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Review

Cancer – the mitochondrial connection

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Abstract: Mitochondria are the powerhouse of the cell. They play a vital role in the energy metabolism and regulate calcium flux and apoptosis. The recent resurgence of interest in mitochondrial studies is largely attributed to the recognition that mitochondrial dysfunctions lead to various physiopathological disorders, especially in the development and progression of cancer. Mitochondrial DNA is very susceptible to mutations, which lead to respiratory dysfunction and are implicated in many cancers. Mitochondria serve as the molecular target for a structurally diverse group of pharmacological agents in cancer chemotherapy. Biochemical and biophysical characterization have helped to identify several important differences between mitochondria of normal and disease state. Such unique alterations in mitochondrial structure and function could become promising target for the development of new generation drugs.

Key words: mitochondria; cancer; apoptosis; mitochondrial DNA-mutations; chemotherapy; drug targeting.

Abbreviations: ETC, electron transport chain; mtDNA, mitochondrial DNA; MTP, mitochondrial transition pore; ROS, reactive oxygen species.

Introduction

With the dawn of mitochondrial research dating back to the mid 19th century, pioneered by Altmann, Benda, Slauter, Mitchell and many others, mitochondrial research have come a long way. Mitochondria are double-membraned dynamic organelles serving as a major source of energy in most eukaryotic cells and they also regulate several biological phenomena, e.g. the urea cycle, fatty acid oxidation, calcium signaling, heme biosynthesis, lipid and amino acid biosynthesis, and most importantly apoptosis. Mitochondria are special organelles having double-stranded circular genome, which partially codes for the respiratory chain complex. Genetic and/or metabolic alterations of mitochondria have been implicated in the development and progression of many diseases, especially cancer. Mitochondrial DNA (mtDNA) mutations whether inherited or acquired result in impaired energy metabolism. mtDNA is highly susceptible to mutation as it lacks introns and histones. Luft et al. (1962) described the first mitochondrial disease in 1962. Mitochondrial disease research surged forward after a long silence of two and a half decades. In the year 1988 identification of mtDNA mutation (Holt et al. 1988; Wallace et al. 1988) and its correlation with disease phenotype was published breaking open the floodgates for future research. Mitochondria are implicated in several types of cancer and they also act as a central player in apoptosis, and are accepted as "motors of cell death" (Bossy-Wetzel & Green 1999). Hence mitochondria have been recognized as a promising and key pharmacological target for the development of mitochondrial medicine as a new field of biomedical research. Mitochondria-targeted drugs can be classified into two categories: (i) drugs whose primary target is the mitochondria; and (ii) drugs having other location as the primary target and mitochondria as the ultimate target. Hence, the identification and development of therapeutic agents for disease states that are linked to mitochondrial dysfunction have started taking a centre stage as a potent strategy for cancer chemotherapy (Szewczyk & Wojtczak 2002).

Mitochondrial research is presently one of the most promising disciplines in biomedicine. The recent surge of interest in the quest of mitochondrial medicine has been fuelled in large part by the recognition of molecular drug targets in mitochondria. Still our understanding of the role of mitochondria in the pathogenesis of several cancers is so poor that new rationales for therapy are badly missing. The identification of novel targets combined with the development of methods for selective delivery of biologically active molecules to mitochondria in the living cells will open up new vistas for mitochondrial function manipulation, which may

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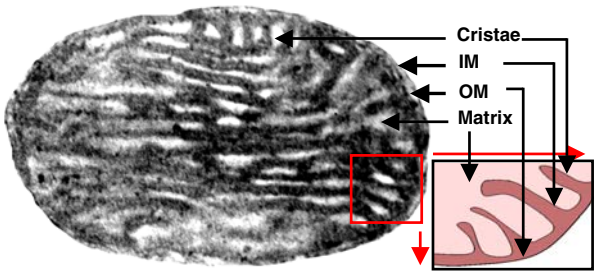

Fig. 1. Transmission electron micrograph showing the ultrastructure of a heart mitochondrion. Heart mitochondria were isolated and fixed with 1% osmium tetraoxide and stained with uranyl acetate and lead citrate and examined with a Philips CM-10, Transmission Electron Microscope operated at 80 kV. Lower inset shows hand drawing of an enlarged part of the mitochondrion. OM, outer membrane; IM, inner membrane.

result in the selective protection, repair and killing of cell.

