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The importance of mitochondria in the tumourigenic phenotype: Gliomas as the paradigm (Review)

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Abstract. Cancer arises from the accumulation of nuclear and cytoplasmic abnormalities, a phenomenon allowing for the expression of the tumourigenic phenotype. Gliomas represent the most frequently diagnosed tumours of the central nervous system in adults. Warburg hypothesized the importance of glycolysis in cancer cells, and implicated additional roles of mitochondria in neoplasia. Recent data have shown the importance of mitochondria in the tumourigenic phenotype, in particular, within the apoptotic process. There have been a variety of studies conducted on brain tumours revealing significant alterations of mitochondria within the tumourigenic phenotype. This review describes some of the more recent findings of mitochondria and gliomas, correlating findings to those observed in other cancers. Alterations in mitochondrial DNA copy number and location, as well as dependence of the cancer cell phenotype on mitochondria are emphasised. In addition to its role in apoptosis, the mitochondrion serves as an important element in the tumourigenic phenotype, and clinical approaches targeting this organelle have potential for the development of effective treatment regimens for patients with glioma and other neoplastic diseases.

Contents

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1. Introduction

Gliomas represent the most common form of intracranial tumour, comprising about 60% of all primary central nervous system (CNS) neoplasms. Glioblastoma, the most common glioma, particularly in adults, is the most malignant and undifferentiated type, and represents 50-60% of all gliomas diagnosed (1). These tumours have a poor prognosis, with less than 15% of patients surviving 2 years. Lower grade tumours, such as the anaplastic astrocytoma and astrocytoma, occur less frequently; for the former, recurrence, typically at higher grade, is the rule; for the latter, the clinical course is more varied, particularly with specific types of low grade tumours which require only surgery for therapy (e.g. pilocytic astrocytoma, ganglioglioma, neurocytoma) (2). Nevertheless, some fraction of the patients diagnosed with astrocytoma will recur, and similarly to the anaplastic type, at a higher grade. As a result, gliomas continue to be a refractory cancer, where therapeutic options are significantly less than optimal, and additional approaches for treatment are required.

Conceptually, understanding the behaviour of tumour cells by analysis of the phenotype is an important aspect necessary to develop preventative measures in addition to treatment modalities which are both effective and safe for patients with cancer. This 'tumourigenic phenotype' has, in the past, been typically evaluated using approaches primarily addressing nuclear-encoded genes. However, it is clear that the influence of cytoplasmic components of the cell can also play a role in the manifestations of malignancy. As a component of the cytoplasm, the mitochondrion has more recently been investigated as a source of influence on the tumourigenic phenotype. There has been considerable evidence implicating the mitochondria in the apoptotic process; moreover, regulation of programmed cell death is considerably affected by the state of the mitochondria. However, other aspects of the mitochondrion *per se* have been noted to be altered in cancer, and may contribute to the neoplastic phenotype. Indeed, changes in mitochondrial DNA (mtDNA), as well as the dependence of cancer cell function on this organelle (despite the use of glycolytic pathways for energy production) suggest that the mitochondria are playing additional roles in the tumourigenic phenotype. This review describes findings of mitochondrial involvement in the tumourigenic phenotype, particularly in gliomas, and correlates the evidence to findings in other cancer types. The

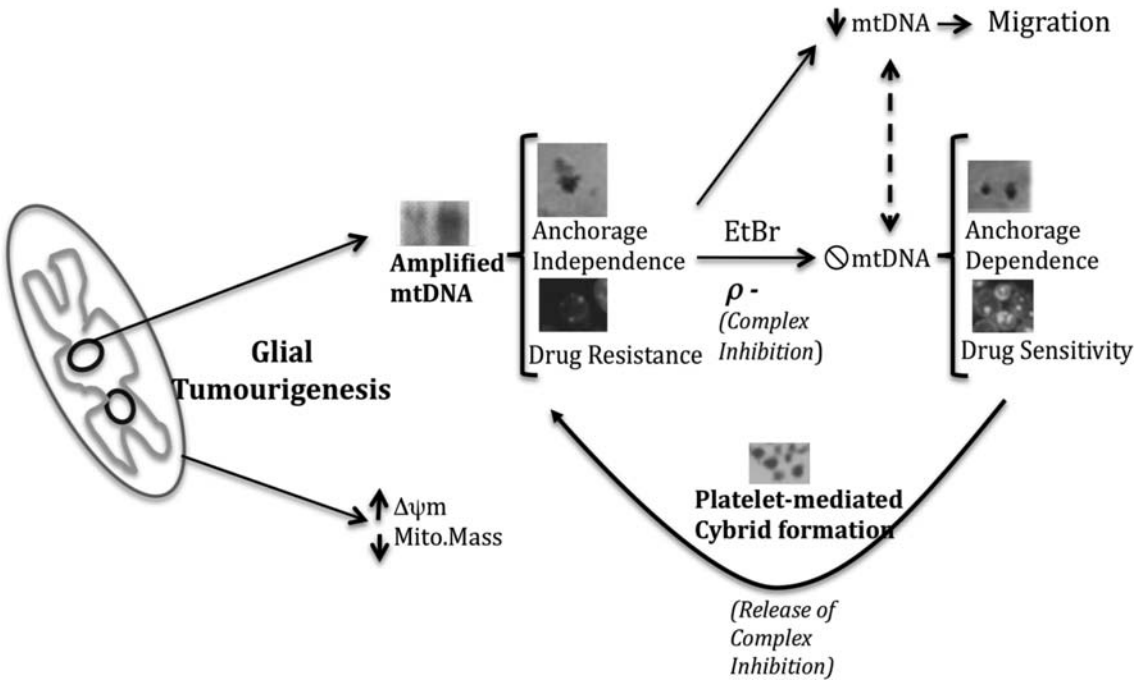

Figure 1. Model of involvement of the mitochondria in the tumourigenic phenotype. Gliomas show evidence of ontologically-early mitochondrial DNA (mtDNA) amplification, associated with mtDNA promiscuity into the nucleus, as well as an increased mitochondrial membrane potential ($\Delta\psi m$) and decreased mitochondrial mass. With significant reductions in mtDNA levels by the formation of ρ^- cells [via treatment with ethidium bromide (EtBr) or complex inhibition], cells lose the ability to grow in an anchorage-independent manner, and become more sensitive to the apoptosis-inducing effects of cytotoxic agents. Less significant reduction (potentially due to the presence of other nuclear or environmental influences) shows a potential for the development of a migratory phenotype. Creating cybrid cells with normal platelets (and thus, normal mitochondria and mtDNA) result in a return of the *de novo* tumourigenic properties, and amplified mtDNA. Similar findings have also been noted in epithelial carcinogenesis.

evidence of mitochondrial involvement in the tumourigenic phenotype in gliomas is summarised in Fig. 1.

2. Alterations of mitochondrial DNA

mtDNA amplification in gliomas. Cancer arises from the accumulation of DNA and cytoplasmic abnormalities, which allows for the expression of the tumourigenic phenotype, which is manifested by certain elements, such as lack of terminal differentiation, immortality, anchorage-independent growth and resistance to cellular death from cytotoxic and further inducing stimuli (3). Gene amplification (of nuclear genes) is an important aspect of this multistep pathway to the neoplastic phenotype, and occurs in most, if not all, tumours (4). Indeed, cytogenetic and molecular cytogenetic data suggest this genetic finding is an intermediate step in the progression of cells in the multistep pathway noted above. Using a molecular subtractive hybridisation approach (5), an amplified cDNA was isolated which revealed a mitochondrial (cf. nuclear) sequence from a glioblastoma cell line. Whilst mtDNA had been observed to be structurally abnormal in the past (6,7), no studies had noted significant changes in copy number. Using mtDNA probes, different stages of primary tissues of glioma (low grade, pilocytic tumours, astrocytomas; intermediate grade, anaplastic astrocytomas; high grade, glioblastomas) as well as tissue culture lines of high grade gliomas, were evaluated to determine the relative ontogeny of the findings (8). Genomic DNA of these tissues revealed frequent amplification of not only the cell lines, but also the primary tumours; of significance was the finding that lower grade tumours also harboured the amplification of mtDNA, suggesting this was an early event in the multistep pathway towards malignancy, in distinct contrast to nuclear DNA amplification in cancer, which is generally considered to be an intermediate step. This correlated with the expression data in a subset of tumours in which RNA was available; the data revealed an increased level of expression of the cDNA in both lower grade and cell line samples.

In gliomas, not only amplification, but also deletions, have been frequently noted. Components of the electron transport chain (including ND1 and ND2, *viz*. amino acid positions 4121-5275) have been reported to be deleted, correlating to diminished expression (8). Indeed, Dmitrenko *et al* (9) noted reduction of mitochondrial gene transcription in glioblastomas compared to that of the normal brain, using a differential cDNA screen. Similarly, they found ND1 to be consistently diminished in expression; further, ATP6, COXII, COXIII and 12S rRNA showed a decreased expression level. Kirches *et al* (10) found variations in the polycytosine tract; indeed, base substitutions were homoplasmic in nature. Similar results were noted by others (11), with the additional finding that there was no association between mtDNA and nuclear instabilities, an observation suggesting that in gliomas there are different distinct DNA repair mechanisms. Nevertheless, both studies suggest that the tumour-associated changes in mtDNA polymorphisms are via the same or similar mechanisms which generate such polymorphisms in an inherited manner. The pathogenic role of such alterations is yet to be determined, and requires additional correlative molecular and clinical studies (12).

findings, *viz*. alterations in the sequences of the y and coding regions of mtDNA in gliomas and the ontologically-early identification of amplification and heterogeneous areas of mtDNA amplification (suggesting deletions in the mitochondrial genome), have served to expand and extend early findings of abnormal mtDNA structure in cancer (13,14). The authors speculated on a possible association with petite mutants in yeast (15,16), where truncated mtDNA is associated with a dysfunction of the mitochondrion and with high degrees of amplification of the remaining DNA (see below).

