their relation to patient survival times in human cancers

Sevtap Savas[1,2], Georgia Skardasi[1], Yajun Yu[1]
[1]Discipline of Genetics; [2]Discipline of Oncology, Faculty of Medicine, Memorial University, St. John's, NL, Canada
E-mail: savas@mun.ca

SWI/SNF is a protein complex that plays important biological roles in chromatin remodeling and the regulation of gene expression. This complex consists of multiple protein subunits, many of which (e.g., *ARID1A*, *BRG1*, *BRM*) are implicated in human diseases, carcinogenesis, and disease susceptibility or clinical outcomes in cancer patients. In a recent analysis using the publically available data from the TCGA samples (1), we demonstrated that the tumor expression levels of a large number of SWI/SNF complex genes were associated with the survival times of patients with low-grade brain glioma and renal clear cell carcinoma (2). In particular, in the latter disease, the reduced expression levels of six SWI/SNF genes (*SMARCC2*, *SMARCD1*, *SMARCD2*, *SMARCD3*, *ACTB* and *BAF45A*) were associated with longer survival times in patients. These novel associations suggest that elevated levels of SWI/SNF complex subunits may adversely affect the disease progression and/or patient survival in renal clear cell carcinoma patients. Further studies in these cancer sites can verify the relationship of the SWI/SNF complex genes, their functions and alterations, and variable patient survival outcomes. In addition, two promoter variants of the *BRM* gene encoding one of the SWI/SNF subunits (Brahma) have been reported to be associated with its gene expression levels, as well as disease risk and/or survival outcomes in several solid cancers, including lung, liver and esophageal cancers (3-5). Brahma is one of the two ATPases of the SWI/SNF complex. We recently examined the association of these variants with the risk and survival outcomes in colorectal cancer for the first time. In this study, we found that individuals carrying both variants had an increased risk of developing colon cancer (6). These promising results should encourage further analyses in additional colorectal patient cohorts. In this presentation, we will discuss select results from our examination of the SWI/SNF complex genes and review the importance of the SWI/SNF complex in cancer research.

1. Cancer Genome Atlas Research Network. *Nature* 2013; 499, pp. 43-49.
2. Savas S and Skardasi G. *Critical Reviews in Oncology/Hematology* 2018; 123: 114-131.
3. Liu G, et al. *Oncogene* 2011; 30(29): 3295-3304.
4. Gao X, et al. *PLOS ONE* 2013; 8(1): e55169.
5. Korpanty GJ, et al. *Oncotarget* 2017; 8(17): 28093-28100.
6. Yu Y, et al. *PLOS ONE* 2018; 13(6): e0198873.

104

Chemical screening-based discovery of a novel drug against glioblastoma-initiating cells

Smile Echizenya[1,2], Yukiko Ishii[3], Satoshi Kitazawa[3], Tomohisa Hatta[4], Tohru Natsume[4], Shin-ichi Watanabe[3], Shinji Hagiwara[3], Toru Kondo[1]
[1]Division of Stem Cell Biology, Institute for Genetic Medicine; [2]Department of Neurosurgery, Graduate School of Medicine, Hokkaido University, Hokkaido; [3]Pharmaceutical & Healthcare Research Laboratories, R&D Management Headquarters, FUJIFILM Corporation, Kanagawa; [4]Molecular Profiling Research Center for Drug Discovery (molprof), National Institute of Advanced Industrial Science and Technology, Tokyo, Japan
Email: tkondo@igm.hokudai.ac.jp

Glioblastoma (GBM)-initiating cells (GICs) comprise a tumourigenic subpopulation of cells that are resistant to radio- and chemotherapies and are responsible for disease recurrence. To identify chemicals that eradicate GICs, we previously established human temozolomide (TMZ, the standard drug for GBM)-resistant GICs (GICRs) that are more homogenous than parental GICs and are thought to contribute recurrence. Using GICRs and normal neural stem cells (NSCs, as a control), we previously performed a small-scale chemical screening and identified 1-(3-C-ethynyl-β-D-ribopentofuranosyl) uridine (EUrd) that selectively killed GICs/GICRs (1). However, the long-term administration of EUrd was toxic to mice. With the goal of identifying chemicals that specifically kill GICs/GICRs, but are not toxic to mice, we performed a large-scale drug screening and found a group of a novel chemotype. We demonstrated that 10580, a potential lead compound, inhibited proliferation, survival and stemness in GICs *in vitro* and *in vivo* by directly inhibiting a key enzyme in the pyrimidine synthesis pathway. Notably, the long-term oral administration of 10580 did not exert any visible side-effects on mice. We further demonstrated that 10580 induced the nuclear export of stem cell factors in GIC in a Crm1 (also known as exportin)-dependent manner. These results suggest that 10580 is a promising novel drug against GICs and other cancer cells that depend on pyrimidine synthesis (2).

1) Tsukamoto et al., Stem Cells 34: 2016-2025, 2016;
2) Echizenya et al., submitted.

105

Immunogenetic influence on acute lymphoblastic leukaemia

Maha Abdullah[1,2], Le Jie Lee[1], Siti Zuleha Idris[1], Norfarazieda Hassan[1], Seow Heng Fong[1], Chang Kian Meng[3], Jasbir Singh Dhaliwal[4], Hishamshah Mohd Ibrahim[5], Raudhawati Osman[6]
[1]Department of Pathology, Faculty of Medicine and Health Sciences; [2]Institute of Bioscience, Serdang, Universiti Putra Malaysia; [3]Hospital Ampang, Kuala Lumpur; [4]Institute for Medical Research, Kuala Lumpur; [5]Paediatric Department, Hospital Kuala Lumpur; [6]Haematology Unit, Hospital Kuala Lumpur, Kuala Lumpur, Malaysia

Acute lymphoblastic leukaemia (ALL) is a malignant disease of lymphoid progenitor cells in the bone marrow and manifests in the blood. ALL is the most common type of cancer among children, but also affects adults. Children respond better to conventional intensive chemotherapy, achieving cure rates of 80%. Adults suffer complete remissions in 80-90% of cases; however, long-term survival remains low at 30-50%. There are two lineages, B-cell and T-cell ALL. ALL forms a heterogeneous group of disease and aetiology is largely unknown. Several risk factors have been identified, including radiation exposure, chemicals (e.g., benzene), infections (HTLV-1, EBV), congenital syndromes (e.g., Down syndrome, Klinefelter), age, race and sex (>males). A weakened immunity due to immunosuppression also may increase the risk of developing ALL by 2-3-fold. Research on ALL has not yielded conclusive results and research in this area continues. The importance of the immune system in preventing cancer is well-established. The immune response is inhibited in cancer patients and various methods are being tested to re-activate immunity. Variations in immune profiles and components influence efficiency in immune function, which subsequently leads to differences in susceptibility to diseases. Gene polymorphisms contribute to this variation. Studies on these factors in ALL are still limited and thus this area of research is worth investigating. We examined gene polymorphisms in HLA-DRB1 in B-cell ALL using PCR-SSO, and natural killer (NK) cell receptor genes in T-cell ALL, using next-generation sequencing. We also determined the levels of regulatory T-cells and soluble HLA-DRB1 proteins in B-cell ALL by flow cytometry and ELISA, respectively. We compared the proliferation rate and proinflammatory cytokine expression in younger and older B-cell ALL patients using flow cytometry and reverse-transcription-polymerase chain reaction (RT-PCR), respectively. We identified several HLA-DRB1 alleles as risk factors in B-cell ALL (n=42) of Malay ethnicity and observed small variant polymorphisms with significant protein effects in 7 of 40 NK cell-related genes analysed in T-cell ALL samples (n=6). Regulatory T-cells were significantly higher In B-cell ALL (n=17) compared to normal controls (n=35), as was soluble HLA-DRB1 proteins (30 B-cell ALL and 31 normal controls). We also revealed significantly increased proliferative rates, but decreased GM-CSF expression levels in younger (2-10 years old) B-cell ALL patients. Thus, a significant variability in immunogenetics was observed among ALL patients. These may be important risk factors or diagnostic markers and help in explaining pathogenesis of the disease.

106

Inhibition of p38 in breast cancer is a questionable strategy

Anna Breitbach, Susanne Gebhard, Annette Hasenburg, Walburgis Brenner
Department of Gynecology, University Medical Center of Johannes Gutenberg University Mainz, Mainz, Germany
E-mail: Brenner@uni-mainz.de

The mitogen-activated protein kinase (MAPK) p38 is more highly expressed in breast cancer tissue than in normal breast specimens and its activity is associated with early relapse in breast cancer. On the other hand, its activity is known to be associated with progression-free survival in breast cancer. In the past, this contradiction has led to extensive analyses concerning the role of p38 in breast cancer progression *in vitro*. However, the majority of studies concerning the influence of p38 inhibition have been performed by using the p38 inhibitorm SB203580. Since SB203580 additionally inhibits other signaling molecules, we compared the effects of SB203580 with those of Skepinone, a more specific p38 inhibitor. In breast cancer cell lines, we quantified p38 activity, cell viability, adhesion and chemotactical migration following treatment with p38 inhibitor. SB203580 and Skepinone treatment of the cells resulted in different cellular effects. We found an enhanced p38 activity following treatment with SB203580, whereas Skepinone reduced p38 activity. SB203580 reduced cell viability, whereas Skepinone enhanced it. The inhibitory effect on cell adhesion and migration, caused by SB203580, was more potent than that caused by Skepinone. These results demonstrate that in breast cancer, p38 apparently has a progressive inhibitory effect and the specific inhibition of p38 should be questioned.

107

Cancer from the perspective of stem cells and misappropriated tissue regeneration mechanisms

Mariusz Z. Ratajczak, Janina Ratajczak
Stem Cell Institute, James Graham Brown Cancer Center, University of Louisville, Louisville, Kentucky, USA

Tumorigenesis can be considered as pathologically misappropriated tissue regeneration. Herein, we aim to address some unresolved issues that support this concept. First, we will discuss the issue of the identity of cancer-initiating cells and the presence of cancer stem cells in growing tumors. We will also aim to determine whether there are rare and distinct populations of cancer stem cells in established tumor cell lines, or are in all of the cancer stem cells. Second, the most important clinical problem with cancer is metastasis, and thus, a challenging question arises: The question of whether, by employing radio-chemotherapy for tumor treatment, we are unintentionally creating a pro-metastatic microenvironment in collateral organs. Specifically, many factors upregulated in response to radio-chemotherapy-induced injury may attract highly migratory cancer cells that survived initial treatment. Third, there is the question of what is the contribution of normal circulating stem cells to the growing malignancy. In addition, there is the question of whether circulating normal stem cells recognize a tumor as a hypoxia-damaged tissue that needs vascular and stromal support and thereby contribute to tumor expansion. Fourth, there is the question of whether it is reasonable to inhibit only one pro-metastatic ligand-receptor axis when cancer stem cells express several receptors for several chemotactic factors that may compensate for the inhibition of the targeted receptor. Fifth, since the majority of aggressive cancer cells mimic early-development stem cells, we would need to determine which properties of embryonic stem cells are retained in cancer cells. We would also need to determine whether it would be reasonable to inhibit cancer cell signaling pathways involved in the migration and proliferation of embryonic stem cells. We will also briefly address some new players in cancerogenesis, including extracellular microvesicles, bioactive phospholipids and extracellular nucleotides.

108

The complement cascade as a mediator of human malignant hematopoietic cell trafficking

Janina Ratajczak, Mariusz Z. Ratajczak
Stem Cell Institute at James Graham Brown Cancer Center, University of Louisville, KY, USA

The complement cascade (ComC) cleavage fragments C3a and C5a regulate the trafficking of normal, differentiated hematopoietic cells, although they do not chemoattract more primitive hematopoietic stem/progenitor cells (HSPCs). By contrast, human myeloid and lymphoid leukemia cell lines and clonogenic blasts from CML and AML patients respond to C3 and C5 cleavage fragments by chemotaxis and increased adhesion. Consistent with this finding, C3a and C5a receptors are expressed by leukemic cells at the mRNA (RT-PCR) and protein (FACS) levels, and these cells respond to C3a and C5a stimulation by phosphorylation of p44/42 MAPK and AKT. However, neither of these ComC cleavage fragments have an effect on cell proliferation or survival. In parallel, we found that inducible heme oxygenase 1 (HO-1) is a negative regulator of ComC-mediated trafficking of malignant cells and that stimulation of these cells by C3 or C5 cleavage fragments downregulates HO-1 expression in a p38 MAPK-dependent manner, rendering cells exposed to C3a or C5a more mobile. We propose that, while the ComC is not directly involved in the proliferation of malignant hematopoietic cells, its activation in leukemia/lymphoma patients (e.g., as a result of accompanying infections or sterile inflammation after radio-chemotherapy) enhances the motility of malignant cells and contributes to their spread in a p38 MAPK–HO-1 axis-dependent manner. Based on this idea, we suggest that inhibition of p38 MAPK or upregulation of HO-1 by available small-molecule modulators would have a beneficial effect on ameliorating expansion of leukemia/lymphoma cells in clinical situations in which the ComC becomes activated. Finally, since we detected the expression of C3 and C5 mRNA in human leukemic cell lines, further study of the potential role of the complosome in regulating the behavior of these cells is needed.

109

Development of small molecule Myc-Max inhibitors as potential therapeutics for prostate cancer

Paul S. Rennie, Nada Lallous, Lavinia A. Carabet, Artem Cherkasov
Vancouver Prostate Centre, University of British Columbia, Vancouver, BC, Canada

In prostate and several other types of cancer, the transcription factor c-Myc has been implicated in cancer progression, therapy resistance and lethal outcomes. Though a valuable therapeutic target, clinically approved anti-Myc drugs have yet to be discovered. Upon activation, Myc forms a heterodimer with the protein Max and together they bind to DNA to activate the transcription of several target genes which promote cell growth, proliferation, and metabolism while blocking differentiation. In this study, we discovered a novel targetable site in the Myc-DNA binding domain of the Myc-Max complex and used a computer-aided rational drug discovery approach to identify small molecules that bind to this site and thereby effectively inhibit Myc-Max activity. Employing our established virtual screening protocols, we identified several candidate compounds that were subsequently evaluated *in vitro* for their ability to inhibit Myc-Max transcriptional activity. In this regard, VPC-70067 was found to effectively inhibit Myc-Max activity with low to mid-micromolar range potency and with minimal off-target cytotoxicity. Additionally, compound VPC-70063, which had an entirely different chemical structure, was our best lead in a panel of *in vitro* assays and became the primary scaffold for optimization efforts. These results lay a foundation for the development of more potent and specific Myc-Max inhibitors that may serve as promising new therapeutics to treat advanced prostate and other malignancies.

110

Ritonavir as a novel antitumor drug candidate

Ladislav Sivak[1], Vladimir Subr[2], Libor Kostka[2], Tomas Etrych[2], Blanka Rihova[1], Marek Kovar[1]

[1]Laboratory of Tumor Immunology, Institute of Microbiology CAS v.v.i., Prague; [2]Department of Biomedicinal Polymers, Institute of Macromolecular Chemistry CAS v.vi., Prague, Czech Republic
E-mail: makovar@biomed.cas.cz

Multidrug resistance (MDR) is a common cause of failure in chemotherapy for malignant diseases. Thus, we aimed to design, synthesize and test the antitumor activity of conjugates based on N-(2-hydroxypropyl)methacrylamide (HPMA) copolymers bearing the cytostatic drug, doxorubicin (Dox), and the inhibitor of P-gp, ritonavir, in MDR tumors overexpressing P-gp. Both Dox and ritonavir were conjugated to HPMA copolymer via pH-sensitive hydrazone bond, enabling the release of these pharmaceutically active compounds in the low pH environment of the tumor and cancer cells. Since ritonavir itself does not contain any suitable functional group for covalent linkage to HPMA copolymer via the hydrazone group, we prepared a ritonavir derivative (RitD) suitable for such a purpose. We proved that such a conjugate is able to overcome MDR both *in vitro* and *in vivo* in P388/MDR and CT26 mouse tumor models expressing high and low levels of P-gp, respectively. More importantly, we found that HPMA copolymer conjugate bearing only RitD exhibited significant antitumor activity *per se*. We determined that RitD, as well as ritonavir, possessed cytostatic and cytotoxic activity in various tumor cell lines *in vivo* and antitumor activity *in vivo*. Moreover, the antitumor activity of HPMA copolymer conjugate bearing RitD synergized with immunotherapy and was able to completely cure BALB/c mice with established and progressively growing s.c. CT26 tumors. Thus, it is demonstrated herein that ritonavir and its derivatives are promising antitumor drugs.

Acknowledgements: This work was supported by grant 19-05649S from GACR and by RVO:61388971.

111

Hit one to eliminate all during cancer treatment: The crucial role of fatty acid synthase (FASN) in ovarian cancer (OC) growth

Astrid Slany[1], Renate Wagner[2,3], Gerald Stübiger[2,4], Mariya Semkova[1], Ramón Colomer[5], María Luz López-Rodríguez[6], Christopher Gerner[1], Thomas W. Grunt[2,3,7]
[1]Department of Analytical Chemistry, Faculty of Chemistry, University of Vienna; [2]Comprehensive Cancer Center; [3]Department of Medicine I; [4]Department of Biomedical Imaging and Image-guided Therapy, Medical University of Vienna, Austria; [5]Spanish National Cancer Research Center (CNIO); [6]Department of Organic Chemistry I, Complutense University of Madrid, Spain; [7]Ludwig Boltzmann Institute for Hematology and Oncology, Medical University of Vienna, Austria
E-mail: thomas.grunt@meduniwien.ac.at

OCs overexpress FASN, the key enzyme in *de novo* lipogenesis. Accordingly, FASN inhibitors have been shown to inhibit OC growth. Nevertheless, there is still a lack of in-depth analysis of the consequences of FASN blockade on the biocybernetic regulatory balance in OC cells. Herein, we combined in a systems biology approach i) tandem mass spectrometry(MS/MS) shotgun proteomics; with ii) antibody microarray kinomics; and with iii) multiple reaction monitoring(MRM)MS/MS targeted metabolomics. SKOV3 cells were cultured for 8 h or 24 h +/- 40 µM FASN inhibitor G28UCM and gene functional classification on the DAVID platform was used to distinguish early E-, late L- and sustained S-responses. E-responses included activated stress pathways (ER stress, UPR), apoptosis and autophagy, as well as the inhibition of nucleoside-, lipid- and central carbon-metabolism, including respiratory chain and electron transport. L-responses comprised the inhibition of DNA replication, ribosome formation, cytoskeleton-/chromatin-remodelling. S-responses included the blockade of signalling, expression, transport, proteasome and OXPHOS. Overall, metabolism and signalling responded prior to stress response and protein downregulation. This was associated with the loss of mitochondrial, membrane and signal lipids, amino acids, biogenic amines and monosaccharides, regardless of unlimited nutrients in the medium analogous to ascites. In summary, membrane integrity was compromised by G28UCM, resulting in defects in molecular uptake/transport. Apparently, cells cannot compensate for nutrient deficiency by importing exogenous nutrients. This dependency of OC to lipogenesis/nutrient uptake should be exploited for chemotherapy.

Supp. by Herzfelder Familienstiftung, Vienna, Austria

112

Comparative evaluation of the antitumor activity of tryptanthrin and its synthetic water soluble analog mostotrin

Alexander Popov[1,2], Anna Klimovich[1], Olga Styshova[1], Taisiya Moskovkina[2], Andrei Schekotihin[3], Natalia Grammatikova[3], Lyubov Dezhenkova[3], Valentin Stonik[1]
[1]G.B. Elyakov Pacific Institute of Bioorganic Chemistry, Far-Eastern Branch, Russian Academy of Sciences, Vladivostok; [2]School of Natural Sciences, Far East Federal University, Vladivostok; [3]G.F. Gauze Research Institute for the Search for New Antibiotics, Moscow, Russia
E-mail: popovam@piboc.dvo.ru

Natural quinazoline alkaloid tryptanthrin (TT) inhibits the growth and survival of tumor cells both in animal models and various tumor cell lines. Moreover, it enhances the effectiveness of the widely used anti-tumor agents(1). Nevertheless, TT reduces the biological reactivity of the organism, adversely affecting its immunological status, probably by direct inhibitory action. However, the main disadvantages of TT are poor solubility and relatively high toxicity. Taking these advantages into consideration, we have synthesized a new therapeutically promising water-soluble alkaloid, designated as mostotrin (MT), as a result of the reaction of TT with Girard's reagent(2). The advantages of MT in comparison with TT include its good solubility in pharmacologically acceptable aqueous media and weaker acute toxicity (about 5 times less than that of TT) as well as a decrease in the immunosuppressive properties (according to the levels of cytokines in the blood plasma). At the same time, a sharp increase of *in vitro* inhibitory activity against tumor cell lines MCF-7, HCT-116 and K-562 and a pronounced increase in antitumor potential *in vivo* in comparison with TT (by more than an order of magnitude) was indicated. About 50% of mice (females), having Ehrlich ascite tumors, survived in the MT-treated group when a dose of 10 mg/kg with a 5-fold treatment was applied intraperitoneally. Combination therapy of MT with doxorubicin was more effective than monotherapy with each of the drugs. Therefore, MT is promising for further studies as an antitumor agent.

(1) Kaur et al., Bioorg Med Chem 25(17): 4533-4552, 2017.
(2) Stonik et al., application RU patent № 2019110477, priority 08.04.2019.

113

Stem cells from a model for genetically predisposed colon cancer

Nitin Telang
Cancer Prevention Research Program, Palindrome Liaisons Consultants, Montvale, New Jersey, USA
E-mail: ntelang3@gmail.com

Germline or somatic mutations in the tumor suppressor adenomatous polyposis coli (APC) gene represent major genetic defects in familial or sporadic colon cancer, and may signify genetic predisposition. The emergence of drug resistant cancer stem cells promote therapy resistant progression of colon cancer. Reliable stem cell models that express APC defects and exhibit quantifiable cancer risk may facilitate the mechanistic evaluation of novel stem cell-targeted therapeutic options. The tumorigenic $Apc^{(-/-)}$ 850^{Min} COL colonic epithelial cell line represented the model. Non-steroidal anti-inflammatory drug sulindac (SUL), selective ornithine decarboxylase inhibitor difluoro-methyl ornithine (DFMO) and select herbal products represented the test compounds. Relative to $Apc^{(+/+)}$ C57 COL cells, $Apc^{(-/-)}$ 850 ^{MIN} COL cells exhibited the loss of homeostatic growth control and increased anchorage-independent (AI) colony formation, indicative of aberrant hyper-proliferation and an enhanced cancer risk. Mechanistically, $Apc^{(-/-)}$ cells exhibited upregulated Apc/β-catenin signaling, and increased cellular expression of early response gene products cyclooxygenase-2 (COX-2) and ornithine decarboxylase (ODC). SUL reduced the cellular expression of Apc target gene products and that of COX-2. DFMO inhibited the cellular expression of β-catenin, cyclin D1, c-Myc and ODC. The combination of SUL+DFMO interacted to induce G_1 phase arrest and inhibit AI colony formation. Herbal extracts reduced AI colony formation, affected cell cycle progression and increased cellular apoptosis. The $Apc^{(-/-)}$ sulindac-resistant (SUL-R) phenotype exhibited tumor spheroid formation and an upregulated cellular expression of the cancer stem cell-specific markers, CD44, CD133 and c-Myc. Collectively, these data provide evidence for the susceptibility of genetically predisposed colon cancer cells to pharmacological and natural agents, and for effectively establishing a model for drug resistant colon cancer stem cells. The present experimental approach may facilitate the identification of testable alternatives for stem cell-targeted treatment for the therapy of resistant colon cancer.

114

Immunomodulatory properties of dietary components, commensal microbiota and antibiotics

Miloslav Kverka[1,2], Klara Kostovcikova[1], Zuzana Jiraskova Zakostelska[1], Stepan Coufal[1], Zuzana Stehlikova[1], Natalie Galanova[1], Alena Fajstova[1], Tomas Hudcovic[3], Tomas Hrncir[3], Martin Kostovcik[1], Helena Tlaskalova-Hogenova[1]
[1]Institute of Microbiology of the CAS, Prague; [2]Institute of Experimental Medicine of the CAS, Prague; [3]Institute of Microbiology of the CAS, Novy Hradek, Czech Republic
E-mail: kverka@biomed.cas.cz

The Gut microbiota is an immune system modulator and disruptions in this system are associated with inflammatory diseases. We used gnotobiotic animal models of inflammation to analyze the immune-modulatory properties of the gut microbiota, dietary components or oral antibiotics. We colonized germ-free mice with complex specific pathogen-free microbiota, obtained from two different animal facilities. We found that they significantly differed in ileal microbiota, cytokine milieu in Peyer's patches, proportions of regulatory T cells and in the sensitivity to acute intestinal inflammation. We then found that the excess of animal protein exacerbated intestinal inflammation. The deleterious effect of the dietary protein was associated with distinct changes in gut bacteria and fungi and a required both protein and microbiota present at the same time. While this effect was not T cell-dependent, it disappeared when the macrophages were depleted with clodronate liposomes. Subsequently, we analyzed the effect of oral metronidazole (M) on intestinal inflammation or delayed-type hypersensitivity (DTH). We found that that M significantly decreased inflammation and pro-inflammatory cytokine production in Peyer's patches. The dampening effect of M on DTH was long-lasting and could be transferred to naive immune-deficient mice by leukocytes. However, while oral M markedly altered the gut microbiota, its DTH-dampening effect was observed even in germ-free animals, suggesting that it is not dependent on the microbiota. Taken together, these results indicate that the gut microbiota, diet and antibiotics are potent modulators of inflammation via a mucosal immune response modulation.

Acknowledgements: Supported by Czech Science Foundation (Grant. No.: 17-09869S, 16-06326S and 17-06632Y).

115

Re-thinking the tumor microenvironment

Luca Vannucci[1], Pavol Lukac[1,4], Paolo Tenti[1,4], Fabian Caja[1,4], Dmitry Stakheev[1,8], O. Chernyavskiy[2], D. Vondrasek[2], Lucie Kubinova[2], Lenka Rajsiglova[1,3], Tomas Hudcovic[1], Hana Kozakova[1], Jiri Dvorak[1], Petr Sima[1], Pavel Vodicka[1], Ludmila Vodickova[1], Pavol Makovicky[5], Peter Makovicky[6], Radislav Sedlacek[6] and Miroslav Levy[7]
[1]Institute of Microbiology of the CAS, v.v.i. Prague/Novy Hradek; [2]Institute of Physiology and [3]Institute of Experimental Medicine of the CAS, v.v.i. Prague; [4]Faculty of Science, Charles University, Prague, Czech Republic; [5]Department of Biology, Pedagogical faculty, Selye Janos University, Komarno, Slovak Republic; [6]Czech Centre for Phenogenomics Institute of Molecular Genetics of the CAS, v.v.i., Vestec; [7]Thomayer's teaching Hospital, Prague; [8]Second Medical Faculty, Charles University, Prague, Czech Republic
E-mail: vannucci@biomed.cas.cz

The establishment of the tumor microenvironment in its early phases needs to be further elucidated. We have found the colon mucosa collagen scaffold particularly reactive to induced immune activation in different animal models (DSS-induced colitis in rat and mouse, AOM-induced carcinogenesis in rat and bacterial colonization of germ-free mice). By 2-photon microscopy, we found dynamic changes in the collagen scaffold related to variations of the tissue immunological state. IL-6, IL-1, IL-10 and TGF-β exhibited different interplay depending on the type of elicited inflammation, both at the local and systemic level. In addition, in human colon cancer samples - normal mucosa, near tumor mucosa and cancer tissue - scaffold changes were related to local immunological features. An increased gene expression of collagen I, LOX2L, IL-1-β, IL-6 and IL-13 was detected, particularly in the near tumor mucosa, a borderline tissue associated to active immune infiltrate. PD-1 and PD-L1 expression may also be modulated by these conditions. We hypothesized that the permissive deregulation of regulatory molecule expression (e.g., TGF-β, IL-10), may overcome the tissue inflammatory threshold normally ruling the homeostatic conditions allowing initial tumor microenvironment establishment.

Acknowledgements: grants RVO 61388971 (CZ), UniCredit Bank s.r.o. (CZ), Generali CEE Holding B.V. (CZ), Michael Cukier Fund (CA), Italinox a.s. and Eurinox a.s. (CZ), Iginio Longo, Roberto and Olga Magini Fund, Manghi Czech Republic (CZ), Paul's Bohemia (CZ) and ARPA Foundation (IT)

116

Colorectal carcinoma diagnosis in Slovakia: A challenge for family doctors

Pavol Makovicky[1], Csaba Podlupski[2], Attila Juhasz[3], Peter Makovicky[3], Luca Vannucci[4]
[1]Department of Biology, Faculty of Education, J. Selye University, Komarno, Slovakia; [2]Department of Gastroenterology, ForLife General Hospital in Komarno, Slovakia; [3]Czech Centre for Phenogenomics, Division BIOCEV, Vestec, Czech Republic; [4]Laboratory of Immunotherapy, Institute of Microbiology, CAS, v.v.i., Prague, Czech Republic
E-mail: vannucci@biomed.cas.cz

Colorectal carcinoma (CRC) is a commonly diagnosed cancer in Europe (13% new cases/year). Slovakia has the highest incidence followed by Hungary. Genetic factors, nutrition, lifestyle and stress, but also level of diagnostics must be taken in account. General focus is commonly put on incidence of CRC, while differences between different regions in one country and role of family doctors in CRC early diagnosis should be better evidenced. The objective of this study was to identify the incidence of the colorectal carcinoma in Komarno, a town in the South of Slovakia on the border with Hungary, and to determine the role of family doctors in early diagnosis of CRC. Biopsies were collected during general screening in the year 2017, from patients living in Komarno. Samples were obtained by colonoscopy performed at the Gastroenterology center of the General For Life Hospital in Komarno, and histologically evaluated. On a total of 84 colonoscopies, 22 colorectal carcinomas were diagnosed (26%). In conclusion, the high incidence of CRC rendered early diagnosis as a great challenge for family doctors in countries at risk. Screening in a wider 50-year-old population by non-invasive occult blood test is the first recommended step, then colonoscopy in positive cases. In families where one member is CRC diagnosed, all relatives need to be included in the prevention program and monitored. The increase of CRC incidence in one region needs consideration of several exogenous factors involved in large bowel tumorigenesis. Therefore, family doctors should have much more intensive impact for early CRC diagnosis, actively promoting extensive two-step screening.

Acknowledgements: RVO 61388971 (CZ), UniCredit Bank s.r.o., GCP Foundation Prague, CZ.

117

Plasma proteomics biomarkers for redefining the HER-2/neu status in breast cancer patients regardless of their hormone receptors

Ayodele A. Alaiya[1], Zakia Shinwari[1], Asma Tulbah[2], Taher Twegieri[3]
[1]Proteomics Unit, Stem Cell & Tissue Re-Engineering Program, King Faisal Specialist Hospital & Research Center, Riyadh; [2]Department of Pathology and Laboratory Medicine and [3]Oncology Center, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia
E-mail: aalaiya@kfshrc.edu.sa

Immunohistochemistry (IHC) and fluorescence *in situ* hybridization (FISH) are currently being routinely used to evaluate HER2 protein expression and its gene amplification, respectively in the selection of patients for antibody-based therapy. However, discrepancies in the accuracy of HER-2/neu status resulting into certain breast cancer patients being erroneously denied appropriate targeted treatment, in particular, the so called borderline or equivocal cases. This study focuses on the discovery of disease-specific surrogate protein markers for redefining the HER2 status. Peripheral blood plasma (PBP), from 30 breast cancer patients classified into IHC/HER2/ER/PR$^+$ (Luminal B), IHC/HER2-/ER/PR+ (Luminal A), IHC/HER2+/ER/PR$^-$ (HER2) and IHC/HER2/ER/PR$^-$ (triple-negative/basal) were analyzed using quantitative label-free liquid chromatography tandem mass spectrometry (LC/MS/MS). We identified 396 plasma proteins of which 73 and 209 were significantly differentially expressed between luminal A versus B subtypes and HER2- and triple negative samples, respectively, with only 44 proteins overlapping between the two datasets. The expression levels of 37/44 were validated with similar expression patterns among 812 breast cancer samples with the Her2 status represented in The Cancer Genome Atlas (TCGA). Furthermore, of clinical importance are some of the 44 proteins sharing similar expression changes between the pairs of Luminal A vs. Luminal B and HER2 vs. basal (TNBC), indicating their potentials as HER2-specific biomarkers, irrespective of the hormone receptor status. Among the identified protein biomarkers are TUFM, SMPX, FKBP1A, MMRN1, STMN1, CCDC57, HIST3H3, RAB35 and TADA2B. Some of these identified proteins have been implicated in PGR, ESR1, PTEN, ERRBB2 and PI3K/AKT molecular pathways using Ingenuity Pathway Analysis (IPA). We identified 44 proteins as potential HER2-specific biomarkers. These proteins would be useful in redefining a subpopulation of patients that are currently labeled as borderline or equivocal for HER2. The effective validation of these sets protein panels would lead to the accurate stratification of breast cancers in the context of HER2 phenotypes for precision therapy for breast cancer patients.

118

De-regulated Stat5 activity during pregnancy represents a risk factor for latent breast cancer development: Studies on a transgenic mouse model

Sharon Laufer[1], Ana Kosenko[1,2], Tatiana Kisliouk[1], Itamar Barash[1]
[1]Institute of Animal Science, ARO, The Volcani Center, Bet-Dagan, Israel; [2]The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Israel
E-mail: itamar.barash@mail.huji.ac.il

The overexpression and enforced activation of Stat5 in the mammary glands of transgenic mice cause parity-dependent latent tumorigenesis. Stat5 activity in the mammary gland is heterogeneous and epithelial cells with hyper Stat5 activity have been located in the center of the developing neoplasia. To unveil the mechanism(s) involved in single-cell-induced tumorigenesis, we examined the association of a high Stat5 activity with the expression of the DNA damage response protein, H2AX. H2AX expression plays a double-edged regulatory role in tumorigenesis. On the one hand, it functions as a suppressor of genomic instability. On the other hand, H2AX overexpression induces tumorigenesis. Stat5 expression and H2AX promoter activity are correlated, and adjacent luminal and basal cells with hyper Stat5 and H2AX activity, respectively, have been located in the pregnant gland. Further analyses supported a model in which the hyper Stat5 activity in individual luminal cells caused paracrine RANKL secretion that induces H2AX promoter de-methylation in their neighboring basal cells. In turn, a deregulated high H2AX expression ensued, subjecting the mammary gland to tumorigenesis. H2AX expression in tumors was higher than in the intact gland. The highest expression characterized the differentiated adenocarcinomas, which preserved the Stat5-dependent pattern of H2AX promoter activity. A negative correlation between the two was detected in the poorly differentiated carcinomas. The distinction between cells overexpressing Stat5 and H2AX was generally preserved, with rare exceptions. The methylation status of the H2AX promoter may mediate its activity. Highly methylated H2AX promoter characterizes all tumors, as compared to moderate methylation in cultured CID-9 cells. Among tumors, total H2AX promoter methylation was higher in the poorly differentiated carcinomas and negatively correlated with its relative activity. The general decrease in GC methylation in position 299 was enhanced in the differentiated adenocarcinomas towards the downstream site 399. Sp1 and CP2 transcription factors that bind this site may mediate the highest H2AX expression of this tumor type and the response to Stat5 effect. Taken together, a deregulated Stat5 activity during pregnancy may be considered as a risk factor for latent breast cancer development. The overexpression of H2AX in individual cells may induce tumor initiation and its continuous expression may affect the tumor phenotype.

119

Role of miR-145-conjugated gold-nanoparticles as a potential adjuvant therapy for epithelial ovarian cancer

Edison Salas[1], Lorena Lobos[2], Francisco Morales[1], Marcelo Kogan[1,5], Maritza Garrido[3,4], Margarita Vega[3,4], Carmen Romero[3,4,5]
[1]Laboratorio de Nanoformulaciones, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile; [2]Centro de Medicina Regenerativa, Facultad de Medicina, Clínica Alemana, Universidad del Desarrollo; [3]Laboratorio de Endocrcinología y Biología Reproductiva, Hospital Clínico Universidad de Chile; [4]Departamento de Obstetricia y Ginecología, Facultad de Medicina, Universidad de Chile; [5]Centro de Estudios de Enfermedades Crónicas (ACCDIS), Santiago, Chile

Ovarian cancer remains a major health concern worldwide, with over 225,000 new cases and 140,000 deaths reported annually. The Globocan study predicts that by 2035, there will be a worldwide increase in incidence to 371,000 (55%) and an increase in deaths of 67% to 254,000 cases. Ovarian cancer is considered to be the most lethal gynecological malignancy. Approximately 80-90% of ovarian cancer cases correspond to serous epithelial ovarian cancer (EOC) and are characterized by high levels of angiogenesis. It has been found that NGF, TRKA, c-Myc, COX-2 and VEGF, among others proteins, are involved in the progression of EOC and are regulated by microRNAs (miRNAs or miRs). Altered miRNA expression profiles have been identified in several malignancies, including EOC. miRNAs may serve as potential indicators of disease, but more importantly, may play a role as potential therapeutic targets. miR-145 expression is decreased during EOC progression and some targets of this miRNA are c-Myc, COX-2 and VEGF. The objective of this study was to evaluate the proliferation, migration and invasion of EOC cells and also to evaluate the formation of clones, using miR-145 associated to gold nano- nanoparticles (miR-145/GNP). GNP were coated with FSH^{33} to achieve tissue specificity with various concentrations of miR-145; finally, we obtained 18 molecules of miR-145/GNP. A viability assay of EOC A2780 by effect of miR-145/GNP was carried out with MTS for 24, 48 and 72 h and we found a statistically significant decrease in viability at 48 and 72 h ($P<0.01$). In addition, the effect of miR-145/GNP on the migration of EOC cells was evaluated by wound-healing assay at 8 and 21 h, and a significant decrease in cell migration was observed ($P<0.01$). Moreover, the proliferation of endothelial cells (Ehay926) was evaluated with conditioned media of A2780 cells treated with miR-145/GNP and a significant decrease compared with the control condition ($P<0.01$) was found. Based on the effect of miR-145/GNP on important biological processes, these results indicate the importance of using an adjuvant therapy with miRs/GNP for EOC.

Acknowledgements: This study was funded by Projects: Fondecyt Nº 1160139 (to CR) and Fondap 15130011, ACCDIS (to MK).

120

Assessment of *in vitro* radiosensitivity in primary immune deficiency patients

A. Vral[1], A. Baeyens[1], K. Claes[2], C. Bonroy[3], V. Bordon[4], E. Beyls[1], E. Duthoo[1], L. Backers[2], S. Vermeulen[1], F. Haerynck[5]
[1]Radiobiology group, Department of Human Structure and Repair, Ghent University; [2]Center for Medical Genetics (CMGG), Ghent University; [3]Department of Laboratory Medicine, Ghent University Hospital; [4]Pediatric Hematology-Oncology and Stem Cell Transplantation, Ghent University Hospital; [5]Center for Primary Immune Diseases (CPIG), Ghent University Hospital, Ghent, Belgium
E-mail: anne.vral@ugent.be

Primary immune deficiency diseases (PIDs) are life-threatening genetic diseases of the immune system. A subset of PIDs involve mutations in genes acting in the repair of DNA double-strand breaks. Affected patients are radiosensitive (RS) and exhibit variable phenotypic expression due to the diversity of causative mutations. Since PID patients can be exposed to radiation for many reasons, e.g., diagnostic imaging, radiotherapy, bone marrow transplant conditioning, radiation may expose RS subjects to serious risks. Surprisingly, radiosensitivity testing is currently not included in the diagnostic routine for PID patients in most European countries. The main goal of this study was to implement nationwide radiosensitivity analysis in the routine diagnostic procedures for PIDs in Belgium. To this aim, two cell-cycle specific *in vitro* radiosensitivity assays will be included in the standard diagnostic procedures in patients with suspected PIDs at the Ghent University Hospital (national reference center for Belgium): i) the G0 cytokinesis-block micronucleus assay (CBMN), which is about to be translated into clinical practice; and ii) the S/G2 CBMN, which has been developed in our center with promising proof-of-concept, but requires further and final optimization before translation to the clinical practice. Both assays will be performed on peripheral blood lymphocytes from PID patients. Radiosensitivity analysis will be the core of two innovative diagnostic and therapeutic algorithms, which will include immunophenotyping, direct genetic analysis and guide optimal patient care. Micronucleus analysis in lymphocytes of patients is currently ongoing and results of a first pilot pre-translational investigation will be presented. The rationale for the inclusion of radiosensitivity testing in such algorithms is the necessity of improving the timely diagnosis and management of patients affected by these life-threatening, heterogeneous and difficult-to-diagnose diseases.

121

The alkylphosphocholine erufosine is a multifaceted antineoplastic agent as its anticancer activities are based on interference with multiple signaling pathways

Martin R. Berger
Toxicology and Chemotherapy Unit, German Cancer Research Center, Heidelberg, Germany

Erufosine (erucylphospho-N,N,N-trimethylpropanolamine; erucylphospho-homocholine) is a membrane-seeking agent with distinct anticancer properties. It belongs to the group of alkylphosphocholines, which are synthetically derived from cell membrane components. They target cell membranes and cause changes in cellular signalling, which is the basis for their diverse effects, including anticancer, antiprotozoal, antibacterial and antiviral activities. Their mechanisms of action differ from those of other classes of anticancer drugs and include multiple signalling pathways. These actions probably initiate from targeting lipid rafts and then altering phospholipase D and C signalling cascades, which in turn will modulate pathways such as the PI3K/Akt/mTor and RAS/RAF/MEK/ERK signalling chains. By feedback coupling, the SAPK/JNK pathway will be affected, as well. Specifically, the changes induced by erufosine block the cell cycle progression, as almost all cyclins and CDKs were concentration dependently down-regulated in oral squamous carcinoma cells (OSCC) upon exposure to erufosine. These findings indicate a pan-cdk/cyclin inhibition by erufosine, which then causes a G_2/M phase cell cycle arrest and subsequently induces programmed cell death with classical hallmarks, such as the stimulation of death receptors, chromatin condensation, caspase activation and PARP cleavage. Significant tumour growth retardation was observed upon treatment with erufosine in xenograft and chemically-induced models. In addition to these genes, erufosine significantly enhanced the levels of CDKI/p27/CIP1 and of p21. Furthermore, investigations on the role of the Rb protein for the antineoplastic activity of erufosine showed that sufficient Rb levels are necessary for the induction of apoptotic signalling cascades in T-cell leukaemia cells. In this regard, induction of ER stress was also observed upon erufosine exposure. The knockdown and pharmacological inhibition of the ER stress sensors, PERK and XBP1, revealed their involvement into the cellular effects of erufosine, including proliferation, apoptosis and autophagy induction. Autophagy was confirmed by increased acidic vacuoles and LC3-B levels. Upon erufosine exposure, calcium influx into the cytoplasm of the OSCC cell lines was observed. Apoptosis was confirmed by nuclear staining, Annexin-V and immunoblotting of caspases. The induction of mitochondrial stress upon erufosine exposure was predicted by gene set enrichment analysis and shown by the effect of erufosine on mitochondrial membrane potential, ATP and ROS production in OSCC cells. Of note, the exact mode of APC action is yet unknown. Nevertheless, the available knowledge on inhibition of AKT phosphorylation, mTOR phosphorylation and cRaf down-regulation render them attractive candidates for modern personalized medical treatment, which is based on various forms of individualized diagnosis and therapy.

122

Immuno-profiling of the microenvironment of colorectal cancer

Apostolos Zaravinos, Constantinos Roufas, Majdi Nagara, Beatriz de Lucas Moreno, Maria Oblovatskaya, Christodoulos Efstathiades, Christos Dimopoulos, Georgios D. Ayiomamitis
Department of Life Sciences, School of Sciences, European University Cyprus, Nicosia, Cyprus.
E-mail: A.Zaravinos@euc.ac.cy

Although the standard therapeutic approaches for the treatment of colorectal cancer (CRC) include the addition of EGFR and VEGF targeted agents to standard chemotherapy, early trial results on PD1 and PD-L1 blockade have yielded promising results, particularly in patients with microsatellite instability (MSI). The intratumoral immune cytolytic activity (CYT) is determined by the expression of the toxins granzyme A (GZMA) and perforin (PRF1), and is significantly elevated upon $CD8^+$ T cell activation and during productive clinical responses to anti-CTLA-4 and anti-PD-L1 immunotherapies. Herein, we hypothesized that CYT is associated with the mutational, structural and neoepitope features of each CRC sample. Colorectal cancer data were extracted from the datasets TCGA-COAD (colon adenocarcinoma, n=480) and TCGA-READ (rectum adenocarcinoma, n=367). Cytolytic activity was calculated as the geometric mean of the genes granzyme A (GZMA) and perforin 1 (PRF1), and patients were stratified to CYT-high and CYT-low subgroups. We investigated recurrent somatic copy number aberrations and somatic point mutations specific for each cytolytic subgroup, and made connections with the cytolytic status. The expression of several immune checkpoint molecules, including PD-1/-2, PD-L1 and CTLA-4, was analyzed with respect to each tumor's CYT and the status of mismatch repair system (MSI^+ or MSS). A high cytolytic activity was associated with an increased mutational load in colon tumors, the count of MHC-I/-II cancer neoepitopes, a high microsatellite instability and a deregulated expression of several inhibitory immune checkpoints. A number of immune checkpoint molecules (IDO1, LAG3, TIGIT, VISTA, PD-1, PD-L1 and CTLA-4) were expressed at significantly higher levels in MSI^+ CRCs compared to MSS tumors. The expression of Treg markers was also significantly higher in CYT-high CRCs. Assessed globally, CYT-low CRCs contained more recurrent somatic copy number alterations. In conclusion, these data highlight the link between different genetic events and the immune microenvironment in CRC, taking into consideration the status of microsatellite instability. We also provide evidence that MSI^+ and CYT-high tumors may be more suitable candidates for combinatorial checkpoint immunotherapy.

123

Correlation of the expression of immune checkpoint molecules with the tumor-infiltrating lymphocyte load, and survival in colorectal cancer patients

Mara Kitsou, Apostolos Zaravinos
Department of Life Sciences, School of Sciences, European University Cyprus, Nicosia, Cyprus
E-mail: A.Zaravinos@euc.ac.cy

Colorectal cancer (CRC) is the second leading cause of cancer-related death with >8% of the newly estimated cases and deaths. Microsatellite unstable colorectal cancers (MSI+ CRCs) expressing PD-L1, respond to anti-PD-1 or anti-PD-L1 checkpoint blockade, whereas microsatellite-stable tumors (MSS) do not respond the same. Adoptive cell therapy using tumor-infiltrating lymphocytes (TILs) is another, highly promising, immunotherapeutic strategy for these patients. Our aim was to examine how the CRC patient's immune landscape correlates to their tumor-infiltrating lymphocytes (TIL load). We extracted next-generation sequencing data and clinicopathological data for 453 colorectal adenocarcinoma patients, from the Cancer Genome Atlas (TCGA-COAD and TCGA-READ datasets) and analysed them computationally. The information for each gene's expression levels as well as each CRC patient's tumor infiltration load (TIL) was extracted from the Human Protein Atlas (HPA) and the Digital Slide Archive (DSA), respectively. The GEPIA2 server was also used for analysing the RNA sequencing expression data of 275 COAD and 92 READ tumors, compared to 350 normal samples from the TCGA and the GTEx projects, respectively. The TIL load was scored as "0", if TILs>1, "1" if the number of TILS ranged between 1 and 15, "2" if the number of TILS ranged between 15 and 215, and "3" if TILS >215 in that particular histological slide. Kaplan-Meier curves were constructed using GraphPad Prism 8 and differences in overall survival between high- or low-gene expressing patients, or between MSI-H, MSI-L and MSS patients, were measured using the log-rank test. The Spearman's test was used to correlate the TIL load with the expression of each immune checkpoint molecule. High expression levels of the immune checkpoints CTLA-4 and TIGIT were significantly associated with the COAD patients' better survival ($p<0.05$). We also found that IDO1 was significantly overexpressed both in COAD and READ tumors, compared to the adjacent normal tissue. On the other hand, LAG3 and VISTA showed a significant decrease in the COAD and READ tumors versus the normal tissues. Furthermore, among COAD patients, the TIL load correlated positively with the expression of CD8, as well as that of the immune checkpoints ADORA2A, CTLA-4, HAVCR2, LAG3, PD-L1, PD-L2, TIGIT, and VISTA ($p<0.005$, Pearson's correlation test). By contrast, among READ patients, such positive correlations between TIL load and immune checkpoint expression were scored only for LAG3 and PD-L2. In conclusion, our data highlight the differential expression of more than one immune checkpoint molecules in colorectal cancer, their positive correlation with the TIL load, as well as the potential use of CTLA-4 and TIGIT as prognostic markers for COAD patient survival.

124

AID/APOBEC-mediated epigenetic changes in colorectal cancer

Christofi Theodoulakis, Apostolos Zaravinos
Department of Life Sciences, School of Sciences, European University Cyprus, Nicosia, Cyprus
E-mail: a.zaravinos@euc.ac.cy

RNA-editing is one potential RNA epigenetic mechanism that was recently found to confer cancer stemness and enhance the oncogenic potential in colorectal cancer. Most mRNA editing events are mediated by ADARs that catalyze the hydrolytic deamination of Adenosine to Inosine (A-to-I editing), and the AID/APOBEC family of enzymes that catalyze cytidine-to-uridine (C-to-U) editing. Herein, we hypothesized that APOBEC-mediated C-to-U editing plays a causative role in the progression of colorectal cancer. We extracted next-generation sequencing data and clinicopathological data for 647 colorectal cancers along with 51 colorectal normal samples from the Cancer Genome Atlas (TCGA-COAD and TCGA-READ datasets) and analysed them computationally to detect single nucleotide polymorphisms (SNPs), insertions and deletions (Indels), copy number variations (CNV) and structural variations (SVs). We further analysed the mutational signatures of each CRC patient. The gene expression levels of the AID/APOBEC family genes were normalized in Transcripts Per Million (TPM) values. We found an increased expression of APOBEC1, -3B and -3C, suggesting an etiologic role in the disease. We also analyzed 102 CRC exome sequencing data from TCGA and identified that the majority of SNVs were C>T transitions, i.e., the mutation type of major preference for AID/APOBEC genes. Of these exome sequencing data, we extracted three mutational signatures and compared them against 30 known and validated mutational signatures from the COSMIC database. Importantly, the first signature results indicated an elevated rate of spontaneous deamination of 5-methyl-Cytosine in these samples. We have also shown that the mutation load is significantly higher in APOBEC-enriched compared to non-APOBEC-enriched colorectal cancers and has a preference to tCw mutations. In conclusion, we postulate that epigenetic changes in mRNA occur during the development of colorectal cancer and can directly drive tumor progression. Further insight into these changes will enable a deeper understanding of the pathophysiology of colorectal cancer and may identify new potential therapeutic targets.

125

The role of RNA editing in cancer development and prognosis

Christofi Theodoulakis, Apostolos Zaravinos
Department of Life Sciences, School of Sciences, European University Cyprus, Nicosia, Cyprus
E-mails: tchris11@ucy.ac.cy; a.zaravinos@euc.ac.cy

Cancer pathogenesis is primarily attributed to genetic mutations which transform a normal cell into a malignant cancer cell. The progression of next-generation sequencing technologies revealed that RNA editing, A-to-I or C-to-U nucleobase modifications mediated by ADAR and AID/APOBEC enzymes, can have a significant contribution in the development and progression of cancer. Transcriptome analysis of various tumours revealed deferential RNA editing levels in each cancer type. Decreased A-to-I editing patterns have been identified in brain, kidney, lung, prostate and testis tumours, with significant global hypo-editing of Alu elements [1]. By contrast, recent studies on multiple cancers found that, elevated editing levels in intergenic, intronic and 3' UTR regions, especially in thyroid, head and neck, breast and lung cancer tissues, are associated with worst patient survival [2, 3]. Editing in a protein coding region has major consequences in the functionality of the affected gene. Such recoding events are found in colorectal, breast, and esophageal cancer, as well as glioblastoma [4-7]. The most well characterised recoding event is the editing of the coding sequence of AZIN1 mRNA. In liver cancer, it potentiates tumour initiation and progression [8]. AZIN1 recoding in colorectal cancer was also found to augment oncogenic potential and stemness; while in esophageal carcinoma it is associated with aggressive tumour behaviour [9, 10]. These data clearly indicate that, the editing levels and targets have different roles in the pathogenesis of cancer and different clinical outcomes in the progression of the disease.

(1) Paz et al., Genome Res 17: 1586-1595, 2007.
(2) Paz-Yaacov et al., Cell Rep 13: 267-276, 2015.
(3) Han et al., Cancer cell 28: 515-528, 2015.
(4) Galeano et al., Oncogene 32: 998-1009, 2013.
(5) Han et al., 211: 613-621, 2014.
(6) Gumireddy et al., Nat Commun 12: 10715, 2016.
(7) Fu et al., PNAS 114: E4631- E4640, 2017.
(8) Chen et al., Nat Med 19: 209-216, 2013.
(9) Qin et al., Cancer Res 74: 840-851, 2014.
(10) Shigeyasu et al., JCI insight 3: e99976, 2018.

126

FGFR2-mediated signaling in luminal breast cancer: Implications for therapy and prognostication

Hanna M. Romanska[1], Lukasz Turczyk[3], Dominika Piasecka[1,3], Dominika Czaplinska[3], Kamila Kitowska[3], Radzisław Kordek[1], Piotr Potemski[2], Rafal Sadej[3]
[1]Department of Pathology and [2]Department of Chemotherapy, Medical University of Lodz; [3]Department of Molecular Enzymology, Medical University of Gdansk, Poland
E-mail: hanna.romanska@gmail.com

Stromal stimuli mediated by growth factor receptors leading to ligand-independent activation of steroid receptors have long been implicated in the development of breast cancer (BCa) resistance to endocrine therapy. Herein, an impact of FGFR2 activation on ER-dependent BCa cell behavior was analyzed in an *in vitro* model (MCF7 and T47D, luminal BCa cell lines and their FGFR2-deficient mutants, MCF^{FGFR2-} and T47D^{FGFR2-}) using western blotting and immunoprecipitation. The evaluation of the clinical significance of the FGFR2-mediated pathway in invasive luminal BCa was carried out in tissue samples of invasive ductal carcinoma (IDC) from 166 women who had undergone surgery, followed by adjuvant hormonal or chemotherapy using immunohistochemistry for FGFR2, RSK and phosho-RSK. We demonstrated that: i) Signaling mediated by FGFR2 caused ER phosphorylation, ubiquitination and subsequent ER proteasomal degradation, which counteracted tamoxifen-promoted ER stabilization (1); ii) FGF7 stimulated the activation of PI3K/AKT, leading to the phosphorylation of ER at Ser167 and the upregulation of Bcl-2, both of which mediated FGF7/FGFR2-driven resistance to the drug (1); iii) FGF7/FGFR2-triggered signaling induced the phosphorylation of PR at Ser294 through RSK2, which resulted in receptor ubiquitination and subsequent degradation via the 26S proteasome pathway (2); iv) in clinical material, the expression of FGFR2 inversely correlated with ER and the expression of PR inversely correlated with activated RSK (RSK-P) (P=0.016); patients with RSK-P(+)/PR(-) tumors had a 3.629-fold higher risk of recurrence (P=0.002) when compared with the remaining cohort and RSK-P(+)/PR(-) was an independent prognostic factor (P=0.006) (3). ER/PR regulation by FGFR2-mediated signaling may thus represent a novel mechanism likely to contribute to the development of the resistance of BCa to endocrine therapy.

(1) Turczyk et al., Neoplasia 19: 791-804, 2017.
(2) Piasecka et al., Oncotarget 7: 86011-86025, 2016.
(3) Czaplinska et al., Tumour. Biol. 37:13721-1373, 2016.

Acknowledgements: Research supported by the National Science Centre Grant 2015/17/B/NZ4/02157.

127

RhoB in relation to radiotherapy in colorectal cancer

Na Liu[1,2], Weiyingqi Cui[1], Xia Jiang[1,3], Zhiyong Zhang[4], Sebastian Gnosa[1], Zaheer Ali[5], Lasse Jensen[5], Jan-Ingvar Jönsson[1], Stéphanie Blockhuys[1], Eric W-F Lam[6], Zengren Zhao[3], Jie Ping[1], Ning Xie[2], Maria Kopsida[1], Xin Wang[4], Xiao-Feng Sun[1*]
[1]Department of Oncology and Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden; [2]Department of Gastroenterology, the Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an; [3]Department of General Surgery, the First Hospital of Hebei Medical University, Shijiazhuang; [4]Xijing Hospital of Digestive Diseases, Xijing Hospital, the Fourth Military Medical University, Xi'an, P.R. China; [5]Department of Medical and Health Sciences, Linköping University, Linköping, Sweden; [6]Department of Surgery & Cancer, Imperial College London, London, UK

The aim of this study was to explore whether Rho protein is involved in the radioresistance of colorectal cancer and to investigate the underlying mechanisms. Rho GTPase expression was measured following radiation treatment in colon cancer cells. RhoB knockout cell lines were established by a CRISPR/Cas9 system. *In vitro* assays and zebrafish embryos were used for analyzing radiosensitivity and invasive ability. Mass cytometry was used for the detection of RhoB downstream signaling factors. RhoB and FOXM1 expression levels were detected by immunohistochemistry in patients with rectal cancer who participated in a radiotherapy trial. RhoB expression was related to radiation resistance. The complete depletion of RhoB protein increased radiosensitivity and impaired radiation-enhanced metastatic potential *in vitro* and in a zebrafish model. Probing signaling using mass cytometry-based single-cell analysis revealed that the Akt phosphorylation level was inhibited by RhoB depletion following radiation. FOXM1 was downregulated in RhoB knockout cells and the inhibition of FOXM1 led to lower survival rates; it also attenuated the migratory and invasive abilities of the cells following radiation. In patients with radiotherapy, RhoB overexpression was related to a high FOXM1 expresssion, a late TNM stage, a high distant recurrence and poor survival, independent of other clinical factors. In conclusion, RhoB plays a critical role in the radioresistance of colorectal cancer through the Akt and FOXM1 pathways.

128

Glutamine and asparagine cross-talk in cancer cells

Ilaria Chiodi, Domenica Berardi, Cecilia Perini, Giorgia Casali, Chiara Mondello
Institute of Molecular Genetics L.L. Cavalli-Sforza, CNR, Pavia, Italy
E-mail: mondello@igm.cnr.it

During tumorigenesis, metabolism is extensively reprogrammed towards anabolism to fulfil the elevated biosynthetic demand of highly proliferating cancer cells. Glutamine is one of the major sources of building blocks for macromolecule biosynthesis and several cancers are addicted to this amino acid to survive. During the study of cancer cell responses to glutamine deprivation, we found that glutamine withdrawal not only impaired cell survival and proliferation, but also altered c-myc expression. Given the involvement of glutamine in non-essential amino acid (NEAA) biosynthesis, we investigated whether a panel of five NEAAs, which are not contained in the culture medium (namely alanine, asparagine, aspartate, glutamate and proline), was able to allow cell survival and c-myc synthesis in the absence of glutamine. The results revealed that asparagine was necessary and sufficient to supply for the lack of glutamine. In fact, cells incubated in the absence of glutamine and in the presence of asparagine were able to survive and proliferate and exhibited a c-myc expression pattern similar to that observed in cells grown in complete medium. Further analysis of the role of asparagine in cancer cell growth revealed an asparagine-dependent modulation of c-myc and glutamine synthetase expression that supports cell proliferation in the absence of exogenous glutamine.

129

Merkel cell polyomavirus oncoproteins induce microRNAs that suppress multiple key genes in autophagy

Satendra Kumar[1*], Hong Xie[1,2*], Hao Shi[1], Jiwei Gao[1], Christofer C. Juhlin[1,3], Viveca Björnhagen[4], Anders Höög[1,3,5], Linkiat Lee[1], Catharina Larsson[1,3], Weng-Onn Lui[1]
[1]Department of Oncology-Pathology, Karolinska Institutet; Cancer Center Karolinska, Karolinska University Hospital, Stockholm, Sweden; [2]Tianjin Life Science Research Center and Department of Pathogen Biology, School of Basic Medical Sciences, Tianjin Medical University, Tianjin, P.R. China; [3]Department of Clinical Pathology and Cytology, Karolinska University Hospital, Stockholm; [4]Department of Reconstructive Plastic Surgery, Karolinska University Hospital, Stockholm; [5]Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden

Viruses can inhibit host autophagy through multiple mechanisms, and the evasion of autophagy plays an important role in immune suppression and viral oncogenesis. Merkel cell polyomavirus (MCPyV) T-antigens are expressed and involved in the pathogenesis of a large proportion of Merkel cell carcinoma (MCC). However, the mechanisms through which MCPyV induces tumorigenesis are not yet fully understood. Herein, we demonstrate that MCPyV T-antigens induce *miR-375*, *miR-30a-3p* and *miR-30a-5p* expression levels, which target multiple key genes involved in autophagy, including *ATG7*, *SQSTM1* (p62) and *BECN1*. In MCC tumors, a low expression of ATG7 and p62 was associated with MCPyV-positive tumors. The ectopic expression of MCPyV small T-antigen and truncated large T-antigen (LT), but not the wild-type LT, resulted in the suppression of autophagy, suggesting the importance of autophagy evasion in MCPyV-mediated tumorigenesis. Torin-1 treatment induced cell death, which was attenuated by autophagy inhibitor, but not by pan-caspase inhibitor, suggesting a potential role of autophagy in the promotion of cell death in MCC. Conceptually, this study demonstrates that MCPyV oncoproteins suppress autophagy to protect cancer cells from cell death, which contributes to a better understanding of MCPyV-mediated tumorigenesis and potential MCC treatment.

130

***H. triquetrifolium* potential anticancer activity: Apoptosis induction and cell cycle arrest of a colon cancer cell line and chemical analysis**

H. Zaid[1,2], H. Mahajna[1], S. Kadan[1,3]
[1]Qasemi Research Center, Al-Qasemi Academic College, Baqa El-Gharbia, Israel; [2]Faculty of Sciences, Arab American University, Jenin, Palestine; [3]Casali Center for Applied Chemistry, Institute of Chemistry, the Hebrew University of Jerusalem, Givat Ram, Jerusalem, Israel

This *in vitro* study aimed to investigate the role of apoptosis, cell cycle modulation and cell cycle arrest in the observed *Hypericum triquetrifolium* (*H. triquetrifolium*) extract-induced cytostatic effects on the human colon cancer cell line, HCT-116. The effects of *H. triquetrifolium* on cell viability were evaluated by MTT and LDH assays. Cells seeded in 96-well plates (20,000 cells/well) were exposed to increasing concentrations (0-1 mg/ml) of *H. triquetrifolium* extracts for 24 h. We considered concentrations that caused <20% cell death as non-toxic concentrations. Annexin-V is capable of detecting cells in early apoptotic stages via membrane-associated processes, by binding to the phosphatidylserine (PS) head groups. Apoptosis assay using Annexin-V staining was performed on the HCT-116 cell line following exposure to 0.064, 0.125, 0.25, 0.5 mg/ml of *H. triquetrifolium* extracts. A significant level of apoptosis (90%) was induced by 0.25 mg/ml of *H. triquetrifolium.* We observed that *H. triquetrifolium* induced cell death via an apoptotic process, as assayed by Annexin V-Cy3 assay, and confirmed by the analysis of caspase-3 activity, suggesting that the *H. triquetrifolium*-induced apoptosis of human colon cells was mediated primarily through the caspase-dependent pathway. RT-PCR analysis revealed that *H. triquetrifolium* extract had no effect on the mRNA levels of Apaf-1 and NOXA. Moreover, we clearly demonstrated that *H. triquetrifolium* attenuated the cell cycle progression machinery in HCT-116 cells. GC/MS analysis of the extract identified 51 phytochemicals; some are reported as apoptosis inducers and cell cycle arrest agents. On the whole, these results suggest that *H. triquetrifolium* seems to be a potent therapeutic agent for colon cancer growth inhibition.

131

Precision antisense antibiotics against multidrug-resistant Gram-negative bacteria

Peter E. Nielsen
Center for Peptide-Based Antibiotics, Department of Cellular and Molecular Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

Multidrug-resistant Gram-negative bacteria pose an increasing threat to human health, and the development of novel antibiotics would be one answer to this challenge. The majority of efforts to date have focused on the development of broad-spectrum antibiotics with, unfortunately, limited success. We would argue that precision, narrow-spectrum antibiotics optimized against resistant strains are more likely to succeed, both by directly addressing the challenge, as well as limiting the risk of the development of new resistance and spreading. Thus, we are aiming at developing precision antisense antibiotics based on peptide nucleic acids (PNA) specifically targeting (essential) bacterial genes (1-2). Using PNA oligomers targeting the *acp*P gene and conjugated to bacterial penetrating peptides (BPP) (3-6), antimicrobials showing (sub)micromolar antibacterial activity against *Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii* and *Pseudomonas aeruginosa* (including multi resistant clinical isolates), as well as *in vivo* activity in a mouse model, have been discovered. Based on these *in vitro*, as well as *in vivo* results, the prospects of developing novel precision antibiotics against infections by multi resistant Gram-negative bacteria will be discussed.

1. Good L & Nielsen PE (1998) *Nature Biotechnol.* 16: 355, 1998.
2. Good L, Awasthi SK, Dryselius R, Larsson O & Nielsen PE *Nature Biotechnol.* 19: 360, 2001.
3. Ghosal, A, Vitali, A, Stach, JEM, Nielsen, PE *ACS Chem. Biol*. 8: 360, 2013.
4. Ghosal, A, Nielsen, PE *Nucleic Acid Ther*. 22: 323-324, 2012.
5. Hansen AM, Bonke G, Larsen CJ, Yavari N, Nielsen PE, Franzyk H. *Bioconjugate Chem.* 27: 863, 2016.
6. Goltermann L, Yavari N, Zhang M, Ghosal A and Nielsen PE *Front. Microbiol.* 10: 1032, 2019.

132

Human acute myeloid leukemia (AML): Leukemia stem cell (LSC) concept, molecular landscape and multilevel targeting

Asterios S. Tsiftsoglou, Stefanos A. Tsiftsoglou
Laboratory of Pharmacology, Department of Pharmaceutical Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece.
E-mail: tsif@pharm.auth.gr

Human myeloid leukemias (HMLs) represent a large class of highly heterogeneous, aggressive and difficult to treat hematological malignancies derived in the bone marrow from genetically aberrant or epigenetically impaired hematopoietic stem cells (HSCs) or early-uncommitted progenitors. Some of these populations are converted into leukemia stem cell (LSC) clones that retain proliferation dormancy, but exhibit reduced or abnormal outputs of differentiated cell progenies (LSC concept) (1). The most challenging issue, in terms of acute myeloid leukemia (AML) therapeutics, is the eradication of LSC chemotherapy-resistant clones, which support disease relapse. Unfortunately, no real progress has been recorded over the past 30 years of combination chemotherapies in treating AML with cytarabine, daunomycin and other reagents, including kinase inhibitors. The complex nature of the molecular pathology of AML is usually characterized by numerous cytogenetic abnormalities (gene fusion pml-rara), chromosomal translocations [(t(8:21), t(15-17)], inversions, as well as other mutations which affect both the structure and functions of important cell cycle regulators (TP53, Npm1), transcription factors (Runx1, Cebra), epigenetic modifying enzymes (EZH2) and cell signaling pathways (2). All these layers of molecular abnormalities indicate that a coordinated multi-regimen targeting strategy could be more effective in eradicating or controlling AML beyond traditional chemotherapies (3). This could include, in addition to chemotherapeutics, potent differentiation inducers, therapeutic monoclonal antibodies against selected cell surface antigens (CD33 and CD123), small molecule inhibitors against signaling mediators (Flt3-Ind inhibitors) and epigenetic regulators (Stat, Dntm-3A and Histone Deacetylase-HDACs inhibitors), as well as other suppressors of human leukemic cell proliferation, as the ones recently developed by our group, and advanced CAR-T/NK cell based immunotherapies. Such coordinated multilevel AML therapeutic approaches could be valuable as strategic alternatives in eradicating resistant LSC clones or controlling their relapse.