Mitochondria and the cell

Mitochondria, the powerhouse of the cell, convert food energy into chemical energy (ATP), which is used to drive the cellular reactions essential to keep the cell alive. Mitochondria are specialized semi-autonomously functioning organelles of the size of bacteria (about 1–2 μm) and are almost found in all eukaryotic cells in very large number, specially in metabolically active organs, such as liver, brain, cardiac and skeletal muscle tissues. Each mitochondrion is delimited by two membranes (Fig. 1), an unfolded smooth outer membrane and a folded (folds called cristae) inner membrane thus creating two separate compartments, the internal matrix space and the narrow intermembrane space. While the outer membrane is permeable to molecules smaller than 5 kDa, the inner membrane is highly impermeable and characterized by an unusually high content of membrane proteins and a unique lipid composition that houses the components of the respiratory chain including ATP synthesis and a whole variety of transport proteins. The inner membrane impermeability is a prerequisite for the establishment of a gradient in the distribution of protons across the inner membrane. The gradient is composed of the electrical component ($\Delta\Psi$) and the proton concentration gradient (ΔpH). The electrochemical proton gradient thus formed, also designated as the protonmotive force (Δp) is the driving force for the backflow of protons through the ATP synthase complex. The Δp is responsible for the synthesis of 90% of cellular ATP (Cross 1994; Wallace 1997).

Mitochondrial DNA

Mammalian cells possess two different and interdependent genomes: (i) the nuclear genome; and (ii) the mitochondrial genome. Although the two genomes are physically distinct, there is a high degree of functional interdependence among them. The mitochondrial genome which was discovered by Nass & Nass (1963) contains 16,569 base pairs in the form of a single, closed circular, double helical chromosome and contains approximately 10^3–10^4 copies of mtDNA. mtDNA codes for two rRNAs, 22 tRNAs, seven subunits of respiratory enzyme complex I, one subunit of complex III, three subunits of complex IV and two subunits of complex V (Penta et al. 2001; Tsang & Lemire 2003). Notably each mitochondrion contains 2–10 copies of its genome and is maternally inherited. mtDNA is extremely compact, introns are absent, hence more susceptible to mutations than nuclear genome. mtDNA undergoes rapid replication even in non-dividing cells, leading to the accumulation of mutations (Modica-Napolitano & Singh 2002). mtDNA repair mechanisms are assumed to be poorly developed or inefficient in comparison to nuclear DNA (Pettepher et al. 1991). Moreover, mtDNA is located in the mitochondrial matrix and therefore lies in close proximity to the electron transport chain (ETC) where reactive oxygen species (ROS) are continuously generated hence increasing its propensity to harbor mutational damage (Yakes & Houten 1997). The accumulation of mutations in mtDNA is approximately ten folds greater than nuclear DNA (Pettepher et al. 1991).

Mitochondria and cancer

Mitochondrial dysfunction is closely related to a plethora of cancers. Cancer cells exhibit resistance to cell death stimuli and abnormal energy metabolism. mtDNA mutations serve as important mediators in the pathogenesis of cancer. Cancer cells acquire resistance towards programmed cell death or apoptosis in conjugation with unlimited replicative potential. Mitochondria are the key to cellular apoptosis. Therefore, targeting the mitochondria and mitochondrial proteins might provide an effective means to circumvent the resistance of most cancer cells towards apoptosis. Importantly several phenotypic, genotypic and bioenergetic differences exist among normal and cancer mitochondria, providing an opportunity to selectively target cancer cell mitochondria.

Phenotypic characteristic of cancer cell mitochondria

Cancer cells have an altered metabolism and mitochondria are directly or indirectly involved with this process. The mitochondria of rapidly growing tumor cells tend to be fewer in number, smaller and having smaller number of cristae in comparison to normal and slowly growing tumors. Luciakova & Kuzela (1992) reported on diminished mitochondrial number in hepatic tumor cell. Interestingly, the usually benign oncocytoma of thyroid, salivary gland, kidney, parathyroid and breast are characterized by the presence of abnormally large number of mitochondria and elevated levels of oxidative enzymes. Alterations in the molecular composition of the inner membranes of the tumor mitochondria have also been reported. Analysis of cancer samples revealed significantly different protein profiles as compared to

Table 1. List of mtDNA mutations in different types of cancer.