In support of these studies in glioma, Penta *et al* (17) noted that breast, liver and colon cancers showed increases in mtDNA expression, with deletions in other tumours including those of the colon, stomach, bladder, head, neck and lung. Carew and Huang (18) also identified mtDNA as being altered in these tumour types, and additionally described findings in ovarian, esophageal, pancreatic, renal cell and prostate cancer, as well as thyroid cancers and haematologic malignancies. Beheshti *et al* (19) found mtDNA amplification in neuroblastoma, and Haugen *et al* (20) found an increased expression of mtDNA-encoded genes in papillary thyroid carcinomas. Jia *et al* (21) showed increases in mtDNA levels with the mdr-1 genotype, and Tarantul *et al* (22) found evidence for amplified mitochondrial sequences in SIV-infected primate non-Hodgkin lymphoma (NHL). Similarly, Nikolaev *et al* (23) showed mtDNA increases in simian NHL, and Nenasheva *et al* (24) found that such sequences increased in SV40-transformed fibroblasts, observations suggesting that such modifications serve as early markers for the development and/or the pathogenesis of malignancy. However, Dani *et al* (25) evaluated breast, colorectal, gastric and head and neck tumours for the mtDNA^{4977} deletion ('common deletion'), and concluded that cells of these tumour types are essentially free of this mutation; similarly, Kiebish and Seyfried (26) did not find significant mtDNA mutations in mouse brain tumours and concluded that these mutations do not play a role in either chemically-induced or spontaneous mouse brain tumours. However, this has not been confirmed by others; indeed, primary cells of these types showed alterations in the mtDNA^{4977} region, amongst other mutations (10,27-31). Furthermore, the D-loop region (where mtDNA initiates replication, and is non-coding) has frequently been associated with alterations in the copy number as well as with mutations in osteosarcoma, uterine serous and gastric carcinomas, and ovarian, endometrial and breast cancers (32-39). Indeed, Duncan *et al* (40) identified a novel human mitochondrial D-loop RNA species which was up-regulated subsequent to cellular immortalisation, supporting the hypothesis that mtDNA alterations represent an early modification in generation of the tumourigenic phenotype. Moreover, associations of D-loop mutations have been noted in the background of the *p53* mutation, which is associated with changes in the expression of mitochondrial proteins (41,42). Of note, others have found a distribution of mutations throughout the mitochondrial genome (43,44). These studies support a role for mtDNA in the tumourigenic phenotype, and suggest alterations in the copy number (particularly increases) as an ontologically-early aspect of the pathway to malignant behaviour.

Promiscuous mtDNA. The discovery of mtDNA amplification, and the hypothesis that this form of pathobiology could recapitulate findings in yeast, suggested the possibility of 'mtDNA escape' in cancer. In yeast, mtDNA escape occurs as a manifestation of communication between the nuclear and mitochondrial genome (45,46), and has been postulated to be related to both genetic and environmental control. mtDNA has been found transposed to the nucleus in cultured cells (HeLa), as well as carcinogen-induced hepatic carcinoma cells (47,48). Indeed, it has been hypothesised that high copy numbers of mtDNA may be required to transfer mitochondrial sequences from mitochondrial sources to the nucleus in cancer, a phenomenon similar to that observed in yeast (49,50).

Using interphase nuclei with fluorescent DNA probes (interphase fluorescent *in situ* hybridisation, or 'interphase FISH'), Liang and Hays (8) evaluated both primary and tissue culture specimens of glial tumours, including lower grade tumours. As noted previously, mtDNA showed increases in copy number in all the lower grade tumours studied, but not the normal brain specimens. When evaluated by the interphase FISH technique using fluorescent probes of the entire mitochondrial genome, at least 69% of the nuclei revealed mitochondrial sequence hybridisation in the specimens investigated. In comparison, when evaluating two normal brain samples, as well as a glioblastoma specimen (which did not reveal increases in mtDNA copy number), <8% of nuclei revealed hybridisation with the mtDNA fluorescent probes. This observation suggested an association with the amplification of mtDNA, and the finding of 'promiscuous' mtDNA sequences in the nuclear compartment. Indeed, this was the first description of an association of the nuclear localisation of mtDNA with mtDNA amplification as an ontologically early event; furthermore, it was the first description of such an event found in primary tissues, rather than in cell culture experiments. Of particular interest is the recent finding by Guescini *et al* (51) who described microvesicles released by glioblastoma cells (as well as astrocytes), which harbour mtDNA. Such microvesicles carried Tsg101, CD9 and Alix markers, suggesting these are true exosomes; hypothetically, mtDNA can be transferred not only within, but also between cells, a finding which serves to provide intriguing potential mechanism(s) of disease processes, such as cancer.

Additional evidence of a role of promiscuous mtDNA in cancer has been reported. Czarnecka *et al* (52) have described cancer as a 'mitochondriopathy', and have noted alterations of mtDNA in various tumours, as well as the insertion of mtDNA sequences into the nuclear genome, with findings in hepatoma and meningioma (53-55). Interestingly, in both human myeloid leukemia HL60 and colorectal carcinoma COLO 320DM cells, amplification of mtDNA was found to be associated with mitochondrial sequences identified in the nucleus; in normal fibroblasts and peripheral blood T-cells, no promiscuous mtDNA sequence was noted (56). Such mtDNA sequences were found to be localised to the double-minute sequences identified by molecular cytogenetic techniques. Similarly, in mouse and rat model systems, mtDNA-like inserts were found at higher frequencies in tissue nuclei when expressed relative to those of normal cells (57). Additionally, Ricchetti *et al* (58) found mtDNA sequences

interrupting intronic regions of MADH2, a suppressor gene in colorectal cancer (59), and Borensztajn *et al* (60) and Turner *et al* (61) showed promiscuous mtDNA in the nuclear genome in both the intron and exon regions, which were associated with clinical phenotypes of disease (Factor VII deficiency and Pallister-Hall syndrome, respectively). These studies suggest that promiscuous mtDNA may represent a mechanism related to the generation of the tumourigenic phenotype. These mtDNA alterations could have significant implications regarding both the developmental mechanisms and detection of malignant cells in patients with cancer. Indeed, Copeland (62), Modica-Napolitano and Singh (63), Saffroy *et al* (64), Chatterjee *et al* (65), and Paul and Mukhopadhyay (66), have noted the relevance of mtDNA mutations and the potential consequences regarding both the diagnosis and treatment of patients with malignancy. In fact, the diversity of changes throughout the mitochondrial genome (67,68), in addition to specific polymorphisms (69,70), have been reported to be associated with the risk for cancer. Jiang *et al* (71) found that there was an increased mtDNA content in saliva associated with head and neck cancer patients. Treatment of these patients with apoptosis-inducing therapy (but not surgery alone) was associated with a decline in mtDNA levels (72). Hence, alterations in mtDNA may serve as a diagnostic tool, reflecting the response to a therapeutic intervention with modalities such as radiation therapy. Indeed, Polyak *et al* have filed a patent to utilise the presence or absence of mtDNA mutations in order to detect cells as a diagnostic approach in cancer patients (U.S. Patent Application 10/053,611).

3. Models of tumour phenotype

Creation of mtDNA-less cells (ϱ^-). Cavalli *et al* (73) employed techniques to decrease the levels of mtDNA (ϱ^- cells). In this manner, the direct effect of mtDNA, and mitochondrially-encoded biomolecules, would be evaluable, and the concepts could potentially be translated to the treatment of malignant disease.

Using a glioblastoma cell line, neoplastic cells were treated with low concentrations of ethidium bromide (EtBr). As EtBr is an inhibitor of mtDNA replication (74), during cell growth and passage, the initial number of mtDNA molecules was decreased by a factor of two upon each doubling. The cells became auxotrophic on uridine and pyruvate, a key characteristic of mtDNA-less cells. The requirement for uridine is related to the need of dihydroorotate dehydrogenase (an enzyme of pyrimidine biosynthesis, located on the inner mitochondrial membrane) to have an active electron transport chain for normal function. The auxotrophic dependence of ϱ^- cells on pyruvate is not definitively known or well described, but most likely relates to the inability of the cell to oxidise cytoplasmically-produced NADH.

Cybrids (cytoplasmic hybrids) of the ϱ^- cells were also created. Cybrids, fusions of ϱ^- cells and normal cells, harbour mitochondria in order to replace this organelle and allow the study of mitochondrial function. In this case, normal platelets were employed, rather than *de novo* enucleated glioma cells and cytoplast transformation, in order to avoid nuclear and cytoplasmic component influences of the tumour cells on the cybrid phenotype, i.e. entirely normal mitochondria with a minimal level of influence from nuclear-encoded genes. By definition, platelets are fragmented aspects of cells, and thus anuclear.

A key aspect of the tumourigenic phenotype is the ability of such cells to grow in an anchorage-independent fashion (75). Both ϱ^+ parent and ϱ^- cells were assessed with regard to their abilities to form colonies in soft agar, an indication of anchorage-independence. Whilst both types of cells were able to attach to plastic tissue culture flasks, the formation of colonies occurred only in the ϱ^+ parent cells that harboured mtDNA, with minimal colony formation found in ϱ^- cells; similar results were noted in xenograft experiments.

Moreover, studies involving sensitivity to cytotoxic agents showed interesting results. Glioblastoma cells were treated with 1,3-*bis*-dichloroethyl-nitrosourea (BCNU), a common drug utilised in the therapy of patients with glioblastoma. ϱ^- cells were more sensitive to the respective agent than parent ϱ^+ cells. No changes were noted in the expression of *bcl-2*, *bax*, *mdr1*, *mrp* and *O^6-alkyltransferase*. Interestingly, removal of functional mitochondria via depletion of mtDNA failed to prevent apoptotic cell death, an observation indicating that actively-translated, mitochondrially-encoded molecules are not, *per se*, required for apoptosis induction by cytotoxic chemotherapeutic agents.

Cybrids created from the ϱ^- cells and normal platelet mitochondria revealed amplified mtDNA levels similar to ϱ^+ cancer cells, suggesting that a cellular influence to maintain increased mtDNA levels was still acting through the ϱ^- state, and was subsequent to platelet fusion. Glioma cybrid cells showed a return of anchorage-independent growth; as well, the relative sensitivity of cells to the effects of cytotoxic drugs increased to the levels of the parental cells.

Mitochondrial proteins have recently been found to be relevant in glioma. Indeed, the isocitrate dehydrogenase genes, IDH1 and IDH2 have been found to be mutated in astrocytomas (76); whilst IDH1 is a cytoplasmic protein, IDH2 resides in the mitochondria. Moreover, further studies (77) have shown IDH2 mutations to be more frequent in astrocytomas with oligodendroglial traits. These data are of interest in defining mitochondrial proteins and processes important in glial tumourigenesis; indeed, Hao *et al* (78) also reported mitochondrial protein alterations associated with tumourigenicity in paraganglioma. This group described alterations in the succinate dehydrogenase (SDH) complex, which is involved both in the electron transport chain and the tricarboxylic acid cycle (TCA); in particular, SDH5 was associated with a loss of SDH-dependent respiration, and a germline loss of function in a family with hereditary paraganglioma, an observation strongly suggesting SDH5 as a tumour susceptibility gene. The other components of this complex have also been found to be associated with this tumour type (79).

The importance of the mitochondria on tumourigenicity has been reported in additional studies. Hayashi *et al* (80) used enucleation studies and revealed that replacement of mitochondria from normal cells (a rat embryonic cell line) supported the growth of such rat glioma cybrids in nude mice. Indeed, growth patterns were identical in xenografts of the *de novo* rat glioma cells and in those of the cybrid subclones, although there was a heterogeneity of mitochondria

tumour cells and normal cells. Interestingly, Griguer) showed CD133 (a neural stem cell marker) up-regulation in ρ⁻ cells; using similar cybrid techniques, it was observed during hypoxic conditions that the reduction of mtDNA levels was associated with a migratory phenotype; in such conditions, nestin (another stem cell marker) was also expressed. Of particular note was the ability to detect mtDNA by PCR, suggesting the cells may not have been entirely ρ⁻, accounting for the migratory phenotype (see below). The use of enucleated glioma cells to create cybrids from the ρ⁻ cells resulted in the return of the CD133 levels of the parent cells. These data suggest that the milieu of glioma cells may be of significant influence, and the mitochondria may respond to generate a differential phenotype (e.g. migration) or cell surface expression (e.g. CD133). Such phenomena have also been noted in other tumour types (see below).

