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

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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 mesenchymal-to-epithelial transition prostate cancer cells, which is highly regulated by EMT. Our previous studies showed that atypical protein kinase C-α (PKC-α) and a5 (PCK-C) inhibition attenuated the activation of the NF-κB pathway by diminishing NF-κB nuclear translocation. The present study demonstrated that the siRNA knockdown of PCK-C and PCK-C downregulated Starkin, PAK1, Vimentin, 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 PCK-C and PCK-C knockdown samples. Immunoprecipitation experiments suggested the direct association of Vimentin with PKC-C and PCK-C, sequentially. Laser-stimulated confocal immunofluorescence and immunogold transmission electron microscope techniques were used to further confirm the relationship of Vimentin with PKC-C. 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 PKC-C/mRNA knockdown. Overall, the results revealed a stronger relationship between PCK-C and Vimentin over PCK-C and Vimentin. Microscopic results also showed PCK-C accumulated along the cell membrane together with Vimentin in addition to the abundant redistribution throughout the cell. In addition, our results suggested that both PCK-C target activation sites (S509, S539 and 906) on Vimentin play a crucial role in Src Vimentin dynamics, which is essential for increased prostate cancer cell motility. We used a novel PKC-C specific inhibitor (K80150) to inhibit Src in vitro experiments on murine models. Excess tumors were analyzed for pathways observed in vivo, in vitro experiments. Immunohistochemical and western blot analysis of the tumor samples confirmed the relationship of PKC-C and Vimentin. In addition, these samples were analyzed for the mRNA expression upon PKC-C inhibition. Overall, the results suggest that both PKC-C 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-C and PCK-C can be effectively targeted using specific inhibitors to develop targeted therapeutics for metastatic prostate carcinoma.

Effect of anti-CD107a on murine gliomas using dendritic cells transduced with HTERT2C2 reconstituted adenovirus

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HTERT2C2, a 27ΔDHT HTERT T-teratocarcinoma polyhedron, has been demonstrated to induce tumor-specific Hela cell killing and the growth of mice melanoma. HTERT2C2 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. Hence, we report that dendritic cells (DCs) transduced with HTERT2C2 can increase T-teratocarcinoma proliferation, and augment the concentration of interleukin-2 (IL-2) and interferon-γ (IFN-γ) in the supernatant of T cells. The T cells co-cultured with rAd27-DDC cells produced 75.5±4.32 pg/ml of IL-2 and 61.35±2.23 pg/ml of IFN-γ, which are higher than those in rAd-EGFP DCs and the normal control DCs groups. The cytotoxic activity of rAd27-D DCs was 59.8±8.95% at a 4:1 effector/target ratio (E/T), while no obvious lysis by rAd-EGFP DCs or DCs was detected, even at the highest E/T ratio (29.3±1.90, 25.7±3.21%). It was demonstrated that the cytotoxic T lymphocytes (CTL) against glioma cells were mainly induced by HTERT2C2 peptides. To further evaluate whether immunological interactions with rAd27-D DCs influence the induction of tumor-specific T cell responses, tumor-bearing mice were immunized twice. The cytotoxicity in the mice elicited by rAd27-D DCs was much higher than the other groups at the E/T ratios of 5:1, 10:1 and 20:1. 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 rAd27-D DCs group were 10.3±1.24 mm3, which was significantly smaller than other groups (P=0.001). The mice administered Ad-27-D DCs exhibited a significantly prolonged survival compared with rAd-EGFP DCs or DCs. These data suggest that HTERT2C2 gene-transduced DCs can efficiently enhance immunity against gliomas in vivo and in vitro.

Key words: dendritic cells, cytotoxic T lymphocytes, immunotherapy, HTERT2C2

The SWNSFN complex subunit genes and their relation to patient survival times in human cancers

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SWNSFN 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., SMARCC1, SMARCB1, SMARCAD1, SMARCBD1, SMARCAD2, SMARCAD3, SMACC) are involved in human diseases, carcinogenesis, and disease susceptibility or clinical outcomes in cancer patients. In a recent analysis using the publicly available data from the TCGA samples (1), we demonstrated that the tumor expression levels of a large number of SWNSFN complex genes were associated with the survival times of patients with low-grade brain gliomas and renal clear cell carcinomas (2). In particular, in the latter disease, the reduced expression levels of six SWNSFN genes (SMARCC2, SMARCD1, SMARCD2, SMARCD3, SMACC, and SMADC) were associated with longer survival times in patients. These novel associations suggest that elevated levels of SWNSFN 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 SWNSFN complex genes, their functions and alterations, and variable patient survival outcomes. In addition, two promotor variants of the BRM gene encoding one of the SWNSFN subunits (BRM) 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). BRM is one of the two ATPases of the SWNSFN complex. We recently examined the association of these variants with the risk and survival outcomes in colorectal cancer patients 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 studies in additional clinical colorectal cancer cohorts. In this presentation, we will discuss recent results from our examination of the SWNSFN subunit associations and review the importance of the SWNSFN complex in cancer research.


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

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Glioblastoma (GBM)-initiating cells (GICs) comprise a tumorigenic 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 glioblastoma (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-ethyl-β-D-ribofuranosyl) 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 1050, a potential lead compound, inhibited proliferation, survival and stenosis 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 1050 did not exert any visible side-effects on mice. We further demonstrated that 1050 induced the nuclear export of stem cell factors in GIC in a Ctr1 (also known as exportin-) dependent manner. These results suggest that 1050 is a promising novel drug against GICs and other cancer cells that depend on pyrimidine synthesis (2).

2) Ezhibiyanova et al., submitted.
Inhibition of p38 in breast cancer is a questionable strategy

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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 inhibitors SB203580. Since SB203580 additionally inhibits other signaling molecules, we compared the effects of SB203580 with those of Skeppinone, a more specific p38 inhibitor. In breast cancer cell lines, we quantified p38 activity, cell viability, adhesion and chemotactatct migration following treatment with p38 inhibitor, SB203580 and Skeppinone treatment of the cells resulted in different cellular effects. We found an enhanced p38 activity following treatment with SB203580, whereas Skeppinone reduced p38 activity. SB203580 reduced cell viability, whereas Skeppinone enhanced it. The inhibitory effect on cell adhesion and migration, caused by SB203580, was more potent than that caused by Skeppinone. These results demonstrate that in breast cancer, p38 apparently has a progressory inhibitory effect and the specific inhibition of p38 should be questioned.

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

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Tumorogenesis 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 we 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 radiochemotherapy for tumor treatment, we are unintentionally creating a pro-metastatic microenvironment in collateral organs. Specifically, many factors are upregulated in response to radiochemotherapy-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 chemokine 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 cancersogenesis, including extracellular microvesicles, bioactive phospholipids and extracellular nucleotides.

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

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The complement cascade (CoMC) cleavage fragments C3a and C5a regulate the trafficking of normal, differentiated hematopoietic cells, although they do not chemotactract more primitive hematopoietic stem/progenitor cells (HSPCs). By contrast, human myeloid and lymphoid leukemia cell lines and chronic leukemic blasts from CML and AML patients respond to C3 and C5 cleavage fragments by chemotaxis and increased adhesion. Consistent with this finding, C5a 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/symptoma patients (e.g., as a result of accompanying infections or sterile inflammation after radiochemotherapy) enhances the mobility 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/symptoma cells in clinical situations in which the CoMC becomes activated. Finally, since we detected the expression of C3 and C5 mRNA in human leukemia cell lines, further study of the potential role of the complement in regulating the behavior of these cells is needed.
Development of small molecule Myc-Max inhibitors as potential therapeutics for prostate cancer
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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 transcriptional activity. In this regard, VPC-78067 was found to effectively inhibit Myc-Max activity with low to mid-micromolar range potency and with minimal off-target cytotoxicity. Additionally, compound VPC-78063, 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 development of more potent and specific Myc-Max inhibitors that may serve as promising new therapeutics to treat advanced prostate and other malignancies.

Ritonavir as a novel antitumor drug candidate
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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 Vc2-hydroxypropylmethoxyamine (HPMA) copolymers bearing the cyclostatin drug, doxorubicin (Dox), and the inhibitor of Pgp, -tonavir, in MDR tumors overexpressing Pgp. Both Dox and -tonavir 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 (Rd) suitable for such a purpose. We proved that such a conjugate is able to overcome MDR both in vitro and in vivo in F98/MDR and C1256 mouse tumor models expressing high and low levels of Pgp, respectively. More importantly, we found that HPMA copolymer conjugate bearing only Rd exhibited significant antitumor activity in vivo. Moreover, the antitumor activity of HPMA copolymer conjugate bearing Rd synergized with immunotherapy and was able to completely cure 95% of mice with established and progressively growing C1256 tumors. Thus, it is demonstrated herein that ritonavir and its derivatives are promising antitumor drugs.

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HIT one to eliminate all during cancer treatment: The crucial role of fatty acid synthase (FASN) in ovarian cancer (OC) growth
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OCs overexpress FASN, the key enzyme in de novo lipogenesis. Accordingly, FASN inhibitors have been shown to induce OC growth. Nevertheless, there is still a lack of in-depth analysis of the consequences of FASN blockade on the biologically regulatory balance in OC cells. Herein, we combined a systems biology approach i) tandem mass spectrometry(MS/MS) shotgun proteomics, with ii) antibody microarray technologies, and with iii) multiple reaction monitoring(MRM)targeted metabolomics. MS/MS cells were cultured for 8 h or 24 h at < 0.1 µM FASN inhibitor G802UCM and gene (functional) classification on the DAVID platform was used to distinguish early Fa, late L- and sustained S- responses. E-responses included activated stress pathways (ER stress, UPR), apoptosis and autophagy, as well as the inhibition of nucleotides, lipids and cellular catabolism, including respiratory chain and electron transport. L-responses comprised the inhibition of DNA replication, ribosome formation, cytoskeleton-actin re-modeling, b-receptor induced the breakdown of signalling, expression, transport, proteasome and COX/ROS. 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, regulation of unlimited nutrients in the medium analogous to nutrients. In summary, membrane integrity was compromised by G802UCM, resulting in defects in cellular uptake. Apparently, cells cannot compensate for nutrient deficiency by importing exogenous nutrients. This dependency of OC to lipogenesis/nutrient uptake should be exploited for chemotherapy.

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Comparative evaluation of the antitumor activity of trypanthrin and its synthetic water soluble analogustropan
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Natural quinazoline alkaloid trypanthrin (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. 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 tropan (MT), as a result of the reaction of TT with Girard's reagent. The advantages of MT in comparison with TT include its good solubility in pharmaceutically 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 MCT-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.

(2) Stonik et al., application RU patent No 2019110477, priority 08.04.2019.
Stem cells from a model for genetically predisposed colon cancer

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Germline or somatic mutations in the tumor suppressor adenomatous polyposis coli (APC) gene regulate growth 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<sup>−/−</sup> 850<sup>−/−</sup> COL cecal epithelial cell line represented the model. Non-stressed anti-inflammatory drug sulindac (SUL), selective ornithine decarboxylase inhibitor difluoro methyl ornithine (DFMO) and select herbal products represented the test compounds. Relative to Apc<sup>+/−</sup> COL 857 COL cells, Apc<sup>−/−</sup> 850<sup>−/−</sup> COL cells exhibited the loss of cell growth control and increased anchorage-independent (AI) colony formation, indicative of aberrant hyper-proliferation and an arrested cancer phenotype. Mechanistically, Apc<sup>−/−</sup> cells exhibited upregulated Apc<sup>−/−</sup> 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 G1 phase arrest and inhibit AI colony formation. Herbs extracts reduced AI colony formation, affected cell cycle progression and increased cellular apoptosis. The Apc<sup>−/−</sup> sulindac-resistant (SUL-R) phenotype exhibited tumor size reduction and an upregulated cell cycle 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.

Immunomodulatory properties of dietary components, commensal microbiota and antibiotics

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The Gut microbiota is an immune system modulator and disruptions in this system affect host health. In human microbiota, ‘bacteria with free lunch’ with complex-specific pathogen-free microbiota, obtained from two different animal facilities. We found that they significantly differ in ileal microbiota, cytokine milieu in Peyer’s patches, proportions of regulatory T cells and in the sensitivity to antibiotics induced inflammation. We then found that the excess of animal protein exacerbated intestinal inflammation. The detectable 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 effects of oral reconstitution (OM) on intestinal inflammation or delayed-type hypersensitivity (DTH). We found that M significantly decreased inflammation and pro-inflammatory cytokine production in Peyer’s patches. The dampening effect of O 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.

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Re-thinking the tumor microenvironment

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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. In IL-6, IL-1, IL-10 and TGF-beta exhibited different interplay depending on the type of cell inflammation, both at the local and systemic level. In addition, in human colon cancer samples - normal mucosa, tumor mucosa and cancer tissue - scaffold changes were related to local immunological features. An increased gene expression of collagen 1, IL-6, IL-1, IL-10 and TGF-beta was detected, particularly in the late tumor mucosa, a borderline tissue associated to active immune infiltration. PD-1 and PD-L1 expression also may be upregulated by these conditions. We hypothesized that the permissive deregulation of regulatory molecule expression (e.g., TGF-beta) may overcome the tissue inflammatory threshold normally regulating the homeostatic conditions allowing initial tumor microenvironment establishment.

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Colorectal carcinoma diagnosis in Slovakia: A challenge for family doctors

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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 Komarne, 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 Komarne. Samples were obtained by colonoscopy performed at the Gastroenterology center of the General For Life Hospital in Komorany, and histologically evaluated. On a total of 84 colorectal carcinomas, 22 colorectal carcinomas were diagnosed (26%). In conclusion, the high incidence of CRC render early diagnosis also 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 increased CRC incidence in one region needs consideration of several exogenous factors involved in large bowel tumorigenesis. Therefore, family doctors should have much more intense impact for early CRC diagnosis, actively promoting extensive two-step screening.

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

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Immunochemistry (IRC) and fluorescence in situ hybridization (FISH) are currently being 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/new status resulting into certain breast cancer patients being erroneously deprived 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 (PPtP), from 30 breast cancer patients classified into IHC2/HER2+PR+ (Luminal B), IHC2/HER2+PR- (Luminal A), IHC2/HER2-PR+ (HER2+) and IHC2/HER2-PR- (triple-negative/basal) were analyzed using quantitative label-free liquid chromatography tandem mass spectrometry (LC-MS/MS).

We identified 106 plasma proteins of which 73 and 209 were significantly differentially expressed between luminal A versus 0 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 81 breast cancer samples with the HER2 status represented in The Cancer Genome Atlas (TCGA). Further, a panel of clinical relevance is some of the 44 proteins during similar expression changes between the pairs of Luminal A vs. Luminal B and HER2 vs. basal (TNBC), indicating their potentials as HER2-specific biomarkers in various subtypes of breast cancer.

Among the identified protein biomarkers are TUFM, SMX1, FKBPL1, MME, STOM1, C12C57, HST331, RB35 and TADA2B. Some of these identified proteins have been implicated in PGR, ER, PR, HER2 and/or inflammatory 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 biomarkers for precision therapy for breast cancer patients.

3-Deoxynolactate Staff activity during pregnancy represents a risk factor for late stage ovarian cancer development: Studies on a transgenic mouse model

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The overexpression and enforced activation of Staff in the mammary glands of transgenic mice cause party-dependent latent tumorigenesis. Staff activity in mammary epithelial tumorogenic and epithelial cells with hyper Staff activity are 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 Staff activity with the expression of the DNA damage response proteins. IDAX. IDAX 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, HDAX overexpression induces tumorigenesis. Staff expression and HDAX promoter activity are correlated, and adjacent laminar and basal cells with hyper Staff activity, respectively, have been located in the pregnant gland. Further analyses supported a model in which the high Staff activity in individual laminar cells caused paracrine RANKL secretion that induces HDAX promoter de-methylation in their neighboring basal cells. In turn, a deregulated high IDAX expression ensued, triggering the mammary gland to tumorigenesis. HDAX expression in tumors was higher than in the intact gland. The highest expression characterized the differentiated adenocarcinomas, which preserved the Staff-dependent pattern of HDAX promoter activity. A negative correlation between the two was detected in the proliferating adenocarcinomas. The distinction between cells overexpressing Staff and HDAX was generally preserved, with rare exceptions. The methylation status of the HDAX promoter may mediate its activity. Highly methylated HDAX promoter characterizes all tumors, as compared to moderate or low HDAX expression in Staff cells. Among the highly methylated HDAX 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 C/EBP transcription factors that bind this site may mediate the highest HDAX expression of this tumor type and the response to Staff activity. Taken together, a deregulated Staff activity during pregnancy may be considered as a risk factor for late breast cancer development.

Role of mIrl-145-associated gold-nanoparticles as a potential adjuvant therapy for epithelial ovarian cancer

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Ovarian cancer remains a major health concern worldwide, with over 225,000 new cases and 149,000 deaths reported annually. The Gynecologic study positions that by 2035, there will be 200,000 deaths in incidence to 370,000 (15%) and as increased in deaths to 479 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 TNFα, COX-2 and VEGF, among others proteins, are involved in the progression of EOC and are regulated by microRNAs (miR-145 or miR-145-5P). Altered microRNA 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. The 145-145 expression is decreased during EOC progression and some targets of this miRNA are e-COX-2 and VEGF. The objective of this study was to evaluate the proliferation, migration and invasion of the EOC cells and also to evaluate the formation of clones, using fold-expression of miR-145-5P and microRNA (miR-145-5P) in EOC cells. MicroRNA (miR-145-5P) of EOC cells was evaluated by western-blowing assay in 5 and 21 h, and a significant decrease in cell migration was observed (P<0.001). In addition, the effect of miR-145-5P on the migration of EOC cells was evaluated by western-blowing assay in 5 and 21 h, and a significant decrease in cell migration was observed (P<0.001). Moreover, the proliferation of endometrial cells (Th2/2/2) was evaluated with conditioned media of 21 and 2788 cells (treated with or without miR-145-5P), and a significant decrease in cell proliferation was observed (P<0.001). Based on the effect of miR-145-5P on important biological processes, these results affirm the importance of using an adjuvant therapy with miR-145-5P for EOC.

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Assessment of in vitro radiosensitivity in primary immune deficiency patients

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Primary immune deficiency diseases (PIDs) are life-threatening genetic diseases of the immune system. A subset of PIDs involves mutations in genes acting in the repair of DNA double-stranded 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.

The main goal of this study was to implement nationwide radiosensitivity analysis in the routine diagnostic procedures for PIDs in Belgium. In 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 GO cyclin-dependent-block micronucleus assay (CBMN), which is about to be translated into clinical practice; and (ii) the SG2 CBNM, which has been developed in our center with promising proof-of-concept, but requires further and final optimization before translation to routine practice. Both tests 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 and direct genetic analysis and guide optimal patient care. Micrometastasis analysis in lymphocytes of patients is currently ongoing and results of a first pilot pretranslational 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.
The alkylphosphocholine erucamide is a multifunctional antitumor agent as its anticancer activities are based on interference with multiple signaling pathways.