Solid tumor	Mutation	Reference
Breast	12S and 16S rRNA, subunit ND1, ND4, ND5, cyt-b, COXI, COXII, COXIII	Parrella et al. (2001) Tan et al. (2002)
Ovary	T→C or G→A transitions, D-loop, 12S rRNA, 16S rRNA, cyt-b	Liu et al. (2001)
Thyroid	Complex-I, Complex-IV, ATPase-6 gene	Yeh et al. (2000)
Kidney	Deletion of ND1 (264bp)	Horton et al. (1996)
Liver	ND5, cyt-c oxidase II and 16S rRNA overexpression	Corral et al. (1989)
Lung	T→C or G→A base transitions, cyt-b	Fliss et al. (2000)
Colorectal	12S rRNA, 16S rRNA, ND1, ND4, ND5, cyt-b, cyt-c oxidase I, II and III	Polyak et al. (1998)
Gastric	deletions, C:G/T:A transition	Alanso et al. (1997)
Brain	D loop, erb-b gene amplification	Liang et al. (1996)
Pancreatic	12S rRNA, 16S rRNA, ND1, ND2, ND4, cyt-b, COX-I, II & III, ATPase-6	Jones et al. (2001)
Esophageal	D loop mutation	Hibi et al. (2001)
Prostate	D loop, 16SrRNA and NADH subunits	Jeronimo et al. (2001)
Leukemia	**Mutation**	**Reference**
Acute and chronic leukemia	cyt-c oxidase I, II and ATPase 8 and proportion of circular dimers	Clayton et al. (1967)
Lymphoma	ND5 overexpression	Carew & Huang (2002)

the normal counterpart (Carew & Huang 2002). Proteomic studies have identified alterations in many of the proteins that reside permanently or transiently in mitochondria. For example, the altered ratios of anti-apoptotic proteins (like Bcl-2) and pro-apoptotic proteins (like Bax and Bak) promote cancer cell survival and growth. However, no universal metabolic alteration is common to all tumors. The full potential of mitochondrial proteomics is yet to be explored and further research is needed to find out other membrane and cytosolic mitochondrial proteins associated with cancer initiation and progression (Verma et al. 2003).

Mitochondrial DNA mutations and cancer

The concept of mitochondrial disease or mitochondrial medicine started with the identification of mtDNA deletions in patients with mitochondrial myopathies (Holt et al. 1988; Wallace et al. 1988). Alterations in the multimeric respiratory chain complexes, comprising the oxidative phosphorylation system have been reported in both solid tumors and hematological malignancies (Duchen 2004; Brandon et al. 2006). Understanding of mitochondrial dysfunction may be suggestive of new approaches for diagnosis and treatment.

Solid tumors

Solid tumors exhibit a range of mtDNA mutations. Breast tumors are characterized by mtDNA aberrations in complex IV and genomic instability is also noticed in certain cases (Duchen 2004). Somatic mutations of the mitochondrial genome were reported in human colorectal cancers (Polyak et al. 1998). Homoplasmic somatic mutations were identified in the 12S, 16S rRNA genes, subunit ND1, ND4L and ND5, cytochrome b and in the subunits of cytochrome c oxidase I, II and III. Nucleotide substitutions are mainly observed while single base pair insertions are also reported (Wallace 1997). Somatic mutations of the mitochondrial genome were reported in colorectal cancer. Microsatellite instability, microsatellite DNA alterations in the non-coding D-loop region and frame shift mutations in complex I were reported in mitochondrial genome in colorectal cancer. Certain tumors like colorectal, liver, pancreatic and breast cancers showed elevated levels of certain mitochondrial genes like subunit ND4 and ND4L, cytochrome b, cytochrome c oxidase II, ATPase 6, 8 and 16S rRNA (Table 1).

In gastric carcinoma (adenocarcinoma) mtDNA loop mutations were also identified (Alanso et al. 1997). Transition and insertion both were detected in the hypervariable region. Tamura et. al. (1999) demonstrated mtDNA mutations in both non-malignant and malignant gastric neoplasms. Maximo et. al. (1999, 2001) detected large mtDNA deletions (4,977 bp) in gastric carcinoma. Burgart et al. (1995) detected somatic mutations (50 bp) in the D-loop region of gastric carcinoma. Increase in mtDNA encoded transcripts for ND5, cytochrome c oxidase II and 16S r RNA were also observed in chemically induced rat hepatomas (Corral 1989). Almost every type of solid tumor is associated with mtDNA mutations as summarized in Table 1.