Earlier studies have shown the importance of mitochondria on tumourigenicity, as manifested by xenograft growth (82-84). Xue *et al* (85), using Boc.Aspartyl(O-methyl)CH_2F, removed mitochondria from neurons and HeLa leukemia cells; such removal did not damage other cellular components, but irreversibly committed these cells to death via an apoptotic mechanism. Hence, cells with inhibited or removed mitochondria, undergo apoptosis more readily compared to control cells. Dong *et al* (86) showed that mitochondrial transcription factor A (mTfam), a key regulator of DNA transcription, was significantly overexpressed (over 10-fold when compared to normal liver) in Morris (mouse) hepatoma cells, with augmentation of downstream mitochondrial gene expression. Moreover, mTfam protein was found in the hepatoma cell nucleus, suggesting a role of nuclear-mitochondrial communication in this neoplastic lesion. Indeed, these findings indicated that the mitochondrial influence on the observed tumourigenic phenotype is complex and involves a myriad of differing proteins interacting in both upstream and downstream pathways. Grandemange *et al* (87) performed a study, in which human dermal fibroblasts acquired transformed properties (morphologic changes, anchorage-independent growth, growth in xenografts) with stimulation of mitochondrial activity, using p43. Moreover, this transformation was associated with an increase in *c-Jun* and *c-Fos* expression, and virtual extinction of the tumour suppressor genes, *p53*, $p21^{WAF1}$ and *Rb*. When mitochondria were then inhibited using chloramphenicol, a loss of the tumourigenic phenotype was observed (although restoration of the tumour suppressor genes did not occur). This study thus identified p43, at least in dermal fibroblasts, as a potential mechanism by which the early involvement of mitochondria in the tumourigenic phenotype may be manifested, and supports the hypothesis of a direct involvement of the mitochondria in maintenance of the tumourigenic phenotype. Similarly, additional studies in other model systems have identified proteins which may be responsible for the importance of the mitochondria in the malignant phenotype. In a clinically-based study, Ambrosone *et al* (88) found an association of manganese superoxide dismutase (MnSOD, a protein transported to the mitochondria) genotypes (with limitations on transport into mitochondria) with breast cancer risk; Roy *et al* (89) noted estrogen-related processes in the nuclear-mitochondrial communication, via estrogen-induced mitochondrial oxidants; Palomares *et al* (90) showed a reduction of glutathione (GSH)-sensitised melanoma cells to cyclophosphamide; Ohnami *et al* (91) demonstrated that *K-ras* impacts the expression of mitochondrial genes; when utilising an antisense approach to pancreatic cancer cells, they identified 11 overexpressed genes, which were all of mitochondrial origin. Indeed, Weinberg *et al* (92) showed the requirement of mitochondria for *K-ras*-mediated tumourigenicity and in addition, the importance of mTfam in the phenotype. This further suggests the importance of the nuclear-mitochondrial communication pathway, where K-ras represents an important mediator and responder of mitochondrial gene expression and of changes in phenotype. Mills *et al* (93) identified CD14 as a potential mediator of apoptosis in HL60 cells. Mitochondria were inhibited using rotenone, antimycin A or oligomycin; all agents increased the expression of CD14 in these leukemic cells, followed by a loss of viability and apoptosis. Magda *et al* (94) studied A549 ρ⁻ cells (lung cancer) induced by exposure to EtBr (although auxotrophy was not reported in the study). This group showed a lower proliferative ability, but a greater level of intermediate filaments expression in the ρ⁻ cells, suggesting a more invasive phenotype. This observation is interesting in light of the hypothesis by Felty and Roy (95) that malignant cell spreading may be, at least in part, ascribable to the contraction and expansion of mitochondria, rather than only to the tension maintenance on the cytoskeleton via binding to the extracellular matrix. Moreover, these A549 ρ⁻ cells, whilst able to grow in mouse xenografts, required longer induction periods with smaller tumours than their ρ⁺ parents. This suggests a clear difference in phenotype, perhaps due to differences in nuclear gene expression (96-98). Similar results have been noted in breast cancer cells (99). Interestingly, ρ⁻ cells increased the expression of MHC-1 in addition to that of glucuronidation genes, a consideration relevant in immune surveillance and detection function; this suggests the relevance of mitochondria to the extrinsic manifestations of apoptosis induction, an observation similar to that made by Mills *et al* (93) with the expression of CD14, and to observations with CD95 (Fas) (100).

Gradual reductions of mtDNA levels in rhabdomyoblasts (transformed muscle cells) using EtBr resulted in changes in mitochondrial membrane potential ($\Delta\psi m$; see also below) and in elevated free Ca^{2+} concentrations in the cytosol (101,102); these changes resulted in the alteration of genes related to Ca^{2+} transport and storage, which could be reverted once cells were allowed to grow in the absence of EtBr. Interestingly, as mtDNA levels were declining (but not absent), there was an increase in the ability of the rhabdomyoblasts to move through a reconstituted Matrigel membrane, an observation suggesting a migratory phenotype which was lost once mtDNA levels were allowed to return to their control values [see also Griguer *et al* (81)]. These data suggest mtDNA levels are affected not only early in the tumourigenic phenotype, but also in ontologically-subsequent manifestations of malignancy, and could serve as a marker identifying a pathway by which the migratory phenotype is induced. Hence, using feedback to the mitochondria and mtDNA levels, the nuclear-mitochondrial communication could effect the expression of nuclear genes in response to the micro-

environment milieu; this occurs in yeast at a variety of ρ^- settings (103,104).

Ethyl-nitrosourea (ENU)-transformed normal human astrocytes. Few studies have been performed evaluating the ontogeny of mitochondrial alterations in gliomas. A model of chemical transformation with normal human astrocytes (NHA) treated with ENU to derive transformed astrocytes was employed to evaluate the ontogeny of malignant changes (105,106). This model system was also tested with citrate, an allosteric inhibitor of phosphofructokinase (PFK), which represents a key regulatory enzyme in the glycolytic process. NHA exposed to ENU became immortal, and at approximately 26 doublings achieved the ability to grow in soft agar and at a low density. When NHA cells were treated with ENU and both pre- and co-treated with the PFK inhibitor, citrate, cells still became immortal, dividing well-past terminal differentiation. However, despite achieving the ability to avoid replicative senescence, these dually-exposed cells did not gain anchorage independence nor the ability to grow at low density. When ENU-transformed clones were subsequently treated with citrate (cf. pre- and co-treatment) and evaluated for growth under low density conditions, there was a decrease in colony formation observed at an added level of 100 μM of this agent, suggesting that in this model, alterations of the malignant phenotype could be accomplished via an inhibition of PFK.

In NHA cells, $\Delta\psi$m was found to progressively decrease with increasing doublings until terminal division. Subsequent to undergoing 3-5 doublings, the ENU-exposed cells exhibited a significantly higher polarized $\Delta\psi$m, when compared to that of NHA cells. Although the $\Delta\psi$m levels of these ENU-exposed cells declined, they were, in general, higher than those observed with NHA, with maintenance of an elevated membrane potential after the development of immortality. Interestingly, in parallel with the lack of anchorage-independence and growth at low density, pre- and co-exposure of cells to citrate did not result in the increased $\Delta\psi$m observed in cells treated with ENU alone. No differences were noted between the ENU/citrate-exposed cells, and the NHA or NHA citrate-exposed cells in $\Delta\psi$m.

With respect to mitochondrial mass, NHA cells showed a very gradual, modest decline with successive doublings. With ENU administration, however, after 10 doublings, there was a substantial reduction in mitochondrial mass, to about 25% of the starting mass measured. Citrate prevented this dramatic drop in mitochondrial mass; even with immortality achieved, the mitochondrial mass of ENU/citrate-treated cells did not differ from NHA cells which were terminally differentiated. When calculating the $\Delta\psi$m/mitochondrial mass ratios, such data revealed a hyperpolarized state of the mitochondrial membrane of the ENU-transformed cells, which was preventable by citrate pre- or co-exposure. These data suggest that hyperpolarization of the mitochondrial membrane represents an early manifestation of the transformed/tumourigenic phenotype, associated with anchorage independence, and may be mitigated by the use of the PFK glycolytic inhibitor, citrate.

Hence, maintenance of the tumourigenic phenotype was found to be related to early changes in $\Delta\psi$m in a glioma model. Noteworthy, is the observation that in neu-initiated mammary epithelial cell tumours with increased $\Delta\psi$m values, the inhibition of glycolytic pathways using short hairpin RNAs of LDH-A, decreased $\Delta\psi$m and also reduced the ability of these cells to proliferate in both *de novo* and hypoxic environments (107), supporting glioma data acquired with the use of citrate as a glycolytic inhibitor of PFK, with associated changes in membrane potential reflective of the overall tumourigenic phenotype (i.e. resistance to apoptosis and growth in xenografts). Other researchers have investigated similar hypotheses (108). Matarrese *et al* (109) using a drug-resistant human T-lymphoblastoid CEM cell line, VBL100 variant (with amplified mtDNA), sensitive to TNF-α, showed that the induction of apoptosis was attributable to the depolarization of $\Delta\psi$m *per se*. Indeed, these authors showed a direct correlation between susceptibility to apoptosis (by various agents) and the state of the mitochondria ($\Delta\psi$m); prevention of the decrease in the membrane potential, and thus of mitochondrial function, using bongokrekic acid (an inhibitor of the $\Delta\psi$m decrease, acting by antagonising the adenine-nucleoside transporter, which, in turn, is responsible for allowing the mitochondrial-membrane permeability transition and thus an attenuation of the potential), showed that the apoptosis-inducing effects could be mitigated by agents which affected the intrinsic (i.e. the mitochondrial), but not the extrinsic pathway of apoptosis.

4. Targeting the mitochondria as a translational therapeutic approach

Previous studies have noted that the involvement of the mitochondria and the susceptibility to apoptosis are separated from ATP production via oxidative phosphorylation, which was diminished (110). Further chemotherapeutic approaches with agents which were noted to have anti-mitochondrial activity have been described. 2',3'-Dideoxycytidine (ddC) an anti-retroviral agent, is a potent inhibitor of DNA polymerase γ and consequently has a distinct anti-mitochondrial activity (111). ddC is currently approved for patients with acquired immune deficiency syndrome (AIDS) as part of the multi-agent cocktail treatment regimen. ddC was evaluated in glioma cell lines (112). Alterations of $\Delta\psi$m were noted, with a decrease in polarized mitochondria from 31-84% in cells treated with ddC; at all ddC doses studied, cell death eventually ensued. Interestingly, at some doses of ddC (between 10 nM and 15 μM), a delay (but not total rescue) in the death of both cell lines could be obtained with supplementation of the medium with glucose, uridine and pyruvate, an observation suggesting that the cells lacked a fully functional respiratory chain; however, cells treated with higher doses of ddC were unresponsive to such supplementation. This suggests that mitochondria (in a functional ρ^- state) are involved in at least one mechanism of the observed ddC-induced cellular death, although since rescue was only partially possible with glucose, uridine and pyruvate, and the cells eventually died, the overall mechanisms effecting apoptosis are likely to be more complex and multifactorial.