(1) Bonnet D and Dick JE: Nat Med 3(7): 730-737, 1997.
(2) Grimwade D, Ivey A and Huntly BJ: Blood 127(1): 29-41, 2016.
(3) Tsiftsoglou AS, Bonovolias ID and Tsiftsoglou SA: Pharmacol Ther 123(3): 264-280, 2009.

133

Genetics of autoimmune diseases: From gene to protein structure and function

Maria I. Zervou[1], Elias Eliopoulos[2], George N. Goulielmos[1]
[1]Section of Molecular Pathology and Human Genetics, Department of Internal Medicine, School of Medicine, University of Crete, Heraklion, Greece; [2]Department of Biotechnology, Agricultural University of Athens, Athens, Greece
E-mail: mzervou@med.uoc.gr

Autoimmune diseases are multifactorial disorders that are increasing in incidence worldwide. They are associated with a complex mode of inheritance and Genome Wide Association Studies (GWAS) in different racial/ethnic populations have demonstrated that a number of genes are involved in the development and progression of each disease. Recent GWAS of a number of complex diseases have successfully identified novel susceptibility loci, with many of these being associated with more than one condition; however, it remains unclear as to whether the association between the various Single Nucleotide Polymorphisms (SNPs) and disease susceptibility can be explained biologically. Over the past years, we have performed various case-control studies in a rather genetically homogeneous population from Greece (1). Considering that most disease-associated SNPs are located in non-coding intronic or other genomic regions, we managed to identify the molecular mechanisms through which these gene polymorphisms lead to various autoimmune disease phenotypes, such as SLE, RA, PS, JIA, MS, T1D and PsA, by performing either sequence analysis or other functional experiments (2,3). Thus, research from our laboratory has successfully elucidated the functional role of *CD40* rs4810485, *PD1* rs11568821, *C1Q* rs292001, *IRF8* rs17445836 and various SNPs of *IRF5* gene. To better understand the putative role of the functional polymorphisms *VEGFR2* rs2305948 (V297I) implicated in SLE and *TYK2* rs34536443, involved in RA, PsA, JIA and endometriosis, we performed a structural biological study (4). The structural data suggested that rs2305948 of *VEGFR2* may cause impairment in cell signaling, thus contributing possibly to SLE pathogenesis, given that the V297I polymorphism may affect the efficiency of trans-autophosphorylation and cell signaling. Moreover, the location of the Pro1104Ala mutation on the 3-dimensional (3D) structure of TYK2 protein may affect the structural and dynamical elements of the molecule, considering that the introduction of a helical former alanine residue to replace the rather constrained helical breaker proline influences the secondary structure of this region due to alteration of the folding properties. Taken together, the integration of genomic data and construction of 3D protein models may provide additional insight into complex autoimmune diseases.

(1) Zervou M et al., Hum Immunol 69: 647-650, 2008; (2) Bertsias G et al., Arthritis Rheum 60: 207-218, 2009; (3) Vazgiourakis V et al., Ann Rheum Dis 70: 2184-2190, 2011; (4) Myrthianou E et al., Scand J Rheumatol 46: 180-186, 2017.

134

Shaping the genetic profile of endometriosis through the correlation of gene polymorphisms with protein functionality

George N. Goulielmos[1], Elias Eliopoulos[2], Michael Matalliotakis[1,3], Charoula Matalliotaki[1,3], Demetrios A. Spandidos[4], Maria I. Zervou[1]
[1]Section of Molecular Pathology and Human Genetics, Department of Internal Medicine, School of Medicine, University of Crete, Heraklion, Greece; [2]Department of Biotechnology, Agricultural University of Athens, Athens, Greece; [3]Third Department of Obstetrics and Gynecology, Aristotle University of Thessaloniki, Thessaloniki, Greece; [4]Laboratory of Clinical Virology, School of Medicine, University of Crete, Greece
E-mail: goulielmos@med.uoc.gr

Endometriosis is a complex gynecological disorder, affecting up to 10% of women of childbearing age, characterized by the presence of functional endometrial tissue at ectopic positions generally within the peritoneum. It is a heritable condition influenced by multiple genetic and environmental factors, with an overall heritability estimated at approximately 50%. However, the exact genomic basis of endometriosis remains unclear. Previous linkage and candidate genetic studies as well as genome-wide association studies (GWAs) and meta-analyses on endometriosis have led to the identification of disease-susceptibility loci implicated in various biochemical and cellular processes such as matrix remodeling, cell cycle regulation and signaling, cell adhesion, transcription regulation, inflammation, immunity, oxidative stress, hormone receptors and metabolism (1). In our laboratory, we have performed various case-control, as well as whole exome sequencing (WES) studies in Greece, aiming to replicate former findings and to detect new endometriosis-associated gene polymorphisms. Notably, genetic association and gene profiling studies provide important information for key molecules relevant to the disease but are less informative of protein-protein interactions, post-translational modification and regulation by targeted subcellular localization. To better understand the association detected of rs11556218 single nucleotide polymorphism (SNP) of the interleukin (*IL)-16* gene with endometriosis, we proceeded with the construction of 3-dimensional (3D) protein model aiming to gain additional insight regarding the functional importance of this SNP. Rs11556218 is located in the exon 6 region, leading to an amino acid change (Asn446Lys) on position 446 of the shorter isomorph 2 (631 amino acids) of Pro-IL-16, which may alter protein structure and function. IL-16 is a proinflammatory cytokine that plays a decisive role in most immune and inflammatory responses, as well as in the pathogenesis of endometriosis (2). Our results demonstrated that rs11556218 is associated with endometriosis in Greek women, probably by resulting in the aberrant expression of IL-16, as suggested by the bioinformatics analysis conducted on the SNP-derived protein sequences, which indicated a possible association between mutation and functional modification of Pro-IL-16 (3). Additionally, progress in next-generation sequencing provides the opportunity to search for less common variants with significant effects, thus assisting the structural/cellular biology results towards a better therapeutic treatment of women with endometriosis in clinical practice.

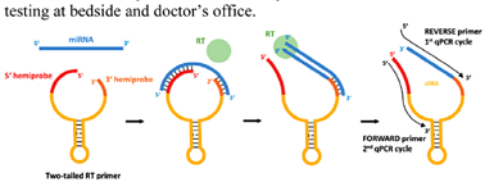
(1) Kobayashi H et al., Mol Med Rep 9: 1483-1505, 2014; (2) Mathy NL et al., Immunology 100: 63-69, 2000; (3) Matalliotakis M et al., Int J Mol Med 41: 1469-1476, 2008.

135

Two-tailed PCR and other ultrasensitive methods for the measurement of molecular cancer biomarkers

Mikael Kubista[1,2], Peter Androvic[2], Lukas Valihrach[2], Andrei Herdean[1], Alexandra Bergman[1], Robert Sjöback[1]
[1]TATAA Biocenter AB, Gothenburg 411 03, Sweden; [2]Laboratory of Gene Expression, Institute of Biotechnology CAS, Biocev, Vestec 252 50, Czech Republic
E-mail: mikael.kubista@tataa.com

We present a highly specific, sensitive and cost-effective system to quantify miRNA and for typing of cell-free DNA based on novel chemistry called Two-tailed PCR (1). Two-tailed PCR takes advantage of target-specific primers composed of two hemiprobes complementary to two different parts of the target molecule connected by a hairpin structure. The introduction of a short hemiprobe that senses the variable sequences confers exceeding sequence specificity while maintaining the very high sensitivity of PCR. Highly similar targets can be distinguished with superior precision, irrespective of the position of the mismatched nucleotide. Furthermore, the target molecule can be very short, rendering two-tailed PCR the preferred method for miRNA profiling, as well as for the analysis of rare sequence variants in cell-free DNA and formalin-fixed paraffin-embedded (FFPE) tissue. Two-tailed RT-qPCR has a dynamic range of 7 logs and a sensitivity sufficient to detect less than ten target miRNA molecules. Two-tailed PCR is readily multiplexed and for less challenging applications such as genotyping Two-tailed PCR can be applied directly on blood samples eliminating the need for extraction. This very smooth and friendly workflow is most suitable to testing at bedside and doctor's office.



(1) Two-tailed RT-qPCR: A novel method for highly accurate miRNA quantification. P Androvic, L Valihrach, J Elling, R Sjoback, M Kubista. Nucleic acids research 45 (15), e144-e144.

136

ALDH1A1: The novel biomarker of pancreatic cancer stem cells

Hong-Quan Duong
Laboratory Center, Hanoi University of Public Health, 1A Duc Thang, Bac Tu Liem, Hanoi, Vietnam

Gemcitabine is the front-line standard chemotherapy used for the treatment of pancreatic cancer; however, intrinsic and acquired resistance to gemcitabine commonly occurs and is still the major obstacle to the successful control of this disease. Aldehyde dehydrogenase 1A1 (ALDH1A1), one of the characteristic features of tumor initiating and/or cancer stem cell properties in multiple types of human cancer, is very important for both intrinsic and acquired resistance to chemotherapy. Since it is known that ALDH1A1 plays an important role in the resistance to chemotherapeutic agents in cancer, we investigated the important function of ALDH1A1 as the novel biomarker for gemcitabine resistance in pancreatic cancer stem cells. This study demonstrated that the combination of ALDH1A1 inhibition and gemcitabine significantly decreased cell survival by the induction of apoptotic cell death and cell cycle arrest at the S-phase. This study also demonstrated that in the gemcitabine-resistant MIA PaCa-2 cells (MIA PaCa-2/GR), the levels of ALDH1A1 expression and SRC phosphorylation were significantly increased compared to the parental MIA PaCa-2 cells (MIA PaCa-2/P). Furthermore, the combination of ALDH1A1 or SRC inhibition and gemcitabine synergistically decreased cell survival by the induction of apoptotic cell death. Importantly, the combination of SRC inhibition and gemcitabine significantly decreased the levels of ALDH1A1 expression, emphasizing the importance of ALDH1A1 for gemcitabine resistance in pancreatic cancer. This study defined that ALDH1A1 is involved in both intrinsic and acquired resistance to gemcitabine and the inhibition of ALDH1A1 may be a novel effective strategy with which to overcome the resistance of pancreatic cancer stem cells to gemcitabine.

Key words: Aldehyde dehydrogenase-1A1 (ALDH1A1), chemoresistance, gemcitabine, pancreatic cancer, SRC, cancer stem cells

137

On the way to cytokine-antibody single-chain fusions for cancer immunotherapy

J. Tomala[1], S. Ludwig[2], H. Yang[2], M. Leff[2], J. Spangler[2], M. Kovar[1]
[1]Laboratory of Tumor Immunology, Institute of Microbiology CAS, Prague, Czech Republic; [2]Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD, USA
E-mail: tomala@biomed.cas.cz

Interleukin-2 (IL-2) is a multifunctional cytokine that is able to potently stimulate immune effector cells (e.g., $CD8^+$ T and NK cells). Unfortunately, its concurrent promotion of regulatory T cells (T_{reg}) and harmful off-target effects have limited its clinical efficacy. Boyman *et al.* (1) reveal methods with which to mitigate these issues by complexing mouse IL-2 to anti-IL-2 mAb S4B6. These IL-2 complexes are superior to free IL-2, they manifest selective stimulatory activity for memory $CD8^+$ T and NK cells and possess significant antitumor activity. However, the potential clinical use of these complexes is limited due to the mouse origin of IL-2 and the dissociation of the complexes at low concentrations. Based on our previous studies, we designed, engineered and produced translationally relevant protein chimera (immunocytokine, IC) consisting of hIL-2 linked to light chain of anti-hIL-2 mAb through a flexible oligopeptide spacer, functionally similar to S4B6 mAb, which circumvent disadvantages of IL-2/S4B6 mAb complexes and exerts sufficient biological activity. We demonstrate that this IC we produced contained both IL-2 and mAb in a single molecule and IL-2 interacted with binding site of mAb. We also demonstrate its biophysical characteristics related to IL-2 receptor and its biological activity *in vitro* and *in vivo*.

1) O. Boyman, M. Kovar, M.P. Rubinstein, C.D. Surh, J. Sprent, Selective stimulation of T cell subsets with antibody-cytokine immune complexes. *Science* **311**, 1924-1927 (2006).

138

Polymer prodrugs for the treatment of experimental solid tumors and immunomodulation

Milada Šírová[1], Iva Malátová[1], Klára Hrabánková[1], Ladislav Sivák[1], Blanka Říhová[1], Martin Studenovský[2], Petr Chytil[2], Tomáš Etrych[2]
[1]Laboratory of Tumor Immunology, Institute of Microbiology of the CAS v.v.i., Prague; [2]Division of Biomedical Polymers, Institute of Macromolecular Chemistry of the CAS v.v.i., Prague, Czech Republic
E-mail: sirova@biomed.cas.cz

Polymer drug delivery systems represent a promising strategy for efficient tumor treatment without severe systemic toxicity. The conjugation of a drug to a synthetic polymer carrier, such as water-soluble *N*-(2-hydroxypropyl) methacrylamide (HPMA) copolymer, allows for tumor-targeted drug delivery via the Enhanced Permeability and Retention (EPR) effect. The HPMA conjugates can be created with various architecture, molecular weight, tunable drug content and controlled drug release. They exhibit an extended circulation time, preferential accumulation in solid tumor tissue, and limited side toxicity of the drug. Complete tumor regression and long-term resistance against the disease was documented in murine syngeneic tumors, such as EL4 T cell lymphoma or CT26 colon carcinoma. Tumor re-challenge of the cured animals provided conclusive evidence of the anticancer immune responses. The conjugates act as endogenous vaccines capable to amplify the anti-cancer immune responses. Importantly, the HPMA copolymers were successfully explored as carriers of various active agents suitable for modulating the tumor microenvironment (TME). Copolymers decorated with organic nitrates were prepared as polymer donors of nitric oxide (NO) with the aim to achieve the tumor-selective accumulation and local generation of NO. *In vivo*, the conjugates potentiated the accumulation of co-administered macromolecular cancerostatics, leading to a better therapeutic outcome. The effect was not observed with the parent low-molecular weight drug (doxorubicin), pointing to the enhanced EPR effect as the main mechanism of action. HPMA copolymer conjugates carrying drugs, suitable for dampening the suppressive activity of myeloid-derived suppressor cells (MDSCs), such as all-trans retinoic acid (ATRA) or cucurbitacin D, have been also studied. In conclusion, the HPMA copolymers may be beneficial as drug delivery systems for the targeted chemotherapy of tumors, as well as for TME modulation.

Supported by Czech Science Foundation (17-08084S), and Ministry of Health of the Czech Republic (16-28600A).

139

Micellar polymer drug delivery systems for the treatment of chemoresistant tumors

Martin Kaňa[1,3], Alena Braunová[2], Tomáš Etrych[2], Jan Bouček[3], Milada Šírová[1]
[1]Institute of Microbiology, Prague; [2]Institute of Macromolecular Chemistry, Prague; [3]Faculty Hospital Motol, Prague, Czech Republic
E-mail: martin.kana@biomed.cas.cz

Polymeric nanotherapeutics have been used as targeted delivery systems of anticancer drugs, improving their biodistribution and decreasing systemic toxicity. Amphiphilic diblock copolymer conjugates based on hydrophilic N-(2-hydroxypropyl)methacrylamide (HPMA) and hydrophobic poly(propylene-oxide) (PPO) were evaluated as an effective system enabling the prolonged circulation of the cytostatic drug (doxorubicin) in the blood, its high accumulation and controlled release in the target solid tumor tissue. In addition, PPO was able to inhibit ABC transporters, namely P-glycoprotein (P-gp), the upregulation of which is the common mechanism of tumor multidrug resistance (MDR). We verified the ability of the HPMA-PPO diblock copolymer to inhibit MDR *in vitro* in Dox-resistant P388/MDR and CT26 cancer cell lines, both overexpressing P-pg. However, our results suggested that the inhibitory activity of HPMA-PPO copolymer was dependent on its physico-chemical characteristics, including the presence of protecting (Boc) groups, residual amounts of HPMA linear chains or triblock (HPMA-PPO-HPMA) copolymers. In particular, the unbound PPO significantly increased the ability of the tested polymer samples to inhibit ABC transporters. To further increase the inhibitory activity of the diblock copolymer, biodegradable disulfide bond (S-S) was incorporated between hydrophylic and hydrophobic blocks. Furthermore, the Diblock-doxorubicin conjugates exhibited a high cytotoxic activity *in vitro*, as well as a high therapeutic efficacy *in vivo* in the CT26 colon carcinoma MDR model. In conclusion, the diblock HPMA-PPO drug carrier appears to be a promising delivery system that should be further studied as a potential treatment of chemoresistant tumors.

140

MMP-9 serum levels and *2127G>T* intron 4 polymorphism in patients with cutaneous melanoma

Tatyana Vlaykova[1], Tanya Tacheva[1], Ekaterina Vachkova[2], Denitsa Vlaykova[1], Georgi Beev[3], Dimo Dimov[1],
[1]Medical Faculty, Trakia University, Stara Zagora, Bulgaria; [2]Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria; [3]Agriculture Faculty, Trakia University, Stara Zagora, Bulgaria
E-mail: tvlaykov@mf.uni-sz.bg

Gelatinase B (MMP-9) is a member of the large family of zinc-dependent proteases, which are able to break down the extracellular matrix (ECM) proteins and to process many other cell surface proteins involved in the regulation of processes, such as inflammation, cell proliferation, survival and apoptosis. There is emerging knowledge about the role of MMP-9 in the progression, aggressiveness and spreading of a variety of cancers, including melanoma. The expression of MMP-9 is influenced by several inflammatory cytokines and growth factors, epigenetic modifications and genetic polymorphisms. However, there is still limited evidence about the effects of genetic variants in MMP-9 gene on the risk and clinical course of melanoma. Thus far, to the best of our knowledge, only one study has reported significant associations of SNPs in MMP-9 with the progression of cutaneous malignant melanoma (Q279R rs2664538, P574R rs2250889, and R668Q rs2274756). In the current study we aimed to explore the possible effect of 2127G>T SNP in intron 4 of the *MMP-9* gene (rs2274755) and to determine the role of the serum levels of the enzyme in the development and clinical course and outcome of skin malignant melanoma. Genotyping was performed by allele-specific TaqMan assay and serum levels were measured by ELISA. The genotype distributions of MMP-9 2127 G>T SNP varied significantly between the patients and controls (P=0.012), as the common G allele homozygosity was associated with a 3.245-fold risk of melanoma compared to the any other genotypes (OR=3.245, 95% CI 1.387-7.592, P=0.007). At the same time, patients with the GG genotype tended to have a longer DFS (mean of 209.6 vs. 65.5 mo, P=0.069) and a longer survival after diagnosis (mean of 189.12 vs. 74.99 months, P=0.103, log rang test). The serum level of MMP-9 was significantly lower in the patients than the controls [13.39±0.85 vs. 10.83±0.71 (SEM) ng/ml, P=0.024]. The values below the cut-off of 14 mg/ml assessed by ROC curve analysis (AUC=0.627, p-0.036) determined skin melanoma with 76.3% sensitivity and only 47.4% specificity. The patients with serum levels of MMP-9 lower than 14 mg/ml, however, had a significantly longer DFS (206.57 vs. 64.33 months, P=0.003) and a longer survival after diagnosis (181.62 vs. 61.37 mo, P=0.005). There was no association between the genotypes and serum levels of MMP-9. In conclusion, the results of this study suggest that the GG genotype of MMP9 2127 G>T SNP may be a risk factor for development of skin melanoma, but may favor the survival of the patients. The higher serum levels of MMP-9 are possibly an unfavorable biomarker for the early recurrence of the disease and for a shorter survival of melanoma patients.

Acknowledgements: The work was financially supported by projects 4/2016 and 1/2018 MF, TrU.

141

Serum 25-hydroxyvitamin D levels in patients with COPD

Dimo Dimov[1], Tanya Tacheva[2], Yanitsa Zhelyazkova[2], Emil Slavov[3], Denitsa Vlaykova[1], Tatyana Vlaykova[1]
[1]Department of Health Care, Faculty; [2]Department of Chemistry and Biochemistry; [3]Department of Biology, Immunology and Medical Genetics, Medical Trakia University, Stara Zagora, Bulgaria
E-mail: dmdimov65@yahoo.com

Over the past decades, vitamin D and its hormonally active form, 1,25-dihydroxyvitamin D, have attracted increasing attention, not only due to their function in calcium and phosphorous homeostasis and bone remodeling, but also due to their pleiotropic roles and extra-skeletal effects. In spite of a number of studies being published on this topic, the associations of serum vitamin D form (25-hydroxyvitamin D, 25(OH)D) with the risk and the clinical course of chronic obstructive pulmonary disease (COPD) remain to be fully established. In this respect, we aimed to assess the associations of serum 25-hydroxyvitamin D levels with several blood and clinical characteristics of Bulgarian patients with COPD in order to explore the possible role of the serum vitamin D as a biomarker in this disease. We measured 25(OH)D levels in 45 patients and 19 control individuals, all from the region of Central-South Bulgaria using a commercial kit of Roche Diagnostics Deutschland GmbH. The serum levels of 25(OH)D of patients with COPD were significantly lower than those of the controls [17.43±1.01 vs. 45.39±4.19 (SEM) ng/ml, P<0.0001]. The levels of the patients depended on the season when the blood samples were obtained: They were higher for the period May-September (23.45±0.97 ng/ml) compared to the period October-April (16.13±2.77 ng/ml, P=0.013). The 25(OH)D levels were inversely associated with the number of white blood cells (Rho=-0.415, P=0.009), neutrophils (Rho=-0.359, P=0.025) and lymphocytes (Rho=-0.325, P=0.043). In patients with severe and very severe COPG (GOLD 3 and 4), there were strong significant positive correlations between the serum vitamin D and lung function spirometric indexes FEV1 % pr. (Rho=0.728, P=0.002) and FVC% (Rho=0.584, P=0.022). In conclusion, the observed inverse correlations in COPD patients of serum 25-hydroxyvitamin D levels with blood inflammatory cells confirm the suggested before immunomodulatory effect of vitamin D. In addition, the positive correlations with the spirometric indexes of patients with severe COPD, propose a role of vitamin D in improving the lung function. Although with several limitations, the current study suggest that the serum 25-hydroxyvitamin D levels may be a useful biomarker for COPD.

Acknowledgements: The study was financially supported by projects 3/2018 MF, TrU and Infrastructural University project 3/2017, TrU.

142

Immunobiological changes in human colorectal cancer and 3D cancer modelling *in vitro*

Pavol Lukac[1,3], Paolo Tenti[1,3], Fabian Caja[1,3], Dmitry Stakheev[1], Jan Svoboda[1], Oleksandr Chernyavskiy[2], David Vondrasek[2], Lenka Rajsiglova[1,3], Pavol Makovicky[4], Miroslav Levy[5], Peter Makovicky[6], Radislav Sedlacek[6], Luca Vannucci[1]
[1]Institute of Microbiology and [2]Institute of Physiology of the CAS, v.v.i. Prague, Czech Republic; [3]Faculty of Science, Charles University, Prague, Czech Republic; [4]Department of Biology, Pedagogical faculty, Selye Janos University, Komarno, Slovak Republic; [5]Thomayer's Teaching Hospital, Prague, Czech Republic; [6]Czech Centre for Phenogenomics, Institute of Molecular Genetics of the CAS, v.v.i., Vestec, Czech Republic

The tumor stroma is an important modulator of cancer cell behaviour. Upon the examination of colorectal cancer specimens from patients by 2-photon microscopy (second-harmonic generation imaging - SHG), we found differences in the stroma organization of mucosa at a distance from the tumor (apparently normal), near the tumor border (transitional) mucosa and tumor. The analysis of mucosa proteins by RT-qPCR revealed the progressive increase in the expression of COL1A1, IL-1β, IL-13 and LOXL2, all involved in tissue remodelling. IL-6 expression exhibited an increase, particularly at the transition from the mucosa to the tumor in association with a higher inflammatory cell infiltrate. IL-6 participation was also immunohistochemically evident. Notably, both PD-1 and PD-L1 expression was increased in the transition mucosa and in the tumor. To model *in vitro* the tumor development, 3D cultures of colorectal tumor cells as spheroids are undergoing. They appear useful for investigating the modality of the expression of PDL-1 and PD-1immune check-point ligand and receptor, respectively, in complex cultures, including fibroblasts. This will help to elucidate their expression during tumor evolution and evaluate immune escape mechanisms in the early stages of cancer development. Spheroids of colorectal cancer cells appear very promising for aiding in the analysis of microenvironment events under controlled system and conditions.

Acknowledgements: Grants RVO 61388971 (CZ), Generali/Czech Insurance Foundation a.s. (CZ), UniCredit Bank s.r.o., Prague (CZ), Di Cursi Donation (CZ), AERO s.r.o. (CZ), Eurinox s.r.o (CZ) and ARPA Foundation (IT).

143

In vitro and *in vivo* evaluation of ferric and ferritin-based nanoparticles from the theranostic perspective

Lenka Rajsiglova[1,5], Pavol Lukac[1,5], Paolo Tenti[1,5], Michal Babic[2], Daniel Jirak[3], Peter Makovicky[4], Radislav Sedlacek[4], Elisabetta Falvo[6], Pierpaolo Ceci[6], Luca Vannucci[1]
[1]Institute of Microbiology, and [2]Institute of Macromolecular Chemistry of the CAS, v.v.i.; [3]Institute of Clinical and Experimental Medicine - IKEM, Prague, Czech Republic; [4]Czech Centre for Phenogenomics, Institute of Molecular Genetics of the CAS, v.v.i., Vestec, Czech Republic, [5]Faculty of Science, Charles University, Prague, Czech Republic; [6]CNR, Rome, Italy

With the progress of nanomedicine, a variety of nanoparticles (NPs) have been widely studied and applied in biomedicine. Depending on their unique properties, NPs have become a hotspot in theranostic approaches. Despite their growing use as carriers in targeted localization, MRI and/or thermotherapy, their possible short and long-term toxic and immunotoxic effects warrant further investigation. In this study, tumour and non-tumour cell lines (CT26 colorectal carcinoma and 3T3 fibroblasts) were challenged with 2 types of NPs. γ-Fe2O3-based NPs (with or without nickel) (polymer coated/naked) were tested *in vitro* for effects on cell viability (MTT, crystal violet assay) and also on apoptosis and ROS production (FACS). Their effect on immune cells *in vivo* was evaluated by FACS analysis on murine splenocytes. Preliminary data revealed a reduced viability in some *in vitro* conditions in all methods; however, almost no significant changes were observed 24 h following administration *in vivo*. The only exception was a slightly reduced percentage of NK/NKT cells and the mild activation of B lymphocytes in the spleen. Another type of NPs (ferritin NPs loaded with chemotherapeutics) was used in a murine model of colorectal carcinoma (Balb/c, CT26), both alone and combined with anti-PD1 antibodies. The monitoring of tumour growth, body weight and survival of the animals suggest a possible advantage of NPs.

Acknowledgements: Grants RVO 61388971 (CZ), UniCredit Bank s.r.o. (CZ), Generali CEE Holding B.V. (CZ), PPT ABRASIVES s.r.o. (CZ), Italinox a.s. (CZ), Lucia and Mario Pini Donation (CZ) and ARPA Foundation (IT).

144

Lysyl oxidases and the formation of the early tumoral niche

Paolo Tenti[1,2], Lenka Rajsiglova[1,2], Pavol Lukac[1,2], Luca Vannucci[1]
[1]Institute of Microbiology of the CAS, v.v.i. Prague, Czech Republic; [2]Faculty of Science, Charles University, Prague, Czech Republic
E-mail: paolo.tenti@biomed.cas.cz

The lysyl oxidases (LOXs) are a family of enzymes deputed to cross-link collagen and elastin, shaping the structure and strength of the extracellular matrix (ECM). Additional functions have also been recently described, suggesting a multifaceted role of LOXs within a complex network of signals regulating a number of cell functions, including survival/ proliferation/ differentiation. Among these signaling pathways, TGF-β and PI3K/Akt/mTOR, in particular, cross-talk extensively with each other and with LOXs also initiating complex feedback loops. According our preliminary data, the tissue microenvironment remodelling begins early, already at the beginning of the carcinogenesis process, as a result of the altered balance of pro-inflammatory and regulatory signals and altered colon mucosa homeostasis. In particular, the thickening of the collagen scaffold and the increase in the tissue stiffness is largely dependent on LOXs activity. Tissue stiffening is a well-known mechanism leading to epithelial-to-mesenchymal transition and metastatic tumoral progression. While the LOX association with advanced and metastatic cancer has been well established, there is suffiicient experimental evidence to also support a significant role of LOXs in promoting the transformation of normal epithelial cells. Working on a mouse experimental model of colitis-induced colorectal carcinoma, we are aiming to better define the role of LOXs in the early establishment of the tumor microenvironment and the formation of the early tumoral niche, also aiming to elucidate the network with crucial signaling pathways possibly involved, e.g. PI3K/Akt/mTOR, IL-13, non-canonical TGF-ß pathways.

Acknowledgements: Grants RVO 61388971 (CZ), UniCredit Bank s.r.o. (CZ), Generali CEE Holding B.V. (CZ), Roberto Di Cursi Fund (CZ), Paul's Bohemia (CZ) and ARPA Foundation (IT).

145

Adjuvant, local radiotherapy effects on circulating immune response parameters in breast cancer patients

Nongnit Laytragoon Lewin[1], Thitiya Luetragoon[2], Levar Shamoun[1], Bengt-Åke Andersson[1], Delmy Oliva[3], Michael Strandeus[3], Sture Löfgren[1], Lars-Erik Rutqvist[4], Freddi Lewin[3]
[1]Department of Laboratory Medicine, Ryhov Hospital, Sweden; [2]Department of Medical Technology, Naresuan University, Thailand; [3]Department of Oncology, Ryhov Hospital, Sweden; [4]Swedish Match AB, Sweden

Radiotherapy (RT) is commonly used for cancer treatment. RT induces DNA damage, cell cycle arrest and cell death. The influence of adjuvant local RT on systemic immune response cell numbers and phenotypes was investigated in female breast cancer patients. Peripheral blood from 93 operated female breast cancer patients, before and directly after 50 gy adjuvant local RT was investigated. The total number and phenotype of white blood cell subpopulations were used as the biomarkers of systemic innate and adaptive immune response in these patients. Decreasing circulating numbers of lymphocytes, $CD3^+$, $CD4^+$ and $CD56^+$ cells were detected in the breast cancer patients. Increasing numbers of immunosuppressive neutrophils and monocytes, $CD13^+$ $CD56^+$ cells were also detected. Following adjuvant local RT, the total numbers of white blood cells, lymphocytes, neutrophils and monocytes decreased without any influence on $CD13^+$ $CD56^+$ cells. It has shown that the neutrophil to lymphocytes ratio (NLR) can indicate a poor prognosis and a short survival time for cancer patients. In spite of decreased numbers of circulating cell numbers, the NLR of the patients was significantly increased following RT. Thus, states of immunosuppression persist in breast cancer patients despite the removal of the visible tumour. Adjuvant RT alters cell numbers and phenotypes of circulating immune response cells. This may be the consequence of systemic immunosuppression or re-distribution of the immune response cells from circulation to local radiation site.

146

Changes in normal brain ECM upon anti-glioblastoma chemotherapy are associated with experimental tumour growth *in vivo*

Mariia A. Kolpakova[1,2], Alexandra Y. Tsidulko[1], Oleg B. Shevelev[3], Anna S. Dotsenko[3], Anastasia V. Suhovskih[1,2], Evgeniy L. Zavjalov[3], Elvira V. Grigorieva[1,2]
[1]Institute of Molecular Biology and Biophysics FRC FTM, Novosibirsk; [2]Novosibirsk State University, Novosibirsk; [3]Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia
E-mail: kolpakovamariia97@gmail.com

Glioblastoma multiforme (GBM) is characterised by a low patient survival mainly due to frequent relapse, in spite of intensive adjuvant chemotherapy with temozolomide (TMZ) and dexamethasone (DXM). As systemic drugs, TMZ and DXM affect not only tumour cells, but also the surrounding normal brain tissue, which may contribute to the disease relapse. The purpose of this study was to investigate the effects of TMZ and DXM therapy on the tumour growth rate in the GBM relapse model. To model the GBM relapse *in vivo*, TMZ (150 mg/m^2) and/or DXM (1 mg/kg) were administered to SCID mice for 6 weeks following the inoculation of GBM U87 cells into the brain. According to MRI, the tumour growth rate in the mice that received TMZ and/or DXM before the U87 glioma cell inoculation was significantly higher compared with that of the control group, particularly for TMZ/DXM combination. Co-culture of the U87 cells with organotypic hippocampus slices *ex vivo* pre-treated with TMZ and/or DXM resulted in the increased proliferation and invasion of the tumour cells compared with the untreated slices. In addition, the TMZ and/or DXM treatments led to significant changes in the expression of key brain ECM components, such as proteoglycans both at the core proteins and polysaccharide chains levels in experimental models *in vivo* and *ex vivo*. In summary, we have shown that TMZ and DXM impair the ability of normal brain tissue to resist tumour growth by changing its ECM structure and composition what may be a possible molecular mechanism of GBM relapse.

Acknowledgements: This work was funded by Russian Science Foundation (RSF grant 16-15-10243). Tsidulko A.Y. was supported by scholarship of Russian Federation President for young scientists (SP-5435.2018.4).

147

Combined photoacoustic and fluorescence label-free microscopy for the *ex vivo* investigation of ocular melanotic lesions

George J. Tserevelakis[1], Kostas G. Mavrakis[1], Efstathios T. Detorakis[2], Danai Pantazopoulou[1], Eleni Karamouzi[1], Eleni Lagoudaki[2], Giannis Zacharakis[1]
[1]Foundation for Research and Technology Hellas, Institute of Electronic Structure and Laser, Heraklion, Crete, Greece; [2]University of Crete, School of Medicine Heraklion, Crete, Greece
E-mail: detorakis@hotmail.com

This study examined the use of an extended field-of-view microscope, combining photoacoustic and fluorescence label-free contrast modalities, for the *ex vivo* investigation of ocular melanotic lesions of the conjunctiva and choroid, surgically excised from human eyes. Examined specimens included both benign (naevi) and malignant (melanoma) lesions. Human bioptic samples had been preserved in paraffin which was removed prior to imaging by a standard deparaffinization and re-hydration process (1,2). A custom-developed hybrid microscopy setup was used, integrating two distinct excitation paths, each of them dedicated for the autofluorescence and photoacoustic imaging mode respectively. The autofluorescence imaging path employed a compact CW diode-pumped laser module emitting at 450 nm, as an excitation source. Back-scattered fluorescence radiation was transmitted through a dichroic mirror. Photoacoustic waves were detected by a 20 MHz central frequency spherically focused ultrasonic transducer, immersed into a tank in a confocal and coaxial configuration with respect to the optical focus. Biopsy samples presented a remarkable spatial overlap of the two signals in the nevus region, indicating a positive correlation between them. The bimodal microscopy approach presented in this study has the potential to contribute in the differentiation between benign and malignant intraocular tumors of the uvea and conjunctiva in surgical biopsies. Future systems may incorporate photoacoustic capabilities for *in vivo* observations, adding to existing methods of clinical differential diagnosis.

(1) Tserevelakis et al. Hybrid photoacoustic and optical imaging of pigments in vegetative tissues. *J. Microsc* 263: 300-306, 2016.
(2) Tserevelakis et al. Delineating the anatomy of the ciliary body using hybrid optical and photoacoustic imaging. *J Biomed Opt* 22: 60501, 2017.

148

Metformin induces caspase-independent apoptosis in human bladder carcinoma T24 cells

Ji Hoon Jang, Tae-Jin Lee, Eon-Gi Sung, Joo-Young Kim
Department of Anatomy, College of Medicine, Yeungnam University, Nam-Gu, Daegu 42415, South Korea
E-mail: jookim@med.yu.ac.kr

Bladder cancer is the sixth most common type of cancer among males worldwide. However, the chemotherapy of this type of cancer is associated with various side-effects. Metformin is well-known for inducing the apoptosis of a number of types of cancer *in vitro*. Furthermore, it is a common anti-diabetic agent used for the treatment of type 2 diabetes mellitus. However, to date, to the best of our knowledge, there are no studies available reporting the molecular mechanisms involved in metformin-induced apoptosis in bladder carcinoma. Thus, these remain to be elucidated. In the present study, treatment with metformin induced apoptosis in human bladder carcinoma T24 cells in a dose-dependent manner. We demonstrated that the degradation of cellular FADD-like interleukin-1-converting enzyme (FLICE)-like inhibitory protein (c-FLIP) was associated with metformin-mediated apoptosis. By contrast, benzyloxycarbonyl-Val-Ala-Asp-fluoromethyl ketone (z-VAD-fmk, a pan-caspase inhibitor) and N-acetyl-L-cysteine (NAC), a reactive oxygen species (ROS) scavenger did not inhibit metformin-induced apoptosis and the degradation of c-$FLIP_L$ protein. Notably, we found that c-$FLIP_L$ protein expression was downregulated by the decreased protein stability of c-$FLIP_L$ in metformin-treated T24 cells. In addition, apoptosis inducing factor (AIF) was released from the mitochondria and translocated to the nucleus. Taken together, these results suggested that metformin-induced apoptosis was regulated by the AIF-mediated caspase-independent pathway in T24 cells, and metformin may thus be a potential agent for the treatment of bladder cancer.

Key words: Bladder cancer, Metformin, Apoptosis, c-FLIP, AIF

149

Reduction of CFU-GM and circulating hematopoietic progenitors in a subgroup of children with chronic autoimmune neutropenia associated with severe infections and delayed recovery

Fabio Timeus[1], Nicoletta Crescenzio[2], Luiselda Foglia[2], Alessandra Doria[2], Maria Giuseppina Stillitano[2], Stefano Vallero[1], Ugo Ramenghi[2], Paola Saracco[2]
[1]Pediatric Onco-Hematology, Regina Margherita Children Hospital, Turin; [2]Pediatric Hematology, University of Turin, Italy
E-mail: fabio.timeus@unito.it

Myelopoiesis was evaluated in 66 pediatric patients with chronic neutropenia who were positive for anti-neutrophil antibodies (median age at diagnosis: 11 months, median neutrophil count at diagnosis: 419/µl). Other causes of neutropenia were excluded. Bone marrow morphology, clonogenic tests and/or the peripheral blood CD34$^+$ cell count and the apoptotic rate were evaluated in 61 patients with neutropenia lasting or >12 months or with severe infections. The circulating CD 34$^+$ cell count and apoptotic rate were evaluated in 5 patients with neutropenia which lasted for a shorter period of time. The median follow-up time was 29 months (range, 7-180 months). Forty-seven patients (71.2%) had a spontaneous recovery after 7-180 months (median, 29 months). The group of patients younger than 24 months at diagnosis (n=50) had a higher probability of recovery (40/50 vs. 7/16 χ^2 p<0.01) with a shorter period of neutropenia (median 26 vs. 47 months, Kaplan-Meier analysis, p=0.001). The CFU-GM was significantly decreased in 26/35 patients (74%) evaluated for clonogenic tests. All patients with normal CFU-GM recovered (9/9 patients), whereas neutropenia persisted in 12/26 patients with reduced CFU-GM (46%, Pearson's χ^2 p=0.02). In 36/55 (65%) patients evaluated by flow cytometry, we observed reduced circulating CD34$^+$ cells compared with the controls of the same age. An increase in the circulating CD34$^+$ cell apoptotic rate was observed in 28/55 patients (51%). Infections requiring hospitalization were observed in 9/18 (50%; Pearson's χ^2, p=0.03) of patients with both decreased circulating CD34$^+$ cells and increased CD34$^+$ apoptotic rates. In the group aged <24 months, we observed a significant correlation between the persistence of neutropenia and a decreased number of circulating CD34$^+$ cells (Pearson's χ^2 p=0.008). In conclusion, reduced CFU-GM and circulating hematopoietic progenitors were observed in a subgroup of children with chronic neutropenia who were positive for anti-neutrophil antibodies and had a higher incidence of severe infections and delayed spontaneous remission.

150

Comparison of the anti-atopic dermatitis effect of DHMEQ and tacrolimus ointments in a mouse model without stratum corneum

Huan He[1], Xiaoxiao Gao[1], Xiaomin Wang[1], Xin Li[1], Xiaoxue Jiang[1], Zhehui Xie[1], Jun Ma[2], Kazuo Umezawa[3], Yuyang Zhang[1]
[1]School of Life Science and Biopharmaceutics, Shenyang Pharmaceutical University, Shenyang, P.R. China; [2]Department of Research and Development; Shenzhen Wanhe Pharmaceutical Co., Ltd., Shenzhen, P.R. China; [3]Department of Molecular Target Medicine, Aichi Medical University School of Medicine, Nagakute, Japan
E-mail: 13614053862@163.com

In previous studies, it was demonstrated that DHMEQ improves DNCB/OX-induced atopic dermatitis-like lesions (1). This study focused on the anti-dermatitis effect of DHMEQ in a mouse model without stratum corneum and the comparison of the effect with that of tacrolimus. Six-week old BABL/C female mice were purchased and randomly divided into the Normal Group, Vehicle, DHMEQ (0.1%), and Tacrolimus (0.1%) group. Atopic dermatitis-like lesions in BABL/C mice were induced by the repeated use of 2,4-dinitrochlorobenzene (DNCB) and oxazolone (OX) on the surface of mouse ears, while medical tape was additionally used to disrupt the stratum corneum, which aimed to accelerate the development of AD-like lesions (2). The mice were then treated externally with DHMEQ ointment and tacrolimus ointment, and the efficacy of the drugs was compared. The results revealed that DHMEQ and tacrolimus significantly improved the dermatitis symptoms of the mice with DNCB/OX-induced AD-like lesions. They also reduced epidermis and dermis thickness and the number of mast cells. However, tacrolimus resulted in a significant decrease in body weight after long-term application. Both drugs significantly inhibited the production of serum total IgE and the expression of the inflammatory factors, IL-4, IL-6, IL-13, IL-1β and interferon (IFN)-γ as well. However, the behavior of the mice treated with tacrolimus was altered, with the mice behaving irritably with jumping movements. In addition, marked inflammatory exudation on the lesioned-skin surface of the mice was found. By contrast, DHMEQ did not result in any adverse responses. Collectively, DHMEQ was found to be safer, gentler and more suitable for long-term use than tacrolimus for the treatment of atopic dermatitis-like lesions.

(1) Jiang X et al., Immunopharmacol Immunotoxicol 39: 157-164, 2017.
(2) Duan J et al., Exp Dermatol 21: 448-452, 2012.

151

Specific hydroxycinnamic acid derivatives synergize with the polyphenol carnosic acid against acute myeloid leukemia cells

Aviram Trachtenberg[1], Katarzyna Sidoryk[2], Suchismita Muduli[1], Somaya Alreate[1], Marcin Cybulski[2], Michael Danilenko[1]
[1]Department of Clinical Biochemistry & Pharmacology, Ben-Gurion University of the Negev, Beer Sheva, Israel; [2]Chemistry Department, Pharmaceutical Research Institute, Warsaw, Poland
E-mail: aviramtr@post.bgu.ac.il

Acute myeloid leukemia (AML) is a devastating blood malignancy characterized by unrestrained proliferation of leukemic blasts. We have previously shown that the combination of curcumin (CUR) and carnosic acid (CA) synergistically induces massive Ca^{2+}-dependent apoptosis in human AML cells both *in vitro* and *in vivo*, without affecting normal hematopoietic cells. In this study, we synthesized a series of hydroxycinnamic acid derivatives, such as hydroxybenzylideneacetones and methyl hydroxycinnamates, and screened these compounds for the ability to cooperate with either CUR or CA in producing antileukemic effects. For all eight hydroxycinnamic acid derivatives tested the growth and viability of AML cells was reduced in a dose-dependent manner. Evaluation of the structure-activity relationship of these compounds in combination with CUR or CA revealed that none cooperated with CUR and that only methyl 4-hydroxycinnamate (KS-3) and methyl 3-methoxy-4-hydroxycinnamate (KS-6) had the ability to synergize with CA to induce a strong and rapid (4-8 h) apoptotic effect, along with a dramatic reduction in cell numbers and viability following 72 h. In addition, we observed striking similarities between the antileukemic features of KS-3+CA and CUR+CA in that the apoptotic effect of both combinations was caused by cytosolic Ca^{2+} accumulation and was not accompanied by elevated ROS levels. By contrast, KS-3+CA had no cytotoxicity in normal peripheral blood mononuclear cells. Thus, we identified new effective combinations of phenolic agents that specifically kill AML cells in a CUR+CA-like mechanism. Importantly, this effect strongly depends on both the position of the hydroxyl group on the aromatic ring and the modification of the carbonyl group in a hydroxycinnamic acid derivative. Synergistically acting combinations of specific phenolic compounds may provide a prototype of novel efficacious and safe therapeutics for AML treatment, particularly, in elderly or unfit patients.

Financial support: Supported by the Israel Science Foundation Grant 226/16.

152

Pharmacogenetics and pharmacogenomics in precision pharmacotherapy

Nikolaos Drakoulis
Clinical Pharmacologist, Research Group of Clinical Pharmacology and Pharmacogenomics, Faculty of Pharmacy, National and Kapodistrian University of Athens, Athens, Greece

From the beginning of the eighties, scientists worldwide have been trying to develop approaches to individualize drug therapy. Soon, high pressure liquid chromatography and gas chromatography were replaced by molecular genetic methodologies. Thus, personalized medicine through genetics can be broadly classified in four general categories with respect to appropriate selection of medications used to treat patients: i) Identification of patients at risk for toxicities associated with poor drug metabolism; ii) identification of toxicities unrelated to dosing; iii) identification of patients who are unlikely to respond to treatment with a particular agent; iv) identification of patients who would be predicted to have a good response to a particular drug with little risk of toxicity. Genetic diversity, including the individual risk of disease, can be often defined by the occurrence of single nucleotide polymorphisms (SNPs) within genes. Most known SNPs probably have little or no effect on gene expression or protein activity. Of critical importance in determining risk of disease are the rarer *functional SNPs* that affect gene expression, alternative splicing patterns and the catalytic activity or binding properties of the gene product. The combination of these *functional polymorphisms* in a number of key genes interacting with environmental factors, determine both the risk of developing common diseases and how patients will respond to treatment. They are actually biological markers. A *Biomarker* is any substance, structure or process that can be measured in the body or its products and influence or predict the incidence of outcome of disease or pharmacotherapy. In addition to personalized medicine through genetics, the use of biomarkers allows physicians to answer important questions related to disease and therapeutic approach. Thus, the ability to determine a *Biomarker* before intake of a therapeutic agent is of certainly more important than to measure it after exposure of individuals to the drug. In conclusion, *Biomarkers* are used to identify the degree of inter-individual variability, hence, can be indicators of susceptibility to effects of exposure or to disease. *Susceptibility Biomarkers* provide insight into the mechanisms of disease development, support biological plausibility, and assist in the choice of pharmacotherapy.

153

Biliary tumorigenic effect on hypopharyngeal cells is significantly enhanced by pH reduction

Dimitra P. Vageli[1], Sotirios G. Doukas[1], Clarence T. Sasaki[1]
[1]The Yale Larynx laboratory, Department of Surgery, Yale School of Medicine, New Haven, CT, USA
E-mail: dimitra.vangeli@yale.edu

Biliary reflux has been considered a potential risk factor in upper aerodigestive tract malignancies (1). However, it is not yet clearly known how pH affects the bile-induced NF-κB-related oncogenic mRNA phenotype previously linked to hypopharyngeal carcinogenesis. We have used our prior *in vitro* model (2,3) to explore the effect of bile, in ranges of strongly acidic (pH 4.0), weakly acidic (pH 5.5) and neutral pH (7.0) on human hypopharyngeal primary cells (HHPCs) in activating NF-κB and related oncogenic mRNA phenotypes. We performed repetitive applications of conjugated primary bile acids with or without unconjugated secondary bile acid, deoxycholic acid (DCA) on HHPC, and we used immunofluorescence, western blotting, luciferase assay, qPCR and PCR microarray analyses to detect NF-κB activation levels and the transcriptional activation of NF-κB-related oncogenic mRNA profiles. The effect of conjugated primary bile acids, including DCA at strongly-acidic pH (4.0) optimally enhances bile-induced NF-κB activation, STAT3 nuclear translocation, Bcl-2 overexpression and the significant overexpression of the oncogenic mRNA phenotype, compared to weakly-acidic pH (5.5) or neutral pH (7.0). As the pH becomes less acidic, the partially activated primary bile acids and activated DCA begin to exert their effects, altough with significantly less intensity compared to bile acids at strongly-acidic pH. These findings suggest that biliary tumorigenic effect is strongly pH-dependent. Thus, controlling pH during reflux events may be therapeutically effective in reducing the potential risk of bile-induced hypopharyngeal cancer.

(1) Langevin et al., Cancer Epidemiol Biomark Prev 22, 1061-1068, 2013.
(2) Sasaki et al., Head Neck 38 Suppl 1: E1381- E1391, 2016.
(3) Vageli et al., Oncotarget 9: 5876-5879, 2018.

154

Temporal characteristics of NF-κB inhibition in blocking bile-induced oncogenic molecular events in hypopharyngeal cells

Dimitra P. Vageli[1], Panagiotis G. Doukas[1], Sotirios G. Doukas[1], Clarence T. Sasaki[1]
[1]The Yale Larynx laboratory, Department of Surgery, Yale School of Medicine, New Haven, CT, USA
E-mail: dimitra.vangeli@yale.edu

Laryngopharyngeal reflux (LPR) has been linked to chronic inflammatory and neoplastic diseases of the upper aero-digestive tract (1). The presence of bile fluid in patients with LPR suggests its possible carcinogenic effect on hypopharyngeal mucosa. We previously demonstrated the key role of NF-κB in mediating acidic bile-induced pre-neoplastic events in hypopharyngeal cells (2), and also that the co-administration of specific NF-κB inhibitor, BAY 11-7082, together with acidic bile, effectively prevented its related oncogenic molecular effects (3,4). We hypothesized that the addition of BAY 11-7082 (10 μM) either before or after the application of acidic bile (400 μM-conjugated bile acids; pH 4.0), is capable of comparably blocking acidic bile-induced oncogenic molecular phenotypes in murine hypopharyngeal primary cells (MHPCs). We performed 15 min of pre- or post-application of BAY 11-7082 on acidic bile-exposed MHPCs and we used immunofluorescence, luciferase assay, western blot analysis and qPCR, to identify changes in NF-κB activation levels and related mRNA and miRNA oncogenic phenotypes. The results *in vitro* revealed that 15 min of pre- or post-application of BAY 11-7082 effectively inhibited acidic bile-induced NF-κB activation, the transcriptional activation of RELA(p65), STAT3, EGFR, IL-6, Bcl-2, WNT5A, the 'upregulation' of 'oncomirs' miR-21, miR-155, miR-192 and the 'downregulation' of 'tumor suppressor' miR-34a, miR-375, miR-451a. Our observations support the understanding that the acidic bile-induced deregulation of the anti-apoptotic or oncogenic factors, Bcl-2, STAT3, EGFR, IL-6, WNT5A, miR-21, miR-155, miR-375, is highly NF-κB-dependent, indicating that even the post-application of an inhibitor can suppress their deregulation. The application of specific NF-κB inhibitor, has the capability of adequately blocking the early oncogenic molecular events produced by acidic bile, whether it is applied pre- or post-exposure. In addition to therapeutic implications, these findings provide a window of observation into the complex kinetics characterizing the mechanistic link between acidic bile and early neoplasia. Although BAY 11-7082 itself may not be suitable for clinical use, the application of other NF-κB inhibitors merits further investigation.

(1) Langevin et al., Cancer Epidemiol Biomark Prev 22, 1061-1068, 2013; (2) Sasaki et al., Head Neck 38 Suppl 1: E1381- E1391, 2016; (3) Vageli et al., Oncotarget 9: 5876-5879, 2018; (4) Doukas et al, J cell Mol Med 22(5): 2922-2934, 2018.

155

Protein kinase C iota promotes pancreatic tumorigenesis

Kristin S. Inman[1,2], Michele L. Scotti Buzhardt[1,3], Michael Leitges[4], Murli Krishna[5], Howard C. Crawford[6], Alan P. Fields[1], Nicole R. Murray[1]
[1]Department of Cancer Cell Biology, Mayo Clinic, Jacksonville, FL, USA; [2]Environmental Health Perspectives, Durham, NC, USA; [3]NeoGenomics Laboratories, Charlotte, NC, USA; [4]Biotechnology Centre of Oslo, University of Oslo, Norway; [5]Department of Pathology/Lab Medicine, Mayo Clinic Jacksonville, FL, USA; [6]Department of Molecular and Integrative Physiology/Internal Medicine, University of Michigan, Ann Arbor, MI, USA
E-mail: murray.nicole@mayo.edu

Pancreatic cancer (PDAC) is a highly lethal disease due to its therapeutic resistance, emphasizing the need for the identification and characterization of more effective molecular targets for pancreatic cancer therapy. We have previously demonstrate that protein kinase C iota (PKCι) is required for PDAC-transformed growth (1), and that a targeted inhibitor of PKCι blocks PDAC transformed growth *in vitro* and *in vivo* (2). PKCι is significantly overexpressed in patients with PDAC, with a high PKCι expression predicting a poor patient survival (1). These findings support the clinical relevance of our studies. However, little is known about the role of PKCι in pancreatic development or pancreatic tumorigenesis. Thus, a transgenic mouse model was developed to investigate the effects of tissue-specific PKCι ablation on pancreatic homeostasis and KrasG12D-mediated pancreatic tumor formation. The effects of PKCι ablation were characterized using histochemical, immunohistochemical and electron microscopic analyses. The tissue-specific ablation of PKCι expression in the pancreas did not significantly affect pancreatic development or function; however, it decreased pancreas size. PKCι ablation significantly altered KrasG12D-driven pancreatic tumor imitation and progression. Taken together, these results reveal that PKCι plays a required role in pancreatic epithelial cell metabolism and PDAC development, suggesting that the targeted inhibition of PKCι may prove to be a novel therapeutic strategy in pancreatic cancer.

(1) Scotti et al., Cancer Res 70: 2064-2074, 2010.
(2) Butler et al., Oncotarget 6: 15297-15310, 2015.

156

Thrombomodulin: Correlation with inflammatory and cardiac parameters in women with breast cancer treated with chemotherapy with doxorubicin

Rodrigo Mendonça Cardoso Pestana[1], Ricardo Simões[1,2], Luciana Maria Silva[3], Heloísa Helena Marques Oliveira[3], Adriano de Paula Sabino[1], Michelle Teodoro Alves[1], Karina Braga Gomes[1]
[1]Department of Clinical and Toxicological Analysis, Faculty of Pharmacy, Federal University of Minas Gerais, Belo Horizonte, Brazil; [2]Alberto Cavalcanti Hospital, Belo Horizonte, Brazil; [3]Ezequiel Dias Fundation, Belo Horizonte, Brazil
E-mail: rodrigomcpestana@hotmail.com

Thrombomodulin is a transmembrane protein expressed on the surface of endothelial cells in all vasculature, which indicates endothelial injury. The aim of this study was to investigate the association between plasma levels of thrombomodulin with cardiac biomarkers (NT-proBNP) and inflammatory plasma parameters (TNF-α, IL-1 and C-reactive protein - CRP) in women with breast cancer treated with doxorubicin (DOXO). Blood samples were collected after the final cycle (T1) and one year after chemotherapy-DOXO based (T2) in 80 women with breast cancer. The Ethics Committee of the Federal University of Minas Gerais and FHEMIG approved the study and all participants signed informed consent forms. Thrombomodulin, TNF-α and IL-1 were determined by Multiplex immunoassays, NT-proBNP by immunometric method and CRP by sandwich enzymatic immunoassay. Statistical analyses were performed using SPSS v.17.0 software. At T1, the median levels of TNF-α were 10.61 pg/ml (IQR 6.42), those of IL-1 were 0.77 pg/ml (IQR 0.21), CRP were 9.35 mg/dl (IQR 10.05), NT-proBNP were 58.85 pg/ml (IQR 93.50) and those of thrombomodulin were 4.08 ng/ml (IQR 2.10). At T2, the median levels of TNF-α were 11.56 pg/ml (IQR 8.18), those of IL-1 were 0.77 pg/ml (IQR 0.25), CRP were 7.55 mg/dl (IQR 5.63), NT-proBNP were 61.40 pg/ml (IQR 5.63) and those of thrombomodulin were 4.60 ng/ml (IQR 3.78). There was a difference in the CRP levels between T1 and T2 (p-value = 0.038). The TNF-α, IL-1 and CRP levels (at T1) were positively correlated (Spearman's correlation, p-value < 0.05) with the thrombomodulin (T2) levels, and the NT-proBNP (T1) levels were also positively correlated with the thrombomodulin levels (T1). Taken together, these data suggest that endothelial injury, evaluated by thrombomodulin levels, is associated with inflammation and cardiotoxicity post-DOXO treatment in women with breast cancer.

Aknowledgements: FAPEMIG: Fundação de Amparo à Pesquisa do Estado de Minas Gerais.

157

Druggable kinases activated by disruptive TP53 mutations in solid tumors

Ágnes Ősz[1], Ádám Nagy[1], Balázs Győrffy[1]
[1]The 2nd Department of Pediatrics, Semmelweis University, Budapest, Hungary
E-mail: gyorffy.balazs@yahoo.com

The tumor suppressor TP53 is the most frequently mutated gene in solid tumors. Although TP53 decides cell fate and governs the initiation of apoptosis, inhibitors targeting mutant TP53 have not yet reached clinical use. Our goal was to identify novel potential therapeutic targets in TP53 mutant solid tumors by the *in silico* analysis of multiple large, independent next-generation sequencing and gene chip datasets. First, gene expression and mutation data from multiple solid tumors were collected from the TCGA and METABRIC databases. Samples were separated based on the TP53 mutation status, mutational type and tumor type to identify targetable genes. Differential gene expression was compared using the Mann-Whitney test between the mutated (disruptive mutations only) and wild-type patient cohorts across all genes. Subsequently, the prognostic value of identified genes was validated in a gene chip-based dataset obtained from the GEO repository. Survival analysis was performed using Cox proportional hazards regression. The significance threshold was set at $P<0.01$. Finally, the false discovery rate was computed to correct for multiple hypothesis testing. The TCGA dataset included 9,720 patients (21 different cancer types), the METABRIC dataset (breast cancer) 1,399 patients, and the GEO dataset (breast, lung, and brain tumors) 7,386 patients. Only genes with a higher expression in the TP53 mutant cohort were selected and the list of the top targets was further filtered to include only druggable kinases. The best performing kinases include MPS1 (P=2.9E-58, FC=2.82), PLK1 (P=2.6E-55, FC=2.55), MELK (P=5.2E-54, FC=2.81), and AURKB (P=2E-53, FC=3.23). Each of these kinases had a significant prognostic power as well. Of the top 2 (MPS1 and PLK1), both have multiple inhibitors available (for other indications) with PLK1 closest to the clinical use. The findings of this study suggest that MPS1 (monopolar spindle 1 kinase) and PLK1 (polo like kinase 1) kinases are the strongest druggable targets in TP53 mutant solid tumors.

158

Vitamin D derivatives and their combinations with clinically relevant agents in the differentiation therapy of acute myeloid leukemia

Haiat Nujedat[1], Alaa Dawod[1], Aviram Trachtenberg[1], Andrzej Kutner[2], George P. Studzinski[3], Michael Danilenko[1]
[1]Clinical Biochemistry and Pharmacology, Ben-Gurion University of the Negev, Beer-Sheva, Israel; [2]Chemistry and Pharmacology, Pharmaceutical Research Institute, Warsaw, Poland; [3]Pathology and Laboratory Medicine, Rutgers-New Jersey Medical School, Newark, NJ, USA
E-mail: misha@bgu.ac.il

Vitamin D derivatives (VDDs) - 1α,25-dihydroxyvitamin D_2/D_3 and synthetic analogs - have the potential for use in the differentiation therapy of acute myeloid leukemia (AML). However, the calcemic toxicity of supraphysiological doses of VDDs limits their clinical use. We report herein that various activators of the transcription factor Nrf2, including the fumaric acid ester (FAE) dimethyl fumarate (DMF), that has been clinically approved for the treatment of multiple sclerosis, synergistically potentiate the differentiation-induced effects of low concentrations of VDDs on AML cells. The stable expression of a dominant-negative Nrf2 mutant precluded the enhancing effects of DMF or its active *in vivo* metabolite monomethyl fumarate (MMF) on the VDD-induced upregulation of vitamin D receptor (VDR) signaling and on cell differentiation. Conversely, the overexpression of wild-type Nrf2 increased cell sensitivity to lower concentrations of these FAEs and various VDDs. Compounds that do not activate Nrf2 failed to upregulate VDR and potentiate VDD-induced cell differentiation. These data suggest that the differentiation-enhancing activity of clinically relevant FAEs is mediated by the Nrf2 signaling pathway. Furthermore, the combination of DMF and a highly potent vitamin D_2 analog PRI-5202 cooperatively inhibited AML progression in mouse models. By contrast, the post-treatment of leukemia-bearing mice with PRI-5202+DMF following cytotoxic induction therapy with cytarabine (arabinocytosine; AraC) reduced the overall therapeutic outcome. Likewise, the addition of MMF reduced the enhanced anti-leukemic effect of AraC+PRI-5202 in a surrogate *in vitro* model of induction/consolidation chemotherapy. Collectively, these results suggest that the activation of Nrf2 signaling is beneficial for VDD-based differentiation therapy, although it may interfere with the conventional chemotherapy of AML.

Acknowledgements: This study was supported by the US-Israel Binational Science Foundation grant 2017112 to MD and GPS.

159

Dark genome and its regulation by long non-coding RNAs

Katerina Pierouli[1], George N. Goulielmos[2], Elias Eliopoulos[1], Dimitrios Vlachakis[1,3]
[1]Genetics and Computational Biology Group, Laboratory of Genetics, Department of Biotechnology, Agricultural University of Athens, Athens; [2]Section of Molecular Pathology and Human Genetics, Department of Internal Medicine, School of Medicine, University of Crete, Heraklion; [3]Division of Endocrinology and Metabolism, Center of Clinical, Experimental Surgery and Translational Research, Biomedical Research Foundation of the Academy of Athens, Athens, Greece
E-mail: dimvl@aua.gr

As regards the human genome, only 2-3% of this translates into proteins, whereas 97-98% of the genome is comprised by sequences that are not translated, and thus these are defined as 'dark DNA', that appear as non-coding RNAs (ncRNAs). There are various types of ncRNAs that address main cell functions and specifically, are interrelated with the regulation of gene expression. They are categorized according to their length, as small, long and very long, as well as according to their location in genome (introns, genes, promoters, enhancers, or intergenic space). In contrast to small ncRNAs whose role is well clarified, since they are involved in RNA interference (RNAi) pathways, in the control of RNA base alteration and in RNA splicing mediation, the role of long non-coding RNAs (lncRNAs) has not yet been clarified. lncRNAs consist of more than 200 nucleotides and are derived from various regions in the genome. Several lncRNAs create RNA-protein, RNA-DNA and RNA-RNA complexes and they are associated with chromatin modification and lead the transcription factors to specific genomic DNA targets. Another function of lncRNAs is the regulation of the mRNA translation levels by suspending the miRNAs and for that reason, they are associated with various diseases, such as cancer, myocardial infractions and Alzheimer's disease. The basic functions of ncRNAs are imprinted in the process of: i) translation; ii) splicing; iii) replication; and iv) gene regulation. More specifically, alternative splicing is an adjustable process during gene expression, leading to the expression of a single gene for the production of multiple proteins. It is remarkable that alternative splicing allows the human genome to direct the synthesis of more proteins than would be expected from the 20,000 genes encoding proteins. For that reason, we analyzed the lncRNA functions in gene expression and genome regulation by using big data processing, recording potential repeated motifs and epigenetic modifications in splicing sites, which lead to alternative splicing of a mRNA producing different proteins. Moreover, we examined the interactions of lncRNAs with the chromatin remodeling complexes, which are responsible for what genes will be expressed and when, recording both cis and trans gene expression. Hence, based on the fact that lncRNAs are associated with various diseases, the non-coding regions and motifs that were identified, could constitute potential drug targets leading in more specialized and personalized treatment.

160

Genome-wide association studies (GWAS) in an effort to provide insight into the complex interplay of nuclear receptor transcriptional networks and the contribution to the maintenance of homeostasis: The role of the glucocorticoid receptor

Thanasis Mitsis[1], George P. Chrousos[2], Elias Eliopoulos[1], Dimitrios Vlachakis[1,3]
[1]Genetics and Computational Biology Group, Laboratory of Genetics, Department of Biotechnology, Agricultural University of Athens, Athens; [2]Division of Endocrinology, Metabolism and Diabetes, First Department of Pediatrics, National and Kapodistrian University of Athens Medical School, "Aghia Sophia" Children's Hospital, Athens; [3]Division of Endocrinology and Metabolism, Center of Clinical, Experimental Surgery and Translational Research, Biomedical Research Foundation of the Academy of Athens, Athens, Greece
E-mail: dimvl@aua.gr

Recent advances in technology and genetic research, including genome-wide association studies, have allowed for the rapid improvement of molecular medicine. Nuclear receptors are a large group of transcription factors and regulate the activity of a broad range of biological processes. A wide variety of disorders are the result of nuclear receptor malfunction, suggesting that more in-depth knowledge on the interplay of nuclear receptors' transcriptional networks can provide new information into essential biological functions. A fine example of a nuclear receptor is the glucocorticoid receptor (GR). The glucocorticoid receptor's structure is emblematic of the Nuclear Receptor superfamily. GR is also involved in several biological functions. Most importantly, the glucocorticoid receptor plays an essential role in the regulation of the stress system, contributing to the maintenance of homeostasis. A comprehensive list of epigenetic factors, receptor cofactors, and enzymes that interact with GR was constructed, in an effort to create a concise network of the various biological functions this receptor partakes in. That information was later applied on a dataset comprised of more than 60.000 full human genome sequences. The dataset was analyzed with the help of computer-aided techniques. Those techniques included data management, data mining, and autonomous learning. A large number of polymorphisms and mutations of the GR gene, along with genes corresponding to the GR-associated factors were drawn from this procedure. Furthermore, evolutionary and structural information emerged from the dataset. These results can have a wide array of implementation. From pharmacogenomics and the production of new personalized medicine to better prognosis, new models for calculating disease risk factors and an extensive understanding of the nuclear receptors' evolution, the information received from such an extensive database of human genome is quite intriguing.