Hematological cancers

The mitochondrial genotype alterations are associated not only with solid tumors; they are also common in hematological cancers (Table 1). Three novel structures of mtDNA, circular dimers, catenated dimers and catenated trimers are assumed to play a key role in the etiology of acute leukemia, chronic leukemia and myelo dysplastic syndrome (MDS) as described by Clayton & Vinograd (1967). They also got similar result in acute and granulocytic leukemias. The percentage of the circular dimers decreased in some leukemia patients following treatment with chemotherapeutic agents, giving some insight about the role of such novel DNA structures in the disease incidence. Granulocytic leukemia patients show a greater percentage of the catenated form than the lymphocytic leukemia. mtDNA mutations were also identified in cytochrome-c oxidase I, II

and ATPase 8 in myelo dysplastic syndrome.

In case of lymphoma, mitochondrial genomic aberrations were identified in murine lymphoma cells (ND5 of complex I is over expressed), leading to metastasis. The degree to which cancer is caused by or is a consequence of mitochondrial genomic alterations is unknown but the substantial data suggest an involvement of mtDNA mutation in the carcinogenesis process. Alteration in expression of mtDNA-encoded polypeptides required for oxidative phosphorylation may be a general characteristic of neoplastic disease. mtDNA alteration can play a pivotal role at multiple stages in the of oncogenesis process (Penta et al. 2001).

Bioenergetics of cancer mitochondria

ATP required for normal cell function comes primarily from glycolysis and tricarboxylic acid cycle. In glycolysis, glucose is converted to pyruvate in the cytoplasm with the production of 2 ATP molecules/glucose molecule. Pyruvate is in turn converted to lactic acid. Normal cells depend mainly on the tricarboxylic acid cycle-mediated process of ATP generation. In tricarboxylic acid cycle, the pyruvate formed from glycolysis donates electrons to the ETC complex of the mitochondria and in this process 38 ATP molecules/glucose molecule are produced. Many cancer cells interestingly exhibit altered glucose metabolism. In most normal cells glycolysis is inhibited in the presence of oxygen. This inhibition is termed as "Pasteur effect". Cancer cells show a bioenergetic versatility by maintaining glycolysis through a range of oxygen concentrations. Conversion of glucose to lactic acid in the presence of oxygen is termed as "Warburg effect" or aerobic glycolysis (Gatenby & Gillies 2004; Kim & Dang 2006). Experimental evidence for the enhanced glycolysis was further affirmed by the wide spread clinical success of positron emission tomography using fluorodeoxyglucose (Czernin & Phelps 2002). The molecular mechanisms leading to constitutive upregulation of aerobic glycolysis in certain cancers are not well defined. The metabolic shift of cancer cells towards aerobic glycolysis may be due to mutational dysfunction in the mitochondrial ETC proteins or it may be due activation of oncogenes that results into increased glucose transportation and glycolytic rate or both remains to be answered.

Mitochondria as cancer drug target

Mitochondrial dysfunction plays an important role in the development and progression of cancer. Recent reports suggest that targeting of mitochondria and mitochondrial proteins have emerged as a novel target for anticancer chemotherapy. Results of various animal studies, clinical trials and *in vitro* studies strongly support the notion that induction of apoptosis is associated with the anticancer activity of these compounds. Moreover, the mode of action of several cancer chemotherapeutic agents revolves around the mitochondria. Several classes of chemotherapeutics target the mitochondria-mediated apoptosis. Thereby, suggesting the importance of understanding the link between mitochondria with apoptosis for efficient cancer therapy (Hail 2005).

Apoptosis and the mitochondria

In the past decade the most significant step in mitochondrial biology was the discovery of its strong involvement in apoptosis and cell survival. Apoptosis is a morphologically distinct form of programmed cell death through which excess, damaged or infected cells are eliminated normally for the maintenance and regulation of tissue system within multicellular organisms. Apoptosis is a highly organized process that involves special characteristic morphological changes, which includes blebbing, chromatin condensation, nuclear fragmentation, loss of adhesion, rounding and cell shrinkage (Zimmermann et al. 2001). Biochemical features associated with apoptosis include internucleosomal cleavage of DNA, leading to an oligonucleosomal "ladder", phosphatidylserine externalizaton and proteolytic cleavage of a number of intracellular substrates (Hengartner 2000).