To more specifically target the mitochondria, compounds which have lipophillic (i.e. diffusible into the cell and past mitochondrial membranes), and cationic (allowing attraction and retention in the anionic environment of the mitochondrion)

istics would be ideal. A compound which fits these criteria includes tyrphostin AG17 [NSC 242557, (3,5-di-tert-butyl-4-hydroxybenzylidene) malononitrile], described by Burger *et al* (113) and available from the Cancer Therapeutics Evaluation Program (CTEP), National Cancer Institute, NIH, USA. This agent also has tyrosine kinase inhibitory activity, which is manifested as a growth retardation mechanism in other studies (114); it has been studied in human glioma cell lines as a potential sensitiser to the effects of cytotoxic chemotherapy (115). Treatment of glioma cell lines demonstrated auxotrophy on uridine and pyruvate after 30 days of exposure, which was not oberved with NHA. Exposure to AG17 after 2 or 3 days was associated with an increased sensitivity to the cytotoxic agent BCNU; in contrast, the sensitivity of NHA cells to BCNU was not modified (LD_{50} of 40 μg/ml for both the AG17-treated and the untreated cells).

In other pharmacologic approaches, Lena *et al* (116) evaluated temozolomide-resistant glioma cells, using agents to induce the mitochondrial permeability transition (MPT). Selected disrupting agents used included betulinic acid, lonidamine and CD437; each was able to dissipate $\Delta\psi m$ within 24 h of treatment. Such dissipation was inhibited by the MPT pore blocker, cyclosporine A. Subsequent evaluation of treated glioma cells revealed a mixture of cell death mechanisms, although the vast majority of cells underwent apoptotic cell death. These experiments suggested that the alteration of the MPT in temozolomide-resistant glioma cells effected an apoptotic phenotype; since evaluated cells included a PTEN hemizygous deletion and a lack of EGFR amplification, this approach might be useful when targeted therapy is otherwise unavailable. Griguer *et al* (117) studied OMX-2, a methyquinone targeting mitochondria; OMX-2 was able to dissipate $\Delta\psi m$ without effecting cell death in a glioma cell line; in contrast, normal cells maintained their membrane potential despite exposure to the agent. Moreover, OMX-2 was able to also potentiate cell death induced by 2-deoxyglucose in glioma cells. Higgins and Pilkington (118) were able to demonstrate an increased sensitivity of glioma cells to the inhibitory effects of dexamethasone and of tricyclic drugs, particularly clomipramine, on respiration. The authors suggest the use of such agents for targeting the mitochondria could be useful in the development of therapies for gliomas. Similarly, the use of glitazones (119) and carvedilol (120) revealed a potential to create apoptosis-sensitisation strategies with other agents (both via targeted and cytotoxic drugs) against glial tumours. Finally, sensitisation of glioma cells using interferon γ was related to the engagement of Fas, when up-regulation of mRNA expression was noted. Given data on ρ^- cells expressing increased levels of Fas, it would be interesting to evaluate the relationship of the cytokine to mitochondrial function (100). Regardless, it would be required to take into account the sensitivity to Fas-based apoptotic mechanisms, given the lack of responsiveness to Fas-engagement in some tumour types (121,122).

Lund *et al* (123) were able to demonstrate the direct effects of ddC on mtDNA copy number. Indeed, there was a significant depletion of mtDNA with ddC treatment in HepG2 hepatocelluar carcinoma cells, without this agent exerting an effect on the metabolism of such cells. These results confirm data acquired in glioma cells, which became auxotrophically-dependent on uridine and pyruvate when treated with ddC. Similarly, Bouayadi *et al* (111) showed that ddC could effect apoptosis in B16 melanoma cells, and related this finding not only to DNA polymerase γ inhibition (found in mitochondria), but also to DNA polymerase ß (which is typically found to be down-regulated in normal cells, but overexpressed in some). This phenomenon was also observed by Louat *et al* (124), and suggests that ddC has a multitude of functions by which its anti-neoplastic activities are manifested. However, it is clear that a significant, and probably dominant, effect of this agent is through the mitochondrion, since the sensitising effects of ddC to the apoptotic phenotype require a decrease in mtDNA levels/transcripts to a critical threshold level (125). Indeed, the relative resistance to ddC has been explored in CEM T-lymphoblast cells (created by incubation at a low concentration of the anti-nucleoside agent) (126); ddC-resistant cells showed an amplification of mtDNA relative to the corresponding *de novo* cells, with significant increases in the expression of mTfam. These data suggest that mTfam may be a mitochondrially-based mechanism of drug resistance to compounds such as nucleoside inhibitors as a manifestation of the tumourigenic phenotype. Previous work has also shown the importance of mTfam in cancer cells (86). Further, it has been shown that mutations in mtDNA polymerase γ promotes tumourigenesis in breast cancer (127). Moreover, ddC has also been noted to have sensitising effects with ionizing radiation. Indeed, Coucke *et al* (128) showed a dose-dependent response of ddC treatment to sensitization with radiation. This is of particular interest since radiotherapy generates reactive oxygen species (ROS) *in vivo*, including the aggressively-reactive hydroxy radical ($^{\bullet}OH$). Inhibition of the mitochondrial processes could potentially diminish this effect. Moreover, Humer *et al* (129) showed a synergistic effect of azidothymidine (which has a similar anti-mitochondrial effect to ddC), with CDDP in melanoma cells. However, these results are confounded by the possible formation of a complex of azidothymidine with the platinum(II) [Pt(II)] metal ion centre in CDDP. Indeed, in experiments focused on evaluations of the synergistic inhibitory effects of these agents on melanoma cell growth, the agents were co-administered in the same medium and Pt(II) readily formed such complexes with thymidine (130). A further complicating factor is that CDDP can also undergo ligand-substitution reactions with selected amino acids (either free or protein-incorporated) or other biomolecules present in the culture medium employed, particularly methionine and protein residues of this amino acid which have a side-chain thioether group sulphur donor atom, for which Pt(II) has a high affinity. Mitochondria must, therefore, harbour further complex mechanisms outside of changes in expression which alter the phenotype of therapeutic resistance as a manifestation of the tumourigenic phenotype.

Clinically, the use of ddC in combination chemotherapy has shown evidence of efficacy. In adult patients with T-cell lymphoma, Besson *et al* (131) treated patients with CHOP induction (cyclophosphamide, hydroxyurea, vincristine and prednisone); in a subset of patients, this was followed by consolidation with etoposide and an anti-nucleoside (typically ddC, but in some cases azidothymidine). Patients treated with

the consolidation regimen showed a significantly increased survival when compared to those with induction only with CHOP (17 vs. 3 months, p=0.004); no major toxicity was noted, although there were some peripheral neuropathy events. Although this study was small (n=29), it showed the potential benefit of addressing multiple targets, including the mitochondria, in neoplastic diseases. In addition, patients with AIDS-related lymphoma were also treated with low doses of methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine and dexamethasone, in addition to ddC, in a single arm study (132). Complete response was noted in 56% of patients, including those with poor prognosis factors (AIDS diagnosis prior to lymphoma; <200/mm^3 CD4 lymphocytes). Interestingly, minimal peripheral neuropathy was noted, and improvements in AIDS-related markers were observed. These observations suggest that ddC may indeed be valuable in the treatment of certain cancers, as noted herein; the key will be to ensure that the therapeutic index, particularly with the anticipated longer time of therapy and when administered in combination with other agents, is wide enough to avoid toxicity.

Lipophillic cations and related compounds should be valuable for the treatment of tumours with an increased anionic $\Delta\psi m$ (133). Indeed, in view of their ability to diffuse into and be retained within the mitochondrial space, this may serve as the mechanism involved in studies which have revealed cellular chemosensitisation to such agents. Targeting mitochondria to enhance the potential sensitivity of cells to cytotoxic agents is of particular interest with respect to the design of new chemotherapeutic and other approaches with available as well as newly-developed novel agents. Indeed, such increases in sensitivity of tumours to chemotherapy could allow the disease process to be more accessible to therapeutic intervention, with limited toxic effects to normal tissues.

With the findings of changes in the tumourigenic phenotype related to the mitochondria, a great deal of interest has been focused on targeting organelle-related functions for the treatment of human malignancy (134). Several studies (110,135) have shown an increased sensitivity to CDDP in ϱ^- U937 and 143B cells; in both studies, the mitochondrial pathway was not found to be the major producer of energy (using mitochondrial inhibitors), and in the latter, increases in sensitivity to CDDP were related to significantly increased caspase 3 activation. Indeed, these 143B ϱ^- cells were also found to have an increased release of cytochrome c into the cytoplasm with exposure to CDDP, an observation suggesting that caspase activation, in addition to increasing the magnitude of the response to CDDP, plays a role in ϱ^- apoptotic sensitivity. Moreover, Cummings and Schnellmann (136) showed at least partial (50%) reliance of CDDP-induced apoptosis on caspase 3 activation in normal renal proximal tubular cells, without changes in ATP concentrations; further potential mechanisms included those speculated to involve either bax or BID proteins, but most likely are not ascribable to caspases 8 or 9, which were inhibited in these studies. Similarly, Schwerdt *et al* (137), showed an increased sensitivity to CDDP when normal collecting duct-derived cells were incubated with mitochondrial inhibitors; again, no apparent modifications in ATP levels were observed. Troyano *et al* (138) showed that ATP levels were maintained in U937 lymphoma cells when mitochondria were inhibited with oligomycin, indicating these cells are not dependent on mitochondria for energy production. However, of much interest was the additional finding that depletion of GSH (a reactive oxygen species scavenger, transported into the mitochondria) increased the sensitivity of cells to CDDP-induced apoptosis when mitochondria were inhibited, an observation similar to those of Kharbangar *et al* (139) and of previous studies conducted with ϱ^- U937 cells. Moreover, with increases in the duration of CDDP exposure, a necrotic phenotype was noted, along with a decline in $\Delta\psi m$. This suggests that GSH may serve as a candidate for moderating sensitivity, as well as being involved in the mechanism of mitochondrially-mediated cell death, and that mitochondria, at the extreme, may also play a role in necrotic cell death. In contrast, Yang *et al* (140) found that in head and neck cancer cell lines which were enucleated, there was no difference in the sensitivity to CDDP when compared to parental cell harbouring nuclei; however, ϱ^- cells were more resistant to the effects of CDDP, i.e., up to five times more resistant to the effects of the drug. Similarly, Lee *et al* (141) found that when evaluating TRAIL-induced apoptosis in SK-Hep1 hepatoma cells, ϱ^- cells were more resistant than parental cells to the effects of this cytokine; a different model (HeLa cells treated with photodynamic therapy or adriamycin) also showed resistance to apoptosis in ϱ^- cells (142). However, these data were in contrast to the observations by Mizutani *et al* (143), which revealed that cybrids derived from mitochondria were more resistant to CDDP than ϱ^- cells, an observation which agrees with previously cited results, as well as with data from Mizumachi *et al* (144) who reported that anthracycline-resistant head and neck cancer cells had a significantly higher mtDNA content. Furthermore, in alternative model systems, photodynamic approaches have revealed increases in cell death with mtDNA depletion (145,146); indeed, in L1210 murine leukemia cells, there was a significant increase in the sensitivity to mitochondrially-related photodamage in ϱ^- cells, a process giving rise to apoptotic death; when made resistant to ddC, CEM lymphoblast cells showed increases in their mtDNA content and expression (126). These differences may arise from the various cell sensitivities to apoptosis (based on grade and stage, and thus nuclear gene expression, e.g. *bax:bak* expression), the effects of CDDP directly on the mitochondria, and/or from alterations in mitochondria during the development of CDDP-resistance, and hence its susceptibility to the Pt(II) ion-based chemotherapy. Several research groups have also proposed this hypothesis (110,140,141,147,148). Nonetheless, sensitivity to apoptotic-inducing stimuli, such as CDDP, can be manifested by the state of the mitochondria, which has direct therapeutic implications with regard to both targeting and combination therapies. In this regard, compounds such as tyrphostin AG17 could be of interest, since this agent exerts little or no effect on normal cells (astrocytes); Wallace and Starkov (149) noted that the selective delivery of compounds to the mitochondria, as speculated with AG17, could be a key opportunity for therapeutic intervention. This compound is of interest as a tyrosine kinase and as a mitochondrial inhibitor in cancer (150), including gliomas.