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Erucamide (erucylphospho-NN,N′-trimethylpropanolamine), erucylphospho-

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

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Colorectal cancer (CRC) is the second leading cause of cancer-related death with 8% of the newly diagnosed cases and deaths. Monoclonal antibody targeting soluble colorectal cancer (MSC-1) expressing PD-L1, response to anti-PD1 or anti-CTLA-4 blockade but whereas microsatellite-stable tumors (MSS) do not respond the same. Adaptive cell therapy using tumor-infiltrating lymphocytes (TIL) load is another, highly promising, immunotherapeutic strategy for these patients. Our aim was to examine how the CRC patient’s immune landscape correlates to tumor microenvironment (TME) factors. We evaluated neo-antigen sequencing data and clinicopathological data for 456 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 GEPAS server was also used for analysing the RNA sequencing expression data of 275 COAD and 92 READ tumors, compared to 356 normal samples from the TCGA and the GTEx projects, respectively. The TIL load was scored as "0", if "TILs < 1" if the number of TILs ranged between 1 and 15, "1" if the number of TILs ranged between 15 and 215, and "2" if TILs >215 in that particular histological subtype. Kaplan-Meier curves were constructed using logrank P 0.05 for all differences in overall survival between high- or low-gene expression patients, or between MSS, MSI-H, and MSI-L patients, respectively. Using the logrank test. The Spearman’s test was used to correlate the TIL load with the expression of each immune checkpoint molecule. High expression levels of PD-L1, PD-L2, PDL1 and TIGIT were significantly associated with the COAD patients’ better survival. We also found that TIL was significantly overexpressed both in COAD and READ tumors, compared to the adjacent normal tissue. On the other hand, LAG-3 and CD27 showed a significant decrease in the COAD and READ tumors versus the normal tissues. Furthermore, among CRC patients, the TIL load was positively correlated with the expression of CD8, as well as that of the immune checkpoints APODZA, CTLA-4, HAVCR, LAG3, PD-L1, PD-L2, TIGIT and VISTA (p<0.05). Predictors correlation was also assessed. By contrast, among READ patients, such positive correlations between TIL load and immune checkpoint expression were scored only for LAG-3 and PD-L2. In conclusion, our data highlight the differential expression of more than one immune checkpoint molecule which suggests a higher positive correlation with the TIL load, as well as the potential use of CTLA-4 and TIGIT as prognostic markers for COAD patient survival.

RNA-editing is one potential RNA epigenetic mechanism that was recently found to lower cancer stiffness and enhance the oncosuppressive 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 APOBE-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 analyzed them computationally to detect single nucleotide polymorphisms (SNPs), insertions and deletions (indels), copy number variations (CNV) and structural variations (SVs). We further analyzed the mutational signatures of each CRC patient. The gene expression levels of the AID/APOBEC family genes were normalized in Transcript Per Million (TPM) values. We found an increased expression of APOBE1-C3, and APOBE3C, suggesting a role in the disease. We also analyzed 102 CRC exome sequencing data from TCGA and identified that the majority of SVs were C7 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 to 129 knowns and validated mutational signatures from the COSMIC database. Importantly, the first signature results indicated an elevated rate of spontaneous deamination of 5-methyl-C to T mutations in these samples. We have also shown that the mutation load is significantly higher in APOBE1-enriched compared to non-APOBE1-enriched colorectal cancers and has a preference to T mutations. Therefore, we conclude that postulate that epigenetic changes in mRNA occur during the development of colorectal cancer and can directly drive tumor progression. Further insight into cancer evolution will help to develop a deeper understanding of the pathophysiology of colorectal cancer and may identify new potential therapeutic targets.

Immunoprofiling of the microenvironment of colorectal cancer

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Although the standard therapeutic approaches for the treatment of colorectal cancer (CRC) including the addition of TGFβ 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-unstable (MSI) CRC. The intratumoral cytotoxic activity (CTA) is determined by the expression of the toxins granulocyte A (GZMA) and perform 1 (PRF1), and patients were stratified to CTLA-4 and CTLA-4 subgroups. We investigated recurrent somatic copy number alterations and somatic point mutations specific for each cytolytic subgroup, and made connections with the cytoplasmic state. The expression of several immune checkpoint molecules, including PD-L1, PD-L1 and CTLA-4, was analyzed with respect to each tumor’s CYC and the status of microsatellite instability (MSI). A high cytolytic activity was associated with an increased mutational load in colorectal tumors, the cohort of MHC-III cancer neoplasia, a high microsatellite instability (MSI) and the expression of several inhibitory immune checkpoints. A number of immune checkpoint molecules (IDO, LAG3, TIGIT, VISTA, PD-1, PD-L1, and PD-L1) were higher in MSI CRCS compared to MSS tumors. The expression of Treg markers was also significantly higher in CTLA-4 CRCs. Assessed globally, CRCs did not receive much emphasis in the generation of T cells in CRCs. 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 CTLA-4 high tumors may be more suitable candidates for combinatorial checkpoint immunotherapy.
The role of RNA editing in cancer development and prognosis
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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 conversion, mRNA, and protein modifications mediated by ADAR and AID/APOBEC enzymes, can have a significant contribution in the development and progression of cancer. Transcriptome analysis of various tumor types revealed differential RNA editing levels in each cancer type. Decreased A-to-I editing patterns have been identified in brain, kidney, lung, prostate and testis tumors, with significant global hyper-editing of A-to-I elements. By contrast, recent studies on multiple cancers found that, elevated editing levels in intronic, 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-characterized recoding event is the editing of the coding sequence of AZIN1 mRNA. In liver cancer, it potentiates tumour initiation and progression [8]. AZIN1 editing 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 indicate that, editing levels and targets have different roles in the pathogenesis of cancer and different clinical outcomes in the progression of the disease.

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FGFR2-mediated signaling in luminal breast cancer: Implications for therapy and prognosis
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Strontium stimuli mediated by growth factors receptors leading to ligand-independent activation of strontium 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, MCF7FGFR2−/− and T47DFGFR2−/−) using western blotting and immunoblotting techniques. 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 366 women who had undergone surgery, followed by adjuvant hormonal or chemotherapy using immunohistochemistry for FGFR2, ERK and phospho-ERK. We demonstrated that: (i) Signaling mediated by FGFR2 caused ER phosphorylation, ubiquitination and subsequent ER pro-apoptotic degradation, which counteracted tumour-cell-protected ER stabilization (1); ii) FGFR2 stimulated the activation of PI3K/AKT, leading to the phosphorylation of ER at Ser67 and the upregulation of p90, both of which mediated FGFR2-induced resistance of breast cancer cells to tamoxifen treatment (2); iii) 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 cellular material, the expression of FGFR2 inversely correlated with ER and the expression of PIR inversely correlated with activated RSK2 (R = -0.160); patients with RSK2/P/PR/PIR had a 3.62-fold higher risk of recurrence (P = 0.002) when compared with the remaining cohort and RSK2/P/PR/PIR was as an independent prognostic factor (P = 0.006) (3). ER/PR regulation by FGFR2-mediated signaling may thus represent a novel molecular pathway likely to contribute to the development of the resistance of IDC to endocrine therapy.

(2) Pleszcz et al., Oncotarget 7: 96011-96025, 2016.

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RhoIL in relation to radiotherapy in colorectal cancer
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The aim of this study was to explore whether Rho protein is involved in the radiosensitivity 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 radiosensitivities and invasive abilities. 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. Prolonged signalling 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 expression, 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 radiosensitivity of colorectal cancer through the Akt and FOXM1 pathways.

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Glutamine and asparagine cross-talk in cancer cells
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During tumorigenesis, metabolism is extensively reprogrammed towards a Warburg-like aerobic glycolysis and demand of highly proliferating cancer cells. Glutamine is one of the major sources of building blocks for macromolecular biosynthesis and several cancers are activated in this amino acid to support the study of the effects of amino acid deprivation to glucose deprivation, we found that glutamine withdrawal not only impaired cell survival and proliferation, but also altered e-myc expression. Given the involvement of glutamine in non-essential amino acid (NEAA) biosynthesis, we investigated whether a panel of five NEAA, 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 absence 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 e-myc and glutamine synthetase expression that supports cell proliferation in the absence of exogenous glutamine.
Merkel cell polyomavirus oncoproteins induce microRNAs that suppress multiple key genes in autophagy

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Viruses can inhibit host autophagy through multiple mechanisms, and the evasion of autophagy plays an important role in immune suppression and viral oncoegenicity. 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-antigens and truncated large T-antigens (LT), but not the wild-type LT, resulted in the suppression of autophagy, suggesting the importance of autophagy evasion in MCPyV-mediated tumorigenesis. Tumor I treatment induced cell death which was attenuated by autophagy inhibitor, but not p53-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.

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

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This in vitro study aimed to investigate the role of apoinotin, 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 (2000 cells/well) were exposed to increasing concentrations (0.1-1 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. Apoiosis 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 (80%) 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-Cy5 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 had no effect on the expression levels of Apopt-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, as well as 60 microRNAs, in cell cycle arrest agents. On the whole, these results suggest that H. triquetrifolium seems to be a potential therapeutic agent for colon cancer growth inhibition.

Precision antitumor antibodies against multidrug-resistant Gram-negative bacteria

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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 were aware 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 were aiming at developing precision antitumor antibiotics based on peptide nucleic acids (PNA) specifically targeting (essential) bacterial genes (1-2). Using PNA oligomers targeting the aceF gene and conjugated to bacterial penetrating peptides (IPP) (3-6), antimicrobials showing (sub)potent antimicrobial activity against Enterobacter coli, Klebsiella pneumoniae, Acinetobacter baumanii and Pseudomonas aeruginos (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 antitumor antibiotics against infections by multi resistant Gram-negative bacteria will be discussed.


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

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Human myeloid leukemias (HMLs) represent a large class of highly heterogeneous, aggressive and difficult to treat hematological malignancies derived from the bone marrows 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) (1-4). 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 cytotoxic, daunorubicin and other reagents, including kinase inhibitors. The complex nature of the molecular pathway of AML is usually characterized by numerous cytopathologc abnormalities (gene fusion pml-ras), chromosomal translocations [(t(8;21), t(15;17)], inversions, as well as other mutations which affect both the unfare and functions of important cell cycle regulators (TOP3, Npmt), transcription factors (Runt, C bpa), epigenetic modifying enzymes (DNMT2) and cell signaling pathways (5). All those layers of molecular abnormalities indicate that a coordinated multistage targeting strategy could be more effective in eradicating or controlling AML beyond chemical therapeutics (6). This could include, in addition to chemotherapeutics, potential differentiation inducers, therapeutic monoclonal antibodies against selected cell surface antigens (CD3 and CD123), small molecule inhibitors against signaling mediators (Fos-induced inhibitors) and epigenic regulators (Stat3, Dnmt3a and Histon Decenyase-HDACs inhibitors), as well as other suppressors of human leukemia cell proliferation, as the ones recently developed by our group, and advanced CAR-T/NC1 cell based immunotherapies. Such coordinated multilevel AML therapeutic approaches would be valuable as strategic alternatives in eradicating resistant LSC clones or controlling their relapse.

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

Maria I. Zervou, Ilia Koropoulou, Giorgos N. Goulis

Aims: To present the recent advances in the field of genetics of autoimmune diseases (AIDs), with a special emphasis on the role of genetic factors in the pathogenesis of these diseases. In this review, we will focus on the latest findings in the field of genetics of autoimmune diseases, including the role of genetics in the pathogenesis of these diseases, the role of genetic factors in the development of autoimmune diseases, and the role of genetic factors in the treatment of these diseases.

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

Mikael Kubbista

Aims: To review the different methods for the measurement of molecular cancer biomarkers.

Shaping the genetic profile of endometriosis through the correlation of gene polymorphisms with genetic factors

George N. Castagna, Ilia Koropoulou, Michael Mastorakos, Chrysovalanis Maxoulis

Aims: To present the latest findings in the field of genetics of endometriosis, with a special emphasis on the role of genetic factors in the pathogenesis of this disease. In this review, we will focus on the latest findings in the field of genetics of endometriosis, including the role of genetics in the pathogenesis of this disease, the role of genetic factors in the development of endometriosis, and the role of genetic factors in the treatment of this disease.

ALDH1A1: The novel biomarker of pancreatic cancer stem cells

Hong-Quan Dong

Aims: To review the latest findings in the field of genetics of pancreatic cancer, with a special emphasis on the role of genetic factors in the development of pancreatic cancer. In this review, we will focus on the latest findings in the field of genetics of pancreatic cancer, including the role of genetics in the development of pancreatic cancer, the role of genetic factors in the treatment of pancreatic cancer, and the role of genetic factors in the prevention of pancreatic cancer.

Germline mutations are of particular importance for the development of pancreatic cancer, however, the role of genetic factors in the development of pancreatic cancer is not well understood. This review will focus on the latest findings in the field of genetics of pancreatic cancer, including the role of genetics in the development of pancreatic cancer, the role of genetic factors in the treatment of pancreatic cancer, and the role of genetic factors in the prevention of pancreatic cancer.
On the way to cytokine-antibody single-chain fusions for cancer immunotherapy

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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 (Treg) and harmful off-target effects have limited its clinical efficacy. Boyman et al. (1) described methods with which to address 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 human cytokine chimera (immunocytokine, IC) consisting of IL-2 linked to light chain of anti-IL-2 mAb through a flexible oligopeptide spacer, functionally similar to S4B6 mAb, which circumvent disadvantages of IL-2/S4B6 mAb complexes and exert sufficient biological activity. We demonstrate that this IC we have produced contains 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.


Polymer prodrugs for the treatment of experimental solid tumors and immunomodulation

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Polymer drug delivery systems represent a promising strategy for efficient tumor targeting and several advantages over the systemic treatment: 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 architectural, 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 EL-4 T cell lymphoma or CT26 colon cancer. Tumor rechallenge of the cured animals provided conclusive evidence of the antitumor 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 nitrogens 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 potentiatisated the accumulation of co-administered macromolecular cancerotoxins, 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 carry advantages for dampening the suppressive activity of myeloid-derived suppressor cells (MDSCs), such as all-trans retinoic acid (ATRA) or erlotinib (Erlotinib). 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 (17-06868S), and Ministry of Health of the Czech Republic (16-28606A).

Micellar polymer drug delivery systems for the treatment of chemoresistant tumors

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Polymeric nanotherapeutics have been used as targeted delivery systems of anticancer drugs, improving their biodistribution and decreasing systemic toxicity. Amphoteric diblock copolymer conjugates based on hydrophobic 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 (N-(asparagine), in the blood, with a 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 up-regulation of which is one of the main mechanisms of tumor multidrug resistance (MDR). We verified the ability of the HPMA-PPO diblock copolymer to inhibit MDR in vitro in Doxor-resistant P388/MDR and CT26 cancer cell lines, both expressing P-gp. However, our results suggested that the inhibitory activity of HPMA-PPO copolymer was dependent on its physico-chemical characteristics, including the presence of protecting (bloc) groups, residual amounts of HPMA linear chains, or P388/MDR, HPMA-PPO-P388 copolymers. In particular, the unbound PPO significantly increased the ability of the tested polymer samples to inhibit ABC transporters. To further increase the in vivo activity of the diblock copolymer, biodegradable disulfide bond (S-S) was incorporated between hydrophilic 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.

MMP-9 serum levels and 2127G→T SNP in 4 polymorphism in patients with cutaneous melanoma

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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 angiogenesis, cell proliferation, cell 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 high influenced by several inflammatory cytokines and grows 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 c.266G>A, P574R c.1724G>A, R664Q c.2074T>G). In the current study we aimed to explore the possible effect of 2127G→T SNP in intron 4 of the 33590 gene (c.2074T>G) 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 2127G→T SNP varied significantly between the patients and controls (P<0.012), as the common G allele homozygosity was associated with a 3.24-fold risk of melanoma compared to the other genotype (OR=3.245, 95% CI 1.387-7.526, P=0.007). At the same time, patients with the GG genotype tended to have a longer DFS (mean of 208 vs. 65.5 mos, P<0.0049) and a longer survival after diagnosis (mean of 159.2 vs. 74.9 mos, P=0.013, log rank test). The serum level of MMP-9 was significantly lower in the patients than in the controls (13.29±3.83 vs. 25.3±7.11, SEM mg/L, P=0.028). The values below the cutoff of 14 mg assessed by ROC curve analysis (AUC=0.671, p=0.001) determined skin melanomas with 56.5% 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 (208±7 vs. 64.33 mos, P=0.002) and a longer survival after diagnosis (181.62 vs. 63.37 mos, P=0.005). There was no association between the genotypes and serum levels of MMP-9. In conclusion, that result of this study suggests that the GG genotype of MMP-9 2127G→T SNP may be a risk factor for development of skin melanomas, but may favor the outcome of patients. The 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.

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Serum 25-hydroxyvitamin D levels in patients with COPD

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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 phosphorus homeostasis and bone remodeling, but also due to their possible role in lung diseases. As a result of a number of studies being published on this topic, the association of serum vitamin D from 25-hydroxyvitamin D, 25(OH)D(0.0) with the risk and the clinical course of chronic obstructive pulmonary disease (COPD) remains 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 ImmunoDiagnostics Deutschland GmbH. The serum levels of 25(OH)D in patients with COPD were significantly lower than those of the controls [17±4.8 (0.0) vs. 29±4.9 (0.0) nmol/L (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 [21±5.9 (0.0)] compared to the period October-April (16±2.7 (0.0) P<0.001). The 25(OH)D levels were inversely associated with the number of white blood cells (P=0.045, P=0.009, neutrophils (P=0.039), lymphocytes (P=0.025) and eosinophils (P=0.035, P=0.045). In patients with severe and very severe COPD (GOLD 3 and 4), there were strong significant positive correlations between the serum levels of 25(OH)D and the spirometric indexes FEV1 % pr. (P=0.026, P=0.026) and FVC % pr. (P=0.034, P=0.024). In conclusion, the observed inverse correlations in COPD patients of serum 25-hydroxyvitamin D levels with blood inflammatory cells confirm the suggested immunomodulatory effect of vitamin D but, additionally, 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.

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Immunological changes in human colorectal cancer and 3D cancer modelling in vitro

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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-PCR revealed the progressive increase in the expression of COL1A1, IL-1β, IL-13 and LOXL2, all involved in tissue remodeling. IL-6 expression increased 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 PDL-1 expression was increased in the transition mucosa and in the tumor. To model in vitro the tumor development, 3D cultures of colorectal tumor cells and stromal underlying tissues appear useful. They 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 modeling the plasticity of the microenvironment under controlled system and conditions.

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Lysyl oxidases and the formation of the early tumoral niche

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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 recently been described, suggesting a multitandem role of LOXs within a complex network of signaling pathways, regulating a number of cell functioning, 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 to our preliminary data, the tissue microenvironment remodeling 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 sufficient 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/mTORIL-13, non-canonical TGFβ pathways.

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

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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 samples 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 CD8+ cells were detected in the breast cancer patients. Increasing numbers of immunosuppressive neutrophils and monocytes, CD14+ CD16+ 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+ CD33+ 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 redistribution of the immune response cells from circulation to local radiation site.

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

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Glioblastoma multiforme (GBM) is characterised by a low patient survival rate 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 resection model. To model the GBM relapse in vivo, TMZ (150 mg/m²) 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 those of the control group, particularly for TMZ/DXM combination. Co-culture of the U87 cells with organotypic hippocampus slices in vitro pre-treated with TMZ and/or DXM resulted in an increased proliferation of 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 polyanionic chains levels in experimental models in vitro 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.

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Combined photoacoustic and fluorescence label-free microscopy for the ex vivo investigation of oral melanotic lesions of the conjunctiva and choroid, surgically excised from human eyes.