In general two partly independent pathways may lead to programmed cell death – the extrinsic and the intrinsic pathway. The extrinsic pathway is initiated by the ligation of death receptors at the cell surface. On the other hand, the intrinsic pathway involves the mitochondria (Chang 2000; Zimmermann et al. 2001). One of the early events in apoptosis is the release of cytochrome c from the inner mitochondrial membrane. Cytochrome c accumulates in the cytosol where it functions as a co-factor in the activation of caspases. Apaf-1, (a human protein homologous to *Caenorhabditis elegans* CED-4) is a receptor of cytochrome c. Cytochrome c along with dATP forms an Apaf-1-caspase-9 complex that initiates apoptotic protease cascade (Nijhawan et al. 1997). dATP, cytochrome c and Apaf-1 assemble into a approximately 1.4 MDa complex, dubbed the "apoptosome" (Adrain & Martin 2001; Adams & Cory 2002). The activation of caspase-9 in turn activates caspase-3, which is an executioner protease that ultimately executes apoptosis (Fig. 2) (Qin et al. 1999). A number of other deadly apoptosis initiators have been identified (Smac/DIABLO, AIF, EndoG and Htr A2) which all reside in the mitochondrial intermembrane space (Green & Evan 2002). Permeabilization of the mitochondrial membrane is an important parameter governing apoptosis (Wang 2001). Apoptosis by the death receptors and death ligands include the TNFR family death receptors (TNFR1, Fas, DR3/WSL) and the TNF related apoptosis inducing ligands. When, these death receptors bind to their respective death ligands, passes on a signal, then the apoptotic process gets activated e.g., Fas receptor-signaling pathway. Ligation of death receptors causes the rapid formation of a death inducing signaling complex, through the receptors death domain (Ashkenazi & Dixit 1998). This domain is responsible for coupling the death receptor either to a cascade of caspases, leading to an induction of

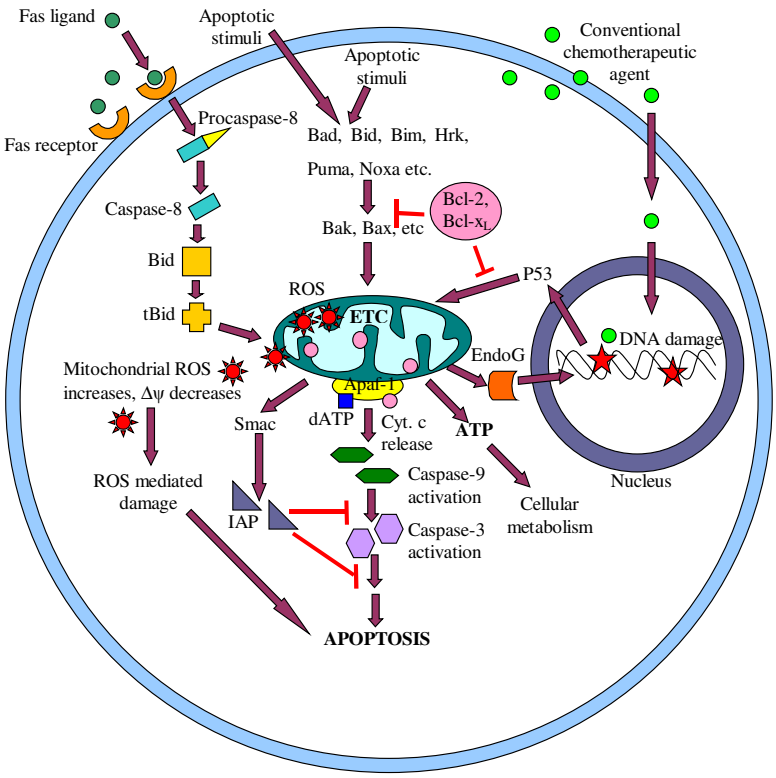

Fig. 2. Multiple apoptotic pathways emanate from the mitochondria.

apoptosis, or to the activation of kinase signaling. The role of respiration in drug sensitivity and induction of apoptosis have been evaluated by several groups by using respiratory deficient ρ° cells lacking mtDNA. Experiments with ρ° cells in certain cases show that tumorigenicity is closely associated with mtDNA (Singh et al. 1999) but contradictory results are also there, proving the existence of a complex cross talk between the nuclear and the mitochondrial genome. Despite its apparent indispensability to several models of apoptosis, the precise mechanism by which cytochrome c is released remains under contention (Bratton & Cohen 2001). Recent models for cytochrome c release can roughly be divided into mitochondrial transition pore (MTP) dependent (Tsujimoto & Shimizu 2000) and MTP independent paradigms (Degterev et al. 2001). The channel, MTP, can be defined as a voltage dependent anionic channel, cyclosporine-A-sensitive and high conductance inner membrane channel. Increase in cytosolic oxidants and inorganic phosphates are all classical triggers of MTP, while pore opening is blocked by cyclosporin-A and bongkrekic acid (Regula et al. 2003). Once triggered, MTP results into mitochondrial swelling, outer membrane ruptures and release of mitochondrial proteins occurs. The propensity towards apoptosis is also governed by the balance between apoptotic and anti-apoptotic members of the Bcl-2 family proteins (Gross et al. 1999). Superoxide is the primary species directly generated from the mitochondrial respiratory chain and leads to induction of MTP opening (Desagher & Martinou 2000). Thus multiple apoptotic pathways emanate from the mitochondrion.