The investigations cited herein have investigated the role that mitochondria play in the tumourigenic phenotype, outside and above the facilitation of apoptosis, and the potential of these phenomena for use as a target in the treatment of glial and other cancers. In gliomas, as well as in other cancers, mitochondrial alterations are ontologically-early processes and manifest throughout the multistep pathway to malignancy, with both nuclear genetic and genomic alterations, as well as those at the level of the mitochondria and its genome. These changes evolve over the lifecycle of the malignant cell and its milieu. Indeed, low grade gliomas show evidence of mtDNA increases, which was also noted by Nenasheva *et al* (24) in other haematologic neoplasms. In contrast, varying results have been reported in hepatocellular, lung, colorectal and gastric cancers, where some, but not all, types showed an amplification of mtDNA (141,151). Of particular interest was the association of the higher levels of mtDNA with less somatic mutations at the D-loop, and that mtDNA reductions occurred (with increases in D-loop mutations) in later cancer stages (141,151). Combined with the data described in gliomas and other tumours, these findings support a model where mtDNA amplification represents an early aspect of the generation and support of the tumourigenic phenotype, potentially ascribable to oxidative stress and altered nuclear-mitochondrial communication (as in petite mutants in yeast), followed by additional manifestations of malignancy, where glycolytic pathways predominate and nuclear mutations support tumourigenicity. While this would not obviate the maintenance of high mtDNA levels, the external and genomic milieu would determine whether the cell is required to have amplification (particularly, for example, in the context of certain types of drug resistance) (21,86,126,138). It has recently been noted that promiscuous mtDNA anomalies are of particular relevance at regulatory sequences in gene-rich locations of the genome, within both introns and exons, including, for example, transcriptional modulators and expressed genes responsible for diverse functions such as thiamine transporters, serine proteases and the T-cell receptor ß-chain (58, Liang and Novak, unpublished data). Hence, alterations in mtDNA copy number and expression, as well as promiscuous mtDNA, is potentially an early event in the tumourigenic phenotype, being noted not only in gliomas but other malignancies as well. Moreover, epigenetic findings with mtDNA depletion have been observed, with aberrant methylation at promoter regions of genes putatively important in cancer (e.g. endothelin B receptor, 0^6-methylguanine-DNA methyltransferase, and E-cadherin) (152). Hence, this area of cancer biology, as well as the mechanisms upstream and downstream of mtDNA amplification, continue to be evaluated to improve our understanding of the mechanisms involved as well as the ramifications of this process in human cancer cells.

Evidence from mouse epidermal growth factor-transformed cells suggests that the initial influence on mtDNA copy number may be attributable to the regulation of nuclear-encoded proteases (e.g. the mitochondrially-located LON protease); similarly, heregulin, which activates the *erb-b2* pathway, can also increase expression of mtDNA genes, such as COXII (153). On the phenotypic level, in colorectal cancer, a lower mtDNA content was associated with a later clinical stage, which in turn was associated with a reduced five year disease-free survival (154). In radiation-induced preleukemic lesions, increases in cytochrome c oxidase activity were noted; with maturation of thymic lymphomas, there was a reduction of COXI/II/III gene expression, data suggesting that the mitochondrial changes occur early in this process (155). Furthermore, in cells which harbour HMGA1 overexpression (associated with advanced malignancy and increased metastatic potential), mtDNA levels (as well as mitochondrial mass) were found to be reduced, although, interestingly, and consistently with the findings cited, the susceptibility to apoptosis was increased (particularly to inhibitors of glycolysis) (156). Indeed, Wu *et al* (157) showed that in gastric cancers mtDNA was depleted in the most infiltrating class of tumours. Collectively, these data suggest that mtDNA alterations, in particular increases in copy number and/or expression, are early in the manifestation of the tumourigenic phenotype, both with respect to ontogeny and stage of disease. With an infiltrating or migratory phenotype, levels of mtDNA appear to become normalised in some circumstances, suggesting a distinct difference in genotype/phenotype. This has been observed in a variety of other studies evaluating the biology of the migratory vs. growth neoplastic phenotypes (158,159). It could thus, be postulated that reversion back to a growth phenotype would effect the specific alterations noted previously, including changes in mtDNA levels and resultant phenotype.

As a future approach for therapy, there have been a significant number of studies now evaluating the targeting of the tumourigenic phenotype via the mitochondria both as therapy (141,151,160-162) as well as in a preventative approach (163-165). Petit *et al* (166), as well as Liminga *et al* (167), postulated that the toxicity of certain anti-mitochondrial compounds might be utilised to treat and/or sensitise cancer cells to the effects of other agents. Joshi *et al* (168) also targeted mitochondria using a novel approach with fatty acid agents (most likely to include the apoptosis-inducing agent ceramide) to effect cell death, which was interestingly dependent on an intact respiratory chain; Fantin *et al* (169) showed, using an approach previously described with AG17, that compound F16 with a delocalised positive charge would accumulate in the anionic mitochondria, effecting cell death in an upstream-independent manner. Indeed, with the data noted with ddC, there is some level of proof-of-concept that this approach could have significant viability in the future, and there are ongoing efforts to use this postulated approach to modify the tumourigenic phenotype in patients with cancer (170,171). Certainly, targeting mitochondria at both the early stages subsequent to the development of immortality, as well as at the time of the cell growth phenotype, would appear to represent the most prudent opportunities for therapy. In addition, if cells which harbour a migratory phenotype have a hyperpolarized $\Delta\psi m$ (169), appropriate use of anti-mitochondrial agents such as lipophillic cations (e.g., AG17 or F16) could have significant utility when administered alone, or (more likely) in combination treatment regimens.

In summary, the mitochondria play a significant role in the tumourigenic phenotype, in addition to the mediation of

the intrinsic pathway of apoptosis, and represent an evolving approach for the therapy of cancer. Gliomas have been shown to represent both this malignant set of behaviours as well as to respond to interventions targeting mitochondria, both *in vitro* and *in vivo*. The ongoing efforts to understand and exploit these biologic findings will hopefully result in the improvement of outcomes for patients who harbour neoplastic disease.