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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 oral melanotic lesions of the conjunctiva and choroid, surgically excised from human eyes. Examined specimens included both benign (norial) and malignant (melanoma) lesions. Human biopsy samples had been preserved in paraffin which was removed prior to imaging by a standard deparaffinization and re-hydration process. 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 diotretic mirror. Photoacoustic waves were detected by a 20 MHz central frequency spherically focused 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 possible concomitance between them. The bimodal microscopy approach presented in this study has the potential to contribute in the differentiation between benign and malignant intraocular tumours 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.


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

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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 results will 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, benzoyloxycarbonyl-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, respectively. Notably, we found that c-FLIP, protein expression was downregulated by the decreased protein stability of c-FLIP, 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
Reduction of CFU-GM and circulating hematopoietic progenitors in a subgroup of children with chronic neutropenia associated with severe infections and delayed recovery

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Myelopoesis 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/mcL). Other causes of neutropenia were excluded. Bone marrow morphology, clonogenic tests and the peripheral blood CD34+ cell count and the apoptotic rate were evaluated in 61 patients with neutropenia lasting >12 months or with severe infections. The circulating CD34+ 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 28 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. 5/16; 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 36/55 patients (74%) evaluated for clonogenic tests. All patients with normal CFU-GM recovered (>59 patients), whereas neutropenia persisted in 12/26 patients with reduced CFU-GM (46%; Pearson’s χ²=0.02). In 36/55 (65%) patients evaluated by flow cytometry, we observed reduced circulating CD34+ cell frequency compared with 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 18/18 patients with Pearson’s C=0.05. Nineteen patients had 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 χ²=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.

Comparison of the anti-inflammatory effect of DHEEQ and tacrolimus in a mouse model without atopic dermatitis

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In previous studies, it was demonstrated that DHEEQ improves DNLXOX-induced atopic dermatitis-like lesions. This study focused on the anti-inflammatory effect of DHEEQ in a mouse model without atopic dermatitis-like lesions. DNLXOX-induced tacrolimus, and the efficacy of the drug was compared. The results revealed that DHEEQ and tacrolimus significantly improved the dermatitis symptoms of the mice with DNLXOX-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 serotonin (5-HT) and the expression of the inflammatory factors, IL-6, IL-13, IL-18, and interferon (IFN)-γ as well. Moreover, the behavior of the mice treated with tacrolimus was altered, with the mice running irritably with jumping movements. In addition, marked inflammatory exudation on the lesioned-skin surface of the mice was found. In contrast, DHEEQ did not result in any adverse effects. Collectively, DHEEQ was found to be safer, gentler and more suitable for long-term use than tacrolimus for the treatment of atopic dermatitis-like lesions.

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

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Acute myeloid leukemia (AML) is a devastating blood malignancy characterized by unregulated proliferation of leukemic blasts. We have previously shown that the combination of carvacrol (C) and carvacrol (CA) synergistically induces massive CA-dependent apoptosis in human AML cells both in vivo and in vitro, without affecting normal hematopoietic cells. In this study, we synthesized a series of hydroxycinnamic acid derivatives, such as hydroxyphenylferulonitrile 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-4) had the ability to synergize with CA to induce a strong and rapid (48 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 cytotoxic Ca2+ accumulation and was not accompanied by cell cycle perturbations. 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 C1R-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 carboxyl 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.

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Findings of the study

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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 metabolisms; 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. Genotype 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 so-called 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 influencing environmental factors, determine both the risk of developing common diseases and how patients will respond to treatment. They are the genetic 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 drug response. In addition to personalized medicine through genetics, the use of biomarkers allows physicians to answer important questions related to disease and therapeutic approaches. 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 individual variability, hence, can be indicators of susceptibility to effects of exposure or to disease. Susceptibility Biomarker provide insight into the mechanisms of disease development, support biological plausibility, and assist in the choice of pharmacotherapy.
Biliary tumorigenic effect on hypopharyngeal cells is significantly enhanced by pH reduction

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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 mRNAs phenotype previously linked to hypopharyngeal carcinogenesis. We have used our in vitro model (2,3) to explore the effect of bile, in range 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 mRNAs phenotypes. We performed comprehensive apoptosis experiments of conjugated primary bile acids with or without unprocessed secondary bile acid, deoxycholic acid (DCA) on HHPCs, and we used immunofluorescence, western blotting, lucerase assay, qPCR and PCR microarray analyses to detect NF-κB activation levels and the transcriptional activation of NF-κB-related oncogenic mRNAs 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 mRNAs 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, although 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.

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Protein kinase C iota promotes pancreatic tumorigenesis

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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 demonstrated that protein kinase C iota (PKCi) is required for PDAC-transformed growth (1), and that a targeted inhibitor of PKCi blocks PDAC transformed growth in vitro and in vivo (2). PKCi is significantly upregulated in patients with PDAC, with a high PKCi expression predicting a poor patient survival (1). These findings support the clinical relevance of our studies. However, little is known about the role of PKCi in pancreatic development or pancreatic tumorigenesis. Thus, a transgenic mouse model was developed to investigate the effects of tissue-specific PKCi ablation on pancreatic adenocarcinoma. KrasG12D/Pten-deficient pancreatic tumor formation. The effects of PKCi ablation were characterized using histological, immunohistochemical and electron microscopic analyses. The tissue-specific ablation of PKCi expression in the pancreas did not significantly affect pancreatic development or function; however, it decreased pancreatic size. PKCi ablation significantly altered KrasG12D-driven pancreatic tumor initiation and progression. Taken together, these results reveal that PKCi plays a required role in pancreatic epithelial cell metabolism and PDAC development, suggesting that the targeted inhibition of PKCi may prove to be a novel therapeutic strategy in pancreatic cancer.

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

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Thrombomodulin is a transmembrane protein expressed on the surface of endothelial cells in all vessels, 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 FHEMG 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 106.1 pg/mL (IQR 6-62), those of IL-1 were 19.4 pg/mL (IQR 16-37), CRP were 9.35 mg/dL (IQR 10-35). At T2, the median levels of TNF-α were 58.85 pg/mL (IQR 35-95.9), those of TNF-α were 4.08 ng/mL (IQR 2.90-5.80), CRP were 5.75 mg/dL (IQR 5.63-6.34). 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 (Pearson’s correlation, p-value < 0.05) with the thrombomodulin levels (T1), 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.

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

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Vitamin D derivatives (VDDs) - 1α,25-dihydroxyvitamin D3 (1,25-D) and 1α,25-dihydroxyvitamin D2 (1,25-D2) and synthetic analogs - have the potential for use in the differentiation therapy of acute myeloid leukemia (AML). The evaluation herein that various activators of the transcription factor Nrf2, including the furanolic 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 to 1α,25-dihydroxymorpholino dimethyl fumarate (MMDF) on the VDD-induced regulation 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 analog PRL-3520 cooperatively inhibited AML progression in mouse models of hematological malignancies. Treatment with PRL-3520-DMF following cytotoxic induction therapy with etarabine/tamoxifen/cyclophosphamide/Amruct reduced the overall therapeutic outcome. Likewise, the combination of MMDF resulted in enhanced anti-leukemic effect of Acr+PRL-3520 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 chemotherapeutics of AML.

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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 homoeostasis: The role of the glucocorticoid receptor

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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 malfunctions, suggesting that more and more knowledge on the interplay of nuclear receptors’ transcriptional networks could provide new insights 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 and the inactivation of proinflammatory factors, GR is involved in numerous physiological processes like cell proliferation, differentiation, and apoptosis. The information was later applied on a dataset comprised of more than 64,000 full human genome sequences. The data were 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 found from this procedure. Furthermore, evolutionary and structural information emerged from the dataset. These results have a wide array of applications. 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.
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Attempts to link exocrine gene polymorphisms to disease-associated protein modified functionality: A structural biology approach

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Pathology physiology 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 discriminate disposition under severity and genetic variation. Genome-wide association studies (GWAS) play a primary role in depicting genetic contributions to disease development, while accommodating the exocrine polymorphisms on the protein structure level, when available, enhances our understanding of protein function modification or deletion. In this framework, we investigated functional polymorphisms by correlation with protein structure function for several multifactorial autonomous or diseases. Cases include protein targets involved in intracellular signaling (TYK2, STAT1, VEGFR2 (1-3), inflammation (IL6, NF-κB) and (4) endomembranes (LAM5, NAT2, SKAP1, GRIEB1) (5). Immune responses and apoptosis (TNF, RANKL, NF-κB, SYK) (6,7), as well as autoimmune diseases (pMHC) (8). Here, based on several examples, we analyzed the sequence of techniques used in order to achieve a national link from gene polymorphism to structure to modified function including metagenomic analysis of SNP polymorphisms, protein crystallography, protein molecular modeling, molecular mechanics and dynamics. Learning, shaping and understanding the target protein interface interaction plays a decisive role in most cases and provides clues for further pharmacological or medical actions.

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TNF and derivative clinical data

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TNF is a central molecule for mammalian and human health optimization and has a plethora of prospective medical applications. Applications cover from combating neoplastic growth, cardiovascular disease, nephropathy, metabolic disease (including diabetes), to modern approaches for countering muscular-skeletal and developmental defects. Most importantly it is not only tumoral the most abundant β-sulfur-containing amino acid in our bodies that promotes a fine balance between health and disease. TNF generates derivatives, such as N-formyl-TNF (NF-TNF) and N-bromotaurine (NBTr) by innate hemeolipid reactions. These molecules prove to be key to our immune defense system as at the same time they may, 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-carbohydrate unwanted usage due to their overwhelming therapeutic failures.

Key words: TNF health uses, taurine derivative clinical data

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Cytoskeletal stressors in cancer cells

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Transmembrane 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 multiple cellular forces capable of altering genomic reprogramming. The interaction of the tumor microenvironment with ECM scaffolds normally activate a cell’s membrane focal adhesion proteins and transmembrane signal receptors (TSRs). The mechanosensors regulate tumour cell growth via signal transaction between the extracellular active domain of cells and the Intracellular Focal Filaments by triggering an avalanche of Protein conformational changes and the execution of TSR pathways requisite the activating force to lay in the low pN set of force values and certainly below the pN 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 electro polar or other type of dispersive interactions and confined local interactions of outward scaffolds with the biological milieu was recognized to be responsible for diverging cell functionality roles. Contracting 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 extracellular matrix, providing the identification of further facets of cytoskeletal approach. Tension of outward scaffolds are also related with sarcomere formation, the electrical charging states and the strength of molecular bonding between outward scaffolds and plaques. In summary, transmembrane signal receptors activate cancer cell growth by localized extracellular mechanical signal transaction from nanoscale scaffolds (1) and ECM, triggering pathways and viability tests in different human cell lines point to coherent mechanical stimulation of >10 pN ligand-binding sites of integrins and EGFRA via a coherent synergistic action. Any local force perturbation in the extracellular matrix may evoke a ligand adhesion binding site in integrins (MIBRA or ADMEAN) still perceived that the stressing force lies within 10 and 500 pN (1). Atomic force microscopy (AFM) and nanodetermination cytoskeletal cell analysis also show an enhanced probability for tethered signal transaction in metastatic tumour cells compared to necrotic ones. The study contributes towards recognizing different cytoskeletal stressing modes in cancer.
Mesenchymal stem cells isolated from the umbilical cord act as potent anticancer agents

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Mesenchymal stem cells (MSCs) possess distinctive features, such as high proliferation rates and the capability of differentiating 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 RCC-specific gene therapy 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; data collection 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 transcription 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 therapy 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-tumorogenic effects displayed by MSCs, which are mediated by different pathways.


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Novel approaches to parvovirus B19 diagnostics in cancer patients

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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 multilayered carbon nanotubes (CNT) and nanoporous composites. For the non-edimentic detection of viral DNA hybridization, non-Faradic 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 method, any probability of contamination of negative samples during amplification can be excluded.

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

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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 PRC1 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 mous L561-Kras124Val/Trp536577 (KP)-mediated LADC tumorigenesis can proceed through both PPK-dependent and PPK-independent pathways. The predominant pathway involves the PPK-dependent transformation of bronchiolar/cylindrical stem cells (BCASs). However, KP mice harboring conditional knockout PPK alleles (KP mice) developed LADC tumors through the PPK-independent transformation of A312 bronchiolar type 2 (AT2) stem cells. The transformed progeny of AT2 cells were blocked by Wnt pathway inhibition in vitro and in vivo. Furthermore, a KPP-deleted genomic signature predicts the sensitivity of human LADC cells to Wnt inhibition, and identifies a distinct subset of primary LADC tumors exhibiting a KP-like genotype. Thus, LADC can develop through both PPK-dependent and PPK-independent pathways, resulting in tumors exhibiting distinct oncogenic signaling and pharmacologic vulnerabilities. These data provide a compelling rationale for the use of PKCα and Wnt pathway-targeted therapeutics in treatment of distinct LADC subtypes that can be distinguished using Wnt pathway gene profiling.
A peptide nucleic acid targeting the mcp-1 gene of Pseudomonas aeruginosa inhibits bacterial induced biological alterations in cystic fibrosis sputum

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Abstract: A peptide nucleic acid (PNA) targeting the mcp-1 gene of Pseudomonas aeruginosa (PA) to inhibit the expression of mcp-1 in cystic fibrosis sputum was designed. The peptide was then conjugated to a lipid and formulated in a liposomal preparation, which was then tested in vitro for its ability to inhibit the expression of mcp-1 in cystic fibrosis sputum. The results showed that the PNA-conjugate was able to inhibit the expression of mcp-1 in cystic fibrosis sputum, suggesting that this therapy could be beneficial for the treatment of cystic fibrosis.

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Identification of deregulated miRNAs in liquid biopsies from colorectal cancer (CRC) patients: Impact on personalized miRNA therapies
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MicroRNAs (miRNAs) are small non-coding RNAs regulating gene expression by sequence-specific 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 cell-free nucleic acids (including miRNAs) present in the blood of cancer patients. It is considered one of the most advanced non-invasive diagnostic systems suitable for early diagnosis, staging, prognosis prediction, therapy response, outcome prediction, and follow-up during therapeutic intervention. 1) We performed NGS of plasma isolated from 30 colorectal carcinoma (CRC) patients and identified a description of 12 deregulated miRNAs, including miR-221, miR-222 and miR-141. These data were further validated by droplet digital RT-qPCR (ddRT-qPCR). 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 experimentally 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-Glycaminoglycane units, are excellent tools. We developed novel delivery strategies for PNA targeting miRNAs, based on the use of PNAs linked to a poly-arginine Arg peptide (0-5), nanoparticles and its novel molecules constructed by a multisubstrate monomeric RNase-inhibitor substrate to be used as non-covalent vector for the gau-Lyn mRNAs (3). As far as the validation of PNA activity, we focused on miRNAs targeting miR-221, miR-222 and miR-141, based on data. Inhibitory anti-miRNA constructs were obtained with the co-administration of oligonucleotides and a PNA targeting miR-221. Further, the increased treatment of glioma (U251) cells with non-specific miR-221 and the PNA targeting miR-221, led to the induction of apoptosis at high levels. In conclusion, the liquid biopsy could be considered as a basis for personalized miRNA based therapy of cancer, and PNAs may be a valuable option to modulate the abundance of deregulated miRNAs, in addition to miRNA replacement molecules mimicking tumor-suppressive miRNA.

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Stress-mediated induction of fetal hemoglobin in beta-thalassemia: Impact of the Samd protein family member 2 (SAMD2) protein
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The in vivo effects of stress on the induction of fetal hemoglobin (HbF) is of key importance for therapeutic protocols in a variety of hemoglobinopathies, including \\
GREAT-SCISD (We have previously reported the strong inducing effect of the TGF-beta inhibitors exinunotropen (exinosin) and evoirenol on HbF production by erythroid precursors (EPCs) from fdSAMD2-knockout mice. We take advantage from the availability of a fdSAMD2 cellular bioassay allowing stratification of the patients with respect to fetal hemoglobin production and response to HbF inducers. The results obtained by HPLC analysis of the EPCs cultures from 38 patients led to the following conclusions: (a) stress increases HbF in cultures from beta-thalassemia patients with different base fetal levels (the cultures from 54.8% of the patients were responsive to stress induction); (b) the cultures from 73.4% of the patients were not responsive to stress or hydroxyurea (HU); (c) stress induced a high induction of HbF in 46.15% of the cultures not responsive to HU; (d) stress displayed higher efficiency than HU in 57.34% of the cultures responsive to both stress and stress cultures. (e) 42.86% of the HbF-treated cultures displayed HbF induction higher than stress. In order to study a possible association between DNA polymorphisms and stress-mediated HbF induction, the lipidoglycan CD151 (CD151A1), the two BCL11A (rs41245076 and rs10898573) and the H3.3-enhanced MYB rs9391273 polymorphisms were analyzed. Both the CD151A1 (rs41245076) and the BCL11A (rs10898573) polymorphisms, an association with stress-mediated HbF induction was found, but only in the human embryonic patients (3:287 and 3:78, respectively). These results indicate that the g-y-globin (red) rs41245076 should be considered a very useful polymorphism for recruitment of beta-thalassemia patients in stress-stimulated clinical trials.

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Non-invasive prenatal detection of beta-thalassemia mutations in maternal plasma using Droplet Digital PCR
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Non-invasive prenatal testing (NIPT) is based on fetal DNA analysis with the aim to identify genetic abnormalities from the maternal plasma during pregnancy. Actually, commercial services can detect only aneuploidy, 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) technology3 to identify the two most common thalassemia mutations in the Mediterranean population (βS79 and βFV3S-I10) and maternally 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 generic DNA samples carrying different genotypes and percentages to simulate fetal and maternal circulating cell-free DNA (cfDNA) cell ratios of 5% to 10% weeks. Then the ddPCR assays were applied to determine the fetal genotypes from 36 maternal blood samples at different gestational ages. The diagnostic outcomes were confirmed for all the samples carrying paternally inherited mutaion 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 allele ratio values that were statistically significant and not overlapping, allowing the correct 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 βFV3S-I10 and βFV30 mutations paternally and maternally inherited suggesting its application also for other single point mutations causing genetic diseases.

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Deregulation of LAR is associated with induction of fetal hemoglobin in thalassemia-carriers treated erythroid cells
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LAR (Le-1 antibody reactive clone) protein is a recently identified repressor of Agyglin gene transcription in erythroid cells and binds to the 5′-GOTATT-3′ sequence of the 5′-region of the gene. The r9009-9530 GCA-polymerase chain reaction in this site is present in 95% of the patients and is associated with a high production of fetal hemoglobin (HbF). The finding that LAR binds less effectively to the GCA motif 5′-GOTATT-3′ binding site might explain the increased basal and inducible levels of HbF in erythroid cells. The present study was undertaken to verify the effects of the IFB inducer indomethacin (IMH) on LAR. We first determined whether MTH was able to inhibit the LAR/BDNA interactions using both nuclear factor kappa B (NF-κB) and recombinant LAR protein. Electrophoretic mobility shift assay demonstrated that MTH strongly interfered with the binding of LAR to the double-stranded oligonucleotides containing the Agyglin LAR binding site. We also performed RT-PCR and western blot analysis of MTH-treated cells demonstrating an increase in LAR mRNA and Agyglin gene expression. LAR expression (analyzed at mRNA and at the protein level) was downregulated in association with the upregulation of Agyglin gene expression and IFB production. LAR downregulation was confirmed in MTH-treated K562 cells, as well as in erythroid precursors cells (EPCs) from β-thalassemia patients. In the analyzed EPCs those which were found as non-responders to MTH demonstrated unchanged LAR content. In order to verify possible MTH/deregulated effects on LAR expression and function, the LAR promoter was studied and at least five Sp1/CR-rich binding sites identified. EMSA was performed using double-stranded oligonucleotides mimicking those 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 LAR promoter. In conclusion, downregulation of LAR expression and functions strongly contribute to induction of Agyglin gene expression and IFB production in erythroid cells.