Mitochondrial free radical generation and cancer

The intrinsic balance between life and death can be influenced by several environmental stresses. Reactive products of oxygen are amongst the most potent and omnipresent threats faced by any living organism. ROS includes free radicals such as superoxide anion ($O_2^{\bullet -}$), hydroxyl radicals (OH^{-}) and the non-radical hydrogen peroxide (H_2O_2). Superoxide and hydrogen peroxide are the products of univalent and bivalent reduction of oxygen during normal aerobic metabolism. Though several biological reactions contribute to the production of these ROS, but mitochondria seem to be quantitatively the most important cellular source (Curtin et al. 2002). Though the mitochondria contain a battery of detoxifying enzymes to combat these superoxide produced, the high ROS generation from mitochondria coupled with resistance to toxicity from oxidation is hypothesized to be the key mediators of the transformed phenotype (Cerutti 1985). mtDNA mutations have been quite frequently observed with cancer phenotypes and it has been postulated that these mutations confer a cell growth advantage to the cancer cells by inhibiting the ETC. A high H_2O_2 level promotes procancer responses (Carew & Huang 2002). On the contrary, ROS are beneficiarily used to induce damage to cancer cells and used as a promising therapeutic strategy. Daunorubicin, daunomycin, adriamycin and other group of anthracyclin drugs are being used to inflict damage to the cancer cells (Muindi et al. 1984). Keeping in mind the dillema of ROS functionality, the significance of mitochondria-

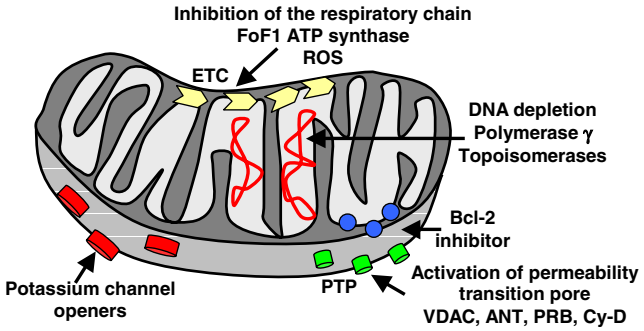

Fig. 3. Schematic illustration of sites of action of mitochondria-targeted drugs.

derived ROS in human cancer progression remains to be appropriately deciphered. On the other hand induction of ROS generation in cancer mitochondria to inflict apoptosis may further be exploited.

Mitochondria – target for established and novel anticancer drug

The development of tumor arises as a consequence of dysregulated proliferation and a suppression of apoptosis, and each of these primary defects provides an obvious opportunity for clinical intervention. Apoptosis is intimately related with mitochondria and this simple fact exemplifies the importance of targeting the mitochondria. Mitochondria-acting drugs may have several preferred sites of action like respiratory chain inhibitors, mtDNA acting drugs, Bcl-2 inhibitors, drugs acting on the MTP pore and potassium channel openers (Fig. 3). mtDNA-acting drugs (Fig. 4) may be DNA intercalating agents like ethidium bromide and dicercalinium (acting by depleting the mtDNA), while there are topoisomerase I and II inhibitors like etoposide and teniposide (inhibiting the cancer cell proliferation). There are drugs/compounds that inhibit the respiratory chain functions like rotenone, myxothiazole, natural stilbenes and flavonoid. Most of the drugs that act on ETC generates ROS, causes redox imbalance and causes cell death. The combination of agents that simultaneously increase ROS production and inhibit ROS elimination might be useful to limit cancer cell proliferation. Increase in the permeability of the mitochondrial membrane to protons or potassium ions by opening the mitochondrial potassium channels leads to the mitochondrial membrane potential ($\Delta\Psi_m$) depolarization and subsequent apoptosis. Cromokalim, NS1619, NS004 and azelaic acid couple membrane potential depolarization, cell proliferation and apoptosis. Bcl-2 inhibitors like genansense (oblimersen sodium, gp3139) antisense oligonucleotide, HA14–1 and NSC365400 have shown promising result. Drugs acting on the opening of the MTP pore like bongkrekic acid, betulinic acid, gossypol, lonidamine and MKT-077 can open the mitochondrial "doors" or channels for membrane potential depolarization and subsequent apoptosis. Many cancers regress after initial chemotherapy – a process called drug resistance or chemoresistance. The chemoresistant cells survive by increasing their apoptotic threshold (Grad et. al. 2001). Mitochondria are central players in drug-induced apoptosis, hence recent efforts to get rid of these chemoresistant cells are by targeting mitochondrial functions. Lonidamine acts on the MTP directly (Ravagan et al. 1999). Use of high doses of etoposide revealed the release of cytochrome c. Etoposide-mediated nuclear changes may also result in the release of nuclear factors that are ultimately responsible for the release of cytochrome c. Etoposide exhibits a similar action like that of lonidamine on the MTP (Robertson et al. 2000). Figure 4 depicts the structure of a few mitochondria-acting drugs. In addition to finding the newer mitochondria-acting drugs, the specific delivery of existing drugs to the mitochondria is equally important and requires attention.