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References

1. Neyns B, D'Haeseleer M, Rogiers A, *et al*: The role of cytotoxic drugs in the treatment of central nervous system gliomas. Acta Neurol Belg 110: 1-14, 2010.
2. Ruiz J and Lesser GJ: Low-grade gliomas. Curr Treat Options Oncol 10: 231-242, 2009.
3. Cavalli LR and Liang BC: Mutagenesis, tumorigenicity, and apoptosis: are the mitochondria involved? Mutat Res 398: 19-26, 1998.
4. Liang BC, Meltzer PS, Guan XY and Trent JM: Gene amplification elucidated by combined chromosomal microdissection and comparative genomic hybridization. Cancer Genet Cytogenet 80: 55-59, 1995.
5. Liang BC: Direct isolation of variably amplified cDNAs from human tumour cell lines. Int J Oncol 7: 611-615, 1995.
6. Firkin FC and Clark-Walker GD: Abnormal mitochondrial DNA in acute leukaemia and lymphoma. Br J Haematol 43: 201-206, 1979.
7. Gianni AM, Dalla-Favera R and Polli E: Restriction enzyme analysis of human leukemic mitochondrial DNA. Leuk Res 4: 155-160, 1980.
8. Liang BC and Hays L: Mitochondrial DNA copy number changes in human gliomas. Cancer Lett 105: 167-173, 1996.
9. Dmitrenko V, Shostak K, Boyko O, *et al*: Reduction of the transcription level of the mitochondrial genome in human glioblastoma. Cancer Lett 218: 99-107, 2005.
10. Kirches E, Krause G, Warich-Kirches M, *et al*: High frequency of mitochondrial DNA mutations in glioblastoma multiforme identified by direct sequence comparison to blood samples. Int J Cancer 93: 534-538, 2001.
11. Vega A, Salas A, Gamborino E, Sobrido MJ, Macaulay V and Carracedo A: mtDNA mutations in tumors of the central nervous system reflect the neutral evolution of mtDNA in populations. Oncogene 23: 1314-1320, 2004.
12. Montanini L, Regna-Gladin C, Eoli M, *et al*: Instability of mitochondrial DNA and MRI and clinical correlations in malignant gliomas. J Neurooncol 74: 87-89, 2005.
13. Clayton DA and Vinograd J: Circular dimer and catenate forms of mitochondrial DNA in human leukaemic leucocytes. Nature 216: 652-657, 1967.
14. Clayton DA and Smith CA: Complex mitochondrial DNA. Int Rev Exp Pathol 14: 1-67, 1975.
15. Ragnini A and Fukuhara H: Genetic instability of an oligomycin resistance mutation in yeast is associated with an amplification of a mitochondrial DNA segment. Nucleic Acids Res 17: 6927-6937, 1989.
16. Traven A, Wong JM, Xu D, Sopta M and Ingles CJ: Interorganellar communication. Altered nuclear gene expression profiles in a yeast mitochondrial DNA mutant. J Biol Chem 276: 4020-4027, 2001.
17. Penta JS, Johnson FM, Wachsman JT and Copeland WC: Mitochondrial DNA in human malignancy. Mutat Res 488: 119-133, 2001.
18. Carew JS and Huang P: Mitochondrial defects in cancer. Mol Cancer 1: 9, 2002.
19. Beheshti B, Braude I, Marrano P, Thorner P, Zielenska M and Squire JA: Chromosomal localization of DNA amplifications in neuroblastoma tumors using cDNA microarray comparative genomic hybridization. Neoplasia 5: 53-62, 2003.
20. Haugen DR, Fluge O, Reigstad LJ, Varhaug JE and Lillehaug JR: Increased expression of genes encoding mitochondrial proteins in papillary thyroid carcinomas. Thyroid 13: 613-620, 2003.
21. Jia L, Liu KZ, Newland AC, Mantsch HH and Kelsey SM: Pgp-positive leukaemic cells have increased mtDNA but no increased rate of proliferation. Br J Haematol 107: 861-869, 1999.
22. Tarantul VZ, Nikolaev AI, Martynenko A, Hannig H, Hunsmann G and Bodemer W: Differential gene expression in B-cell non-Hodgkin's lymphoma of SIV-infected monkey. AIDS Res Hum Retroviruses 16: 173-179, 2000.
23. Nikolaev AI, Martynenko AV, Kalmyrzaev BB, *et al*: [Amplification of transcription of separate mitochondrial genes in human and monkey B-cell non-Hodgkin's lymphoma]. Mol Biol (Mosk) 35: 120-127, 2001 (In Russian).
24. Nenasheva VV, Nikolaev AI, Dubovaia VI, *et al*: [Analysis of expression of a series of lymphoma-specific genes in human fibroblasts immortalized by SV40 virus]. Mol Biol (Mosk) 38: 265-275, 2004 (In Russian).
25. Dani MA, Dani SU, Lima SP, *et al*: Less DeltamtDNA4977 than normal in various types of tumors suggests that cancer cells are essentially free of this mutation. Genet Mol Res 3: 395-409, 2004.
26. Kiebish MA and Seyfried TN: Absence of pathogenic mitochondrial DNA mutations in mouse brain tumors. BMC Cancer 5: 102, 2005.
27. Kamalidehghan B, Houshmand M, Panahi MS, Abbaszadegan MR, Ismail P and Shiroudi MB: Tumoral cell mtDNA approximately 8.9 kb deletion is more common than other deletions in gastric cancer. Arch Med Res 37: 848-853, 2006.
28. Poetsch M, Dittberner T, Petersmann A and Woenckhaus C: Mitochondrial DNA instability in malignant melanoma of the skin is mostly restricted to nodular and metastatic stages. Melanoma Res 14: 501-508, 2004.
29. Chung KL, Shim S, Kim J and Song E: Analysis of mitochondrial DNA mutation in hepatoma. J Biochem 33: 417-421, 2000.
30. Polyak K, Li Y, Zhu H, *et al*: Somatic mutations of the mitochondrial genome in human colorectal tumours. Nat Genet 20: 291-293, 1998.
31. Dai JG, Xiao YB, Min JX, Zhang GQ, Yao K and Zhou RJ: Mitochondrial DNA 4977 BP deletion mutations in lung carcinoma. Indian J Cancer 43: 20-25, 2006.
32. Zhu W, Qin W, Bradley P, Wessel A, Puckett CL and Sauter ER: Mitochondrial DNA mutations in breast cancer tissue and in matched nipple aspirate fluid. Carcinogenesis 26: 145-152, 2005.
33. Guo XG and Guo QN: Mutations in the mitochondrial DNA D-loop region occur frequently in human osteosarcoma. Cancer Lett 239: 151-155, 2006.
34. Pejovic T, Ladner D, Intengan M, *et al*: Somatic D-loop mitochondrial DNA mutations are frequent in uterine serous carcinoma. Eur J Cancer 40: 2519-2524, 2004.
35. Maximo V, Soares P, Seruca R, Rocha AS, Castro P and Sobrinho-Simoes M: Microsatellite instability, mitochondrial DNA large deletions, and mitochondrial DNA mutations in gastric carcinoma. Genes Chromosomes Cancer 32: 136-143, 2001.
36. Liu VW, Shi HH, Cheung AN, *et al*: High incidence of somatic mitochondrial DNA mutations in human ovarian carcinomas. Cancer Res 61: 5998-6001, 2001.
37. Wang Y, Liu VW, Xue WC, Tsang PC, Cheung AN and Ngan HY: The increase of mitochondrial DNA content in endometrial adenocarcinoma cells: a quantitative study using laser-captured microdissected tissues. Gynecol Oncol 98: 104-110, 2005.
38. Tan DJ, Bai RK and Wong LJ: Comprehensive scanning of somatic mitochondrial DNA mutations in breast cancer. Cancer Res 62: 972-976, 2002.
39. Lievre A, Blons H, Houllier AM, *et al*: Clinicopathological significance of mitochondrial D-loop mutations in head and neck carcinoma. Br J Cancer 94: 692-697, 2006.
40. Duncan EL, Perrem K and Reddel RR: Identification of a novel human mitochondrial D-loop RNA species which exhibits upregulated expression following cellular immortalization. Biochem Biophys Res Commun 276: 439-446, 2000.
41. Zhou S, Kachhap S and Singh KK: Mitochondrial impairment in p53-deficient human cancer cells. Mutagenesis 18: 287-292, 2003.
42. Gochhait S, Bhatt A, Sharma S, Singh YP, Gupta P and Bamezai RN: Concomitant presence of mutations in mitochondrial genome and p53 in cancer development - a study in north Indian sporadic breast and esophageal cancer patients. Int J Cancer 123: 2580-2586, 2008.

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ciak JP, Wang W, Markowitz ME, *et al*: Mitochondrial is a cancer biomarker. J Mol Diagn 7: 258-267, 2005.
44. Ivanova R, Lepage V, Loste MN, *et al*: Mitochondrial DNA sequence variation in human leukemic cells. Int J Cancer 76: 495-498, 1998.
45. Campbell CL and Thorsness PE: Escape of mitochondrial DNA to the nucleus in yme1 yeast is mediated by vacuolar-dependent turnover of abnormal mitochondrial compartments. J Cell Sci 111: 2455-2464, 1998.
46. Thorsness PE and Weber ER: Escape and migration of nucleic acids between chloroplasts, mitochondria, and the nucleus. Int Rev Cytol 165: 207-234, 1996.
47. Shay JW and Werbin H: New evidence for the insertion of mitochondrial DNA into the human genome: significance for cancer and aging. Mutat Res 275: 227-235, 1992.
48. Corral M, Paris B, Baffet G, *et al*: Increased level of the mitochondrial ND5 transcript in chemically induced rat hepatomas. Exp Cell Res 184: 158-166, 1989.
49. Netter P and Robineau S: The differential overamplification of short sequences in the mitochondrial DNA of ϱ^- petites in *Saccharomyces cerevisiae* stimulates recombination. Gene 83: 25-38, 1989.
50. Liang BC: Evidence for association of mitochondrial DNA sequence amplification and nuclear localization in human low-grade gliomas. Mutat Res 354: 27-33, 1996.
51. Guescini M, Genedani S, Stocchi V and Agnati LF: Astrocytes and glioblastoma cells release exosomes carrying mtDNA. J Neural Transm 117: 1-4, 2010.
52. Czarnecka AM, Felice VD, Zummo G and Cappello F: Cancer as a 'Mitochondriopathy'. J Cancer Mol 3: 71-79, 2007.
53. Bianchi NO, Bianchi MS and Richard SM: Mitochondrial genome instability in human cancers. Mutat Res 488: 9-23, 2001.
54. Yamauchi A: Rate of gene transfer from mitochondria to nucleus: effects of cytoplasmic inheritance system and intensity of intracellular competition. Genetics 171: 1387-1396, 2005.
55. Sorenson MD and Fleischer RC: Multiple independent transpositions of mitochondrial DNA control region sequences to the nucleus. Proc Natl Acad Sci USA 93: 15239-15243, 1996.
56. Hirano T, Shiraishi K, Adachi K, Miura S, Watanabe H and Utiyama H: Co-localization of mitochondrial and double minute DNA in the nuclei of HL-60 cells but not normal cells. Mutat Res 425: 195-204, 1999.
57. Hadler HI, Devadas K and Mahalingam R: Selected nuclear LINE elements with mitochondrial-DNA-like inserts are more plentiful and mobile in tumor than in normal tissue of mouse and rat. J Cell Biochem 68: 100-109, 1998.
58. Ricchetti M, Tekaia F and Dujon B: Continued colonization of the human genome by mitochondrial DNA. PLoS Biol 2: E273, 2004.
59. Eppert K, Scherer SW, Ozcelik H, *et al*: MADR2 maps to 18q21 and encodes a TGFbeta-regulated MAD-related protein that is functionally mutated in colorectal carcinoma. Cell 86: 543-552, 1996.
60. Borensztajn K, Chafa O, Alhenc-Gelas M, *et al*: Characterization of two novel splice site mutations in human factor VII gene causing severe plasma factor VII deficiency and bleeding diathesis. Br J Haematol 117: 168-171, 2002.
61. Turner C, Killoran C, Thomas NS, *et al*: Human genetic disease caused by de novo mitochondrial-nuclear DNA transfer. Hum Genet 112: 303-309, 2003.
62. Copeland WC, Wachsman JT, Johnson FM and Penta JS: Mitochondrial DNA alterations in cancer. Cancer Invest 20: 557-569, 2002.
63. Modica-Napolitano JS and Singh KK: Mitochondria as targets for detection and treatment of cancer. Expert Rev Mol Med 4: 1-19, 2002.
64. Saffroy R, Chiappini F, Debuire B and Lemoine A: Emerging concepts in the analysis of mitochondrial genome instability. Curr Genomics 5: 309-325, 2004.
65. Chatterjee A, Mambo E and Sidransky D: Mitochondrial DNA mutations in human cancer. Oncogene 25: 4663-4674, 2006.
66. Paul MK and Mukhopadhyay AK: Cancer-the mitochondrial connection. Biologia 62: 371-380, 2007.
67. Ray AJ, Turner R, Nikaido O, Rees JL and Birch-Machin MA: The spectrum of mitochondrial DNA deletions is a ubiquitous marker of ultraviolet radiation exposure in human skin. J Invest Dermatol 115: 674-679, 2000.
68. Cai Q, Shu XO, Wen W, *et al*: Genetic polymorphism in the manganese superoxide dismutase gene, antioxidant intake, and breast cancer risk: results from the Shanghai Breast Cancer Study. Breast Cancer Res 6: R647-R655, 2004.
69. Darvishi K, Sharma S, Bhat AK, Rai E and Bamezai RN: Mitochondrial DNA G10398A polymorphism imparts maternal Haplogroup N a risk for breast and esophageal cancer. Cancer Lett 249: 249-255, 2007.
70. Canter JA, Kallianpur AR, Parl FF and Millikan RC: Mitochondrial DNA G10398A polymorphism and invasive breast cancer in African-American women. Cancer Res 65: 8028-8033, 2005.
71. Jiang WW, Masayesva B, Zahurak M, *et al*: Increased mitochondrial DNA content in saliva associated with head and neck cancer. Clin Cancer Res 11: 2486-2491, 2005.
72. Jiang WW, Rosenbaum E, Mambo E, *et al*: Decreased mitochondrial DNA content in posttreatment salivary rinses from head and neck cancer patients. Clin Cancer Res 12: 1564-1569, 2006.
73. Cavalli LR, Varella-Garcia M and Liang BC: Diminished tumorigenic phenotype after depletion of mitochondrial DNA. Cell Growth Differ 8: 1189-1198, 1997.
74. Nass MM: Differential effects of ethidium bromide on mitochondrial and nuclear DNA synthesis in vivo in cultured mammalian cells. Exp Cell Res 72: 211-222, 1972.
75. Nikiforov MA, Hagen K, Ossovskaya VS, *et al*: p53 modulation of anchorage independent growth and experimental metastasis. Oncogene 13: 1709-1719, 1996.
76. Yan H, Parsons DW, Jin G, *et al*: IDH1 and IDH2 mutations in gliomas. N Engl J Med 360: 765-773, 2009.
77. Hartmann C, Meyer J, Balss J, *et al*: Type and frequency of IDH1 and IDH2 mutations are related to astrocytic and oligodendroglial differentiation and age: a study of 1,010 diffuse gliomas. Acta Neuropathol 118: 469-474, 2009.
78. Hao HX, Khalimonchuk O, Schraders M, *et al*: SDH5, a gene required for flavination of succinate dehydrogenase, is mutated in paraganglioma. Science 325: 1139-1142, 2009.
79. Burnichon N, Briere JJ, Libe R, *et al*: SDHA is a tumor suppressor gene causing paraganglioma. Hum Mol Genet 19: 3011-3020, 2010.
80. Hayashi J, Tagashira Y, Watanabe T and Yoshida MC: Effect of mitochondrial DNA transmitted cytoplasmically from non-tumorigenic to tumorigenic rat cells on the phenotypic expression of tumorigenicity. Cancer Res 44: 3957-3960, 1984.
81. Griguer CE, Oliva CR, Gobin E, *et al*: CD133 is a marker of bioenergetic stress in human glioma. PLoS One 3: e3655, 2008.
82. Hayashi J, Werbin H and Shay JW: Effects of normal human fibroblast mitochondrial DNA on segregation of HeLaTG mitochondrial DNA and on tumorigenicity of HeLaTG cells. Cancer Res 46: 4001-4006, 1986.
83. Hayashi J, Takemitsu M and Nonaka I: Recovery of the missing tumorigenicity in mitochondrial DNA-less HeLa cells by introduction of mitochondrial DNA from normal human cells. Somat Cell Mol Genet 18: 123-129, 1992.
84. Zinkewich-Peotti K, Parent M and Morais R: On the tumorigenicity of mitochondrial DNA-depleted avian cells. Cancer Lett 59: 119-124, 1991.
85. Xue L, Fletcher GC and Tolkovsky AM: Mitochondria are selectively eliminated from eukaryotic cells after blockade of caspases during apoptosis. Curr Biol 11: 361-365, 2001.
86. Dong X, Ghoshal K, Majumder S, Yadav SP and Jacob ST: Mitochondrial transcription factor A and its downstream targets are up-regulated in a rat hepatoma. J Biol Chem 277: 43309-43318, 2002.
87. Grandemange S, Seyer P, Carazo A, *et al*: Stimulation of mitochondrial activity by p43 overexpression induces human dermal fibroblast transformation. Cancer Res 65: 4282-4291, 2005.
88. Ambrosone CB, Freudenheim JL, Thompson PA, *et al*: Manganese superoxide dismutase (MnSOD) genetic polymorphisms, dietary antioxidants, and risk of breast cancer. Cancer Res 59: 602-606, 1999.
89. Roy D, Felty Q, Narayan S and Jayakar P: Signature of mitochondria of steroidal hormones-dependent normal and cancer cells: potential molecular targets for cancer therapy. Front Biosci 12: 154-173, 2007.
90. Palomares T, Bilbao P, del Olmo M, Castro B, Calle Y and Alonso-Varona A: In vitro and in vivo comparison between the effects of treatment with adenosine triphosphate and treatment with buthionine sulfoximine on chemosensitization and tumour growth of B16 melanoma. Melanoma Res 9: 233-242, 1999.
91. Ohnami S, Matsumoto N, Nakano M, *et al*: Identification of genes showing differential expression in antisense K-ras-transduced pancreatic cancer cells with suppressed tumorigenicity. Cancer Res 59: 5565-5571, 1999.