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

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Shwachman-Diamond syndrome (SDS) is an inherited disease caused by mutations of a gene encoding for SHG5 protein. SDS patients present several hematological disorders, including neutropenia and myelodysplastic syndrome (MDS), with increased risk of leukemia evolution. In lymphoblastic cells of SDS patients, the already reported upregulated miR34a phosphorylation is also associated with the downregulation of PTEN, thus validating inhibitors of miR34a phosphorylation. Since PTEN expression might be under the post-transcriptional control of microRNAs, this study was undertaken to verify the miRNANome in SDS patients and find a possible correlation with PTEN gene expression. Lymphoblastic 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 microRNAs were up-regulated in SDS cell cultures compared to controls, according to a 1.5-2.0 fold threshold. The differential miRNA expression was further validated by droplet digital RT-qPCR. When the 11 up-regulated microRNAs were compared with the 81 validated PTEN-regulating microRNAs (identified using the mirTarget2 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: (1) upregulation of miR-34a-3p and miR-744-3p is variable among SDS cell lines; (2) PTEN downregulation was found in SDS cells to varying degrees; (3) 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.

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

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Detection of Autologous Blood Transfusions (AIT) 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 AIT is available. The present study was performed to determine whether the miRNA-based analysis of the global miRNA profile might be useful for the detection of AIT. Blood (500 ml) was drawn from 6 healthy subjects (T2) and then infused after 35 days (T3). Blood samples for microarray analysis of microRNA were taken 5 days before (T1) and 16 days after blood withdrawal (T3), at day of infusion (T5) and at 3 days (T6) and 15 days (T7) after infusion. For these subjects the withdrawn blood was stored at -80°C, while for the remaining three at -8°C prior to infusion. Global microRNA profiling was performed for a total of 39 RNA samples (extracted from plasma), using the Agilent Human microRNA array v2.1.0 (na. 4872A). This chip represents 2490 microRNAs, sourced from the miBase database (Release 21). Microarray run was performed on the GeneSpring GX 11 software (Agilent Technologies). Differentially expressed microRNAs were selected following determination of the fold-change analysis, taking T1 as a reference sample. Both up- and downregulated microRNAs were considered. Microarray cluster analysis of informative miRNAs found in plasma from AIT samples at T6 and T8 demonstrated that all the ABT T6 and T8 samples are clustered together and differ from T1 and T3 control samples. These results lead to conclude that the miRNANome analysis might be considered a one-step approach for the detection of AIT.

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The possible low cancer risk in uterine leiomyomas through the regulatory role of microRNAs: Preclinical data


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Leiomyomas have increased the role of microRNAs (miRNAs) as regulatory factors in the etiology and pathophysiology of major psychiatric disorders, such as schizophrenia spectrum disorders, bipolar disorder, depression etc. Reports have indicated a low cancer risk in patients with schizophrenic spectrum disorders and this link 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 suppressive activity of antipsychotics and mainly the First Generation Antipsychotics (FGAs) on this, an impact on specific gene expression associated with the development of cancer. miRNAs are a large group of small non-coding RNAs that regulate gene expression mainly in the Central Nervous System (CNS). While the development of cancer is characterized by induced gene expression (that lead to uncontrolled cell proliferation, the development of schizophrenia is characterized by the opposite phenomenon and specific genetic factors in whose genes products suppress cellular proliferation and increase apoptosis. Therefore, the suppressive role of miRNA expression on cell growth in the development of both disorders and their comorbidity might emerge as a common pathophysiological factor for both disorders and may interfere with this positive correlation. This hypothesis is based on their pharmacodynamic and genomic function, different subtypes are associated with the expression of one gene and no type can act as many targets. In our previous study, we tested the implications of miR-155, miR-161, and miR-155 in possible biomarkers for cancer and schizophrenia (1,2). The scope of this review was to examine the role of CRISPR-Cas9 in the model organisms and animal models with the possibility of regulating miR-208 and miR-203-3p in possible biomarkers for cancer and schizophrenia (1,2). The scope of this review was to examine the role of CRISPR-Cas9 in the model organisms and animal models with the possibility of regulating miR-155, miR-161, and miR-155 in possible biomarkers for cancer and schizophrenia (1,2). The scope of this review was to examine the role of CRISPR-Cas9 in the model organisms and animal models with the possibility of regulating miR-155, miR-161, and miR-155 in possible biomarkers for cancer and schizophrenia (1,2).


Monoclonal antibodies to human norovirus that was growing in cell culture

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Monoclonal antibodies are the main tools for detection of antigens in a large variety of applications in the clinical and literature. Norovirus is a member of the Caliciviridae 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 caliciviruses and mumps 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 60% of pepsin in serum-free media. Norovirus from fecal samples cause CRV-1; 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 Gc2–density gradient ultracentrifugation. Then, 100 μg of purified virus in 6.5 ml of PBS was mixed with Complete Freund’s Adjuvant and injected into fixed pathet of Balb/c mice. After 1 week, injection of purified virus was repeated and after 3 days poplar lymph nodes were removed and the lymphocytes were fixed with Sp6-9 mouse myeloma. Hybridomas were directed by indirect ELISA with purified virus as an antigen and mouse IgG1-HRP conjugate. In total, 29 clones were chosen and frozen in liquid nitrogen, and 1,2 Mab’s were produced as ascitic fluids. The Mabs were purified by protein A chromatography and sandwich ELISA was used as well as HRP-conjugates of Mab’s to generate common calicivirus epitope. Very soon after the isolation of human norovirus and animal caliciviruses were then obtained by the panel.
Endolysosomal reticulum stress in the evasion of immunosurveillance and resistance to anticancer therapy of breast cancer cells

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The induction of endolysosomal 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 endolysosomal reticulum stress complex class I and II molecules by ER α-mannosidases (ERα-MAP2) and the assembly of the antigen-MHC class I molecules complex and transport to the cellular surface by ER glycoporins [protein disulfide isomerase (PDI) and endoplasmic reticulum oxido-reductase 1 alpha (ERO1)]. This 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 unrecognised by the immune system and therefore avoid elimination. This hypothesis was tested by performing genomics-wide association studies in MCF7 and MDA-MB-231 breast cancer cells, in which PDA1 had been silenced, treated with the ER stress inhibitor, etoposide, or interferon gamma. Our results indicate that the genes upregulated in the PDA1-silenced cells are primarily involved in physiological mechanisms directly associated with the antigen presentation process or indirectly through pathways modulating calcium metabolism.

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

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Despite intense research efforts, cancer remains difficult to treat due to chemoresistance and after recurrence. The p53 tumor suppressor is crucial for cancer development, DNA damage and the chemotherapeutic response. We crucially show p53 aimlass activation models and investigated their predictive power for the stratification of cancer patients. The comparison of model simulations obtained by knockdown tests mimicking mutations with the omics type of experimental data demonstrated a significant rate of successful predictions in osteosarcoma, colon cancer and melanoma 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 29 genes that were associated with survival. In patients with osteosarcoma, p53 FEN1 and MM22 exhibited the highest inverse correlation, whereas in osteosarcoma patients with a p53 mutated status FEN1 and MDM2 was significantly associated with survival. Using DRUGSERV, experimental and commercial drugs targeting FEN1 and MM22 were identified. Testing revealed that drugs that target FEN1 (epirubicin and etoposide) have a cytotoxic effect, whereas mirtazapine and brilintus, which target MDM2 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 drugs in pathways required for chemoresistance. We consider that open further training 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.

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Polyomaviruses' antitumor activity tested on Hela cancer cell line

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Cancer cell-activated antitumor resistance requires alternative cytotoxic compounds.1 The antitumor activity of polyomaviruses (PoMAs) such as hepa- or hepa-tissue-morophile, stronger than that of well-known cytostatics (E-Rasumampecini, gemcitabine, cisplatinum), is demonstrated.2,3 This enhanced activity of PoMAs is accomplished via decreasing of the mieloidemia activity by inhibiting ATP synthesis, thus activating apoptosis mechanisms inside cancer cells.4,5 We tested 10 multidomain/tumorgen PoMAs previously synthesized via a two-step self-assembling method. All PoMAs were characterized by physicochemical 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 oncomiophage type 16 of the Human Papilloma Virus) compared with normal HRECC (Human Embryonic Renal Cell) cells. We found that, at the tested concentrations, heteropolyomaviruses had significantly stronger antitumor effect that heteropolyomaviruses, our results prove that these are real solid cytotoxic-like behaviors and their significant antitumor activity recommends them for further studies in this field.

Carcinogenesis: A systems biology approach
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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 modeling work at the intersection of development and cancer biology (2,3,4). 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 stimuli 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 signaling 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 hypoplastic 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 vivo (2) and in vitro (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 therapies of cancer, and their prevention. Hopefully the present work will motivate new approaches for cancer research.


Management of RSV infection in children: New advances and challenges
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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 therapy. 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 licensed 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.

Modulation of the tumor microenvironment by liposomal prednisolone enhances the antitumor activity of liposomal doxorubicin in an in vivo model of murine melanoma
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In solid cancers, tumor cells create an immunosuppressive niche by the induction of regulatory T cells and polarization of the tumor associated macrophages (TAM) as an alternative, proinflammatory 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 cytoplastic 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 B16F10 melanoma-bearing mice via the inhibition of the angiogenic/inflammatory efficacy of TAMs, while DOX exerted a direct cytostatic 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 cytoxic 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-bearing mice induced a more prominent reduction (by 82%) 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 progenitor production, as well as the expression level of the phosphatase c-iran, a main regulator of melanoma progression. Additionally, the combination therapy reduced the activation of matrix metalloproteinases, 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.

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

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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 siamstatin (LCL-SIM) with 5-FU encapsulated in LCL (LCL-5FU) on C26 colorectal carcinomas 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 cytoxic effects on C26 colon carcinomas 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 regimens of treatment administration were used and the main pro-tumor processes, such as angiogenesis, inflammation, oxidative stress and apoptosis were investigated. Our results indicated a potent anti-angiogenic activity in vivo exerted by LCL-SIM based on anti-angiogenic and cytoxic effects on C26 colon carcinomas. 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 anti-tumor effects. Taken together, our data indicate that this combined therapy has the potential to become a successful colorectal cancer targeted therapy.

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

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Inflammatory bowel diseases (IBD) are associated with the inhibition and replacement of the normal gut microbiota (bacterial microflora or depletion of its composition. Under these conditions, the diversity of bacteria, producers of short-chain fatty acids (SCFA) (mainly butyrate and propionate), exhibiting anti-inflammatory and protective properties, is often significantly reduced. Currently, there are various methods of microbiota transplantation (FMT) is considered the most effective. FMT is performed by introducing the local microbial community of a healthy donor into the patient’s intestinal tract. Metabotypes 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 faces. The quantitative results obtained, namely 57.3±2.66 mmol/g for acetate, 17.29±1.84 mmol/g for propionate and 14.99±0.59 mmol/g for butyrate, as well as their percentage ratio 58.6±5.99%; 19.54%±4.51; 25.5±5% (acetate: propionate: butyrate, respectively) demonstrated good agreement 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 faces compared with the control group. Long-term patient observations (4-30 days) demonstrated that FMT treatment affected the SCFA ratios, but did not reach the profiles of healthy donors. The patterns of metabolite profiles for both diseases differed, with an increase 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 bactera strains responsible for their production revealed a relationship between changes in fatty acid profiles and the corresponding bactera-producer profiles, as well as with patient recovery. On the metagenomic and metabolic levels, we clearly observed the amelioration of the microbiota to a healthy one and a trend to reach a healthy ratio in the case of SCFA for both diseases. Using metabolomics analysis, we demonstrated changes in metagenomic and metabolic levels in IBD patients. Further validation of impact of specific metabolites and microflora in therapy of IBD will help to develop targeted personalized approaches with which to improve quality of life of IBD patients.

Residential radon exposure and lung cancer risk in Kazakhstan

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Lung cancer is a serious health concern and is one of the most common cancers worldwide and it is also the most common type of cancer in the Republic of Kazakhstan. Radon exposure has been considered as a risk factor for lung cancer. Kahash was characterized for having a high indoor radon concentration. To date, a large amount of evidence has been accumulated on the involvement of mRNAs in the carcinogenesis of various malignant neoplasms, including lung cancer. Recent data have indicated that mRNAs are engaged in the regulation of cellular processes induced by radiation and, consequently, mRNAs 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 mRN-196 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 mRN-196-3p 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 mRN-196-3p 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 Kazakhstani 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 1.046 - 4.61, respectively. Individuals with Arg/Pro variant of TP53 gene exposed to a high level of radon had an OR of 4.69 (95 CI 2.6-8.54) when compared with individuals living in areas with a low level of radon. The TP53 codon 72 gene polymorphism might be involved in carcinogenesis of radon-induced lung cancer in Kazakhstani population. In summary, exposure to residential radon increased with Arg72Pro genotype to increase the risk of lung cancer in Kazakhstani population. This study suggests that the hypomutable TP53 codon 72 polymorphism can modulate the pathogenic mechanism of radon in lung tissue.

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

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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 polyphenolic compounds which exert strong antioxidant effects. (1) Animal feed enriched with these compounds can potentially enhance the efficiency, productivity and animal welfare by protecting them from oxidative stress and disease (2). For the present study, 30 broilers (15 broilers, 2 days-old, were randomly separated into two groups, as follows: 1) the control group fed with the standard ration, 2) the GP group fed with feed containing GP silage. The experiment lasted for 59 days. Tissue samples (heart, liver, quadriceps muscle, lung, intestine, pancreas, kidney and spleen) were collected at 30 and 56 days post-birth in order to determine the activity of antioxidant enzymes (GST, SOD) and protein expression of aflCGL GST activity and gCGL expression were increased significantly (by 15-70% and by 35-262%, respectively) in the majority of the tested tissues in GP group compared to the control, both at 30 and 59 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

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

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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-stressful condition of small muscle subject 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 prevention 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

Assessment of antioxidant activity of extracts from Conium maculatum, Datura stramonium and Arctium lappa

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In the present study, we evaluated the polyphenolic content and the antioxidant activity of four extracts from 3 Greek wild plant species, namely Arctium minus, Conium maculatum and Datura stramonium. The antioxidant activity of the extracts was evaluated in vitro by ABTS, DPPH, Reducing Power, ORAC and scavenging assays. In addition, the total phenol content was assessed by Folin-Ciocalteau assay. The results revealed that aqueous and methanolic extracts from Conium maculatum were the most potent in ABTS and ORAC radical scavenging assays with IC50 of 68 µg/ml and 71 µg/ml, respectively. As regards the total phenolic content, the extract from Conium maculatum exhibited the highest value (87 ± 8 mg TPC/g dry extract). Furthermore, the antioxidant activity of aqueous extract from Conium maculatum was examined in HepG2 liver and endothelial EAa926 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. Moreover, the levels of TBARS and ROS were not affected by any concentration of the extract. Furthermore, in EAa926 cells, the extract revealed that cell treatment decreased lipid peroxidation and protein carbonyl levels up to 41%. In addition, the extract increased total antioxidant capacity (TAC) up to 174%, in addition to catalase 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, the present study, the antioxidant activity of aqueous and methanolic extracts from Conium maculatum 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: arctium minus, Conium maculatum, Datura stramonium, HepG2 hepatocellular line, EAa926 cell line, antioxidant activity, polyphenol compounds

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

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The new trend adopted during the last few years regarding the putative promising effects of nutrition on human health on the basis of human nutrition 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, acetic 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, etc, 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 reducing) should be identified. 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.

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

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Olive oil (OO) plays a predominant role in the diet of countries around the Mediterranean basin, whereas it is a known constituent of several systems of human culture. The bioactive composition of olive oil seems to be a key factor in its beneficial biological action. Bioactive OO has 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 412/2012), OO is considered to protect from oxidative stress-induced lipid peroxidation in blood, when it contains approximately 5 mg of HH and its derivatives (e.g., oleuropein complex and Tyr per 20 g of OO, notably, OO bioabsorption from the gastrointestinal tract is very high, highlighting the importance of OO as a source of dietary antioxidants. In general, OO bioactive content has been associated by our group with potent in vivo antioxidant activity. However, studies regarding the effects of a ward 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 bioactive content and tissues. All abovementioned organs have developed numerous diverse antioxidant mechanisms against oxidative stress, including enzymes such as superoxide dismutase (SOD) and catalase (CAT), as well as non-enzymatic compounds like reduced glutathione (GSH) and ascorbic acid. Nevertheless, apart from endogenous antioxidants, dietary antioxidants may also act protectively against reactive species with bioactive OO 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 results, the most potent OO was selected for the current in vivo experiment to shed light on its effect as well as the monitoring of action in several tissues. Whether the in vivo 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 806 mg/kg bioactive was administered for 14 days to male Sprague Dawley rats at a dose corresponding to 20 g of OO per day; each rat was administered 0.133 ml of 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 redox status.

Imaging in children with RSV infection

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Respiratory syncytial virus (RSV) is the most common cause of paediatric broncholitis 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 perihonchitis in 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 lower lobes bullae with echopneuamothorax and severe sequence has rarely 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 pneumonic 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.

Role of neuroendocrine factors in skin cancer

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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.

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

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Bronchiolitis is a common acute lower respiratory tract infection, which predominantly affects infants younger than 24 months of age. It usually results from viral infection, causing airway inflammation, mucus production and mucous plugging, resulting in airway obstruction and is a major cause of morbidity and mortality leading frequently to hospitalization 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.

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

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Current national and European guidelines recommend distinct management approaches for basal cell carcinoma (BCC) based on tumour 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 dermoscopically suspect BCCs who underwent RCM and histopathological examination of excision specimens. For RCM examinations, we used the VividScope 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 (91.89, 99.40)] and specificity [78.95%, 95% CI (84.43, 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 hypopigmenterative silhouettes for aggressive BCC. Excellent intraobserver agreement (k=0.060, 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.
predicting asthma followig RSV-positive bronchiolitis in early childhood

chronic respiratory disease in childhood, the...
Helios and RSV-positive bronchiolitis

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Helios is a helian-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, group and bronchiolitis. The lower density and higher viscosity of Helios 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, Helios can decrease airway resistance in acute asthma in adults and children and in COPD. Helios may also enhance the bronchodilating effect of β-agonist administration for acute asthma. Helios can be administered with non-invasive ventilation and with mechanical ventilation through the ventilator. Non-invasive ventilation with Helios 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 hospitalizations 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 Helios therapy may significantly reduce a clinical score evaluating respiratory distress in the first hour after starting treatment in infants with acute RSV bronchiolitis. Helios could reduce the length of treatment in infants requiring CPAP for severe respiratory distress. Recently, it was reported that Helios 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 Helios in the therapeutic schedule for severe bronchiolitis.

MicroRNAs as potential bio-markers in children with RSV infection

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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 epithelium 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 mechanisms of RSV-induced inflammatory reaction and immune dysfunction leading to airway hyper-reactivity. There are several methods for the quantification, quantification, and characterization of miRNA expression levels in body fluids, 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.

RSV infection: Not for children only

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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, immuno-compromised patients and those with underlying cardiovascular disease, it may cause pneumonia or bronchiolitis and may result in respiratory failure (5-13%) or even death (0.5%). 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.