161

Attempts to link exonic gene polymorphisms to disease-associated protein modified functionality: A structural biology approach

Elias Eliopoulos[1], Maria I. Zervou[2], George N. Goulielmos[2], Michael Matalliotakis[2,3], Dimitrios Vlachakis[1]
[1]Department of Biotechnology, Agricultural University of Athens, Athens, Greece; [2]Section of Molecular Pathology and Human Genetics, Department of Internal Medicine, School of Medicine, University of Crete, Heraklion, Greece; [3]Third Department of Obstetrics and Gynecology, Aristotle University of Thessaloniki, Thessaloniki, Greece
E-mail: eliop@aua.gr

Disease pathophysiology stems from a broad spectrum of environmental influences and genetic factors. Genetic association studies take into account candidate gene polymorphisms, and hence target at unraveling the association between disease predisposition and/or severity and genetic variation. Genome-wide association studies (GWAS) play a primary role in depicting genetic contributions to disease development, while accommodating the exonic polymorphisms on the protein structure level, when available, enhances our understanding of protein function modification or depletion. In this framework, we investigated functional polymorphisms by correlation with protein structure-function for several multifactorial autoimmune or other diseases. Cases include protein targets involved in intracellular signaling (TYK2, STAT1, VEGFR2) (1-3), inflammation (ILs, NF-κB) (4) endometriosis (LAMA5, NAT2, SKAP1, GREB1) (5), immune responses and apoptosis (TNF, RANKL, NF-κB, SIVA) (6,7), as well as autoinflammatory diseases (pyrin) (8). Herein, based on several examples, we analyzed the sequence of techniques used in order to achieve a rational link from gene polymorphism to structure to modified function including metagenomic analysis of SNP polymorphisms, protein crystallography, protein molecular modeling, molecular mechanics and dynamics. Locating, shaping and understanding the target protein interaction interface plays a decisive role in most cases and provides clues for further pharmacological or medical actions.

(1) Lesgidou N. et a1., Bioinformatics 34(17): i781-i786, 2018; (2) Myrthianou E. et al., Scand J Rheumatol. 46: 180-186, 2017; (3) Goulielmos G. et al., J Rheumatol 46: doi 10.3899/jrheum.181346, 2019; (4) Matalliotakis M. et al., Int J Mol Med. 4: 1469-1476, 2018; (5) Matalliotaki et al., Mol Med Reports doi 10.3892/ mmr.2019.10247, 2019; (6) Douni E. et al., Mol Genet. 21: 784-798, 2012; (7) Goulielmos GN et al., Biochem Biophys Res Commun 402: 141-146; 2910; (8) Fragouli E. et al., Clin Genetics 73: 152-159, 2008.

162

N-Bromotaurine and its stable analogue molecule (Bromamine T-BAT) exert a therapeutic effect against cancer and inflammation *in vitro* and *in vivo*

Stella Baliou[1], Markus Nagl[2], Antony M. Kyriakopoulos[3], Vassilis Zoumpourlis[1]
[1]Institute of Biology, Medicinal Chemistry & Biotechnology (IBMCB), National Hellenic Research Foundation, Athens, Greece; [2]Division of Hygiene and Medical Microbiology, Medical University of Innsbruck, Innsbruck, Austria; [3]NASCO AD Biotechnology Laboratory, Pireas, Greece
E-mail: smpaliou@eie.gr

N-Bromotaurine is a natural molecule, which arises from the neutrophil myeloperoxidase halide system of metabolism during inflammation and constitutes the reaction product of taurine with hypobromous acid (HOBr). In order to unravel novel therapeutic options in cancer, we used N-Bromotaurine as the agent with known anti-inflammatory property and anti-microbial ability. The tumor-suppressive effect of N-Bromotaurine was observed in various cancer cell types. In the case of skin cancer, we demonstrated that N-Bromotaurine can bypass the glucocorticoid receptor (GR)-resistance of cancer cells when used in combination with cisplatin, despite the frequently mentioned glucocorticoid unresponsiveness due to GR impairment (1). However, due to the poor stability of the N-Bromotaurine molecule, an analogue molecule, named Bromamine T (BAT), was subsequently used. We demonstrated that cancer cell proliferation was suppressed with the use of BAT, whilst the anticancer effect of BAT appeared to be superior to that elicited by taurine. Flow cytometry and western blot analysis highlighted the intrinsic apoptotic pathway used by cancer cells following their exposure to BAT. Additional experiments proved that BAT triggered oxidative burst in cancer cells. Following treatment of the cancer cells with BAT, the phosphorylation of two main arms of the MAPK family (JNK1/2 and p38) were stimulated. This suggested that BAT induced ROS accumulation, which triggered the phosphorylation of stress-related MAPK kinases by eliciting pro-apoptotic signals in cancer cells. In parallel, we indicated that BAT exerted anti-inflammatory effects by suppressing the mRNA expression levels of cytokines in LPS-induced macrophages. Most importantly, the *in vivo* experiments revealed that tumor formation and the distribution of immune populations in mice were impaired following treatment with BAT. This study has produced results that demonstrate BAT as an emerging anti-proliferative agent with favorable efficacy.

(1) Logotheti S, Khoury N, Vlahopoulos SA, Skourti E, Papaevangeliou D, Liloglou T, Gorgoulis V, Budunova I, Kyriakopoulos AM, Zoumpourlis V. N-bromotaurine surrogates for loss of antiproliferative response and enhances cisplatin efficacy in cancer cells with impaired glucocorticoid receptor. Transl Res. 2016 Jul; 173: 58-73. e2. doi: 10.1016/j.trsl.2016.03.009. Epub 2016 Mar 21. PubMed PMID: 27063960.

Acknowledgements: The research work was supported by State Scholarships Foundation-IKY (2018-2021).

163

Taurine and derivative clinical data

A.M. Kyriakopoulos[1], M. Nagl[2], W. Gottardi[2], S. Baliou[3], V. Zoumpourlis[3]
[1]NASCO AD Biotechnology Laboratory, Pireas, Greece; [2]Division of Hygiene and Medical Microbiology, Medical University of Innsbruck, Innsbruck, Austria; [3]Institute of Biology, Medicinal Chemistry & Biotechnology (IBMCB), National Hellenic Research Foundation, Athens, Greece
E-mail: rnd@nascoad.com

Taurine is a central molecule for mammalian and human health optimization and has a plethora of prospective medical applications. Applications cover from combating neurodegeneration, cardiovascular disease, nephrology, metabolic disease (including diabetes), to modern approaches for counteracting muscular-skeletal and developmental defects. Most importantly it is not only taurine, the most abundant β-sulphur-containing amino acid in our bodies that promotes a fine balance between health and disease. Taurine generates derivatives, such as N-chlorotaurine (NCT) and N-bromotaurine (NBrT) by innate halogenation reactions. These molecules prove to be key to our immune defense system as at the same time may, they act as anti-infectious, anti-inflammatory and anti-cancer agents. Moreover, these are natural drugs produced by our immune system. As we have progressed towards medical applications, even at the elementary clinical stage, we present valuable clinical findings that prove an unimaginable future use of taurine derivatives. These findings range from anti-viral to anti-bacterial efficacy and to the anti-inflammatory potential of these agents. These agents may lead to a medical breakthrough that may enable us to face previously undiagnosed and difficult to cure medical conditions, and may thus be of great clinical importance and complexity. The most significant value of these findings is that they co-exist in an era of post-antibiotic and post-corticosteroid unwanted usage due to their overwhelming therapeutic failures.

Key words: Taurine health uses, taurine derivative clinical data

164

Cytoskeletal stressing modes in cancer cells

V. Gavriil[1], E. Sarantopoulou[1], Z. Kollia[1], M. Goulielmaki[2], V. Zoumpourlis[2], A.C. Cefalas[1]
[1]Theoretical and physical chemistry Institute, National Hellenic Research Foundation, Athens, Greece; [2]Institute of Biology, Medicinal Chemistry & Biotechnology (IBMCB), National Hellenic Research Foundation, Athens, Greece
E-mail: ccefalas@eie.gr

Tumorigenesis is a multifaceted process involving genomic changes activated also by cell-extracellular matrix (ECM) interactions between scaffolds and cytoskeletal structures expressed by the stressing of mechanosensors, similar to integrins, from multipart cellular forces capable of altering genomic reprogramming. The interactions of the tumor microenvironment with ECM scaffolds normally activate a cell's membrane focal adhesion proteins and transmembrane signal receptors (TSRs). The mechanosensors regulate tumor cell growth via signal transaction between the extracellular active domain of cells and the intracellular F-actin filaments by triggering an avalanche of phosphorylation reactions. Protein conformational changes and the excitation of TSR pathways requisite the activating force to lay in the low pN set of force values and certainly below the nN gauge (1). Apart from random mechanical responses and other strong chemical affinities, the binding efficiency (strength of bonding) between outward scaffolds in the ECM and cell proteins can be restrained either via short or long-range electrical polar or other types of dispersive interactions and confined local interactions of outward scaffolds with the biological milieu was recognized to be responsible for diverging cell functionality routes. Contradicting results from tumor cells exposed to outward scaffolds for variable toxicity levels elevated safety attention. Nevertheless, currently, there is a lack of knowledge on the specific pathways through which outward scaffolds interact with eukaryotic cells, precluding the identification of a universal therapeutic approach. Toxicities of outward scaffolds are also related with surfactants morphology, the electrical charging states and the strength of molecular bonding between outward scaffolds and phenotypes. In this study, transmembrane signal receptors activate cancer cell growth via localized extracellular mechanical signal transduction from nanosize outward scaffolds. AKT and ERK signalling pathways and viability tests in different human cell lines point to coherent mechanical stimulation of $>10^4$ ligand adhesion binding sites of integrins and EGFR via a coherent synergistic action. Any local force perturbation in the extracellular matrix may well activate one ligand adhesion binding site in integrins (MIDAS or ADMIDAS site) provided that the stressing force lays within 10 and 500 pN (1). Atomic force microscopy (AFM) and nanoindentation cytoskeletal cell analysis also show an enhanced probability for unstable signal transaction in metastatic tumour cells compared to non-metastatic ones. The study contributes towards recognizing different cytoskeletal stressing modes in cancer cells.

1) Tiny Rare-Earth Fluoride Nanoparticles Activate Tumour Cell Growth via Electrical Polar Interactions V. V. Semashko, M.S. Pudovkin, A.C. Cefalas, et al, *Nanoscale Research Letters* 13, 370 (2018).

165

Existing immunotherapy approaches to prostate cancer treatment and novel combinations that circumvent therapeutic resistance

Maria Adamaki[1], Vassilios Zoumpourlis[1]
[1]Institute of Biology, Medicinal Chemistry & Biotechnology (IBMCB), National Hellenic Research Foundation, Athens, Greece
E-mail: madamaki@eie.gr

Prostate cancer (PCa), an age-related disease predominantly affecting males over the age of 60, is the most frequently diagnosed type of cancer and the third cause of cancer-related mortality among Caucasian males. Considering that approximately one-fifth of the global population is estimated to be ≥60 years old by 2050, this undoubtedly highlights the profound socio-economic consequences of the disease and the need for devising effective treatments. In recent years, immunotherapy, in the form of active immunotherapy, has emerged as a promising treatment modality for the management of PCa. Active immunotherapeutic agents exert their anticancer effects by engaging with the host immune system, thereby eliciting an effective immune response against cancer cells. In light of the discovery of cancer-specific antigens and adoptive cellular targeting, such as dendritic cell-based immunotherapy, active immunotherapy approaches include the development of antigen-specific vaccines, as well as non-antigen specific vaccines, immunomodulating agents, such as checkpoint inhibitors and adjuvants that enhance antigen immunogenicity when combined with an antibody. In addition, multiple immunologic platforms that include combinations of vaccines and immune checkpoint inhibitors are currently being evaluated as alternate approaches in overcoming therapeutic resistance. Overall, it appears that immunotherapy renders cancer cells more susceptible to chemotherapy and radiotherapy, thereby increasing overall survival in patients with PCa and therefore holds much promise for a more effective treatment of the disease.

166

Mesenchymal stem cells isolated from the umbilical cord act as potent anticancer agents

Maria Goulielmaki[1], Ioannis Christodoulou[1], Vassilis Zoumpourlis[1]
[1]Institute of Biology, Medicinal Chemistry & Biotechnology (IBMCB), National Hellenic Research Foundation (NHRF), Athens, Greece
E-mail: mgoulielmaki@eie.gr

Mesenchymal stem cells (MSCs) possess distinctive features, such as high proliferation rates and the capability of differentiation into multiple non-hematopoietic cell types, which can be isolated from both adult and fetal tissues (1). Furthermore, MSCs are able of migrating towards tumors within the organism, where they interact with the local supportive microenvironment, a property that renders them as highly appropriate candidates for use in cancer cytotherapy protocols. Towards this purpose, both naïve (unmodified) and genetically-modified MSCs (GM-MSCs) have been employed both *in vitro* and *in vivo*, although with variable results (2). Based on the already published research work on the field, we performed a small-scale meta-analysis using a four-step strategy: The compilation of a relevant publication library; deconstruction of literature methodology and reported findings; classification and organization of extracted experimental data; and data consolidation and statistical analysis (3). In turn, based on the observations and conclusions of our analysis, we evaluated the paracrine effects of various MSC populations on the proliferation and survival of selected cancer cell lines representing distinct cancer types *in vitro* and *in vivo*. Subsequently, we examined the transcriptome of two cell lines by RNA microarrays in order to exploit the expression pathways and regulatory networks contributing to the observed anticancer activity. The interpretation of the meta-analysis results led us to the deduction that the outcome of MSC-mediated cancer cytotherapy approaches is largely dependent on various parameters. Furthermore, we were able to highlight a set of optimal conditions, where the tumor suppressive action of MSC predominates. MSCs derived from Wharton's Jelly (WJ-MSCs) were found to possess a tumor suppressive behavior, both *in vitro* and *in vivo.* mRNA analysis of cancer cells revealed a significant target dependence of the anti-tumorigenic effects displayed by MSCs, which are mediated by different pathways.

(1) Weissman et al. Annu Rev Cell Dev Biol. 17: 387-403, 2001.
(2) Studeny et al. J Natl Cancer Inst. 96: 1593-1603, 2004.
(3) Christodoulou et al. Stem Cell Res Ther. 9: 336, 2018.

Acknowledgements: This research work was supported by the Hellenic Foundation for Research and Innovation (HFRI) and the General Secretariat for Research and Technology (GSRT), under the HFRI PhD Fellowship grant (GA. no. 2400).

167

Novel approaches to parvovirus B19 diagnostics in cancer patients

Andrei Babenka[1], Marina Yermalovich[2], Inna Anufrionak[3], Valentina Egorova[4], Ihar Lipnevich[5], Nina Krylova[5], Halina Grushevskaya[5]
[1]Belarussian State Medical University, Minsk, Belarus; [2]Republican Research and Practical Center for Epidemiology and Microbiology, Minsk, Belarus; [3]N. N. Alexandrov National Cancer Center, Lesnoy, Belarus; [4]M. Tank Belarusian State Pedagogical University, Minsk, Belarus; [5]Belarussian State University, Minsk, Belarus

In recent years, research data on the detection of parvovirus B19 in tumor tissue of different localization have come to light. Some authors consider this phenomenon as evidence of the possible role of parvovirus in the development of the pathological process, others as an accidental detection of a co-infection. A number of studies have demonstrated that parvovirus B19 DNA is often found in the tumor tissue of cancer patients, even in the absence of an immune response and corresponding IgM in the blood. Of note, the data on the frequency of occurrence of B19 in similar groups of patients differ significantly. It may be connected with errors during the tests using amplification due to the low viral DNA copy number in tissue samples. We offer a highly sensitive biosensor-based approach for parvovirus DNA diagnostics in tumor tissue samples. This method is based on the platform of quantum graphene-like structures, including multiwalled carbon nanotubes (CNT) and nanoporous composites. For the non-edimetric detection of viral DNA hybridization, non-Faradaic type electrochemical impedance DNA nanosensors were used. The developed method is completely PCR-free and does not require any amplification of the target DNA sequence. The technique is designed to work with targets that have a low number of copies in the sample. We used 148 samples of lung tumor tissue and 70 samples of intestinal tumor tissue as model samples for comparing the nanosensor-based PCR-free method with qPCR data. As a result, we have shown that our method is 100% concordant to the certified qPCR method. In the case of our novel approach, any probability of contamination of negative samples during amplification can be excluded.

168

Protein kinase Cι and Wnt/β-catenin signaling: Alternative pathways to *Kras*/*Trp53*-driven lung adenocarcinoma

Ning Yin[1], Yi Liu[1], Andras Khoor[2], Xue Wang[3], Aubrey E. Thompson[1], Michael Leitges[4], Verline Justilien[1], Capella Weems[1], Nicole R. Murray[1], Alan P. Fields[1,*]
Departments of [1]Cancer Biology, [2]Pathology and [3]Health Sciences Research, Mayo Clinic Florida, Jacksonville, Florida, USA; [4]Memorial University of Newfoundland, St. John's, Newfoundland, Canada
*E-mail: fields.alan@mayo.edu

Lung cancer is the leading cause of cancer-related mortality worldwide. The most prevalent form of lung cancer, lung adenocarcinoma (LADC), is a heterogeneous disease characterized by high relapse rates and a poor prognosis. The major oncogenic drivers of LADC are mutant *KRAS* and the loss of the tumor suppressor *TP53*, which occur in ~30% and 95% of LADCs, respectively. However, few therapeutic intervention strategies effectively target mutant *KRAS/TP53*-driven LADC. We recently demonstrated that *PRKCI* is an oncogene and downstream effector of oncogenic *KRAS* in LADC that can be therapeutically targeted for the treatment of LADC. Herein, we identified and characterized two distinct tumorigenic pathways to *Kras/Trp53*-driven LADC in mice. Specifically, we found that mouse *LSL-Kras^{G12D}/Trp53$^{fl/fl}$* (KP)-mediated LADC tumorigenesis can proceed through both *Prkci*-dependent and *Prkci*-independent pathways. The predominant pathway involves the *Prkci*-dependent transformation of bronchioalveolar stem cells (BASCs). However, KP mice harboring conditional knockout *Prkci* alleles (KPI mice) developed LADC tumors through the *Prkci*-independent transformation of Axin2^{+} alveolar type 2 (AT2) stem cells. The transformed growth of KPI, but not KP tumors is blocked by Wnt pathway inhibition *in vitro* and *in vivo*. Furthermore, a KPI-derived genomic signature predicts the sensitivity of human LADC cells to Wnt inhibition, and identifies a distinct subset of primary LADC tumors exhibiting a KPI-like genotype. Thus, LADC can develop through both *Prkci*-dependent and Prkci-independent pathways, resulting in tumors exhibiting distinct oncogenic signaling and pharmacologic vulnerabilities. These data provide a compelling rationale for the use of PKCι and Wnt pathway-targeted therapeutics in treatment of distinct LADC subtypes that can be distinguished using Wnt pathway gene profiling.

169

Targeting microRNAs in cystic fibrosis (CF)

Roberto Gambari
Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy
E-mail: gam@unife.it

Cystic fibrosis (CF) is a lethal autosomal recessive genetic disease caused by a variety of mutations of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Since the demonstration that microRNAs are deeply involved in CF, increasing attention has been dedicated to the possible alteration of CFTR gene expression by targeting those miRNAs involved in the downregulation of CFTR and associated proteins. In this case, peptide nucleic acids (PNAs) appear to be of great interest, since they are capable of sequence-specific and efficient hybridization with complementary DNA and RNA. Our group has demonstrated that the PNA-mediated inhibition of miR-145-5p (1) and miR-101-3p (which downregulate CFTR) leads to an increase in CFTR expression in Calu-3 cells. In addition, to the direct interaction with CFTR, miRNAs may regulate CFTR by binding to the 3'UTR of mRNAs coding CFTR regulators, such as NHERF1, NHERF2 and ezrin. Notably, the PNA-mediated targeting of miR-335-5p, one of the miRNAs involved in NHERF1 regulation, was found to be associated with the specific inhibition of miR-335-5p, an increase in NHERF1 and an increase in CFTR expression. NGS was performed to verify whether PNA-mediated effects may be accompanied by the co-inhibition of other miRNAs. The results obtained sustain the concept that specific miRNA inhibition (in our case inhibition of miR-145-5p, miR-101-3p and miR-335-5p) may be accompanied by the co-inhibition of other miRNAs (for instance miR-155-5p in the case of PNA-mediated inhibition of miR-145-5p) involved in cystic fibrosis (2). In addition to the PNA-mediated targeting of CFTR-inhibiting miRNAs (anti-miRNA therapeutic approaches), miRNA-replacement therapy may also be considered. This may be a key strategy for inhibiting *Pseudomonas aeruginosa*-dependent inflammatory responses, with relevant clinical implications. In this respect, miR-93-5p was demonstrated to be downregulated during the *P. aeruginosa* infection of CF cells (3). Accordingly, the transfection of CF cells with pre-miR-93-5p was found to be associated with anti-inflammatory effects, including a decrease in IL-8 mRNA content and IL-8 protein release.

Acknowledgements: This study was supported by Fondazione Fibrosi Cistica (FFC), Project 'MicroRNA Therapeutics in CF: Targeting CFTR and inflammation networks (MICRORNA-CF)' FFC#3/2016, FAR-UNIFE.

(1) Fabbri E, et al., Molecules 23: pii:E71, 2017; (2) Finotti A, et al., Am J Respir Crit Care Med, 2019; (3) Fabbri E, et al., Am J Respir Cell Mol Biol 50: 1144-1155, 2014.

170

A peptide nucleic acid targeting the *acpP* gene of *Pseudomonas aeruginosa* inhibits bacterial induced biological alterations in cystic fibrosis cells

Elisabetta D'Aversa[1], Chiara Tupini[1], Enrica Fabbri[1], Monica Borgatti[1], Ilaria Lampronti[1], Alessia Finotti[1], Peter E. Nielsen[2], Roberto Gambari[1]
[1]Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy; [2]Department of Cellular and Molecular Medicine & Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, The Panum Institute, University of Copenhagen, Copenhagen, Denmark
E-mail: gam@unife.it

One of the major clinical features of Cystic Fibrosis (CF) is the chronic infection generally sustained by the Gram-negative bacterium *Pseudomonas aeruginosa (P.aeruginosa)*[(1)]. An excessive lung inflammation, with a huge infiltrate of neutrophils in the bronchial lumen, CF is associated with *P.aeruginosa* infection and occurs mainly due to the release of the chemokine interleukin IL-8[(2)]. The identification of new effective antibacterial drugs, able to reverse, at least partially, the *P.aeruginosa*-induced alteration of biological effects on CF cells, is considered a promising therapeutic strategy to prevent progressive lung tissue deterioration. Recently, antisense antibacterial Peptide Nucleic Acids (PNAs) targeting the mRNA translation initiation region of *ftsZ* (an essential bacterial gene involved in cell division) or *acpP* (an essential bacterial gene involved in fatty acid synthesis) of *P.aeruginosa* were discovered, and showed promising bactericidal activity[(3)]. The aim of the present study was to determine the effects of one of these anti-*acpP* PNA peptide conjugates (PNA-3969) on *P.aeruginosa* (PAO-1 strain) induction of the biological effects on CF cells. The first conclusion of the present study is that this PNA protects NuLi-1 cells, CF IB3-1 and CuFi-1 cells from the bacterial induced upregulation of the pro-inflammatory cytokine IL-8. Moreover, the anti-PAO-1 PNA-3969 protects IB3-1 cells from bacterial-induced cytotoxicity and pro-apoptotic effects, with an efficiency approaching that of the antibacterial agent gentamycin. Of interest, cooperative effects were obtained when PNA-3969 and gentamycin were used in combination at sub-optimal concentrations. In conclusion, this type of antisense antibiotics should be considered of great interest for further discovery of novel and specific antibiotics against pulmonary infections in cystic fibrosis patients.

Acknowledgements: This work was supported by grants from the Italian Cystic Fibrosis Research Foundation (grant nos. 15/2004 and 17/2010 to R.G.).

(1) Rimessi A. et al., Am J Respir Cell Mol Biol 59: 428-436, 2018.
(2) Bezzerri V. et al., J Immunol 187: 6069-6081, 2011.
(3) Ghosal A, Nielsen PE. Nucleic Acid Ther 22: 323-334, 2012.

171

New synthetic isoxazole derivatives as potent inducer of fetal hemoglobin (HbF) in β-thalassemia

Ilaria Lampronti[1], Cristina Zuccato[1], Lucia Carmela Cosenza[1], Chiara Tupini[1], Chiara Gemmo[1], Riccardo Baruchello[2], Daniele Simoni[2], Roberto Gambari[1]
[1]Department of Life Sciences and Biotechnology, Ferrara University, Ferrara, Italy; [2]Department of Chemical and Pharmaceutical Sciences, Ferrara University, Ferrara, Italy
E-mail: lmi@unife.it; gam@unife.it

Several examples linking anti-proliferative effects to erythroid differentiation using many cellular model systems (for instance K562 cells) do exist. Moreover, in most cases, inducers of K562 erythroid differentiation are capable of stimulating fetal hemoglobin (HbF) production in erythroid precursor cells (ErPCs) isolated from β-thalassemia patients. In this study, new anti-proliferative 4,5,6,7-tetrahydro-isoxazolo-[4,5-c]-pyridines and 3,4-isoxazolediamide derivatives[(1)] were screened for induction of erythroid differentiation in K562 cells and stimulation of HbF induction in ErPCs. The original structures of geldanamycin and radicicol, considered natural Hsp inhibitors, were modified because both of them exhibit several drawbacks, including poor solubility, significant hepatotoxicity and intrinsic chemical instability or deprivation of *in vivo* activity. These potential HbF inducers were tested first on the K562 cell line and second on erythroid precursors derived from patients affected by β-thalassemia and SCD (Sickle Cell Disease) in order to select novel bioactive analogues. Increase of HbF was determined by HPLC and γ-globin mRNA expression was analyzed by RT-qPCR. Overall, the data obtained indicate that: (a) Isoxazol derivatives increase HbF in cultures from β-thalassemia and SCD patients with different basal HbF levels; (b) Isoxazol derivatives increase the overall Hb content/cell; (c) Isoxazol derivatives selectively induce γ-globin mRNA accumulation, with only a minor effect on β-globin and no effect on α-globin mRNAs; (d) there is a strong correlation between the increase by Isoxazol derivatives of HbF and the increase in γ-globin mRNA content; (e) in several cases these compounds were found more active than hydroxyurea, a well-known HbF inducers in β-thalassemia and SCD.

Acknowledgements: RG is funded by Fondazione Cariparo (Cassa di Risparmio di Padova e Rovigo), by UE THALAMOSS Project (Thalassemia Modular Stratification System for Personalized Therapy of beta-Thalassemia; n. 306201-FP7-HEALTH-2012-INNOVATION-1), by Wellcome Trust (Innovator Award 208872/Z/17/Z) and by AIFA (AIFA-2016-02364887).

(1) Baruchello R et al, J Med Chem, 54: 8592-8604, 2011.

172

A PNA-based masking strategy for CFTR upregulation by targeting miR-145-5p binding sites of CFTR mRNA

Shaiq Sultan[1], Enrica Fabbri[1], Anna Tamanini[2], Jessica Gasparello[1], Alex Manicardi[3], Roberto Corradini[3], Alessia Finotti[1], Chiara Tupini[1], Ilaria Lampronti[1], Maria Cristina Dechecchi[2], Giulio Cabrini[2], Roberto Gambari[1], Monica Borgatti[1]
[1]Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy; [2]Laboratory of Molecular Pathology, Department of Pathology and Diagnostics, University-Hospital of Verona, Verona, Italy; [3]Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parma, Italy
E-mail: gam@unife.it

MicroRNA miR-145-5p is involved in the post-transcriptional regulation of the Cystic Fibrosis Transmembrane conductance Regulator (*CFTR*) gene, deeply altered in Cystic Fibrosis (CF). We have recently reported the effects of a Peptide Nucleic Acid (PNA) targeting miR-145-5p (1,2). An octaarginine-anti-miR145 PNA conjugate was delivered to Calu-3 cells, exerting sequence-dependent inhibition of miR-145-5p, associated with enhancement of the expression of the miR-145 regulated *CFTR* gene, analyzed at mRNA (RT-qPCR, Reverse Transcription quantitative Polymerase Chain Reaction) and CFTR protein (western blotting) level. In addition to this anti-miRNA strategy, PNAs can be employed as useful tools to perform efficient 'masking' of miRNA binding sites, thus preventing molecular interactions between miRNAs and target 3'UTR binding sites. In this context, we designed, synthesized and tested a PNA 100% complementary to the CFTR mRNA 3'UTR, (N-term, 5')-R8-CCA GTT ATC ATT ATC TAA-Gly-NH2(C-term, 3') (R8-PNA-145mask). Calu-3 cells were treated with increasing concentrations of the R8-PNA-145mask (0.5-4 μM) for three days, and RNA and proteins were isolated from treated cells to analyze the *CFTR* gene expression. The results obtained demonstrated that the treatment of Calu-3 cells with the R8-PNA-145mask leads to an increase of *CFTR* expression. Therefore, direct inhibition of miR-145-5p with antisense PNAs and/or PNA-dependent prevention of miR-145-5p binding to the CFTR mRNA 3'UTR might be efficient strategies to enhance CFTR expression, a key action in therapeutic protocols for CF.

Acknowledgements: Supported by Fondazione Fibrosi Cistica, Project "MicroRNA Therapeutics in CF: Targeting CFTR and inflammation networks (MICRORNA-CF)" and FAR fund of Ferrara University.

(1) Fabbri E, et al., Molecules, 2017;
(2) Finotti A, et al., Am J Respir Crit Care Med, 2019.

173

Sirolimus-mediated induction of fetal hemoglobin in beta-thalassemia: Impact of the XmnI rs74482144 polymorphism

Cristina Zuccato[1], Lucia Carmela Cosenza[1], Giulia Breveglieri[1], Chiara Gemmo[1], Ilaria Lampronti[1], Enrica Fabbri[1], Nicoletta Bianchi[1], Monica Borgatti[1], Jessica Gasparello[1], Alessia Finotti[1], Marco Prosdocimi[2], Roberto Gambari[1]
[1]Department of Life Sciences and Biotechnology, Ferrara University, Ferrara, Italy; [2]Rare-Partners Srl, Milano, Italy
E-mail: gam@unife.it

The *in vivo* effects of sirolimus on the induction of fetal hemoglobin (HbF) is of key importance for therapeutic protocols in a variety of hemoglobinopathies, including β-thalassemia and sickle-cell disease (SCD). We have previously reported the strong inducing effect of the mTOR inhibitors rapamycin (sirolimus) and everolimus on HbF production by erythroid precursors (ErPCs) from β-thalassemia patients. We took advantage from the availability of a β-thalassemia cellular bio-bank allowing stratification of the patients with respect to fetal hemoglobin production and response to HbF inducers. The results obtained by HPLC analysis of the ErPCs cultures from 38 patients led to the following conclusions: (a) sirolimus increases HbF in cultures from β-thalassemia patients with different basal HbF levels (the cultures from 51.4% of the patients were responsive to sirolimus treatment); (b) the cultures from 37.8% of the patients were not responsive to sirolimus or hydroxyurea (HU); (c) sirolimus was able to induce HbF in 46.15% of the cultures not responsive to HU; (d) sirolimus displayed higher efficiency than HU in 57.14% of the cultures responsive to both sirolimus and HU; (e) 42.86% of HU-treated cultures displayed HbF induction higher than sirolimus. In order to study a possible association between DNA polymorphisms and sirolimus-mediated HbF induction, the γ-globin *Xmn*I (rs74482144), two BCL11A (rs1427407 and rs10189857) and the HS1L-cMYB rs9399137 polymorphisms were analyzed. Both homozygous *Xmn*I (T/T,+/+) and heterozygous *Xmn*I (T/C,+/-) cultures (from 11 patients, 29.9% of the 38 patients analyzed) had high sirolimus-mediated HbF induction. The BCL11A rs1427407 G>T polymorphism was not associated with high HbF induction. Concerning the HS1L-cMYB T>C rs9399137 and the BCL11A A>G rs10189857 polymorphisms, an association with sirolimus-mediated HbF induction was found, but only in the homozygous patients (3/38 and 7/38, respectively). These results indicate that the γ-globin *Xmn*I rs74482144 should be considered a very useful polymorphism for recruitment of β-thalassemia patients in sirolimus-based clinical trials.

Acknowledgements: UE FP7 THALAMOSS Project (grant no. 306201-FP7-Health-2012-INNOVATION-1), by Wellcome Trust and by AIFA (Agenzia Italiana del Farmaco).

174

Non-invasive prenatal detection of beta-thalassemia mutations in maternal plasma using Droplet Digital PCR

Elisabetta D'Aversa[1], Giulia Breveglieri[1], Patrizia Pellegatti[2], Giovanni Guerra[2], Roberto Gambari[1], Monica Borgatti[1]
[1]Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy; [2]Operative Unit of Laboratory Analysis, University Hospital S. Anna, Ferrara, Italy
E-mail: monica.borgatti@unife.it, gam@unife.it

Non-invasive prenatal testing (NIPT) is based on fetal DNA analysis with the aim to identify genetic abnormalities from the maternal plasma during pregnancy[(1)]. Actually, commercial NIPT kits can detect only aneuploidies, small deletions or insertions but not single point mutations causing genetic diseases. In this study, we have developed two genotyping assays, based on innovative and sensitive droplet digital PCR (ddPCR) technology[(2)] to identify the two most common thalassemia mutations in the Mediterranean population ($\beta^0$39 and β^+IVSI-110) maternally and/or paternally inherited on fetal DNA. First, the two genotyping assays were optimized and validated, in terms of amplification efficiency and hybridization specificity, using mixtures of two genomic DNAs carrying different genotypes and percentages to simulate fetal and maternal circulating cell-free DNA (ccfDNA) at different gestational weeks. Then the two ddPCR assays were applied to determine the fetal genotype from 36 maternal blood samples at different gestational ages. The diagnostic outcomes were confirmed for all the samples carrying paternally inherited mutation by DNA sequencing. In the case of maternally or both parents inheriting the mutation a precise dosage of normal and mutated alleles was required to determine the fetal genotype. In particular, we identified two diagnostic ranges for allelic ratio values that were statistically distinct and not overlapping, allowing the corrected fetal genotype determination in all the samples analyzed. In conclusion, we have developed a simple and sensitive diagnostic approach, based on ddPCR, for non-invasive pre-natal determination of β^+IVSI-110 and $\beta^0$39 mutations paternally and maternally inherited suggesting its application also for other single point mutations causing monogenic diseases.

Acknowledgements: This work was supported by the Italian Ministry of Health under the project no. 098/GR-2009-1596647-Young Investigators-2009 and FIR 2017-University of Ferrara to MB. The QX200™ Droplet Digital™ PCR system (Bio-Rad Laboratories, Hercules, CA, USA) was funded by Ferrara University Grant 'Bando per l'acquisizione di strumenti per la ricerca di ateneo-2015'.

(1) Breveglieri G, et al. Mol Diagn Ther 23: 291-299, 2019.
(2) D'Aversa E, et al. Mol Med 24: 14, 2018.

175

Identification of dysregulated miRNAs in liquid biopsies from colorectal cancer (CRC) patients: Impact on personalized miRNA therapeutics

Alessia Finotti, Roberto Gambari
Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy
E-mail: alessia.finotti@unife.it

MicroRNAs (miRNAs) are small non-coding RNAs regulating gene expression by the sequence-selective targeting of mRNAs, leading to translational repression or mRNA degradation. In cancer, miRNAs are associated with tumor onset and progression. Liquid biopsy of cancer is based on the analysis of circulating tumor cells and/or cell-free nucleic acids (including miRNAs) in peripheral blood of cancer patients and it is considered one of the most advanced non-invasive diagnostic systems suitable for early diagnosis, staging, prognosis, prediction of therapy responses, therapy outcome, and follow-up during therapeutic intervention (1). We performed NGS of plasma isolated from 35 colorectal carcinoma (CRC) patients and identified a short-list of 12 dysregulated miRNAs, including miR-221, miR-222 and miR-141. These data were further validated by droplet digital RT-PCR (dd-RT-PCR). Despite the fact that patient-to-patient heterogeneity was found, this study provides a list of novel potential targets for the development of therapeutic protocols for CRC. The association between the miRNAs expressed in tumors and their plasma content was validated in experimental mice xenografted with tumor cell lines derived from CRC patients (2). For miRNA targeting, peptide-nucleic acids (PNAs), DNA analogues in which the sugar-phosphate backbone has been replaced by N-(2-aminoethyl)glycine units, are excellent tools. We developed novel delivery strategies for PNAs targeting miRNAs, based on i) the use of PNAs linked to a poly-arginine R8 peptide tail; ii) nanoparticles; and iii) novel molecules constituted by a macrocyclic multivalent tetraargininocalix[4]arene to be used as non-covalent vector for anti-miRNA PNAs (3). As far as the validation of PNA activity, we focused on PNAs targeting miR-221, miR-222 and miR-155 in glioblastoma cells. Increased pro-apoptotic effects were obtained with the co-administration of these PNAs. In addition, synergistic effects were found with the co-administration of corilagin and a PNA targeting miR-221. Finally, the combined treatment of glioma U251 cells with the pro-apoptotic pre-miR-124 and the PNA targeting miR-221, led to the induction of apoptosis at very high levels. In conclusion, the liquid biopsy approach may be considered the basis for a personalized miRNA based therapy of cancer, and PNAs may be a relevant therapeutic tool for the inhibition of oncomiRNAs, also in combination with miRNA replacement molecules mimicking tumor-suppressor miRNAs.

Acknowledgements: This study was funded by AIRC IG13575, and FAR fund from University of Ferrara.

(1) Finotti A, et al., Int J Oncol 53(4): 1395-1434, 2018;
(2) Gasparello J, et al., J Exp Clin Cancer Res 37: 124, 2018;
(3) Gasparello J, et al., Sci Rep 9: 3036, 2019.

176

Downregulation of LYAR is associated with induction of fetal hemoglobin in mithramycin-treated erythroid cells

Lucia Carmela Cosenza, Jessica Gasparello, Matteo Zurlo, Cristina Zuccato, Roberto Gambari, Alessia Finotti
Department of Life Sciences and Biotechnology, Ferrara University, Ferrara, Italy
E-mail: alessia.finotti@unife.it

LYAR (Ly-1 antibody reactive clone) protein is a recently identified repressor of Aγ-globin gene transcription in erythroid cells and binds to the 5'-GGTTAT-3' sequence of the 5'-region of the gene. The rs368698783 G>A polymorphism of this site is present in β-thalassemia patients and is associated with a high production of fetal hemoglobin (HbF). The finding that LYAR binds less efficiently to the G>A mutated 5'-GGTTAT-3' binding site might explain the increased basal and induced levels of HbF in erythroid cells. The present study was undertaken to verify the effects of the HbF inducer mithramycin (MTH) on LYAR. We first determined whether MTH was able to inhibit the LYAR/DNA interactions using both nuclear factors from K562 cells and recombinant LYAR protein. Electrophoretic mobility shift assay demonstrated that MTH strongly interfered with the binding of LYAR to the double-stranded oligonucleotides containing the Aγ-globin LYAR binding site. We also performed RT-qPCR and western blot analysis of MTH-treated cells demonstrating an association between LYAR mRNA and Aγ-globin gene expression. LYAR expression (analyzed at mRNA and at the protein level) was downregulated in association with the upregulation of γ-globin gene expression and HbF production. LYAR downregulation was confirmed in MTH-treated K562 cells, as well as in erythroid precursor cells (ErPCs) from β-thalassemia patients. In the analyzed ErPCs those which were found as non-responders to MTH demonstrated unchanged LYAR content. In order to verify possible MTH-dependent effects on LYAR transcription, the LYAR promoter was studied and at least five Sp1 CG-rich binding sites identified. EMSA was performed using double-stranded oligonucleotides mimicking these binding sites and the effects of MTH addition were determined, demonstrating that MTH is able to inhibit the interactions between Sp1 and the Sp1 binding sites present within the LYAR promoter. In conclusion, downregulation of LYAR expression and functions might strongly contribute to induction of Aγ-globin gene expression and HbF production in erythroid cells.

Acknowledgements: Supported by the UE FP7 THALAMOSS Project (THALAssaemia MOdular Stratification System for personalized therapy of beta-thalassemia; grant n.306201-FP7-Health-2012-INNOVATION-1), by Wellcome Trust and by AIFA (Agenzia Italiana del Farmaco).

177

Upregulation of miR-34a-3p and miR-744-3p is associated with downregulation of PTEN in lymphoblastoid cells from Shwachman-Diamond Syndrome patients

Jessica Gasparello[1], Chiara Papi[1], Giulia Breveglieri[1], Chiara Tupini[1], Elisabetta D'Aversa[1], Valentino Bezzerri[2], Marco Cipolli[2], Roberto Gambari[1], Monica Borgatti[1], Alessia Finotti[1]
[1]Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy; [2]Cystic Fibrosis Center, Azienda Ospedaliero Universitaria Ospedali Riuniti di Ancona, Ancona, Italy
E-mail: alessia.finotti@unife.it

Shwachman-Diamond syndrome (SDS) is an inherited disease caused by mutations of a gene encoding for SBDS protein. SDS patients present several hematological disorders, including neutropenia and myelodysplastic syndrome (MDS), with increased risk of leukemic evolution. In lymphoblastoid cells of SDS patients, the already reported upregulated mTOR phosphorylation(1) is possibly associated with the downregulation of PTEN, a validated inhibitor of mTOR phosphorylation. Since PTEN expression might be under the post-transcriptional control of microRNAs(2), this study was undertaken to verify the miRNome in SDS patients and find a possible correlation with *PTEN* gene expression. Lymphoblastoid cell cultures derived from 7 SDS patients and 4 control subjects were studied by Next Generation Sequencing (NGS) and western blot analysis. In total, 11 miRNAs were upregulated in SDS cell cultures compared with controls, according to a 1.5-fold threshold. The differential miRNA expression was further validated by droplet-digital RT-PCR. When the 11 upregulated miRNAs were compared with the 81 validated PTEN-regulating miRNAs (identified using the miRTarBase software: *www. mirtarbase.mbc.nctu. edu.tw*), two microRNAs were identified: miR-34a-3p and miR-744-3p. In order to relate the PTEN expression with miR-34a-3p and miR-744-3p levels, PTEN was analyzed by RT-qPCR and western blot analysis. The results obtained supported the following conclusions: (a) upregulation of miR-744-3p and miR-34a-3p is variable among SDS cells; (b) PTEN downregulation was found in SDS cells to varying degrees; (c) a correlation does exist between the miR-34a-3p and miR-744-3p levels and *PTEN* gene expression. Consequently, miR-34a-3p and miR-744-3p are possible targets for increasing PTEN expression in SDS patients.

Acknowledgements: This study was supported by a FIR and a FAR fund of Ferrara University to AF, and by a fund from the Italian Ministry of Health (MOH) to VB, MB and GB.

(1) Bezzerri V, et al., Sci Rep 6: 33165, 2016.
(2) Li W, et al., J Exp Clin Cancer Res 37: 223, 2018.

178

Analysis of the miRNome as a possible tool for the detection of autologous blood transfusion misuse in sport

Jessica Gasparello[1], Nicola Lamberti[2], Chiara Papi[1], Enrica Fabbri[1], Lucia Carmela Cosenza[1], Ilaria Lampronti[1], Fabio Manfredini[2], Roberto Gambari[1], Alessia Finotti[1]
[1]Department of Life Sciences and Biotechnology, Ferrara University, Ferrara, Italy; [2]Department of Biomedical and Surgical Specialties Sciences - Section of Sport Sciences, Ferrara University, Ferrara, Italy; [3]Blood Transfusion Service Azienda Ospedaliera Universitaria Ferrara, Ferrara, Italy
E-mail: alessia.finotti@unife.it

Detection of Autologous Blood Transfusions (ABT) is a key issue in the field of anti-doping for the performance enhancing effects of this prohibited method and the consequent unfair use in sport. Unfortunately, at present no direct detection method of ABT is available. The present study was performed to determine whether the microarray-based analysis of the global miRNA pattern might be useful for the detection of ABT. Blood (500 ml) was drawn from 6 healthy subjects (T2) and then infused after 35 days (T5). Blood samples for microarray analysis of miRNAs were taken 5 days before (T1) and 10 days after blood withdrawal (T3), at day of infusion (T5), and at 3 days (T6) and 15 days (T8) after infusion. For three subjects the withdrawn blood was stored at -80°C, while for the remaining three at +4°C prior to infusion. Global microRNA profiling was performed for a total of 39 RNA samples (extracted from plasma), using the Agilent Human microRNA microarray v.21.0 (no. G4872A). This chip represents 2549 microRNAs, sourced from the miRBase database (Release 21). Microarray results were analyzed using the GeneSpring GX 13 software (Agilent Technologies). Differentially expressed miRNAs were selected following determination of the fold-change analysis, taking T1 as a reference sample. Both up- and downregulated miRNAs were considered. Microarray cluster analysis of informative miRNAs found in plasmas from ABT-trained athletes at T6 and T8 demonstrated that all the ABT T6 and T8 samples are clustered together and differ from T1 and from control samples. These results support the concept that the miRNome analysis might be considered a one-step approach for the detection of ABT.

Acknowledgements: This work was supported by the World Anti Doping Agency Science grant # ISF17D05FM.

179

The possible low cancer risk in schizophrenic patients, through the regulatory role of microRNAs: Preliminary data

E. Rizos[1], N. Siafakas[2,1], E. Skourti[3], C. Papageorgiou[4], J. Tsoporis[5], Th. Parker[5], D.A. Spandidos[6], E. Katsantoni[7], K. Tsamakis[1], N. Nikolakakis[1,8], V. Zoumpourlis[3]
[1]National and Kapodistrian University of Athens, School of Medicine, 2nd Department of Psychiatry, University General Hospital "ATTIKON", Athens; [2]National and Kapodistrian University of Athens, Microbiology Laboratory, University General Hospital "ATTIKON", Athens; [3]Unit of Biomedical Applications, Institute of Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation, [4]National and Kapodistrian University of Athens, School of Medicine, 1st Department of Psychiatry, Eginition Hospital, Athens, Greece; [5]Keenan Research Centre, Li Ka Shing Knowledge Centre, Institute of Biomedical Science, St. Michael's Hospital, Toronto, Canada; [6]Laboratory of Clinical Virology, Medical School, University of Creta, Heraklion; [7]Biomedical Research Foundation, Academy of Athens, Hematology-Oncology Division, Athens; [8]Department of Psychiatry and Behavioral Sciences, Faculty of Medicine, University of Crete, Heraklion, Greece
E-mail: erizos@med.uoa.gr

Interest has increased into the role of microRNAs (miRNAs) as regulatory factors in the aetiology and pathophysiology of major psychiatric disorders, such as schizophrenic spectrum disorder, bipolar disorder, depression etc. Reports have indicated a low cancer risk in patients with schizophrenic spectrum disorder and this risk seems to be related to the duration and age of onset of schizophrenia; specifically, duration seems to be inversely correlated with cancer risk. Moreover, other clinical reports have indicated the apoptotic activity of antipsychotics and mainly the First Generation Antipsychotics (FGAs) and thus, an impact on specific gene expression associated with the development of cancer. miRNAs are a large group of small non-coding oligonucleotides that regulate gene expression mainly in the Central Nervous System (CNS). While the development of cancer is characterized by increased gene expression that leads to uncontrolled cell proliferation, the development of schizophrenia is characterized by the opposite phenomenon and specifically, by the reduced expression of genes whose products suppress cellular proliferation and increase apoptosis. Therefore, the regulatory role of miRNA expression is crucial in the development of both of disorders and moreover, miRNAs emerge as a significant etiopathogenetic factors and possible biological markers for both disorders and may intermediate this possible negative correlation. This hypothesis is based on their pleiotropic and epistatic function; different subtypes can affect the expression of one gene and one type can act on many targets. In our previous study, we found an implication of let-7, mir-98 and mir-183 as possible biomarkers for cancer and schizophrenia (1,2). The scope of this review was to examine role of miRNAs in the rare co-morbidity of SZ and Ca and to highlight the possible molecular pathways which may be involved on both of these disorders (3). Moreover, new future medicinal strategies could be tested based on miRNA analysis, for both of the disorders, cancer and schizophrenic spectrum disorder.

(1) Rizos et al., Oncol Rep 28: 2200-2204, 2012; (2) Rizos et al., PLoS One 10(4): 1-11, 2015; (3) Rizos et al., Mol Med Rep 14: 4942-4946, 2016.

180

Monoclonal antibodies to human norovirus that was growing in cell culture

Vladimir Sologub, Irina Koromyslova
Research Center for Molecular Diagnostics and Therapy, Department of Biotechnology, Moscow, Russia

Monoclonal antibodies are the main tools for detection of antigens in a large variety of applications in the clinic and literature. Norovirus is a member of the Calicivirus family where many animal models with different biological but with the same morphological or physical properties were described. Replication in cell culture was shown for feline calicivirus and mouse norovirus, but attempts to cultivate human norovirus in cell culture were mainly unsuccessful. We have made successful attempt to cultivate Human Norovirus in monolayer cell culture. The cell culture was the high level of *in vitro* passage fibroblast-like human melanoma. The main property of the cells was their stability at 10 µg/ml of trypsin in serum-free media. Norovirus from fecal samples cause CPE in the cell monolayer after 3-4 days of cultivation at 37°C. Production of the virus was achieved by sandwich ELISA with pair of Mab's produced to Feline and Rabbit Caliciviruses and did not discriminate rabbit and feline viruses. Virus, at the level of 12th passage, was concentrated by ultracentrifugation and purified by $CsCl_2$ density gradient ultracentrifugation. Then, 100 µg of purified virus in 0.5 ml of PBS was mixed with Complete Friend's Adjuvant and injected into food patties of Balb/c mice. After 1 week, injection of purified virus was repeated and after 3 days popliteal lymph nodules were removed and the lymphocytes were fused with Sp/2-0 mouse myeloma. Hybridomas were detected by indirect ELISA with purified virus and gout anti-mouse IgG-HRP conjugate. In total, 29 clones were chosen and frozen in liquid nitrogen, and 12 Mab's were produced as ascetic fluids. The Mab's were purified by protein A chromatography and sandwich ELISA was used as well as HRP-conjugates of Mab's to generate common Calicivirus epitopes. Very sensitive detection of human norovirus and animal caliciviruses were then obtained by the panel.

181

HLA polymorphism in Transylvanian population in comparison with other populations

Mihaela Laura Vică[1], Gheorghe Zsolt Nicula[1], Cosmina Ioana Bondor[2], Costel Vasile Siserman[3,4], Horea Vladi Matei[1,4]
[1]'Iuliu Haţieganu' University of Medicine and Pharmacy, Department of Cell and Molecular Biology, Cluj-Napoca, Romania; [2]'Iuliu Haţieganu' University of Medicine and Pharmacy, Department of Medical Informatics and Biostatistics, Cluj-Napoca, Romania; [3]'Iuliu Haţieganu' University of Medicine and Pharmacy, Department of Legal Medicine, Cluj-Napoca, Romania; [4]Legal Medicine Institute, Cluj-Napoca, Romania
E-mail: mvica@umfcluj.ro

The major histocompatibility complex (MHC) is a large gene complex involved in the continuity of the short arm of chromosome 6. Known as the human leukocyte antigen (HLA), the human MHC-encoded glycoproteins are specialized in the presentation of short peptides to T cells[(1)]. Their importance in the immune system, drug response, susceptibility to infection and human transplantation is derived from the huge polymorphism at the HLA loci[(2,3)]. As a significant number of human diseases are more often observed among individuals carrying particular HLA alleles, determination of HLA profiles in various ethnic groups is important since distribution of HLA alleles is unique for any given ethnic group. Our study aimed to determine HLA (A, B, C and DRB1) profiles in a population group from Transylvania, Romania and to compare them with various European population groups. HLA alleles from 2,794 individuals were examined in the process making use of PCR methods, with HLA allele frequencies data from several European population groups serving as reference in comparisons with the local sample. The distribution of HLA alleles in the studied population group was heterogeneous, as Hardy-Weinberg equilibrium was statistically significant ($p<0.01$). The most common alleles found in our sample were A*02 (0.27%), B*35 (0.14%), C*07 (0.25%) and DRB1*11 (0.19%), while the most common haplotype was A*01-B*08-C*07-DRB1*03 (1.26%). This analysis was the first to determine the frequencies of these genes in a significant sample for this region. Comparisons based on univariate/multivariate analysis indicated that populations genetically closer to the Transylvanian one were those in close vicinity (Serbia, Wallachia, Hungary and Croatia). Our study confirms that a genetic imbalance appeared over the centuries due to massive population migrations and provides an incipient database for subsequent genetic population studies.

(1) Holoshitz, Discov Med 16: 93-101, 2013; (2) Trowsdale and Knight, Annu Rev Genomics Hum Genet 14: 301-323, 2013; (3) Blackwell et al., Clin Microbiol Rev 22: 370-385, 2009.

182

Endoplasmic reticulum stress in the evasion of immunosurveillance and resistance to anticancer therapy of breast cancer cells

Rashed Al-Hammad[1], Sasiprapa Khunchai[2], Nopprarat Tongmuang[3], Thawornchai Limjindaporn[3,4], Pa-thai Yenchitsomanus[3], Marija Krstic-Demonacos[5], Constantinos Demonacos[1]
[1]Faculty of Biology, Medicine and Health, Division of Pharmacy and Optometry, University of Manchester, Manchester, UK; [2]Department of Anatomy, Faculty of Medical Science, Naresuan University, Phitsanulok, Thailand; [3]Division of Molecular Medicine, Research Department, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand; [4]Department of Anatomy, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand; [5]College of Science & Technology, School of Environment and Life Sciences, University of Salford, Salford, UK
E-mail: constantinos.demonacos@manchester.ac.uk

The induction of endoplasmic reticulum stress due to tumor inflammatory microenvironmental conditions is a common characteristic of all solid tumors. ER stress, as a pro-survival mechanism, contributes to resistance to anticancer therapy. Accumulating evidence indicates that ER stress-responsive proteins are involved in the crosstalk between the tumor and the immune system of the host. The interaction between ER and tumor immunity includes the regulation of the stability of the major histocompatibility complex class I and II molecules by ER aminopeptidases (ERAP1/2) and the assembly of the antigen-MHC class I molecules complex and transport to the cellular surface by ER chaperones [protein disulphide isomerase (PDI) and endoplasmic reticulum oxidoreductase 1 alpha (ERO1A)]. It is thus possible that apart from the induction of pro-survival mechanisms, ER stress can promote tumor proliferation by interfering with the process of antigen presentation enabling cancer cells to remain unrecognizable by the immune system and avoid elimination. This hypothesis was tested by performing genome-wide association studies in MCF-7 and MDA-MB-231 breast cancer cells, in which PDIA1 had been silenced, treated with the topoisomerase inhibitor, etoposide, or interferon gamma. Our results indicate that the genes upregulated in the PDIA1-silenced cells are primarily involved in physiological mechanisms directly associated with the antigen presentation process or indirectly through pathways modulating calcium metabolism.

183

Bioinformatics and clinical practice: Predictive models in the stratification of cancer patients and the identification of novel therapies

Kun Tian[1], Emyr Bakker[2], Michelle Hussain[3], Alice Guazzelli[1], Hasen Alhebshi[1], Parisa Meysami[1], Constantinos Demonacos[4], Jean-Marc Schwartz[4], Luciano Mutti[5], Marija Krstic-Demonacos[1]
[1]School of Environment and Life Sciences, University of Salford, Salford, UK; [2]School of Medicine, University of Central Lancashire, Preston, UK; [3]School of Medicine, University of Cardiff, Cardiff, UK; [4]Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK; [5]Sbarro Institute for Cancer Research and Molecular Medicine, Center for Biotechnology, College of Science and Technology, Temple University, Philadelphia, USA
E-mail: M.Krstic-Demonacos@salford.ac.uk

Despite intense research efforts, cancer remains difficult to treat due to chemotherapeutic resistance. The p53 tumor suppressor is crucial for cancer development, DNA damage and the chemotherapeutic response. We created Boolean p53 interactome models and investigated their predictive power for the stratification of cancer patients. The comparison of model simulations obtained by knockout tests mimicking mutations with the omics type of experimental data demonstrated a significant rate of successful predictions in osteosarcoma, colon cancer and mesothelioma cell lines, as well as in 71 patients with mesothelioma. Model analysis allowed the identification of deregulated pathways, the prediction of therapeutic schemes and the linking of the affected pathways with the clinical state of the patients. Model validations demonstrated successful predictions ranging from 52 to 85%, depending on the drug, algorithm or sample used for validation. Patients were stratified depending on their p53 status and therapy received, and their clinical outcomes and simulation comparisons were then used to identify 30 genes that were associated with survival. In patients with the wild-type p53, FEN1 and MMP2 exhibited the highest inverse correlation, whereas in untreated patients with a p53 mutated status, SIAH1 was negatively associated with survival. Using DRUGSURV, repositioned and experimental drugs targeting FEN1 and MMP2 were identified. Testing revealed that drugs that target FEN1 (epinephrine and myricetin) have a cytotoxic effect, whereas marimastat and batimastat, which target MMP2 have an inhibitory effect on mesothelioma cell migration. In summary, the p53 model has predictive properties with versatile potential for use in cancer treatment by identifying pathways crucial for tumor growth, by facilitating patient stratification and by the identification of shifts in pathways required for chemoresistance. We consider that upon further testing in animal models and wider database analysis, clinical decisions and personalized therapy can be devised based on individual patient genetic profiles and previous chemotherapeutic treatments.

184

Polyoxometalates' antitumor activity tested on HeLa cancer cell line

Gheorghe Zsolt Nicula[1], Olga Soriţău[2], Dan Rusu[3], Miuţa Filip[4], Mihaela Laura Vică[1], Rodica Ungur[5], Horea Vladi Matei[1], Costel Vasile Siserman[6], Ştefana Bâlici[1]
[1]Iuliu Haţieganu University of Medicine and Pharmacy, Faculty of Medicine, Department of Cell and Molecular Biology, Cluj-Napoca; [2]The Oncology Institute Prof. Dr. Ion Chiricuţă, Radiotherapy, Tumor and Radiobiology Laboratory, Cluj-Napoca; [3]Iuliu Haţieganu University, Faculty of Pharmacy, Department of Physical-Chemistry, Cluj-Napoca; [4]Babeş-Bolyai University, Raluca Ripan Institute for Research in Chemistry, Analytical and Environmental Chemistry Laboratory, Cluj-Napoca; [5]Iuliu Haţieganu University of Medicine and Pharmacy, Faculty of Medicine, Department of Medical Rehabilitation, Cluj-Napoca; [6]Iuliu Haţieganu University of Medicine and Pharmacy, Faculty of Medicine, Department of Legal Medicine, Cluj-Napoca, România
E-mail: gnicula@umfcluj.ro

Cancer cell-acquired antitumor resistance requires alternative cytostatic compounds[(1)]. The antitumor activity of polyoxometalates (POMs) such as hepta- or hexa-tungsten/molybdate, stronger than that of well-known cytostatics (5-fluorouracil, gemcitabine, cisplatin), is demonstrated[(2)]. This enhanced activity of POMs is accomplished via decreasing of the mitochondrial activity by inhibiting ATP synthesis, thus activating apoptosis mechanisms inside cancer cells[(3)]. We tested 10 molybdenum/tungsten POMs previously synthesized via a two-step self-assembling method. All POMs were characterized by physic-chemical methods including thermal analysis, UV and FT-IR spectroscopy and atomic absorption. Their antitumor activity was investigated on HeLa cervical cancer cells (originally infected with oncogenic serotype 16 of the *Human Papilloma Virus*) compared with normal HUVEC (Human Umbilical Vein Endothelial Cells) cells. We found that, at the tested concentrations, heteropolyoxomolibdates had significantly stronger antitumor effects than heteropolyoxotungstates. Our results prove that these POMs exhibit cytostatic-like behaviors and their significant antitumor activity recommends them for further studies in this field.

(1) Prudent et al., Biochim Biophys Acta 1804(3): 493-498, 2010.
(2) Ogata et al., Br. J. Cancer 98(2): 399-409, 2008.
(3) Rusu and Bâlici, Polioxometalaţii. Aplicaţii biomedicale, Casa Cărţii de Ştiinţă Ed., 2013.

185

Carcinogenesis: A systems biology approach

Fernando Méndez-López
Autonomus University of Nuevo Leon, School of Public Health, Mexico
E-mail: luis.mendezl@uanl.mx

Cancer is a degenerative chronic disease that can be interpreted as a robust developmental process intrinsic to human aging. Motivated by the systems dynamics view of cellular differentiation and morphogenesis (1), in this contribution I present the conclusions and perspectives derived from the modelling work at the intersection of development and cancer biology (2,3). I will discuss an interpretation of epithelial carcinogenesis based on an integrative model inspired by a mechanistic understanding of development in terms of gene regulatory networks (GRNs) and the study of similitudes observed at the level of molecular, cellular and histologic events during the carcinogenesis of epithelial tissues (3,4). We developed a Boolean network model of the GRN and cell signalling processes underlying senescence, inflammation and EMT in carcinomas, that reproduced the expected gene expression state of epithelial, senescent and mesenchymal phenotypes as attractors, that recapitulate the commonly observed progression by which epithelial cells first become hyperplasic atypical lesions and then lose differentiation through progression to high-grade carcinomas (2). The model reproduced the transitions between the cellular phenotypes involved in epithelial carcinogenesis *in vitro* (2) and *in vivo* (3,4). Moreover, it predicted that the transition into the mesenchymal-like phenotype is accelerated by inflammation, consistent with the poor prognosis of pro-inflammatory conditions (2). By linking inflammation, and aging, this interpretation provides a novel mechanistic relationship, and suggests modulation strategies for the therapeutics of cancer, and their prevention. Hopefully the present work will motivate new approaches for cancer research.

(1) Huang et al., Sem. Cell Dev. Biol 20: 869-876, 2009.
(2) Méndez-López, et al. BMC Sys Bio 11: 1-24, 2017.
(3) Méndez-López, et al. Arc Med Res, *41*: 261-268, 2010.
(4) Méndez-López, et al., Arc Med Sci, 13: 228-237, 2017.

186

Management of RSV infection in children: New advances and challenges

Simon B. Drysdale
St George's, University of London, London, UK

Respiratory syncytial virus (RSV) causes a large burden of disease globally and can present as a variety of clinical syndromes in children of all ages. The current management of RSV bronchiolitis is purely supportive with feeding support and oxygen supplementation. Ribavirin is currently the only licensed drug for the specific treatment of RSV infection but due to toxicity and evidence of only minimal clinical benefit it is reserved for use in severely immunocompromised children. Multiple types of novel therapeutic molecules have been developed aimed at various RSV targets. There are, therefore, several antiviral medications to treat RSV infection in development although none have yet progressed beyond Phase 2 clinical trials and few have recruited children. There is currently no licenced RSV vaccine but passive immunisation with a monoclonal antibody, palivizumab, reduces hospitalisation due to RSV infection by up to 80% in some groups of high-risk infants. There are more than 40 RSV vaccine or monoclonal antibody candidates currently in development. A maternal RSV vaccine recently completed a Phase 3 trial and showed 44% efficacy against hospitalization for RSV lower respiratory tract infection in infants. A new long acting monoclonal antibody, having shown excellent promise in a Phase 2 trial in infants, is about to be investigated in a Phase 3 clinical trial. The development of an efficacious treatment or prophylactic agent against RSV infection could revolutionise the care and outcome of many vulnerable infants.

187

Understanding the burden of RSV and influenza infections in real-time: Partnering for Enhanced Digital Surveillance of Influenza-like Disease and the Effectiveness of Antivirals and Vaccines (PEDSIDEA)

Barbara Rath[1,2,3]
[1]Vienna Vaccine Safety Initiative, Berlin, Germany; [2]Université Bourgogne Franche-Comté, Besancon, France; [3]University of Nottingham School of Medicine, Nottingham, UK

The clinical impact and burden of respiratory syncytial virus (RSV) infections in different age and risk groups has drawn the interest of public health stakeholders and regulatory agencies in recent years. With RSV vaccines and antivirals in development, it will be important to: i) Diagnose RSV infections in a timely manner, ii) differentiate RSV from other forms of acute respiratory infections, and iii) communicate the test results back to patients and parents/caretakers along with information on the individual disease risk and severity. The Vienna Vaccine Safety Initiative is an international non-profit research organization which, in collaboration with academic institutions and public health agencies in Europe and the United States, developed digital tools and programs to improve the quality of care for children and adults with influenza-like illness (ILI) acute respiratory viral infections (ARI). Taking a person-centered approach, we developed the ViVI Disease Severity Score ('ViVI Score'), a mobile application enabling healthcare professionals to measure disease severity at the point of care within minutes, providing a uniform approach to define *ad hoc* severity at any given time point, based on extensive literature review as well as WHO Criteria for uncomplicated and complicated ILI. In collaboration with the Robert Koch Institute, the ViVI Score was validated in a Cohort of 6000 children (age 0-18) in Berlin, Germany and subsequently used in a European pilot project 'Partnering for Enhanced Digital Surveillance of Influenza-like Disease and the Effectiveness of Antivirals and Vaccines' (PEDSIDEA). The PEDSIDEA program has since been implemented in community clinic networks in the Unites States for the real-time digital surveillance of RSV and influenza disease incidence and severity at the point of care. The talk will highlight the challenges of implementing person-centered quality improvement programs in busy acute care settings and the opportunities arising for an improved understanding for the 'real-life' impact and the burden of RSV and influenza infections inside and outside of the hospital.

188

Modulation of the tumor microenvironment by liposomal prednisolone enhances the antitumor activity of liposomal doxorubicin in an *in vivo* model of murine melanoma

Emilia Licarete[1,2], Denise-Minerva Drotar[1], Ioana Stejerean[1], Laura Patras[1,2], Valentin Florian Rauca[1,2], Lavinia Luput[1,2], Alina Sesarman[1,2], Manuela Banciu[1,2]
[1]Department of Molecular Biology and Biotechnology, Babes-Bolyai University, Cluj-Napoca, Romania; [2]Molecular Biology Centre, Institute for Interdisciplinary Research in Bio-Nano Sciences, Babes-Bolyai University, Cluj-Napoca, Romania
E-mail: emilia_licarete@yahoo.com

In solid cancers, tumor cells create an immunosuppressive niche by the induction of regulatory T cells and polarization of the tumor associated macrophages (TAMs) to an alternative, protumor phenotype (M2 macrophages). M2-polarized TAMs secrete chemokines and growth factors to maintain the immunosuppressive state of the tumor microenvironment and support tumor angiogenesis, drug resistance and metastasis. Additionally, several studies have demonstrated that M2-polarized TAMs decreased the cytotoxic effects induced by chemotherapeutic drugs, such as doxorubicin (DOX) on tumor cells. We have previously demonstrated that prednisolone phosphate (PLP) encapsulated in long circulating liposomes (LCL), exerts potent strong antitumor effects on B16.F10 melanoma-bearing mice via the inhibition of the angiogenic/inflammatory capacity of TAMs, while DOX exerted a direct cytotoxic effect on the tumor cells. Based on these findings, the aim of the present study was to investigate whether the administration of LCL-PLP concomitant with LCL-DOX could improve the therapeutic outcome of the cytotoxic agent in B16F10 melanoma tumors *in vivo*. Our results indicated that the administration of 10 mg/kg of LCL-PLP concomitant with 5 mg/kg of LCL-DOX to B16F10 melanoma-tumor bearing mice induced a more prominent reduction (by 85%) of the tumor volume compared to the drugs administered alone. Furthermore, we found that LCL-PLP potentiated the cytotoxic activity of LCL-DOX mainly by inhibiting angiogenic proteins production, as well as the expression level of the phosphoprotein c-Jun, a main regulator of melanoma progression. Additionally, the combination therapy reduced the activation of matrix metalloproteinase-2, suggesting its anti-metastatic potential. In conclusion, the concomitant administration of LCL-PLP and LCL-DOX may be a promising strategy with which to increase the therapeutic outcome of DOX treatment in melanoma.