92. Weinberg F, Hamanaka R, Wheaton WW, *et al*: Mitochondrial metabolism and ROS generation are essential for K-ras-mediated tumorigenicity. Proc Natl Acad Sci USA 107: 8788-8793, 2010.
93. Mills KI, Woodgate LJ, Gilkes AF, *et al*: Inhibition of mitochondrial function in HL60 cells is associated with an increased apoptosis and expression of CD14. Biochem Biophys Res Commun 263: 294-300, 1999.
94. Magda D, Lecane P, Prescott J, *et al*: mtDNA depletion confers specific gene expression profiles in human cells grown in culture and in xenograft. BMC Genomics 9: 521, 2008.
95. Felty Q and Roy D: Estrogen, mitochondria, and growth of cancer and non-cancer cells. J Carcinog 4: 1, 2005.
96. Akimoto M, Niikura M, Ichikawa M, *et al*: Nuclear DNA but not mtDNA controls tumor phenotypes in mouse cells. Biochem Biophys Res Commun 327: 1028-1035, 2005.
97. Li M, Wang J, Ng SS, *et al*: The four-and-a-half-LIM protein 2 (FHL2) is overexpressed in gliomas and associated with oncogenic activities. Glia 56: 1328-1338, 2008.
98. Ismail A and Bateman A: Expression of TBX2 promotes anchorage-independent growth and survival in the p53-negative SW13 adrenocortical carcinoma. Cancer Lett 278: 230-240, 2009.
99. Yu M, Shi Y, Wei X, *et al*: Depletion of mitochondrial DNA by ethidium bromide treatment inhibits the proliferation and tumorigenesis of T47D human breast cancer cells. Toxicol Lett 170: 83-93, 2007.
100. Liang BC: Increased expression but not sensitivity to Fas/CD95 in glioblastoma cells depleted of mitochondrial DNA. Exp Ther Med 1: 1049-1055, 2010.
101. Biswas G, Anandatheerthavarada HK and Avadhani NG: Mechanism of mitochondrial stress-induced resistance to apoptosis in mitochondrial DNA-depleted C2C12 myocytes. Cell Death Differ 12: 266-278, 2005.
102. Amuthan G, Biswas G, Zhang SY, Klein-Szanto A, Vijayasarathy C and Avadhani NG: Mitochondria-to-nucleus stress signaling induces phenotypic changes, tumor progression and cell invasion. EMBO J 20: 1910-1920, 2001.
103. Butow RA and Avadhani NG: Mitochondrial signaling: the retrograde response. Mol Cell 14: 1-15, 2004.
104. Del Giudice L, Massardo DR, Pontieri P and Wolf K: Interaction between yeast mitochondrial and nuclear genomes: null alleles of RTG genes affect resistance to the alkaloid lycorine in ρ0 petites of *Saccharomyces cerevisiae*. Gene 354: 9-14, 2005.
105. Liang BC, Miller L and Weller A: Ethyl-nitrosourea transformed astrocytes exhibit mitochondrial membrane hyperpolarization and constrained apoptosis. Apoptosis 4: 89-97, 1999.
106. Hiraga S, Arita N, Ohnishi T, *et al*: Transformation of type 1 astrocytes with N-ethyl-N-nitrosourea: establishment of an in vitro system and the role of the p53 gene. Glia 13: 51-63, 1995.
107. Fantin VR, St-Pierre J and Leder P: Attenuation of LDH-A expression uncovers a link between glycolysis, mitochondrial physiology, and tumor maintenance. Cancer Cell 9: 425-434, 2006.
108. Halabe Bucay A: The biological significance of cancer: mitochondria as a cause of cancer and the inhibition of glycolysis with citrate as a cancer treatment. Med Hypotheses 69: 826-828, 2007.
109. Matarrese P, Testa U, Cauda R, Vella S, Gambardella L and Malorni W: Expression of P-170 glycoprotein sensitizes lymphoblastoid CEM cells to mitochondria-mediated apoptosis. Biochem J 355: 587-595, 2001.
110. Liang BC and Ullyatt E: Increased sensitivity to cis-diammine-dichloroplatinum induced apoptosis with mitochondrial DNA depletion. Cell Death Differ 5: 694-701, 1998.
111. Bouayadi K, Hoffmann JS, Fons P, Tiraby M, Reynes JP and Cazaux C: Overexpression of DNA polymerase beta sensitizes mammalian cells to 2',3'-deoxycytidine and 3'-azido-3'-deoxythymidine. Cancer Res 57: 110-116, 1997.
112. Ullyatt E and Liang BC: 2',3'-Dideoxycytidine is a potent inducer of apoptosis in glioblastoma cells. Anticancer Res 18: 1859-1863, 1998.
113. Burger AM, Kaur G, Alley MC, *et al*: Tyrphostin AG17, [(3,5-Di-tert-butyl-4-hydroxybenzylidene)- malononitrile], inhibits cell growth by disrupting mitochondria. Cancer Res 55: 2794-2799, 1995.
114. Ara G and Teicher B: Relationship of cellular energy parameters to cytotoxicity for AG-17, lonidamine and cyclocreatine in four human tumour cell lines. Int J Oncol 8: 865-873, 1996.
115. Liang BC and Ullyatt E: Chemosensitization of glioblastoma cells to bis-dichloroethyl-nitrosourea with tyrphostin AG17. Clin Cancer Res 4: 773-781, 1998.
116. Lena A, Rechichi M, Salvetti A, Bartoli B *et al*: Drugs targeting the mitochondrial pore act as cytotoxic and cytostatic agents in temozolomide-resistant glioma cells. J Transl Med 7: 13, 2009.
117. Griguer CE, Oliva CR, Gillespie GY, Gobin E, Marcorelles P and Yancey Gillespie G: Pharmacologic manipulations of mitochondrial membrane potential (DeltaPsim) selectively in glioma cells. J Neurooncol 81: 9-20, 2007.
118. Higgins SC and Pilkington GJ: The in vitro effects of tricyclic drugs and dexamethasone on cellular respiration of malignant glioma. Anticancer Res 30: 391-397, 2010.
119. Perez-Ortiz JM, Tranque P, Burgos M, Vaquero CF and Llopis J: Glitazones induce astroglioma cell death by releasing reactive oxygen species from mitochondria: modulation of cytotoxicity by nitric oxide. Mol Pharmacol 72: 407-417, 2007.
120. Erguven M, Yazihan N, Aktas E, *et al*: Carvedilol in glioma treatment alone and with imatinib *in vitro*. Int J Oncol 36: 857-866, 2010.
121. Shima Y, Nishimoto N, Ogata A, Fujii Y, Yoshizaki K and Kishimoto T: Myeloma cells express Fas antigen/APO-1 (CD95) but only some are sensitive to anti-Fas antibody resulting in apoptosis. Blood 85: 757-764, 1995.
122. Westendorf JJ, Lammert JM and Jelinek DF: Expression and function of Fas (APO-1/CD95) in patient myeloma cells and myeloma cell lines. Blood 85: 3566-3576, 1995.
123. Lund KC, Peterson LL and Wallace KB: Absence of a universal mechanism of mitochondrial toxicity by nucleoside analogs. Antimicrob Agents Chemother 51: 2531-2539, 2007.
124. Louat T, Servant L, Rols MP, *et al*: Antitumor activity of 2',3'-dideoxycytidine nucleotide analog against tumors up-regulating DNA polymerase beta. Mol Pharmacol 60: 553-558, 2001.
125. Hentosh P and Tibudan M: 2-Chloro-2'-deoxyadenosine, an antileukemic drug, has an early effect on cellular mitochondrial function. Mol Pharmacol 51: 613-619, 1997.
126. Bjerke M, Franco M, Johansson M, Balzarini J and Karlsson A: Increased mitochondrial DNA copy-number in CEM cells resistant to delayed toxicity of 2',3'-dideoxycytidine. Biochem Pharmacol 75: 1313-1321, 2008.
127. Singh KK, Ayyasamy V, Owens KM, Koul MS and Vujcic M: Mutations in mitochondrial DNA polymerase-gamma promote breast tumorigenesis. J Hum Genet 54: 516-524, 2009.
128. Coucke PA, Li YX, Copaceanu ML, *et al*: Cell line specific radiosensitizing effect of zalcitabine (2',3'-dideoxycytidine). Acta Oncol 36: 199-205, 1997.
129. Humer J, Ferko B, Waltenberger A, Rapberger R, Pehamberger H and Muster T: Azidothymidine inhibits melanoma cell growth in vitro and in vivo. Melanoma Res 18: 314-321, 2008.
130. De Napoli L, Lacovino R, Messere A, *et al*: Synthesis of platinum(II) complexes of thymidine and 1-methylthymine (1-MeThy); crystal structure of cis-[PtCl(1-MeThy)(PPh3)2]. J Chem Soc Dalton Trans 1999: 1945-1950, 1999.
131. Besson C, Panelatti G, Delaunay C, *et al*: Treatment of adult T-cell leukemia-lymphoma by CHOP followed by therapy with antinucleosides, alpha interferon and oral etoposide. Leuk Lymphoma 43: 2275-2279, 2002.
132. Levine AM, Tulpule A, Espina B, *et al*: Low dose methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, and dexamethasone with zalcitabine in patients with acquired immunodeficiency syndrome-related lymphoma. Effect on human immunodeficiency virus and serum interleukin-6 levels over time. Cancer 78: 517-526, 1996.
133. Modica-Napolitano JS, Koya K, Weisberg E, Brunelli BT, Li Y and Chen LB: Selective damage to carcinoma mitochondria by the rhodacyanine MKT-077. Cancer Res 56: 544-550, 1996.
134. Nguyen DM and Hussain M: The role of the mitochondria in mediating cytotoxicity of anti-cancer therapies. J Bioenerg Biomembr 39: 13-21, 2007.
135. Yen HC, Tang YC, Chen FY, Chen SW and Majima HJ: Enhancement of cisplatin-induced apoptosis and caspase 3 activation by depletion of mitochondrial DNA in a human osteosarcoma cell line. Ann NY Acad Sci 1042: 516-522, 2005.
136. Cummings BS and Schnellmann RG: Cisplatin-induced renal cell apoptosis: caspase 3-dependent and -independent pathways. J Pharmacol Exp Ther 302: 8-17, 2002.
137. Schwerdt G, Freudinger R, Schuster C, Weber F, Thews O and Gekle M: Cisplatin-induced apoptosis is enhanced by hypoxia and by inhibition of mitochondria in renal collecting duct cells. Toxicol Sci 85: 735-742, 2005.