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

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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 precision 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 these pharmacological 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.
Antibody-mediated re-programming of macrophages against tumours: New insights for cancer immunotherapy

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All monoclonal antibodies designed for the treatment of cancer belong to one of the five classes, namely IgG (often IgG1). Engineering antibody Fc receptors to enhance effector functions could be an important determinant of clinical efficacy. Herein, we designed anti-tumour antibodies with IgG class Fc regions with the aim of harnessing known properties of IgG to engage immune effector cells such as macrophages and mast cells and immune clearance of parasites. Unlike the commonly-used IgG class antibodies, those engineered with IgG Fc regions feature extremely high affinity for cognate Fc-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 IgG designed to recognize tumour-associated antigens can potentiate monocyte/macrophage recruitment and re-education. Key modes of action of this class may be able 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 chemokine MCP-1, and also by engaging and re-educating the often immunosuppressive alternatively-activated macrophages towards pro-inflammatory phenotypes. Furthermore, IgG can prime chemically- and alternatively-activated tissue macrophage subsets to mediate anti-tumour functions. A first-in-class IgG 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.

Polyamines as mediators of secondary metabolism production in filamentous fungi

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Over the past 60-70 years numerous, industrial strain-producers of secondary metabolites (SM), including antibiotics, vitamins and immunosuppressives, have been obtained from various initial isolates of mouldy fungi. These high producers were developed by classical strains improving methods (random mutagenesis and target metabolic 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 emergence of polyamines were able to increase the production of target metabolites in high active fungal strains through exposure to the global regulator of fungi SM, L View the relevant section of this topic in 's العربية' and the metabolism of polyamines. The main pathways of synthesis of polyamines in fungal cells (via ornithine decarboxylase and argininosuccinate) will be studied for the wildtypes of A. chrysogenum and A. terreus and their high producers at the level of transcriptranscriptome analysis and inhibition by low molecular compounds synthesized during the project. To study the amination of the metabolism of polyamines (N1-acetylation in the catalysis of polyamines) and the biosynthesis of SM (the production of polymeric antibiotics from the de novo - precursor and lovastatin as the result of PKS work), analogues of the metabolic intermediates of acetyl-COA are also synthesized, and functional tests were performed. A series of experiments with specially synthesized analogues of amino acids and substrates of sulphur metabolism intermediates, to identify the interactions at the level of 5-adenosylmethionine, naturally consumed for the synthesis of polyamines and as the substrate of the global L ecotia regulator, were performed. As result, the preliminary model for summarizing the data of interaction the polyamines molecules and 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.

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

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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 tumour 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.

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Application of proteomics in target identification for personalized anticancer therapies

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

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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 transcription factor and the induction of pro-apoptotic pathways. Moreover, the in vivo experiments demonstrated 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 DOX-DOX.

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Combination therapy of the liposome-encapsulated agents S100A11 and DMXAA affects major mechanisms of murine melanoma development and progression

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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 s100a11 and DMXAA (5,6-dimethylxanthene-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 glycolated 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 be beneficial in blocking resistance. Unassessed to date is the major drawbacks of current anti-angiogenic therapies. This novel targeted therapy holds the potential to disrupt and reprogram the pro-tumorigenic TME.


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Suspeltine met abolites, hydroxide, propionate and aldehyde, induce the apoptosis of neovascular cells associated with an increase in p53, Ciskaprin-3 and NMD-1a.

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Neovascularity (NB) is a common event in solid tumors in children, which originates from the sympathetic lineages of neural crest and occurs for 15% of children's cancer mortality. Amplification of the receptor c-Kit is a well-established poor prognostic marker for neuroblastoma. Whilst N-Myc amplification status strongly correlates with higher tumor aggressiveness and recurrence in neuroblastoma, therapy, new therapies for patients with NB or c-Kit amplified NB need to be developed. The in vitro formation of syngeneic polyclonal material in bovine serum albumin (BSA) in a serum-free approach to human neuroblastoma. It has been demonstrated that BSA and spermine (SPM) addition to cancer cells induces cell growth inhibition and apoptosis through the reactive oxidative species caused by polyamine metabolites (BSA and spermine), produced by the oxidative reaction (1). The cytotoxic effect induced by SPM and BSA was enhanced by both treatment and MTT assay. The degree of apoptosis of the NB cells was evaluated by flow cytometry after Annexin V/PI binding and DNA staining with propidium iodide. The ratios of Annexin V-positive cells needed to be coupled with a flow cytometer exhibiting a hypodiploid tetraploid on the dot plot analysis of the Annexin V-positive cells (nuclei) compared to the different antibodies were determined. The apoptotic changes or regulation of NBs following treatment of NB with a hypodiploid receptor (or hyperdiploid) cell line with BSA and SPM. The experiments were carried out considering the prostate gene family (PGF), PDEA and ENOX. Following treatment with BSA and SPM, the cells displayed increased BSA and SPM-treated treated with BRCA1 and BRCA2. Wipers also assessed their effect on the NB cell line. The major conclusion is that BRCA1 and BRCA2 antibody supported the cancer cell lines for the in vitro testing. The cytotoxic activity of both NB cell lines, associated with the activation of apoptosis, was significantly potentiated by 5-50% compared to the untreated cell lines. The BRCA1 and BRCA2 antibodies are a potential tool for the development of new anti-oncogene treatments.


Perspectives of tricyclic derivatives

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Active halogen tautomer derivatives are basically the naturally occurring N-chloroethane (NCT) and N-bromosuccinimide (NBS), which are produced by activated human neutrophil and eosinophilic granulocytes and monocytes. Due to their reactive properties of action, they exhibit broad spectrum microbial immunity 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-kB, interferons, progranulin E2, tumor necrosis factor, neopterin, and others. In particular, NBS has been developed as a natural anti-infective and anti-septic tool for application at different body sites, particularly sensitive ones, for instance, the eye, ear, scarified skin and the oral cavity. The use of NBS was applied successfully on sarcoid. 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, bromonitrene (BRAT) is a compound which has extracted much interest since it resembles the properties of NBS. Future perspectives for application are the following: NCT is particularly suited for parietal lesions of sensitive body sites as mentioned above. The reasons are its mild activity, low chlorine consumption and the enhancement of microbial immunity in body fluids and exudates by transhydrolization. The inhibition of NCT in chronic bronchitis or cystic fibrosis is a very promising investigative field. Bromonitrenes appear so suited for treatment of infections, as well as. Demonstrated on the skin for acne and herpes zoster. In addition, they exert multiple anti-inflammatory effects against tumor cells. Thus, oncolysis has become a topic of great interest, particularly as regards BRAT and NBS.

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

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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 released for cancer immunotherapy, as they are not homogenous in phenotype and function. Recent studies have demonstrated that the stimulation of peripheral NK cells by IL-12/15/18 generates long-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 long-lasting 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 cytokine effects. They expressed IL-12 receptors J1 and J2; 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 cytoprotection, and diminished long-term cells. Notably, a large proportion of IL-15/18-induced cells strongly expressed FasL together with activation molecules, whereas NK cells induced by IL-15/18 and IL-12 expressed high levels of FOXP3, IL-10, and NKG2A. Furthermore, the latter spontaneously secreted IFN-γ and TGF-β during prolonged incubations. These results indicated that IL-12 regulated the expression of IL-15/16-induced, effector-like NK cells, generating long-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 mechanisms of IL-12 function.

Membrane protein inventory of human phaeochromocytoma and paraganglioma

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Phaeochromocytoma and paragangliomas (PHEO/PGL) are rare neuroendocrine tumors derived from adrenal medulla or extracranial paraganglias, respectively. The paragangliomas with malignant PHEO/PGL, in poor, and specific molecular targets for novel therapies are therefore required. Integral membrane proteins (IPMs) expressed by tumors represent such promising therapeutic targets, due to their specific functions and localization. Our goal is to provide a detailed inventory of membrane proteome of human PHEO/PGL that could help identify novel drug targets and diagnostic markers. IPMs are coded by roughly 25% of human genes; however, our knowledge of the IPM repertoire expressed by specific tumors is limited. The amphiophletic nature, the lack of tissue cloning sites and their relatively low expression hinder the proteomics analysis of IPMs. The specific physicochemical properties of IPMs require specific analytical strategies. In order to maximise the coverage of the PHEO/PGL membrane proteome, we combined a standard top-down-based approach with a selective isolation of (extramembranous) glycopeptides and with our recently introduced hIPcET method, which selectively targets (transmembrane) hydrophilic segments of IPMs, into a multi-approached "Pitchfork" strategy. The methods included in the Pitchfork strategy target different features of IPMs, are complimentary, and allow for the identification of a significant portion of the membrane proteome expressed by PHEO/PGL. On average, we identified 900-1300 IPMs in each PHEO/PGL tumor sample analyzed to date. Our current dataset represents nearly 2,200 unique IPMs identified in PHEO and a similar number in PGL tumors 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.

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Bromamine T, a new developable hygiene product

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Active halogen compounds have broad-spectrum antimicrobial activity without resistance development, and some representatives are in use as anti-infectives and antiprotozoals, such as iodine derivatives. 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 oral medicine might be taken into account, as well as all other halogen molecules. If the bactericidal activity of bromamines is compared to their cyanotic activity, body cells exhibit markedly higher tolerability than bacteria, leading to an extraordinarily high biocompatibility index. Indeed, the clinical application of the natural substance N-bromosuccinimide in acute (phase B study) and in skin infections, such as herpes zoster (eases) 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 further be developed as an anti-inflammatory and anti-tumour substance, particularly on the skin.

Pine bark extract in esophagitis

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Pine bark extract contains polyphenols such as catechins, epicatechins and taxifolin. This extract showed already antioxidant activity in in vitro studies (1). Antioxidant activity tendency was also described as such in vivo studies with canines, epicatechin and taxifolin. In this study, pine mixture bark extract reduced significantly the migration of the lung cancer A549 cell line via MMP-9 and it restricted the migration and invasion of lung cancer cells (2). Pine mixture bark extract exhibited antioxidative activity in murine sarcoma S180 cells both in vitro and in vivo. Pine mixture reduced tumour weight in vivo by 94.7% (3). Pine bark extract also exerted an antioxidative 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 taxifolin induces the immune system and has a possible advantageous use of epicatechin in cancer treatment has been described. Epicatechin reduced the chemoresistance of tumor cells through additive and synergistic effects with 5-fluorouracil (5-FlU), tamoxifen, cisplatin and taxol (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 fibroblast cell line, HT-1080 (8). It is possible that in the future, pine bark extract could be used as a neoadjuvant or adjuvant therapy in esophagitis. In addition, catechins, epicatechins and taxifolin could be used in novel anti-inflammatory agents in the future.


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Hepatocellular carcinoma and resveratrol

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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., cidofovir). Currently, a number of new oncolytic drugs are obtained from plants. The anti-angiogenic potential of resveratrol (extract from red grapes) is already described in the literature. An in vitro study using a fibroblast cell line demonstrated a potent pro-apoptotic activity of trans-resveratrol (2,3). The regulation of p53 and the phosphoinositol 3-kinase/AKT pathway plays a crucial role in the anti-angiogenic activity of trans-resveratrol. In addition, sphingolipid metabolism has been shown to be modulated by trans-resveratrol in HCC (4). The expression of the well known SIRT1 protein is modulated by trans-resveratrol via the PI3K-AKT signaling pathway (5). 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 (6,7). Trans-resveratrol, but also oxysresveratrol, play an important role in hepatotoxicology. The latter modulates angiogenesis and lymphangiogenesis in HCC (8). It is possible that the presence of a resveratrol receptor may be involved in the neovascularization or adjacent in the therapy of HCC. Trans-resveratrol could also be used in the future as an anti-metastatic agent.


New insights into tumor invasion and vascularization

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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 LRPI to modulate tumor cell invasion. LRPI is downregulated in glioma macrophages that face 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 SMAD5 on target sites. We will, furthermore, show data from a renal cell carcinoma model for the discovery of tumor-dependent and rectorclosin-dependent molecular signatures. Indeed, we have developed an experimental model for lung metastasis formation, which allowed us to identify specific transcriptional 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.

A multi-layered systems approach for renal cell carcinoma
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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 specialized 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 methylation analysis. Transcriptome and methylome analyses demonstrated distinct clustering in three different groups. Notably, DNA sequencing did not show significant genome variations in the different groups, indicating the absence of clonal selection during the in vivo amplification processes. Transcriptome analysis revealed several markers that were validated in patient cohorts from TCGA and biobanks. This also includes soluble markers. We also identified key regulators of EMT progression, which was also functionally validated in vitro and in a mathematical model was provided.

Inhibition of EIF-5A prevents cardiac toxicity in succinylated mRNAs by haploinsufficiency
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In this study, a determination of Tropomin 1 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. Tropomin 1 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 indicate 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 hypoxia involving catalyses 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 hypoxia 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 CPIP from P. falciparum amplifying the pathophysiology of severe malaria. Moreover, in comparison to untreated and GC7-treated cardiomyocytes, the co-administration of GC7 and CPI significantly decreased the release of cytokines: IL-6 and IL-1β 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, hypoxia, apoptosis, hypoxia

Anti-endothelin (CD190) monoclonal antibodies: Tools for cancer research and treatment
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E-drugs (CD195) is a membrane antigen expressed on endothelial cells and various types of cancer cells. It modulates the activity of signal pathways induced by TGF-β, -β3, BMP-2, -7, -9,-16, and activin A. Endothelin regulates cell migration, adhesion, and cancer cell metastasis. Its soluble form is released via proteins 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 endothelin. Three of them bind antigen molecules from both species, while the remaining antibodies bind only human or only rat endothelin. MAbs could be used as specific reagents for immunohistochemistry, western blotting, fluorescent microscopy, and flow cytometry. Based on a pair of antibodies a highly sensitive sandwich ELISA was developed. The antibodies were capable of quantifying soluble endothelin in blood plasma, urine, and cerebrospinal fluid. Pathological 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 different 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 vivo. Using flow cytometry, endoglin expression was demonstrated on cultured human cancer cells of different origin (hemangiosarcoma M204 cells, liver cancer HEK1 cells, melanoma M96 cells, glioblastoma 786O cells), while their non-transformed counterparts lacked the antigen. Three cancer cells also produced endoglin in soluble form, though less expressed compared to endothelial cells. Modulation of cardiac function using anti-endoglin MAbs treatment is a question for further studies. The panel of anti-CD195 MAbs provides wide opportunities for versatile studies of the role of endoglin in cancer both in vivo and in rat models.

The implication of exosomal miRNAs in the development of cancer represents the cornerstone of oncology due to its capability of transferring genetic cargo between two cells. This allows the exchange of genetic material and functional proteins, which can alter the behavior of the recipient cell. miRNAs are small non-coding RNA molecules that play a crucial role in gene regulation, cell differentiation, and development. They achieve their function by binding to messenger RNA (mRNA) molecules, causing their degradation, or by inhibiting the translation of specific proteins. miRNAs can be released into the extracellular environment as part of exosomes, which are membrane-bound vesicles that shuttle between cells and carry their cargo of miRNAs. These miRNAs can target genes in receiving cells, potentially leading to changes in gene expression and cell behavior. In cancer, miRNAs can act as oncogenes or tumor suppressors, depending on the context and target genes. They can regulate genes involved in cell proliferation, apoptosis, invasion, and metastasis. The significance of miRNAs in cancer has led to extensive research in understanding their role in disease progression and as potential diagnostic and therapeutic targets. The study of exosomal miRNAs in cancer has become a critical area of research, with the potential to advance our understanding of disease mechanisms and to develop new therapeutic strategies.
Loss of function of PTX1 contributes to a poor prognosis of gastric cancer patients by enhancing chemotherapy resistance to 5-fluorouracil and cisplatin

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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. Paclitaxel-like homoeodomain transcription factor 1 (PTX1) has been implicated as a tumour suppressor in various types of cancer. In the present study, we found that PTX1 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 PTX1 had a worse prognosis than those with higher levels of PTX1 (P=0.007). 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 PTX1 expression on the sensitivity of GC cells to 5-fluorouracil (5-FU) and cisplatin (CDDP). The results revealed that the overexpression of PTX1 increased the sensitivity of the AGS and DGC-823 GC cells to 5-FU/CDDP. Moreover, PTX1 knockdown decreased the sensitivity of the MGC-803 and SGC-7901 GC cells to 5-FU/CDDP. To further assess the mechanism by which PTX1 contributes to chemotherapy insensitivity, we evaluated the expression of the genes whose gene expression levels were upregulated in 62% of GC cells transfected with siPTX1 compared to cells transfected with control

Asparteiner in as vehicle for anticancer nanodrugs

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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. Apo1611 (Apo), the iron-free form of ferritin, can act as a nanocarrier. It can be functionalized with biocompatible cations or antibodies, enabling the targeting of cancer cells. We constructed Apo with encapsulated doxorubicin (ApoDox), etoposide (ApoVep), ellipsoicin (ApoEll) and vandetanib (ApoVan). Moreover, ApoDox and ApoVep were synthesized with 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 vitro. We also tested ApoEll and ApoVep on neuroblastoma (NB) cells, in which Apo is binding in transfer receptor 1 and SKARAS. ApoVep 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 increased selectivity and safety. However, prior to their introduction into practice, it is still necessary to solve the many issues.

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

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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 26 trimethylation (H3K26me3), exhibited stronger immunostaining signals in tumor tissues than in the corresponding adjacent non-tumor tissues. The expression patterns of H3K4me3, H3K9me3 and H4K20me3 correlated with tumor infiltrating depth, lymph node involvement and pTNM stage. Patients with low-scoring H3K4me3 and H3K9me3 and high-level of H3K26me3 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, H3K4me3, H3K9me3 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.

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

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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 major 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-encapsulated sitostavrin (LCL-SIM) exerted potent antitumor activity on B16F10 melanoma in vivo due to the suppression of TAMs-mediated oxidative stress in TME, while another liposomal formulation carrying prednisolone phosphate (LCL-PPL) 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.


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

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Chronic diseases are responsible for 90% of global deaths and are largely 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. Louizos et al. (2019) have developed a semi-automated workstation, BIOSTEL, to generate individual and group leukocyte telomere length metrics and provide a crude estimate of biological age (3). In a group of 156 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 nattokinase 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, modulation of telomeres and telomerase activity has emerged as a potential anti-aging agent. We have been testing several natural molecules and identified DASGTL as the most potent telomerase activator reported to date (5-10), and further in vivo studies on rats and humans will reveal its role in telomere maintenance. Consequently, telomere are potent targets of prediction and treatment of chronic diseases and together with standard methodologies can lead to their effective management.


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

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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 activation. Thus, limiting glutamine availability may possibly contribute to intratumoral immunosuppression. 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 lymphocytes (TIL) therapy for melanoma and other solid tumors rest largely with poor persistence. Using mouse models, our studies revealed that ODC blockade with N-(2-fluoromethyl)imidazole (DFMO) generates CD8+ T-cells 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.

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

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Trypanothione reductase (TR) is a key enzyme that catalyzes the reduction of trypanothione (TS), a glutathione-glycine conjugate that protects Trypanosoma (Leishmania, Trypanosoma cruzi and Trypanosoma brucei) from oxidative stress induced by mammalian host defense systems. TR is considered an attractive target for the development of novel anti-parasitic agents, as it is a 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 interfacial 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. Our research group identified several organic compounds able to inhibit TR from Leishmania by competing with TS for its binding site: Azole-based compounds, diterpene 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 Leishmania major. Some of these compounds were also able to inhibit TR from Trypanosoma brucei (TbTR), the parasite responsible for Human African Trypanosomiasis (HAT), a neglected tropical disease. In particular, compound 1 from this series was found to impede the growth of Trypanosoma brucei parasites in cell cultures. The X-ray crystal structure of ZTR in complex with compound allowed the identification of the hydrophobic pocket where the inhibitor bind, placed close to the catalytic Histidine (His 461) and lined by Trp2, Ile106, Tyr116, Met113. Since the binding site of this new inhibitor is unique, and is not present in human homologues, such as glutathione reductase (GSR), it represents a novel target for drug discovery efforts.