Acknowledgements: This study was supported by grants of the Romanian Ministry of Research and Innovation, CNCS-UEFISCDI, project number PN-III-P4-ID-PCE-2016-0342, contract number 91/2017 within PNCDI-III.

189

Novel therapies based on long-circulating liposomal simvastatin in colorectal cancer

Lavinia Luput[1,2], Emilia Licarete[1,2], Alina Sesarman[1,2], Valentin Florian Rauca[1,2], Laura Patras[1,2], Alina Porfire[3], Dana Muntean[3], Marcela Achim[3], Ioan Tomuta[3], Laurian Vlase[3], Manuela Banciu[1,2]
[1]Department of Molecular Biology and Biotechnology, Faculty of Biology and Geology, Babes-Bolyai University, Cluj-Napoca, Romania; [2]Molecular Biology Centre, Institute for Interdisciplinary Research in Bio-Nano-Sciences, Babes-Bolyai University, Cluj-Napoca, Romania; [3]Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, University of Medicine and Pharmacy 'Iuliu Hatieganu', Cluj-Napoca, Romania
E-mail: lavinia_luk@yahoo.com

Chemotherapy for colorectal cancer is largely based on 5-fluorouracil (5-FU) administration and has several limitations, such as the chemoresistance of cancer cells to 5-FU, severe side-effects and a low therapeutic index. Therefore, in the present study, we aimed to investigate a novel combined therapy for colorectal cancer based on the administration of long-circulating liposomal simvastatin (LCL-SIM) with 5-FU encapsulated in LCL (LCL-5-FU) on C26 colon carcinoma *in vivo* model. To achieve this goal, the anti-tumor activity of LCL-SIM on colon carcinoma microenvironment was tested, also the underlying molecular mechanisms of anti-tumor activity of LCL-SIM such as angiogenesis, inflammation and cytotoxic effects on C26 colon carcinoma were evaluated on C26 murine colon carcinoma-bearing mice. Furthermore, we evaluated the potential of LCL-SIM to sensitize the colon cancer cells to LCL-5-FU administration. Thus, different regiments of treatment administration were used and the main pro-tumor processes, such as angiogenesis, inflammation, oxidative stress and resistance to apoptosis were investigated. Our results indicated a potent antitumor activity *in vivo* exerted by LCL-SIM based on anti-angiogenic and cytotoxic effects on C26 colon carcinoma cells. The results of the investigation of combined therapy showed the potential of LCL-SIM to sensitize colon cancer cells to LCL-5-FU administration, the capacity to inhibit angiogenesis and antioxidant effects. Taken together, our data indicate that this combined therapy has the potential to become a successful colorectal cancer targeted therapy.

190

Residential radon exposure and lung cancer risk in Kazakhstan

Rakhmetkazhi I. Bersimbaev, Olga V. Bulgakova, Assiya A. Kusainova, Asemgul Zh. Kausbekova, Akmaral A. Aripova
L.N. Gumilyov Eurasian National University, Astana, Kazakhstan
E-mail: ribers@mail.ru

Lung cancer is a serious health concern and is one of the most common types of cancer worldwide; it is also the most common type of cancer in the Republic of Kazakhstan. Radon exposure has been classified as the second cause of lung cancer. Kazakhstan is characterized for having a high indoor radon concentration. To date, a large amount of evidence has been accumulated on the involvement of microRNAs in the carcinogenesis of various malignant neoplasms, including lung cancer. Recent data have indicated that miRNAs are engaged in the regulation of cellular processes induced by radiation and, consequently, miRNAs can potentially be used as biomarkers to assess the degree of exposure to radiation in human. The aim of the present study was to determine the alterations in free circulating miR-19b and the level of p53 protein in the plasma of patients with lung cancer exposed to high doses of radon. It was shown that the plasma miR-19b-3p level was significantly higher in the lung cancer patient groups compared with the healthy control. No other statistically significant differences were found in the expression level of plasma miR-19b-3p between patients diagnosed with lung cancer exposed to radon and not exposed to radon. We found that *TP53* codon 72 Arg/Pro polymorphism was associated with the lung cancer risk in the Kazakh population. Arg/Pro and Pro/Pro variants conferred an odds ratio (OR) of 6.95 (95% confidence interval (CI) 2.41 - 20.05) and 1.45 (95 % CI 0.46 - 4.64), respectively. Individuals with Arg/Pro variant of *TP53* gene exposed to a high level of radon had an OR=8.6 (95% CI 2.6–28.59) when compared with individuals living in areas with a low level of radon. The *TP53* codon 72 gene polymorphism might be involved in pathogenesis of radon-induced lung cancer in Kazakh population. In summary, exposure to residential radon interacted with Arg72Pro genotype to increase the risk of lung cancer in Kazakh population. This study supports the hypothesis that *TP53* codon 72 polymorphism can modulate the pathogenic mechanism of radon in lung tissue.

191

Impact of fecal microbiota transplantation on short chain fatty acid levels in feces of patients with inflammatory bowel diseases

Elena Zhgun, Yuri Kislun, Alexander Pavlenko, Tatiana Kalachniuk, Elena Ilina
Federal Research and Clinical Center of Physical-Chemical Medicine, Moscow, Russia
E-mail: al.androva@gmail.com

Inflammatory bowel diseases (IBD) are associated with the inhibition and replacement of the normal intestinal microflora or depletion of its composition. Under these conditions, the diversity of bacteria, producers of short-chain fatty acids (SCFA) (namely butyrates and propionates), exhibiting anti-inflammatory and protective properties, is often significantly reduced. Currently, there are various methods of microbiota correction, among which fecal microbiota transplantation (FMT) is considered the most effective. FMT is performed by introducing the fecal microbial community obtained from a healthy donor into the patient's intestinal tract. Metabolites produced by intestinal microflora can provide an unbiased evaluation of the intestinal microbiota health and, consequently, the success of FMT treatment. Using the gas chromatography technique, we analyzed SCFA from 60 healthy donor feces. The quantitative results obtained, namely 57.33±26,66 μmol/g, for acetate, 17.297±10,8 μmol/g for propionate and 14.659±9,09 μmol/g for butyrate, as well as their percentage ratio 58.6±6.5%:19.9%±5.4%:21.5±5.5% (acetate: propionate: butyrate, respectively) demonstrated good accordance with the literature data. In a limited group of IBD patients (n=15) before and after FMT treatment, we determined the SCFA concentrations and ratios, respectively. The data revealed a significant imbalance in the ratios of SCFA in patient feces compared with the control group. Long-term patient observations (0-30 days) demonstrated that FMT treatment affected the SCFA ratios, but did not reach the profiles of healthy donors. The patterns of metabolic profiles for both diseases differed, with a clear decrease in the butyrate level due to the acetate increase in the case of ulcerative colitis, and due to the propionate increase in the case of Crohn's disease. The superpositioning of patient SCFA molar ratio profiles on the taxonomic profiles of the bacteria species responsible for their production revealed a relationship between changes in fatty acid profiles and the corresponding bacteria-producer profiles, as well as with patient recovery. On the metagenomic and metabolic levels, we clearly observed the amelioration of the microflora to a healthy one and a trend to reach a healthy ratio in the case of SCFA for both diseases. Using multi-omics analysis, we demonstrated changes in metagenomic and metabolic levels in IBD patients. Further validation of input of specific microbes and metabolites in therapy of IBD will help to develop targeted personalized approaches with which to improve quality of life of IBD patients.

192

Feed supplemented with wine by-products enhances the antioxidant defense system of broiler tissues

Sotiria Makri[1], Aristidis S. Veskoukis[1] and Demetrios Kouretas[1]
[1]Department of Biochemistry and Biotechnology, University of Thessaly, ViopolisMezourlo, 41500, Larisa, Greece
E-mail: sotirina_m@hotmail.com

Grape pomace (GP) is a byproduct of winery industry that on the one hand is a polluting factor when discarded in the environment due to its high organic load, while on the other hand is rich in polyphenols which exerts significant antioxidant effects (1). Animal feed enriched with these compounds can putatively enhance the efficiency, productivity and animal welfare by protecting them from oxidative stress-related diseases (2). For the present study, 30 broilers (Hubbard), 2 days-old, were randomly separated into two groups, as follows: i) the control group fed with the standard ration; ii) the GP group fed with feed containing GP silage. The experiment lasted for 50 days. Tissue samples (heart, liver, quadriceps muscle, lung, intestine, pancreas, kidney and spleen) were collected at 30 and 50 days post-birth in order to determine theactivity of antioxidant enzymes (GST, SOD) and protein expression ofγ-GCL. GST activity and γ-GCL expression were increased significantly (by 15-76% and by 35-262%, respectively) in the majority of the tested tissues in GP group compared to the control, both at 30 and 50 days. SOD activity was not affected. To the best of our knowledge, this study is the first time to evaluate these key antioxidant enzymes in a large variety of tissues of productive animals in order to reveal potential mechanisms with which to enhance their welfare and improve the taste and quality of their meat products. The results of this study indicate that feed supplemented with GP byproducts improves the tissue redox status in broilers, mainly due to their polyphenolic compounds. Finally, this experimental approach would contribute to the development of a low-cost intervention in order to cope with pathological conditions related to oxidative stress, while the harnessing of these byproducts may reduce the environmental pollution caused from their uncontrolled disposal (3,4).

Key words: oxidative stress, grape pomace, polyphenols, broilers

Acknowledgements: The present study was implemented by the State Scholarship Foundation funded by the «Scholarship Program for postgraduated studies of second cycle" from the resources of the Operational Program "Human Resources Development, Education and Lifelong Learning" 2014-2020 with co-funded from The European Social Fund (ESF) and the Greek State.

(1) Makri S., et al., Food Chem. Toxicol. 102 (2017) 24-31; (2) Lykkesfeldt J., et al., Vet J. (2007); 173(3): 502-511; (3) Kerasioti E., et al., Toxicol Reports. (2017); 4: 364-372; (4) Makri S., et al., *In vivo* 32: 291-302 (2018).

193

Effects of developmental stages and sex on the redox status of farm animals

Zoi Skaperda[1], Aristidis S. Veskoukis[1], Angeliki Argyriadou[2], Georgios Arsenos[2], Demetrios Kouretas[1]
[1]Department of Biochemistry and Biotechnology, University of Thessaly, Viopolis Mezourlo, Larissa, [2]Veterinary Faculty, Aristotle University, Thessaloniki, Greece
E-mail: zoiskap94@gmail.com

Nowadays, humans worldwide depend utterly on animals for the production of meat, fat, milk and other essential products. Therefore, the demand for livestock products has increased and world livestock production is facing major challenges that endanger the welfare and health of animals and, consequently, their productivity. Contemporary concerns over farm animal welfare have emerged as a result of producers' efforts to greatly increase the performance of their livestock. The progressive exploitation of the biological capacity of the animals to produce economic output, raise increasing worries about the extent to which they are stressed. To this end, the present study evaluates the redox status of farm animals (i.e., goats) from farms of Greece using modern research tools. In the current condition, we evaluated the levels of some widely used biomarkers for the assessment of animal redox status, namely thiobarbituric acid-reactive substances (TBARS, lipid peroxidation biomarker), protein carbonyls (protein oxidation biomarkers) and total antioxidant capacity (TAC) in blood plasma, reduced glutathione (GSH) and catalase activity in erythrocytes. These biomarkers were also measured in tissues of the animals and specifically in liver, in the non-substantial stress psoas major muscle, in the quadriceps muscle subjected to intermediate stress and in the most stressed muscle of the diaphragm. The analysis was made both at the developmental stage as indicated by animal on body weight, and gender. We report significant differences between the aforementioned independent variables. This study is expected to contribute mainly to the promotion of the welfare of farm animals, the promotion of Greek meat production and the improvement of its quality and commerciality through the scientific data that will emerge.

Key words: redox status, livestock, biomarkers, developmental stages

194

Personalized nutrition regimen and the redox biomarker issue: Five premises that should be followed

Aristidis S. Veskoukis, Demetrios Kouretas
Department of Biochemistry and Biotechnology, University of Thessaly, Viopolis, Mezourlo, Larissa, Greece
E-mail: dkouret@uth.gr

The new trend adopted during the last few years regarding the putative promising effects of nutrition on human health on the basis of blood and tissues redox profile is the personalized regimen. Specific antioxidants are expected to act differently on human subjects (either healthy individuals or those who suffer from redox-related pathologies). The holistic evaluation of the action of exogenously administered antioxidants depends on the redox biomarkers measured, the genetic background and the baseline redox profile of the subjects. Specifically, the following 5 premises should be followed. Premise 1: Assessment of the baseline values of the most influential blood antioxidants (i.e., GSH, vitamins, uric acid etc). Premise 2: Antioxidant evaluation of foods in order not to neglect the essential role of nutrition *per se*. Premise 3: It is crucial to select the appropriate redox biomarkers in order to correctly assess the impact of nutrition on blood and tissue redox status. Premise 4: Apart from redox biomarkers on the biochemical level, the evaluation of them on the molecular level (genes, protein expression, study molecular pathways of cell culture) is of utmost importance. Premise 5: After clustering the biomarkers in a network with enhanced translational potency, the baseline blood redox profile (oxidative vs reductive stress) should be estimated. All these premises in combination could offer valuable knowledge in order to correctly build a scheme of administered antioxidants that are highly possible to assist human health.

195

Assessment of antioxidant activity of extracts from *Conium divaricatum*, *Ruta graveolens* and *Artemisia arborescens*

Demetrios Ladas[1], Eftichia Kontozisi[1], Christina Anna Kamvoukou[1], Paraskevi Kouka[1], Efthalia Kerasioti[1], Fotis Tekos[1], Dimitrios Kouretas[1], Serko Haroutounian[2], Dimitrios Stagos[1*]
[1]Department of Biochemistry and Biotechnology, University of Thessaly, Larissa, Greece; [2]Department of Pharmacy, University of Athens, Athens, Greece
[*]E-mail: stagkos@bio.uth.gr

In the present study, we evaluated the polyphenolic content and the antioxidant activity of four extracts derived from 3 Greek wild plant species, namely *Artemisia arborescenses*, *Conium divaricatum* and *Rutagraveolens*. The antioxidant activity of the extracts was evaluated *in vitro* by DPPH, ABTS, Reducing Power, $OH^{\cdot}$ and $O_2^{\cdot}$ scavenging assay. In addition, the total phenol content was assessed by Folin-Ciocalteu assay. The results revealed that aquatic and methanolic extracts from *Conium divaricatum* were the most potent as regards antioxidant activity. Specifically, aquatic extract from *Conium divaricatum* exhibited IC_{50} values of 60 μg/ml, 28 μg/ml and $RP_{0.5AU}$ of 44 μg/ml for DPPH, $O_2^{\cdot}$ scavenging assays and reducing power assay, respectively. Moreover, the methanolic extract from *Conium divaricatum* was the most potent in ABTS and $OH^{\cdot}$ radical scavenging assays with IC_{50} 85 μg/ml and 70 μg/ml, respectively. As regards the total phenolic content, the aquatic extract from *Conium divaricatum* exhibited the highest value (87.8 mg TPC/g dry extract). Furthermore, the antioxidant activity of aquatic extract from *Conium divaricatum* was examined in HepG2 liver and endothelial EA.hy926 cell lines. The results revealed that in HepG2 cells, the extract at 3 μg/ml, 6 μg/ml and 12 μg/ml protected proteins and lipids from oxidation, in a dose-dependent manner. However, the levels of glutathione and ROS were not affected by any concentration of the extract. Furthermore, in EA.hy926 cells, the results revealed that extract treatment decreased lipid peroxidation and protein carbonyl levels up to 61%. In addition, the extract increased total antioxidant capacity (TAC) up to 174%. In addition, glutathione levels (GSH) increased in a dose-dependent manner, namely 31.69, 35.24 and 47.26% at 12, 25 and 50 μg/ml, respectively. In conclusion, in the present study, the antioxidant activity of aquatic and methanolic extracts derived from *Conium divaricatum* was assessed for the first time, to the best of our knowledge; however, further studies are required to elucidate the molecular mechanisms through which this activity is mediated.

Key words: *Artemisia arborescenses*, *Conium divaricatum*, *Ruta graveolens*, HepG2 hepatic cell line, EA.hy926 cell line, antioxidant activity, polyphenol compounds

196

Evaluation of the effects of a highly rich in biophenols olive oil sample on blood and tissue redox status of rats

Paraskevi Kouka[1], Aristidis S. Veskoukis[1], Maria Halabalaki[2], Alexios-Leandros Skaltsounis[2], Ioannis Taitzoglou[3], Demetrios Kouretas[1]
[1]Department of Biochemistry and Biotechnology, University of Thessaly, Larissa, Greece; [2]Department of Pharmacognosy and Natural Products Chemistry, Faculty of Pharmacy, University of Athens, Athens, Greece; [3]Department of Physiology, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University, Thessaloniki, Greece
E-mail: vkoyka@hotmail.com

Olive oil (OO) plays a predominant role in the diet of countries around the Mediterranean basin, whereas it is a known constituent of several sectors of human culture. The biophenolic composition of olive oil seems to be a key factor in its beneficial biological action. Biophenols have gained scientific interest lately due to the major beneficial health effects observed after consumption. Of note, according to a health claim on OO polyphenols approved by the European Food Safety Authority (EFSA; Commission Regulation EU 432/2012), OOs are considered to protect from oxidative stress-induced lipid peroxidation in blood, when they contain approximately 5 mg of HT and its derivatives (e.g., oleuropein complex and T) per 20 g of OO. Notably, OO biophenol absorption from the gastrointestinal tract is very high, highlighting the importance of OO as a source of dietary antioxidants. In general, OO biophenolic content has been associated by our group with potent *in vitro* antioxidant activity. However, studies regarding the effects of a total OO on redox status *in vivo* are either observational, or lack in-depth analysis in order to provide an overall picture of the interaction between the whole OO high biophenolic content and tissues. All aerobic organisms have developed numerous diverse antioxidant mechanisms against oxidative stress, including enzymes such as superoxide dismutase (SOD1) and catalase (CAT), as well as non-enzymatic compounds like reduced glutathione (GSH) and uric acid. Nevertheless, apart from endogenous antioxidants, dietary antioxidants may also act protectively against reactive species with biophenols being the most abundant. As regards OO extracts, previous studies by our team have shown that they potentially stimulate endogenous mechanisms, such as the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) pathway. Following a series of in vitro screening studies[(1-3)], the most potent OO, was selected for the current in vivo experiment to shed light on its effect as well as the mechanism of action in several tissues. Whether the *in vitro* findings correspond to those *in vivo*, is an active field of research and is of particular interest to our research group. To this end, in the current study, an OO with 800 mg/kg OO biophenols was administered for 14 days to male Wistar rats at a dose corresponding to 20 g OO/per day; thus each rat was administered 0.313 ml OO per day. The dose was equivalent to the human daily safe consumption, according to EFSA. Sequentially, blood and eleven tissues were collected and analyzed to allow a wide range screening regarding the effect of OO consumption on rat redox status.

(1) Kouka P, et. al, Int J Mol Med 40: 703-712, 2017.
(2) Kouka P, et.alPhytomedicine 47: 135-142, 2018.
(3) Kouka P, et. al, Oxid Med Cell Longev 2019: 13, 2019.

197

Fertility & work stress related in health care workers

Emanuele Cannizzaro[1], Caterina Ledda[2], Serena Matera[2], Ermanno Vitale[2], Andrea Marconi[2], Diana Cinà[3], Venerando Rapisarda[2]
[1]Promotion of Health, Maternal-Infant, Internal and Specialized Medicine of Excellence "G. D'Alessandro", University of Palermo, Italy; [2]Occupational Medicine, Department of Clinical and Experimental Medicine, University of Catania, Italy; [3]Clinical Pathology and Clinical Molecular Biology Unit, "Garibaldi Centro" Hospital of Catania, Catania, Italy
E-mail: emanuele.cannizzaro@unipa.it

Stress identifies a condition in which the individual does not feel able to match the expectations or demands of his environment. This condition can materialize both for problems in the living environment and the work environment. In particular, in the workplace, it occurs when the worker is not able to meet the demands of the job; therefore, inherent in the content and/or context of the job (1). Recent studies have reported a correlation between occupational stress and female infertility (2). This study aimed to determine the concentrations of hormones involved in a woman's fertility in the context of the perceived stress associated with occupational habits. For this purpose, we recruited 40 nurses of child-bearing age, who were non-smokers and non-alcoholics, without any pathology. The women, after the calculation of the day of ovulation (Ogino-Knaus method), were subjected to blood sampling at 8:00 a.m. and at 2:00 p.m. The following were determined: Luteinizing hormone (LH), stimulating follicle hormone (FSH), prolactin (PRL), estradiol and progesterone and plasma cortisol levels. Stress determination was performed through the Karasek questionnaire. The study sample was divided into two groups: a group with 20 nurses, in charge of shift work, even at night; and one of 20 nurses, only involved in daytime work. The two groups were uniform for age, length of service, etc. The average concentrations of LH, FSH, PRL, estradiol and progesterone were within normal ranges. A significant difference ($p<0.05$) was observed in the LH values, which were lower in shift workers and PRL, and always higher in shift workers. As regards plasma cortisol levels, a significantly higher average concentration ($p<0.05$) was observed in shift workers, compared with non-shift workers, in both sample sets. The results relating to the Karasek questionnaire show significantly ($p<0.05$) stress conditions in shift workers working on active guard. In women, various causes have been identified that lead to infertility, often resolved also through a thorough psychological and psychosomatic analysis (3). The cause of the rise and fall of these hormones has been described in the literature and can also be attributed to stress (2). From the multivariate statistical re-elaboration, the related work stress appears to be a risk factor for raising cortisol and PRL levels and lowering LH levels. From the data that have emerged, despite the small sample size, it is evident there are significant associations that encourage further studies on the subject.

(1) European Agency for Safety and Health at Work (2004) Factsheet 22: Work-related stress; (2) Bratlid D. World J Pediatrics 7(2): 101-102, 2011; (3) Sheiner E, et al., Occup Med 53(4): 265-269, 2003.

198

Chronodisruption in health care workers

Caterina Ledda[1], Lucia Rapisarda[2], Angelo Savoca[3], Ermanno Vitale[1], Diana Cinà[4], Venerando Rapisarda[1]
[1]Occupational Medicine, Department of Clinical and Experimental Medicine, University of Catania, Italy; [2]Spinal Unit, "Cannizzaro" Hospital, Catania, Italy; [3]SPRESAL, Provincial Health Authority of Catania; [4]Clinical Pathology and Clinical Molecular Biology Unit, "Garibaldi Centro" Hospital of Catania, Catania, Italy
E-mail: cledda@unict.it

Oxidative damage is the main threat to the integrity of the genome in most living organisms and is therefore considered an essential factor in the process of carcinogenesis, although direct causal connections are still lacking (1-2). The molecular mechanisms underlying the process of tumor transformation induced by oxidative damage have not yet been completely clarified. Recent studies point out that shift work can compromise the ability of DNA to repair the damage of natural oxidative processes. Furthermore, night work seems to be a risk factor for certain neoplastic diseases such as breast, prostate, etc. (3). In the present study, the urinary concentrations of 8-hydroxy-deoxyguanosine (8-OH-dG) and 6-sulfatoxymelatonin (aMT6) were determined in a group of operators who also performed night work. The 8-OH-dG allows for the evaluation of oxidative damage; through the concentration of aMT6, it is possible to determine the concentration of secreted melatonin. Twenty healthcare shift workers (HCSWs) and 20 non-shift workers (HCNSWs) were recruited. The study was proposed as part of the periodic health surveillance visit. Each was asked to collect the 24h urine. The values of 8-OH-dG and aMT6 were corrected for urinary creatinine concentration (CREU). The study participants were all women, with a mean age of 46.5±7.8 and 47.4±6.4, respectively in HCSWs and HCNSWs. The average number of night shifts/month was 3.7 for HCSWs. The urinary 8-OH-dG concentration was significantly ($p<0.05$) greater in HCSWs (10.3±4.1 nmol / 24 h) than in OSNs (6.3±3.7 nmol/24 h). The mean values of aMT6 (expressed in ng/mgCREU) were significantly ($p<0.05$) lower in HCSWs (20.5±7.7) than in HCNSWs (44.5±10.8). In HCSWs, the detection of high urinary concentrations of 8-OH-dG, a by-product responsible for repairing oxidative stress damage, would appear to be due to the low amount of aMT6 found. Melatonin, a hormone that regulates the sleep-wake cycle, is one of the promoters of the nucleotide excision DNA repair mechanism (1). Therefore, it appears that at low melatonin levels, the repair mechanism does not go into action, and free radicals are not countered, generating altered levels of 8-OH-dG.

(1) Proietti S, et al., Cell Mol Life Sci 2017; 74(21): 4015-4025.
(2) Mayor S. BMJ (Online) 2017; 357.
(3) Schwartzbaum J, et al., Scand J Work Environ Health 2007; 33(5): 336-343.

199

Adipokines in shift workers

Venerando Rapisarda[1], Serena Matera[1], Ermanno Vitale[1], Lucia Rapisarda[2], Diana Cinà[3], Caterina Ledda[1]
[1]Occupational Medicine, Department of Clinical and Experimental Medicine, University of Catania; [2]Spinal Unit, "Cannizzaro" Hospital, Catania; [3]Clinical Pathology and Clinical Molecular Biology Unit, "Garibaldi Centro" Hospital of Catania, Catania, Italy
E-mail: vrapisarda@unict.it

All the molecules produced and secreted by adipose tissue with autocrine, paracrine and/or endocrine functions are defined as adipokines; these can be hormones, cytokines, chemokines, regulators of lipid metabolism, regulators of glucose homeostasis, growth factors, complement proteins, vascular homeostasis proteins, acute phase inflammatory proteins/stress response or components of the extracellular matrix (1). The aim of the present study was to evaluate the presence of some adipokines in healthcare workers (HCWs) and their excretion levels in shift workers. In the present cross-sectional study, the following adipokines were determined in 50 shift healthcare workers (HCSWs) and 50 non-shift workers (HCNSWs): Leptin, adiponectin, interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α). For each HCWs, socio-demographic, occupational and anthropometric data were recorded. Recruitment was carried out in the context of health surveillance. In this study, 100 HCWs were examined, 54% were women, and 50 (50%) were OST; the average age was 42.4±8.2 years, with a working age of 11.0±7.5 years. The average BMI was 21.77±2.13. The two groups were homogeneous in terms of age, sex, seniority, BMI, smoking habits and alcohol consumption. A statistically significant difference ($p<0.001$) was found between the average concentration of leptin in males (7.3±2.0 ng/ml) compared to females (23.8±4.0 ng/ml), regardless of whether they were shift workers or not. No statistically significant difference was found between leptin values detected in HCNSW and HCSW males, while in the female population, a statistically significant difference ($p<0.001$) was found between HCNSWs and HCSWs (26.3±4.3 and 21.3±3.6 ng/ml, respectively). The average of the adiponectin values was significantly lower in HCWs (9.4±5.1 µg/ml) compared to HCNSWs (15.8±3.1 µg/ml). The adiponectin levels were inversely related to BMI. No statistically significant differences were found for IL-6 and TNF-α between HCSWs and HCNSWs. Adiponectin is a multimeric hormone whose levels are inversely related to the mass of adipose tissue. Its production is inhibited by pro-inflammatory cytokines, but also by hypoxia and oxidative stress (2,3). Adiponectin plays a protective role against the metabolic complications of obesity (1). In HCSW levels, circulating adiponectin levels were significantly lower than in HCNSWs. Similarly, circulating levels of leptin are reduced in the obese (1); although in the present study, sex differences (male vs. female and among them, HCSWs vs. HCNSWs) were observed.

(1) Shea SA, et al., J Clin Endocrinol Metab 2005;90(5): 2537-2544.
(2) Yamashita K, et al., Nutr Diabetes 2012; 2(APRIL).
(3) Angelousi A, et al., Eur J Clin Invest 2018; 48(6).

200

Prevention of RSV infections: An update on vaccines

Maria Theodoridou
'Aghia Sophia' Children's Hospital, University of Athens School of Medicine, Athens, Greece

Respiratory syncytial virus (RSV) is an important cause of acute lower respiratory tract infections (ALRI) in infants and causes significant disease in the paediatric and elderly population. While vaccines are among the most cost-effective health interventions for infectious diseases, a safe and effective vaccine against RSV is still not available. For more than 50 years, live-attenuated vaccine approaches have been unsuccessful due the difficulty of researchers to balance immunogenicity and vaccine safety. Recent breakthroughs in determining the structure and antigenic content of the fusion (F) glycoprotein of the virus as well as novel live-attenuated and chimeric virus vaccines' candidates have enhanced the interest in the research of the vaccines' development. There are now more than 60 vaccine candidates, 16 of which are in clinical development. The strategic focus of the World Health Organization (WHO) meeting in Geneva in 2015 was on the development of high quality, safe and efficacious preventive interventions against RSV for global use and included i) maternal/passive immunization to prevent RSV-associated infections in infants less than 6 months of age; and ii) paediatric immunization in infants over six months of age and young children, once protection from maternal antibodies wanes. As the number of clinical trials is growing and industries are moving rapidly, it is likely that a vaccine against RSV will be commercially available in 5 to 10 years. The prevention of RSV-associated infections with the vaccine will become a reality and its focus should be placed on children in their first six months of life, when the risk of severe RSV infections is highest.

201

Imaging in children with RSV infection

Georgia Papaioannou
Department Paediatric Radiology, "Mitera" Children's Hospital, Athens, Greece

Respiratory syncytial virus (RSV) is the most common cause of paediatric bronchiolitis and pneumonia worldwide, initially affecting infants and young children. Albeit its association with a high mortality and prolonged morbidity in certain populations and underlying conditions, i.e., premature children, bronchopulmonary dysplasia, congenital heart disease and Down syndrome, it may require hospitalization with severe sequelae in previously healthy, full-term affected children. Chest radiographs in children with RSV infection and acute respiratory symptoms may be normal or reveal non-specific findings, which are also encountered in other viral infections: Most commonly perihilar opacities and hyperinflation, and rarely consolidation and bronchial cuffing or air-leak. Among these findings, hyperinflation is considered the most specific for RSV infection, whereas central pneumonia and peribronchitis in young children with RSV infection are the commonest findings. Radiography is commonly obtained to rule out other entities, such as bacterial pneumonia and foreign body aspiration. Guidelines suggest the performance of chest radiograph in presence of significant respiratory distress or hospitalization. In newborns affected with RSV infection, the radiological pattern on chest radiography may be a predictor of clinical outcome. RSV encephalitis with encephalopathic symptoms and severe sequelae has sporadically been reported. It usually develops within 1-2 days from the onset of the clinical symptoms and the mechanism of its rapid evolution remains unclear. On brain MRI performed in neurologic involvement, abnormal findings may mimic non-specific findings encountered in other viral and limpic encephalitis. Although imaging cannot set the diagnosis of RSV infection, it is important to identify the possible pattern of viral disease, in order to avoid unnecessary administration of antibiotic therapy and predict possible late effects.

202

High-flow warm humidified oxygen via nasal cannula and RSV-positive bronchiolitis among children admitted to PICU

Alexia Papatheodoropoulou
Paediatric Intensive Care Unit (PICU), "Aglaia Kyriakou" Children's Hospital, Athens, Greece

Bronchiolitis is a common acute lower respiratory tract infection, which predominantly affects infants younger than 24 months of age. It causes airway inflammation, mucus production and mucous plugging, resulting in airway obstruction and is a major cause of morbidity and mortality leading frequently to hospitalizations and pediatric intensive care unit (PICU) admissions. To date, effective pharmacotherapy is lacking and conventional treatment consists of supportive therapy in the form of fluids, supplemental oxygen and respiratory support. Traditionally oxygen delivery is as a dry gas at 100% concentration via low-flow nasal prongs. However, the use of heated, humidified, high-flow nasal cannula (HFNC) therapy enables delivery of higher inspired gas flows of an air/oxygen blend, up to 12 l/min in infants and 30 l/min in children. Its use provides some level of continuous positive airway pressure to improve ventilation in a minimally invasive manner. HFNC has emerged as a new method to provide humidified air flow to deliver a non-invasive form of positive pressure support with titratable oxygen fraction. High-flow does not significantly reduce time on oxygen compared with standard therapy, suggesting that early use of HFNC does not modify the underlying disease process in moderately severe bronchiolitis and does not reduce admission rates or length of stay to the PICU. However, HFNC plays a role as a rescue therapy to reduce the proportion of children requiring high-cost intensive care. This reduces the need for invasive respiratory support thus potentially lowering costs, with clinical advantages and fewer adverse effects. Despite wide clinical use, there remains a lack of evidence on the comparative effectiveness and safety of these interventions. Further research is required to determine the role of HFNC in the management of bronchiolitis in infants. The results of the ongoing studies on HFNC will contribute to the evidence in future.

203

Role of neuroendocrine factors in skin cancer

Constantin Caruntu[1,2], Daniel Boda[2,3], Ana Caruntu[4,5], Sabina Zurac[6,7], Carolina Constantin[7,8], Monica Neagu[7,8,9]
[1]Department of Physiology, "Carol Davila" University of Medicine and Pharmacy, Bucharest; [2]Department of Dermatology, 'Prof. N. Paulescu' National Institute of Diabetes, Nutrition and Metabolic Diseases, Bucharest; [3]Dermatology Research Laboratory, 'Carol Davila' University of Medicine and Pharmacy, Bucharest; [4]Department of Oral and Maxillofacial Surgery, 'Carol Davila' Central Military Emergency Hospital, Bucharest; [5]Department of Oral and Maxillofacial Surgery 'Titu Maiorescu' University, Faculty of Medicine, Bucharest; [6]Department of Pathology, 'Carol Davila' University of Medicine and Pharmacy, Bucharest; [7]Department of Pathology, Colentina Clinical Hospital, Bucharest; [8]Immunology Department, 'Victor Babes', National Institute of Pathology, Bucharest; [9]Faculty of Biology, University of Bucharest, Bucharest, Romania

Skin cancer is the most common type of malignancy; non-melanoma skin cancer encompasses approximately 40% of all malignancies and its incidence has markedly increased over the past decades. The other major type of skin cancer, melanoma, is less frequent; however, it has a very aggressive course, accounting for more than 75% of all skin cancer-related deaths. Various researchers suggest the involvement of neuroendocrine factors in the appearance and progression of skin cancer. Acting directly by modifying the proliferation or metastasis capacity of tumor cells, or indirectly by modulation of immune response and cutaneous inflammatory processes, modification of the adhesion molecules expression or tumor microenvironment, neuropeptides, neurohormones and neurotransmitters can have a significant impact on skin cancer. Studying the cellular and molecular mechanisms through which neuroendocrine factors could influence the clinical course of the disease may open up new areas of biomedical research and may lead to the development of novel potential approaches for the treatment of skin cancer.

Acknowledgements: This study was partially funded by a grant of the Romanian Ministry of Research and Innovation (CCCDI-UEFISCDI; project no. 61PCCDI/2018 PN-III-P1-1.2-PCCDI-2017-0341), within PNCDI-III.

204

In vivo reflectance confocal microscopy for basal cell carcinoma diagnosis and subtyping

Daniel Boda
Department of Dermatology, "Prof. N. Paulescu" National Institute of Diabetes, Nutrition and Metabolic Diseases, Bucharest, Romania

Current national and European guidelines recommend distinct management approaches for basal cell carcinoma (BCC) based on tumor location, size and histopathological subtype. *In vivo* reflectance confocal microscopy (RCM) is a non-invasive skin imaging technique which may alter the diagnostic pathway for BCC patients. This study aimed to determine the sensitivity and specificity of RCM for BCC diagnosis, assess the predictive values of several confocal criteria in correctly classifying BCC subtypes, and evaluate the intraobserver reliability of RCM diagnosis for BCC. We conducted a retrospective study in two tertiary care centers in Bucharest, Romania. We included adults with clinically and dermoscopic suspect BCCs who underwent RCM and histopathological examination of excision specimens. For RCM examinations, we used the VivaScope 1500 and histopathology of the surgical excision specimen was the reference standard. Of the 123 cases included in the analysis, BCC was confirmed in 104 and excluded in 19 cases. RCM revealed both a high sensitivity [97.1%, 95% CI (91.80, 99.40)] and specificity [78.95%, 95% CI (54.43, 93.95)] for detecting BCC. Several RCM criteria were highly predictive for BCC subtypes: Cords connected to the epidermis for superficial BCC, large tumor islands, peritumoral collagen bundles and increased vascularization for nodular BCC and hyporefractile silhouettes for aggressive BCC. Excellent intraobserver agreement (κ=0.909, $P<0.001$) was observed. These data suggest that RCM may be used for preoperative diagnosis and BCC subtype classification in patients with suspected BCCs in tertiary care centers.

205

Immune biomarkers in skin cancer therapy

Monica Neagu[1,2,3], Carolina Constantin[1,3], Constantin Caruntu[4], Mihaela Surcel[1,2], Sabina Zurac[1,3]
[1]'Victor Babes' National Institute of Pathology, Immunology Department, Bucharest; [2]Faculty of Biology, University of Bucharest, Bucharest; [3]Colentina University Hospital, Pathology Department, Bucharest; [4]'Carol Davila' University of Medicine and Pharmacy, Bucharest, Romania

With the advent of the new era of immunotherapy, new rapidly emerging aspects are highlighted from both fundamental research and clinical applications. As we are gaining experience from clinical application in terms of adverse effects types, the management of them, acquiring resistance, overcoming resistance, using adjuvant therapies, the discovery of the best prognosis/efficacy biomarkers becomes of outmost importance. For example, when immunotherapy was first installed, tissue PD-1, PD-L1 expression was tested as an efficacy biomarker; however, the practice has shown that it was far from being accurate. A combination of several biomarkers, such as tissue PD-L1 protein expression associated with PD-L1 mRNA, and circulating PD-L1 would be suitable for monitoring therapy. In this light, we are searching for several proteomic/genomic biomarkers in patients diagnosed with cutaneous melanoma. Several proteomic technologies were used to identify and quantify biomarkers from patient sera: Multiplex, protein microarray, mass-spectrometry. Genomic technologies, such as CGH-array were used to identify copy number variations in genetic material extracted from tumors. Our experience in circulatory biomarkers has shown that peripheral immune cells have a particular pattern in melanoma, whether in patients or in mouse experimental models (1,2). A Th1-type immune signature is associated with a good prognosis. Circulatory Tregs announce an immune suppressive pattern for patients with a worse outcome of the disease. Another finding reported by us is that inflammatory markers govern the prognosis (3). Using multiplexing technology (4) and mass spectrometry (5), we found that key players in this process are IL-6 that if increased in the sera of patients, will prognosticate a bad outcome of the disease, independent of the stage the patient is diagnosed in. Another player is the chemokine IL-8 that is increased in advanced stages and has a negative correlation with vitamin D (3). Tumors are highly heterogeneous in terms of protein and gene expression. Using CGH-array technology, we have shown differences in the same tumors in terms of CNV (6,7). Using protein microarray technology (8) we have shown that several proteins are overexpressed in the sera of melanoma patients. Out of all these, leptin, a hormone appending mainly to the adipose tissue was found increased 10-30-fold in comparison to normal serum. Applying the STRING program, we have shown that leptin, among other, is an inducer of IL-6. In conclusion, as the recently developing immunotherapy is more efficient in combination; thus, the biomarkers that should monitor therapy, should also be used in combination.

Acknowledgements: PN-III-P1-1.2-PCCDI-2017-0341/2018, *EPITRAN* COST ACTION 16120/2018. 7PFE/16.10.2018.

1) Gheorghiță Isvoranu, et al (2019). Natural killer cells monitoring in cutaneous melanoma - new dynamic biomarker. Oncol Lett 17, pp. 4197-4206; 2) Monica Neagu et al. (2013). Immune parameters in prognosis and therapy monitoring of cutaneous melanoma patients - experience, role and limitations, BioMed Research International, Vol 2013, Article ID 107940. 3) Monica Neagu, et al. (2019). Inflammation - key process in skin tumorigenesis, Oncol Lett 17, pp.4068-4084. 4) Cristiana Tanase, et al. (2019). Updates in immune-based multiplex assays, Journal of Immunoassay and Immunochemistry. DOI: 10.1080/15321819.2019.1565064. 5) Carolina Constantin, et al. (2017). Surface-Enhanced Laser Desorption/Ionization Mass Spectrometry for Biomarker Discovery in Cutaneous Melanoma. Current Proteomics 14 (2), pp. 100-111. 6) Jantsch MF, et al. (2018). Positioning Europe for the EPITRANSCRIPTOMICS challenge. RNA Biol 1-3. doi: 10.1080/15476286.2018.1460996; 7) Carmen Dumitru, et al (2017). Innovative array-based assay for omics pattern in melanoma, Journal of Immunoassay and Immunochemistry 38(4), pp. 343-354. 8) Clara Matei, et al. (2014). Protein microarray for complex apoptosis monitoring of dysplastic oral keratinocytes in experimental photodynamic therapy, Biol Res 47(1), pp. 33-41.

206

Updating taxonomy changes: RSV is now known as Orthopneumovirus

Ioannis N. Mammas, Demetrios A. Spandidos
Department of Clinical Virology, School of Medicine, University of Crete, Heraklion, Greece

The field of virus naming is run by the International Committee on Taxonomy of Viruses (ICTV), which authorizes and organizes the classification and naming of virus species. Thus far, among the 4,958 viruses, which have been formally described, current taxonomy is vastly incomplete and in several places even wrong. It was in 1956 when *Respiratory Syncytial Virus* (RSV), first isolated in nasal secretions from chimpanzees with rhinorrhea and coryza, was initially named *Chimpanzee Coryza Agent* (CCA). The following year, CCA was identified in children with respiratory tract infection and gained its name, RSV, when electronic microscopy revealed that RSV is related to the presence of syncytia. Syncytia are formed by the fusion of infected host cells with neighboring cells, leading to the formation of multi-nucleate enlarged cells. Although the formation of syncytia is the hallmark of the cytopathic effect caused by RSV, which causes the host cellular membranes to merge, syncytia are not pathognomonic for RSV. They can also be formed when host cells are infected with several other types of viruses, such as HSV-1, HIV and *Metapneumovirus*. Until 2016, the ICTV classified *Pneumoviruses* such as RSV and *Metapneumovirus* in the *Paramyxoviridae* family. However, it was realized that the *Pneumoviruses* are only distantly related to *Paramyxoviruses* and in fact are more closely related to *Filoviruses*, such as Ebola virus (e.g., for the RDRP or L gene). Thus in 2016, the *Pneumoviruses* were re-classified as a separate family, the *Pneumoviridae*. As part of the ongoing effort to add more systematics to virus classifications, and having already a genus termed *Metapneumovirus*, ICTV decided to rename the genus with the earliest known *Pneumoviruses* as the *Orthopneumovirus* genus. *Human Orthopneumovirus* was classified in the genus *Orthopneumovirus* within the family *Pneumoviridae*.

207

Predicting asthma following RSV-positive bronchiolitis in early childhood

Chryssie Koutsaftiki
Paediatric Intensive Care (PICU), "Penteli" Children's Hospital, Penteli, 15236, Greece

Although asthma is the most common paediatric chronic disease in childhood, delay in its diagnosis is prevalent, resulting in suboptimal asthma management. Viral respiratory infections induced respiratory viruses are closely linked to wheezing illnesses in children of all ages. Moreover, respiratory viruses are detected in most exacerbations of asthma throughout childhood. Severe respiratory infection induced by respiratory syncytial virus (RSV) is associated with subsequent development of asthma later in childhood. To date, there has been progress in understanding the pathogenesis of viral respiratory illnesses, and this has provided new insight into how these processes may differ in asthma. Several host factors, including respiratory allergy and virus-induced interferon responses, viral virulence factors, genetic risk factors, and environmental exposures modify the risk of virus-induced wheezing and promote more severe wheezing illnesses and the risk for progression to asthma. Treatments that inhibit inflammation have efficacy for RSV-induced wheezing, whereas the anti-RSV mAb palivizumab decreases the risk of severe RSV-induced illness and subsequent recurrent wheeze. RSV positive lower respiratory tract infection (LRTI) in prematurely born infants is associated with increased healthcare utilisation and cost of care in the first and second year. It has been suggested that prematurely born infants have a genetic predisposition to RSV infection-related respiratory morbidity and subsequent respiratory morbidity. Single-nucleotide polymorphisms in genes coding for IL-8, IL-19, IL-20, IL-13 mannose-binding lectin, IFNG and a RANTES polymorphism have been associated with subsequent wheeze following RSV LRTI in term-born infants. Large studies have confirmed a comparable increased risk of first asthma hospitalisation following RSV disease in the first 2 years of life in preterm or born with a low birth weight as well as in term children. Children hospitalized with bronchiolitis, caused by other viruses than RSV, develop recurrent wheezing at substantially higher rates during a 3-year follow-up period than do children with RSV-induced bronchiolitis. However, other researchers have reported that in otherwise healthy preterm infants RSV prevention has no major effect on lung function and asthma development later on in life. Further understanding of the role of RSV in asthma pathogenesis will enable our understanding of the impact of future vaccines against RSV in asthma prevention.

208

Thrombocytosis and RSV infection in hospitalised children with bronchiolitis

Ioannis N. Mammas, Demetrios A. Spandidos
Department of Clinical Virology, School of Medicine, University of Crete, Heraklion, Greece

In healthy children normal count platelet ranges between 250,000 µl and 450,000 µl, while an elevated platelet count >2 SD defines a condition of thrombocytosis. On a clinical level, thrombocytosis is classified 'mild' at a platelet count between >500,000 µl and <700,000 µl; 'moderate' at a platelet count between >700,000/µl and <900,000/µl; 'severe' at a platelet count >900,000/µl; and 'excessive' at a platelet count >1,000,000/µl. Thrombocytosis is usually discovered incidentally and can be classified as primary or essential or as secondary or reactive. Secondary thrombocytosis is observed in patients with a variety of clinical conditions, such as acute and chronic infections, iron deficiency, bleeding, haemolytic anaemias, collagen vascular and renal diseases, Langerhans cell histiocytosis and Kawasaki disease. The most frequent cause of secondary thrombocytosis in childhood is acute respiratory tract infections and in the vast majority of children it is mild and transient. To date, several authors have reported significantly higher mean platelet count in patients with respiratory syncytial virus (RSV) infection than in patients with other acute respiratory tract infections. Thrombocytosis is more likely to occur in younger patients who have clinical manifestations of wheezing and dyspnoea and have been diagnosed with RSV-positive bronchiolitis. Moreover, thrombocytosis has been suggested as an early marker of RSV infection. Excessive thrombocytosis has also been detected at an early stage of RSV-positive bronchiolitis. It has been proposed that patients with thrombocytosis have a more severe clinical course and a longer duration of hospitalization. However, other authors have found that platelet counts are not associated with disease severity and clinical outcome. Routine prophylactic anti-platelet treatment or further investigations are not necessary in children with RSV-positive bronchiolitis and thrombocytosis.

209

Heliox and RSV-positive bronchiolitis

Alexia Papatheodoropoulou
Paediatric Intensive Care Unit (PICU), 'Aglaia Kyriakou' Children's Hospital, Athens, Greece

Heliox is a helium-oxygen gas mixture, which can facilitate gas exchange and limit peak inspiratory pressures through reduced resistance to gas flow and decreased turbulent flow. It has been used for a number of decades for the treatment of adults and children with a variety of upper and lower airway conditions. These conditions include respiratory distress syndrome, acute exacerbation of chronic obstructive pulmonary disease (COPD), acute lung injury, asthma, croup and bronchiolitis. The lower density and higher viscosity of Heliox relative to nitrogen-oxygen mixtures can significantly reduce airway resistance when an anatomic upper air-flow obstruction is present and gas flow is turbulent. Clinically, Heliox can decrease airway resistance in acute asthma in adults and children and in COPD. Heliox may also enhance the bronchodilating effects of β-agonist administration for acute asthma. Heliox can be administered with non-invasive ventilation and with mechanical ventilation through the ventilator. Non-invasive ventilation with Heliox has been proposed as a promising therapeutic option for children with various respiratory pathologies who do not respond to conventional treatment. Bronchiolitis is a common, self-limiting, seasonal viral respiratory tract infection in infancy accounting for a significant number of hospitalization and PICU admission in this age group. Acute viral bronchiolitis is associated with airway obstruction and turbulent gas flow. Supportive care remains the mainstay of treatment, concentrating on fluid replacement, gentle suctioning of nasal secretions, prone position (if in hospital), oxygen therapy and respiratory support if necessary. Current evidence suggests that the addition of Heliox therapy may significantly reduce a clinical score evaluating respiratory distress in the first hour after starting treatment in infants with acute RSV bronchiolitis. Heliox could reduce the length of treatment in infants requiring CPAP for severe respiratory distress. Recently, it was reported that Heliox could result in improvement of oxygenation when used with high flow nasal cannula in infants with RSV acute bronchiolitis during the initial phase of the therapy. However, it has no effect in the reduction in the rate of intubation, in the rate of emergency department discharge, or in the length of treatment for respiratory distress. Further studies are required to provide the necessary information as to the appropriate place for Heliox in the therapeutic schedule for severe bronchiolitis.

210

MicroRNAs as potential bio-markers in children with RSV infection

Chryssie Koutsaftiki
Paediatric Intensive Care (PICU), "Penteli" Children's Hospital, Penteli, Greece

MicroRNAs (miRNAs) are small, endogenous, non-coding single-stranded RNA molecules, with characteristic, complex secondary structures that are conserved evolutionarily in plants, invertebrates and vertebrates. In humans, miRNAs are involved in post-transcriptional gene regulation and play a significant role in the inflammatory response mounted against several pathogens. Their role in viral infections is implicated in the modulation of antiviral defense, through modulation of both host innate and adaptive immune response. To date, it has been shown that respiratory syncytial virus (RSV) can modulate the host innate immune response by dysregulation of host miRNAs related to the antiviral response, a feature that also affects the memory immune response to RSV. The abnormal expression of non-coding miRNAs can be detected from the peripheral blood and airway tract epithelial of RSV infected infants. Understanding alterations in miRNA expression profiles and identifying miRNA targets genes, and their contribution to the pathogenesis of RSV, may aid in the clarification of the mechanisms of virus-host interaction, the mechanism of RSV-induced inflammatory reaction and immune dysfunction leading to airway hyper-reactivity. There are several methods for the purification, quantification, and characterization of miRNA expression profiles in biofluids, whole blood samples and tissue samples obtained from *in vivo* studies. In the future, miRNAs may become a potential bio-marker of detecting severe RSV infection and a novel target of early intervention and therapeutic strategy in recurrent wheezing or asthma related to RSV infection. Further understanding of their role and the molecular mechanisms that are involved could lead to development of new antiviral treatments against RSV.

211

RSV infection: Not for children only

Eleftheria Kozanidou, Vasileios Achtsidis
2nd Department of Internal Medicine, "St Panteleimon" General Hospital of Nikaia, Piraeus, Greece

Respiratory syncytial virus (RSV) is the most commonly identified cause of lower respiratory tract infections in young children. However, RSV was not recognized as a potentially serious health concern in older adults until the 1970s, when outbreaks of the virus occurred in long-term care facilities. Since then, additional studies in non-hospitalized and hospitalized adults have suggested that RSV is an important cause of illness among elderly individuals. Although the majority of children are infected with RSV by the age of 2 years, RSV re-infections occur throughout life. Previous studies, using viral cultures or serology for diagnosis, have led to widely variable assessments of the incidence and effects of the disease. More recent epidemiological data indicate that RSV infection is an important illness in elderly and high-risk adults, with a disease burden similar to that of non-pandemic influenza A. In adults, RSV usually causes mild influenza-like signs and symptoms. However, in older adults, immunocompromised patients and those with underlying cardiopulmonary disease, it may cause pneumonia or bronchiolitis and may result in respiratory failure (8-13%) or even death (2-5%). RSV accounts for approximately 10,000 deaths annually in the United States in individuals over the age of 65 years. This has stimulated interest in vaccines and other treatments for RSV required not only in children, but also in older and high-risk adult population. A careful immunization strategy including children will be expected to protect both children and adults from RSV-associated morbidity and mortality. However, additional data regarding virus-specific epidemiology and disease effects, particularly in community-dwelling elderly persons and high-risk adults are still required.

212

RSV and precision medicine: Time for a more precise approach to diagnosis, treatment and prevention

Maria Theodoridou
'Aghia Sophia' Children's Hospital, University of Athens School of Medicine, Athens, Greece

Precision medicine, over recent years, has become very popular, incorporating novel taxonomies and stratification of patients derived using large-scale data including clinical, lifestyle, genetic and further biomarker information, thus going beyond the classical 'signs-and-symptoms' approach. Its role in paediatric healthcare involves the selection of targeted diagnostic, therapeutic and prevention strategies matched to precise molecular, epidemiological and clinical profiling of each patient. Respiratory syncytial virus (RSV) infection represents a good paradigm of the significance of presicion medicine in the management approach of children infected with RSV. A new terminology was recently adopted, while the use of molecular techniques has revealed new diagnostic and prognostic markers on bronchiolitis in children; these diagnostic tools are based on the individual characteristics of each patient. A number of precision medicine trials are recently completed, underway or in development on the management and prevention of RSV-positive bronchiolitis. All of future pharmaceutical agents against RSV should be precise, where a greater understanding of individual data will lead to personalized treatment. Applying such co-ordinated diagnostic, clinical and research efforts constitutes an important step in advancing paediatric care, improving outcomes and limiting RSV global predominance.

213

Antibody-mediated re-programming of macrophages against tumours: New insights for cancer immunotherapy

Sophia N. Karagiannis[1]
[1]St. John's Institute of Dermatology, Division of Genetics and Molecular Medicine, King's College London & NIHR Biomedical Research Centre at Guy's and St. Thomas's Hospitals and King's College London, Guy's Hospital, King's College London, London, UK

All monoclonal antibodies designed for the treatment of cancer belong to one of the five classes, namely IgG (often IgG1). Engineering antibody Fc regions to enhance effector functions could be an important determinant of clinical efficacy. Herein, we designed anti-tumour antibodies with IgE class Fc regions with the aim of harnessing known properties of IgE to engage immune effector cells such as macrophages and mast cells and mediate immune clearance of parasites. Unlike the commonly-used IgG class antibodies, those engineered with IgE Fc regions feature extremely high affinity for cognate Fcε-receptors on monocytes and macrophages, an effector cell subset often found infiltrating tumour lesions. In several *in vitro* and *in vivo* pre-clinical model systems, our findings suggest that IgE designed to recognize tumour-associated antigens can potentiate monocyte/macrophage recruitment and re-education. Key modes of action of this class may be abile to mobilise tissue-resident macrophages in the tumour microenvironment towards tumour lesions via a cascade that enhances the local production of TNFα and the macrophage chemoattractant MCP-1, and also by engaging and re-educating the often immunosuppressive alternatively-activated macrophages towards pro-inflammatory phenotypes. Furthermore, IgE can prime classically- and alternatively-activated tissue macrophage subsets to mediate anti-tumour functions. A first-in-class IgE antibody has reached clinical testing in patients with solid tumours. On the whole, our findings may provide opportunities to extend the current IgG-only class of monoclonal antibodies for cancer immunotherapy.

214

Polyamines as mediators of secondary metabolite production in filamentous fungi

Gulgina Nuraeva, Alexander Zhgun
Research Center of Biotechnology RAS, Moscow, Russia
E-mail: zzhgun@mail.ru

Over the past 60-70 years numerous, industrial strain-producers of secondary metabolites (SM), including antibiotics, statins and immunosuppressants, have been obtained from various initial isolates of mycelial fungi. These high producers were developed by classical strain improving methods (random mutagenesis and target metabolite selection), and have currently reached their technological limits for improvements. Further use of traditional technology does not lead to an increase in the production of necessary SM. Recently, it was shown that exogenously-introduced polyamines were able to increase the production of target metabolites in high active fungal strains through exposure to the global regulator of fungi SM, LaeA - S-adenosylmethionine-dependent histone methylase, chromatin remodelling factor. The aim of this study was to investigate the association between secondary metabolic pathways of filamentous fungi (exemplified by beta-lactam NRPS-dependent metabolism in *Acremonium chrysogenum* and polyketide pathway of lovastatin biosynthesis in *Aspergillus terreus*) and the metabolism of polyamines. The main pathways of synthesis of polyamines in fungal cells (via ornithine decarboxylase and through agmantinase) will be studied for the wild-types of *A. chrysogenum* and *A. terreus* and their high producers at the level of comparative transcriptome analysis and inhibition by low molecular compounds synthesized during the project. To study the intersection of the metabolism of polyamines (N1-acetylation in the catabolism of polyamines) and the biosynthesis of SM (the production of cephalosporin C from the deacetyl- precursor and lovastatin as the result of PKS work), analogues of the metabolite intermediates of acetyl-CoA are also synthesized, and functional tests were performed. A series of experiments with specially synthesized analogues of amino acids, and analogues of sulphur metabolism intermediates, to identify the intersections at the level of S-adenosylmethionine, mutually consumed for the biosynthesis of polyamines and as the substrate of the global LaeA regulator, were performed. As result, the preliminary model for summarizing the data of intersection the polyamines metabolism and fungi SM (at the levels of acetylation, methylation, global regulation, etc.) with the aim of studying the possibilities of further increasing pharmaceutically important compounds in highly active fungal strains was developed.

Acknowledgements: The authors are grateful to leading researcher of the Research Center of Biotechnology, RAS Michael Eldarov for his essential advices.

This study was supported by RFBR Grant 19-04-01173.

215

Influence of the mesenchymal phenotype on the response to VEGF-targeted agents in the treatment of colorectal cancer

Annette K Larsen[1-4], Lila Louadj[1-3], Sandrine Thouroude[1-3], Aimery de Gramont[1,2,5], Benoist Chibaudel[1,2,5], Jean-Paul Thiery[6], Jérôme Denis[1-3,7], Anaïs Bouygues[1-3]
[1]Cancer Biology and Therapeutics, Centre de Recherche Saint-Antoine, Paris; [2]Institut National de la Santé et de la Recherche Médicale U938, Paris; [3]Institut Universitaire de Cancérologie, Paris; [4]Centre National de la Recherche Scientifique, Paris; [5]Institut Hospitalier Franco-Britannique, Levallois-Perret, France; [6]Yong Loo Lin School of Medicine, National University of Singapore; [7]Department of Oncology and Endocrine Biochemistry, Pitié-Salpêtriére; Paris, France
E-mail: annette.larsen@sorbonne-universite.fr

Vascular endothelial cell growth factor (VEGF) is a validated target for treatment of metastatic colorectal cancer (mCRC) with bevacizumab and aflibercept being approved for first- and second-line treatment, respectively. However, there are currently no clinically validated biomarkers to predict which patients are likely, or not, to respond to VEGF-targeted agents. Recently, different CRC subtypes have been identified, including a mesenchymal subgroup with a high microvascular density and poor prognosis. Clinical findings from other cancer types, such as ovarian cancer suggest that the tumor microvascular density may be predictive for the response to VEGF-targeted agents. We herein i) use different strategies to define the mesenchymal vs. the epithelial phenotype in CRC, including RT-qPCR, immunohistochemistry, epithelial-mesenchymal transition (EMT) score and new generation sequencing (NGS). We then ii) compared the expression of epithelial and mesenchymal markers in tumor cells and their corresponding tumor xenografts. Finally, we determine if the mesenchymal phenotype is iii) associated with higher vascularization and iv) is predictive of the response to VEGF-targeted agents.

This project was financed in part by Sanofi-Genzyme.

216

Application of proteomics in target identification for personalized anticancer therapies

George Mihai Nitulescu
Faculty of Pharmacy, 'Carol Davila' University of Medicine and Pharmacy, Bucharest, Romania
E-mail: george.nitulescu@umfcd.ro

Despite numerous efforts and important breakthroughs, cancer remains one of the major causes of mortality worldwide. New targeted drugs have emerged to attack the very mechanism of cancer development, giving hope to patients and doctors. However, cancer is associated with complex mechanisms, and it is not governed by a single mechanism, but a plethora of different subtypes distinguished by their molecular mechanisms; in addition, not all cancers are similar as regards therapeutic solutions. The solution seems to be in the molecular profiling of cancer patient samples allowing for a greater degree of personalized medicine. The progresses recorded in proteomics techniques will enable larger scale, sensitive and quantitative protein analyses. Measuring the levels of target proteins and genes can provide the most effective therapy for an individual's condition, given there is a specific drug available. However, the outcomes of proteomics research for target identification has to be supported by the pharmaceutical research and by the drug development programs in order to provide a new drug for patients. A number of new treatments tailored to target a specific change are still in clinical trials, and some have proven their efficacy only in preclinical models. The rate of success depends on teamwork among various types of scientists, with backgrounds in molecular biology, biochemistry, chemistry, physiology, pharmacology, toxicology and medicine, as well as a tight collaboration between academia and the industry. Even more, it is essential for a good communication and goal sharing between drug manufacturers, regulators, healthcare providers and political decision makers. Proteomics is one of the promising strategies in the cancer war and will hopefully make a great impact in the near future.

Key words: proteomics, target identification, precision medicine, anticancer drugs

217

Mitochondrial network collapse is an essential process in cancer cell death induction by cold atmospheric plasma-activated liquids

Yoshihiro Suzuki-Karasaki
Plasma ChemiBio Laboratory, Nasushiobara, Tochigi, Japan
E-mail: suzuki.pcbl@gmail.com

Cold atmospheric plasma (CAP)-activated liquids (PALs) preferentially kill a variety of cancer cell types, while displaying minimal damages in non-transformed cells. This high cancer-selective properties makes them a promising cancer-targeting weapon. Although the effect is attributed to CAP-induced reactive oxygen species (ROS), the mode of actions of PALs are not yet fully understood. We have previously reported that helium plasma-activated medium (PAM) induces mitochondrial network collapse in cancer cells, but not in normal cells (1). PAM treatment results in the accumulation of ROS within the mitochondria matrix, which leads to excessive fragmentation, swelling, and clustering of the organelles. To determine the generality of the mitochondrial network aberration, we examined the effect of different types of PALs on the mitochondrial morphology. We found that regardless of the sources of liquids, types of plasma jets and gasses employed for CAP generation, all PALs tested caused mitochondrial network collapse and the potency correlated with the anticancer effect. Moreover, we found that intracellular nitric oxide (NO) played a vital role in the collapse and cell death caused by PALs. We have previously demonstrated that the disruption of membrane potential and Ca^{2+} homeostasis contributes to the anticancer activity of PALs and the mitochondrial network (2). In an expansion of the previous work, the present study revealed a closed functional relationshop among membrane potential, Ca^{2+}, and NO in regulating the mitochondrial network in cancer cells. Thus, the modulation of these parameters commonly leads to massive impacts on the mitochondrial integrity in cancer cells and can be exploited as a promising target for cancer control.

(1) Saito et al., Oncotarget 7: 19910-19927, 2016.
(2) Tokunaga et al., Int J Oncol 52: 697-708, 2018.

218

Air plasma-activated medium induces an iron-dependent cell death in human malignant tumor cells

Manami Suzuki-Karasaki[1], Ando Takashi[2], Yushi Ochiai[1,3], Miki Suzuki-Karasaki[1], Yukihiro Yoshida[4], Toyoko Ochiai[1,5], Yoshihiro Suzuki-Karasaki[1*]
[1]Plasma ChemiBio Laboratory, Nasushiobara, Tochigi; [2]Department of Orthopedic Surgery, Yamanashi University School of Medicine, Yamanashi; [3]Department of Neurological Surgery, Nihon University School of Medicine, Tokyo; [4]Department of Orthopedic Surgery, Nihon University School of Medicine, Tokyo; [5]Department of Dermatology, Nihon University Hospital, Tokyo, Japan
E-mail: suzuki.pcbl@gmail.com

Malignant melanoma (MM), osteosarcoma (OS) and glioblastoma (GB) are representatives of malignant tumor cells that are highly resistant to multidisciplinary treatments, including chemotherapy. Cold atmospheric plasma (CAP)-activated medium (PAM) has emerged as a new promising anticancer tool as it preferentially injuries malignant cells. However, the conventional PAM contains the potential hepato- and neurotoxic substance phenol red (PhR). We found that helium plasma-PAM without PhR was less effective than conventional He-PAM, while air bubbling potentiated the effect. Therefore, we developed another new CAP-based agent, Air-PAM by irradiating air plasma to PhR-free DMEM. This agent potently killed the conventional He-PAM-resistant human OS and GB cells. Mechanistically, Air-PAM induces explicitly a caspase-independent cell death, which was characterized by the numerous subfragment of the nuclei and excessive clustering of fragmented mitochondria. Air-PAM had minimal cytotoxicity in human fibroblasts. Strikingly, the cell death was entirely blocked by iron chelators, such as bipyridyl and deferoxamine while augmented by Fe^{2+} and buthinine sulfoximine. The ferroptosis activator erastin not always mimicked the effect of Air-PAM and the ferroptosis inhibitor ferrostatin-1 did not necessarily inhibit the cell death caused by Air-PAM. Finally, Air-PAM reduced the growth of mice and human OS cells in mice by displaying minimal adverse effects. Our results suggest that Air-PAM has unique anticancer properties capable of triggering massive iron-dependent cell death in a tumor-selective manner; therefore, it may be exploited as a promising anticancer agent.

219

Co-encapsulation of curcumin and doxorubicin in long circulating liposomes enhances the efficacy of colon cancer treatment

Alina Sesarman[1-3*], Lucia Tefas[3], Bianca Sylvester[3], Emilia Licarete[1,2], Valentin Florian Rauca[1,2], Lavinia Luput[1,2], Laura Patras[1,2], Sebastian Porav[1,4], Manuela Banciu[1,2], Alina Porfire[3]
[1]Faculty of Biology and Geology and [2]Molecular Biology Centre, Institute for Interdisciplinary Research in Bio-Nano-Sciences, "Babes-Bolyai" University; [3]Faculty of Pharmacy, University of Medicine and Pharmacy 'Iuliu Hatieganu'; [4]National Institute of R&D of Isotopic and Molecular Technologies, Cluj-Napoca, Romania
*E-mail: sesarman@gmail.com

The aim of this study was to prepare long circulating liposomes (LCL) co-encapsulating curcumin (CURC) and doxorubicin (DOX) and to determine whether this new formulation can be exploited more efficiently than liposomal DOX for future colorectal cancer therapy. Therefore, the physicochemical properties of LCL-CURC-DOX were assessed and the mechanisms of anticancer activity were investigated in *in vitro* and *in vivo* models of C26 colon carcinoma. Our results proved that the developed nanoformulation based on the co-encapsulation of CURC and DOX in LCL exerted the highest anti-proliferative effects on C26 cells, mediated mainly by the inhibition of the NF-κB-dependent production of angiogenic and inflammatory proteins. Moreover, LCL-CURC-DOX met the quality profile of a product suitable for intravenous administration and passive tumor targeting, exhibiting the highest efficacy in inhibiting the growth of C26 colon carcinoma *in vivo,* among all treatments tested. Mechanistically, this high therapeutic efficacy of LCL-CURC-DOX was achieved by the suppression of the main protumor processes, i.e., inflammation, angiogenesis, oxidative stress, invasion and resistance to apoptosis, and by the dysregulation of the Th1/Th2 cell axis which favored the antineoplastic phenotype of tumor microenvironment (TME). Our findings suggest that LCL-CURC-DOX could be beneficial in the management of colon cancer, as it demonstrated a high therapeutic efficacy *in vivo*, surpassing that of LCL-DOX.