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no A, Fernandez C, Sancho P, de Blas E and Aller P: t of glutathione depletion on antitumor drug toxicity (apoptosis and necrosis) in U-937 human promonocytic cells. The role of intracellular oxidation. J Biol Chem 276: 47107-47115, 2001.
139. Kharbangar A, Khynriam D and Prasad SB: Effect of cisplatin on mitochondrial protein, glutathione, and succinate dehydrogenase in Dalton lymphoma-bearing mice. Cell Biol Toxicol 16: 363-373, 2000.
140. Yang Z, Schumaker LM, Egorin MJ, Zuhowski EG, Guo Z and Cullen KJ: Cisplatin preferentially binds mitochondrial DNA and voltage-dependent anion channel protein in the mitochondrial membrane of head and neck squamous cell carcinoma: possible role in apoptosis. Clin Cancer Res 12: 5817-5825, 2006.
141. Lee MS, Kim JY and Park SY: Resistance of rho(0) cells against apoptosis. Ann NY Acad Sci 1011: 146-153, 2004.
142. Singh KK, Russell J, Sigala B, Zhang Y, Williams J and Keshav KF: Mitochondrial DNA determines the cellular response to cancer therapeutic agents. Oncogene 18: 6641-6646, 1999.
143. Mizutani S, Miyato Y, Shidara Y, *et al*: Mutations in the mitochondrial genome confer resistance of cancer cells to anticancer drugs. Cancer Sci 100: 1680-1687, 2009.
144. Mizumachi T, Suzuki S, Naito A, *et al*: Increased mitochondrial DNA induces acquired docetaxel resistance in head and neck cancer cells. Oncogene 27: 831-838, 2008.
145. Kessel D and Sun HH: Enhanced responsiveness to photodynamic therapy-induced apoptosis after mitochondrial DNA depletion. Photochem Photobiol 70: 937-940, 1999.
146. Morgan J, Potter WR and Oseroff AR: Comparison of photodynamic targets in a carcinoma cell line and its mitochondrial DNA-deficient derivative. Photochem Photobiol 71: 747-757, 2000.
147. Hirama M, Isonishi S, Yasuda M and Ishikawa H: Characterization of mitochondria in cisplatin-resistant human ovarian carcinoma cells. Oncol Rep 16: 997-1002, 2006.
148. Cullen KJ, Yang Z, Schumaker L and Guo Z: Mitochondria as a critical target of the chemotherapeutic agent cisplatin in head and neck cancer. J Bioenerg Biomembr 39: 43-50, 2007.
149. Wallace KB and Starkov AA: Mitochondrial targets of drug toxicity. Annu Rev Pharmacol Toxicol 40: 353-388, 2000.
150. Levitt ML and Koty PP: Tyrosine kinase inhibitors in preclinical development. Invest New Drugs 17: 213-226, 1999.
151. Lee HC, Yin PH, Lin JC, *et al*: Mitochondrial genome instability and mtDNA depletion in human cancers. Ann NY Acad Sci 1042: 109-122, 2005.
152. Xie CH, Naito A, Mizumachi T, *et al*: Mitochondrial regulation of cancer associated nuclear DNA methylation. Biochem Biophys Res Commun 364: 656-661, 2007.
153. Sun Y, Lin H, Zhu Y, Ma C, Ye J and Luo J: Induction or suppression of expression of cytochrome C oxidase subunit II by heregulin beta 1 in human mammary epithelial cells is dependent on the levels of ErbB2 expression. J Cell Physiol 192: 225-233, 2002.
154. Lin PC, Lin JK, Yang SH, Wang HS, Li AF and Chang SC: Expression of beta-F1-ATPase and mitochondrial transcription factor A and the change in mitochondrial DNA content in colorectal cancer: clinical data analysis and evidence from an in vitro study. Int J Colorectal Dis 23: 1223-1232, 2008.
155. Verlaet M, Duyckaerts C, Rahmouni S, *et al*: Transient modifications of respiratory capacity in thymic cells during murine radioleukemogenesis. Free Radic Biol Med 33: 76-82, 2002.
156. Dement GA, Maloney SC and Reeves R: Nuclear HMGA1 nonhistone chromatin proteins directly influence mitochondrial transcription, maintenance, and function. Exp Cell Res 313: 77-87, 2007.
157. Wu CW, Yin PH, Hung WY, *et al*: Mitochondrial DNA mutations and mitochondrial DNA depletion in gastric cancer. Genes Chromosomes Cancer 44: 19-28, 2005.
158. Hehlgans S, Haase M and Cordes N: Signalling via integrins: implications for cell survival and anticancer strategies. Biochim Biophys Acta 1775: 163-180, 2007.
159. Harradine KA and Akhurst RJ: Mutations of TGFbeta signaling molecules in human disease. Ann Med 38: 403-414, 2006.
160. Marin-Hernandez A, Gracia-Mora I, Ruiz-Ramirez L and Moreno-Sanchez R: Toxic effects of copper-based antineoplastic drugs (Casiopeinas) on mitochondrial functions. Biochem Pharmacol 65: 1979-1989, 2003.
161. Preston T and Singh G: Mitochondrial signaling and cancer. Adv Cell Ageing Gerontol 7: 103-130, 2001.
162. Teo JW, Thayalan P, Beer D, *et al*: Peptide deformylase inhibitors as potent antimycobacterial agents. Antimicrob Agents Chemother 50: 3665-3673, 2006.
163. Sun SY, Hail N Jr and Lotan R: Apoptosis as a novel target for cancer chemoprevention. J Natl Cancer Inst 96: 662-672, 2004.
164. Hail N Jr: Mitochondria: A novel target for the chemoprevention of cancer. Apoptosis 10: 687-705, 2005.
165. Hail N Jr and Lotan R: Cancer chemoprevention and mitochondria: targeting apoptosis in transformed cells via the disruption of mitochondrial bioenergetics/redox state. Mol Nutr Food Res 53: 49-67, 2009.
166. Petit T, Izbicka E, Lawrence RA, Nalin C, Weitman SD and Von Hoff DD: Activity of MKT 077, a rhodacyanine dye, against human tumor colony-forming units. Anticancer Drugs 10: 309-315, 1999.
167. Liminga G, Jonsson B, Nygren P and Larsson R: On the mechanism underlying calcein-induced cytotoxicity. Eur J Pharmacol 383: 321-329, 1999.
168. Joshi B, Li L, Taffe BG, *et al*: Apoptosis induction by a novel anti-prostate cancer compound, BMD188 (a fatty acid-containing hydroxamic acid), requires the mitochondrial respiratory chain. Cancer Res 59: 4343-4355, 1999.
169. Fantin VR, Berardi MJ, Scorrano L, Korsmeyer SJ and Leder P: A novel mitochondriotoxic small molecule that selectively inhibits tumor cell growth. Cancer Cell 2: 29-42, 2002.
170. Fulda S and Kroemer G: Targeting mitochondrial apoptosis by betulinic acid in human cancers. Drug Discov Today 14: 885-890, 2009.
171. Gogvadze V, Orrenius S and Zhivotovsky B: Mitochondria as targets for chemotherapy. Apoptosis 14: 624-640, 2009.