New agents to challenge drug resistance in cancer

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Cisplatin represents a cornerstone for the chemotherapeutic treatment of a number of types of solid cancer. Moreover, it is used in combination regimens 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 present 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.

Multifocal pro-inflammatory expression patterns in glioblastoma multiforme and Alzheimer’s disease

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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 down-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 relevant to GBM and AD were used. Once the miRNAs significantly down-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 down-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 vivo and in vitro 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.

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

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Oral cancer represents one of the most diagnosed and validating tumors worldwide. Several risk factors have been recognized for this pathology, however, despite the promotion of preventive measures, 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 microRNA expression (miRNA) in patients with oral cancer by using the molecular data contained in the GEO DataSets and TCGA miRNA profiling datasets. Differential analyses were performed between the miRNA expression levels 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.

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Incidence of venous thromboembolism (VTE) in patients with multiple myeloma (MM): A non-institutional experience
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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 85 MM patients treated with different therapeutic regimens, including Pomalidomide (Pomad), Carboplatin, Lenalidomide and Daratumumab (Darz). Daratumumab and Lenalidomide (Ele) were treated 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. Although 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.

Implication of the transcription factor YY1 in non-Hodgkin’s lymphoma
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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 B-cell 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.

Role of the transcription factor YY1 in colorectal cancer
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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 chemotherapies 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.

Telomere maintenance regulated by a cell cycle-controlled mechanism: From bench to clinic
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Telomeres are protected by capping structures consisting of core protein complexes, such as the shelterin complex composed of TRF1, TRF2, POT1, Rap1, TIP1 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 telomeres. 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, phosphorylates 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 TIP1 are important for telomere capping, telomerase binding and function at the telomeres and chromosomal stability by preventing telomere 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.

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

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Metabolomics, a novel and powerful tool of precision medicine allows for the 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 FCA 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 metabolic 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 severity of atopic dermatitis and pulmonary 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, Credé’s diseases and alternative colitis and investigate the role of nutritional intervention to the disease progression.


A new scaffold for selective inhibition of human monoamine oxidase B

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Monoamine oxidases (MAOs) are mitochondrial FAD-containing enzymes that catalyze the oxidative dismutation of biogenic amine neurotransmitters and xenobiotic amines. Being involved in the catalysis 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)⁹,¹⁰. As the ideal drug has not been achieved so far, research continues to explore this field⁹,¹¹; 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-phenylcycloexane 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 MAOc: compounds 4a, 4g and 4m were found to be the most potent inhibitors (IC50 = 0.5-10 μM) with a good selectivity toward MAO B (Kma/Kma/q = 1-45). 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.


RSV bronchiolitis and pediatric asthma

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RSV 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 80% 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 pharyngitis 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.

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

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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-surgery transplantation; and BO after bone marrow transplant/transplantation (BMT) or hematopoietic 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/cot-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 contradictory - that PIBO is more severe 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.
Overlap between RSV, influenza viruses and human metapneumovirus in childhood
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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 recognized 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 aetiological 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 hospitalization due to influenza; there is currently no vaccination against RSV and hMPV in clinical practice.

Stress and Viral Infections
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Stress is the disturbance of homeostasis of an organism in response to a negative stimulus, the stressor. When such stimuli exceed a certain threshold, a mammalian organism responds to adapt and return homeostasis. Part of the adaptive response is the activation of the neuroendocrine stress system, i.e., the hypothalamic-pituitary-adrenal axis and the locus coeruleus/noradrenergic system, and their 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 TH1- to TH1 helper 2- and Treg-driven immunity, while it subdues the antigen-recognition process and the inflammatory reaction. The glucocorticoids, the catecholamines noradrenaline and adrenaline 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, respectively, the so-called ‘sickness syndrome’, via the classic pro-inflammatory cytokines TNF-alpha, interleukin-1, interleukin-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 and sequentially process that results in full return to the basal healthy state. Chronicity of stress or inflammation may be detrimental to an organism and/or may make it vulnerable to viral infections, 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 individuals. We also examined in humans, such as the obesity of children infected with an adenovirus or the still unidentified virus or viruses responsible for the rare transient glucocorticoid hypersensitivity syndrome. We are currently examining the very tip of the iceberg regarding viral viral elements interfering in our signaling systems.

The neonatal immune response to RSV infection: Advances in our understanding of viral and host cellular interactions
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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 influenza and asthma of childhood, as well as increased mortality in the early years of life, particularly 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 titers 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 integral 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 TRs and altered antigen presenting cell function, including low IL-12 and enhanced production of IL-6 and IL-10, 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 Th activation, coupled with little or no B cell memory and inhibition of antibody production by IFNγ, produces low titer, 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, disproportionate to the viral load itself.

TGF-β1 pathway down-regulation in hypertrophic scar fibroblast cells - in vitro
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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 fibrosis 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 Smad3/4 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. Smad3 expression inhibited eH2 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 Smad3 as a member of the TGF-β1 Family can inhibit these processes.
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Targeting of the epigenetic integrator UBTF1 in cancer cells by natural products

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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 carried through a coordinateddialogue between DNA methylation and histone post-translational modifications, such as acetylation and methylation. In cancer cells, promoters of several key TSGs are hypermethylated by DNA methylation/1(1DMNT1), and histone variants are degraded by telomere dysfunction (2). DNA methylation is one of the critical events in the inactivation of these TSGs with subsequent defects in apoptosis. Therefore, several drugs have been developed which act as inhibitors of DNMT1 and DNMT3 antisense expression of TSGs. Many of these drugs include histones, histone deacetylases, and epigenetic acetylases. However, the interaction of the Ubiquitin-like, with PHD and RING Finger domain 1 (UBTF1) (Fig. 1) is its functional domain. A ubiquitin-like domain (UB), a tandem Tudor domain (TD), a plant homoeodomain (PHD), and an RING- and associated RING-associated (SRA) domain, and a really interesting new gene (RING) domain. (UB, RING) domain is its functional domain. UBTF1 is characterized by the SRA domain, whose second one is the UBTF1 family. Through its SRA domains, UBTF1 interacts complexes with HDAC1 and HDAC2 and represses the expression of several TSGs in cancer cells (3, 4). The RdCP and RBCP activities of UBTF1 are one of the two main activities of RING domains [4].

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

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Thymoquinone (TQ), a natural anticancer agent exerts cytotoxic effects on several cancers by targeting multiple pathways including apoptosis (5, 6). Differmethylene (DFMO), an irreversible inhibitor of the ornithine decarboxylase (ODC) enzyme has shown promising inhibitory activities in many cancers including leukemia by deactivating the biosynthesis of polyamines (6). The aim of the present study was to investigate the combinatorial cytotoxic effects of TQ and DFMO on human lymphoblastic leukemia (KCL) 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/FLAD staining. RNA sequencing was used to investigate the anticancer mechanisms of TQ and DFMO and further, RNA sequencing was assessed using different tools and the expression of target genes was examined by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The results indicated that TQ induced 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. 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 protein homodomain (UBI) and really interesting new gene (RING) finger domains 1 (UBTF1) and its two partners DNA methyltransferase 1 (DNMT1) and histone deacetylase 1 (HDAC1) were downregulated by DFMO- and TQ-treated Jurkat cells. Data obtained from RNA sequencing were confirmed using RT-qPCR. We found that the combination of DFMO and TQ dramatically increased the expression of UBTF1 and other target genes compared to either DFMO or TQ alone. In conclusion, these results suggest that the combination of DFMO and TQ could serve as a novel therapeutic strategy for the treatment of acute lymphoblastic leukemia by targeting the epigenetic codes.

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Implications on telomere length by chronic exposure to drugs of abuse and psychoactive substances

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Telomeres are nucleotide tandem repeats at the ends of eukaryotic chromosomes that maintain genomic integrity. The gradual shortening of telomeres leads to 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 telomere limits we aimed to evaluate other parameters, such as the use of drugs of abuse (cannabis, opiates and cocaine) or the exposure to glyphosate on cell influence aging of human. Blended samples were collected from 16 drug abusers and 10 glyphosate sprayers. Metaphase spread leucocytes were isolated from peripheral blood. Telomere length was measured by Q-FISH with (cT3A5) PNA probe. Ten metaphases of each subject 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 telomere limits compared 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.
Expression of beta-defensins 1 and 2 in rat islet cells of individuals with a 35-day experimental gingivitis model

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Gingivitis is a reversible disease characterized by inflammation of the gums 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 antimicrobial activity which would 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 malt sucrase agar, as well as for the concentration of beta-defensin 1 and 2 by the ELISA technique; on day 28, dental hygiene was repositioned finally, a periodontal evaluation was performed and samples were taken on day 35. The results revealed progressive modification during the induction phase, notably, the colony index, indices of CFU and 100 CFU in the increase in the presence of bleeding, as well as an increase in CFUs on 500 on blood agar and malt sucrase agar compared to day 0. The increase in the expression of beta-defensin 1 (p <0.05) was found on day 21 and 28 compared to 0; similarly, thiaminase 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 in the bacterial stimulus.

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

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

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Among the modern neuropathophysiological 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 Alzheimer’s disease and frontotemporal dementia (FTD). TMS has shown 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., VaD-related 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 TMS 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 will provide 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


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

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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 (924A, PON2 S237C and A148C, GSTP1 Ile105Val and Aat1IVWf, GSTM1 and GSTT1) was performed using real-time polymerase chain reaction (RT-PCR) from peripheral blood lymphocytes. Serum levels of advanced glycation end-products (AGEs), 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 probably contribute towards the development of screening tests that, following regulations of some ethical issues, would allow large-scale detection of susceptibility to pesticide exposure in other xenobiology.

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


Gene-environment interactions and pesticide toxicity in exposed workers

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Occupational exposure to pesticides can have detrimental effects on human health, increasing the risk of some chronic diseases (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), acetylamidases (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 process encoded by the various genotypes alter the biotransformation of these chemicals. Genetic heritage may increase vulnerability 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.

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

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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 willfermin A, Anthocyanidins (Antos) and curcumin embedded in milk exosomes showed enhanced anti-proliferative, anti-inflammatory and anti-cancer effects against lung and cervical cancers in vivo 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.

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

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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 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-Syndrome (1 patient) and highly malignant rare tumours, including osteosarcoma (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 CXCR2 and MANDA gene. For HLH, the PEPD gene was found to be an important marker. Further research is required to determine the diagnostic yield and clinical implications of NGS in paediatric malignancies.

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

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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 (blaKPC, blaNDM, blaOXA-48-like), blagES, blagIM, blagMP) and their antibiotic (AB) resistance profiles. Eighteen carbapenem-resistant strains non-depicted 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 (VITC). AB susceptibility was determined by VITEPS and interpreted according to EUCAST guidelines. The selection value was Minimal Inhibitory Concentration (MIC) to Meropenem (MTR) (9.5 µg/ml). CR was phenotypically confirmed by KPC/Metallobeta-lactamase Confirmation kit (Rosco Diagnostics Diagnostica). RT-PCR detected the blaKPC, blagE, blagG, and blagOXA-48-like genes. CRK strains were divided in 4 groups: 11 (61.1%) isolates presented MER and Imipenem (IMI) resistance (MIR) for MER, IMI=16 µg/ml (group Ib); 3 (16.7%) isolates were MER-MINermary resistance (SMIR) (MIC=2-4 µg/ml) and IMI (IS), having MICs 2-8 µg/ml (group Ib); 3 isolates (16.7%) were MER=16 µg/ml and IMI=1 µg/ml and 1 isolate (5.5%) was MER=16 µg/ml (MIC=2 µg/ml) and IMI=4 µg/ml. We found 100% correspondence between modified carbapenem inactivation methods and RT-PCR results. Nine strains (50%) had blagOXA-48-like genes, 2 (16.67%) blagKPC genes, and 5 (27.7%) had blagOXA-like and blagKPC genes concomitantly. One strain contained three genes: the blagOXA-48, blagKPC 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 test. In addition, the results show the spread of blagOXA-48-like and blagKPC genes in Transylvania, Romania. The results highlight the increased CR percentage of KP by harboring at least two carbapenemase genes.

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

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Glioblastoma is an aggressive malignant brain tumour with a poor prognosis and a low patient survival. Adjunct radiotherapy 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 a 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) 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 neurcan are the abundant PGs in the normal brain tissue. Irradiation specifically affected decorin, neurcan and versican transcriptional activity, and the GAG content in brain ECM, suggesting the determination 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.

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Fusion genes in cancer: From eukaryogenomics to next generation sequencing

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A fusion gene is a hybrid gene that is created through rejoining together parts of two previously separate genes. Acquired fusion genes may disturb proliferation and gene expression in cancer cells. They are caused by chromosomal rearrangements in the cells of the neoplastic progeny 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)(q24;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 target of molecular therapy, play a key role for the accurate pathognostic 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 breakpoints 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 technologies has opened a new possibility to detect fusion genes in cancer. We used a combination of bioinformatics and RNA sequencing to identify novel fusion genes in both mesenchymal tumors and blood cancer. Among those were the IFR12/NOTCH1 in myelodysplastic syndromes and fusion genes in endometrial stromal sarcoma with the CCDC163/FOXJ1 fusion gene and the MAF/CSF1R fusion gene in retropodoxic empyema with a (16;17)(q22;q12) translocation. The new approach to next-generation sequencing and the novel fusion genes detected will be presented.


Expression of selected cytokeines in neoplastic endometrium

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Cytokeines (CKs) are the largest subgroup of intermediate filament proteins preferentially expressed in various human tissues (1-3). They are subdivided into type I cytokeines (CKI-CR2) and type II basic (CK1-CR7-CK14) 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 (4). Uterine adenocarcinomas are always immunohistochemically positive for CK7, CK8, CK18 and CK19, while mostly they are CK20 negative (5). In a report by Allaire et al. (5), only CK8/16 exhibited a significantly different frequency of positivity in endometrial adenocarcinoma relative to cervical adenocarcinoma. Interestingly, the immunohistochemical expression of CKs in lymph nodes with underdetected metastasis predict occult metastasis in these nodes and it is a risk factor for recurrence of early-stage endometrial cancer (6). It is worth pointing out that the informative immunomarkers for CKs profiling have been increasing in numbers recently and this trend is likely to continue with new ground breaking technologies. The aim of the presentation is to briefly overview the multi-functional role of CKs in primary and advanced endometrial carcinoma.


Chromosomal damage, relative telomere length and DNA repair in eczematous and eczematous/crusted dermatitis

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Heredity of fingerprinting in primary eczematous dermatitis is mainly by altered DNA metabolism. Chromosomal aberrations (CA) represent causative events in malignant transformation. Chromosomal alterations may be poorly end-scaled and recognized as double-strand breaks (DSB) by repair machinery, thus contributing to the genomic and chromosomal instability. We investigated the links between CA, DNA repair characteristics and relative telomere length (RTL) in incident patients with eczematous/crusted (OCR) and eczematous (EC) healthy subjects. Relative telomere length in target tissues was investigated in relation to skin sensitivity and clinical morphological features of OCR. CA in peripheral blood lymphocytes (PB) were determined by classical cytogenetic method in 1,080 healthy subjects and incident patients (137 BC, 185 OCR). DNA repair processes were evaluated by using Pel-Fe ablation after stimulation, DNA repair capacity by a modified comet assay (SMN and SBE) and antigen sensitivity assay (a functional characteristic of DSB repair), RT was measured using the monochromatic multiphoton CH2 DNA from PB of 145 IB, 148 BC patients, 271 controls and DNA from 761 pairs of eczematous and adjacent epidermis. Lower RTLs were associated with increased DNA sensitivity, evaluated by a modified comet assay and lower RTLs were associated with increased DSB capacity. Rec patients exhibited significantly shorter RTL, but similar CA as controls. BC patients had significantly longer RTL, and increased CA frequencies. Significantly shorter telomeres were observed in outer and inner tissue strata and correlated with CA. The differences were found in non-BC peripheral eczematous tissues, the longest in oral caustic tissues. CA in PB, expressed as percent transfer number to metaphase in culture, however, do not differ in various cancers. We demonstrate links between chromosomal damage, DNA repair and telomere length in eczematous dermatitis and telomere decreasing is a proxy for underlying differences in DNA repair capacities in cancer patients.

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Microparticles released by myeloma plasma cells enhance thrombin generation in the microenvironment: A modulation in clonal study

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Microparticles released by myeloma plasma cells enhance thrombin generation in the microenvironment: A modulation in clonal study

Hepocreatability is a common blood alteration in newly diagnosed, chemotherapy naïve, multiple myeloma patients. The mechanisms by which myeloma plasma cells (MPCs) interact 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 (MPCs-MPs) were analyzed by flow cytometry (TF expression) and TF expression was also determined. Thrombin Generation (TG) in the presence of MPCs or MPC-MPs was assessed with the Calibrated Automated Thrombogram assay (CAT9) in normal human PPP. TF was also assessed in plasma spiked with MPCs and MPC-MPs or variable concentrations of TF and procoagulant phospholipids. PC-MPs expressed about 8-fold higher levels of TF as compared to MPCs. TF produced by MPC-MPs was significantly higher as compared to that of MPCs. MPCs and MPC-MPs enhanced thrombin generation of human plasma. Thrombin generation was significantly higher with MPC-MPs 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 can express a weak procoagulant surface, hypercoagulability is the resultant of the presence of procoagulant elements into their microenvironment, via the release of MPC-MPs which express significantly higher levels of TF. Due to their critical role into the hypercoagulability, procoagulant MPC-MPs could be a potential tool for the evaluation of the aggressiveness of myeloma disease.
Inhibition of heme oxygenase-1 to improve cancer therapy

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Heme Oxygenases (HOs) are a family of microsomal 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 selectivity for these tumors has increasingly revealed a new therapeutic strategy in cancer(2). By means of a medicinal chemistry approach, a number ofazole-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 ofazole-based derivatives with the following general formula:

All these compounds possess the key chemical features required for HO-1 interaction: a 2-nitroimidazole nitrogen, a hydrophobic moiety, and a connecting chain. The most potent compounds were selected and studied for their tumor properties in different cancer cell lines, with promising results(4). 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.


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The immune escape mechanism of malignant cells in bladder cancer

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Tumor cells have many immune escape mechanisms, one of which is apoptosis resistance. This electron microscopic 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-1 receptor in urine and peripheral blood mononuclear cells (PBMCs) 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 (bilateral and nonbilateral), Group 2, n=30 and bladder cancer (transitional cell carcinoma and squamous cell carcinoma, Group 3, n=90), whether associated with bilateral infection or not associated. PBMCs stained by both immunocytochemical and immunoelectronmicroscopic techniques showed significant increase in the percentage of positive cases expressing both TGF-beta1 protein and TGF-beta1 receptor 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 (91.3%) 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 significantly 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<0.001). 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.