Targeting drugs to mitochondria

Development of methods for selective delivery of drugs to the mitochondria along with the identification and validation of new molecular drug targets in mitochondria will open up new chapters for selective protection, repair and eradication of cells. Two important features are currently targeted to give mitochondria-specific drugs a selective advantage. The high membrane potential generated across the inner membrane may be used to target drugs specifically to the mitochondria. The mitochondrial protein import machinery/mitochondrial metabolite transporter can also be important for drug targeting (Weissig et al. 2004).

Mitochondrial membrane potential and protein import machinery

The synthesis of ATP by oxidative phosphorylation via the respiratory chain leads to the generation of inside negative membrane potential (Liberman et al. 1969). Positively charged dyes with proper physico-chemical properties can enter the mitochondria. According to the Nernst equation an increase of 61.5 mV of membrane potential cause a ten-fold increase in the accumulation of membrane permeant cation.

Mitochondria synthesize a small set of proteins and the rest are imported from the cytosol. The mitochondrial membrane is rich in protein translocases and a variety of chaperons. Cytosolic proteins destined for the mitochondria possess an amino terminal called MLS peptide. MLS peptides exhibit a characteristic feature of displaying a net positive charge and have the ability to form amphiphilic α-helices (Modica-Napollitano & Singh 2002). Therefore the mitochondrial $\Delta\Psi_m$ and protein import machinery may be exploited to selectively deliver molecules to the mitochondria.

Mitochondriotropics

Mitochondriotropics are chemical entities that specifically target and localize in the mitochondria mostly