Financial support: From Romanian National Authority for Scientific Research and Innovation, CNCS-UEFISCDI, project no. PN-II-RU-TE-2014-4-0220 and PN-III-P4-ID-PCE-2016-0342.

220

Combination therapy of the liposome-encapsulated agents Simvastatin and DMXAA affects major mechanisms of murine melanoma development and progression

Valentin Florian Rauca[1,2], Laura Patras[1,2], Lavinia Luput[1,2], Alina Sesarman[1,2], Emilia Licarete[1,2], Vlad Toma[1], Augustin C. Mot[3], Manuela Banciu[1,2]
[1]Department of Molecular Biology and Biotechnology; [2]Molecular Biology Centre, Institute for Interdisciplinary Research in Bio-Nano Sciences and [3]Department of Biochemistry and Biochemical Engineering of Babes-Bolyai University, Cluj-Napoca, Romania
E-mail: valirauca@yahoo.co.uk

Recent advances in molecular oncology research define melanoma as the result of numerous alterations in various interconnected signaling pathways affecting cell growth and apoptosis, deregulating angiogenesis and the response to oxidative stress (1). The tumor microenvironment (TME) plays a pivotal role in facilitating the aberrant communication between cells within the tumor stroma. Our previous *in vitro* studies provided confirmatory evidence for the ability of co-administered simvastatin and DMXAA (5,6-dimethylxanthenone-4-acetic acid) to suppress the aggressive phenotype of B16.F10 melanoma cells co-cultured with tumor associated macrophages under hypoxia-mimicking conditions *in vitro* (2). Therefore, the aim of the present study was to evaluate the effectiveness of the two therapeutic agents incorporated in polyethylene glycol-coated long circulating liposomes on an *in vivo* murine melanoma model. The results revealed that the combined liposomal drug therapy inhibited tumor growth more than single liposomal therapy. A switch to an apoptotic state was suggested by low levels of anti-apoptotic Bcl-xL and high levels of pro-apoptotic Bax, while the overall suppression of inflammatory and angiogenic proteins favored an anti-angiogenic state of TME. The modulatory effect on TME oxidative stress parameters suggests that this therapeutic approach might break the barrier of drug resistance, one of the major drawbacks of current anti-angiogenic therapies. This novel targeted therapy holds the potential to disrupt and reprogram the pro-tumorigenic TME.

(1) Lopez-Bergami P. et al., Photochem Photobiol 84(2): 289-306, 2008; (2) Rauca V.F. et al., PLoS One 13(8): e0202827, 2018.

Acknowledgements: This work was supported by grants of the Romanian Ministery of Research and Innovation, CNCS– UEFISCDI, project number PN-III-P4-ID-PCE-2016-0342, contract number 91/2017 within PNCDI-III.

221

Spermine metabolite, hydrogen peroxide and aldehyde, induce the apoptosis of neuroblastoma cells associated with an increase in p53, Caspase-3 and miR-34a

Enzo Agostinelli[1,2], Yuta Kanamori[1], Alessia Finotti[3], Laura Di Magno[4], Gianluca Canettieri[4], Shinji Ohkubo[1], Fabio Timeus[5], Jessica Gasparello[3], Italia di Liegro[6], Roberto Gambari[3]
[1]Department of Biochemical Sciences, University of Rome "La Sapienza", Rome; [2]International Polyamines Foundation ONLUS, Rome; [3]Department of Life Sciences and Biotechnology, Biochemistry and Molecular Biology Section, University of Ferrara, Ferrara; [4]Department of Molecular Medicine, University of Rome 'La Sapienza', Rome; [5]Paediatric Onco-haematology, Regina Margherita Children's Hospital, Turin; [6]Department of Biomedicine, Neurosciences and Advanced Diagnostics (Bi.N.D.), University of Palermo, Palermo, Italy
E-mail: enzo.agostinelli@uniroma1.it

Neuroblastoma (NB) is a common malignant solid tumour in children, which originates from the sympathoadrenal lineage of neural crest and accounts for 15% of childhood cancer mortality. Amplification of the oncogene N-Myc is a well-established poor prognostic marker for neuroblastoma. Whilst N-Myc amplification status strongly correlates with higher tumour aggression and resistance to treatment. Therefore, new therapies for patients with N-Myc amplified NB need to be developed. The *in situ* formation of cytotoxic polyamine metabolites by bovine serum amino oxidase (BSAO) is a recent approach in cancer enzymotherapy. It has been demonstrated that BSAO and spermine (SPM) addition to cancer cells induces cell growth inhibition and apoptosis through the oxidative stress caused by polyamine metabolites, H_2O_2 and aldehydes, produced by the oxidative reaction (1). The cytotoxic effect induced by BSAO and SPM was evaluated by both clonogenic and MTT assays. The detection of apoptosis of the NB cells was evaluated by flow cytometry after Annexin V-FITC labelling and DNA staining with propidium iodide. The percentages of Annexin V-positive cells matched quite well with those of cells exhibiting a hypodiploid sub-G1 peak. An increase in mitochondrial membrane depolarization (MMD) was found in the NB cells treated with the enzymatic system. The mitochondrial membrane potential activity was examined by flow cytometric assays, labelling cells with the probe JC1 dye. We also analysed, by RT-qPCR, the transcript of some genes involved in the apoptotic process, to determine the possible down- or upregulation of mRNAs following treatment of the SJ-N-KP and the N-Myc-amplified IMR-5 cell lines with BSAO and SPM. The experiments were carried out considering the pro-apoptotic genes p53, PUMA and Caspase-3. Following treatment with BSAO and SPM, both cell lines displayed increased mRNA levels for all the pro-apoptotic genes. Notably, the level of the pro-apoptotic Sirt-1 inhibitor microRNA, miR-34a, significantly increased in the SJ-N-KP and IMR5 cells treated with BSAO and SPM. Western blot analysis with PARP and Caspase-3 antibody supported the concept that BSAO/SPM treatment induced high levels of apoptosis in the NB cell lines. The major conclusion is that BSAO/SPM treatment leads to the anti-proliferative and cytotoxic activity of both NB cell lines, associated with the activation of apoptosis. Moreover, these findings suggest that enzymatic spermine metabolite may be a powerful tool for the development of novel anticancer treatments.

(1) Amendola et al. Reactive oxygen species spermine metabolites generated from amine oxidases and radiation represent a therapeutic gain in cancer treatments (2013) International Journal of Oncology, 43 (3), pp. 813-820.

222

Perspectives of taurine derivatives

Markus Nagl
Division of Hygiene and Medical Microbiology, Medical University of Innsbruck, Austria
E-mail: m.nagl@i-med.ac.at

Active halogen taurine derivatives are basically the naturally occurring N-chlorotaurine (NCT) and N-bromotaurine (NBrT), which are produced by activated human neutrophilic and eosinophilic granulocytes and monocytes. Due to their oxidative mechanisms of action, they exhibit broad spectrum microbicidal activity against bacteria, fungi, viruses and protozoa, as well as anti-inflammatory properties, such as the downregulation of pro-inflammatory factors and cytokines such as NF-κB, interleukins, prostaglandin E2, tumor necrosis factor, neopterin and others. In particular, NCT has been developed as a natural anti-infective and antiseptic for topical application at different body sites, particularly sensitive ones, for instance, the eye, ear, ulcerated skin and the urinary bladder. NBrT was applied successfully on acne. Since both natural compounds have to be cooled for longer storage, which is only sufficient for NCT, synthetic active halogen compounds have been created that possess a higher stability. In particular, bromamine T (BAT) is a compound which has attracted much interest since it resembles the properties of NBrT. Future perspectives for application are the following: NCT is particularly suited for purulent infections of sensitive body sites as mentioned above. The reasons are its mild activity, low chlorine consumption and the enhancement of microbicidal activity in body fluids and exudates by transhalogenation. The inhalation of NCT in chronic bronchitis or cystic fibrosis is a very promising investigative field. Bromamines appear to be suited for treatment of infections, as well, as demonstrated on the skin for acne and herpes zoster. In addition, they exert multiple anti-proliferative effects against tumor cells. Thus, oncology has become a topic of great interest, particularly as regards BAT and NBrT.

223

IL-12 regulates the expansion of effector-like NK cells induced by IL-15/18 and alters their phenotypes and functions

Naoto Oka[1], Tzvetanka Markova[2], Magdalena Pencheva[2], Wen Li[3], Kyousuke Yamanishi[3], Hiromichi Yamanishi[4], Haruki Okamura[3]
[1]Department of Otorhinolaryngology-Head and Neck Surgery, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan; [2]Department of Pharmacology, Medical University, Sofia, Bulgaria; [3]Department of Psycho-immunology, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan; [4]Hirakata General Hospital for Developmental Disorders Hirakata, Osaka, Japan
E-mail: haruoka@hyo-med.ac.jp

Recent progress in cancer immunotherapy has been greatly encouraging; however, the limited efficacy prompts researchers to improve therapy. The exhaustion of effector lymphocytes may bring about the tumor evasion of immune attack. NK cells are critical for cancer immunotherapy, as they are not homogeneous in phenotype and function. Recent studies have demonstrated that the stimulation of peripheral NK cells by IL-12/15/18 generates longer-living, memory-like NK cells. In the present study, we demonstrated that this process could be separated into phases of effector-like cells induced by IL-15/18 and longer-living cells differentiated by IL-12. Freshly prepared splenic NK cells expressed IL-15Rs and IL-18Rs, and rapidly began to proliferate by the stimuli of combination of IL-15 and IL-18 (IL-15/18). These proliferating cells highly expressed various activation markers and exerted potent cytotoxic effects. They expressed IL-12 receptors, β1 and β2; however, they did not secrete cytokines, while they had a high potential to produce IFN-γ in response to IL-12. IL-12 strongly activated STAT4 in the cells activated by IL-15/18, upregulated p21 and p27, and led to withdrawal from the cell cycle suppressing cell expansion. In parallel, IL-12 rapidly induced IFN-γ production, greatly altered the expression of surface molecules, reduced cytotoxicity, and generated longer-living cells. Notably, a large proportion of IL-15/18-induced cells strongly expressed PD-1 together with activation molecules, whereas NK cells induced by IL-15/18 and IL-12 expressed high levels of TIM-3, LAG-3 and NKG2A. Furthermore, the latter spontaneously secreted IL-10 and TGF-β during prolonged incubation. These results indicated that IL-12 regulated the expansion of IL-15/18-induced, effector-like NK cells, generating longer-living cells. In addition, peripheral NK cells were suggested to be differentially regulated by various ligands of immune checkpoint molecules at different stages of differentiation. IL-12 signaling may be involved in the terminal differentiation of NK cells and influences the population size of effector NK cells. These findings may also give suggestion to understanding of mechanism of exhaustion of NK cells.

224

Membrane protein inventory of human pheochromocytoma and paraganglioma

Ondrej Vit[1], Karel Pacak[2], Jiri Petrak[1]
[1]BIOCEV, First Faculty of Medicine, Charles University in Prague, Czech Republic; [2]National Institutes of Child Health and Human Development (NICHD), Bethesda, Maryland, USA
E-mail: jpetr@lf1.cuni.cz

Pheochromocytoma and paraganglioma (PHEO/PGL) are rare neuroendocrine tumors derived from adrenal medulla or extradrenal paraganglia, respectively. The prognosis of patients with malignant PHEO/PGL is poor, and specific molecular targets for novel therapies are therefore required. Integral membrane proteins (IMPs) expressed by tumors represent such potential therapeutic targets, due to their specific functions and localization. Our goal is to provide a detailed inventory of membrane proteome of human PHEO and PGL that could help identify novel drug targets and diagnostic markers. IMPs are coded by roughly 25% of human genes; however, our knowledge of the IMP repertoire expressed by specific tumors is limited. Their amphipathic nature, the lack of trypsin cleavage sites and their relatively low expression hinder the proteomics analysis of IMPs. The specific physicochemical properties of IMPs require specific analytical strategies. In order to maximize the coverage of the PHEO/PGL membrane proteome, we combined a standard trypsin-based approach with a selective isolation of (extramembrane) glycopeptides and with our recently introduced hPTC method, which selectively targets (transmembrane) hydrophobic segments of IMPs, into a multi-pronged 'Pitchfork' strategy. The methods included in the Pitchfork strategy target different features of IMPs, are complementary, and allow for the identification of a significant portion of the membrane proteome expressed by PHEO/PGL. On average, we identified 900-1300 IMPs in each PHEO/PGL tumor sample analyzed to date. Our current dataset represents nearly 2,200 unique IMPs identified in PHEO and a similar number in PGL tumor samples. Among the identified proteins, we observed several proteins expressed in tumor tissue, but not in healthy adrenal medulla. Such proteins are currently studied in detail as potential drug targets or disease markers.

Financial support: The Ministry of Education, Youth and Sports (Inter-Action LTA USA 17148) and the Ministry of Health of the Czech Republic (AZV NC 19-01-00083) supported the work.

225

Bromamine T, a new developable hygiene product

Waldemar Gottardi
Division of Hygiene and Medical Microbiology, Medical University of Innsbruck, Austria
E-mail: waldemar.gottardi@i-med.ac.at

Active halogen compounds have broad-spectrum microbicidal activity without resistance development, and some representatives are in use as anti-infectives and antiseptics, such as iodine and hypochlorous acid. The bromine line has been under-represented thus far; however, there are some features that render these molecules interesting. In particular, their bactericidal activity at even low micromolar concentrations is very strong, and their sufficient fungicidal and virucidal properties have also been shown. Consumption effects by organic material have to be taken into account, as with all active halogen molecules. If the bactericidal activity of bromamines is compared to their cytotoxic activity, body cells exhibit a markedly higher tolerability than bacteria, leading to an extraordinarily high biocompatibility index. Indeed, the clinical application of the natural substance N-bromotaurine in acne (phase II study) and in skin infections, such as herpes zoster (cases) was very well tolerated and effective. Due to higher stability, synthetic compounds, such as bromamine T are of interest and have exhibited similar clinical properties in first case applications. Moreover, the anti-inflammatory effects of bromamines are pronounced, and recently, their anti-proliferative and anti-tumour activity has been shown. Due to these features, bromamine T should be further developed as an anti-infective and anti-tumour substance, particularly on the skin.

226

Hepatocellular carcinoma and resveratrol

Pawel Slodnik[1], Christoph Zeller[1]
[1]Praxis Am Bahnhof Ruti, Ruti, Switzerland
E-mail: pawel.slodnik@yahoo.de

Hepatocellular carcinoma (HCC), as a primary liver tumor, has a poor prognosis. There are a number of risk factors for the development of this type of cancer, such as infection with hepatitis virus, alcoholism, non-alcoholic steatohepatitis, diabetes type 2, congenital disorders and other liver diseases (1). Over the past years, HCC has exhibited increasing resistance against standard therapeutic procedures (e.g., cisplatin). Currently, a number of new oncologic drugs are obtained from plants. The anti-neoplastic potential of trans-resveratrol (extract from red grapes) is already described in the literature. An *in vivo* study using a fibrosarcoma cell line demonstrated a potent pro-apoptotic activity of trans-resveratrol (2,3). The regulation of p53 and the phosphoinositide 3 kinase/protein kinase B pathway plays a crucial role in the anti-neoplastic activity of trans-resveratrol. In addition, sphingolipid metabolism has been shown to be modulated by trans-resveratrol in human HCC (4,5). The expression of the well known SIRT1 protein is modulated by trans-resveratrol via the PI3K/AKT signaling pathway (6). One of the main reasons for the poor prognosis of patients with HCC is metastasis. Trans-resveratrol has also exhibited anti-metastatic activity in HCC through the modulation of SP-1 (7). Trans-resveratrol, but also oxyresveratrol, play an important role in hepato-oncology. The latter modulates angiogenesis and lymphangiogenesis in HCC (8). It is possible that in the future, resveratrol could be used as a neoadjuvant or adjuvant in the therapy of HCC. Trans-resveratrol could also be used in the future as an anti-metastatic agent.

(1) Hepatocellular carcinoma: a review. J. Balogh et al. J Hepatocell Carcinoma. 2016 Oct 5; 3: 41-53; (2) Resveratrol induces apoptosis and alters gene expression in human fibrosarcoma cells. P. Slodnik et al. Anticancer Res. 2015 Feb; 35(2): 767-774; (3) Pro-apoptotic effects of pycnogenol on HT1080 human fibrosarcoma cells. P. Slodnik et al. Int J Oncol. 2015 Apr; 46(4): 1629-1636; (4) Influence of Resveratrol on Sphingolipid Metabolism in Hepatocellular Carcinoma Cells in Lipid Overload State. Charytoniuk et al. Anticancer Agents Med Chem. 2018 Dec 24; (5) Resveratrol inhibited the progression of human hepatocellular carcinoma by inducing autophagy via regulating p53 and the phosphoinositide 3-kinase/protein kinase B pathway. B. Zhang et al. Oncol Rep. 2018 Nov; 40(5): 2758-2765; (6) Resveratrol inhibits proliferation and migration through SIRT1 mediated post-translational modification of PI3K/AKT signaling in hepatocellular carcinoma cells. R. Chai. Mol Med Rep. 2017 Dec; 16(6): 8037-8044; (7) The Antimetastatic Effects of Resveratrol on Hepatocellular Carcinoma through the Downregulation of a Metastasis-Associated Protease by SP-1 Modulation. CB Yeh et al. PLoS One. 2017 Mar 20; 12(3): e0174494; (8) Oxyresveratrol prevents murine H22 hepatocellular carcinoma growth and lymph node metastasis via inhibiting tumor angiogenesis and lymphangiogenesis. Y. Liu et al. J Nat Med. 2018 Mar; 72(2): 481-492.

227

Pine bark extract in oncology

Pawel Slodnik[1], Christoph Zeller[1]
[1]Praxis am Bahnhof Ruti, Ruti, Switzerland
E-mail: pawel.slodnik@yahoo.de

Pine bark extract contains polyphenols such as catechin, epicatechin and taxifolin. This extract showed already antineoplastic activity in *in vitro* studies (1). Antineoplastic activity was also described in *in vitro* studies with catechin, epicatechin and taxifolin. In this study, *Pinus massoniana* bark extract was found to inhibit the migration of the lung cancer A549 cell line via MMP-9 and to restrict the migration and invasion of lung cancer cells (2). *Pinus massoniana* bark extract exhibited antineoplastic activity in murine sarcoma S180 cells both *in `vitro* and *in vivo*. *Pinus massoniana* reduced tumor weight *in vivo* by 9-67% (3). Pine bark extract also exerted an inhibitory effect on hepatitis c virus replication, known as one of the risk factors of hepatocellular carcinoma (4). Catechins are known to exert preventive and therapeutic effects on prostate cancer. It has also been shown that catechins induce the immune system (5). In the literature, the possible adjuvant use of epicatechin in cancer treatment has been described. Epicatechin induced the chemosensitization of tumor cells through additive and synergistic effects with 5-fluorouracil (5-FU), temozolomide, cisplatin and tamoxifen (6). In the literature, it has been shown that taxifolin inhibits scar cell carcinoma and induces apoptosis, cell cycle arrest and the suppression of the PI3K/AKT/mTOR pathway (7). The PI3K/AKT pathway also plays a crucial role in apoptosis induced by pine bark extract in the fibrosarcoma cell line, HT1080 (1). It is possible that in the future, pine bark extract could be used as a neoadjuvant or adjuvant therapy in oncology. In addition, catechin, epicatechin and taxifolin could be used as novel antineoplastic agents in the future.

1) Pro-apoptotic effects of pycnogenol on HT1080 human fibrosarcoma cells. Slodnik P et al. Int J Oncol. 2015 Apr; 46(4): 1629-1636; 2) Pinus massoniana bark extract inhibits migration of the lung cancer A549 cell line. Mao P et al. Oncol Lett. 2017 Feb; 13(2): 1019-1023; 3) Antitumor effects of Pinus massoniana bark extract in murine sarcoma S180 both in vitro and in vivo. Zhang JH et al. Am J Chin Med; 4) Inhibitory effects of Pinus massoniana bark extract on hepatitis C virus in vitro. C. Wang et al. Pharm Biol. 2015 Mar; 53(3): 451-456; 5) The Possibility of Preventive and Therapeutic Use of Green Tea Catechins in Prostate Cancer. Rogovskii VS et al. Anticancer Agents Med Chem. 2019 Apr 4; 6) Green tea polyphenol epigallocatechin-3-gallate (EGCG) as adjuvant in cancer therapy. Lecumberri E et al. Clin Nutr. 2013 Dec; 32(6): 894-903; 7) Taxifolin inhibits the scar cell carcinoma growth by inducing apoptosis, cell cycle arrest and suppression of PI3K/AKT/mTOR pathway. Zhou W et al. J BUON. 2019 Mar-Apr; 24(2): 853-858.

228

New insights into tumor invasion and vascularization

Andreas Bikfalvi
Angiogenesis and Tumor Microenvironment Laboratory, INSERM U1029, University Bordeaux, France
E-mail: andreas.bikfalvi@u-bordeaux.fr

Tumor development is governed not only by genetic mechanisms, but also by microenvironmental cues (1). Tumors adapt to environmental constraints by inducing survival mechanisms and tumor cell migration. We will illustrate this by discussing results from the laboratory on brain tumor pathophysiology. Indeed, we have uncovered 2 different mechanisms that promote tumor cell invasion. The first is related to CXCR3 activation in tumor cells (2). We have shown that CXCR3 interacts closely with LRP1 to modulate tumor cell invasion. LRP1 is downregulated at the invasive front in glioblastoma (GBM) cells, which increases the number of CXCR3A at the cell surface and modifies its conformation. Another mechanism is related to thrombospondin-1 (TSP1) which, in the invasive front, triggers invasion by interacting with CD47 on tumor cells (3). In GBM, TSP1 does not activate TGFβ1 but on the contrary TGFβ1 activates TSP1 through binding of SMAD3 on target sites. We will, furthermore, show data from a renal cell carcinoma model for the discovery of tumor-dependent and metastasis-dependent molecular signatures. Indeed, we have developed an experimental model for lung metastasis formation, which allowed us to identify specific transcriptomic and epigenetic signatures for primary tumor and metastasis formation. Finally, we have developed a new system for the construction of artificial blood vessels to study the tumor-vessel interface (4). Data from this study will also be presented.

1) Laplane L, Duluc D, Larmonier N, Pradeu T, Bikfalvi A. The Multiple Layers of the Tumor Environment. Trends Cancer. 2018 Dec; 4(12): 802-809.
2) Boyé K, Pujol N, D Alves I, Chen YP, Daubon T, Lee YZ, Dedieu S, Constantin M, Bello L, Rossi M, Bjerkvig R, Sue SC, Bikfalvi A, Billottet C. The role of CXCR3/LRP1 cross-talk in the invasion of primary brain tumors. Nat Commun 2017, Nov 17; 8(1): 1571.
3) Daubon T, Léon C, Clarke K, Andrique L, Salabert L, Darbo E, Pineau R, Guérit S, Maitre M, Dedieu S, Jeanne A, Bailly S, Feige JJ, Miletic H, Rossi M, Bello L, Falciani F, Bjerkvig R, Bikfalvi A. Deciphering the complex role of thrombospondin-1 in glioblastoma development. Nat Commun 2019, Mar 8; 10(1): 1146.
4) Andrique L., G. Recher, K. Alessandri, N. Pujol, M. Feyeux, P. Bon, L. Cognet, P. Nassoy, A. Bikfalvi. A model of guided cell self-organization for rapid and spontaneous formation of functional vessels. Science Adv (in press).

229

A multi-layered systems approach for renal cell carcinoma

Lindsay Cooley[1], Wilfried Souleyreau[1], Justine Rudewicz[1,2], Macha Nikolski[2], Kim Clarke[3], Francesco Falciani[3], Emeline Ribot[4], Sebastian Benzekry[5], Cécile Tang[5], Maeva Dufies[6], Diether Lambrechts[7], Gilles Pages[6], Andreas Bikfalvi[1]
[1]INSERM U1029 University of Bordeaux, Pessac, France; [2]The Bordeaux Bioinformatics Center (CBiB), University of Bordeaux, Bordeaux, France; [3]University of Liverpool, Liverpool, UK; [4]RMSB CNRS, University of Bordeaux, Bordeaux, France; [5]INRIA Bordeaux, France; [6]Institute for Research on Cancer and Aging, Nice (IRCAN), Nice, France; [7]Center for Cancer Biology, VIB, Leuven, Belgium

The therapeutic options of renal cell carcinoma after metastatic spread are limited. Furthermore, there is a lack of markers that predict the response to targeted treatment in order to stratify patients accordingly. To discover molecular pathways and markers in renal cancer development and spread, we developed a mouse model to generate sequentially more aggressive and specialised cell lines. Multiple cell lines for primary tumor growth, survival in the blood circulation and lung metastasis or metastatic spread from the primary tumor were generated and analysed using a multi-layered approach, which included large-scale transcriptome, genome and methylome analysis. Transcriptome and methylome analysis demonstrated distinct clustering in three different groups. Notably, DNA sequencing did not show significant genomic variations in the different groups, indicating the absence of clonal selection during the *in vivo* amplification process. Transcriptome analysis revealed several markers that were validated in patient cohorts from TCGA and biobank. This also includes soluble markers. We also identified key regulators of RCC progression, which was also functionally validated *in vivo* and a mathematical model was provided.

230

Anti-endoglin (CD105) monoclonal antibodies: Tools for cancer research and treatment

Ilya Smirnov[1,2], Irina Gryazeva[1], Anastasia Stolbovaya[1,2], Agnia Pinevich[1], Olga Shashkova[1], Marina Samoylovich[1]
[1]Russian Research Center for Radiology and Surgical Technologies named after acad. A.M. Granov, Hybridoma Technology Laboratory; [2]The Research Institute of Obstetrics Gynecology and Reproductology named after D.O. Ott, Saint-Petersburg, Russia
E-mail: smirnov.iv.mail@gmail.com

Endoglin (CD105) is a membrane antigen expressed on endothelial cells and various types of cancer cells. It modulates the activity of signal pathways induced by TGF-β1, -β3, BMP-2, -7, -9, -10, and activin A. Endoglin regulates cell migration, adhesion, and cancer cell metastasis. Its soluble form is released via protein cleavage. Endoglin expression is upregulated in tumor blood vessels compared to the vessels of normal tissues. We developed 18 monoclonal antibodies (MAbs) against human and rat endoglin. Three of them bind antigen molecules from both species, while the remaining antibodies bind only human or only rat endoglin. MAbs could be used as specific reagents for immunohistochemistry, western blotting, fluorescence microscopy, and flow cytometry. Based on a pair of antibodies a highly sensitive sandwich ELISA was developed. The antibodies were capable of quantifying soluble endoglin in blood plasma, urine, and cerebrospinal fluid. Published evidence indicates the level of soluble endoglin is increased in patients with certain types of cancer. Using a western blot assay followed by SDS-PAGE we demonstrated heterogeneity of soluble endoglin isolated from biological fluids. The nature of this phenomenon is yet to be determined. Anti-endoglin MAbs are considered potential tools for anti-angiogenic cancer treatment. We showed that the two antibodies against non-overlapping epitopes have multiple effects on functional properties of EA.hy926 endothelial cells. They inhibit cell migration in the presence of TGF-β1, increase monocyte adhesion to endothelium, and reduce the rate of endoglin shedding *in vitro*. Using flow cytometry, endoglin expression was demonstrated on cultured human cancer cells of different origin (osteosarcoma Mg63 cells, liver cancer HEPG2 cells, melanoma MeWo, glioblastoma T98G cells), while their non-transformed counterparts lacked the antigen. These cancer cells also produced endoglin in soluble form, though less extensively compared to endothelial cells. Modulation of cancer cell properties via anti-endoglin MAbs treatment is a question for further studies. The panel of anti-CD105 MAbs provides wide opportunities for versatile studies of the role of endoglin in cancer both *in vitro* and in rat models.

Financial support: This work was supported by Russian Science Foundation [grant number 17-15-01230].

231

Inhibition of EIF-5A prevents cardiac symptoms in complicated malaria through apoptosis

Kirsten Heiss[2,3], Ann-Kristin Mueller[2,3], Rolf Fimmers[4], Jan Matthes[5], James Thujon Njuguna[6], Annette Kaiser[1]
[1]Medical Research Centre, University Duisburg-Essen, Essen, Germany; [2]Centre for Infectious Diseases, Parasitology Unit, University Hospital Heidelberg, Heidelberg, Germany; [3]German Center for Infectious Diseases (DZIF), Heidelberg, Germany; [4]Institut für Medizinische Biometrie, Informatik und Epedimologie, Sigmund Freud, Bonn, Germany; [5]Institut für Pharmakologie, Köln, Germany; [6]Astel Diagnostics, Kampala, Uganda
E-mail: kaiser@microbiology-bonn.de

In this study, a determination of Troponin I and creatine kinase activity in whole-blood samples in a cohort of 100 small infants at the age of 2-5 years from Uganda with complicated *Plasmodium falciparum* malaria suggests the prevalence of cardiac symptoms in comparison to non-infected patients. Troponin I and creatine kinase activity increased during infection. Different reports revealed that complicated malaria coincides with hypoxia in children. The obtained clinical data prompted us to further elucidate the underlying regulatory mechanisms of cardiac involvement in human cardiac ventricular myocytes. Complicated malaria is the most common clinical presentation and may induce cardiac impairment by hypoxia. Eukaryotic initiation factor 5A (eIF-5A) is involved in hypoxia inducible factor (HIF-1α) expression. EIF-5A is a protein post-translationally modified by hypusination involving catalysis of the two enzymes deoxyhypusine synthase (DHS) and deoxyhypusine hydroxylase. Treatment of human cardiomyocytes with GC7, an inhibitor of DHS, catalyzing the first step in hypusine biosynthesis, led to a decrease in proinflammatory and proapoptotic myocardial caspase-1 activity in comparison to untreated cardiomyocytes. This effect was even more pronounced following the co-administration of GC7 and GPI from *P. falciparum* simulating the pathology of severe malaria. Moreover, in comparison to untreated and GC7-treated cardiomyocytes, the co-administration of GC7 and GPI significantly decreased the release of cytochrome *c* and lactate from damaged mitochondria. In sum, the co-administration of GC7 prevented cardiac damage driven by hypoxia *in vitro*. Our approach demonstrates the potential of the pharmacological inhibitor, GC7, to ameliorate apoptosis in cardiomyocytes in an *in vitro* model simulating severe malaria. These regulatory mechanisms are based on blocking EIF-5A hypusination.

Key words: cardiomyocytes, GC7, hypusine, apoptosis, hypoxia

232

The implication of exosomal miRNAs in the development of cancer represents the cornerstone of onco-regulation by Bio Immune(G)ene Medicine [BI(G)MED]

Carme Parés Santilari
Department of Clinical Research, European Bio Immune(G)ene Medicine Association (EBMA), Barcelona, Spain

Exosomes are a type of extracellular vesicle (EV) of endosomal origin that serve as transfer vehicles between cells. Their presence in the extracellular space has been known from the eighties; however, their important role in very different biological processes, such as in cancer has become evident more recently. Exosomes derived from tumors play a key communicating role not only between the cancer cell and the tumor microenvironment, but also with distant cells. Among the exosomal cargo there are proteins, lipids, chemokines and nucleic acids, particularly miRNAs. Recent literature has described how the presence of these miRNAs, whose expression is particularly high in exosomes, is responsible for tumor growth and aggressiveness, neo-angiogenesis, the promotion of inflammation, therapy resistance as well as metastasis formation. The miRNAs associated with each step in the process of cancer formation have been identified; thus, they can be applied as objective factors in the treatment of cancer. The therapeutic use of miRNAs still remains subject to controversy due to technical factors; however, we propose its use by means of Bio Immune(G)ene Medicine [BI(G)MED]. BI(G)MED is a novel nanobiotherapy that uses a maximum of cell molecular resources, particularly at the epigenetic level related to miRNAs, with the aim of restoring cell homeostasis. This is made possible by using ultra-low doses of molecules ($1:10^5$ to $1:10^{12}$ Mol), according to the Hormesis principle, to provide a 'soft' and progressive epigenetic regulation. The results are often spectacular, particularly in the field of oncology.

233

Loss function of PITX1 contributes to a poor prognosis of gastric cancer patients by enhancing chemotherapy resistance to 5-fluorocytosine and cisplatin

Xiaohui Shen, Fengchang Qiao, Pihai Gong, Zhujiang Zhao, Hong Fan
Department of Medical Genetics and Developmental Biology, Medical School of Southeast University, The Key Laboratory of Developmental Genes and Human Diseases, Ministry of Education, Southeast University, Nanjing, P.R. China
E-mail:891624930@qq.com

Gastric cancer (GC) is the third leading cause of cancer-related mortality worldwide, and systemic chemotherapy is the major treatment strategy for patients with advanced GC. Paired-like homeodomain transcription factor 1 (PITX1) has been implicated as a tumour suppressor in various types of cancer. In the present study, we found that PITX1 expression was downregulated at the transcriptional level in 44% (14/32) of GC patients. The result of Kaplan-Meier curve analysis indicated that GC patients with lower levels of PITX1 had a worse prognosis than those with higher levels of PITX1 (*P=0.027). A poor prognosis following chemotherapy is the general outcome owing to recurrent resistance. A cell counting kit-8 assay was performed to examine the effect of PITX1 expression on the sensitivity of GC cells to 5-fluorocytosine (5-FU) and cisplatin (CDDP). The results revealed that the overexpression of PIXT1 increased the sensitivity of the AGS and BGC-823 GC cells to 5-FU/CDDP. Moreover, PIXT1 knockdown decreased the sensitivity of the MCG-803 and SGC-7901 GC cells to 5-FU/CDDP. To further assess the mechanism by which PITX1 contributes to chemotherapy insensitivity, a total of co-expressed genes, 1620 genes were screened by a KEGG analysis, the biological processes of which were primarily implicated in necroptosis and apoptosis. PDCD5, a cell apoptosis-related gene, was thus a candidate gene according to the results of CHIP-Seq and GO analysis. A luciferase reporter assay revealed that the transcription activity of the motif was significantly higher in AGS cells transfected with pPITX1 than in cells transfected with pcDNA3.1 plasmids without the motif region. An EMSA was applied to certify the physical interaction of PITX1 with PDCD5. Furthermore, the expression level of PDCD5 in GC cells was positively regulated by PITX1. Finally, we found that PDCD5 promoted the apoptosis of GC cell lines, as shown by flow cytometry and Annexin V staining. Collectively, these data indicated that PITX1 enhanced the cytotoxicity of 5-FU and CDDP in GC cells, partially by targeting the PDCD5 promoter to induce cell apoptosis.

234

Methylation pattern of H3K9me3, H3K36me3 and H4K20me3 correlated with patient prognosis contributes to esophageal squamous cell carcinoma

Menghan Zhou[1,2], Yiping Li[1], Shaofeng Lin[3,4], Yanyan Qian[1], Zhujiang Zhao[1], Hong Fan[1]
[1]Department of Medical Genetics and Developmental Biology, Medical School of Southeast University, Nanjing; [2]Institute of Life Science, Southeast University, Nanjing; [3]Department of Thoracic Surgery, Jiangsu Cancer Hospital, Nanjing; [4]Department of Oncology, Fujian Provincial Cancer Hospital, Fuzhou, P.R. China
E-mail: mh_zhou@hotmail.com

Esophageal squamous cell carcinoma (ESCC) is characterized by a high mortality rate for unclear diagnoses and delayed therapy. Multiple cancer-related biological processes are associated with histone methylation, which have emerged as transcriptional regulators in a variety of human cancers. In the present study, we evaluated potential roles of four histone lysine trimethylation markers for ESCC prognosis by immunohistochemistry, in ESCC tissue microarrays which contain 135 cases. The results revealed that histone 3 lysine 4 trimethylation (H3K4me3), histone 3 lysine 9 trimethylation (H3K9me3) and histone 4 lysine 20 trimethylation (H4K20me3), but not histone 3 lysine 36 trimethylation (H3K36me3), exhibited stronger immunostaining signals in tumor tissues than in the corresponding adjacent non-tumor tissues. The expression patterns of H3K36me3, H3K9me3 and H4K20me3 correlated with tumor infiltrating depth, lymph node involvement and pTNM stage. Patients with low-scoring H3K9me3 and H4K20me3 and a high level of H3K36me3 were more likely to have a better clinical outcome. We then performed a ChIP-Seq assay of H3K9me3 in ESCC cell line EC9706 and found that some enriched gene segments played a critical role in tumor proliferation and migration. Collectively, H3K9me3, H4K20me3 and H3K36me3 exhibited a close association with clinical features and the combination of these markers is believed to further enhance evaluations of ESCC prognosis and treatment.

235

Apoferritin as a vehicle for anticancer nanodrugs

T. Cerna[1,2], J. Hrabeta[1], Z. Heger[3], S. Dostalova[3], R. Indra[2], V. Adam[3], M. Stiborova[2], T. Eckschlager[1*]
[1]Department of Pediatric Hematology Oncology, 2nd Medical Faculty, Charles University and University Hospital Motol, Prague; [2]Department of Biochemistry, Faculty of Science, Charles University, Prague; [3]Department of Chemistry and Biochemistry, Mendel University and Central European Institute of Technology, Brno, Czech Republic
*E-mail: tomas.eckschlager@lfmotol.cuni.cz

One of the approaches to decrease the adverse effects of anticancer drugs is their encapsulation inside a nanocarrier. This allows for a targeted delivery to tumour tissue, while avoiding the healthy cells. Apoferritin (Apo), the iron-free form of ferritin, can act as a nano-carrier. It can be functionalized with biomolecules e.g., antibodies, enabling the targeting of cancer cells. We constructed Apo with encapsulated doxorubicin (ApoDox), etoposide (ApoVp), ellipticine (ApoElli) and vandetanib (ApoVan). Moreover, ApoDox and ApoDox were modified by antibodies. ApoDox modified with anti-PSMA was tested on prostate cancer cells and non-cancer cells both *in vitro* and *in vivo*. Free Dox was more toxic than ApoDox in both cell lines, but anti-PSMA-modified ApoDox retained the potency of a free drug, but was associated with a lower incidence of side-effects *in vivo*. We also tested ApoElli and ApoVan on neuroblastoma (Nbl) cells, in which Apo is binding on transferrin receptor 1 and SCARA5. ApoVan was more efficient than the free drug and had the same efficiency under hypoxic conditions. Apo nanotransporters particularly modified by antibodies exhibited promising effects due to increasing selectivity and safety. However, prior to their introduction into practice, it is still necessary to solve the many issues.

Financial Support: Supported by grants GACR 17-12816S and 18-10251S.

236

Combined melanoma-targeted therapies to suppress tumor microenvironment cell-mediated processes

Emilia Licarete[1], Valentin Florian Rauca[1], Alina Sesarman[1], Lavinia Luput[1], Laura Patras[1], Manuela Banciu[1]
[1]Faculty of Biology and Geology and Molecular Biology Centre, Institute for Interdisciplinary Research in Bio-Nano-Sciences, Babes-Bolyai University, Cluj-Napoca, Romania
E-mail: manuela.banciu@ubbcluj.ro

Tumor microenvironment (TME) cells can regulate all processes involved in cancer progression, such as angiogenesis, inflammation and oxidative stress. Moreover, these stromal cells form a genetically stable population and therefore, it is less likely to develop resistance to anticancer therapies. Thus, TME cell-targeted therapies may enhance the efficacy of melanoma cell-targeted therapies, when they are administered in combination. Thus, due to the natural tropism of long-circulating liposomes (LCL) for the main key cell players in TME- tumor-associated macrophages (TAMs) (1), we investigated whether protumor processes favorable for tumor development coordinated by TAMs may be significantly affected by LCL-encapsulated anticancer drugs. More specifically, we demonstrated that LCL-incorporated simvastatin (LCL-SIM) exerted potent antitumor activity on B16.F10 melanoma *in vivo* due to the suppression of TAMs-modulated oxidative stress in TME, while another liposomal formulation carrying prednisolone phosphate (LCL-PLP) inhibited the growth of the same tumor via the inhibition of TAM-associated angiogenesis. Therefore, taking advantage of their suppressive actions of TAM protumor activities in the melanoma microenvironment, both liposomal formulations presented above were exploited in combined therapies to enhance the antitumor activity of different melanoma-targeted treatments that inhibit cancer cell proliferation and progression (2). Our data suggested that each TAM-targeted therapy increased the antitumor activity of melanoma cell-targeted treatments and prevented the settlement of cancer cell resistance to the applied therapies.

(1) Alupei et al., Cancer Lett. 356(2 Pt B): 946-952, 2015.
(2) Rauca et al., PLoS One 13(8): e0202827, 2018.

Financial support: PN-III-P4-ID-PCE-2016-0342, contract number 91/2017 within PNCDI-III.

237

Clinical application of telomere length measurements: A challenge for precision medicine

Dimitris Tsoukalas[1,2,3], Gerasimos Tsilimidos[1], Persefoni Fragkiadaki[2], Athanasios Alegakis[2], Evangelia Sarandi[1,2], Eleni Vakonaki[2], Aristidis Tsatsakis[2]
[1]Metabolomic Medicine Clinic, Health Clinics for Autoimmune and Chronic Diseases, Athens, Greece; [2]Laboratory of Toxicology, Medical School, University of Crete, Heraklion, Greece; [3]Clinical Pharmacy and University of Medicine and Pharmacy, Faculty of Pharmacy, Craiova, Romania;
E-mail: research@metabolomicmedicine.com

Chronic diseases are responsible for 70% of global deaths and are mainly caused by modifiable risk factors. In addition, chronic conditions are related to aging, a multifactorial process where modifiable metabolic factors accelerate it. A complementary approach to track aging and the onset of chronic diseases related to aging is the analysis of telomeres, the protective caps of chromosomes (1). Telomeres shorten each time cells divide, and the pace of telomere attrition is a robust marker of aging and aging-related diseases. Leucocyte telomere length measurement has been associated with known metabolic risk markers, such as HDL cholesterol, triglycerides, insulin resistance, adiposity, cardiovascular disease, increased mortality, shorter lifespan and negative health effects in humans (2). We have developed a semi-automated worksheet, BIOTEL, to generate individual and group leukocyte telomere length statistics and provide a crude estimation of biological age (3). In a group of 150 healthy individuals, age and sex were found to affect telomere length and mostly, the length of short telomeres. Ongoing clinical studies from our group will determine the environmental and metabolic factors that accelerate telomere attrition as novel anti-aging targets. Preliminary results revealed that supplementation with nutraceuticals was positively associated with longer telomeres in a study of 47 participants suggesting a potential role in healthy aging (4). The activation of telomerase has been shown to contribute to telomere length maintenance and stability. Thus, modulators stabilizing telomeres and increasing telomerase expression/activity have been proposed as potent anti-aging agents. We have been testing several natural molecules and identified 08AGTL as the most potent telomerase activator reported to date, (up to 10-fold), and future *in vivo* studies on rats and humans will unravel its mode of action. Conclusively, telomeres are potent targets of prediction and treatment of chronic diseases and together with standard methodology can lead to their effective management.

(1) Calado et al. N. Engl. J. Med. 361, 2353-2365, 2012; (2) Rode et. al. J. Natl. Cancer Inst. 107, 1-8 2015; (3) Tsatsakis et al. Front. Genet. 10, 1-8, 2019; (4) Tsoukalas et al. Int. J. Mol. Med. 1-9, 2019.

238

Cues to the development of T cell memory to improve cancer immunotherapies

R.E. Hesterberg[1,2], A. Elmarsafawi[3], P.K. Epling-Burnette[1]
[1]Department of Immunology, Moffitt Cancer Center & Research Institute, Tampa, Florida; [2]Cancer Biology PhD Program, University of South Florida, Tampa, Florida; [3]Morsani College of Medicine, University of South Florida, Tampa, Florida, USA

Understanding the adaptation of $CD8^+$ T-cells to the altered nutrient availability is an area of general importance to immunology. Glutamine is known to play a critical role in T-cell proliferation, cytokine production and metabolism; thus, limitations in glutamine availability may possibly contribute to intratumoral immune suppression. Metabolic flux studies from our group have demonstrated that the catabolism of glutamine, rather than arginine, contributes to the production of ornithine, which is then converted by enzyme ornithine decarboxylase (ODC) into putrescine, followed by spermidine, and spermine. Herein, we demonstrate that glutamine-derived polyamines control a critical decision by $CD8^+$ T-cells to undergo cellular differentiation into an effector versus memory fate. Moreover, the decision point controlled by polyamines occurs early after activation and reprograms the cells for future functional processes. Limitations to the clinical success of emerging cancer therapies, such as chimeric antigen receptor (CAR) T-cells (CAR-T) for B-cell precursor acute lymphoblastic leukemia and B-cell lymphomas and tumor infiltrating lymphocyte (TIL) therapy for melanoma and other solid tumors rest largely with poor persistence. Using mouse models, our studies revealed that ODC blockade with α-difluoromethylornithine (DFMO) generates $CD8^+$ T-cell with superior oxidative metabolism and improved antigenic recall response following vaccination *in vivo*. Understanding how the polyamine circuit directs effector and memory fate decisions by $CD8^+$ T-cells will have important implications in tumor immune surveillance and autoimmunity. Changing the longevity of human $CD8^+$ T cells is critical for improving adoptive T cell immunotherapies for cancer.

239

New compounds inhibiting trypanothione reductase: An attractive target to develop drugs against Human African Trypanosomiasis

Theo Battista[2,3], Lorenzo Turcano[1], Esther Torrente[1], Antonino Missineo[1], Matteo Andreini[1], Steven Harper[1], Alberto Bresciani[1], Annarita Fiorillo[2,3], Gianni Colotti[3], Andrea Ilari[3]
[1]IRBM Science Park S.p.A., Pomezia; [2]Dipartimento di Scienze Biochimiche, "Sapienza" Università di Roma, Roma; [3]Istituto di Biologia e Patologia Molecolari (IBPM)-CNR, Roma, Italia
E-mail: theo.battista@uniroma1.it

Trypanothione reductase (TR) is a key enzyme that catalyses the reduction of trypanothione (TS_2), a glutathione-spermidine conjugate that protects Trypanosomatids (*Leishmania*, *Trypanosoma cruzi* and *Trypanosoma brucei*) from oxidative stress induced by mammalian host defence systems. TR is considered an attractive target for the development of novel anti-parasitic agents, as it is essential for parasite survival, but has no close homologue in man. The trypanothione binding site resides in a large cavity at the interface between the two monomers, formed by the residues of the FAD binding domain of one subunit and those of the interface domain of the other. The reduction of trypanothione takes place via a mechanism in which two electrons are transferred from NADPH via FAD to the Cys52-Cys57 disulfide bridge and then to TS_2. Our research group identified several organic compounds able to inhibit TR from *Leishmania* by competing with TS_2 for its binding site: Azole-based compounds, diaryl sulfide compounds, chalcone-based compounds and carboxamide compounds. Recently, we identified, by screening an in-house library containing 120,000 compounds and setting up a new assay in which NADPH oxidation was coupled to a luminescence assay, new inhibitors of trypanothione reductase from *Leshmania infantum*. Some of these compounds were also able to inhibit TR from *Trypanosoma brucei* (*Tb*TR), the parasite responsible for Human African Trypanosomiasis (HAT), a neglected tropical disease. In particular, compound1 from this series was found to impede the growth of *Trypanosoma brucei* parasites in cell cultures. The X-ray crystal structure of *Tb*TR in complex with compound1 allowed the identification of the hydrophobic pocket where the inhibitor bind, placed close to the catalytic Histidine (His 461') and lined by Trp21, Val53, Ile106, Tyr110, Met113. Since the binding site of this new inhibitor is unique, and is not present in human homologs, such as glutathione reductase (hGR), it represents a novel target for drug discovery efforts.

240

New agents to challenge drug resistance in cancer

Mariafrancesca Hyeraci[1], Maria Pia Rigobello[2], Simona Samaritani[3], Lisa Dalla Via[1]
[1]Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Italy; [2]Department of Molecular Medicine, University of Padova, Italy; [3]Department of Chemistry and Industrial Chemistry, University of Pisa, Italy
E-mail: lisa.dallavia@unipd.it

Cisplatin represents a cornerstone for the chemotherapeutic treatment of a number of types of solid cancer. Moreover, it is used in combination regiments for the treatment of ovarian, bladder, lung and head and neck cancer (1). Nevertheless, the therapeutic outcome of cisplatin chemotherapy can be impaired by two major drawbacks: Dose-limiting side-effects, mainly neurotoxicity and nephrotoxicity, and acquired or intrinsic resistance. Platinum-based drug resistance is the consequence of multifactorial events, including decreased drug accumulation, by either active efflux/sequestration/secretion or impaired uptake, detoxification of platinum species by GSH conjugates, metallothioneins and other antioxidants, enhanced repair of DNA damage and inhibition of apoptosis (2). The circumvention of cisplatin resistance constitutes an important task for the researchers and considerable efforts have been undertaken over the past years, to tackle this unsolved problem. Herein, we report the biological profile of some new metal-based agents that may be taken into consideration to exploit new strategies for overcoming cisplatin resistance in tumors. In particular, the cytotoxicity on both sensitive and resistant human tumor cell lines was studied along with the ability to be accumulated inside cells. Furthermore, to elucidate the molecular mechanisms of action, the effects on different intracellular targets were investigated, in comparison to those of cisplatin. Finally, a correlation between the cell effects and the resistance phenomenon was attempted.

(1) Kelland, Nat Rev Cancer 7: 573-584, 2007.
(2) Shen et al., Pharmacol Rev 64: 706-721, 2012.

241

Methylation profiling of genes involved in inflammation and immune response in melanoma

Saverio Candido[1], Rossella Salemi[1], Luca Falzone[1], Giuseppe Gattuso[1] Silvia Vivarelli[1], Massimo Libra[1]
[1]Department of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy
E-mail: luca.falzone@unict.it

Chronic inflammation is a long-term process that substantially contributes to the cancer development. Conversely, cancer cells are able to sustain the inflammatory microenvironment that plays a crucial role in cancer progression, inducing tumor cell proliferation, invasion and metastasis. Several inflammatory and immunity factors may suppress the activity of innate and adaptive immune cells that could be normally capable to recognize and kill the tumor cells. Over the past years, targeting tumor-related immunosuppressive factors represents the most promising challenge of cancer treatment. However, in spite of the introduction of several immune-checkpoint inhibitors into clinical practice, numerous patients still succumb to cancer, due to disease progression, including those with cutaneous melanoma. The failure of therapy may be associated with the deregulation of cancer genes involved in several immunological functions. In turn, such a deregulation may be mediated by epigenetics mechanisms of which DNA methylation plays an important role. On this basis, we performed computational analysis using our EpiMethEx R package (R Project) in order to identify specific methylation patterns of melanoma tumor cell associated with tumor-related inflammation and immunosuppression. EpiMethEx is able to perform cyclic correlation analysis between gene expression and methylation levels of each gene obtained from omics databases. Notably, EpiMethEx stratifies the methylation probesets according to their relative gene positions (from gene promoter to untranslated regions and CpG Islands) in order to retrieve information about the methylation status of extended genomic region. The Cancer Genome Atlas (TCGA) Cutaneous Melanoma datasets were used to obtain a set of modulated genes highly associated with methylation events that may be involved in melanoma-related inflammation and immunotherapeutic response. To this aim, the EpiMethEx data were filtered according to a list of genes related to inflammation and immunity obtained from recent literature revision. The results indicated that 102 of 693 (15%) inflammation genes may be regulated by at least one methylation hotspot within the gene sequence. Furthermore, only 89 genes (13%) showed DNA methylation extended to large gene regions particularly in promoter. Finally, only 14 genes showed global gene methylation. Notably, 6 of 13 major Human Leukocyte Antigen (HLA) genes, involved in immune recognition of cancer cells, showed expression levels highly correlated with their DNA methylation patterns. This analysis allows the identification of putative methylation biomarkers involved in immune tumor microenvironment useful to predict therapeutic response and prognosis of melanoma patients. Validation studies should be performed in melanoma samples in order to evaluate the predictive values of selected methylation hotspots.

242

Identification of pro-inflammatory microRNAs as diagnostic and prognostic biomarkers for oral cancer

Luca Falzone[1], Silvia Vivarelli[1], Rossella Salemi[1], Saverio Candido[1,2], Massimo Libra[1,2]
[1]Department of Biomedical and Biotechnological Sciences; [2]Research Center for Prevention, Diagnosis and Treatment of Cancer, University of Catania, Catania, Italy
E-mail: luca.falzone@unict.it

Oral cancer represents one of the most diagnosed and invalidating tumors worldwide. Several risk factors have been recognized for this pathology; however, despite the promotion of screening strategies, oral lesions are often not correctly diagnosed as tumors. Moreover, several studies have tried to identify new diagnostic and prognostic biomarkers for oral cancer without obtaining good levels of sensitivity and specificity. On these bases, there is an urgent need for the identification of novel biomarkers for oral cancers. For this purpose, in the present study, we aimed to identify the microRNAs (miRNAs or miRs) involved in inflammatory processes and in turn, in the development and progression of oral cancers by analyzing the molecular data contained in the GEO DataSets and TCGA miRNA profiling datasets. Differential analyses were performed between the miRNA expression levels observed in tumor and normal samples. Furthermore, several bioinformatics and prediction tools were used to establish the functional roles of these miRNAs, particularly regarding their involvement in the inflammatory and tumor pathways. The results of this study allowed us to identify a set of miRNAs strictly related to the presence of oral cancers and therefore, used as diagnostic biomarkers for this pathology. Furthermore, some of these miRNAs were also associated to the prognosis of patients. Overall, the integrated computational analysis herein proposed allowed for the identification of miRNAs with diagnostic and prognostic significance for oral cancer that need to be validated in liquid biopsy samples before being used as biomarkers in clinical practice.

Acknowledgements: This study was supported in part by the Lega Italiana per la Lotta contro i Tumori (LILT).

243

Inverse microRNA expression patterns in glioblastoma multiforme and Alzheimer's disease

Luca Falzone[1], Manuela Pennisi[1], Silvia Vivarelli[1], Rossella Salemi[1], Sofia Basile[1], Saverio Candido[1]
[1]Department of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy
E-mail: luca.falzone@unict.it

An inverse association between glioblastoma multiforme (GBM) and Alzheimer's disease (AD) has been already demonstrated. By contrast, the molecular mechanisms associated with their development are very similar, including their common association with an inflammatory pattern. However, the role of miRNA expression in both GBM and AD is still debated. The aim of the present study was to identify a set of miRNAs significantly de-regulated in both GBM and AD, and hence to define whether the identified miRNAs exhibit an inverse correlation within the two pathologies. For this purpose, miRNA expression profiling datasets derived from GEO DataSets and relative to GBM and AD were used. Once the miRNAs significantly de-regulated in both pathologies were identified, DIANA-mirPath pathway prediction analysis and STRING Gene Ontology enrichment analysis were performed to establish their functional roles in the onset of each pathology. The results allowed for the identification of a set of miRNAs found de-regulated in both GBM and AD, whose expression levels were inversely associated in the two pathologies. In particular, the strong negative association observed between the miRNA expression level in GBM compared to AD suggests that although the molecular pathways beyond the development of the two pathologies are the same, they appear to be inversely regulated by miRNAs. Although the identification of this miRNA dataset can be further used for diagnostic, prognostic and therapeutic purposes, further functional *in vitro* and *in vivo* evaluation is required in order to validate the diagnostic and therapeutic potential of the identified miRNAs, as well as their involvement in the development of GBM and AD.

244

Anti-proliferative effects of cell-free supernatants from *Lactobacillus rhamnosus* GG in cancer cells

Rossella Salemi[1], Saverio Candido[1,2], Luca Falzone[1], Silvia Vivarelli[1], Maria Santagati[1], Massimo Libra[1,2]
[1]Department of Biomedical and Biotechnological Sciences; [2]Research Center for Prevention, Diagnosis and Treatment of Cancer, University of Catania, Catania, Italy
E-mail: rossellasalemi@alice.it

Due to its anti-inflammatory properties, *Lactobacillus rhamnosus* GG (LGG) is one of the most studied and well characterized among probiotics. Several studies demonstrated that probiotics may exert a protective anti-tumor effect, and in particular, in colorectal carcinogenesis. However, the mechanisms through which probiotics, including LGG, may prevent colon cancer (CRC) remain debated. On the one hand, it has been hypothesized that probiotics may prevent CRC development through the regulation of cell proliferation and apoptosis; on the other hand, it has been shown that LGG may play a role in the reduction of proliferation and metastasis. Nevertheless, the biological effects of LGG metabolic products on tumor growth remain to be deeply elucidated. On these bases, the potential role of cell-free supernatants (CFS), obtained from LGG (CFS-LGG), in the control of the viability of cultured human colorectal carcinoma and melanoma cells was investigated. To this aim, Caco-2, HT-29, HCT-116 and A375 cells were treated with CFS-LGG or with unconditioned cell medium. A significant reduction in cell viability was observed following treatment with CFS-LGG in the tumor cells in a dose-dependent manner when compared to the untreated cells. Notably, our results further demonstrated that such a cell growth reduction was linked to a cell cycle block at G2/M and not to apoptosis. Finally, combined treatment with both CFS-LGG and chemotherapeutic compounds led to a significant reduction in tumor proliferation when compared to treatment with the antitumor agents alone. Overall, the results of the present study indicate that the CFS-LGG may include active molecules with anticancer potential.

245

Incidence of venous thromboembolism (VTE) in patients with multiple myeloma (MM): A monoinstitutional experience

Salvatore Santo Signorelli[1]
[1]Department of Clinical and Experimental Medicine, University of Catania, Catania, Italy
E-mail: ssignore@unict.it

Venous thromboembolism (VTE) may be diagnosed in cancer patients, including those with haematological disorders. The release of inflammatory cytokines, the upregulation of pro-thrombotic mediators and the downregulation of antithrombotic factors play a crucial role in the emerging risk of VTE haematological malignancies, such as multiple myeloma (MM). Additionally, current therapeutic strategies for MM may be associated with pro-thrombotic conditions leading to VTE. On these bases, to better understand the risk factors of VTE in MM patients, in the present study, we investigated the prevalence of VTE in 105 MM patients treated with different therapeutic regimens, including Pomalidomide (PomaD), Carfilzomib, Lenalidomide and Desamethasone (KRd), Daratumumab (Dara), Elotuzumab and Lenalidomide (EloRd) in a single institution. Finally, the efficacy of VTE prophylaxis in MM patients was analysed based on different therapeutic protocols administration. The results showed a very low VTE incidence in MM patients treated with anti-MM novel drugs. A positive thromboprophylaxis effect in preventing VTE in MM patients prescribed anti-MM novel drugs was also observed. Altough the incidence of VTE in MM patients is very low, our data strongly support the notion that ASA prophylaxis remains a good option to prevent VTE in a subset of MM patients.

246

Role of the transcription factor YY1 in colorectal cancer

Silvia Vivarelli[1], Luca Falzone[1], Saverio Candido[1], Massimo Libra[1]
[1]Department of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy
E-mail: silvia.vivarelli7@gmail.com

Colorectal cancer (CRC) is the third most diffused malignancy worldwide. The formation of CRC is a multistep process which may arise from the accumulation of genetic and epigenetic alterations, including specific molecular alterations with functional effects on signaling pathways modulating cell survival. The transcription factor Yin Yang 1 (YY1), found differentially expressed in a number of cancer types, has been found to either stimulate or inhibit cancer growth, although the mechanisms responsible for such diverging effects need to be further elucidated. In the present study, we aimed to evaluate whether YY1 plays a role in modulating the efficacy of chemotherapy in CRC and its effects on the apoptotic pathway. On this ground, YY1-silenced colon cancer cell lines were generated and their survival rates were compared with those of parental wild-type ones. YY1 silencing in HT-29 and SW620 colon cancer cell lines conferred resistance to chemotherapy. In line with this result, cleaved caspases were less produced in YY1-silenced clones compared with their parental cells. Finally, the performed *in silico* analysis suggested the presence of several YY1 putative binding sites located on the promoters of potential target genes belonging to apoptotic, as well as to metastasis regulatory pathways. On these bases, the regulatory role of YY1 on potential direct targets involved in such resistance mechanism will be further analyzed. The validation of YY1 interaction with the promoter of a direct target may have important implications for the management of CRC patients.

247

Implication of the transcription factor YY1 in non-Hodgkin's lymphoma

Silvia Vivarelli[1], Luca Falzone[1], Saverio Candido[1], Massimo Libra[1]
[1]Department of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy
E-mail: silvia.vivarelli7@gmail.com

Non-Hodgkin's lymphomas (NHLs) are a heterogeneous group of lymphoproliferative malignancies exhibiting a variable response to treatments, depending on their nature, grade and stage. Although effective therapeutic approaches have been developed, there is still the need to reduce the frequency of NHL refractory to anti-cancer therapy. On this ground, the objective of this study was to explore the potential involvement of the transcription factor Yin Yang 1 (YY1) in the development, as well as in the prognosis of NHL. YY1 has been found mainly overexpressed in NHL, although its prognostic role remains unclear. YY1 plays an essential role in all stages of B-cell differentiation and a previous study performed in our laboratory highlighted that YY1 may be useful as a biomarker of NHL transformation, as well as a potential target for therapeutic interventions. Towards the use of up-to-date bioinformatics approaches, a novel *in silico* study on NHL patient datasets was performed, in order to uncover the YY1 correlation with the expression of different genes involved in the regulation of apoptosis. The results strongly highlighted the potential direct involvement of YY1 in apoptotic Bcl2-family genes modulation. Further *in vitro* validation was performed through the generation of YY1-silenced NHL cell lines. The effects of YY1 silencing on cellular growth following anti-cancer treatments were examined. Although consistency between the *in silico* analyses and the *in vitro* validation corroborated the potential role of YY1 in NHL development, some arisen discrepancy may be explained by the diverse complexity of the two approaches used.

248

Telomere maintenance regulated by a cell cycle-controlled mechanism: From bench to clinic

Maria Thanasoula[1], Dimitris Tsoukalas[1,2,3], Evangelia Sarandi[1,2], Anca Docea[4], Maria A. Buga[4], Aristidis Tsatsakis[2]
[1]Metabolomic Medicine Clinic, Health Clinics for Autoimmune and Chronic Diseases, Athens, Greece; [2]Laboratory of Toxicology, Medical School, University of Crete, Heraklion, Greece; [3]Clinical Pharmacy and [4]Toxicology, University of Medicine and Pharmacy, Faculty of Pharmacy, Craiova, Romania
E-mail: mariathanasoula84@gmail.com

Telomeres are protected by capping structures consisting of core protein complexes, such as the shelterin complex composed of TRF1, TRF2, POT1, Rap1, TPP1 and TIN2. Following their replication and elongation in the S-phase, telomeres become transiently uncapped in G2 and are sensed as DNA damage. This triggers the DNA damage response (DDR), which includes accumulation of DDR proteins at the telomere. Herein, we investigated whether this telomere capping process is controlled by the cell cycle surveillance machinery. Our results indicated that telomere capping is monitored at the G2/M transition by 2 independent DDR pathways both activated in the presence of dysfunctional telomeres. These initiate from the activation of 2 main DDR kinases, ATM and ATR which in turn activate either the p53/p21-dependent DDR (1), or phosphorylate CHK1 and CHK2 kinases (2). Both pathways lead to G2/M arrest preventing mitotic entry of cells with persisting uncapped and short telomeres that often result in end-to-end fusions and genomic instability. In separate studies, we found that TRF1 and TPP1 are important for telomere capping, telomerase binding and function at the telomeres and chromosomal stability by preventing telomeric fusions and chromosome breaks (3,4), while Rap1 was found to play a role in telomere integrity and length, as well as in transcriptional regulation (5). Our current aim is to integrate this knowledge about telomere stability and maintenance into human clinical studies on anti-aging and health improvement.

(1) Thanasoula et al., Curr Biol, 23;20: 521-526, 2010.
(2) Thanasoula et al., EMBO J, 31: 3398-3410, 2012.
(3) Martínez, Thanasoula et al. Genes Dev., 23: 2060-2075, 2009.
(4) Tejera et al., Dev Cell, 18: 775-789, 2010.
(5) Martinez, Thanasoula et al., Nat Cell Biol, 12: 768-780, 2010.

249

Metabolomics application in precision medicine: Prediction and treatment of chronic diseases

Evangelia Sarandi[1,2], Dimitris Tsoukalas[1,2,3], Gerasimos Tsilimidos[1], Daniela Calina[3], Aristidis Tsatsakis[2,3]
[1]Metabolomic Medicine Clinic, Health Clinics for Autoimmune and Chronic Diseases, Athens; [2]Laboratory of Toxicology, Medical School, University of Crete, Heraklion, Greece; [3]University of Medicine and Pharmacy, Faculty of Pharmacy, Craiova, Romania
E-mail: esarandi6@hotmail.com

Metabolomics, a novel and powerful tool of precision medicine allows fort he discrimination of cellular metabolic alterations as a result of environmental factors and specific genetic background. Thus, the analysis of metabolites provides a detailed overview of the phenotype through precise data of nutritional deficiencies, metabolic imbalances, environmental toxins, microbiome status, and uncovers underlying genetic predispositions that can be modified through diet, lifestyle, supplements or medications (1,2). Critical signs of systemic dysfunction at the molecular level can be revealed years before clinical symptoms appear. We have demonstrated that targeted metabolomic analysis in patients with atopic dermatitis and asthma can show significant biochemical disruptions at the TCA cycle and fatty acids metabolism. Significant correlations were found between 5 urinary organic acids and pulmonary function markers (3). Personalized intervention with diet and nutritional supplements according to the metabolomic test results reduced the skin lesions and lung inflammation, respectively, within a few weeks from the start of treatment in most cases (4). Of note, the intake of 150 g of cooked fish twice a week for 6 months reduced the eNO marker of bronchial inflammation by 14 units in patients with asthma. Our overall aim is to demonstrate the predictive value of key metabolites in chronic diseases, and a methodology to target their metabolic causes. Ongoing clinical studies will define the metabolic fingerprint of rheumatoid arthritis, Hashimoto's thyroiditis, psoriasis, Crohn's diseases and ulcerative colitis and investigate the role of nutritional interevention to the disease progression.

(1) Catrina et al Nat. Rev. Rheum. 13, 79-86, 2017.
(2) Tsoukalas et al. IJMM 43, 233-242 2019.
(3) Papamichael et al. Nutr. Res. 61, 31-40, 2018.
(4) Papamichael et al. J Hum Nutr Diet 24, 184, 2018.