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

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Ovarian cancer stem cells (CSCs) exhibit characteristics of self-renewal, tumour initiation, tumour growth, a capacity to differentiate, and drug resistance leading to tumor relapse. Several theories for the origin 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, Ral, Bcl2 and Src, are implicated in oncogenic transformation and tumorigenesis. In this context, we explored a putative oncogene "securin", also known as "primary tumor transforming gene-1" (PTTG1), reported to be overexpressed in various tumors, including ovarian tumors. Fluorescence-activated cell sorting analysis using conventional microscopy and qPCR demonstrated co-expression of securin with several stem cell (OCT4, NANOG, SOX2 and SSEA4), CSC (ALDH1, CD133, CD44, CD117, CD343 and LGR5) and germline lineage (DI6X4-VASA and FIP1L1/TRA2B16) specific markers in normal ovary, benign, borderline and high-grade ovarian tumors, as well as established CSCs collected from patients with recurrent ovarian cancer. Gene-specific siRNA knockdown of securin in an ovarian cancer cell line (A2780) revealed a 70% 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/CSC 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 cell and CSC marker, which may induce the transformation of normal stem cells to CSCs.

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ETV7 as novel mediator of chemoresistance in breast cancer

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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 responses toward 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-Dox and MCF7-ETV7 cells stably overexpressing ETV7, and we tested the sensitivity of these cells to the chemotherapeutic drugs Doxorubicin and 5-Flouracil (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 determined that ETV7 expression led to the downregulation of DAC15, a co-activator 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 DAC15 and we also found that DNA methylation may be a factor in ETV7-mediated transcriptional repression at the DAC15 promoter. These findings of an inverse correlation between ETV7 and DAC15 expression in breast cancer cells in terms of Doxorubicin resistance, correlated well with recurrent responses of breast cancer patients with recurrent disease, based on our analyses of reported genome-wide expression groups. Moreover, we demonstrated that ETV7-mediated Doxorubicin resistance involves increased Doxorubicin efficacy via nuclear uptake, significantly reduced by knockdown of ETV7. Consistent with this observation, we could anticipate an increase in ABC transporter and the Bcl2 anti-apoptotic proteins 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−) 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 CSC vulnerabilities and the improvement of combinatorial regimens with the aim of avoiding resistance and relapse in breast cancer.

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

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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 cytokeratin 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-scale 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 cytokeratin 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 ± 0.8 ng/ml, p<0.0001), CA 15-3 (12.9 ± 9.0 kU/l, p<0.0037) and CYFRA 21-1 (1.3 ± 1.0 µg/l, p<0.0009). TPA and CYFRA 21-1 exhibited a statistically significant difference in clinical stage III compared to clinical stage II, and when comparing the groups 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 (0.8783). We concluded that CYFRA 21-1 overcomes TPA as both a 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.

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

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The purpose of this study was to evaluate the prognostic value of contrast-enhanced Magnetic Resonance Imaging (MRI) texture features in patients 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-LZGHE) were shown to be significant in relation to OS. The multivariate Cox regression analyses suggested two features (GLZLM-LZE and GLZLM-LZGHE) 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.

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

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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 CD11b, CD117, 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 von Willebrand factor and 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, etoposide, irinotecan) and angiogenesis-targeted agents (nimotuzumab and regorafenib). Taken together, our results indicate that increased expression of Slug in epithelial phenotype is associated with a higher invasive potential without detectable changes in growth or drug sensitivity.

Key words: chondroma, biopsy, organside, PD-L1, prognostic markers, immunotherapy

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

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Immunotherapy targeting the programmed cell death receptor ligand 1 (PD-L1) is an emergent treatment for chondromas. 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 analyzed PD-L1 expression and response to immuno-oncology treatments in 24 patient-derived organsals from primary patients by comparing sensitivity and specificity of two antibodies (HIC 28.6 and E1L3N) and correlating results with clinical parameters. PD-L1 expression was evaluated by intensity and percentage of positive tumour cells and lymphocytes. Furthermore, we developed a spherical model from patient-derived cells to assess the individual response to antibody treatment. E1L3N and 28.6 antibodies showed a significant linear correlation (R²=0.49 beta=0.63, p=0.001). However, E1L3N was more sensitive and specific for cell membranes (ROCC=0.898 area) and yielded higher tumour scores (p=0.001). E1L3N detected PD-L1 in 34% of chondromas, with positive tumour cells varying from 1-15% of tumour area. In 84.6% of cases, tumour-infiltrating lymphocytes present at the neoplastic lateral margins were also positive for PD-L1 (R²=0.556, p=0.001). PD-L1 expression was associated with greater tumour diameter (p=0.014). Spheroids generated from chondroma 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 chondromas 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 predict individual treatment responses even in patients with low or no immunohistostenochial PD-L1 expression.

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Role of Cathepsin B circular RNAs in tumor cells

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Pancreatobiliary Adenoma Carcinoma (PACAC) 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 5- and 10-year survival rates for PACAC 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 a tumor-associated marker in this cell maintenance. The role of circular RNAs encoded by the Cathepsin B gene (CTSB) remains unknown. It is now recognized that most protein coding genes not only produce linear mRNA but also produce circular RNAs and that their output ratio from linear to circular is dependent on the efficiency of circular RNA processing. Thirteen circular RNAs have been identified which are produced from the CTSB gene: Circular RNAs (circRNAs) are now known to be recognized as belonging to a significantly important regulatory layer: the functions of these CTSB-coding circular RNAs have yet to be identified. From our data, it was found that CTSBPR mediated the knockdown of Cathepsin B (CTSB) in pancreatic cancer cells, revealing significant changes in the expression profiles of various circular RNAs. We observed a 15.9-fold upregulation of hsa_circRNA_0042484 and a 20.5-fold downregulation of hsa_circRNA_104002 in the MIA PaCa-2 cells in which CTSB was knocked down and a 4.4-fold upregulation of hsa_circRNA_081609 and a 3.8-fold downregulation of hsa_circRNA_104069 in the Panc-1 cells in which CTSB was knocked down. This variation in differential up- or down-regulation of the expression of circRNAs may be attributed to the varying phenotypes of MIA PaCa-2 and Panc-1 pancreatic cancer cells. Notably, the Panc-1 cells in which CTSB was knocked down failed to establish tumors in nude mice. From the further analysis of CTSB circRNA, we observed that CTSB hsa_circRNA_0033530 had the highest number of esaurientin initiation factor 44-3 (EIF4A3) binding sites. We thus concluded that circular RNAs play a significant role in tumorigenesis.

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Autophagy dependence of small molecule angiotensin inhibitors in colorectal cancer

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Autophagy plays an important role in the response of tumor cells to environmental stress. Small molecule angiotensin 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 angiotensin inhibitors. We here aimed to establish the influence of mTOR on the activity of nintedanib and regorafenib, two small molecule angiotensin inhibitors with clinical activity in metastatic CRC. Our results showed that nintedanib and regorafenib displayed comparable activity toward a panel of 12 well-characterized CRC cell lines, with average IC50 values between 2 and 2.6 µM. However, the activity profile towards the different tumor cell line was markedly different. Immunochemistry and western blot analysis of the morphological marker LC3 as well as the Autophagy Bbox2 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, genotoxic models with attenuated expression of the autophagy regulator Becn1 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 angiotensin inhibitors. We propose that tumor cells with high autophagic flux may be selectively sensitive to nintedanib.

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

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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, two features 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 = 0.005, respectively), homogeneity (p = 0.018, p = 0.003, respectively), energy (p = 0.002, p = 0.002, respectively) and entropy (p = 0.032, p = 0.025, respectively) of gray-level co-occurrence matrix, JRE (p = 0.016, p = 0.005, respectively), LRLGE (p = 0.002, p = 0.001), respectively, and contrast (p = 0.001, p = 0.010, respectively). Furthermore, sphericity (p = 0.002, p = 0.007), compactness (p = 0.003), LRLGE (p = 0.014, LIZGE (p = 0.003), LRLGE (p = 0.15), were significantly associated with progression-free survival, while entropy (p = 0.031) and energy (p = 0.015) from histogram-based matrix, dissimilarity (p = 0.03), SRE (p = 0.011), SRLGE (p = 0.024), RP (p = 0.03), LZE (p = 0.033), LZIGE (p = 0.033) and LZIGE (p = 0.033) were significantly associated with overall survival. In conclusion, our study provides preliminary evidence that several textural features derived from CT images were prognostic factors and predictive markers for CRC patients who are candidates for targeted therapy (bevacizumab and cetuximab).

Discrimination of pluripotent adenosomas and craniopharyngioma on MRE: From image features to texture features

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The aim of the study was to explore the differences of MRI scanning between primary adenosomas and craniopharyngioma from MR image features to 3D-based texture features. A total number of 131 patients were introduced into this study (pluripotent adenosomas, 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 pluripotent adenosomas and craniopharyngiomas. 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 pluripotent adenosomas and craniopharyngioma and represent practical diagnostic value. In addition, the two types of features are associated with each other.
Epigenetic analysis of depression-like behavior in interleukin-1β-deficient mice

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Interleukin-1β (IL-1β) is an interleukin-1-producing cytokine that is involved in the immune system. Inflammatory cytokines such as IL-1β are known to play a role in the pathophysiology of depression-like behavior. We investigated the expression of IL-1β in the hippocampus of mice treated with an antidepressant drug. Our results showed that the expression of IL-1β was significantly reduced in the hippocampus of mice treated with the antidepressant drug compared to the control group. This finding suggests that the inhibition of IL-1β expression may be a potential therapeutic target for depression-like behavior.

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

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Cancer mortality is closely associated with the presence of drug-resistant, invasive subpopulations of tumor cells. We demonstrated that the functional and mechanistic interactions between the two phenotypes are incompletely understood. We have developed a panel of 4-isosine CRC cell lines comprising of the parental HCT116 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 vasculogenesis/vascular mimicry and has been associated with a highly invasive and metastatic cancer phenotype. Cellular growth in murine models revealed that two of the resistant cell lines had acquired the capacity to form cellular networks as vitronectin in control to parental cells. Further work will include antibody arrays, EU-LSA, and nuclear DBA-2E2 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.


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

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Colorectal cancer was the second most common cause of cancer mortality in 2018, mainly due to resistance to therapy. Chemotherapy often kills only differentiated cancer cells, while the more undifferentiated cells, the mesenchymal and stromal cells, can survive after therapy, giving rise to therapy-resistant tumors. 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 GSK3β 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 renal 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 88 primary colon cancer cells. We initially examined the photon radiation effect on cell viability of 88 primary colon cancer cells and of commercially available colon cancer RKO, observing that photon radiation increases the viability of RKO but not that of 88 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 apoptosis and proliferation, as well as the generation of ROS.

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MicroRNA biomarkers in sleep apnea

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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 from hypotension, coronary heart disease, heart failure and stroke. Vice versa SA is highly prevalent in patients with CD. Several discoveries in the pathogenesis of SA have led to novel ideas to manage it, 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 mRNA as potential biomarkers. The aim of the study was to improve diagnostics in SA and to evaluate mRNA 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 ambulances. The group comprised 130 men and 64 women with a median age of 62.5 years. Venous blood was obtained using K-EDTA tubes. miRNAs were isolated from 200 μl plasma by miNeat<sup>®</sup> Nucleic Acid Plasma Kit (Qiagen) using miRNeasy Mini Kit (QIAamp) in a spin column and exogenous normalizer. RT was performed using Taqman<sup>®</sup> MicroRNA Reverse Transcription Kit (Thermo Fisher Scientific<sup>®</sup>). Quantification of mRNA was carried out using a TaqMan<sup>®</sup> MicroRNA Assays (Thermo Fisher Scientific<sup>®</sup>) and measured by real-time PCR on LightCycler<sup>®</sup> 96 System (Roche). The relationship of circulating miRNA levels and echocardiographic characteristics of patients will be presented. A variety of potential biomarkers, including miRNA or proteins, have to be compared to establish the single or combination of markers for clinic.

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

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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 Weihai Central Hospital prior to curative treatment. In terms of interest (OR) that was used around the tumor, and texture analysis was conducted on both PET and CT images within the same ROI. Convolutional rectiﬁable 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, based on the PET dataset; 6 for OS and 14 for PFS respectively, based on the CT dataset, including skewness and volume). Multivariate analysis identiﬁed kurtosis (HR 3.319,994, p = 0.009, volume (HR 25.282, 95% CI 0.032-76.8, p = 0.002) in PET and skewness (HR 13.047, p = 0.039, GLNI1GLRLM (HR 11.164, p = 0.032) HZEGEGLZM (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.

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

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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 progression. In this study, we conducted a meta analysis for ESCC by the integrated analysis of accumulated datasets and the identification of 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 (lincRNA) network of ESCC. A total of 81 interaction lncRNAs were identified, and 67 of these participated in the lincRNA network. Functional analysis revealed that these 67 key lincRNAs mainly dominated in cellular biological process. We then analyzed the associations between the expression levels of these key lincRNAs and the clinicopathological features and survival of ESCC patients from TCGA. In total, 18 of these lincRNAs were associated with tumor grade, TNM stage and lymphatic metastasis status (p < 0.05). In addition, 15 key lincRNAs were found to be associated with the survival of ESCC patients from TCGA (p < 0.05). Finally, 5 key lincRNAs 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-regulation were well-behaved between GEO, TCGA and RT-qPCR were completely consistent. In addition, we also found that some of these 5 key lincRNAs were significantly associated with tumor TMS stage and lymph-node metastases (p < 0.05). Clinically relevant analysis and the above bioinformatic analysis support the conclusion, and prove that our bioinformatics analysis is credible. Overall, this study provides further insight into the lincRNA functional features of ESCC through bioinformatics integrative analysis of GEO and TCGA datasets, and reveals the potential diagnosis and prognosis biomarkers for ESCC.

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

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FOLFOXIRI chemotherapy displays significant survival improvements in patients with pancreatic cancer. However, toxicities have hampered enrollment for the use of FOLFOXIRI in full doses. In order to increase the tolerability, many researchers have focused on the modification of FOLFOXIRI. On the other hand, hyperthermia (HT) has been considered as an effective auxiliary treatment for cancer therapy. To date, at least to the best of our knowledge, there are no studies available evaluating the combination of deep hepatic regional hyperthermia (DHRT) with modified-FOLFOXIRI for pancreatic cancer patients. In this study, we conducted a retrospective review of pancreatic cancer patients treated with the combination of new form modified-FOLFOXIRI and DHRT (FOLFOXIRI+DHRT). Patients underwent chemotherapy that included low-dose mitomycin, cisplatin on day 1 and 5-FU or capcitabine (CAP) or tegafur, gimeracil andoteracil potassium (TA-5), for a 2-week schedule. Generally, DHRT treatment was performed weekly, 45 min for each time during chemotherapy. The patients receiving FOLFOXIRI+DHRT, as the first line chemotherapy combined with DHRT, exhibited an improvement in OS and PFS. 17 months (95% CI 1.17-5.03 months) and 4 months (95% CI 1.8-4.29 months), respectively. Overall, this combination regimen was safe; 17/45 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 advance in hyperthermia, these are as follows: Tumor stiffening, a key determinant of tumor progression, is reversed by nonmesothermal induced photothermal therapy. Scientists in Yunnan investigated the evolution of tumor stiffness, as well as the growth and progression under the effect of mild hyperthermia and thermal ablation generated by light-sensitive multi-wall carbon nanotubes in an epithelial carcinoma mouse xenograft. This study highlights non invasive hyperthermia as a promising adjuvant strategy for the reversal of tumor stiffening and normalizes the mechanical tumor environment (2).

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

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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 of 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 of phases I and II stages (173.91±28.08 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 (183.75±37.81 pg/ml), P<0.05. The EGF-EGF levels were positively correlated with the serum-EGF levels with a correlation coefficient of 0.95 (P<0.05). The sensitivity and specificity of EBC-EGF test were 80.09% and 89.62%, 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

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

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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 remains 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) and tumorgenesis 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. Mechanically, 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 a 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 CyclinA, 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 CyclinA and induced the expression of p21. In conclusion, this study elucidates a novel epigenetic mechanism through which miR-155 accelerates the growth of liver cancer cells. In vivo, the miR-155 silence markedly decreased the expression of CDK2, CDK2/CyclinA/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
Normalization of serum vitamin D levels improves glycosmic parameters in patients with type 2 diabetes mellitus

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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. Hypoglycemia in T2DM contributes to complications. Factors that regulate glycemic control are complex. Vitamin D (25-hydroxyvitamin D3) is known for its effect on bones. Vitamin D also 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 supplementation (50,000 IU 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, total serum insulin 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). This treatment 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.

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

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MicroRNAs (miRNAs) have played important roles in the regulation of gene expression in many cancers, but their roles in esophageal squamous cell carcinomas (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 Genomic Atlas (TCGA) for microRNA expression profiles to identify ESCC-specific key miRNAs related to the ESCC patient tumour biologic grade and lymphatic metastasis from TCGA. Next, the key miRNAs potential genes regulatory functions and relationships with ESCC patients' clinical characteristics and overall survival were analyzed, respectively. Finally, these key miRNAs were validated using luciferase and FC-PCR was used to validate the bioinformatics analysis results' reliability and validity. Thirty-five ESCC-specific miRNAs from the TCGA database were identified (false-discovery rate < 2.10^-6). 20 of them were involved in the miRNome/miRNA co-expression network construction, and 17 were related to ESCC patients' tumor biologic grade, TCGA stage and lymphatic metastasis (p < 0.05). Additionally, six miRNAs (including miR-200b-3p, miR-31-5p, miR-181d-5p, miR-182-5p and miR-434-3p) were correlated with patients' overall survival (log-rank p0.05). miR-130b-5p, miR-182-5p and miR-215-5p were selected for verification of the expression levels in ESCC patients' tissues samples via qRT-PCR. We found that the fold-changes between FC-PCR and TCGA were completely consistent. Results also suggested that miR-130b-5p, miR-15b-5p and miR-92b-5p were significantly correlated with tumor differentiation degrees (p < 0.05). miR-15b-5p was significantly correlated with tumor TNM stage (p < 0.05), and miR-130b-5p was significantly correlated with lymphatic metastasis (p < 0.05). miR-182-5p, miR-15b-5p and miR-191-5p ESCC patients' clinical characteristics and the TCGA bioinformatics analysis were similar. Our study revealed the landscape of ESCC-related key miRNAs. In conclusion, key miRNAs worth 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.

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

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Mefeninol is a widely used antibacterial 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 mefeninol on the expression and secretion of different cytokines and chemokines from mouse colon SMCs (CSCMs), following induction of inflammation with lipopolysaccharide (LPS) in vitro. CSCMs 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 µM mefeninol for 24 h. Expression and secretion of tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), macrophage colony stimulating factor (M-CSF), T-cell activation gene-3 (TAC-1) and stromal cell-derived factor-1 (SDF-1) was evaluated by ELISA. LPS-treated CSCMs demonstrated a significantly increased expression of TNF-α, IL-1β, M-CSF, TAC-1 and SDF-1 when compared with the control group (P < 0.05). Co-treatment with mefeninol (5 and 10 µM) significantly reduced their expression by approximately 20-40% when compared with LPS treatment alone (P < 0.05). Furthermore, secretion of TNF-α, IL-1β, M-CSF and TAC-1 into the conditioned media was significantly decreased by mefeninol (5 and 10 µM; P < 0.005). In addition, mefeninol decreased LPS-induced phosphorylation of LPS-induced factor-4 (LPS-induced factor-4) phosphorylation. These data suggest that mefeninol may provide beneficial anti-inflammatory effects on CSCMs and it may be utilized as an adjunct therapy for patients suffering from IBD.

Key words: IBD, mefeninol, smooth muscle

Antioxidum activity of cannabidiol and cannabizapran extracts on a variety of human malignant cells

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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 order to study the antioxidantum activity native hemocromies from Helix lucorum (HBB) and Helix aspersa (HAl), as well as Rana sesea (R1B) 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 T25, HeLa,780, MHS and MDA-MB. The methods used were WST-1 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 IC50 values varying from 0.12 to 22.35 µM (depending on the cancer cell line, the cells were stained, the fibroblastic sub-β1 fraction by Nicotin's flow cytometric method, and an increase in the number of cells in X, G2 and M (G2/M-arrest). The results obtained for native hemocromies and their isoforms indicated that the structural subunits: R1VH (molecular weight 420 kDa), isolated from R. venosa hemocromie and Bio-HAl (isolated from H. aspersa hemocromie) have the highest antioxidant potential with effective concentrations between 500 and 1000 µM. In conclusion, given their activity and the absence of toxic effects, it can be said that cannabidiol as well as cannabizapran extracts are promising candidates for their use as add-ons to standard therapeutic regimes and could be used as an 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 Bulgaria national program BioActiveMed (D01-217/20 November 2018; governmental decision No. 658/14 September 2018).
Intratumoral subpopulation of cancer stem cells as predictive marker in cervical squamous cell carcinoma patients receiving chemoradiotherapy

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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 value of CSC proportion in cervical scrape samples from CSCC patients for short-term outcomes of concurrent chemoradiotherapy. Study group consisted of 55 patients with CSCC at FIGO stages IIa-IVb. Informed consent was obtained from all the patients. CSCs were detected by flow cytometry as CD45^CD44^CD24^ cells before the treatment and 24 h after local ELI radiation exposure at a cumulative dose of 10 Gy (to point A in the standard dose fractionation mode (2 Gy/day). The degree of tumor regression was assessed 3.6 months after the full course of the treatment including external and intrauterine irradiation. Weekly intratumoral injections of cisplatin (49 mg/m²) were performed in the period of external irradiation. Complete tumor regression was achieved in 25 patients, and partial regression was observed in 30 patients. The CSC proportion in patients with complete regression decreased on average by 2.01±1.4% after irradiation, while in patients with partial regression this indicator increased insignificantly (P=0.03±0.3%). 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 in 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.

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

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Cutaneous T-cell lymphoma (CTCLs) is 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 cytoxic efficacy and internalization rate in 2 CTCL cell lines, MCT-7 and M1, 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 cytoxic efficacy of micellar curcumin over its standard ethanol solution. IC₅₀ values varied from 29.7 to 29.3μM, depending on the cell line, with M1 demonstrating higher sensitivity. The internalization rate was determined by fluorescent microscopy and UN-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 expression, compared to the standard ethanolic solution. Curcumin downregulated WT-1, ALK, p-JAK2, p-JAK3, p-GSK-3β and p-PLCγ1, p-STAT5 and p-STAT3. The upregulation of pro-apoptotic proteins, such as p21, Waf1/Cip1, Bad and Bax was observed. 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.

Products of sperminolysis by bovine semen anionic elodes cause membrane permeabilization to granzyme cell mitochondria. A new physiological mechanism for regulating cell death induction in tumor mitochondria

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To elucidate of sperminolysis by bovine semen anionic elodes (BAE) induces extrinsic and extracellular cell death in tumor cells (I), electron microscopy observations have shown that mitochondria in these cells exhibit dramatic alterations, suggesting involvement of mitochondrial pathways of cell death (II). To investigate the role of the major extracellular enzyme produced by BAE, sperminolysis, preliminary experiments on BAE have also provided evidence that sperminolysis by BAE induces membrane alterations attributable to mitochondrial membrane permeability transition (MPT) and mitochondrial membrane permeabilization (MOMP) with the opening of a protein pore across both the mitochondrial membrane or only the outer membrane, respectively. This aim of this study was to confirm the previous results obtained by BEA and to elucidate the involvement in the membrane alterations of MPT or MOMP, or both, and thus to determine whether they can lead to apoptosis. We also wished to obtain information about the proteins involved in MPT or MOMP in tumor cells. The results confirmed that mitochondria damage by sperminolysis and BAE was responsible for the extracellular and the apoptosis of tumor cells. BAE underwent membrane potential collapse, mitochondria swelling, the loss of mitochondrial and oxidative stress, indicative of MPT induction. Furthermore, immunoblotting and densitometry experiments demonstrated the release of the proapoptotic factors, cytochrome c and Smac/DIABLO, indicative of an "apoptosis promoter" scenario in the BAE extracellular activation pathway. These results demonstrate that sperminolysis and BAE induce MPT in both BAE and stimulate the induction of MOMP. Consequently, our data indicate that the release of these proteins, involved in pore opening by MPT or MOMP, in normal or cancerous cells, increases the sensitivity to apoptotic stimuli, particulate in the formation of the pore. In conclusion, these results demonstrate that tumor cells exposed to the extracellular products of sperminolysis cell death by intrinsic apoptosis and this treatment can be considered as a potential tool for therapeutic interventions against cancer.


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

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

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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, lysine 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 hydroxyl lysine 370 of the tumor suppressor p53 and has been shown to play a regulatory role in a number of cancer and noncancer 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 trans-2-cyclohexylpropene and irreversible inhibitors. Our group previously described a series of 3,5-diamino-1,2,4-triazines that are effective reversible inhibitors of LSD1. The compound series produced a cell type specific cynosity 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 new causative factor in prostate cancer, which is infected by radiation factor probing. Inhibition of SMOX by the pan peptidase oxidase inhibitor MDL 72527 is capable of reducing these effects. However, MDL 72527 is an irreversible inhibitor of SMOX with unacceptable low potency. To date, few inhibitors of SMOX have been identified, and no specific inhibitors of the enzyme have been identified. Currently we are initiating hit-to-lead optimization studies intended to reveal new 3,5-diamino-1,2,4-triazine-based LSD1/SMOX inhibitors with improved potency and selectivity compared to the parent compound.

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

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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 (NIIH 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 weighting method. Heterogeneity was assessed by calculating the I² 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 sizes (GRADE criteria). Data showed considerable heterogeneity (I²=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 increased crude odds ratio of 1.59 (95% CI: 0.71-3.57, P=0.62) and considerable heterogeneity (I²=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.

Polyamine Metabolism in the Pathogenesis of Non-alcoholic Steatohepatitis

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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 catalytic 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-field 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 acetyl spermidine 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.

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

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Macrophages are phagocytic cells but they participate in different processes such as elimination of pathogens, thermogenesis regulation in adipose tissue, necroinflammation in vivo, among others. The expression of O-GlcNAc is increased in macrophages. This is a non-canonical glycosylation that involves the binding of N-acetylglucosamine (GlcNAc) to the product of the biosynthetic pathway of the hexosamines to serine and threonine residues of cytoplasmic, extra cellular 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 treatment of macrophages with GlcNAc-N-acetylglucosamine in the production of ROS. Our results showed that the production of ROS is increased in macrophages of cell line RAW 264.7 increased by 21.52% stimulated with LPS/GlcNAc/Thimerosal G and 22.6% with LPS/GlcNAc with respect to cellsstimulated only with LPS. In contrast, in an increase in the expression of O-GlcNAc and OGT was observed in both conditions as well as a decrease in OGA compared to control. On the other hand, for the cell line PM-2, it was increased by 6.13% stimulated with LPS/GlcNAc/Thimerosal G 1.99% with LPS/GlcNAc with respect to cells stimulated only with LPS. In the cytotoxicity assays, the expression of O-GlcNAc and OGT was increased. Notably, we found that there was a relationship between the OGT enzyme to the membrane in both conditions to relate to control. There were no changes in the levels and location of OGA. No changes were detected in viability in any conditions in the cell types. Our study shows that GlcNAc-N-acetylglucosamine does not have a significant role in the production of ROS; however, changes in the expression and localization of O-GlcNAc and OGT that may be related to other processes of macrophage biology should be enlightened.

Key words: O-GlcNAcylation, superoxide anion, macrophage

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

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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 CRC in 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 dysregulated by expression by RNA whole transcriptome sequencing. Lastly, we measured the secretion of cytokines characteristic of effector T helper cell (Th) subsets by ELISA from 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 TGFβ1, IL-10, IL-1β, and TNFα than those of CA patients. Furthermore, TCGA analysis of normal adjacent tissue identified several candidate genes involved in inflammatory and immune-related pathways in AAs, which may contribute to the observed differences in tumor immunology between AA and CA patients. We found an association of cytokine expression in AAs with higher expression of GZMB (5 years). In conclusion, we demonstrated a differential immunological profile of AA compared to CA cancer patients. This would suggest, for the AAs-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

Role of p16 INK4a in uveal melanoma

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Uveal melanoma is the most common intraocular tumor in adults. Despite the overall excellent prognosis, a 20%–70% of patients with uveal melanoma develop metastases within 5–30 years (1). The diagnosis of metastatic disease is often a medium to late stage, with approximately 13 months (2). No treatment regimen for metastatic disease has been successful to date for p16 INK4a, located at chromosome 9p21, in a tumor suppressor gene, whose role has been clearly defined in many malignant tumors. p16 frequently presents germline mutation in familial uveal 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 composition, presents 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 (TMA) built with 2 mm cores derived from 34 uveal melanomas, from 2003 to 2018. We observed a variable overexpression of p16 in 6 of 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 context of unknown primary tumor.


The pheomelanin P343 exclusively eradicates human cancer cells


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The pheomelanin derivative P343 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 PARP, it acts as PARP inhibitor. Recently, we identified its exclusive cytotoxic activity in human cancer cells. This activity was independent of PARP inhibition or caspase activity causing apoptotic cell death. We found that P343 exclusively eradicates human cancer cells without harming normal acinar cells via a high affinity of the drug molecules to the interaction with NaMDA in other chemotheraphic conditions. Our data indicates that P343 in MCAs is a macromolecule (MHC)-binding melanin 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. This activity is mediated by melanin synthesis and paracrine factors produced by the melanin synthesis, thereby preventing its transport 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 mitotic arrest followed by immediate self-destruction of the dividing cells. Human cancer cells resistant to apoptosis-inducing agents, were eradicated by "mitotic catastrophe cell death" induced by P343. This phaeomelanin derivative was shown to be present in many human cancer cells, indicating that, despite being hypoxia and triple-negative breast cancer, melanoma and paracrine factors, glioblastomas, squamous and hematogenous malignancies. After examining the pharmacokinetics and bioavailability of P343, we tested its therapeutic efficacy in vivo. We will present the results of the recently tested P343 in xenografts of paracrine cancer PACNI. The results of IV treatment with P343 were examined 30 days after treatment. About 80-90% of P343's anti-cancer effects were established 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 Pharmaceud, preclinical C30, Israel. The reports are available at request.

Elucidation of the catalytic cycle of type II DNA topoisomerase using a structural and molecular biological approach
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Type II topoisomerases play essential roles in DNA replication, chromosome segregation and recombination, and are important antibacterial and antitumor targets (1). Bacterial topoisomerase II is an increasing and now widely recognized threat, and the limited number of new antibacterials developed in recent years is a matter of serious concern. One of the approaches to 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 be vital in the rational design and development of new molecular approaches to combat bacterial infections, while at the same time keeping the realistic side-effects of the drugs at an acceptable minimum.

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Prediction application of mRNA markers for colorectal polyps by membrane array
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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. The precancerous stage of cancer can be diagnosed and treated earlier, the incidence and mortality of colorectal cancer would be greatly reduced. Unbiased membrane microarray analysis on the tumors and normal tissues of patients with polyps and found that the differentially expressed 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 MUC 2 were significantly associated with polyp size, the number of polyps and the malignancy of polyps (P<0.01). Additionally, we found that genes, including HLA-A2, RAP1B, TDX1, 12B4 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

PCAL and PCT monitoring in the study of osteoarthritis
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Anamnestic arthroses are among the most feared complications following colorectal surgery. Their clinical significance must be underestimated due to their association with increased morbidity, mortality and oncologic recurrence (1). The tissue damage can achieve an inflammatory pressure and/or a chemical necrosis, in addition directly proportional to plasma clastoprotein (P Cal), a protein contained in neutrophil granulocytes. In addition, the serum level of procalcitonin (PCT), which is significantly increased in patients with endotoxin and sepsis infections, could represent an early biomarker of septic complication and acute 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 (CRP) and white blood cell count (WBC), in order to evaluate the use of P Cal and PCT in more efficient in terms of specificity and sensitivity than other markers, in the early assessment of osteoarthritis. The prospective and observational study, conducted from September, 2017 to July, 2018, involved 2) patients enrolled in general emergency surgery departments of the AOO “G. Malagolfo” and “M. Invernizzi”. All participants were subjected to laparoscopic intestinal surgery, apart from a case that was performed laparoscopically (5%). The intervention guaranteed the reintroduction of intestinal continuity without performing a protective stoma. Blood samples were collected in patients in the 1st, 3rd and 5th postoperative day (POD). P Cal was superior to PCT in the detection of anamnestic arthroses. Furthermore, the best diagnostic accuracy was obtained when the WBC, P CRP and P Cal measurements of POD 3 were combined. In conclusion, procalcitoxin and procalcitonin can be useful markers in the early diagnosis of anamnestic arthroses for the better management of the postoperative phase prior to the risk of anamnestic arthroses. The key words: plasma calprotectin, procalcitonin, anamnestic arthroses, leucinemia, colorectal surgery.

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Modeling and simulation approaches for the description of pharmacokinetics and pharmacodynamics of glucocorticoid
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In recent years, the use of modeling and simulation approaches, such as pharmacokinetics (PK) and pharmacodynamics (PD) models were taken from the previous modeling exercise (1), while several administration regimes were investigated. Several levels of prednisolone clearance were explored regarding the usual 2 mg/kg and PD. Also, the properties of the PD model were examined by altering EC50 and (1) PK and PD model parameters. The model was implemented in Monolix 2016R1 using also Mphine and Simcys, by writing the appropriate code in the R Language. Concentration-time and effect-time plots for the generated virtual patients were created. Decrease of prednisolone clearance from the body led to higher peak plasma concentrations as well as more intense and longer duration of the effect (namely, increase of (DCO) inhibition). A higher and more intensive effect was also observed with decrease of IC50 and increase of IC50. The concentration-time and effect-time profiles were further simulated assuming different dosage schemes. In conclusion, the pharmacokinetic-pharmacodynamic models were performed using a joint PK/PD model. Without any real patient interventions, the use of in silico models allowed the prediction of prednisolone plasma levels and the anticipated efficacy under several different dosage scenarios. Modeling and simulations would assist clinical ontologists in setting the appropriate dosage regimes, even with newest advanced therapies, such as with epacadostat.
Role of RNA-binding proteins in the link between diabetes and cancer

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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 modification of adenosine at the nitrogen-6 position (m6A) in RNA in response to elevated glucose levels. In line with these changes, there were alterations in N6-methyladenosine (m6A) levels in high-glucose-treated SW620 cells compared to control cells exposed to normal glucose conditions. Our study also suggests that the glucose-responsive m6A modification may potentially be regulated by the combined action of more than one RBP of the m6A machinery in a complex manner. Given that m6A RNA methylation has been increasingly implicated in human cancer[2], altered m6A levels caused by dysregulation of the RBPs involved in m6A modification may play at least a partial role in diabetes-induced cancer, which warrants further 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.


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

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We have recently put forward a research application to verify the mechanisms of estrogen-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). Fischbach and Rudzinski are arylated 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 Fischbach and Rudzinski may have estrogenic and disruptive effects on ER-expressing breast cancer cells by inhibiting activation of cell cycle- and EMT-related genes via an ER-dependent pathway.

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

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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 SAOS2, an a HDAC inhibitor, and inhibited the colony and tumor spheroid formation. In addition, GA decreased the mitochondrial membrane potential (MMP, AMF), and increased the number of cells in apoptosis stage, and induced DNA fragmentation. Western blot analysis, GA demethylated the expression of HDAC1 and 2, leading to the upregulation of acetylated histone expression at the protein level, resulting in downregulation of the expression of cell cycle-related genes such as PCNA, Cyclin D1 and E2. Upregulation of the expression of cell cycle arrest genes, p21, p27, and regulation of the expression of apoptosis intrinsic pathway-related genes, such as Bcl-2, cleaved-Caspase3 and PARP, in both cell lines. Furthermore, oral administration of GA for 5 weeks on PC-3 cell-derived tumor xenograft mice model decreased the tumor size, damaged the tumor skin, and downregulated the expression of HDAC1 and 2. PCNA in tumor mass, confirmed by histological analysis. Taken together, GA inhibited 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.

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

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Although the effects of stem cells expressing anti-cancer genes on tumor growth have been previously demonstrated in various preclinical models, renal cancer cells (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) and interferon-beta (IFN-β) using genetically engineered neural stem cells (NSCs) in a cellular and metastasis model of renal cell carcinoma (RCC). The CD5-CD method has been shown to reduce the risk of normal tissues because it selectively targets cancer cells via the CD gene, which converts produg 5-Fu to drug 5-fluorouracil. Moreover, we used NSCs 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 A949 cells, a cell line of RCC, and identified the A949-specific migration ability of NSCs. We also confirmed that the proliferation of A949 cells was significantly induced by therapeutic NSCs in the presence of 5-Fu. Furthermore, we established an A949 metastasis model. In the animal experiment, the weight of the lungs increased in response to cancer metastasis, but was normalized by NSCs expressing CD and/or IFN-β genes, while the incidence of liver metastasis was suppressed by the NSCs. 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.

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(2) Sihn IJ et al., J Cell Biochem In press, 2019.
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Myocardial differentiation appeared to be hindered by cigarette smoke components in mouse embryonic stem cells

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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, g3-p53 and cyclin D1 protein expression, which are cardiode differentiation and cell-cycle markers, respectively. In addition, exposure of mouse embryoid stem cells (mESCs) to cigarette smoke components inhibited myocardial differentiation and development through the expression of HDAC2, g3-p53, 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 cardiogenicity, 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.

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

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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 population without being manifested. Thus, its late detection and consequent treatment dictates 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.


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

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The interaction between oncopgenes and epigenetic modifiers is mediated through regulatory loops and circuits involving target genes. The oncopgene 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 (NHBL). We have extended this analysis to non-coding RNAs encoded in conserved genomic loci. To this end, we evaluated the immunohistochemical expression of the oncopgenes MYC and TCL1A and the epigenetic modifier EZH2, the catalytic subunit of the polycomb repressive complex 2, in 75 tissues of five NHBL 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 15 reactive lymph node (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, TCL1A and EZH2-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.

Autoantibody biomarkers differentiate pancreatic ductal adenocarcinoma from chronic pancreatitis

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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 adenocarcinomas 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 testing) 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 InnoLisa-Magix software from which neighbourhood background signal was subtracted were extracted. Following this, we generated 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.
Chromosomal studies on the Egyptian fresh water snail Biomphalaria alexandrina by using transmission and scanning electron microscope

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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 colchicin 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 mitotic phases: interphase, prophase, metaphase and anaphase. Our study showed a diploid number of chromosomes 2n=56 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)

Ultrastructural study of neutrophil apoptotic changes in hepatitis C patients

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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 (50 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/Fluorescein isothiocyanate (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 dUTP 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 Annexin V cells was decreased in the 2 patient groups versus the control group while in Group II Annexin V cells were significantly decreased and Annexin V 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.
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