250

A new scaffold for selective inhibition of human monoamine oxidase B

Maria Luisa Di Paolo[1], Michael S. Christodoulou[2], Alessandra M. Calogero[3], Luca Pinzi[4], Giulio Rastelli[4], Daniele Passarella[5], Graziella Cappelletti[3], Lisa Dalla Via[6]
[1]Dipartimento di Medicina Molecolare, Università di Padova; [2]DISFARM, Sezione di Chimica Generale e Organica "A. Marchesini", Università di Milano, Milano; [3]Dipartimento di Bioscienze, Università di Milano, Milano; [4]Dipartimento di Scienze della Vita, Università di Modena e Reggio Emilia, Modena; [5]Dipartimento di Chimica, Università di Milano, Milano; [6]Dipartimento di Scienze del Farmaco, Università di Padova, Padova, Italy
E-mail: marialuisa.dipaolo@unipd.it

Monoamine oxidases (MAOs) are mitochondrial FAD-containing enzymes that catalyze the oxidative deamination of biogenic amine neurotransmitters and xenobiotic amines. Being involved in the catabolism of neurotransmitters, MAOs are well-known and constitute attractive pharmacological targets in various neurological, psychiatric and neurodegenerative diseases, such as Parkinson's disease (PD) and Alzheimer's disease (AD)[(1,2)]. As the ideal drug has not been achieved so far, researchers continue to explore this field[(3)], in particular in search for reversible MAO B inhibitors. In the frame of a screening campaign of a huge library of natural and synthetic compounds, 2-phenyloxazole emerged as a potential scaffold and a library of 12 derivatives was prepared. Most of the compounds were found to act as competitive inhibitors of MAOs: compounds **4a**, **4g** and **4m** were found to be the most potent inhibitors (K_i = 0.5-1 μM), with a good selectivity toward MAO B ($K_{iMAO\text{-}A}/K_{iMAO\text{-}B}$>45). Molecular docking analysis allowed rationalizing the experimentally observed binding affinity and selectivity. Compound **4a** was also able to inhibit MAO activity in NGF-differentiated PC12 cells. Our results indicate that **4a** may be considered a promising scaffold for the design of novel effective and selective MAO-B inhibitors, with potential pharmacological applications.

(1) Youdim et al., Nat Rev Neurosci 7: 295-309, 2006.
(2) Fisar, Prog Neuropsychopharmacol Biol Psych 69: 112-124, 2016. (3) Carradori et al., Exp Op Ther Pat 28: 211-226, 2018.

251

RSV bronchiolitis and paediatric asthma

Paraskevi Korovessi
Paediatric Department, "Penteli" Children's Hospital, Penteli, Greece

Bronchiolitis is most often described as a virus-induced inflammation of small bronchioles and their surrounding tissue, in infants and young children, with Respiratory syncytial virus (RSV) being the most common cause. Recent data indicate rhinovirus (RV) as the second most common viral agent, mainly after the age of 12 months, having distinct genetics, pathogenetic mechanisms, clinical characteristics, and responses to treatment compared to RSV. There is compelling evidence that infants in their early months of life, with severe RSV infection, have a subsequent increased risk of developing recurrent wheezing and asthma, with a prevalence of up to 30% compared with non-RSV groups. This significant increase in asthma frequency seems to be predominantly related to long-term changes in neuroimmune control of airway tone rather than to allergic sensitization. RSV possesses the ability to counteract host defense systems through complex mechanisms that facilitate viral replication. Large epidemiologic, observational studies demonstrated that the vast majority of infants hospitalized for RSV bronchiolitis does not constitute an "at-risk" group (atopy, family history), suggesting that viral or host factors, not included in the classical risk factors, may be accountable for disease severity and play a possible role. To address the potential causality between RSV infection and subsequent asthma, prospective studies with RSV-immunoprophylaxis have also been performed, suggesting that long-term effects of RSV prophylaxis appear less likely in infants with atopic family history and that Palivizumab decreased the parent's reported recurrent wheeze, but the incidence of physician-diagnosed asthma at childhood was found similar. Further prospective, follow-up studies are needed to clarify the risk factors and long-term respiratory outcome of children hospitalized for severe RSV in order to elucidate the pathophysiological mechanisms through which RSV causes recurrent wheezing/asthma and consequently plan an evidence-based prevention strategy.

252

Post-infectious bronchiolitis obliterans caused by respiratory syncytial virus in children

Gregory Grivas[1], Christina Lymperatou[1], Paraskevi Korovessi[2]
[1]Paediatric Intensive Care Unit (PICU), "Penteli" Children's Hospital, Penteli, Greece; [2]Paediatric Department, "Penteli" Children's Hospital, Penteli, Greece

Bronchiolitis obliterans (BO) is a chronic and irreversible obstructive lung disease leading to the obstruction and/or obliteration of the small airways. Three main BO entities are distinguished: post-infectious BO (PIBO); BO post-lung transplantation; and BO after bone marrow transplantation (BMT) or hematopoietic stem cell transplantation (HSCT). Most BO in children is post infectious. PIBO is mainly associated with adenovirus infections, although other viruses, such as measles, influenza, parainfluenza, and respiratory syncytial virus (RSV), may also be implicated. The aim of this presentation is to perform a review of the literature to evaluate PIBO caused by RSV infection. After searching the pubmed database, we included 10 manuscripts. Reviewing three case series from China, it was found that RSV was the cause (single/co-infection) of PIBO in 4.3%, 6.2% and 15.4%, retrospectively. In two case series from the Iberian Peninsula, PIBO was attributed to RSV in 4.5 % and 30%, while in a case series from Turkey, RSV was identified in 15%. In two case reports, one from Brazil and one from Australia, PIBO was the result of co-infection with RSV and adenovirus. In a case report from Japan, a child underwent lung volume reduction surgery (LVRS) after the development of severe emphysema due to bronchiolitis obliterans caused by RSV. In a case report from France, a toddler with RSV-induced PIBO developed severe chronic respiratory failure and was in need of chronic non-invasive mechanical ventilation. Although RSV is the agent most often associated with acute viral bronchiolitis, there are few reports in the literature concerning RSV as the cause of PIBO. However, in some case series, this virus has been detected in up to 30% of patients with PIBO. Additionally, there is growing evidence - although still controversial - suggesting greater severity and worse outcomes in children with mixed compared to single respiratory virus infections. The occurrence of simultaneous infection by adenovirus and RSV has been associated with worst outcomes.

253

Overlap between RSV, influenza viruses and human metapneumovirus in childhood

Ioannis N. Mammas, Demetrios A. Spandidos
Department of Clinical Virology, School of Medicine, University of Crete, Heraklion, Greece

Both children and adults are at risk of seasonal influenza-like illness and upper and lower respiratory tract infections (RTIs) caused by respiratory viruses, such as influenza viruses, human metapneumovirus (hMPV) and respiratory syncytial virus (RSV), which represent leading causes of morbidity and mortality, worldwide. Influenza and RSV infection have classically been characterized as diseases that result in hospitalization at the opposite ends of life, the former in old ages and the latter in infancy. Although it has been clear that infections with both viruses occur throughout life, recent advances in viral diagnostic techniques for both diseases have expanded the range of illnesses recognised as being caused by these viruses and have blurred the distinction between the more non-severe presentations of both infections. RSV infection has been associated with morbidity and mortality comparable to influenza. Influenza viruses and RSV have varying degree of seasonal overlap. hMPV, discovered in 2001, has been demonstrated as the aetiologic agent of a substantial proportion of upper and lower RTIs across all age groups in both healthy and immunocompromised hosts throughout the world. Co-infections with RSV and influenza viruses or hMPV are frequently observed in young children. More sensitive and specific diagnostic tests that establish the cause of LRTIs in children have the potential to reduce overall antibiotic use. In addition, rapid identification of viral infections can help infection control and nosocomial transmission. Vaccination has been proven a protective factor against hospitalizations due to influenza; there is currently no vaccination against RSV and hMPV in clinical practice.

254

The neonatal immune response to RSV infection: Advances in our understanding of viral and host cellular interactions

Eri Koutsounaki[1,2]
[1]Neonatal Department, "Alexandra" Maternity Hospital, Athens, Greece; [2]Neonatal Department, "Helena Venizelou" Maternity Hospital, Athens, Greece

Neonatal susceptibility to respiratory syncytial virus (RSV) is highly related to recurrent hospitalization during the first year of life of premature neonates, to wheezing exacerbations of infancy and asthma of childhood, as well as increased mortality in the early years of life, paraticularly in developing settings. Maternal antibody can reduce the burden of RSV infection in infants. Transplacental transport of specific antibodies, more massively during the third trimester of pregnancy, is considered to provide with adequate titters for up to the fourth month of age the term neonate. Passive immunity is considered as compromised, but still significant in premies. The degree to which breast feeding also contributes to passive immunity and to immune system education is currently under study. Once infection is established, the innate immune response plays its bivalent role in viral load elimination and in priming the secondary response. Premature or compromised immune response produces reduced levels of antiviral cytokines, such as interferons. In infants, reduced signaling from TLRs and altered antigen presenting cell function, including low IL-12 and enhanced production of IL-6 and IL-23, coupled with a reduced activation of regulatory T cells, may result in an adaptive response that is skewed toward Th2 and Th17 and away from protective Th1 and CTL. Impaired Tfh activation, coupled with little or no B cell memory and inhibition of antibody production by IFNγ, produces low titter, low affinity antibody. The result may be a poorly protective and dysregulated immune response that leads to bronchiolitis in susceptible infants. Thus, growing host defense mechanisms and bilateral allergic sensitization may confer to respiratory exacerbations triggered by RSV particles, disproportional to the viral load itself.

255

Stress and Viral Infections

George P. Chrousos
First Department of Paediatrics, 'Aghia Sophia' Children's Hospital, University of Athens School of Medicine, Athens, Greece

Stress is the disturbance of homeostasis of an organism in response to a negative stimulus, the stressor. When such as stimulus exceeds a certain threshold, a mammalian organism responds to adapt and reinstall homeostasis. Part of the adaptive response is the activation of the neuroendocrine stress system, i.e., the hypothalamic-pituitary-adrenal axis and the locus caeruleus/norepinephrine system and its sympathetic and parasympathetic limbs. Generally, stress triggers acutely an early systemic inflammatory response called 'neurogenic inflammation'. Soon after, however, stress suppresses innate immunity and causes a shift from T-helper 1- to T-helper 2- and T-helper 17- and T-reg-driven immunity, while it subdues the antigen-recognition process and the inflammatory reaction. The glucocorticoids, the catecholamines norepinephrine and epinephrine and peripheral corticotropin-releasing hormone represent key hormones involved in the regulation of immunity and inflammation. A child with a viral infection has an activated immune and inflammatory response, i.e., the so-called 'sickness syndrome', via the classic pro-inflammatory cytokines TNF-alpha, interleukin-1, intereukin-6, etc., followed by an activated 'stress syndrome' via the hypothalamic-pituitary-adrenal axis and the autonomic nervous system. The natural history of the response to a viral infection is for immune and stress changes to take place in a highly coordinated relatively brief process that results in full return to the basal health state. Chronicity of stress or inflammation may be detrimental to an organism and/or may make it vulnerable to viral illnesses, such as those caused by common cold viruses. On the other hand, viruses may interact with the host endocrine signaling pathways. We have concrete evidence for some of them. For instance, HIV-1 has two accessory proteins, Vpr and Tat, which interact with the glucocorticoid and PPAR gamma signaling pathways, causing, respectively, glucocorticoid hypersensitivity and PPAR-gamma resistance. The former participates in the immunosuppression of the host by HIV-1, while the latter in the insulin resistance and lipodystrophy of the infected individual. We have other examples in Nature, such as the obesity of chicken infected with an adenovirus or the still unidentified virus or viruses responsible for the rare transient glucocorticoid hypersensitivity syndrome. We are only cognizant of the very tip of the iceberg regarding viral elements interfering in our signaling systems.

256

TGF-β1 pathway de-regulation in hypertrophic scar fibroblast cells - *in vitro*

Harun Muayad Said
Department of Molecular Medicine, Graduate School of Health Sciences, Dokuz Eylul University, Izmir, Turkey
E-mail: harun.m.said@gmail.com

Hypertrophic scars of the skin as a human fibro-proliferative disorder is characterized by an increased contractility and excess of extracellular matrix synthesis. Transforming growth factor (TGF)-β1 plays a beneficial role in wound healing despite the fact that the chronic stimulation of it leads to fibrosis. The downregulation/inhibition of the TGF-β1 pathway can control this fibro-proliferative disorder. In this study, intracellular TGF-β1 signaling in fibroblasts derived from hypertrophic scars and normal skin were examined. Analyses were carried out by contraction assay on collagen gels, with further gene expression analysis by both western blot and northern blot analyses of the extracellular matrix genes, as well as members of the TGF-β1 pathway. The ectopic expression of Smad7 or dominant-negative Smads3/4 completely inhibited contractility of scar-derived and normal fibroblasts after suspension in collagen gels. Constitutive Smad2/3 phosphorylation, as well as phosphorylation of Smad3 appeared in both cell types with the fact of being predominant in hypertrophic scar-derived fibroblasts. Smad7 expression inhibited α1(I) collagen and α-smooth muscle actin expression. Primary cultivated hypertrophic scar-derived fibroblasts were derived from human tissue. Soluble TGF-β1 receptor can be inhibited. In conclusion, autocrine TGF-β1/Smad signaling is involved in the contractility and fibroblasts matrix gene expression in both of normal and hypertrophic scars. The negative feedback loop of Smad7 as a member of the TGF-β Family can inhibit these processes.

257

Targeting of the epigenetic integrator UHRF1 in cancer cells by natural products

Mahmoud Alhosin[1,2]
[1]Biochemistry Department, Faculty of Science, King Abdulaziz University; [2]Cancer Metabolism and Epigenetic Unit, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia
E-mail: malhaseen@kau.edu.sa

The epigenetic silencing of tumor suppressor genes (TSGs) is a common characteristic in human cancer cells and is considered one of the main mechanisms involved in the regulation of TSGs. This process is mainly ensured through a coordinated dialogue between DNA methylation and histone posttranslational modifications such as acetylation and methylation. In cancer cells, promoters of several key TSGs are hypermethylated by DNA methyltransferase 1 (DNMT1), and histone proteins are deacetylated by histone deacetylase 1 (HDAC1) enzymes and the consequence is the inhibition of these TSGs with subsequent defects in apoptosis. Therefore, several drugs have been developed which act as inhibitors of DNMT and HDACs, leading to the re-expression of TSGs. Many, if not all, human cancers including leukemia, breast, bladder, gastric, colorectal and astrocytoma express high levels of the **U**biquitin-like, with **PH**D and **R**ING **F**inger domains **1** (UHRF1) (1,2). UHRF1 has five functional domains: A ubiquitin-like domain (UBL), a tandem Tudor domain (TTD), a plant homeodomain (PHD), an SET- and RING-associated (SRA) domain, and a really interesting new gene (RING) domain (2). UHRF1 uses its domains to fulfil multiple important roles. UHRF1 is characterized by a SRA domain which is found only in the UHRF family. Through its SRA domain, UHRF1 constitutes a complex with HDAC1 and DNMT1 and represses the expression of several TSGs including $p16^{INK4A}$, hMLH1 and BRCA1 (1,2). UHRF1 is one of a macro-molecular protein complex called 'ECREM' for 'Epigenetic Code Replication Machinery', which would be able to duplicate the epigenetic code by acting at the DNA replication fork and by recruiting the right enzymatic activity including DNMT1 and HDAC1 at the right moment (1,2). There is evidence to indicate that via its domains, UHRF1 is the driver of this replication process by ensuring a coordinated crosstalk between DNA methylation and histone modifications. This crosstalk would allow cancer cells to maintain the repression of TSGs during cell division (3). Several studies have demonstrated that the downregulation of UHRF1 expression in cancer cells by natural pharmacological active compounds, such as thymoquinone (TQ) and epigallocatechin-3gallate (EGCG), the major biologically active compounds of black seed oil and green tea respectively lead to the re-expression of TSGs, cell proliferation inhibition and apoptosis induction (4-6). This suggests that preventing UHRF1 to exert its role in the duplication of the DNA methylation and histone code is a key event for inducing apoptosis. In this presentation, I present the increasing role of UHRF1 in tumorigenesis and its activity and/or expression as a target of the natural products TQ and EGGC and their underlying molecular mechanisms.

(1) Alhosin et al., J Exp Clin Cancer Res CR **35**(1): 174, 2016; (2) Alhosin et al., J Exp Clin Cancer Res CR **30**: 41, 2011; (3) Bronner et al., Genes 10(1), 65, 2019; (4) Alhosin et al., Oncotarget 9(47): 28599-28611, 2018; (5) Achour et al., Biochem Biophys Res Commun. 430(1): 208-212, 2013; (6) Alhosin et al., Biochem Pharmacol 79(9): 1251-1260, 2010.

258

Thymoquinone and Difluoromethylornithine synergistically induce apoptosis of lymphoblastic leukemia cells through the modulation of epigenetic pathways

Ryan A. Sheikh[1,#], Syed Shoeb Razvi[1,#], Jalaluddin A. Khan[1], Mazin A Zamzami [1,2], Hani Choudhry [1,2], Mahmoud Alhosin[1,2]
[1]Biochemistry Department, Faculty of Science, King Abdulaziz University; [2]Cancer Metabolism and Epigenetic Unit, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia
[#]Equal first authors
E-mail: malhaseen@kau.edu.sa

Thymoquinone (TQ), a natural anticancer agent exerts cytotoxic effects on several tumors by targeting multiple pathways including apoptosis[(1-3)]. Difluoromethylornithine (DFMO), an irreversible inhibitor of the ornithine decacarboxylase (ODC) enzyme has shown promising inhibitory activities in many cancers including leukemia by decreasing the biosynthesis of the intracellular polyamines[(4,5)]. The aim of the present study was to investigate the combinatorial cytotoxic effects of TQ and DFMO on human lymphoblastic leukemia-Jurkat cells and to determine the underlying mechanisms. Cell proliferation was determined by WST-1 assay, apoptosis rate was assessed by flow cytometry using annexin-V/7AAD staining, RNA sequencing was used to investigate the anticancer mechanisms of TQ and DFMO-treated Jurkat cells and gene expression was assessed using different tools, and the expression of target genes was examined by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The combination of DFMO and TQ significantly reduced cell viability compared to either DFMO or TQ alone. Under the same experimental conditions, the combination of both drugs resulted in significant synergistic effects on apoptosis when compared to either DFMO or TQ alone. RNA sequencing showed that many key epigenetic players including ubiquitin-like containing plant homeodomain (PHD) and really interesting new gene (RING) finger domains 1 (UHRF1) and its two partners DNA methyltransferase 1 (DNMT1) and histone deacetylase 1 (HDAC1) were downregulated in TQ or DFMO-treated Jurkat cells. Data obtained from RNA sequencing were confirmed using RT-qPCR. We found that the combination of DFMO and TQ dramatically decreased the expression of UHRF1, DNMT1 and HDAC1 genes compared to either DFMO or TQ alone. In conclusion, these results suggest that the combination of DFMO and TQ could be a promising new strategy for the treatment of acute lymphoblastic leukemia by targeting the epigenetic code.

Acknowledgements and Funding: This work was supported by the Deanship of Scientific Research (DSR), at King Abdulaziz University, Jeddah, under grant no. (D-99-130-1438). The authors, therefore, gratefully acknowledge the technical and financial support from DSR.

(1) Alhosin et al., Oncotarget 9(47): 28599-28611, 2018; (2) Alhosin et al., Invest New Drugs 30(5): 1813-1819, 2012; (3) Alhosin et al., Biochem Pharmacol 79(9): 1251-1260, 2010; (4) Elmets et al., Cancer Prev. Res. 3, 8-11, 2010; (5) Arisan et al., Curr. Pharm. Des. 20, 180-188, 2014.

259

Implications on telomeres length by chronic exposure to drugs of abuse and pesticides

Vasiliki Karzi, Elena Vakonaki, Manolis N. Tzatzarakis, Aristides M. Tsatsakis
Laboratory of Toxicology, Medical School, University of Crete, Voutes, Heraklion, Greece

Telomeres are nucleotide tandem repeats at the ends of eukaryotic chromosomes that maintain genomic integrity. The gradual shortening of telomeres leads the cell to senescence and apoptosis and can be affected by life style. Having already created a database to determine the biological age through the length of the telomeric limbs we aimed to evaluate whether other parameters, such as the use of drugs of abuse (cannabis, opiates and cocaine) or the exposure to glyphosate can influence cell aging of abuser or farmer. Blood samples were collected from 16 drug abusers and 10 glyphosate sprayers. Metaphase spread leukocytes were isolated from peripheral blood. Telomere length was measured by Q-FISH with (C3TA2)3 PNA probe. Ten metaphases of each individual were measured and analyzed by Image-J. Basic statistical tests such as independent samples t-test were used. A reduction in telomere length of drug abusers especially for opiate users was found that was by far greater than their chronological age. Regarding the use of glyphosate, there was a tendency to reduce short telomeric limbs compare to the general population. In conclusion, these findings indicate an association between telomere length and drug abuse or spraying with glyphosate, which lead to premature biological aging. However, a broader study with a wider variety of drugs and pesticides should be followed so that we can create a new database with the effects of each substance on cellular aging.

260

Telomeres and Telomerase biomarkers and advances in reproductive research

Persefoni Fragkiadaki[1,2], Charikleia Kalliora[3], Eleni Vasilopoulos[4], Dimitris Tsoukalas[1,5,6], Eleni Vakonaki[1,2], Elissavet Renieri[1], Evangelia Sarandi[6], Dimitrios Spandidos[7], Aristidis Tsatsakis[1,2]

[1]Laboratory of Toxicology, Medical School, University of Crete, Heraklion, Greece; [2]Spin-Off Toxplus S.A., Heraklion, Greece; [3]Department of Obstetrics and Gynecology, University of Pennsylvania, Philadelphia, USA; [4]Department of Biology, Temple University, Philadelphia, USA; [5]Department of Clinical Pharmacy, University of Medicine and Pharmacy, Faculty of Pharmacy, Craiova, Romania; [6]Metabolomic Medicine, Health Clinics for Autoimmune & Chronic Diseases, Athens, Greece; [7]Laboratory of Clinical Virology, School of Medicine, University of Crete, Heraklion, Greece
E-mail: tsatsaka@uoc.gr

There is a strong link between aging and infertility in both genders, with an earlier onset in females. Recent studies indicate that telomere length, which can be affected by various lifestyle factors, can affect the pace of aging and onset of age-associated diseases. It has been suggested that female human fertility decreases with increased maternal age and that various adverse factors, including alterations in telomerase activity, can contribute to age-associated infertility in women. The fact that telomerase activity is regulated in a time- and location-dependent manner in both embryo and placental tissues highlights it potential importance to the successful completion of pregnancy. Since maternal age is a crucial determining factor for the success of *in vitro* and *in vivo* fertilization, numerous studies have focused on telomere length and telomerase activity and the correlation with mammalian fertilization, as well as the following cleavage and pre-implantation developmental processes. Associations between telomerase activity and pregnancy complications have been previously observed. It has also been claimed that the majority of female infertility factors are associated with shorter telomere length, except for endometriosis, premature ovarian failure, and clear cell carcinoma. The last three showed longer telomere length and Polycystic Ovarian Syndrome, revealing disagreement in several studies, leading to ambiguous conclusions. The study of telomere biology during reproduction can improve our understanding of the significance of telomere length and telomerase activity in fetal development and their lifelong consequences on illnesses and aging processes in different populations. This understanding may lead to improved prevention policies and may reveal novel therapeutic strategies with the ultimate aim of more live births and a reduction of female-caused infertility.

261

Expression of beta-defensins 1 and 2 in total saliva of individuals with a 35-day experimental gingivitis model

Saira Karina Ramírez-Thomé[1,2], Risk Díaz-Castillejos[1], María Cristina Franco-Arellanes[1], Victoria Jiménez-Castillo[1,2,3], Yael Ruíz- Pichardo[1], Ana Patricia Vargas-Casillas[4], Carlos Josué Solórzano-Mata[1,2]
[1]Faculty of Odontology and [2]Faculty of Medicine, Autonomous University Benito Juárez of Oaxaca; [3]Technological Institute of Oaxaca; [4]Faculty of Odontology, National Autonomous University of Mexico, Mexico
E-mail: universidad99@hotmail.com

Gingivitis is a reversible disease characterized by inflammation of the gum in the presence of a mature bacterial plaque. It is estimated that the prevalence rate is very high worldwide, since more than three quarters of the population suffer from it. Its importance lies in the fact that it can evolve into chronic periodontal disease with the consequent loss of dental organs. The beta-defensins are antimicrobial peptides present in saliva with pro-inflammatory and antibacterial activity which could be important in early stages of the disease. The objective of the present study was to identify the expression of beta-defensin 1 and 2 in the total saliva of patients with experimental gingivitis in a 35-day model. Following the approval of the ethics committee in Research, 10 clinically healthy individuals participated, who underwent clinical history, periodontal evaluation and prophylaxis, in the induction phase of experimental gingivitis on days 0, 7, 14, 21 and 28 periodontal evaluation. Saliva sampling was performed for the identification of CFU on blood agar and *mitis salivarius* agar, as well as for the concentration of beta-defensin 1 and 2 by the ELISA technique; on day 28, dental hygiene was reinstituted finally, a periodontal evaluation was performed and samples were taken on day 35. The results revealed progressive modification during the induction phase, the O'Leary index, Sillnes index and Löe index, the increase in the presence of bleeding, as well as an increase in CFUs >500 on blood agar and *mitis salivarius* agar compared to day 0. Likewise, an increase in the expression of beta-defensin-1 ($p>0.05$) was found on day 21 and 28 compared to 0, similarly, fluctuations in beta-defensin 2 concentrations were found without finding statistically significant differences. This study demonstrates that both defensins participate in the early stages of inflammation in gingivitis and its importance as a defense mechanism to the bacterial stimulus.

Key words: experimental gingivitis, beta-defensins, innate immunity

Acknowledgements: This project was supported by PFCE-2015 y 2019.

262

Neural plasticity in Vascular Cognitive Impairment: Translational findings from Transcranial Magnetic Stimulation

Manuela Pennisi
Department of Biomedical and Biotechnological Sciences (BIOMETEC), University of Catania, Catania, Italy

Among the modern neurophysiological techniques, transcranial magnetic stimulation (TMS) is a translational non-invasive tool able to evaluate *in vivo* and in real time the cortical excitability and the underlying transmission pathways involved in different neuropsychiatric disorders, including dementia [1]. Recently, there has been a significant growth in the literature exploiting TMS in Vascular Cognitive Impairment (VCI), which is an "umbrella term" encompassing any degree of vascular-related cognitive decline [2]. TMS in VCI points at enhanced cortical excitability and synaptic plasticity, which seems to correlate with disease process and progression and suggests the progressive involvement of glutamate-mediated compensation in response to vascular lesions [3]. These alterations might eventually promote adaptive plasticity that allows the preservation of motor programming and execution [4]. Moreover, recent findings suggest a specific TMS profile related to VCI subtypes (i.e., VCI-no dementia, vascular dementia, and mixed dementia), thus possibly predicting cognitive deterioration of the so-called "brains at risk" [3]. This finding will be of pivotal importance when designing trials of disease-modifying drugs or non-pharmacological approaches, including neuromodulatory interventions. In demented patients, TMS may select the responders for specific drugs in the attempt to restore maladaptive plasticity. Although a single TMS index has low specificity, a panel of measures can support VCI diagnosis, follow its progression, and identify early markers thereof [1]. These advances could make VCI a potentially preventable cause of both vascular and degenerative dementia. The present talk provides a perspective on this cutting-edge topic by further understanding how cortical electrophysiology, synaptic plasticity, and network connectivity act and interact in the pathogenesis and pathophysiology of VCI and its subtypes.

References

[1] Cantone M, et al. Clin Neurophysiol 2014;125(8): 1509-1532.
[2] Moorhouse P, Rockwood K. Lancet Neurol 2008;7(3): 246-255.
[3] Lanza G, et al. Behav Neurol 2017; 2017: 1421326.
[4] Guerra A, et al. Clin Neurophysiol 2015; 126(5): 906-913.

263

Relationship between genetic polymorphisms and pesticide-induced oxidative damage in exposed workers

Teodoro M[1], Caccamo D[1], Briguglio G[1], Costa C[2], Fenga C[1]
[1]Department of Biomedical and Dental Sciences and Morphofunctional Imaging - Occupational Medicine Section - University of Messina; [2]Department of Clinical and Experimental Medicine - Occupational Medicine Section - University of Messina, Italy

Pesticides include a wide and mixed category of compounds used to prevent and overcome weeds or pests. These chemicals can produce a detrimental effect on the human body. Literature data suggest oxidative stress as one of the key mechanisms for the harmful health effects of exposure to pesticides; by modifying the physiological homeostasis, pesticides can give rise to an excess of oxidant metabolites, leading to severe intracellular damage (1). The study population comprised 63 farmers employed in Eastern Sicily, manipulating chlorpyrifos and occasionally minor amounts of other compounds. Genotyping of polymorphisms PON1 *Q192R*, PON2 *S331C* and *A148G*, GSTP1 *Ile105Val* and *Ala114Val*, GSTM1 and GSTT1 was performed using real-time polymerase chain reaction (RT-PCR) from peripheral blood lymphocytes. Serum levels of advanced glycation end-product (AGE), advanced oxidation protein product (AOPP), reactive oxygen metabolites (ROMs) and biological antioxidant potential (BAP) were thus determined. Preliminary data showed the relationship between the serum marker levels and the presence of polymorphisms suggesting that genetic polymorphisms can alter the effects of individual exposure to pesticides. Further research on the variability of many DNA repair genes and their combinations is of utmost importance to assess their actual role as determinants of pesticide toxicity. A good knowledge of these complex mechanisms could prospectively lead to the development of screening tests that, following resolutions of some ethical issues, would allow large-scale detection of susceptibility to pesticide exposure or other xenobiotics.

Key words: pesticides, genetic polymorphisms, gene-environment interactions, occupational health

1) Kaur G, Jain AK, Singh S: J Genet. CYP/PON genetic variations as determinant of organophosphate pesticides toxicity, 2017 Mar; 96 (1): 187-201.

264

Gene-environment interactions and pesticide toxicity in exposed workers

Fenga C[1], Teodoro M[1], Briguglio G[1], Costa C[2]
[1]Department of Biomedical and Dental Sciences and Morphofunctional Imaging - Occupational Medicine Section - University of Messina, Italy; [2]Department of Clinical and Experimental Medicine - Occupational Medicine Section - University of Messina, Italy

Occupational exposure to pesticides can have detrimental effects on human health, increasing the risk of onset for several disorders (1). Oxidative stress and epigenetic modifications are probably the key mechanisms by which the biological effects may be explained. Some individuals can be more susceptible to pesticide-induced health effects because of the occurrence of genetic polymorphisms (2). A gene is defined as a polymorphism in which multiple alleles occur at a locus with a frequency greater than 1%. The most common gene polymorphisms involved in the metabolism of organophosphorus compounds were investigated. Accordingly, cytochrome P450, glutathione transferases (GST), acetyltransferases (NAT2) and paraoxonases (PON) play a crucial role in pesticide metabolism and their polymorphisms may be associated with different classes of risk within the general population, since the proteins encoded by the various genotypes alter the biotransformation of these chemicals. Genetic heritage may increase susceptibility for the onset of chronic diseases, especially in workers already exposed to other pollutants. To understand the pathogenesis of pesticide-induced diseases further studies are needed to evaluate the role of genetic impact and to improve preventive actions useful in the protection of the subset of "vulnerable subjects" both in occupational and environmental settings.

1) Povey AC. Gene-environmental interactions and organophosphate toxicity. Toxicology. 2010 Dec 30; 278(3): 294-304. doi: 10.1016/ j.tox.2010.02.007.
2) Lee BW, London L, Paulauskis J, Myers J, Christiani DC. Association between human paraoxonase gene polymorphism and chronic symptoms in pesticide-exposed workers. J Occup Environ Med. 2003 Feb; 45(2): 118-122.

265

Milk-derived exosomes – A nano platform for delivery of small and large molecules

Ramesh Gupta, Farrukh Aqil, Jeyaprakash Jeyabalan, Al-Hassan Kyakulaga, Ashish Agrawal, Neha Tyagi, Raghuram Kandimalla, David J. Schultz, Wendy Spencer, Radha Munagala
University of Louisville, Louisville, Kentucky, USA; 3P Biotechnologies Inc., Louisville, Kentucky, USA

Oral delivery of both pharmaceuticals and nutraceuticals has been challenging due to limited bioavailability in most cases. Attempts made in the past several decades using nanoparticles of synthetic and natural lipids and polymers have resulted in limited success; thus, the translatability of nano formulations to millions of people remains elusive. Exosomes are endogenous nanovesicles that have been suggested as a potential drug carrier and could potentially overcome challenges for oral delivery of drugs. We have found that bovine milk has an abundance of exosomes that can be loaded with both small molecules and macromolecules such as siRNA. Our data have shown that oral delivery of an exosomal formulation of the widely used chemotherapeutic drug, paclitaxel (ExoPAC), is more efficacious against lung cancer than the drug alone administered intraperitoneally. ExoPAC was well tolerated based on lack of systemic and immune toxicities. Increased efficacy and bioavailability of exosome formulations is further supported from data derived from other studies in which individual plant bioactives such as withaferin A, Anthocyanidins (Anthos) and curcumin embedded in milk exosomes showed enhanced anti-proliferative, anti-inflammatory and anti-cancer effects against lung and cervical cancers *in vitro* and *in vivo* compared to naked compounds. The higher activities of these agents in exosomal formulations resulted, presumably, due to higher stability, cell uptake and/or tissue delivery of the payload. Finally, milk exosomes embedded with mutated KRAS siRNA showed significant downregulation of the target gene and growth inhibition of human A549 lung cancer tumors in nude mice. Together, these data suggest that milk exosomes provide a functional nano platform for delivery of small molecules as well as biologics.

Supported from R41-CA-189517, KSTC-184-512-15-209, R44CA221487-02 and Duggan endowment.

266

Next-generation sequencing for the identification of molecular markers in diverse childhood malignancies

Susanne Heinsohn, Kai Lehmberg, Udo zur Stadt
University Medical Centre Hamburg-Eppendorf, Clinic and Policlinic for Paediatric, Haematology and Oncology, Hamburg, Germany
E-mail: heinsohn@uke.de

The development and advances of next-generation sequencing (NGS) and the subsequent analysis tools have gained popularity in scientific researches, as well as in clinical diagnostic applications, and have thus defined the genomic landscape of diverse childhood malignancies. Clinical NGS assays are often run on tumour specimens without a matched normal specimen, which complicates the differentiation of germline from somatic variants. In our NGS studies, we also analysed healthy family members, and in most cases, the whole family. In addition, we used a maximum of 0.1% population frequency as cut off for the bioinformatics pipeline resulting in high sensitivity for classification of somatic variants found. Our aim was to detect somatic mutations that may be of prognostic relevance for diagnosis, disease survival or predictive for primary or secondary drug resistance, as well as for monitoring minimal residual disease. Overall, 91 specimens were analysed: 42 patients and 49 related family members. The patient disorders analysed included, haemophagocytic lymphohistiocytosis (HLH) (9 patients), von Willebrand disease (2 patients), unexplained immunodeficiency disease (13 patients), Hyper IgE-Syndrom (1 patient) and highly malignant rare tumours, including osteosarcomas (5 patients) and rhabdoid tumours (ATRT) (12 patients). From most patients, we analysed additionally diverse healthy family members. Several novel variants were found, mostly in patients with immunodeficiency disease, among them two stop mutation in CXCR3 and MNDA gene. For HLH, the PEPD gene was found to be an important marker. Further research is required to determine the exact diagnostic yield and clinical implications of NGS in paediatric malignancies.

Financially supported by Fördergemeinschaft Kinderkrebszentrum Hamburg e.V.

267

OXA-48 and other carbapenemases as resistance determinants in *Klebsiella pneumoniae* strains isolated from blood cultures: Molecular and phenotypic determination

Manuela Tompa[1,2], Stanca Lucia Pandrea[1,2], Monica Ioana Ciontea[1], Mircea Grigorescu[1], Ronald Tompa[2], Stanca Maria Pandrea[2], Lia Monica Junie[2*]
[1]Central Laboratory, Regional Institute of Gastroenterology and Hepathology, Cluj Napoca, Romania; [2]Microbiology Department, University of Medicine and Pharmacy Iuliu Hatieganu, Cluj-Napoca, Romania
[*]E-mail: monicajunie@yahoo.com; mjunie@umfcluj.ro

Rapid dissemination of Carbapenem *Klebsiella pneumoniae* (CRK) represents a medical problem in terms of hospital infection control and antimicrobial chemotherapy. The objectives were to determine carbapenem resistance (CR), the presence of the genes that codify carbapenemases in the CRK strains (*bla*KPC, *bla*NDM, *bla*OXA-48-like, *bla*GES, *bla*VIM, *bla*IMP) and their antibiotic (AB) resistance profiles. Eighteen carbapenem-resistant strains non-duplicated *Klebsiella pneumoniae* from blood samples were isolated during September 2016-September 2017, from hospitalized patients. The isolates identification was performed by Vitek 2 Compact system (V2Cs). AB susceptibility was determined by V2Cs and interpreted according to EUCAST guidelines. The selection value was Minimal Inhibitory Concentration (MIC) to Meropenem (MER) ≥0.5 µg/ml. CR was phenotypically confirmed by KPC/Metallo-beta-Lactamase Confirmation kit (Rosco Diagnostica Denmark). RT-PCR detected the *bla*KPC, *bla*GES, and *bla*OXA-48-like genes. CR strains were divided in 4 groups: 11 (61.1%) isolates presented MER and Imipenem (IMI) resistance (MICs for MER, IMI>=16 µg/ml (group I); 3 (16.67%) isolates were MER-Intermediary sensitivity (IS) (MIC 2-4 µg/ml) and IMI- (IS), having MICs 2-8 µg/ml (group II); 3 isolates (16.67%) were MER-R (MICs >=16 µg/ml) & IMI-IS with MIC 4-8 µg/ml (group III); 1 isolate (5.56%) was MER-(IS) (MIC 2 µg/ml) and IMI-R (MICs >=16 µg/ml. We found 100% correspondence between modified carbapenem inactivation methods and RT-PCR results. Nine strains (50%) had *bla*OXA48-like genes, 3 (16.67) *bla*KPC genes, and 5 (27.78%) had *bla*OXA48-like and *bla*KPC genes simultaneously. One strain contained three genes: the *bla*OXA-48, *bla*KPC and the GES-genes. In conclusion, our study demonstrates the importance of gene detection in *Klebsiella pneumoniae* isolates and their correlation with the phenotypic CR tests. In addition, the results show the spread of the *bla*OXA-48-like and *bla*KPC genes in Transylvania, Romania. The results highlight the increased CR percentage of KP by harboring at least two carbapenemase genes.

268

Effect of glioblastoma radiotherapy on the extracellular matrix of normal mouse brain tissue in an experimental system *in vivo*

Maxim O. Politko[1], Anna I. Prokaeva[1,2], Oxana A. Pashkovskaya[3], Alexander A. Zheravin[3], Galina M. Kazanskaya[3], Alexander V. Volkov[3], Mariia A. Kolpakova[1,2], Alexandra Y. Tsidulko[1], Anastasia V. Suhovskih[1,2], Svetlana V. Aidagulova[4], Elvira V. Grigorieva[1,2]
[1]Institute of Molecular Biology and Biophysics FRC FTM; [2]Novosibirsk State University; [3]Meshalkin National Medical Research Center; [4]Novosibirsk State Medical University, Novosibirsk, Russia
E-mail: kolpakovamariia97@gmail.com

Glioblastoma is an aggressive malignant brain tumour with a poor prognosis and a low patient survival. Adjuvant radiochemotherapy is a conventional strategy used to prevent disease relapse; however, it affects both target glioma cells and normal brain tissue, where one of the main components is proteoglycan (PG)- and glycosaminoglycan (GAG)-based extracellular matrix (ECM). In this study, we aimed to examine the effect of radiotherapy on PG expression, and the structure and composition of normal brain ECM. The irradiation of the brains of 2-month-old C57BL/6 mice was performed using an ElektaAxesse clinical linear accelerator. The expression of main PGs was determined by RT-qPCR, and the composition and content of their GAG chains was assessed by immunohistochemical analysis. Multiple (3 times by 7 Gy/day) irradiation of the experimental animals did not affect the histological structure of normal mouse brain tissue; however, it resulted in significant molecular changes in PG expression levels. Decorin, biglycan and neurocan were the most abundant PGs in the normal brain tissue. Irradiation specifically affected decorin, neurocan and versican transcriptional activity, and the GAG content in brain ECM, suggesting the deterioration of its structure and composition upon irradiation. Taken together, the obtained results demonstrate the influence of multiple irradiation on PG expression and the ECM composition of normal brain tissue, potentially leading to a formation of a tumour susceptible niche and glioblastoma relapse.

Acknowledgements: The reported study was funded by RFBR, project number 18-29-01036. Tsidulko A.Y. was supported by scholarship of Russian Federation President for young scientists (SP-5435.2018.4).

269

Fusion genes in cancer: From cytogenetics to next generation sequencing

Ioannis Panagopoulos
Section for Cancer Cytogenetics, Institute for Cancer Genetics and Informatics, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway
E-mail: ioannis.panagopoulos@rr-research.no

A fusion gene is a hybrid gene that is created through melting together parts of two previously separate genes. Acquired fusion genes may disturb proliferation regulation so as to unleash cancer. They are caused by chromosomal rearrangements in the cells of the neoplastic parenchyma such as translocations, inversions, deletions or insertions. A characteristic example in sarcomas is the *EWSR1-FLI1* fusion gene which is the result of the chromosomal translocation t(11;22)(q23;q12). It codes for an abnormal transcription factor that plays a critical role in the development of Ewing sarcoma. Cancer-specific fusion genes can be the targets of molecular therapy, play a key role for the accurate pathogenetic diagnosis and classification of neoplasms, and have prognostic impact. The identification of novel fusion genes in various neoplasms therefore, not only has obvious research importance, but also potentially major clinical significance. The "traditional" methodology to detect fusion genes in tumors began with cytogenetic analysis which aimed to find the chromosomal rearrangement, followed by utilization of fluorescence *in situ* hybridization techniques to identify the probe which spans the chromosomal breakpoint. Eventually, molecular cloning was performed to localize the breakpoint more precisely and identify the genes fused by the chromosomal rearrangement. Although laborious, the above-mentioned sequential approach is quite robust and reliable and a number of fusion genes have been cloned by such means. The introduction of next generation sequencing has opened up new possibilities to detect fusion genes in cancer. We used a combination of banding cytogenetics and RNA sequencing to identify novel fusion genes in both mesenchymal tumors and blood cancer[(1)]. Among them were the *IRF2BP2-CDX1* in mesenchymal chondrosarcoma with t(1;5)(q42;q32), the recurrent *ZC3H7B-BCOR* fusion gene in endometrial stromal sarcomas with the t(X;22)(p11;q13) chromosomal aberration, and the *KAT6B-KANSL1* fusion gene in retroperitoneal leiomyoma with a t(10;17)(q22;q21)[(2-4)]. The new sequential approach and the novel fusion genes detected will be presented.

(1) Panagopoulos et al., Int J Biochem Cell Biol 53:462-5, 2014; (2) Nyquist et al., PLoS One 7:e49705, 2012; (3) Panagopoulos et al., Genes Chromosomes Cancer 52:610-8, 2013; (4) Panagopoulos et al., PLoS One 10:e0117010, 2015.

270

Expression of selected cytokeratins in neoplastic endometrium

Danuta Vasilevska[1], Anna Semczuk-Sikora[2], Dorota Lewkowicz[3], Maciej Jóźwik[4], Michał Bogusiewicz[5], Andrzej Semczuk[5]
[1]Centre of Obstetrics and Gynecology, Vilnius University Hospital "Santaros Klinikos", Vilnius, Lithuania; [2]Department of Pathology of Pregnancy, Lublin Medical University, Lublin, Poland; [3]Department of Clinical Pathology, Lublin Medical University, Lublin, Poland; [4]Department of Gynecology and Gynecologic Oncology, Bialystok Medical University, Bialystok, Poland; [5]II[nd] Department of Gynecology, Lublin Medical University, Lublin, Poland
E-mail: andrzej.semczuk@umlub.pl

Cytokeratins (CKs) are the largest sub-group of intermediate filament proteins preferentially expressed in various human tissues[(1-2)]. They are sub-divided into type I acidic (CK9-CK28) and type II basic (CK1-CK8 and CK71-CK74) on the basis of biochemical properties[(2)]. CKs are resistant to degradation, show great fidelity of expression and are very antigenic. In general, CK profiling is especially valuable for carcinomas of poorly differentiated histology, for tumors spreading over several organs as well as for metastases of an unknown primary origin[(3)]. Uterine adenocarcinomas are always immunohistochemically positive for CK7, CK8, CK18 and CK19, while mostly they are CK20 negative[(4)]. In a report by Alkushi *et al*[(5)], only CK8/18 revealed a significantly different frequency of positivity in endometrial adenocarcinoma relative to cervical adenocarcinomas. Interestingly, the immunohistochemical expression of CKs in lymph nodes with undetected metastasis predict occult metastasis to these nodes and it is a risk factor for recurrence of early-stage endometrial cancer[(6)]. It is worth pointing out that the innovative immunomarkers for CKs profiling have been increasing in numbers recently and this trend is likely to continue with new ground-breaking techniques. The aim of the presentation is to briefly overviews the multi-functional role of CKs in primary and advanced endometrial carcinomas.

(1) Moll, Subcell Biochem 31: 205-262, 1998; (2) Coulombe and Omary, Curr Opin Cell Biol 14: 110-122, 2002; (3) Karantza, Oncogene 13: 127-138, 2011; (4) Chu and Weiss, Histopathology 40: 403-439, 2002; (5) Alkushi et al., Virchows Arch 442: 271-277, 2003; (6) Yabushita et al., Gynecol Oncol 80: 139-144, 2001.

271

Chromosomal damage, relative telomere length and DNA repair in colorectal and breast carcinogenesis

P. Vodička[1,2,6], M. Kroupa[1,6], L. Vodičková[1,2,6], Z. Polívková[3], L. Mušák[4], M. Dušinská[5], S. Vodenková[1,2], S. Rachakonda[5], V. Vymetálková[1,2], A. Naccarati[1,7], R. Kumar[8], K. Hemminki[8]
[1]Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Prague; [2]First Faculty of Medicine, Charles University, Prague; [3]Third Faculty of Medicine, Charles University, Prague, Czech Republic; [4]Biomedical Center Martin, Division of Molecular Medicine, Comenius University in Bratislava, Jessenius Faculty of Medicine, Martin, Slovakia; [5]NILU, Lillestroem, Norway; [6]Faculty of Medicine and Biomedical Center in Pilsen, Charles University, Pilsen, Czech Republic; [7]HUGEF Turin, Italy; [8]Division of Molecular Genetic Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany
E-mail: pavel.vodicka@iem.cas.cz

Human cancers arise from cells unable to maintain genomic and chromosomal integrity, mainly due to altered DNA repair mechanisms. Chromosomal aberrations (CAs) represent causative events in malignant transformation. Critically shortened telomeres may be poorly end-capped and recognised as double-strand breaks (DSBs) by repair machinery, thus contributing to the genomic and chromosomal instability. Herein, we investigated the links between CAs, DNA repair characteristics and relative telomere length (RTL) in incident patients with colorectal cancer (CRC), breast cancer (BC) and healthy subjects. Moreover, telomere length in target tissues was investigated in relation to several molecular and clinicopathological features of CRC. CAs in peripheral blood lymphocytes (PBL) were determined by classical cytogenetic method in 1,800 healthy subjects and incident cancer patients (157 BC, 101 CRC). DNA repair gene variants were analysed using TaqMan allelic discrimination assay, DNA repair capacity by a modified comet assay (NER and BER) and mutagen sensitivity assay (a functional characteristic of DSB repair). RTL was measured using the monochrome multiplex PCR in DNA from PBL of 143 CRC, 148 BC patients, 271 controls and in DNA from 701 pairs of colon tumors and adjacent mucosa. Lower CAs were associated with high activity *EPHX1* genotype and variant allele in *XPD* Lys751Gln. Binary pair-wise interactions of DNA repair variants significantly modulated CAs. Increased CAs and CSAs emerged in subjects bearing splicing A variant in CCND1 G870A. Telomere shortening in cancer patients correlated with decreased DSB capacity. CRC patients exhibited significantly shorter RTL, but similar CAs as controls. BC patients had significantly longer RTL and increased CAs frequency. Significantly shorter telomeres were observed in colon tumor tissues and correlated with tumor localization. The shortest TLs were found in MSI-H proximal colon tumors, the longest in rectal cancer tumors. CAs in PBL represent perspective transient marker in carcinogenesis; however, they do differ in various cancers. We demonstrate links between chromosomal damage, DNA repair and telomere length in carcinogenesis and telomere shortening may be a proxy for underlying differences in DNA repair capacities in cancer patients.

Grant support: *GACR 19-10543S, 17-16857S, 18-09709S, AZV 15-27580A, NV18-00199*

272

Microparticules released by myeloma plasma cells enhance thrombin generation in the microenvironment: A modelization *in vitro* study

L. Papageorgiou[1], P. Van Dreden[2], M. Dimopoulos[3], M. Mohty[4], G.T. Gerotziafas[5,6]
[1]Hôpital Tenon Hôpitaux Universitaires de l'Est Parisien, Service Hématologie Biologique, Paris, France; [2]Diagnostica Stago, Clinical Research, Gennevilliers, France; [3]National and Kapodistrian University, Department of Clinical Therapeutics, Athens, Greece; [4]Saint Antoine Hospital, Hôpitaux, Department of Hematology and Cell Therapy, Paris, France; [5]Saint Antoine Hospital, Hôpitaux, Department of Hematology and Cell Therapy, Paris, France; [6]Sorbonne Universities, Faculty of Medicine, Cancer, Haemostasis and Angiogenesis, Paris, France

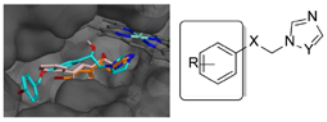
Hypercoagulability is a common blood alteration in newly diagnosed, chemotherapy naïve, multiple myeloma patients. The mechanisms by which myeloma plasma cells (MPCs) interfere with their microenvironment during the blood coagulation process have been poorly investigated. The aim was to identify the principal vectors related to myeloma plasma cells (MPCs) which boost thrombin generation in the microenvironment. TF and annexin V expression by MPCs and myeloma cell-derived microparticles (MPC-dMPs) were analyzed by flow cytometry, TF activity (TFa) and TF gene expression was also determined. Thrombin Generation (TG) in the presence of MPCs or MPC-dMPs was assessed with the Calibrated Automated Thrombogram assay (CAT®) in normal human PPP. TG was also assessed in plasma spiked with MPCs and MPC-dMPs or variable concentrations of TF and procoagulant phospholipids. PC-dMPs expressed about 8-fold higher levels of TF as compared to MPCs. TFa produced by MPC-dMPs was significantly higher as compared to that of MPC. MPCs and MPC-dMPs enhanced thrombin generation of human plasma. Thrombin generation was significantly higher with MPC-dMPs compared to MPCs. Presence of TF and procoagulant phospholipids in the microenvironment resulted in significant amplification of thrombin generation induced by MPCs. In conclusion, this study reports for the first time that the inherent procoagulant properties of myeloma plasma cells are necessary, but not sufficient, to induce hypercoagulability. Since myeloma plasma cells represent a weak procoagulant stimulus, hypercoagulablity is the resultant of the presence of procoagulant elements into their microenvironment, via the release of MPCS-dMPs which express significant levels of TF. Due to their critical role into the hypercoagulablity, procoagulant MPC-dMPs could be a potential tool for the evaluation of the aggressiveness of myeloma disease.

273

Inhibition of heme oxygenase-1 to improve cancer therapy

Loredana Salerno, Sebastiano Intagliata
Department of Drug Sciences, University of Catania, Catania, Italy
E-mail: l.salerno@unict.it

Heme Oxygenases (HOs) are a family of microsomial enzymes responsible for the catabolism of heme into free iron, carbon monoxide, biliverdin, and bilirubin. Among the three isoforms identified so far, HO-1 is inducible by a variety of stimuli, and is considered a survival molecule in various stress-related conditions(1). By contrast, growing evidences suggest that HO-1 expression increases in a number of solid and blood cancers, promoting carcinogenesis, tumor progression, and chemo-resistance. Therefore, HO-1 selective inhibition is increasingly recognized as a new therapeutic strategy in cancer(2). By means of a medicinal chemistry approach, a number of azole-based compounds that inhibit HO-1 in a non-competitive manner have been developed so far. From these studies, the main chemical features needed for HO-1 inhibition have been revealed(3). Based on these premises, in recent years, we developed different series of azole-based derivatives with the following general formula.



All these compounds possess the key chemical features required for HO-1 interaction: a N-3 imidazole nitrogen, a hydrophobic moiety, and a connecting chain. The most potent compounds were selected and studied for their antitumor properties in different cancer cell lines, with promising results.[3] These compounds may be regarded as novel tools in elucidating the pathophysiological roles of HO-1 and HO-2, and might have useful therapeutic applications in cancer therapy. Results obtained so far will be presented at the meeting.

(1) Pittalà, V. et al. Curr. Med. Chem. 2013, 20, 3711-3732.
(2) Furfaro, A. L.et al. Oxid. Med. Cell. Longev. 2016, 1958174.
(3) Salerno, L. et al. Eur. J. Med. Chem. 2019, 167, 439-453.

274

The immune escape mechanism of malignant cells in bladder cancer

Amira H. Mohamed
Theodor Bilhharz Research Institute, Department of Electron Microscope, Cairo, Egypt
E-mai: Amira7121956@yahoo.com

Tumor cells have many immune escape mechanisms, one of which is apoptosis resistance. This electron microscope study tried to clarify this role through the development of targeted therapy that will sensitize tumor cells to apoptosis. In this study, expression of TGF-beta-1 protein and TGF-beta-R-1 receptor in urine and peripheral blood mononuclear cells (PBMNCs) were examined by the light and electron microscopy using immunocytochemical and immunoelectronmicroscopic techniques. Samples were obtained from 20 healthy controls (Group 1) and 120 patients who were classified according to the cytopathologic examination of their urine into 2 main subgroups chronic cystitis (bilharzial and nonbilharzial, Group 2, n=30) and bladder cancer (transitiona cell carcinoma and squamous cell carcinoma, Group 3, n=90), whether associated with bilharzial infection or not associated. PBMNCs stained by both immunocytochemical and immunoelectronmicroscopic techniques showed significant increase in the percentage of positive cases expressing both TGFbeta-1 protein and TGF-beta-R-1 receptors in bladder cancer in comparison with the control (P<0.01 and P<0.05, respectively) and with chronic cystitis (P<0.05). By light and electron microscopic examination, 82 out of 90 bladder cancer cases (91.1%) revealed remarkable apoptotic changes represented by cell shrinkage, surface blebs, nuclear chromatin condensation and vacuolated cytoplasm. Urine examination of the exfoliated necrotic malignant epithelial (urothelial) cells in paraffin sections stained by both immunocytochemical and immunoelectronmicroscopic techniques revealed a statistically significant decrease in the percentage of positive cases expressing TGF-beta-R1 receptor in bladder cancer in comparison with either chronic cystitis cases or controls (P<0.01), while TGF-beta-1 protein was significantly increased (P<0.01). In conclusion, this work helps in better understand one of the escape mechanisms of tumor cells that may facilitate the reverse of tumor escape from the immune system. It also draws attention to TGF-beta-1 protein that can be used as attractive target for anticancer therapy, and the absence of TGF-beta-R1 can be considered a marker for malignant transformation.

275

Securin: A novel marker and regulator of cancer stem cells in ovarian cancer

Seema Parte[1,2], Irma Virant-Klun[3], Manish Patankar[4], Surinder K. Batra[5], Alex Straughn[2], Sham S. Kakar[1,2]
[1]Department of Physiology, University of Louisville, Louisville; [2]James Graham Brown Cancer Center, University of Louisville, Louisville, USA; [3]Department of Obstetrics and Gynecology, University Medical Centre Ljubljana, Ljubljana, Slovenia; [4]Department of Obstetrics and Gynecology, University of Wisconsin-Madison, Madison; [5]Department of Biochemistry and Molecular Biology, University of Nebraska, Omaha, NE, USA
E-mail: sham.kakar@louisville.edu

Ovarian cancer stem cells (CSCs) exhibit characteristics of self-renewal, tumor initiation, tumor growth, a capacity to differentiate, and drug resistance leading to tumor relapse. Several theories for the origination of CSCs have been proposed; however, the origin of CSCs still remains unclear. Studies suggest that epithelial ovarian cancer arises from the surface epithelium, while normal ovarian stem cells are reported to exist in a similar location, thus suggesting a common connection. Several oncogenes, such as: Myc, Ras, Raf, Bcl2 and Src, are implicated in oncogenic transformation and tumorigenesis. In this context, we explored a putative oncogene "securin", also known as 'pituitary tumor transforming gene 1' (PTTG1), reported to be overexpressed in various tumors, including ovarian tumors. Fluoro-immunohistochemical analysis using confocal microscopy and qPCR demonstrated co-expression of securin with several stem cell (OCT4, NANOG, SOX2 and SSEA4), CSC (ALDH1, CD24, CD44, CD117, CD133 and LGR5) and germinal lineage (DDX4/VASA and IFITM3/FRAGILIS)-specific markers in normal ovary, benign, borderline and high-grade ovarian tumors, as well as ascite-derived CSCs collected from patients with recurrent ovarian cancer. Gene-specific siRNA knockdown of securin in an ovarian cancer cell line (A2780) revealed a 90% downregulation of securin, accompanied by downregulation of several stem cell and CSC genes. Further, self-renewal signaling pathways, downstream targets, and EMT signaling genes were concomitantly downregulated. Collectively, our results suggest securin as a novel marker for stem cells/CSCs that also regulates the expression of several stem cell and CSC-related genes through the regulation of self-renewal pathways. To the best of our knowledge, this is the first study proposing securin/PTTG1 as a novel stem cell and CSC marker, which may induce the transformation of normal stem cells to CSCs.

276

ETV7 as novel mediator of chemoresistance in breast cancer

Laura Pezzè[1], Federica Alessandrini[1], Erna Marija Meskyte[1], Stefano Pontalti[1], Kalina Badowska[1], Daniel Menendez[2], Michael A. Resnick[2], Yari Ciribilli[1*]
[1]Laboratory of Molecular Cancer Genetics, Department CIBio (Cellular, Computational and Integrative Biology), University of Trento, Povo (TN), Italy; [2]Genome Integrity and Structural Biology Laboratory, National Institute of Environmental Health Sciences (NIHES), NIH, Research Triangle Park, NC
[*]E-mail: yari.ciribilli@unitn.it

Breast cancer (BC) treatment often includes Doxorubicin and/or other DNA damaging agents as adjuvant as well as neoadjuvant chemotherapy. Despite their cytotoxicity, cancer cells can develop drug resistance to these drugs. Uncovering pathways and mechanisms involved in drug resistance is an urgent and critical aim for breast cancer research oriented to improve treatment efficacy. We have recently demonstrated that different chemotherapeutic drugs, and particularly Doxorubicin, induce the expression of ETV7, a poorly studied transcriptional repressor member of the ETS family. We generated MCF7, MDA-MB-231 and T47D BC-derived cells stably overexpressing ETV7 and we tested the sensitivity of these cells to the chemotherapeutic drugs Doxorubicin and 5-Flouorouracil (5-FU). We observed a reduction in the sensitivity of these BC cells overexpressing ETV7 to both drugs, also highlighted by a diminished cell death. We have also demonstrated that the ETV7 expression led to the downregulation of DNAJC15, a co-chaperone protein whose low expression was previously associated with drug resistance in breast and ovarian cancer. We identified the binding site for ETV7 within the promoter of *DNAJC15* and we also found that DNA methylation may be a factor in ETV-mediated transcriptional repression at the *DNAJC15* promoter. These findings of an inverse correlation between ETV7 and DNAJC15 expression in breast cancer cells in terms of Doxorubicin resistance, correlated well with treatment responses of breast cancer patients with recurrent disease, based on our analyses of reported genome-wide expression arrays. Moreover, we demonstrated that ETV7-mediated Doxorubicin resistance involves increased Doxorubicin efflux via nuclear pumps, which could be rescued in part by DNAJC15 upregulation. Consistent with this observation, we could appreciate an increase in ABC transporters and the BCL2 anti-apoptotic protein expression following ETV7 overexpression. These effects were also accompanied by the observation that alteration of ETV7 expression could significantly affect the population of breast cancer stem cells ($CD44^{+}/CD24^{low}$ cells) in different BC cell lines. With this study, we propose a novel role for ETV7 in breast cancer stem cell plasticity and associated resistance to conventional chemotherapy. We, therefore, suggest that an in-depth investigation of this mechanism could lead to the identification of novel breast CSCs vulnerabilities and the improvement of combinatorial regimens with the aim of avoiding resistance and relapse in breast cancer.

277

Determination of the effectiveness of serum cytokeratins as tumor markers for early-stage breast cancer

Marie Karlikova[1], Ondrej Topolcan[1], Andrea Narsanska[2], Radek Kucera[1], Hana Rezackova[1], Vladislav Treska[2]
[1]Department of Immunochemistry, University Hospital and Faculty of Medicine, Pilsen; [2]Department of Surgery, University Hospital and Faculty of Medicine, Pilsen, Czech Republic

Cytokeratins are specific to epithelial cells and are involved in cancer progression. According to a number of studies, they are overexpressed in breast cancer tissue and also in peripheral blood. Serum levels of cytokeratine fragments (mainly TPA, less CYFRA 21-1) are used as additional tumor markers in breast carcinoma, although their use has not been validated in large-cohort prospective studies and therefore is not recommended in official guidelines. In this study, we focused on the following clinical issues: i) Whether pre-surgery serum levels of cytokeratins reflect the clinical stage and the lymph-node status, and whether they can thus assist in patient prognosis. ii) Determination of the use of cytokeratins patient follow-up. We investigated serum TPA, CYFRA 21-1 and MonoTotal levels (both pre-surgery and at follow-up) together with CA 15-3 and CEA in the sera of 206 patients with invasive breast cancer. A group of 46 women with benign breast disease served as the control group. We found a statistically significant difference between the malignant and benign groups as regards the serum levels of CEA (1.3 vs. 0.8 ng/ml, $p<0.0001$), CA 15-3 (12.0 vs. 9.0 kIU/l, $p=0.0037$) and CYFRA 21-1 (1.3 vs 1.0 µg/l, $p=0.0289$). TPA and CYFRA 21-1 exhibited a statistically significant difference in clinical stage III compared to clinical stage II, and also when comparing the group of patients with positive and negative lymph nodes. In the follow-up, CYFRA 21-1 had the best AUC under the ROC curve (0.9115), followed by TPA (0.9072) and CA 15-3 (0.07873). We concluded that CYFRA 21-1 overcomes TPA both as prognostic marker and marker of follow-up. The best combination of markers for follow-up seems to be CA 15-3 and CYFRA 21-1, increasing the sensitivity of individual markers.

278

Patient-derived organoids as a potential model to predict response to PD-1/PD-L1 checkpoint inhibitors

Giosue Scognamiglio[1], Annarosaria De Chiara[1], Flavio Fazioli[2], Michele Gallo[2], Laura Aversa[2], Rosa Camerlingo[3], Roberto Pili[4], Filomena de Nigris[5]
[1]Division of Pathology, Istituto Nazionale Tumori, Fondazione G. Pascale, IRCSS, Naples, Italy; [2]Division of Sarcoma Surgery, Istituto Nazionale Tumori, Fondazione G. Pascale, IRCSS, Naples, Italy; [3]Stem Cell Division, Istituto Nazionale Tumori, Fondazione G. Pascale, IRCSS, Naples, Italy; [4]Department of Hematology/Oncology, Indiana University Medical School, Indianapolis, IN, USA; [5]Department of Precision Medicine, University of Campania L. Vanvitelli, Naples, Italy
E-mail: filomena.denigris@unicampania.it

Immunotherapy targeting the programmed cell death receptor ligand 1 (PD-L1) is an emergent treatment for chordomas. Determination of patient eligibility largely depends on PD-L1 immunohistochemistry, but prediction of response is complicated by variability in expression and detection of PD-L1. To address this challenge, we retrospectively analysed PD-L1 expression in the surgical specimens of 24 chordoma patients by comparing sensitivity and specificity of two antibodies (IHC 28-8 and E1L3N) and correlating results with clinical parameters. PD-L1 expression was scored by intensity and percentage of positive tumour cells and lymphocytes. Furthermore, we developed a spheroid model from patient-derived cells to assess the individual response to antibody treatment. E1L3N and 28.8 antibodies showed a significant linear correlation ($R^2=0.69$ beta=0.83, $p=0.001$). However, E1L3N was more sensitive and specific for cell membranes (ROC=0.896 area) and yielded higher tumour scores ($p=0.001$). E1L3N detected PD-L1 in 54% of chordomas, with positive tumour cells varying from 1-15% of the tumour area. In 84.6% of cases, tumour-infiltrating lymphocytes present at the neoplastic lobules margins were also positive for PD-L1, ($R^2=0.556$, $p=0.001$). PD-L1 expression was associated with greater tumour diameter ($p=0.014$). Spheroids generated from chordoma biopsies showed significant dose-dependent treatment effects (decreased the diameter and PD-L1-expression, and increased apoptosis) with Nivolumab. Marked differences in detection of PD-L1 in chordomas indicate that standardization of diagnostic immunohistochemistry with a focus on sensitivity and antibodies such as E1L3N is needed. Spheroid models constitute a novel approach to predicting individual treatment responses even in patients with low or no immunohistochemical PD-L1 expression.

Key words: chordoma, biopsy, organoids, PD-L1, prognostic markers, immunotherapy

279

Prognostic value of contrast-enhanced MRI texture in the primary central nervous system lymphoma

Wen Guo[1], Chaoyue Chen[2,3], Xuelei Ma[2,3]
[1]West China School of Medicine, West China Hospital, Sichuan University; [2]Depatment of Biotherapy, Cancer Center, West China Hospital, Sichuan University; [3]State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, Sichuan University, and Collaborative Innovation Center for Biotherapy, Chengdu, Sichuan, P.R. China
E-mail: guowen.lisa@qq.com

The purpose of this study was to evaluate the prognostic value of contrast-enhanced Magnetic Resonance Imaging (MRI) texture features in patents with primary central nervous system lymphoma (PCNSL). This retrospective study included 52 patients diagnosed with PCNSL. The texture feature statistics of tumor tissue were retrieved from contrast-enhanced MRI prior to any anti-tumor treatment. Receiver operating characteristics curve analyses were performed to obtain their optimal cut-off values, based on which we dichotomized each texture parameter. The Kaplan-Meier analysis was conducted to compare overall survival (OS) in subgroups. Multivariate Cox regression analysis was used to determine whether the features could be independent prognostic factors. The number of features extracted from MRI images was 47, five of which (GLCM-Contrast, GLCM-Dissimilarity, GLCM-Homogeneity, GLZLM-LZE, GLZLM-LZHGE) were shown to be significant in relation to OS. The multivariate Cox regression analyses suggested two features (GLZLM-LZE and GLZLM-LZHGE) could be considered as independent predictors while the remaining features could not. The texture features of contrast-enhanced Magnetic Resonance Imaging (MRI) could potentially serve as prognostic biomarkers for PCNSL patients.

280

Expression of the EMT-associated transcription factor Slug/SNAI2 in epithelial colon cancer cells is accompanied by increased invasiveness and VEGF secretion but not by altered drug sensitivity

Katyana Amilca[1-3], Anaïs Bouygues[1-3], Sandrine Thouroude[1-3], Lila Louadj[1-3], Stine Christensen[1-3], Michèle Sabbah[1-4], Jerôme Denis[1-3,5], Annette K. Larsen[1-4]
[1]Cancer Biology and Therapeutics, Centre de Recherche Saint-Antoine (CRSA), Paris, France; [2]Institut National de la Santé et de la Recherche Médicale (INSERM) U938, Paris, France; [3]Institut Universitaire de Cancérologie (IUC), Faculté de Médecine, Sorbonne Université, Paris, France; [4]Centre National de la Recherche Scientifique, Paris, France; [5]Departement d'Oncogénétique et d'angiogénétique moléculaire CHU Hôpital Pitié-Salpêtrière, Paris, France
E-mail: annette.larsen@sorbonne-universite.fr

The epithelial to mesenchymal transition (EMT) in tumor cells has been associated with increased invasiveness and drug resistance. The EMT factor Slug/SNAI2 is closely associated with the EMT phenotype in colorectal cancer (CRC) patients. However, genetic models present conflicting data regarding the activity of Slug, most likely due to differences in the cellular background. In the present study, we characterize a series of Slug-transfected cells using the highly epithelial HT-29 cell line as parental cells. Slug expression was associated with an altered morphology but no detectable differences in cell growth. The epithelial markers CDH1, CDH17, CK7 and CK20 were downregulated whereas the mesenchymal markers vimentin and fibronectin were increased. Slug expression was accompanied by increased migration and invasion that, at least in part, was associated with increased secretion of the vascular endothelial growth factor (VEGF), a potent pro-migratory factor for CRC cells. In clear contrast, no detectable differences in drug sensitivity were observed for any of the anticancer agents tested, including both cytotoxic (5-FU oxaliplatin, irinotecan) and angiokinase-targeted agents (nintedanib and regorafenib). Taken together, our results indicate that increased expression of Slug in an epithelial phenotype is associated with a higher invasive potential without detectable changes in growth or drug sensitivity.

281

Role of Cathepsin B circular RNAs in tumor cells

Manu Gnanamony[1], Christopher S. Gondi [2,3,4]
Departments of, Pediatrics[1], Pathology[2], Surgery[3] and Internal Medicine[4], University of Illinois College of Medicine, Peoria, IL, USA
E-mail: gondi@uic.edu

Pancreatic Ductal Adeno Carcinoma (PDAC) is the third most common cause of cancer-related mortality in the United States and accounts for over 95% of all pancreatic cancers. The combined 1- and 5-year survival rates for PDAC are very poor, at 25% and 7% respectively. A major hallmark of pancreatic cancer is tumor recurrence and extremely poor response to chemotherapy. Cathepsin B is known to be one of the factors involved in stem cell maintenance. The role of circular RNAs encoded by the Cathepsin B gene (CTSB) remains a unknown. It is now recognized that most protein coding genes not only produce linear mRNA, but also produce circular RNAs and this output ratio from linear to circular is dependent on the efficiency of pre-mRNA processing. Thirteen circular RNAs have been identified which are produced from the CTSB gene. Circular RNAs (cirRNAs) are now slowly being recognized as belonging to a significantly importantly regulatory layer. The functions of these CTSB coded circular RNAs have yet not been identified. From our data, it was found that CRISPR mediated the knockdown of Cathepsin B (CTSB) in pancreatic cancer cells, revealing significant changes in the expression profiles of various circRNAs. We observed a 15.9-fold upregulation of hsa_circRNA_042488 and a 20.5-fold downregulation of hsa_circRNA_101692 in the MIA PaCa-2 cells in which CTSB was knocked down and a 4.2-fold upregulation of hsa_circRNA_081069 and a 3.8-fold downregulation of hsa_circRNA_104169 in the PANC-1 cells in which was CTSB knocked down. This variation in differential up- or downregulation of the expression of circRNAs may be attributed to the varying phenotype of MIA PaCa-2 and PANC-1 pancreatic cancer cells. Notably, the PANC-1 cells in which CTSB was knocked down for failed to establish tumors in nude mice. From the further analysis of CTSB circRNA, we observed that CTSB hsa_circRNA_0083350 had the highest number of eukaryotic initiation factor 4A-3 (EIF4A3) binding sites. We thus concluded that circular RNAs play a significant role in tumorigenesis.

Acknowledgements: This study was supported by The McElroy Foundation, Springfield IL, USA and the Theresa Tracy Strive to Survive Foundation, East Peoria IL, USA.

282

Autophagy dependence of small molecule angiokinase inhibitors in colorectal cancer

Lila Louadj[1-3], Paul Mésange[1-3], Michèle Sabbah[1-4], Annette K. Larsen[1-4]
[1]Cancer Biology and Therapeutics, Centre de Recherche Saint-Antoine (CRSA), Paris, France; [2]Institut National de la Santé et de la Recherche Médicale (INSERM) U938, Paris, France; [3]Institut Universitaire de Cancérologie (IUC), Faculté de Médecine, Sorbonne Université, Paris, France; [4]Centre National de la Recherche Scientifique (CNRS), Paris, France
E-mail: annette.larsen@sorbonne-universite.fr

Autophagy plays an important role in the response of tumor cells to environmental stress. Small molecule angiokinase inhibitors target multiple receptor tyrosine kinases on cells in the tumor environment that mediate downstream signaling pathways which can be integrated at the level of mTOR, a central regulator of cell metabolism. Currently, little is known about the interplay between autophagy and angiokinase inhibitors. We here aimed to establish the influence of autophagy on the activity of nintedanib and regorafenib, two small molecule angiokinase inhibitors with clinical activity in metastatic CRC. Our results showed that nintedanib and regorafenib displayed comparable activity towards a panel of 12 well-characterized CRC cells, with average IC_{50} values between 2 and 2.6 µM. However, the activity profile towards the different tumor cell line was markedly different. Immunocytochemistry and western blot analysis of the autophagic marker LC3 as well as the Autophagy Blue™ fluorescence assay showed that nintedanib, but not regorafenib, triggered a strong autophagic response. Interestingly, addition of the autophagy inhibitor 3-methyladenine decreased the cytotoxic activity of nintedanib up to 3-fold, but had no influence on the activity of regorafenib. In agreement, genetic models with attenuated expression of the autophagy regulator Beclin 1 showed up to 3-fold decreased sensitivity to nintedanib, but similar sensitivity to regorafenib. Taken together, our results indicate that autophagy contributes to the cytotoxic activity of at least some angiokinase inhibitors. We propose that tumor cells with high autophagic flux may be selectively sensitive to nintedanib.

Financial support: This research was financed in part by Boehringer Ingelheim.

283

Contrast-enhanced CT-based textural parameters as potential prognostic factors of survival for colorectal cancer patients receiving targeted therapy

Xuelei Ma[1,2], Jing Yang[1,2], Tao Zhang[3]
[1]Depatment of Biotherapy, Cancer Center, West China Hospital, Sichuan University; [2]State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, Sichuan University and Collaborative Innovation Center for Biotherapy; [3]West China School of Medicine, West China Hospital, Sichuan University, Chengdu, P.R. China
E-mail: drmaxuelei@gmail.com

This study was designed to estimate the clinical significance of the contrast-enhanced computed tomography textural features for prediction of survival in colorectal cancer patients receiving targeted therapy (bevacizumab and cetuximab). The LIFEx software was used to extract the textural parameters of the tumor lesions in the contrast-enhanced computed tomography. Progression-free and overall survival was estimated using the Kaplan-Meier method. Univariate and multivariate analyses using the Cox proportional hazards model were performed to assess the prognostic value of textural parameters. In total, 80 colorectal cancer patients receiving targeted therapy (bevacizumab 42; cetuximab 38) were included. In the multivariate analysis, 8 textural parameters were revealed to be independent predictors of progression-free and overall survival, including skewness ($p<0.001$, $p=0.003$, respectively), kurtosis ($p<0.001$, $p=0.007$, respectively), homogeneity ($p=0.018$, $p=0.003$, respectively), energy ($p=0.005$, $p=0.002$, respectively) and entropy ($p=0.032$, $p=0.025$, respectively) of gray-level co-occurrence matrix, LRE ($p=0.016$, $p=0.003$, respectively), LRHGE ($p=0.002$, $p=0.001$, respectively), and contrast ($p=0.001$, $p=0.010$, respectively). Furthermore, sphericity ($p=0.002$), compacity ($p=0.007$), LRLGE ($p=0.014$), LZGE ($p=0.005$) and SZLGE ($p=0.015$) were significantly associated with progression-free survival, while entropy ($p=0.013$) and energy ($p=0.015$) from histogram-based matrix, dissimilarity ($p=0.023$), SRE ($p=0.011$), SRLGE ($p=0.024$), RP ($p=0.003$), LZE ($p=0.0033$), LZLGE ($p=0.033$) and LZHGE ($p=0.033$) were significantly associated with overall survival. In conclusion, our study provides preliminary evidence that several radiomic parameters derived from CT images were prognostic factors and predictive markers for CRC patients who are candidates for targeted therapy (bevacizumab and cetuximab).

284

Discrimination of pituitary adenomas and craniopharyngioma on MRI: From image features to texture features

Hanyue Xu[1], Wen Guo[1], Xuelei Ma[2]
[1]West China School of Medicine, West China Hospital, Sichuan University; [2]State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, Sichuan University and Collaborative Innovation Center for Biotherapy, Chengdu, P.R. China
E-mail: xhydoris@gmail.com

The aim of the study was to explore the differences of MRI scanning between pituitary adenomas and craniopharyngioma from MR image features to 3D-based texture features. A total number of 131 patients were introduced into this study (pituitary adenomas, 68; craniopharyngioma, 63) with pre-surgery MRI image. Qualitative MR features and MRI texture features of the lesion were evaluated using Chi-square test, Fisher's exact test or the Mann-Whitney U test. Multivariate logistic regression analyses were performed to assess their ability as independent predictors. Accuracy measures were calculated substantially for the significant features. Five MRI features were suggested to be significantly different between pituitary adenomas and craniopharyngioma. One of these features, i.e., cystic alteration, was considered an independent practical predictor. Three texture features from contrast-enhanced images (Histo-Skewness, GLCM-Contrast and GLCM-Energy), two texture features from T2WI (Histo-Skewness and GLCM-Contrast) were significantly associated with discrimination between two types of diseases. Two texture features (Histo-Skewness and GLCM-Contrast) were significantly associated with cystic alteration. Both MRI image features and texture features could make significant discrimination between pituitary adenoma and craniopharyngioma and represent practical diagnostic value. In addition, the two types of features are associated with each other.

285

Epigenetic analysis of depression-like behavior in interleukin-18-deficient mice

Kyosuke Yamanishi[1], Keiichiro Mukai[2], Takuya Hashimoto[2], Masahiro Miyauchi[2], Kaoru Ikubo[2], Noriko Uwa[2], Yuko Watanabe[3], Chiaki Yamanishi[3], Haruki Okamura[1], Hisato Matsunaga[1,2]
[1]Department of psychoneuroimmunology and [2]Department of Neuropsychiatry, Hyogo College of Medicine, Nishinomiya, Hyogo; [3]Hirakata General Hospital for Developmental Disorders, Hirakata, Osaka, Japan
E-mail: k-yama@hyo-med.ac.jp

Interleukin-18 (IL-18) is an interferon γ-inducing inflammatory cytokine and related to not only to the immune system, but also to non-immunological functions (1). We previously investigated interleukin-18-deficient (*Il18*$^{-/-}$) mice which exhibited not only metabolic disorders, such as dyslipidemia, but also depression-like behavioral changes induced by hippocampal abnormalities (2,3). However, the mechanisms responsible for these behavioral changes remain to be clarified. It has been shown that the remodeling of chromatin via histone acetylation is related to memory impairment. Therefore, in this study, we hypothesized that there may be a close asociation between IL-18 and histone acetylation. *Il18*$^{-/-}$ male mice were generated from C57Bl/6 mice and as controls, littermate C57Bl/6 *Il18*$^{+/+}$ male mice were used. At 12 weeks of age, the hippocampus of each mouse was extracted. The expression levels of acetylated H3 lysine 9, H3 lysine 14, H4 lysine 5, H4 lysine 8, H4 lysine 12, and H4 lysine 16 were examined. To determine the response of IL-18, recombinant IL-18 was administered intracerebrally twice a week. In the hippocampus of the *Il18*$^{-/-}$ mice, only histone H4 lysine 16 (H4K16) acetylation was significantly increased compared to that of the *Il18*$^{+/+}$ mice. Therefore, these results suggested that in the hippocampus, increased H4K16 acetylation promoted granule cell proliferation as previously described. Taken together, the deficiency of IL-18 may lead to increased H4K16 acetylation to compensate for hippocampal impairment.

Acknowledgements: This study was supported by Japan Society for the Promotion of Science KAKENHI Grant Number JP17K16404.

(1) Okamura et al. Nature. 378: 88-91, 1995.
(2) Yamanishi et al. Transl Res. 2016 Jul; 173: 101-114.e7.
(3) Yamanishi et al. Neuroscience. 2019 Apr 11. pii: S0306-4522(19): 30241-30246.

286

Molecular analysis causing depressive-like behaviors in interleukin-18-deficient mice: Gene expression profiles in the brain

Takashi Ikawa[1], Kyosuke Yamanishi[2], Keiichiro Mukai[3], Takuya Hashimoto[3], Masahiro Miyauchi[3], Kaoru Ikubo[3], Noriko Uwa[3], Yuko Watanabe[1], Chiaki Yamanishi[1], Haruki Okamura[2], Hisato Matsunaga[2,3]
[1]Hirakata General Hospital for Developmental Disorders, Hirakata, Osaka; [2]Department of Psychoneuroimmunology and [3]Department of Neuropsychiatry, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan
E-mail: d1.kawataka457@gmail.com

Interleukin-18 (IL-18) is an interferon γ-inducing inflammatory cytokine and is related to not only to the immune system, but also to non-immunological functions. IL-18-deficient (*Il18*$^{-/-}$) mice exhibit diabetes mellitus. We previously investigated *Il18*$^{-/-}$ mice, demonstrating that these mice also exhibit obesity, dyslipidemia and non-alcoholic steatohepatitis (1). Moreover, we also found *Il18*$^{-/-}$ mice exhibited depressive-like behavioral changes (2). Hence, IL-18 plays a number of important roles related to immunity, energy homeostasis and psychiatric disorders. In this study, we analyzed gene expression profiles in the brains of *Il18*$^{-/-}$ mice in order to identify the genes responsible for causing depressive-like behaviors, as well as other impairments. Using whole genome microarray analysis, the gene expression profiles in the brains of *Il18*$^{+/+}$ and *Il18*$^{-/-}$ mice at 6 and 12 weeks of age were examined and compared. Subsequently, we categorized genes using Ingenuity Pathway Analysis (IPA). A total of 2,805 genes were extracted in the microarray at 12 weeks of age. Surprisingly, the molecules from IPA core analysis related to 'depression' were shown, and 13 molecules related to depression were isolated. We confirmed their expression and correlation by RT-qPCR. Cyclin D1, CCAAT enhancer binding protein delta, endothelin 1, FosB proto-oncogene and NADPH oxidase 4 at 6 weeks of age had an influence on fibroblast growth factor receptor 1, corticotropin releasing hormone, chromogranin A, and protein tyrosine phosphatase, non-receptor type 1 among 13 genes at 12 weeks of age. Therefore, our results suggested that 5 molecules extracted at 6 weeks of age were affected by IL-18 impairment and responsible genes inducing the depression-like phenotype at 12 weeks of age.

Acknowledgements: This study was supported by Japan Society for the Promotion of Science KAKENHI Grant Number JP17K16404.

1) Yamanishi et al. Transl Res. 2016 Jul; 173: 101-114.e7;
2) Yamanishi et al. Neuroscience. 2019 Apr 11. pii: S0306-4522(19) 30241-6.

287

Chemotherapeutic stress is accompanied by pro-invasive signaling in colorectal cancer models

Sandrine Thouroude[1-3], Patrick Van Dreden[4], Grigoris T. Gerotziafas[1-3,5], Annette K. Larsen[1-3,6]
[1]Cancer Biology and Therapeutics, Centre de Recherche Saint-Antoine (CRSA); [2]Institut National de la Santé et de la Recherche Médicale (INSERM) U938; [3]Institut Universitaire de Cancérologie (IUC), Faculté de Médecine, Sorbonne Université; [4]Research and Development, Diagnostica Stago, Gennevilliers; [5]Service d'Hématologie Biologique, Hôpital Tenon, Hôpitaux Universitaires Est Parisien; [6]Centre National de la Recherche Scientifique, Paris, France.
E-mail: annette.larsen@sorbonne-universite.fr

Cancer mortality is closely associated with the presence of drug-resistant, invasive subpopulations of tumor cells[1]. However, the functional and mechanistic interactions between the two phenotypes are incompletely understood[2]. We have developed a panel of 4 isogenic CRC cell lines comprised of the parental HCT-116 cells and three independently derived sublines resistant to 5-fluorouracil, oxaliplatin and SN-38. All resistant cell lines showed increased migration and invasion. Tumor vascularization is needed for tumor growth as well as for dissemination of tumor cells. Capillary endothelial cells usually provide tumor blood flow. However, increasing evidence suggests that some tumor cells are able to form vascular structures that are connected with the endothelial cells and are able to sustain blood flow. This process is known as vasculogenic/vascular mimicry and has been associated with a highly invasive and metastatic cancer phenotype. Cellular growth in matrigel revealed that two of the resistant cell lines had acquired the capacity to form cellular networks *in vitro* in contrast to the parental cells. Further work will include antibody arrays and ELISA assays to identify key players in the tumor cell signaling network. Taken together, our results indicate that prolonged chemotherapeutic stress can be accompanied by an increased invasive potential. We further suggest that this may not be limited to colorectal cancer cells but is likely applicable to a wide range of other tumor types.

(1) M Ayadi et al., Oncotarget 6: 18518-18533, 2015.
(2) AE Escargueil et al., Curr Pharm Des 22: 6625-6644, 2016.

288

Lithium chloride increases sensitivity to photon radiation treatment in primary mesenchymal colon cancer cells

Francesca Cammarota[1], Antonietta Aversano[1], Gianluca Ametrano[2], Paolo Delrio[3], Patrizia Riccio[1], Mimmo Turano[4], Valeria Valente[1], Paola Izzo[1], Giovanna Maria Pierantoni[1], Marina De Rosa[1]
[1]Department of Molecular Medicine and Medical Biotechnology, University Federico II of Naples; [2]UOC Radiation Oncology, Istituto Nazionale per lo Studio e la Cura dei Tumori – Fondazione "Giovanni Pascale"; [3]Division of Gastrointestinal Surgical Oncology, "Istituto Nazionale Tumori - IRCCS - Fondazione G. Pascale"; [4]Department of Biology, University of Naples Federico II; Naples, Italy
E-mail: marina.derosa@unina.it

Colorectal cancer was the second most common cause of cancer mortality in 2018, mainly due to resistance to therapy. Chemoradiotherapy often kills only differentiated cancer cells, while the more undifferentiated cells, the mesenchymal and stem cells, can survive after therapy, giving rise to therapy-resistant tumours. We previously isolated two primary colon adenocarcinoma cell cultures that had undergone epithelial-to-mesenchymal transition. Since GSK3β is an important regulator of cell survival that promotes tumorigenesis in colon cells, we explored the effects of the specific GSK-3β inhibitor LiCl on cell motility and plasticity, demonstrating that LiCl reduced cell migration, stemness features and cell plasticity (1,2). Although radiation therapy is more often used to treat people with rectal cancer than individuals with colon cancer, it may be offered for colon cancer treatment in specific cases. Thus, we investigated the effect of X-ray alone or in combination with LiCl pre-treatment, on the viability of T88 primary colon cancer cells. We initially examined the photon radiation effect on cell viability of T88 primary colon cancer cells and of commercially available colon cancer RKO, observing that photon radiation treatment affects the viability of RKO but not that of T88 cells which appear completely unresponsive. Furthermore, as expected, we observed that LiCl sensitises primary colon cancer cells to photon radiation treatment. Finally, we explored the molecular basis of this response by analysing the expression of proteins involved in the apoptotic mechanism and in death escape, such as Bax, Bcl-2, p53 and survivin proteins, under cell treatments. We found that LiCl downregulates survivin and Bcl-2, but upregulates p53, BAX and beta-catenin proteins and effects are maximum in irradiated cells pre-treated with LiCl. In our opinion, these preliminary data suggest that LiCl could represent an interesting drug useful to sensitise colon cancer cells resistant to photon radiation.

1) Int J Oncol. 2015 46(5): 1913-1923.
2) Int J Oncol. 2018 53(6): 2379-2396.

289

MicroRNA biomarkers in sleep apnea

Rezackova Hana[1,3], Houfkova Katerina[4], Slouka David[2], Windrichova Jindra[1], Pesta Martin[1,4], Kucera Radek[1,3], Topolcan Ondrej[1,3]
[1]Laboratory of Immunoanalysis, Faculty Hospital in Pilsen; [2]Department of Otolaryngology, Faculty Hospital in Pilsen; [3]Central Isotopic Laboratory, Faculty of Medicine in Pilsen, Charles University in Prague; [4]Department of Biology Faculty of Medicine in Pilsen, Charles University in Prague, Prague, Czech Republic
E-mail: rezackovah@fnplzen.cz

Disordered breathing linked to sleep apnea (SA) is associated with hypoxia, oxidative stress endothelial dysfunction and sympathetic activation serving as mediators of cardiovascular disease (CD) and heart muscle damage. Patients with SA often suffer hypertension, coronary heart disease, heart failure and stroke. Vice versa SA is highly prevalent in patients with CD. Several discoveries in the pathogenesis and treatment of CD help to manage SA, but there are no biomarkers for diagnosis, to stratify the patients and set the risk of complications. As SA is associated with risk of cardiac muscle damage, we chose cardio-specific miRNA as potential biomarkers. The aim of the study was to improve diagnostics in SA and to evaluate miRNA specific for the myocardium for the diagnosis and risk of CD. Three circulating miRNAs: miR-1-3p, miR-133a-3p and miR-499a-5p were measured and compared to the clinical status. There was enrolled cohort of 194 patients with SA at the time of diagnosis in specialized ambulance. The group comprised 130 men and 64 women with a median age of 62.5 years. Venous blood was obtained using K_3EDTA tubes. miRNAs were isolated from 200 µl plasma by miRNeasy Serum/Plasma Kit (Qiagen) with cel-miR-39 (Qiagen) as a spike-in control and exogenous normalizer. RT was performed using TaqmanMicro RNAReverse Transcription kit (Thermo Fisher Scientific). Quantification of miRNAs was carried out using TaqMan MicroRNA Assays (Thermo Fisher Scientific) and measured by real-time PCR on LightCycler®96 System (Roche). The relationship of circulating miRNAs levels and clinicopathological characteristics of patients will be presented. A variety of potential biomarkers, including ncRNA or proteins, have to be compared to establish the single or combination of markers for clinic.

290

Integrated analysis highlights multiple long non-coding RNAs reveal the potential roles in progression of human esophageal squamous cell carcinoma

Cheng-Yun Li[1*], Wen-Wen Zhang[1], Ji-Lian Xiang[2], Xing-Hua Wang[3], Jun-Ling Wang[1], Jin Li[1]
[1]Department of Toxicology, School of Public Health, Lanzhou University, Lanzhou; [2]Third people's hospital of Gansu province, Lanzhou, Gansu; [3]Gansu Wuwei Tumor Hospital, Wuwei, Gansu, P.R. China
[*]E-mail: 13919995713@163.com

Esophageal squamous cell carcinoma (ESCC) is a prevalent aggressive malignant tumor with a poor prognosis. The investigation of the molecular changes occurring in ESCC, as well as the identification of novel biomarkers for ESCC diagnosis and prognosis is of utmost importance. Long non-coding RNAs (lncRNAs) have been reported to play a critical role in tumor progress. In this study, we conducted our data mining analysis for ESCC by the integrated analysis of accumulated datasets and the identification the differentially expressed lncRNAs from Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA) databases. We identified intersection ESCC tumor tissues differentially expressed genes (lncRNAs, miRNAs and mRNAs) between the GEO and TCGA datasets. Based on these intersection lncRNAs, we constructed the lncRNA competitive endogenous RNA (ceRNA) network of ESCC. A total of 81 intersection lncRNAs were identified, and 67 of these participated in the ceRNA network. Functional analysis revealed that these 67 key lncRNAs mainly dominated in cellular biological process. We then analyzed the associations between the expression levels of these 67 key lncRNAs the clinicopathological features and survival of ESCC patients from TCGA. In total, 31 of these lncRNAs were associated with tumor grade, TNM stage and lymphatic metastasis status ($P<0.05$). In addition, 15 key lncRNAs were found to be associated with the survival of ESCC patients from TCGA ($p<0.05$). Finally, 5 key lncRNAs were randomly selected for validation of their real expression levels in 30 newly diagnosed patients with ESCC by RT-qPCR. The results suggested that the fold changes of up- and downregulation trends between GEO, TCGA and RT-qPCR were completely consistent. In addition, we also found that some of these 5 key lncRNAs were significantly associated with tumor TNM staging and lymph-node metastasis ($p<0.05$). Clinically relevant analysis and the above bioinformatics analysis results in relative agreement, and prove that our bioinformatics analysis is credible. Overall, this study provides further insight into the lncRNA functional features of ESCC through bioinformatics integrative analysis of GEO and TCGA datasets, and reveals the potential diagnosis and prognosis biomarkers for ESCC.

Key words: esophageal cancer, lncRNA, clinical features, overall survival

Acknowledgements: This study was financially supported by the Fundamental Research Funds for the Central Universities (lzujbky-2018-13), National Natural Science Foundation of China (81803188), Gansu Province Science and Technology Project (1606RJYA270).

291

The prognostic value of 18F-FDG PET/CT radiomics features in patients with primary gastric diffuse large B-cell lymphoma

Hongbin Liang[1], Ruofan Zhou[2], Xuelei Ma[3]
[1]No. 202, Central Xincheng Commercial Building, North section 1, Guanghan, Sichuan; [2]West China School of Medicine, West China Hospital, Sichuan University; [3]State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, Sichuan University and Collaborative Innovation Center for Biotherapy, Chengdu, P.R. China
E-mail: 782880812@qq.com

The aim of the study was to determine whether radiomics features from 18fluorodeoxyglucose (FDG) positron emission tomography/computed tomography (PET/CT) could contribute to prognoses in primary gastric diffuse large B-cell lymphoma (PG-DLBCL). This retrospective study included 35 patients who underwent PET/CT at West China Hospital prior to curative treatment. Regions of interest (ROIs) were drawn around the tumor, and texture analysis was conducted on both PET and CT images within the same ROIs. Conventional metabolic tumor parameters and textural features from PET and CT were evaluated. The extracted features were correlated with overall survival (OS) and progression-free survival (PFS). Univariate and multivariate analyses were conducted to assess the prognostic value of radiomics parameters. In the univariate model, 15 radiomics features extracted from PET and CT datasets were significantly associated with survival (5 for OS and 2 for PFS respectively, from the PET dataset; 8 for OS and 14 for PFS respectively, from the CT dataset, including skewness and volume). Multivariate analysis identified kurtosis (HR 33.994, p=0.009), volume ml (HR 25.382, p=0.013), GLNUGLRLM (HR 14.642, p=0.002) in PET and spherity (HR 13.047, p=0.030), GLNUGLRLM (HR 11.164, p=0.032) HGZEGLZLM (HR 11.207, p=0.008) in CT as independent prognostic factors. The textural features of the 18F-FDG PET/CT are possibly useful for survival prediction in PG-DLBCL. However, studies with a larger cohort are needed to confirm clinical prognostication of these parameters.

292

Modified-FOLFIRINOX combined with deep regional hyperthermia in pancreatic cancer: A retrospective study on Chinese patients and advances in hyperthermia

Meng He, Jinghua Sun, Danyi Zhao, Hongmei He, Bing Wang, Lingling Xu, Yu Shang, Shanling Ren, Yang Zhang, Tao Wu
Department of Oncology, Second Affiliated Hospital of Dalian Medical University, Dalian, P.R. China
E-mail: dlykdxhemeng@163.com

FOLFIRINOX chemotherapy displays significant survival improvements in patients with pancreatic cancer. However, toxicities have hampered enthusiasm for the use of FOLFIRINOX in full doses. In order to increase the tolerability, many researchers have focused on the modification of FOLFIRINOX. On the other hand, hyperthermia (HT) has been considered as an effective ancillary treatment for cancer therapy. To date, at least to the best of our knowledge, there are no studies available evaluating the combination of deep regional hyperthermia (DRHT) with modified-FOLFIRINOX for pancreatic cancer patients. In this study, we conducted a retrospective review of pancreatic cancer patients treated with the combination of new form modified-FOLFIRINOX and DRHT (BSD2000). Patients underwent chemotherapy that included low-dose irinotecan, oxaliplatin on day 1 and 5-FU or capecitabine (CAP) or tegafur, gimeracil and oteracil potassium (TS-1), for a 2-week schedule. Generally, DRHT treatment was performed weekly, 45 min for each time during chemotherapy. The patients receiving mFOLFIRINOX as the first line chemotherapy combined with DRHT exhibited an improvement in OS and PFS, 17 months (95% CI 1.97-32.03 months) and 4 months (95% CI 0–8.29 months), respectively. Overall, this combination regimen was safe; 17.6% patients suffered from grade 3/4 toxicities. In conclusion, the efficacy in the treatment of pancreatic cancer was encouraging; however, further studies are required to prove its merit, compared with conventional treatment (1). As regards the advances in hyperthermia, these are as follows: Tumor stiffening, a key determinant of tumor progression, is reversed by nanomaterial-induced photothermal therapy. Scientists in France investigated the evolution of tumor stiffness, as well as tumor integrity and progression, under the effect of mild hyperthermia and thermal ablation generated by light-exposed multi-walled carbon nanotubes in an epidermoid carcinoma mouse xenograft. This study highlights nanohyperthermia as a promising adjuvant strategy for the reversal of tumor stiffening and normalize the mechanical tumor environment (2).

1) He, M., et al., Modified-FOLFIRINOX combined with deep regional hyperthermia in pancreatic cancer: A retrospective study in Chinese patients. International Journal of Hyperthermia, 2019. **36** (1): p. 394-402.
2) Marangon, I., et al., Tumor stiffening, a key determinant of tumor progression, is reversed by nanomaterial-induced photothermal therapy. 2017. **7**(2): p. 329.

293

Laminin-511: PKM2, the linkage between cancer metabolism and DNA repair

Yi Wang[1,2], Ping Shuai[1], Yuping Liu[1], Shaoping Deng[1,3]
[1]Health Management Center; [2]Center for Translational Medicine and [3]Institute of Organ Transplantation, Sichuan Academy of Medical Science & Sichuan Provincial People's Hospital, P.R. China
E-mail: w_yi@yahoo.com

With a high risk of recurrence and therapeutic resistance, glioblastoma is considered the most malignant and lethal subtype of brain tumours. Our previous studies have indicated that DNA double-strand breaks repair played a central role in the recurrences of gliobastoma after radiotherapy. We also revealed several key regulators, such as GSK3β and 53BP1, played critical role in the DNA repair signalling pathway. Emerging evidence has shown that pyruvate kinase M2 (PKM2), which played essential role in tumour metabolisms, could mediate DNA damage repair. Two main repair pathways, namely homologous recombination (HR) and non-homologous end joining (NHEJ) exist. Currently, no studies have shown by which pathway PKM2 mediated DNA damage repair. Therefore, our study aimed to reveal the significance of PKM2 in DNA double-strand break repair and metabolism. We showed whether PKM2 was phosphorylated and translocated into the nucleus. Then, we aimed to demonstrate by which pathway PKM2 mediated DNA damage repair. Predominantly, PKM2 resides in the cytoplasm, which functions as the main regulator in tumour metabolism. In the current study, we observed the PKM2 translocation from cytoplasm to nuclear under the treatment of ionizing radiation, and thus it played the function of the initiation of HR repair in the nucleus of glioblastoma cells. In addition, by CRISPR/Cas9 technology, DNA double-strand breaks HR repair was abrogated in PKM2 null cells. Furthermore, we explored the phosphorylation site of PKM2. More importantly, *in vitro* and *in vivo* data clearly indicated that inhibition of PKM2, not only abolished the tumour metabolism, but also enhanced radio-sensitivity after ionizing radiation therapy. Based on these results, we concluded that the phosphorylation of PKM2 was indispensable for DNA double-strand breaks HR repair. Therefore, PKM2, which plays a critical role in tumour metabolism, is a potential target for improving radiation sensitivity of glioblastoma.

294

EGF in exhaled breath condensate as diagnostic method for non-small cell lung cancer

Jin-Liang Chen[1], Su-Mei Yao[1], Xue-Dong Lv[1], Qi-Chang Yang[2], Jian-rong Chen[1],
[1]Department of Respiraology and [2]Biochemistry laboratory, Second Affiliated Hospital of Nantong University, Nantong, P.R. China

Lung cancer is one of the most common malignant tumors in human beings. It is very important to find a highly sensitive and specific marker. This study investigated the clinical significance of combined detection of epidermal growth factor (EGF) in exhaled breath condensate (EBC) and serum in patients with non-small cell lung cancer (NSCLC). Between October 17, 2013 to June 5, 2017, EBC samples from 155 NSCLC patients and 115 healthy subjects were collected with a breath condenser. Blood samples of the two groups were also collected. Each sample was analysed by enzyme-linked immunosorbent assay method. The EGF level in EBC from NSCLC group (197.86±60.67 pg/ml) was higher than that of the healthy group (124.75±36.09 pg/ml), P<0.05. The EGF level in EBC in phase III and IV stages of NSCLC group (212.17±35.41 pg/ml) was higher than that in phase I and II stages (173.91±38.08 pg/ml), P<0.05. The EGF level in EBC of the death group (241.05±27.19 pg/ml) was higher than those of the survival group (188.75±37.07 pg/ml), P<0.05. The EBC-EGF levels were positively correlated with the serum-EGF levels with a correlation coefficient of 0.495 (P<0.05). The sensitivity and specificity of EBC-EGF test were 80.0% and 89.6%, respectively. In conclusion, detection of EGF level in EBC has important value in assisting diagnosis, disease monitoring and prognosis of NSCLC.

Key words: exhaled breath condensate, non-small cell lung cancer, epidermal growth factor, detection

295

Study of the epigenetic mechanisms through which miR-155 promotes the cellular growth of liver cancer

Xiaoru Xin[1], Dongdong Lu[1]
[1]School of Life Science and Technology, Tongji University, P.R. China
E-mail: 15801751095@163.com

MicroRNA 155 (miR-155) is known to be highly upregulated in human hepatocellular carcinoma (HCC). Studies have shown that miR-155 is closely related to hepatocarcinogenesis. However, its mechanisms of action as regards the progression of liver cancer remain largely unknown. Furthermore, the epigenetic modification of Histone H3 is also associated with the functions of oncogenes. In this study, we aimed to explore the epigenetic mechanisms of action of miR-155 as regards the acceleration of the malignant progression of liver cancer cells. RT-PCR, western blotting, chromatin immunoprecipitation (CHIP) assay, RNA immunoprecipitation (RIP) and tumorigenesis tests *in vitro* and *in vivo* were performed. miR-155 was found to be overexpressed in the liver cancer tissues of patients with liver cancer metastasis. Moreover, miR-155 accelerated the malignant progression of liver cancer cells *in vitro* and *in vivo*. Mechanistically, miR-155 inhibited the expression of H3F3A by targeting the H3F3A 3'UTR region. In addition, miR-155 reduced the tri-methylation of H3K27 by inhibiting the expression of H3F3A. Notably, H3K27me3 could play the role of a transcription factor as it inhibited the transcription of cyclin-dependent kinase2 (CDK2); the expression of CDK2 then increased. Notably, miR-155 enhanced the interplay between CDK2 and CyclinE, and this was followed by an increase in the phosphorylation of CDK2. Furthermore, the expression of p21 (WAF1/CIP1) was reduced in human liver cancer cells. Of significance, our observations also revealed that pre-miR-155 increased the interplay between CDK2 and CyclinE by forming circRNA. In conclusion, this study elucidates a novel epigenetic mechanism through which miR-155 accelerates the growth of liver cancer cells via the miR-155-H3F3A-H3K27me3-CDK2-CDK2/CyclinE-p21 signaling pathway. This study suggests that miR-155 may be a potential therapeutic target for liver cancer in the future.

Key words: miR-155, H3F3A, H3K27me3, CDK2, p21

296

Study of the molecular mechanisms through which pyruvate kinase M2 (PKM2) regulates the cellular proliferation of liver cancer

Yanan Lu[1], Dongdong Lu[1]
[1]School of Sciences and Technology, Tongji University, P.R. China

Pyruvate kinase M2 (PKM2) is a key glycolytic enzyme, which possesses protein kinase activity and plays an important role in modulating gene expression during tumorigenesis. To date, the functions and regulatory mechanisms of PKM2 have not yet been elucidated. In this study, we focused on the functions and molecular mechanisms of PKM2 during hepatocarcinogenesis. We selected 56 cases of human liver cancer (including cancer tissues and para-cancerous tissues), and performed H&E staining, immunohistochemistry, RT-PCR and immunoblotting to investigate the expression of PKM2 and analyze its clinicopathological significance. Stable Hep3B cell lines were constructed by infection with rLV, rLV-PKM2, pGFP-V-RS and pGFP-V-RS-PKM2, respectively and the growth ability was then determined in *vitro* and *in vivo*, including growth curve, colony information, BrdU staing, sphere formation and Transwell assay. Finally, the functions of PKM2 were explored in the four cell lines using cellular and molecular methods, including immunoprecipitation, CHIP-3C luciferase reporter assay and rescue experiments. This study found that PKM2 was overexpressed in liver cancer tissues and liver cancer tissues of patients with liver cancer metastasis, and PKM2 accelerated the growth and metastatic ability of liver cancer cells *in vitro* and *in vivo*. Mechanically, our results first showed that the interaction among CREBBP, P300, CARM1 and PKM2 was increased when PKM2 was overexpressed in Hep3B cells. Moreover, the expression of NF-κB was enhanced due to the methylation modification of PKM2. PKM2 enhanced the transcription and translation through β-catenin dependent on NF-κB. Nuclear PKM2 promoted the trimethylation of histone H3 on the fourth lysine (H3K4me3), which enhanced the transcription of long non-coding RNA HULC. In particular, PKM2 increased looped HULC in Hep3B cells. PKM2 enhanced the entering of LEF, TCF4 and β-catenin into HULC loops mediated by CTCF, and then increased the interaction among LEF, TCF4 and β-catenin. Thereby, the transcriptional activity of β-catenin was enhanced in liver cancer cells. Therefore, PKM2 activated β-catenin activity through long-non-coding RNA HULC. Our results revealed that PKM2 promoted the binding ability of β-catenin-LEF-TLF4 to the S100A4 promoter region in liver cancer cells, and promoted the expression of S100A4 in human liver cancer cells. Moreover, PKM2 promoted the expression of MMP9 through S100A4 in liver cancer cells, thereby promoting the metastatic ability. Therefore, PKM2 promoted metastasis through S100A4-MMP9 in liver cancer cells. On the other hand, PKM2 promoted the binding of RNAPolII and P300 to long non-coding RNA HOTAIR through β-catenin in liver cancer cells, and then promoted the transcriptional activity of long non-coding RNA HOTAIR and increased its expression in liver cancer cells. Moreover, PKM2 promoted the interplay among EZH2, SUZ12, EED and RbAp46/48 dependent on HOTAIR, and then enhanced the interaction of EZH2, SUZ12, EED and RbAp46/48 with histone H3, which led to increased methylation of Histone H3 at lysine 27, including H3K27me1, H3K27me2 and H3K27me3. Finally, PKM2 increased the loading of H3K27me1, H3K27me2 and H3K27me3 on the promoter region of the tumor suppressor gene p63 dependent on HOTAIR, inhibiting the expression of p63 in liver cancer cells. Rescue experiments revealed that excessive p63 abrogated the oncogenic functions of PKM2 in liver cancer cells. In conclusion, PKM2 accelerates growth via the PKM2- H3K4me3-HULC-LEF/TCF-HOTAIR-p63 signaling pathway. Thus, PKM2 may be a therapeutic target for liver cancer in the future.

Key words: liver cancer, PKM2, liver cancer, liver cancer metastasis, NF-κB, β-catenin, S100A4, MMP9, HOTAIR, p63

297

Normalisation of serum vitamin D levels improves glycemic parameters in patients with type two diabetes mellitus

Mahmoud A. Alfaqih[1], Abdullah Araidah[2]
[1]Department of Physiology and Biochemistry, Faculty of Medicine, Jordan University of Science and Technology, Irbid, Jordan; [2]Department of Medical Laboratory Sciences, Faculty of Allied Medical Health, Jordan University of Science and Technology, Irbid, Jordan
E-mail: maalfaqih@just.edu.jo

Diabetes mellitus (DM) is a worldwide epidemic. In Jordan, the prevalence of DM is increasing. DM is classified into type I DM (T1DM) caused by complete absence of insulin and type II DM (T2DM), caused by insulin resistance. Hyperglycemia in T2DM contributes to complications. Factors that regulate glycemic control are complex. Vitamin D (25-hydroxycholecalciferol) is known for its effect on bone; however, vitamin D has extra-skeletal effects, including mediating insulin action. Vitamin D levels may contribute to glycemic control in T2DM. A case control study was used to test this association. A total of 250 subjects were recruited; of these, 125 were T2DM patients actively treated for their disease, and another 125 patients were subjects free of T2DM at the time of their recruitment. The serum levels of vitamin D were measured using commercially available kits. We found that serum vitamin D levels were significantly lower in T2DM patients ($P<0.05$). Following the above evaluation, vitamin D oral supplementation (50,000 I.U weekly) was administered to patients with low vitamin D levels (n=26) for 3 months. Prior to the intervention, we measured HbA1c, fasting blood glucose (FBG), total serum cholesterol, serum triglycerides and serum insulin which were re-assessed following the intervention. Vitamin D supplementation significantly increased vitamin D serum levels in patients with low vitamin D levels ($P<0.05$). The above increase was accompanied by a significant reduction in HbA1c, FBG, total serum cholesterol, serum triglyceride and serum insulin levels ($P<0.05$). We concluded that the normalization of serum vitamin D levels in T2DM patients may improve glycemic parameters in Jordan.

298

Systematic analyses of a novel microRNA-associated signature as the diagnosis biomarker for esophageal squamous cell carcinoma

Cheng-Yun Li[1*], Wen-Wen Zhang[1], Ji-Lian Xiang[2], Xing-Hua Wang[3], Jun-Ling Wang[1], Jin Li[1]
[1]Department of Toxicology, School of Public Health, Lanzhou University, Lanzhou, P.R. China; [2]Third people's hospital of Gansu province, Lanzhou, Gansu, P.R. China; [3]Gansu Wuwei Tumor Hospital, Wuwei, Gansu, P.R. China
[*]E-mail: 13919995713@163.com

MicroRNAs (miRNAs) have played important roles in the regulation of gene expression in many cancers, but their roles in esophageal squamous cell carcinoma (ESCC) are still unclear. The aim of this study was to determine the potential ESCC-specific key miRNAs from large samples dataset in the Cancer Genome Atlas (TCGA). Integrative bioinformatics analysis was used to identify ESCC-specific key miRNAs related to the ESCC patient tumor histological grade and lymphatic metastasis from TCGA. Next, the key miRNAs potential genes regulatory functions and relationships with ESCC patients' clinical characters and overall survival were analyzed, respectively. Finally, three key miRNAs were selected randomly and RT-qPCR was used to validate the bioinformatics analysis results' reliability and validity. Thirty-five ESCC-specific miRNAs from the TCGA database were investigated (fold-change >2, $p<0.05$), 28 of them were involved in the miRNAs-mRNAs co-expression network construction, and 17 were related to ESCC patients' tumor histological grade, TNM stage and lymphatic metastasis ($p<0.05$). Additionally, six miRNAs (including miR-200b-3p, miR-31-5p, miR-15b-5p, miR-141-3p, miR-135b-5p and miR-195-5p) were correlated with ESCC patient's overall survival (log-rank $p<0.05$). MiR-135b-5p, miR-15b-5p and miR-195-5p were selected for verification of the expression levels in 51 ESCC patients' tissue samples via RT-qPCR. We found that the fold-changes between RT-qPCR and TCGA were completely consistent. Results also suggested that miR-135b-5p, miR-15b-5p and miR-195-5p were significantly correlated with tumor differentiation degrees ($p<0.05$), miR-195-5p was significantly correlated with tumor TNM stage ($p<0.05$), and miR-135b-5p was significantly correlated with lymph-node metastasis ($p<0.05$). MiR-135b-5p, miR-15b-5p and miR-195-5p ESCC patients' clinical relationships and the TCGA bioinformatics analysis were similar. Our study revealed the landscape of ESCC-related key miRNAs. In conclusion, these key miRNAs are worthy of further investigation as potential novel biomarkers for the diagnosis, classification and prognosis for ESCC.

Key words: esophageal cancer, miRNA, clinical features, overall survival

Ethical approval and informed consent
This study was approved by institutional review board and was performed in compliance with hospital ethics and clinical practice guidelines. All patients signed the informed consent.

Acknowledgements: This study was financially supported by the Fundamental Research Funds for the Central Universities (lzujbky-2018-13), National Natural Science Foundation of China (81803188), Gansu Province Science and Technology Project (1606RJYA270).

299

Metformin reduces the expression of cytokines and chemokines in rat intestinal smooth muscle cells

Ahmed Al-Dwairi
Jordan University of Science and Technology, Department of Physiology and Biochemistry, College of Medicine, Irbid, Jordan
E-mail: andwairi7@just.edu.jo

Metformin is a widely used antidiabetic agent known to exert several anti-inflammatory effects in different tissues independently from its hypoglycemic effect. Inflammatory bowel disease (IBD) is a chronic incurable condition characterized by relapsing inflammation of the gut. Intestinal smooth muscle cells (SMCs) are affected structurally and functionally during IBD due to excessive production of different inflammatory mediators. The aim of the present study was to investigate the effect of metformin on the expression and secretion of different cytokines and chemokines from mouse colon SMCs (CSMCs) following induction of inflammation with lipopolysaccharide (LPS) *in vitro*. CSMCs from male BALB/c mice were isolated and cultured in Dulbecco's modified Eagle's medium and treated with LPS (1 µg/ml) and 0, 5, 10 or 20 mM metformin for 24 h. Expression and secretion of tumor necrosis factor-α (TNF-α), interleukin-1α (IL-1α), macrophage colony stimulating factor (M-CSF), T-cell activation gene-3 (TCA-3) and stromal cell-derived factor-1 (SDF-1) was evaluated by ELISA. LPS-treated CSMCs demonstrated a significantly increased expression of TNF-α, IL-1α, M-CSF, TCA-3 and SDF-1 when compared with the control group ($P<0.05$). Co-treatment with metformin (5 and 10 mM) significantly reduced their expression by approximately 20-40% when compared with LPS treatment alone ($P<0.05$). Furthermore, secretion of TNF-α, IL-1α, M-CSF and TCA-3 into the conditioned media was significantly decreased by metformin (5 and 10 mM; $P<0.05$). In addition, metformin decreased levels of LPS-induced nuclear factor-κB phosphorylation. These data suggest that metformin may provide beneficial anti-inflammatory effects on CSMCs and it may be utilized as an adjunct therapy for patients suffering from IBD.

Key words: IBD, metformin, smooth muscle

300

Antitumor activity of cannabidiol and snail/rapana extracts on a variety of human malignant cells

Antonios Trochopoulos[1], Ervin Ivanov[1], Lyudmila Velkova[2], Aleksandar Dolashki[2], Pavlina Dolashka[2], Spiro M. Konstantinov[1]
[1]Faculty of Pharmacy, Medical University of Sofia, Sofia, Bulgaria; [2]Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria
E-mail: konstantinov.spiromihaylov@gmail.com

Malignant diseases are a major problem for public health. Natural sources and their compounds provide a huge variety of chemical structures and mechanisms of action. In our study, the antineoplastic activity native hemocyanins from Helix lucorum (HlH) and Helix aspersa (HaH), as well as Rapana venosa (RvH) was determined on human bladder cancer cells (T-24). Different Cannabidiol (CBD) extracts were tested regarding their cytotoxic efficacy on 4 different cell lines, namely T-24, HuT-78, MJ and MDA-MB. The methods used were MTT-dye reduction assay and flow cytometry. Results for cannabidiol showed dose-dependent cytotoxicity as measured by the conversion of the tetrazolium salt MTT in all cell lines with IC_{50} values varying from 0.12 to 22.35 µM (depending on the extract and the cell line tested), the formation of the apoptotic subG1 fraction by Nicoletti's flow cytometric method, and an increase in the number of cells in S, G2 and M (G2/M-arrest). The results obtained for native hemocyanins and their isoforms indicated that the structural subunits: RVHII (molecular weight 420 kDa, isolated from R. venosa hemocyanin) and βc-HaH (isolated from H. aspersa hemocyanin) have the highest antitumor potential with effective concentrations between 500 and 1000 µg/ml. In conclusion, given their activity and the absence of toxic effects, it can be said that cannabidiol as well as snail/rapana extracts are promising candidates for their use as add-ons to standard therapeutic regimes and could be used as urinary bladder instillation during and after transurethral resection (TUR) for non-muscle-invasive urothelial carcinoma of the bladder.

Acknowledgements: Substantial part of the experimental study was supported by the Bulgarian national program BioActiveMed (D01-217/30 November 2018; governmental decision No. 658/14 September 2018).

301

Intratumoral subpopulation of cancer stem cells as predictive marker in cervical squamous cell carcinoma patients receiving chemoradiotherapy

E.I. Selivanova, I.A. Zamulaeva, L.S. Mkrtchyan, O.N. Matchuk, G.Z. Kulieva, L.I. Krikunova
A. Tsyb Medical Radiological Research Center - Branch of the National Medical Research Radiological Center of the Ministry of Health of the Russian Federation, Obninsk, Russia
E-mail: selivanova_50@inbox.ru

Radioresistance of cancer stem-like cells (CSCs) is considered as one of the possible causes of recurrence after radio- or chemoradiotherapy of various malignancies including cervical squamous cell carcinoma (CSCC). The aim of this study was to evaluate the predictive value of CSC proportion in cervical scrape samples from CSCC patients for short-term outcomes of concurrent chemoradiotherapy. Study group consisted of 35 patients with CSCC at FIGO stages IB-IVA. Informed consent was obtained from all the patients. CSCs were detected by flow cytometry as $CD45^{-}CD44^{+}CD24^{low}$ cells before the treatment and 24 h after low-LET radiation exposure at a cumulative dose of 10 Gy to point A in the standard dose fractionation mode (2 Gy daily). The degree of tumor regression was assessed 3-6 months after the full course of the treatment including external and intracavitary irradiation. Weekly intravenous infusions of cisplatin (40 mg/m^2) were performed in the period of external irradiation. Complete tumor regression was achieved in 25 patients, and partial regression was observed in 10 patients. The CSC proportion in patients with complete regression decreased on average by 3.0±1.4% after irradiation, while in patients with partial regression this indicator increased on average by 3.0±3.7%. As a result of multiple regression analysis, two independent indicators were found to affect the degree of tumor regression: the stage of the disease and the change in the proportion of CSCs after the first irradiation session ($R=0.56$, $p<0.002$ for the model as a whole). The proportion of CSCs prior to treatment did not have prognostic value.

Acknowledgements: This work was supported by the grant from the Russian Scientific Foundation no. 18-75-10025 in part on CSC study and by State assignment of the Ministry of Health of the Russian Federation in part on cervical cancer diagnostics and treatment.

302

New curcumin nanoformulation and its *in vitro* effect on cutaneous T-cell lymphoma cells

Antonios Trochopoulos[1], Maya M. Zaharieva[2], Krasimira Yoncheva[1], Ivanka Pencheva-El Tibi[1], Spiro M. Konstantinov[1], Martin Berger[3]
[1]Faculty of Pharmacy, Medical University of Sofia; [2]Department of Infectious Microbiology, Institute of Microbiology "Stephan Angeloff", Bulgarian Academy of Sciences, Sofia, Bulgaria; [3]Unit of Toxicology and Chemotherapy, German Cancer Research Center, Heidelberg, Germany
E-mail: trochopoulos.antonios@outlook.com

Cutaneous T-cell lymphomas (CTCLs) are a group of heterogeneous, life-threatening, extranodal T-cell lymphoproliferative neoplasms. With inflammation playing a key role in the progression of diseases, curcumin, a natural pigment with proven anti-inflammatory and antineoplastic properties, as well as insignificant toxicity, could serve as a therapeutic agent. In this study, two formulations of curcumin (standard ethanolic and micellar solution) were compared regarding their cytotoxic efficacy and internalization rate in 2 CTCL cell lines, HuT-78 and MJ, as well as their modulating effect on the NF-κB p65 (Total/Phospho) using a corresponding ELISA kit. Western blot analysis was performed to provide further insight into the mechanisms of action of curcumin, investigating its modulatory effects on proteins involved in the proliferation and progression of the disease. The results demonstrated the superiority of the cytotoxic efficacy of micellar curcumin over its standard ethanol solution. IC_{50} values varied from 29.76 to 2.934 μM, depending on the cell line, with MJ demonstrating higher sensitivity. The internalization rate was determined by fluorescent microscopy and UV-spectrophotometric analysis, again showing the advantage of micellar curcumin over its standard form. In addition, the nanoformulation exhibited stronger inhibitory properties on the NF-κB p65 measured by ELISA. Curcumin downregulated WT-1, ALK, p-JAK2, p-JAK3 p-GSK-3β and p-PLCγ1, p-STAT3 and p-STAT5. The upregulation of pro-apoptotic proteins, such as p21 Waf1/Cip1, Bad and Bax was observed. Our results demonstrate that the new curcumin nanoformulation is superior to its standard ethanolic solution, by means of cytotoxic efficacy and internalization rate. The performed ELISA and western blot analysis on the untreated and treated CTCL cells, shed more light into the mode of action of this pleiotropic natural substance, a favourable treatment option for CTCL.

303

Products of spermine oxidation by bovine serum amine oxidase cause membrane permeabilization in hepatoma cell mitochondria: A new physiological mechanism for regulating cell death induction in tumor mitochondria

Antonio Toninello[1], Silvia Grancara[1], Lisa Dalla Via[2], Aida Nelly Garcia-Argaez[2], Salim Merali[3], Carlos Barrero[3], Yuta Kanamori[4], Enzo Agostinelli[4]
[1]Department of Biomedical Sciences, University of Padova, Padova, Italy; [2]Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Padova, Italy; [3]School of Pharmacy, Temple University, Philadelphia, PA, U.S.A.; [4]Department of Biochemical Sciences "A. Rossi-Fanelli", Sapienza University of Rome, Rome, Italy
E-mail: antonio.toninello@unipd.it

Te oxidation of spermine by bovine serum amine oxidase (BSAO) induces cytotoxicity and consequent cell death in tumor cells (1). In particular, electron microscopy observations have shown that mitochondria in these cells exhibit significant damage. Recent investigations (Toninello *et al* 5th Int. Conf. on Polyamines, Taipei, 2018 and 23rd Int. Symp. on Molecular Medicine, Bangkok, 2019) performed on hepatoma cell mitochondria (HCM) have provided evidence that the first step leading to cell death is mitochondrial damage. This should be mainly due to reactive oxygen species (ROS) produced by spermine oxidation. Preliminary experiments on HCM have also provided evidence that spermine and BSAO induce membrane alterations attributable to mitochondrial permeability transition (MPT) and/or the mitochondrial outer membrane permeabilization (MOMP) with the opening of a protein pore across both the mitochondrial membranes or only the outer membrane, respectively. Thus, aim of this study was to confirm the previous results obtained in HCM, and to distinguish the involvement in the membrane alteration of MPT or MOMP, or both, and also to determine whether they can lead '*in vivo*; to apoptosis. We also wished to obtain information about the proteins involved in MPT or MOMP in tumor cells. The results confirmed that mitochondrial damage by spermine and BSAO was responsible for the cytotoxicity and the apoptosis of tumor cells. HCM underwent membrane potential collapse, matrix swelling, the loss of metabolites and oxidative stress, indicative of MPT induction. Furthermore, immunoblotting and densitometry experiments demonstrated the release of the pro-apoptotic factors, cytochrome *c* and Smac DIABLO, indicative of an activation '*in vivo*' of the caspase-dependent apoptotic pathway. These results demonstrate that spermine and BSAO induce MPT in HCM, but do not exclude the induction of MOMP. Quantitative analyses by mass spectrometry of a proteomic approach on human adenocarcinomas (LoVo) cells, treated with spermine and BSAO, revealed that several proteins involved in pore opening by MPT or MOMP, in normal mitochondria, underwent downregulation. This suggests that these proteins participate in the formation of the pore. In conclusion, these results demonstrate that tumor cells exposed to the oxidation products of spermine undergo cell death by intrinsic apoptosis and this treatment can be considered as a potential tool for therapeutic interventions against cancer.

1) Agostinelli E, Belli F, Molinari A, Condello M, Palmigiani P, Dalla Vedova L, Marra M, Seiler N and Arancia G: Toxicity of enzymatic oxidation products of spermine to human melanoma cells (M14): sensitization by heat and MDL 72527. Biochim Biophys Acta 1763: 1040-1050, 2006.

304

KDM1A and SMOX: Dual inhibition of regulatory pathways for cancer metastasis

Steven Holshouser[1], Matthew Dunworth[2], Tracy Murray Stewart[1,2], Yuri K. Peterson[1], Pieter Burger[1], Huan-Huan Chen[1], Robert A. Casero, Jr. [2], Patrick M. Woster[1]
[1]Department of Drug Discovery and Biomedical Sciences, Medical University of South Carolina, Charleston, SC, USA; [2]Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins School of Medicine, Baltimore, MD, USA
E-mail: Holshou@musc.edu

Lysine and arginine residues on nucleosomal histone protein tails undergo reversible mono-, di- and, in the case of lysine, trimethylation that serves to regulate gene expression. Unlike histone acetylation, which activates gene transcription, histone methylation can either activate or silence gene expression, depending on the specific chromatin mark involved. The primary function of the flavin-dependent amine oxidase lysine-specific demethylase, (LSD1, also known as KDM1A), is to remove methyl groups from the activating chromatin marks. LSD1 is also known to demethylate lysine 370 of the tumor suppressor p53 and has been shown to play a regulatory role in a number of cancer and non-cancer disease states. Overexpression of LSD1 has been observed in a variety of tumor cell lines, and promotes the aberrant silencing of tumor suppressor genes (1). For these reasons, LSD1 is regarded as an attractive target for therapeutic intervention. A number of LSD1 inhibitors have been described, including tranylcypromine-based irreversible inhibitors. Our group previously described a series of 3,5-diamino-1,2,4-triazoles that are effective reversible inhibitors of LSD1. The compound series produced a cell type-specific cytotoxicity in a panel of 5 tumor cell lines, and effectively increased cellular levels of methylated histones residues. The closely related flavin-dependent amine oxidase known as spermine oxidase (SMOX), was discovered and first characterized by our group. During the course of our enzyme specificity determinations, our laboratory discovered that a subset of these compounds had activity against SMOX that was superior to the currently used agent MDL 72527. SMOX has recently been implicated as a causative factor in gastric cancer initiated by infection with *Helicobacter pylori*. Inhibition of SMOX by the pan polyamine oxidase inhibitor MDL 72527 is capable of reducing these effects. However, MDL 72527 is an irreversible inhibitor of SMOX with unacceptably low potency. To date, few inhibitors of SMOX have been identified, and no specific inhibitors of the enzyme have been identified. Currently we are initializing hit-to-lead optimization studies intended to reveal new 3,5-diamino-1,2,4-triazole-based LSD1/SMOX inhibitors with improved potency and selectivity compared to the parent compound.

(1) Steven Holshouser, Matthew Dunworth, Tracy Murray-Stewart, Yuri K. Peterson, Pieter Burger, Joy Kirkpatrick, Huan-Huan Chen, Robert A. Casero Jr. and Patrick M. Woster. *Medchemcommunications.* Dual Inhibitors of LSD1 and Spermine Oxidase. 2019.

305

Evaluation of chronic prostatitis as possible a risk factor for prostate cancer

G. Perletti[1,2], E. Monti[1], V. Magri[3]
[1]Department of Biotechnology and Life Sciences, Università degli Studi dell'Insubria, Busto Arsizio; [2]Urology Secondary Care Clinic; [3]ASST-Nord, Milan, Italy

Inflammation is a risk factor for several types of cancer. We performed a systematic review of the literature and meta-analysis to investigate a potential association between a history of clinical chronic prostatitis (NIH category II or III) and a histologically confirmed diagnosis of prostate cancer. Thorough worldwide database search retrieved 2,794 records. After abstract/title and subsequent full-text screening, we retrieved 16 articles written in English, reporting the data of 15 case-control studies involving 422.943 patients. Crude odds ratios and 95% confidence intervals (CI) were calculated. For analysis of pooled data, we adopted a random-effects model and the inverse variance weighing method. Heterogeneity was assessed by calculating the I^2 value. Pooled analysis of data from the 15 studies included in this review resulted in a significant odds ratio of 1.83 (95% CI: 1.43-2.35). The overall quality of the data is low, mainly due to the presence of bias, confounders and extreme effect size outliers (GRADE criteria). Data showed considerable heterogeneity (I^2=91%). Both the Egger's test and the Begg's test for funnel plot asymmetry (publication bias analysis) did not reach statistical significance. The 'trim and fill' method applied to funnel plots imputed 3 missing studies and the resulting adjusted odds ratio estimate (OR=2.12; 95% CI: 1.38-3.22) was higher than the unadjusted one (OR=1.83). Five studies reported data assessed in 8,015 males of African descent. A non-significant crude odds ratio of 1.59 (95% CI: 0.71-3.57; P=0.26), and considerable heterogeneity (I^2=90%) resulted from pooled analysis of clinical data in this population. In conclusion, our study confirms meta-analysis data published previously, and strengthens the hypothesis that a history of chronic prostatitis can significantly increase the odds for prostate cancer in the general population, though such association remains uncertain in males of African descent.

306

Changes in *O*-GlcNAcylation modifies the production of superoxide anion in macrophages

Cynthia Díaz-Hernández[1], Daniela Illescas-Barbosa[1], Victoria Jiménez-Castillo[1,2,3], José Antonio Castellanos-Martínez[2], Beatriz Xóchitl Ávila-Curiel[2], Berenice Fernández-Rojas[1], Pedro Antonio Hernández-Cruz[1], Anayetzin Torres-Rivera[4], Carlos Josué Solórzano-Mata[1,2*]
[1]Faculty of Medicine and [2]Faculty of Odontology, Autonomous University Benito Juárez of Oaxaca; [3]Technological Institute of Oaxaca; [4]Technological Institute of Mexico State, México
[*]E-mail: csolorzano.cat@uabjo.mx

Macrophages are phagocytic cells but they participate in different processes such as elimination of pathogens, thermogenesis regulation in adipose tissue, iron recycling in spleen and liver, neuronal plasticity, cardiac electrical conduction, wound healing and regulation of the immune response. The cytotoxic role of macrophages depends largely on the formation of reactive oxygen species such as superoxide anion (O^-_2), hydrogen peroxide, peroxynitrite, among others for respiratory burst. The O^-_2 is produced by NOX2, which catalyzes the electron transfer from NADPH to oxygen; the NADPH used comes from the pentose pathway associated with an increase in glucose and molecular oxygen consumption. *O*-GlcNAcylation is a non-canonical glycosylation that involves the binding of N-acetylglucosamine (*O*-GlcNAc), the product of the biosynthetic pathway of the hexosamines to serine and threonine residues of cytoplasmic, nuclear and mitochondrial proteins catalyzed by the enzymes OGT and OGA. *O*-GlcNAcylation regulates diverse cellular functions such as proliferation, migration, cell death and cellular signaling; however, its role in the production of free radicals or reactive species of oxygen has not been studied. The objective of this study is to examine the impact of the treatments that favor *O*-GlcNAcylation in the production of O^-_2. Our results showed that the production of O^-_2 in murine macrophages of cell line RAW 264.7 increased by 21.52% stimulated with LPS/Glucosamine/ThiametG and 22.36% with LPS/Glucosamine with respect to cells stimulated only with LPS. In cytochemistry, an increase in the expression of *O*-GlcNAc and OGT was observed in both conditions as well as a decrease in OGA compared to the control. On the other hand, for the cell line J774.1, it was increased by 6.13% stimulated with LPS/Glucosamine/ThiametG and 1.99% with LPS/Glucosamine with respect to cells stimulated only with LPS. In the cytochemistry assays, the expression of *O*-GlcNAc and OGT was increased. Notably, we found that there was a relocation of the OGT enzyme to the membrane in both conditions in relation to the control. There were no changes in the levels and location of OGA. No changes were detected in viability in any condition in the cell types. Our study shows that *O*-GlcNAcylation does not have a significant role in the production of O^-_2; however, changes in the expression and localization of *O*-GlcNAc and OGT that may be related to other processes of macrophage cell biology should be enlightened.

Key words: *O*-GlcNAcylation, superoxide anion, macrophage

Acknowledgements: This project was partially supported by the Program for Strengthening Educational Quality (PFCE 2019).

307

Polyamine Metabolism in the Pathogenesis of Non-alcoholic Steatohepatitis

Connor Quinn, Mario Rico, Carmen Merali, Salim Merali
Temple School of Pharmacy, Philadelphia, Pennsylvania, USA

Non-alcoholic fatty liver disease (NAFLD) has become one of the most prominent forms of chronic liver disease worldwide, mirroring the obesity epidemic. Those with the progressive variant of NAFLD, non-alcoholic steatohepatitis (NASH), are at significantly increased risk of morbidity and mortality. There are currently no approved pharmacologic therapies for NASH. The pathophysiology of NASH involves deranged lipid metabolism, cell death, inflammation, and wound healing. In addition, NASH is at-least 6 times more prevalent in obese subjects as compared to lean subjects, and insulin resistance is prominent in NASH subjects. Polyamines play a crucial role in energy and lipid metabolism. This is evident from our previous studies, which showed the activation of the catabolic enzyme, spermidine/spermine N1-acetyltransferase (SSAT) increases polyamine flux resulting in the reduction of the SSAT substrate, acetyl-CoA in adipose tissue. Consequently, the SSAT transgenic mice showed improved glucose utilization, fat oxidation, and improved energy homeostasis. Although these studies suggest the role of polyamine metabolism in obesity and insulin resistance, the potential implication in NASH has yet to be studied. To address this gap in knowledge, we performed global proteomics analysis of liver in the GOLD standard mouse model of NASH as compared to normal mice. This study revealed mice with NASH had 2-fold increased levels of SSAT and decreased levels of polyamine oxidase and spermidine synthase as compared to normal controls. These results indicate the polyamine levels are reduced in NASH. Therefore, we quantified polyamine levels and showed decreased levels of spermine, spermidine, and increased levels of acetylspermidine and acetylspermine in the NASH livers as compared to normal controls. These findings provide a novel mechanism for NASH that can be targeted for therapeutics.

308

Betula etnensis Raf. (Betulaceae) extract induces HO-1 expression and ferroptotic cell death in human colon cancer cells

Giuseppe Antonio Malfa[1], Barbara Tomasello[1], Rosaria Acquaviva[1], Carlo Genovese[2], Alfonsina La Mantia[1], Francesco Paolo Cammarata[3,4], Monica Ragusa[5], Marcella Renis[1], Claudia Di Giacomo[1]
[1]Department of Drug Science, Section of Biochemistry, University of Catania, Catania; [2]Department of Biomedical and Biotechnological Sciences, Microbiology Section University of Catania, Catania; [3]Institute of Bioimaging and Molecular Physiology, National Council of Research (IBFM-CNR), Cefalù (PA); [4]National Institute of Nuclear Physics, South National Laboratory (LNS-INFN), Catania; [5]Department of Experimental and Clinical Medicine, University "Magna Graecia" of Catanzaro, Catanzaro, Italy

Betula etnensis Raf. (Birch Etna) belonging to the Betulaceae family grows on the eastern slope of Etna. A number of bioactive compounds present in Betula species are considered promising anticancer agents. In this study, we evaluated the effects of *B. etnensis* Raf. bark methanolic extract on a human colon cancer cell line (CaCo2). In order to elucidate the mechanisms of action of the extract, the cellular redox status, cell cycle and heme oxygenase-1 (HO-1) expression in ferroptosis induction were evaluated. Cell viability and proliferation were examined by MTT assay and cell cycle analysis, while cell death was evaluated by Annexin V assay and lactic dehydrogenase (LDH) release. Cellular redox status was assessed by measuring thiol groups (RSH) content, reactive oxygen species (ROS) production, lipid hydroperoxide (LOOH) levels and (γ-glutamyl cysteine synthetase) γ-GCS and HO-1 expression. The extract significantly reduced the viability of CaCo-2 cells, inducing necrotic cell death in a concentration-depending manner. In addition, an increase in ROS levels and a decrease in the RSH content without the modulation of γ-GCS expression were detected, with an augmentation in LOOH levels and a marked increase in HO-1 expression. These results suggest that the *B. etnensis* Raf. extract promotes an oxidative cellular microenvironment, resulting in CaCo2 cell death by ferroptosis mediated by HO-1 hyperexpression.

Key words: Colon cancer, *Betula etnensis* Raf., oxidative stress, heme oxigenase-1, ferroptosis, thiol groups

309

Gene expression signature and immunohistochemical characterization of MDA-MB-231 triple-negative breast cancer xenograft model exposed to proton radiations

Barbara Tomasello[1*], Francesco P. Cammarata[2,3*], Valentina Bravatà[2,3], Luigi Minafra[2,3], Giusi I. Forte [2,3], Roberta Tringali[1], Giuseppe A. Malfa[1], Claudia Di Giacomo[1], GAP Cirrone[3], Pietro Pisciotta[2,3], Giuseppe Broggi[4], Rosario Caltabiano[4], Rosaria Acquaviva[1], Giorgio Russo[2,3]
[1]Department of Drug Science, Section of Biochemistry, University of Catania, Catania; [2]Institute of Molecular Bioimaging and Physiology - National Research Council - (IBFM-CNR), Cefalù; [3]National Institute for Nuclear Physics, Laboratori Nazionali del Sud, INFN-LNS, Catania; [4]Department of Medical, Surgical and Advanced Technological Sciences "G.F. Ingrassia", Section of Anatomic Pathology, University of Catania, Catania, Italy
*Contributed equally

Hadron therapy represents an effective treatment against both inaccessible area-located or conventional radiotherapy (RT)-resistant cancers. Proton RadioTherapy (PRT) offers various potential advantages over conventional (photon/electron based) RT due to greater precision regarding both the administered dose and distribution to the tumor when compared to healthy tissue. However, a number of issues need to be further dealt with, such as cell molecular networks activated in response to unconventional RT and the current controversies regarding RT administration to patients with triple-negative breast cancer (BC) (with ER-/PR-/HER2- receptor status). Herein, using *in vitro* experiments, we report as a 'proof of concept', specific cell and dose-dependent gene signatures, able to drive cell fate after proton exposure, highlighting the involvement of specific genes, some of these associated to radiation cell response, and others whose role was unclear. Our study aimed to compare the *in vitro* and *in vivo* molecular response induced by PRT in a MDA-MB-231 triple-negative breast cancer xenograft model focusing on immunohistochemical characterization and gene expression profile (GEP) analyses. For histological evaluation, specimens were stained with hematoxylin and eosin (H&E) and immunohistochemical analyses tested estrogen receptors (ERs), progesterone receptors (PgRs), c-erb-B2 (HER-2), MIB-1, p53, CD44, CD133 and CASP-3. In addition, we performed whole-genome cDNA microarray gene expression analyses to examine the biological processes activated in MDA-MB-231 xenograft mouse models following PRT, highlighting specific key genes involved in cell response to the radiation. We described specific pathways depending on the dose delivered and able to control some key cellular process, such as cell cycle and cell death. In particular, the preliminary *in vitro* results revealed factors involved in MDA-MB-231 radioresistance, supporting proliferation and chromatin remodeling. Ongoing *in vivo* analyses seem to confirm this behavior as sustained by the high level of MIB-1. Moreover, immunohistochemical analyses allowed us to characterize the triple-negative cell line and to define the response to proton treatment. In conclusion, we detailed the GEPs induced by different irradiation conditions, highlighting specific gene signatures able to modify radioresistance/radiosensitivity balance. Our results demonstrate the multiple advantages of xenograft tissue microarrays for preclinical signature characterization of PRT response useful to understand the molecular mechanism activated, to monitor tumor regression/response and discover new molecular target for combined treatment.

Acknowledgements: This work was supported by National Institute for Nuclear Physics (INFN)-LNS -funded ETHICS project.

310

Role of p16 INK4a in uveal melanoma

Gennaro Ilardi[1*], Rosa Maria Di Crescenzo[*1], Daniela Russo[1], Silvia Varricchio[1], Francesco Merolla[1], Francesco Martino[1], Alessandra Borzillo[1], Sara Pignatiello[1], Giorgio Borrelli[1], Marialuisa Alessandra Vecchione[1], Stefania Staibano[1]
[1]Department of Advanced Biomedical Sciences, Pathology Section, University of Naples "Federico II", Naples, Italy
*Co-first author
E-mail: gennaro.ilardi@gmail.com

Uveal melanoma is the most common intraocular tumor in adults. Despite the effectiveness of local therapy, >50% of patients with uveal melanoma develop metastases within 5-10 years (1). From the diagnosis of metastatic disease, the median overall survival is approximately 13 months (2). No treatment regimen for metastatic disease has been successful thus far. p16 INK4a, located at chromosome 9p21, is a tumor suppressor gene, whose role has been clearly defined in many malignant tumors. p16 frequently present germline mutation in familial cutaneous melanoma, and it is assumed that the loss of p16 tissue expression (by deletion, mutation, etc.) plays a central role in the malignant transformation of melanocytes (3). The immunohistochemical evaluation of p16 is currently used in pathological practice to aid the discrimination between dysplastic nevi and melanoma. Uveal melanoma differs from cutaneous melanoma, for its genetic background. Only 2% of uveal melanomas present germline mutations of p16, and immunohistochemical expression of p16 INK4a is frequently positive in contrast to cutaneous melanoma (4). We examined p16 INK4a expression on paraffin-embedded tissue sections, on tissue microarrays (TMAs) built with 2 mm cores derived from 54 uveal melanomas FFPE blocks, collected from 2005 to 2018. We observed a variable overexpression of p16 in our uveal melanoma cases. This prompted us to hypothesize a possible role of p16 expression as useful tool in differential diagnosis between cutaneous and uveal melanoma metastases, in the case of unknown primary tumor.

1. Piperno-Neumann, S. *et al.* Uveal Melanoma: A European Network to Face the Many Challenges of a Rare Cancer. *Cancers (Basel).* **11**, 817 (2019).
2. Krantz, B. A., Dave, N., Komatsubara, K. M., Marr, B. P. & Carvajal, R. D. Uveal melanoma: Epidemiology, etiology, and treatment of primary disease. *Clinical Ophthalmology* (2017). doi:10.2147/OPTH.S89591.
3. Koh, S. S. & Cassarino, D. S. Immunohistochemical Expression of p16 in Melanocytic Lesions: An Updated Review and Meta-analysis. *Arch. Pathol. Lab. Med.* **142**, 815-828 (2018).
4. Lamperska, K. *et al.* Expression of p16 in sporadic primary uveal melanoma. *Acta Biochim. Pol.* **49**, 377-385 (2002).

311

Tumor biology and cancer health disparity: Gene expression, cytokine secretion, and tumor immunology

Jenny Paredes[1], Ping Ji[2], Jone Garai[3], Marzia Spagnardi[1], Xuefeng Wang[4], Ellen Li[5], Jovanny Zabaleta[3], Laura Martello[1], Jennie Williams[2]
[1]SUNY Downstate Medical Center, Department of Medicine, Division of Gastroenterology and Hepatology, Brooklyn, USA; [2]Stony Brook University, Department of Family, Population and Preventive Medicine, Stony Brook, USA; [3]Stanley S. Scott Cancer Center, Louisiana State University Health Sciences Center, New Orleans, USA; [4]Department of Biostatistics and Bioinformatics, H. Lee Moffitt Cancer Center & Research Institute, Tampa, USA; [5]Stony Brook University, Department of Medicine, Stony Brook, USA
E-mail: jennie.williams@stonybrookmedicine.edu

In the United States of America, colorectal cancer (CRC) is the third most common cancer among African Americans (AAs). When compared to Caucasian Americans (CAs), AAs present with more advanced CRC disease and lower survival rates. Here, we investigated whether differences in the tumor immunology of AA and CA CRC patients are associated with the observed disparities between these populations. We examined the gene expression profile of tumor (N=40) and non-involved adjacent normal (N=40) tissues by RNA whole transcriptome sequencing using samples from AA (N=20) and CA (N=20) CRC patients. Using The Cancer Genome Atlas (TCGA) database, we also examined the gene expression between AA and CA CRC patients, comparing the genes that we found to be significantly disproportionately expressed by RNA whole transcriptome sequencing. Lastly, we measured the secretion of cytokines characteristic of effector T helper cell (Th) subsets by ELISA in plasma from each participant (N=40, 20 per cohort). Our results indicated that the immune profiles of AA patients differed significantly from those of CA patients. AAs exhibited a significantly higher gene expression of FOXP3, IL-1β, IL-8 ($P<0.05$) and differential cytokine production patterns linked to Th cell subsets. In CAs, we observed significantly a higher expression of markers of antitumor activity of GZMB and IFN-γ ($P<0.05$) and targets for immunotherapy (PD-L1, CTLA4). The TCGA database also showed a significantly higher expression of GZMB and PD-L1 in CAs and a 50% higher survival rate in AA patients with high expression of GZMB (5 years). In conclusion, we demonstrated a differential immunological profile of AA as compared to CA CRC patients. This would suggest, for the AA population, deficiencies within the appropriate immune defense mechanisms. As such, these differences could be used to guide new therapeutic strategies.

Key words: cancer disparities, tumor biology, RNASeq

312

The phenanthridine PJ34 exclusively eradicates human cancer cells

A. Castiel[1], L. Visochek[3], T. Golan[1], S. Izraeli[1], T. Peretz[2], M. Cohen-Armon[3]
[1]Cancer Research Center, Sheba Medical Center, Ramat Gan; [2]Sharet Oncology Institute, Hadassah Medical Center, Jerusalem; [3]The Tel-Aviv University, Tel-Aviv, Israel

The phenanthridine derivative PJ-34 is produced as a water-soluble molecule. It is a stable molecule that permeates cell membranes. Due to its affinity to the NAD binding site of PARP1 it acts as a PARP1 inhibitor. However, recently, we identified its exclusive cytotoxic activity in human cancer cells. This activity was independent of PARP1 inhibition or caspase activity causing apoptotic cell death. We found that PJ34 exclusively eradicates human cancer cells without harming normal somatic cells via inhibition of kinases interfering with the interaction of NuMA with other proteins in mitotic spindles of human cancer cells. NuMA is a microtubule (MT)-binding protein that plays a role in the formation and maintenance of the spindle poles and the alignment and the segregation of chromosomes during mitotic cell division. PJ34 prevents NuMA binding to a-tubulin, thereby preventing its transfer along the microtubules to the spindle poles. Improper poles prevent a proper segregation and alignment of the chromosomes in the mitotic spindle. This abnormality induces mitosis arrest followed by immediate self-destruction of the dividing cells[(1)]. Human cancer cells resistant to apoptosis-inducing agents, were eradicated by 'mitotic catastrophe cell death' induced by PJ34[(1)]. This phenanthridine has been tested in many human cancer cells including lung, ovary, triple-negative breast cancer, colon and pancreatic cancers, glioblastoma, squamous and hematological malignancies. After examining the pharmacokinetics and bioavailability of PJ34, we tested its therapeutic efficacy *in vivo*. We will present the results of recently tested PJ34 in xenografts of pancreatic cancer PANC1. The results of IV treatment with PJ34 were examined 30 days after treatment. About 80-90% of PANC1 cancer cells were eradicated in the tumors. Normal cells that had infiltrated into the tumors (stroma) were not eradicated. No harm to normal tissues has been detected. Growth, development and weight gain of the treated mice were not impaired during and 30 days after treatment. These experiments were performed by Pharmaseed, preclinical CRO, Israel. The reports are available at request.

1) Leonid Visochek, Dikla Atias, Asher Castiel, Leonid Mittelman, Michael Elkin,Talia Golan, Shai Izraeli, Tamar Peretz, Malka Cohen-Armon 2017 Exclusive destruction of mitotic spindles in human cancer cells. Oncotarget 8(13): 20813-20824.

313

Elucidation of the catalytic cycle of type II DNA topoisomerase using a structural and molecular biological approach

Ivan Laponogov[1,2], Xiaosu Pan[2], Dennis A. Veselkov[4], Isabelle Marie Thérèse Crevel[2], Jogitha Selvarajah[2], Art Branstrom[3], Ryan Cirz[4], Heinz E. Moser[4,5], Galyna Skamrova[1], Tishant Umrekar[1,6], Mark L. Fisher[2], Mark R. Sanderson[1]
[1]Randall Centre for Cell and Molecular Biophysics, King's College London, Guy's Campus, UK; [2]Molecular and Clinical Sciences Research Institute, St. George's University of London, London, UK; [3]PTC Pharmaceuticals, South Plainfield, NJ, USA; [4]Achaogen Pharmaceuticals Inc., San Francisco, USA; [5]Novartis Institute for Biomedical Research, Emeryville, USA; [6]Institute of Structural and Molecular Biology, Birkbeck College and UCL, London, UK

Type II topoisomerases play essential roles in DNA replication, chromosome segregation and recombination, and are important antibacterial and anticancer targets (1). Bacterial drug resistance is an increasing and now widely recognised threat, and the limited number of new antibacterials developed in recent years is a matter of serious concern. One of the approaches to combat this growing threat is to deeply investigate the mechanisms of action of currently available antibacterials, as well as to examine the mechanisms through which bacteria are developing drug resistance and may potentially develop drug resistance to known drugs in the future. This knowledge will in turn be used in the rational drug design and the general development of appropriate molecular frameworks to combat bacterial infections, while at the same time keeping the negative side-effects of the drugs at an acceptable minimum. This is of particular importance when both bacteria and humans share similar drug targets, such as is the case for type II topoisomerases (in humans topo IIα and topo IIβ are targeted by anticancer drugs i.e., doxorubicin and etoposide), whereas in prokaryotes Gyrase and topoisomerase IV (topoIV) may be targeted by quinolones and quinazolinediones. Type II topoisomerases are involved in the regulation of DNA supercoiling in both bacteria (Gyrase) and eukaryotes and also in the decatenation of bacterial daughter chromosomes during cell division (topo IV). Type II topoisomerases perform their biological functions by binding double-stranded DNA (termed G-segment or Gate-DNA), temporarily cleaving it and passing another double-stranded DNA (called T-segment) in the ATP-assisted process via the cleavage region, thus changing the linking number in steps of ±2. Subsequently, the G-segment is resealed and released. Quinolones, doxorubicin and etoposide are found to be able to disrupt this process, ultimately resulting in cell death (hence their anti-bacterial or anti-cancer action). Herein, we present our studies on the topo II catalytic cycle and the protein-DNA-drug interactions which are involved in the action of clinically used and newly developed quinolone antibacterials (2-3), as well as the recent structures of the ATPase domain-DNA complexes (4).

1) Hauk, G. & Berger, J.M. (2016). Curr. Opin. Struct. Biol. 36, 85-96; 2) Veselkov, D.A. et al. (2016). Acta Cryst. D Structural Biology 72, 488-496; 3) Laponogov, I. et al. (2016) Open Biology 6(9), 160157; 4) Laponogov, I et al. (2018) Nature Communications 9, 2579 (2018).

314

PCAL and PCT monitoring in the study of anastomotic dehiscence

E. Giambò[1], E. Cucinotta[1]
[1]Department of Human Pathology of the Adult and Evolutive Age "Gaetano Barresi", Section of General Surgery, University of Messina, Messina, Italy

Anastomotic leaks are among the most feared complications following colorectal surgery. Their clinical significance must not be underestimated due to their association with increased morbidity, mortality and neoplastic recurrence (1). The tissue damage can activate an inflammatory process and, consequently, an increase in neutrophil levels directly proportional to plasma calprotectin (P Cal), a protein contained in neutrophil granulocytes. In addition, the serum level of procalcitonin (PCT), which is significantly increased in patients with severe bacterial and sepsis infections, could represent an early biomarker of septic complications following abdominal surgery (2,3). The present study considers the use of P Cal and PCT in association and/or comparison with other markers of inflammation, such as C-reactive protein (PCR) and white blood cell count (WBC), in order to evaluate whether the use of P Cal and PCT is more efficient in terms of specificity and sensitivity than other markers, in the early assessment of anastomotic dehiscence. The prospective and observational study, conducted from September, 2017 to July, 2018, involved 23 patients enrolled in general and emergency surgery department of the AOU 'G. Martino' Hospital, in Messina. All participants were subjected to laparotomic intestinal surgery, apart from a case that was performed laparoscopically (5%). The intervention guaranteed the restoration of intestinal continuity without performing a protective stoma. Blood samples were collected in patients in the 1st, 3rd and 5th post-operative day (POD). P Cal was superior to PCR in the detection of anastomotic leaks. Furthermore, the best diagnostic accuracy was obtained when the PCR, WBC and P Cal measurements at POD 3 were combined. In conclusion, calprotectin and procalcitonin can be useful markers in the early diagnosis of anastomotic dehiscence for the better management of the post-operative phase prior to the risk of anastomotic loss.

Key words: plasma calprotectin, procalcitonin, anastomotic leak, dehiscence, colorectal surgery

1) Hyman N, Manchester TL, Osler T, Burns B, Cataldo PA. Anastomotic leak after intestinal anastomosis: it's later than you think. Ann. Surg. 2007 e 254-258, 245.
2) Garcia-Botello S, Canovas de Lucas R, Tornero C et al. Implementation of a postoperative multimodal rehabilitation protocol in elective colorectal surgery. A prospective randomized controlled study. Cir Esp. 2011; 89: 159-166.
3) Reisinger K. W., Poeze M., Hulsewé K. W. E., et al. Accurate prediction of anastomotic leakage after colorectal surgery using plasma markers for intestinal damage and inflammation. 2014 e 10.1016.2014.06.011., 219(4): 744-751.

315

Prediction application of mRNA markers for colorectal polyps by membrane array

Bin Feng[1], Man Feng[2], Huiyong Shi[3]
[1]Internal Medicine-Oncology, [2]Department of Pathology and [3]Center of Endoscopy, Affiliated Hospital of Shandong Academy of Medical Sciences, Shandong First Medical University, Jinan, Shandong, P.R. China
E-mail: shiurulin@gmail.com

Colorectal cancer is the most common type of cancer worldwide. Despite advances being made in medical instruments and medicines, the mortality rate of patients with colorectal cancer is increasing each year. Therefore, if the precancerous stage of cancer can be diagnosed and treated earlier, the incidence and mortality of colorectal cancer would be greatly reduced. We performed microarray analysis on the tumors and normal tissues of patients with polyps and found that the differentiated expression of eight genes in polyp tissue exhibited statistical significance. In addition, a high-sensitivity membrane-array method from colorimetric to chemiluminescence to detect the colorectal polyp-related mRNA markers from the tissue and peripheral blood of the patient could be an earlier prediction tool. The statistical analysis of the correlation between the experimental data and the patient clinicopathological characteristics revealed that MUC 5AC and MUC2 were significantly associated with polyp size, the number of polyps and the malignancy of polyps ($P<0.01$). Additionally, we found that genes, including H2AFZ, RAP1B, TBX19, E2F4 and MMP1, were highly expressed in all polyp tissues. The preliminary results indicated that the accuracy of membrane-arrays was sufficient to predict the colorectal polyps from normal individuals with the advantages of time-saving, cost-effectiveness and high-throughput. Thus, the constructed colorimetric membrane-array could be a promising approach for the future distinguish benign colorectal polyp with malignant potential.

Key words: mRNA markers, colorectal polyp, membrane array

316

Modeling and simulation approaches for the description of pharmacokinetics and pharmacodynamics of epacadostat

Sofia Konstantinidou[1], Vangelis Karalis[2]
[1]Oncology Unit, 3rd Department of Medicine, Medical School, National and Kapodistrian University of Athens, Onassis Foundation Scholar; [2]Department of Pharmaceutical Technology, School of Pharmacy, National and Kapodistrian University of Athens, Athens, Greece
E-mail: sissykonstantinidou@gmail.com

In recent years, the use of modeling and simulation approaches, such as population pharmacokinetic (PK) and pharmacodynamic (PD) methods in clinical trials and everyday clinical practice, has been emerging. PKPD modeling, not only describes drug kinetics and effects, but also explains patient variability and defines individualized dosage regimens, allowing thus determination of the most desired benefit/risk ratio. The aim of this study was to apply population PKPD modeling in order to describe the C-t profile and the PD effect of epacadostat, which is a selective inhibitor of the enzyme indoleamine 2,3-dioxygenase 1 (IDO1) and is being developed as an orally active immunotherapy to treat advanced malignancies. The utilized PKPD model was based on the published results of Shi and colleagues (1). PK and PD model parameters were taken from the previous modeling exercise (1), while several administration regimens were investigated. Several levels of epacadostat clearance were explored regarding their impact on PK and PD. Also, the properties of the PD model were examined by altering IC50 (i.e. the epacadostat concentration causing 50% of maximal inhibition of IDO1), the maximum fraction of inhibition (Imax) and the bioconversion rate from tryptophan to kynurenine. Initially, a relatively low between subject variability (set at 10%) was used in order to avoid hampering the effects of our in silico interventions. The modeling and simulation work was performed in Monolix® 2019R1 using also Mlxplore and Simulx, by writing the appropriate code in the R language. Concentration-time and effect-time plots for the generated virtual patients were created. Decrease of epacadostat clearance from the body led to higher peak plasma concentrations as well as more intense and longer duration of the effect (namely, increase of IDO1 inhibition). A higher and more attenuated effect was also observed with decrease of IC50 and increase of Imax. The concentration-time and effect-time profiles were further simulated assuming different dosage schemes. In conclusion, several simulations were performed using a joint PKPD model. Without any actual patient interventions, the use of in silico methods allowed the prediction of epacadostat plasma levels and the anticipated efficacy under several different dosage scenarios. Modeling and simulation could assist clinical oncologists on setting the appropriate dosage regimens, even with newest advanced therapies, such as with epacadostat.

(1) Shi et al., J Clin Pharmacol 57: 720-729, 2017.

317

Role of RNA-binding proteins in the link between diabetes and cancer

Benjamin S. Minch, Kirsty M. Ponla, Jinsil Kim
Department of Biological Sciences, Biola University, La Mirada, CA, USA
E-mail: jinsil.kim@biola.edu

Recent evidence established that there is a significantly increased risk of cancer in individuals with diabetes compared to those without the condition[(1)]. However, the biological mechanisms underlying the association between diabetes and cancer remain elusive. Hyperglycemia (high blood glucose levels) is a key metabolic abnormality that characterizes diabetes, and could potentially play a role in the pathogenic process of cancer. The aim of this study was to investigate the effects of hyperglycemic conditions on cancer, focusing on the role of RNA-binding proteins (RBPs). The results of cell proliferation and migration assays support cancer-promoting effects of hyperglycemic conditions in SW620 colorectal cancer cells. Analysis of selected RBPs revealed the transcript- and protein-level expression changes of the RBPs involved in the methylation of adenosine at the nitrogen-6 position (N6A) in RNA in response to elevated glucose levels. In line with these changes, there were alterations in N6-methyladenosine (m^6A) levels in high glucose-treated SW620 cells compared to control cells exposed to normal glucose conditions. Our study also suggests that the glucose-responsive m^6A modification may potentially be regulated by the combined action of more than one RBP of the m^6A machinery in a complex manner. Given that m^6A RNA methylation has been increasingly implicated in human cancer[(2)], altered m^6A levels caused by dysregulation of the RBPs involved in m^6A modification may play at least a partial role in diabetes-induced cancer, which warrants future exploration. Collectively, our study provides potentially important insight into the role of RBPs that could add to the understanding of the mechanisms explaining the link between diabetes and cancer.

(1) Ohkuma et al., Diabetologia 61: 2140-2154, 2018.
(2) Chen et al., Mol Cancer 18: 103, 2019.

318

Molecular mechanisms of pesticides as endocrine-disrupting chemicals on the progression and migration of estrogen receptor expressing breast cancers

Ryeo-Eun Go, Kyung-Chul Choi
Laboratory of Biochemistry and Immunology, College of Veterinary Medicine, Chungbuk National University, Cheongju, Chungbuk, Republic of Korea
E-mail: kchoi@cbu.ac.kr

We have recently put forward a research application to verify the mechanisms of endocrine disrupting chemicals (EDCs) in reproductive tissues compared to endogenous steroid hormones. EDCs are environmental chemicals that interfere with the endocrine systems and adversely affect hormone balance or disrupt normal function in the organs that hormones regulate or modulate, leading to detrimental effects in the reproductive and developmental processes (1,2). Of particular relevance to women and children are EDCs, which are associated with an increased risk and incidence of reproductive dysfunction, breast cancer, and ovarian cancers (3). Fenhexamid and fludioxonil are antifungal agents (pesticides) used for agriculture, and are present at measurable amounts in fruits and vegetables. In this study, the effects of these pesticides on cancer cell viability, epithelial-mesenchymal transition (EMT) and metastasis were examined in breast cancer cells with estrogen receptors (ERs). In addition, tumour-progressive effects of these pesticides were evaluated in xenografted mouse models injected with human breast cancers. Taken together, these results suggest that fenhexamid and fludioxonil may have estrogenic and disruptive effects on ER-expressing breast cancer cells by inducing alterations in the expression of cell cycle- and EMT-related genes via an ER-dependent pathway.

Acknowledgements: This work was supported by the Global Research and Development Center (GRDC) Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science and ICT, Republic of Korea (2017K1A4A3014959).

(1) Go RE et al., Environ Toxicol 32: 2225-2233, 2017.
(2) Go RE et al., Environ Toxicol 30: 234-242, 2017.
(3) Go RE et al. Toxicol Appl Pharmacol 289: 48-57, 2015.

319

A phenolic acid, gallic acid, inhibited the progression and migration of prostate cancer by decreasing the expression of histone deacetylase 1 and 2

Yin-Gi Jang, Ji-Hyung Kang, Kyung-Chul Choi
Laboratory of Biochemistry and Immunology, College of Veterinary Medicine, Chungbuk National University, Cheongju, Chungbuk, Republic of Korea
E-mail: kchoi@cbu.ac.kr

Gallic acid (GA) has been known to possess the diverse biological activities involving an anti-cancer activity. Histone deacetylase (HDACs), which are in control of tumor suppressor gene transcription, are overexpressed in various tumors leading to tumor development, progression and poor prognosis. HDAC1, 2 and 3, classified as HDAC class I, have been known to be highly expressed in prostate cancer (PCa) and their upregulation is strongly associated with PCa progression (1, 2). The aim of this study was to demonstrate the effect of GA on the inhibition of PCa progression by modulating the expression of HDAC1 and 2 in PCa cell lines such as LNCaP and PC-3 cells. Results showed that, GA decreased the cell viability of only PCa cell lines, not of normal cells, contrary to SAHA, as a HDAC inhibitor, and inhibited the colony and tumor spheroid formation. In addition, GA decreased the mitochondrial membrane potential (MMP, $\Delta\Psi m$), and increased the number of cells in apoptosis stages, and induced DNA fragmentation. In western blot analysis, GA downregulated the expression of HDAC1 and 2, leading to the upregulation of acetyl-p53 expression at the protein level, resulting in downregulation of the expression of cell cycle-related genes such as *PCNA*, *Cyclin D1* and *E1*, upregulation of the expression of cell cycle arrest gene, *p21*, and regulation of the expression of apoptosis intrinsic pathway-related genes, such as *Bax*, *Bcl-2*, cleaved-*Caspase-3* and *PARP-1* in both cell lines. Furthermore, oral administration of GA for 8 weeks on PC-3 cell-derived tumor xenograft mice model decreased the tumor size, damaged the tumor structure, and downregulated the expression of HDAC1, 2 and PCNA in tumor mass, confirmed by histological analysis. Taken together, GA hindered PCa progression by the inhibition of HDAC1 and 2 expression, suggesting the possibility of GA to be used as a HDAC inhibitor and anti-PCa drug.

Acknowledgements: This work was also supported by a National Research Foundation of Korea (NRF) grant funded by the Ministry of Education, Science and Technology (MEST) of the Republic of Korea (2017R1D1A1A09000663).

(1) Jang YG et al., J Steroid Biochem Mol Biol In press, 2019.
(2) Jang YG et al et al., Nutrients 10: E1784, 2018.

320

Therapeutic gene delivery of cytosine deaminase and interferon-beta via engineering stem cells resulted in the inhibition of progression of renal cell carcinoma

Gyu-Sik Kim, Sung-Moo Lee, Kyung-Chul Choi
Laboratory of Biochemistry and Immunology, College of Veterinary Medicine, Chungbuk National University, Cheongju, Chungbuk, Republic of Korea
E-mail: kchoi@cbu.ac.kr

Although the effects of stem cells expressing anti-cancer genes on tumor growth have been previously demonstrated in various kinds of cancer (1-3), relatively few studies have investigated their inhibitory effects on cancer metastasis. In this study, we examined the inhibitory effects of cytosine deaminase (CD)/5-fluorocytosine (5-FC) and interferon-beta (IFN-β) using genetically engineered neural stem cells (hNSCs) in a cellular and metastasis model of renal cell carcinoma (RCC). The CD/5-FC method has the advantage of minimizing damage to normal tissues because it selectively targets cancer cells via the *CD* gene, which converts prodrug 5-FC to drug 5-fluorouracil. Moreover, we used hNSCs as a tool to effectively deliver the anti-cancer genes to the tumor site. These stem cells are known to possess tumor-tropism because of chemoattractant factors expressed in cancer cells. Therefore, we ascertained the expression of these factors in A498 cells, a cell line of RCC, and identified the A498-specific migration ability of hNSCs. We also confirmed that the proliferation of A498 cells was significantly reduced by therapeutic hNSCs in the presence of 5-FC. Furthermore, we established an A498 metastasis model. In the animal experiment, the weight of the lungs increased in response to cancer metastasis, but was normalized by hNSCs expressing *CD* and/or *IFN-β* genes, while the incidence of liver metastasis was suppressed by the hNSCs. Overall, the results of this study demonstrate that stem cells expressing anti-cancer genes have the potential for use as an alternative to conventional therapy for metastatic cancer.

Acknowledgements: This work was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (MEST) (2016R1D1A1A09919809).

(1) Kim GS et al., Oncol Lett 17: 2576-2582, 2019.
(2) Shin HJ et al, J Cell Biochem In press, 2019.
(3) Heo JR et al, Cancer Res Treat 51: 797-811.

321

Myocardial differentiation appeared to be hindered by cigarette smoke components in mouse embryonic stem cells

Cho-Won Kim, Kyung-Chul Choi
Laboratory of Biochemistry and Immunology, College of Veterinary Medicine, Chungbuk National University, Cheongju, Chungbuk, Republic of Korea
E-mail: kchoi@cbu.ac.kr

The heart is the first organ formed in the developing fetus, and abnormal development of the heart is a major cause of fetal death. The adverse effects of cigarette smoke on the heart have been well established, but it is not well understood how cigarette smoke components regulate signaling molecules and cardiac-specific functions during the early differentiation stage of the embryonic heart (1). In this study, we identified changes in the size of mouse embryoid bodies (mEBs) in response to treatment with cigarette smoke extract (CSE) via regulation of HDAC2, p53, p21 and cyclin D1 protein expression, which are cardiac differentiation and cell-cycle markers, respectively. In addition, exposure of mouse embryonic stem cells (mESCs) to cigarette smoke components inhibited myocardial differentiation and development through the expression of HDAC1, HDAC2, GATA4, NKX2-5, TBX5, HAND1 and Troponin I. Long-term exposure studies showed that CSE and nicotine may delay the development of mouse cardiomyocytes from mESCs and inhibit the contractibility, which is a fundamental function of the heart (2,3). Taken together, these findings suggest that cigarette smoke components, including nicotine, may affect abnormal myocardial differentiation and development.

Acknowledgements: This research was supported by grants from the Ministry of Food and Drug Safety in 2019 (19183MFDS163).

(1) Kim CW et al., Environ Toxicol 34: 689-698, 2019.
(2) Kim CW et al, Toxicol In Vitro 52: 161-169, 2018.
(3) Kim CW et al, Reprod Toxicol 73: 8-19, 2017.

322

Silent male breast cancer: The natural reservoir of the disease in autopsy specimens

Zacharoula Sidiropoulou[1], Ana Paula Vasconcelos[2], Cristiana Couceiro[2], Carlos Dos Santos[3], Ana Araujo[1], Inês Alegre[1], Claudia Santos[1], Filipa Costa[1], Rita Sampaio[4], Vasco Fonseca[5], Carlos Neves[1], Fátima Cardoso[6], Pere Gascon[7]
Departments of [1]General Surgery and [2]Radiology, Hospital São Francisco Xavier, Lisbon; [3]Instituto Nacional de Medicina Legal e Ciências Forenses, I.P.-Delegação Sul, Lisbon; [4]Department of Pathology, Hospital São Francisco Xavier, Lisbon; [5]Department of Medical Oncology, Hospital São Francisco Xavier, Lisbon; [6]Breast Unit, Centro Champalimaud, Lisbon, Portugal; [7]Laboratory of Molecular and Translational Oncology-CELLEX, Barcelona, Spain

Breast cancer epidemiological patterns (1) vary in European countries; presenting different incidence rates (49-148 new cases per 100,000 women) with a narrower, but still variable, range of mortality (15-36 new cases per 100,000 women). Male breast cancer is a very rare disease, comprising approximately 1% of breast cancers, and data are generally scant. The aim of the present study was to quantify the natural reservoir of male breast cancer. The intention was to identify the cases of existing cancers, those that had not clinically manifested themselves and verify whether the natural reservoir of silent breast cancer is superior to the actual incidence, a hypothesis that could not be verified since no silent breast cancer was detected in the subjects studied. The hypothesis was tested in 27 recruited male gender cadavers, achieving with this number the null hypothesis. The findings did not identify any silent breast cancer despite the fact that male breast cancer's molecular surrogate (usually ER, PR, and AR positive, Luminal B-like/HER2-negative and 56% patients of T1 tumours) is usually of good prognosis and it could be hypothesized that the disease is present in the general popuation without being manifested. Thus, its late detection and consequent treatment dictate the disease course [5.1% with metastatic disease (M1) and OS 2.6 years] (2). Therefore, we can conclude that the actual cases of breast cancer manifest themselves and thus we accept the null hypothesis that the natural reservoir of silent breast cancer is not superior to the actual incidence of the disease.

1) Ferlay J, e. a., 2013. Cancer incidence and mortality patterns in Europe: Estimates for 40 countries in 2012.. *Eur J Cance,* Volume 49, pp. 1374-1403.
2) Giordano, S., 2018. Breast Cancer in Men. *The new england journal of medicine,* Volume 378, pp. 2311-2320.

323

Interplay between oncogenes and non-coding RNAs in subtypes of non-Hodgkin B-cell lymphomas

G. Malpeli[1], S. Barbi[2], C.M. Croce[3], C. Visco[4], A. Scarpa[2], A. Zamò[5]
[1]Department of Surgery, Dentistry, Paediatrics and Gynaecology, University of Verona, Verona, Italy; [2]Department of Diagnostics and Public Health, University of Verona, Verona, Italy; [3]Department of Molecular Virology, The Ohio State University, Columbus, USA; [4]Department of Medicine, University of Verona, Verona, Italy; [5]Department of Oncology, University of Turin, Turin, Italy

The interaction between oncogenes and epigenetic modifiers is mediated through regulatory loops and circuits involving target genes. The oncogene TCL1A is a co-activator of transformation and survival of lymphoma cells by regulating multiple signaling pathways. MYC controls 15% of genes encoded in the human genome including many non-coding RNAs. We previously identified microRNAs associated with MYC in non-Hodgkin B-cell lymphomas (NHBCL). We have extended this analysis to non-coding RNAs encoded in conserved genomic loci. To this end, we evaluated the immunohistochemical expression of the oncogenes MYC and TCL1A and the epigenetic modifier EZH2, the catalytic subunit of the polycomb repressive complex 2, in 75 tissues of five NHBCL subtypes, Burkitt's lymphoma (BL), diffuse large B-cell lymphoma (DLBCL), primary mediastinal B-cell lymphoma (PMBL), mantle cell lymphoma (MCL) and follicular lymphoma (FL), and in 11 reactive lymph nodes (rLN) as reference. The expression analysis of non-coding RNAs was performed by microarrays. Overall, MYC^+, $TCL1A^+$ and $EZH2^+$ cells were present in decreasing order of frequency in BL, DLBCL, PMBL, MCL and FL, in agreement with the aggressiveness of the lymphoma subtype. New and known MYC-, EZH2- and TCL1A-related non-coding RNAs were identified. The regulatory network that integrates MYC, TCL1A, EZH2 and non-coding RNAs highlights potential pathways to be explored in the context of future clinical approaches.

324

Autoantibody biomarkers differentiate pancreatic ductal adenocarcinoma from chronic pancreatitis

Metoboroghene O. Mowoe[1], Marc Bernon[2], Karan Gandhi[2], Sean Burmeister[2], Hisham Ali[2], Miriam Kahn[2], Christo Kloppers[2], Eduard Jonas[2], Jonathan Blackburn[1]
[1]Department of Integrated Biomedical Sciences, Division of Chemical and Systems Biology, University of Cape Town, Cape Town; [2]Surgical Gastroenterology Unit, Division of General Surgery, Groote Schuur Hospital, University of Cape Town, Cape Town, South Africa
E-mail: m_mowoe@yahoo.co.uk

Pancreatic ductal adenocarcinoma (PDAC) is a leading cause of cancer deaths worldwide. Late diagnosis in a large percentage of cases is often due to uncharacteristic symptoms and late presentation, resulting in poor prognosis and a 5-year survival below 5%. Chronic pancreatitis (CP) is a known risk factor for PDAC. The dual expression of potential biomarkers in CP and PDAC presents an obstacle to early diagnosis as raised protein biomarkers of CP could be a source of false positives. Previous attempts to develop protein biomarkers have proved moderately successful due to insufficient sensitivity and specificity. Recently, autoantibodies have proven to be one of the more effective blood-based biomarkers as they are believed to reflect the body's initial humoral response to cancer. Thus, increases in their concentrations may be detectable months or years before clinical symptoms are evident. Patients undergoing surgical resections for pancreatic ductal adenocarcinoma or chronic pancreatitis were included. Blood samples, from which sera were extracted, were collected pre-operatively and stored at -80°C. We used the CT (cancer testis) 100+ microarray with 123 antigens belonging primarily to the CT antigen family, which was developed by Blackburn *et al* (2005), to screen the sera of PDAC and CP patients. A total of 197 patients with PDAC and 6 patients with CP were analysed. Intensity values using the Inopsys-Mapix software from which neighbourhood background signal was subtracted were extracted. Following this, we generated a top 10 list of antigens that were differentially expressed in PDAC and CP patients, based on ROC analyses with the highest AUC scores. These antigens, in combination or isolation, may prove to be effective biomarkers improving diagnosis and prognostic assessment in the future.

325

Chromosomal studies on the Egyptian fresh water snail *Biomphalaria Alexandrina* by using transmission and scanning electron microscope

Samah Ibrahim[1], Amira H. Mohamed[2], Shadia M. El-Dafrawy[1]
[1]Departments of Medical Malacology and [2]Electron Microscopy Theodor Bilharz Research Institute, Imbaba, Giza, Egypt
E-mail: Amira7121956@yahoo.com

The present work is the first chromosomal study of *Biomphalaria alexandrina* snails by using Transmission Electron Microscope (TEM) and Scanning electron microscopy (SEM). Preparation of chromosomes and karyotypes of snails were made according to the method described by Barsiene *et al* (2000). Pooled snails (about 10 snails for each sample) were placed directly in colchicine at room temperature. Snails were dissected, separated and treated with KCl. Tissues were then carefully minced in the hypotonic solution, centrifuged and prepared for TEM and SEM examination. TEM allows the accurate study of the four meiotic phases; Interphase, prophase, metaphase and anaphase. Our study showed a diploid number of chromosomes 2n=36 as indicated from metaphase preparation and SEM examination. Chromosomes were arranged in a descending manner according to the total length of 12-2 µm. We could detect four types of chromosomes, 5 metacentric pairs, 4 submetacentric pairs, 3 acrocentric pairs and 6 telocentric pairs. Thus, high resolution SEM has proven to be an appropriate tool for chromosomal study.

Key words: *Biomphalaria alexandrina*, karyotype, freshwater snail, transmission electron microscope (TEM), scanning electron microscope (SEM)

326

Ultrastructural study of neutrophil apoptotic changes in hepatitis C patients

Amira H. Mohamed
Theodor Bilhharz Research Institute, Department of Electron Microscope, Cairo, Egypt
E-mail: Amira7121956@yahoo.com

Hepatitis C Virus (HCV) patients frequently manifest with neutropenia. Dysregulation of neutrophil apoptosis has been implicated in pathogenesis of Hepatitis C patients. The aim of this study was to explore neutrophil apoptosis and the factors relevant to its pathogenesis to determine its implication in shortened neutrophil survival in HCV patients thus contributing to the neutropenia from which they suffer. The study was carried out on 70 subjects who were divided into: Group I (30 chronic HCV patients without neutropenia), Group II (30 chronic HCV patients with neutropenia) and 10 normal controls, matched for age and sex. Neutrophils were separated using Percoll density for detection of apoptosis by flow cytometry using Annexin V-FITC/propidium iodide dye to discriminate between normal, apoptotic and necrotic cells. Late apoptosis was tested by detection of DNA fragmentation using terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling test (TUNEL). Soluble Fas (sFas) expression of neutrophils was determined in the serum by ELISA. The morphological features of apoptosis were examined using electron microscopy. The results showed that the level of An+ve/PI-ve cells were decreased in the 2 patient groups versus the control group while in Group II An+ve/PI+ve cells were significantly decreased and An-ve/PI+ve cells were significantly increased. The number of TUNEL-positive cells was significantly increased in Group I in comparison to both the control and Group II. sFas was significantly more increased in the neutropenic group than in the other two groups. EM detected apoptotic cells in all neutropenic patients and in 35 non-neutropenic patients. In conclusion, our study indicates the significance of sFas and EM examination in detecting apoptosis in such patients, while Annexin V and TUNEL tests suggest factors other than apoptosis influencing neutrophil.

AUTHOR INDEX

Abdullah M, 105
Acevedo-Duncan M, 101
Achim M, 189
Achtsidis V, 211
Acquaviva R, 308, 309
Adam V, 235
Adamaki M, 165
Agostinelli E, 221, 303
Agrawal A, 265
Aidagulova SV, 268
Alaiya AA, 117
Al-Dwairi A, 299
Alegakis A, 237
Alegre I, 322
Alessandrini F, 276
Alfaqih MA, 297
Al-Hammad R, 182
Alhebshi H, 183
Alhosin M, 257, 258
Ali H, 324
Ali Z, 127
Alreate S, 151
Alves MT, 156
Ametrano G, 288
Amilca K, 280
Andersson B-Å, 145
Andreini M, 239
Androvic P, 135
Anufrionak I, 167
Apostolatos C, 101
Aqil F, 265
Araidah A, 297
Araujo A, 322
Argyriadou A, 193
Aripova AA, 190
Arsenos G, 193
Aversa L, 278
Aversano A, 288
Ávila-Curiel BX, 306
Ayiomamitis GD, 122

Babenka A, 167
Babic M, 143
Backers L, 120
Badowska K, 276
Baeyens A, 120
Bakker E, 183
Bâlici Ș, 184
Baliou S, 162, 163
Banciu M, 188, 189, 219, 220, 236
Barash I, 118
Barbi S, 323
Barrero C, 303
Baruchello R, 171
Basile S, 243
Batra SK, 275
Battista T, 239
Beev G, 140
Benzekry S, 229
Berardi D, 128
Berger M, 302
Berger MR, 121
Bergman A, 135
Bernon M, 324
Bersimbaev RI, 190
Beyls E, 120
Bezzerri V, 177
Bianchi N, 173
Bikfalvi A, 228, 229
Björnhagen V, 129
Blackburn J, 324
Blockhuys S, 127
Boda D, 203, 204
Bogusiewicz M, 270
Bondor CI, 181
Bonroy C, 120
Bordon V, 120
Borgatti M, 170, 172, 173, 174, 177
Borrelli G, 310
Borzillo A, 310
Bouček J, 139
Bouygues A, 215, 280
Branstrom A, 313
Braunová A, 139
Bravatà V, 309
Breedy S, 101
Breitbach A, 106
Brenner W, 106
Bresciani A, 239
Breveglieri G, 173, 174, 177
Briguglio G, 263, 264
Broggi G, 309
Buga AM, 248
Bulgakova OV, 190
Burger P, 304
Burmeister S, 324

Cabrini G, 172
Caccamo D, 263
Caja F, 115, 142
Calina D, 249
Calogero AM, 250
Caltabiano R, 309
Camerlingo R, 278
Cammarata FP, 308, 309
Cammarota F, 288
Candido S, 241, 242, 243, 244, 246, 247
Canettieri G, 221
Cannizzaro E, 197
Cappelletti G, 250
Carabet LA, 109
Cardoso F, 322
Caruntu A, 203
Caruntu C, 203, 205
Casali G, 128
Casero RA Jr, 304
Castellanos-Martínez JA, 306
Castiel A, 312
Ceci P, 143
Cefalas AC, 164
Cerna T, 235
Chen C, 279
Chen H-H, 304
Chen J-L, 294
Chen J-R, 294
Cherkasov A, 109
Chernyavskiy O, 115, 142
Chibaudel B, 215
Chiodi I, 128
Choi K-C, 318, 319, 320, 321
Choudhry H, 258
Christodoulou I, 166
Christodoulou MS, 250
Christofi T, 124, 125
Chrousos GP, 160, 255
Christensen S, 280
Chytil P, 138
Cinà D, 197, 198, 199
Ciontea MI, 267
Cipolli M, 177
Ciribilli Y, 276
Cirrone GAP, 309
Cirz R, 313
Claes K, 120
Clarke K, 229
Cohen-Armon M, 312
Colomer R, 111
Colotti G, 239
Constantin C, 203, 205
Cooley L, 229
Corradini R, 172
Cosenza LC, 171, 173, 176, 178
Costa C, 263, 264
Costa F, 322
Couceiro C, 322
Coufal S, 114
Crawford HC, 155
Crescenzio N, 149
Crevel IMT, 313
Croce CM, 323
Cucinotta E, 314
Cui W, 127
Cybulski M, 151
Czaplinska D, 126

Dalla Via L, 240, 250, 303
Danilenko M, 151, 158
D'Aversa E, 170, 174, 177
Dawod A, 158
De Chiara A, 278
De Gramont A, 215
De Lucas Moreno B, 122
De Nigris F, 278
De Paula Sabino A, 156
De Rosa M, 288
Dechecchi MC, 172
Delrio P, 288
Demonacos C, 182, 183
Deng S, 293
Denis J, 215, 280
Dennison C, 101
Detorakis ET, 147
Dezhenkova L, 112
Dhaliwal JS, 105
Di Crescenzo RM, 310
Di Giacomo C, 308, 309
Di Liegro I, 221
Di Magno L, 221
Di Paolo ML, 250
Díaz-Castillejos R, 261
Díaz-Hernández C, 306
Dimopoulos C, 122
Dimopoulos M, 272
Dimov D, 140, 141
Docea A, 248
Dolashka P, 300
Dolashki A, 300
Doria A, 149
Dos Santos C, 322
Dostalova S, 235
Dotsenko AS, 146

Doukas PG, 154
Doukas SG, 153, 154
Drakoulis N, 152
Drotar D-M, 188
Drysdale SB, 186
Dufies M, 229
Dunworth M, 304
Duong H-Q, 136
Dušinská M, 271
Duthoo E, 120
Dvorak J, 115

Echizenya S, 104
Eckschlager T, 235
Efstathiades C, 122
Egorova V, 167
El-Dafrawy SM, 325
Eliopoulos E, 133, 134, 159, 160, 161
Elmarsafawi A, 238
Epling-Burnette PK, 238
Etrych T, 110, 138, 139

Fabbri E, 170, 172, 173, 178
Fajstova A, 114
Falciani F, 229
Falvo E, 143
Falzone L, 241, 242, 243, 244, 246, 247
Fan H, 233, 234
Fazioli F, 278
Feng B, 315
Feng M, 315
Fenga C, 263, 264
Fernández-Rojas B, 306
Fields AP, 155, 168
Filip M, 184
Fimmers R, 231
Finotti A, 170, 172, 173, 175, 176, 177, 178, 221
Fiorillo A, 239
Fisher LM, 313
Foglia L, 149
Fong SH, 105
Fonseca V, 322
Forte GI, 309
Fragkiadaki P, 237, 260
Franco-Arellanes MC, 261

Galanova N, 114
Gallo M, 278
Gambari R, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 221
Gandhi K, 324
Gao J, 129
Gao X, 150
Garai J, 311
Garcia-Argaez AN, 303
Garrido M, 119
Gascon P, 322
Gasparello J, 172, 173, 176, 177, 178, 221
Gattuso G, 241
Gavriil V, 164
Gebhard S, 106
Gemmo C, 171, 173
Genovese C, 308
Gerner C, 111
Gerotziafas GT, 272, 287
Giambò F, 314
Gnanamony M, 281
Gnosa S, 127
Go R-E, 318
Golan T, 312
Gomes KB, 156
Gondi CS, 281
Gong H, 102
Gong P, 233
Gottardi W, 163, 225
Goulielmaki M, 164, 166
Goulielmos GN, 133, 134, 159, 161
Grammatikova N, 112
Grancara S, 303
Grigorescu M, 267
Grigorieva EV, 146, 268
Grivas G, 252
Grunt TW, 111
Grushevskaya H, 167
Gryazeva I, 230
Guazzelli A, 183
Guerra G, 174
Guo W, 279, 284
Gupta R, 265
Győrffy B, 157

Haerynck F, 120
Hagiwara S, 104
Halabalaki M, 196
Haroutounian S, 195
Harper S, 239
Hasenburg A, 106
Hashimoto T, 285, 286
Hassan N, 105
Hatta T, 104
He H, 150, 292
He L, 102
He M, 292
Heger Z, 235
Heinsohn S, 266
Heiss K, 231
Hemminki K, 271
Herdean A , 135
Hernández-Cruz PA, 306
Hesterberg RE, 238
Hill R, 101
Holshouser S, 304
Höög A, 129
Houfkova K, 289
Hrabánková K, 138
Hrabeta J, 235
Hrncir T, 114
Hudcovic T, 114, 115
Hussain M, 183
Hyeraci M, 240

Ibrahim S, 325
Idris SZ, 105
Ikawa T, 286
Ikubo K, 285, 286
Ilardi G, 310
Ilari A, 239
Ilina E, 191
Illescas-Barbosa D, 306
Indra R, 235
Inman KS, 155
Intagliata S, 273
Ishii Y, 104
Ivanov E, 300
Izraeli S, 312
Izzo P, 288

Jang JH, 148
Jang Y-G, 319
Jensen L, 127
Jeyabalan J, 265
Ji P, 311
Jiang X, 127, 150
Jiménez-Castillo V, 261, 306
Jirak D, 143
Jiraskova Zakostelska Z, 114
Jonas E, 324
Jönsson J-I, 127
Jóźwik M, 270
Juhasz A, 116
Juhlin CC, 129
Junie LM, 267
Justilien V, 168

Kadan S, 130
Kahn M, 324
Kaiser A, 231
Kakar SS, 275
Kalachniuk T, 191
Kalliora C, 260
Kamvoukou CA, 195
Kaňa M, 139
Kanamori Y, 221, 303
Kandimalla R, 265
Kang J-H, 319
Karagiannis SN, 213
Karalis V, 316
Karamouzi E, 147
Karlikova M, 277
Karzi V, 259
Katsantoni E, 179
Kausbekova AZh, 190
Kazanskaya GM, 268
Kerasioti E, 195
Khan JA, 258
Khoor A, 168
Khunchai S, 182
Kim C-W, 321
Kim G-S, 320
Kim J, 317
Kim J-Y, 148
Kisliouk T, 118
Kislun Y, 191
Kitazawa S, 104
Kitowska K, 126
Kitsou M, 123
Klimovich A, 112
Kloppers C, 324
Kogan M, 119
Kollia Z, 164
Kolpakova MA, 146, 268
Kondo T, 104
Konstantinidou S, 316
Konstantinov SM, 300, 302
Kontozisi E, 195
Kopsida M, 127
Kordek R, 126
Koromyslova I, 180
Korovessi P, 251, 252
Kosenko A, 118
Kostka L, 110

Kostovcik M, 114
Kostovcikova K, 114
Kouka P, 195, 196
Kouretas D, 192, 193, 194, 195, 196
Koutsaftiki C, 207, 210
Koutsounaki E, 254
Kovar M, 110, 137
Kozakova H, 115
Kozanidou E, 211
Krikunova LI, 301
Krishna M, 155
Kroupa M, 271
Krstic-Demonacos M, 182, 183
Krylova N, 167
Kubinova L, 115
Kubista M, 135
Kucera R, 277, 289
Kulieva GZ, 301
Kumar R, 271
Kumar S, 129
Kusainova AA, 190
Kutner A, 158
Kverka M, 114
Kyakulaga A-H, 265
Kyriakopoulos AM, 162, 163

La Mantia A, 308
Ladas D, 195
Lagoudaki E, 147
Lallous N, 109
Lam EW-F, 127
Lamberti N, 178
Lambrechts D, 229
Lampronti I, 170, 171, 172, 173, 178
Laponogov I, 313
Larsen AK, 215, 280, 282, 287
Larsson C, 129
Laufer S, 118
Laytragoon Lewin N, 145
Ledda C, 197, 198, 199
Lee L, 129
Lee LJ, 105
Lee S-M, 320
Lee T-J, 148
Leff M, 137
Lehmberg K, 266
Leitges M, 155, 168
Levy M, 115, 142
Lewin F, 145
Lewkowicz D, 270
Li C-Y, 290, 298
Li E, 311
Li J, 290, 298
Li W, 223
Li X, 150
Li Y, 234
Liang H, 291
Libra M, 241, 242, 244, 246, 247
Licarete E, 188, 189, 219, 220, 236
Limjindaporn T, 182
Lin S, 234
Lipnevich I, 167
Liu N, 127
Liu Y, 168, 293
Lobos L, 119
Löfgren S, 145
López-Rodríguez ML, 111
Louadj L, 215, 280, 282
Lu D, 295, 296
Lu Y, 296
Ludwig S, 137
Luetragoon T, 145
Lui W-O, 129
Lukac P, 115, 142, 143, 144
Luput L, 188, 189, 219, 220, 236
Lv X-D, 294
Lymperatou C, 252

Ma J, 150
Ma X, 279, 283, 284, 291
Magri V, 305
Mahajna H, 130
Makovicky P, 115, 116, 142, 143
Makri S, 192
Malátová I, 138
Malfa GA, 308, 309
Malpeli G, 323
Mammas IN, 206, 208, 253
Manfredini F, 178
Manicardi A, 172
Marconi A, 197
Markova T, 223
Martello L, 311
Martino F, 310
Matalliotaki C, 134
Matalliotakis M, 134, 161
Matchuk ON, 301
Matei HV, 181, 184
Matera S, 197, 199
Matsunaga H, 285, 286
Matthes J, 231
Mavrakis KG, 147
Méndez-López F, 185
Mendonça Cardoso Pestana R, 156
Menendez D, 276
Meng CK, 105
Merali C, 307
Merali S, 303, 307
Merolla F, 310
Mésange P, 282
Meskyte EM, 276
Meysami P, 183
Minafra L, 309
Minch BS, 317
Missineo A, 239
Mitsis T, 160
Miyauchi M, 285, 286
Mkrtchyan LS, 301
Mohamed AH, 274, 325, 326
Mohd Ibrahim H, 105
Mohty M, 272
Mondello C, 128
Monti E, 305
Morales F, 119
Moser HE, 313
Moskovkina T, 112
Mot AC, 220
Mowoe MO, 324
Muduli S, 151
Mueller A-K, 231
Mukai K, 285, 286
Munagala R, 265
Muntean D, 189
Murray NR, 155, 168
Murray Stewart T, 304
Mušák L, 271
Mutti L, 183

Naccarati A, 271
Nagara M, 122
Nagl M, 162, 163, 222
Nagy Á, 157
Narsanska A, 277
Natsume T, 104
Neagu M, 203, 205
Neves C, 322
Nicula GZ, 181, 184
Nielsen PE, 131, 170
Nikolakakis N, 179
Nikolski M, 229
Nitulescu GM, 216
Njuguna JT, 231
Nujedat H, 158
Nuraeva G, 214

Oblovatskaya M, 122
Ochai T, 218
Ochiai Y, 218
Ohkubo S, 221
Oka N, 223
Okamura H, 223, 285, 286
Oliva D, 145
Oliveira HHM, 156
Osman R, 105
Ősz Á, 157

Pacak K, 224
Pages G, 229
Pan X, 313
Panagopoulos I, 269
Pandrea SL, 267
Pandrea SM, 267
Pantazopoulou D, 147
Papageorgiou C, 179
Papageorgiou L, 272
Papaioannou G, 201
Papatheodoropoulou A, 202, 209
Papi C, 177, 178
Paredes J, 311
Parés Santilari C, 232
Parker Th, 179
Parte S, 275
Pashkovskaya OA, 268
Passarella D, 250
Patankar M, 275
Patras L, 188, 189, 219, 220, 236
Pavlenko A, 191
Pellegatti P, 174
Pencheva M, 223
Pencheva-El Tibi I, 302
Peng Y, 102
Pennisi M, 243, 262
Peretz T, 312
Perini C, 128
Perletti G, 305
Pesta M, 289
Peterson YK, 304
Petrak J, 224

Pezzè L, 276
Piasecka D, 126
Pierantoni GM, 288
Pierouli K, 159
Pignatiello S, 310
Pili R, 278
Pinevich A, 230
Ping J, 127
Pinzi L, 250
Pisciotta P, 309
Podlupski C, 116
Politko MO, 268
Polívková Z, 271
Ponla KM, 317
Pontalti S, 276
Popov A, 112
Porav S, 219
Porfire A, 189, 219
Potemski P, 126
Prokaeva AI, 268
Prosdocimi M, 173

Qian Y, 234
Qiao F, 233
Quinn C, 307

Rachakonda S, 271
Ragusa M, 308
Rajsiglova L, 115, 142, 143, 144
Ramenghi U, 149
Ramírez-Thomé SK, 261
Rapisarda L, 198, 199
Rapisarda V, 197, 198, 199
Rastelli G, 250
Ratajczak J, 107, 108
Ratajczak MZ, 107, 108
Rath B, 187
Ratnayake WS, 101
Rauca VF, 188, 189, 219, 220, 236
Razvi SS, 258
Ren S, 292
Renieri E, 260
Renis M, 308
Rennie PS, 109
Resnick MA, 276
Rezackova H, 277, 289
Ribot E, 229
Riccio P, 288
Rico M, 307
Rigobello MP, 240
Říhová B, 110, 138
Rizos E, 179
Romanska HM, 126
Romero C, 119
Roufas C, 122
Rudewicz J, 229
Ruíz-Pichardo Y, 261
Russo D, 310
Russo G, 309
Rusu D, 184
Rutqvist L-E, 145

Sabbah M, 280, 282
Sadej R, 126
Said HM, 256
Salas E, 119
Salemi R, 241, 242, 243, 244
Salerno L, 273
Samaritani S, 240
Samoylovich M, 230
Sampaio R, 322
Sanderson MR, 313
Santagati M, 244
Santos C, 322
Saracco P, 149
Sarandi E, 237, 248, 249, 260
Sarantopoulou E, 164
Sasaki CT, 153, 154
Savas S, 103
Savoca A, 198
Scarpa A, 323
Schekotihin A, 112
Schultz DJ, 265
Schwartz J-M, 183
Scognamiglio G, 278
Scotti Buzhardt ML, 155
Sedlacek R, 115, 142, 143
Selivanova EI, 301
Selvarajah J, 313
Semczuk A, 270
Semczuk-Sikora A, 270
Semkova M, 111
Sesarman A, 188, 189, 219, 220, 236
Shamoun L, 145
Shang Y, 292
Shashkova O, 230
Sheikh RA, 258
Shen X, 233
Shevelev OB, 146
Shi H, 129, 315
Shinwari Z, 117
Shuai P, 293
Siafakas N, 179
Sidiropoulou Z, 322
Sidoryk K, 151
Signorelli SS, 245
Silva LM, 156
Sima P, 115
Simões R, 156
Simoni D, 171
Šírová M, 138, 139
Siserman CV, 181, 184
Sivák L 110, 138
Sjöback R, 135
Skaltsounis A-L, 196
Skamrova G, 313
Skaperda Z, 193
Skardasi G, 103
Skourti E, 179
Slany A, 111
Slavov E, 141
Slodnik P, 226, 227
Slouka D, 289
Smirnov I, 230
Sologub V, 180
Solórzano-Mata CJ, 261, 306
Soriţău O, 184
Souleyreau W, 229
Spagnardi M, 311
Spandidos DA, 134, 179, 206, 208, 253, 260
Spangler J, 137
Spencer W, 265
Stagos D, 195
Staibano S, 310
Stakheev D, 115, 142
Stehlikova Z, 114
Stejerean I, 188
Stiborova M, 235
Stillitano MG, 149
Stolbovaya A, 230
Stonik V, 112
Strandeus M, 145
Straughn A, 275
Stübiger G, 111
Studenovský M, 138
Studzinski GP, 158
Styshova O, 112
Subr V, 110
Suhovskih AV, 146, 268
Sultan S, 172
Sun J, 292
Sun X-F, 127
Sung E-G, 148
Surcel M, 205
Suzuki-Karasaki M, 218
Suzuki-Karasaki Y, 217, 218
Svoboda J, 142
Sylvester B, 219

Tacheva T, 140, 141
Taitzoglou I, 196
Takashi A, 218
Tamanini A, 172
Tang C, 229
Tefas L, 219
Tekos F, 195
Telang N, 113
Tenti P, 115, 142, 143, 144
Teodoro M, 263, 264
Thanasoula M, 248
Theodoridou M, 200, 212
Thiery J-P, 215
Thompson EA, 168
Thouroude S, 215, 280, 287
Tian K, 183
Timeus F, 149, 221
Tlaskalova-Hogenova H, 114
Toma V, 220
Tomala J, 137
Tomasello B, 308, 309
Tompa M, 267
Tompa R, 267
Tomuta I, 189
Tongmuang N, 182
Toninello A, 303
Topolcan O, 277, 289
Torrente E, 239
Torres-Rivera A, 306
Trachtenberg A, 151, 158
Treska V, 277
Tringali R, 309
Trochopoulos A, 300, 302
Tsamakis K, 179
Tsatsakis AM, 237, 248, 249, 259, 260
Tserevelakis GJ, 147
Tsidulko AY, 146, 268
Tsiftsoglou AS, 132
Tsiftsoglou SA, 132
Tsilimidos G, 237, 249
Tsoporis J, 179
Tsoukalas D, 237, 248, 249, 260
Tulbah A, 117
Tupin C, 171

Tupini C, 170, 172, 177
Turano M, 288
Turcano L, 239
Turczyk L, 126
Twegieri T, 117
Tyagi N, 265
Tzatzarakis MN, 259

Umezawa K, 150
Umrekar T, 313
Ungur R, 184
Uwa N, 285, 286

Vachkova E, 140
Vageli DP, 153, 154
Vakonaki E, 237, 259, 260
Valente V, 288
Valihrach L, 135
Vallero S, 149
Van Dreden P, 272, 287
Vannucci L, 115, 116, 142, 143, 144
Vargas-Casillas AP, 261
Varricchio S, 310
Vasconcelos AP, 322
Vasilevska D, 270
Vasilopoulos E, 260
Vecchione MA, 310
Vega M, 119
Velkova L, 300
Vermeulen S, 120
Veselkov DA, 313
Veskoukis AS, 192, 193, 194, 196
Vică ML, 181, 184
Virant-Klun I, 275
Visco C, 323
Visochek L, 312
Vit O, 224
Vitale E, 197, 198, 199
Vivarelli S, 241, 242, 243, 244, 246, 247
Vlachakis D, 159, 160, 161
Vlase L, 189
Vlaykova D, 140, 141
Vlaykova T, 140, 141
Vodenková S, 271
Vodička P, 115, 271
Vodičková L, 115, 271
Volkov AV, 268
Vondrasek D, 115, 142
Vral A, 120
Vymetálková V, 271

Wagner R, 111
Wang B, 292
Wang J-L, 290, 298
Wang X, 127, 150, 168, 311
Wang X-H, 290, 298
Wang Y, 293
Watanabe S-I, 104
Watanabe Y, 285, 286
Weems C, 168
Williams J, 311
Windrichova J, 289
Woster PM, 304
Wu T, 292

Xiang J-L, 290, 298
Xie H, 129
Xie N, 127
Xie Z, 150
Xin X, 295
Xu H, 284
Xu L, 292

Yamanishi C, 285, 286
Yamanishi H, 223
Yamanishi K, 223, 285, 286
Yang H, 137
Yang J, 283
Yang Q-C, 294
Yao S-M, 294
Yenchitsomanus P-T, 182
Yermalovich M, 167
Yin N, 168
Yoncheva K, 302
Yoshida Y, 218
Yu Y, 103

Zabaleta J, 311
Zacharakis G, 147
Zaharieva MM, 302
Zaid H, 130
Zamò A, 323
Zamulaeva IA, 301
Zamzami MA, 258
Zaravinos A, 122, 123, 124, 125
Zavjalov EL, 146
Zeller C, 226, 227
Zervou MI, 133, 134, 161
Zhang T, 283
Zhang W-W, 290, 298
Zhang Y, 150, 292
Zhang Z, 127
Zhao D, 292
Zhao Z, 127, 233, 234
Zhelyazkova Y, 141
Zheravin AA, 268
Zhgun A, 214
Zhgun E, 191
Zhou M, 234
Zhou R, 291
Zoumpourlis V, 162, 163, 164, 165, 166, 179
Zuccato C, 171, 173, 176
zur Stadt U, 266
Zurac S, 203, 205
Zurlo M, 176

25th International Symposium on Molecular Medicine

26-28 March 2020
Park Plaza Hotel
Santiago, Chile

SPANDIDOS PUBLICATIONS

WORLD ACADEMY OF SCIENCES

UNIVERSIDAD DE CHILE

conferences@worldacademyofsciences.com

www.worldacademyofsciences.com

26th International Symposium on Molecular Medicine

7-9 May 2020

Grand Hotel Duca D'Este
Tivoli, Rome
Italy

SPANDIDOS PUBLICATIONS

SAPIENZA UNIVERSITÀ DI ROMA

IPFO International Polyamines Foundation

WORLD ACADEMY OF SCIENCES

conferences@worldacademyofsciences.com

www.worldacademyofsciences.com