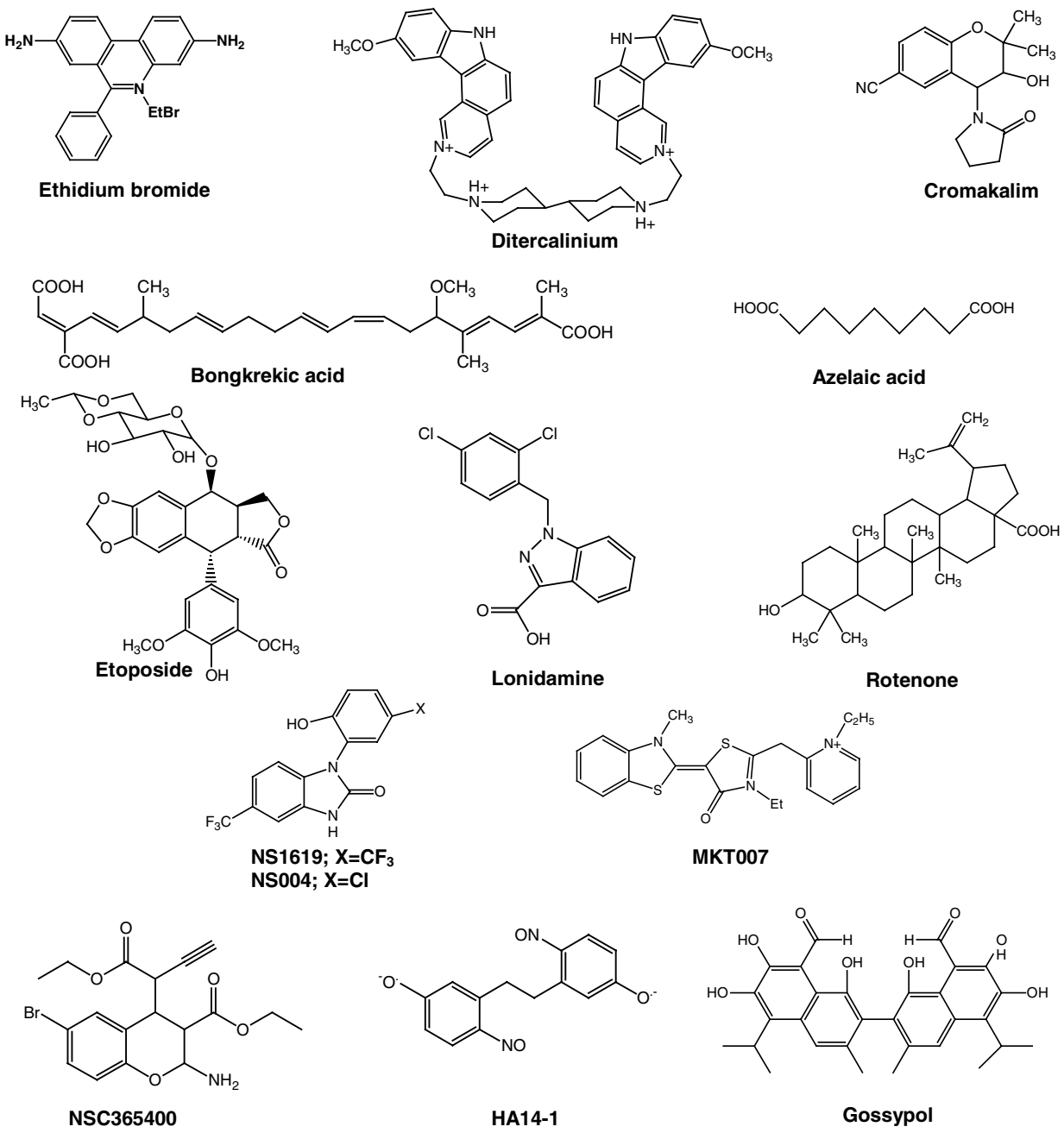

Fig. 4. Structure of some mitochondria-acting drugs.

utilizing the mitochondrial $\Delta\Psi_m$ and mitochondrial protein import machinery. Phosphonium cations were the first described mitochondriotrophic cationic amphiphiles (Green & Evan 2002). Other important and popular mitochondriotrophics are rhodamine 123 (Bernal et al. 1983), cyanine dyes (Oseroff et al. 1986), victoria blue (Morgan et al. 1998) and dequalinium chloride (Weiss et al. 1987) and their derivatives. Mitochondriotrophics are characterized by two characteristics; firstly they are amphiphilic, secondly the entire structure has their π electron charge density extended over at least three atoms or more, i.e., having a delocalized positive charge hence called "delocalized cations" (Weissig & Torchilin 2001).

Mitochondriotropics as chemotherapeutic agents

The increase in membrane potential in cancer can very well be explored for targeting mitochondria. Rhodamine 123 (Lampidis et al. 1983) and tetraphenyl phosphonium chloride (Rideout et al. 1994) both exhibited anticancer activity and has been used to target cancer cells as they specifically localize in the mitochondria driven by the mitochondrial $\Delta\Psi_m$. Rhodamine 123 accumulates in the mitochondria of the cancer cells to a greater extent as compared to the normal cells. Rhodamine 123 affects electron transport and ATP synthesis. MKT-077, a mitochondria-specific

cationic rhodocyanine derivative, selectively kills cancer cells, in part because of its ability to inhibit respiration. MKT-077 accumulates to a much greater concentration in the mitochondria of CX-1 carcinoma cells as compared to normal cells (Modica-Napolitano et al. 1996). MKT-077 also successfully inhibited the growth of renal and prostate xenografts in nude mice and is currently in the phase I clinical trails (Koya et al. 1996). Another important molecule, called AA1, showed ten fold higher activity against mice colon carcinoma cells as compared to normal (Sun et al. 1994). AA1 is also effective against murine bladder, human melanoma and human ovarian tumors. Dequalinium chloride belongs to other mitochondriotropics recently introduced (Weiss et al. 1987). It was found to be 125–fold more cytotoxic towards colon cancer cells as compared to the normal counterpart. Other important mitochondriotropics exhibiting the anticancer property include meta-iodobenzyl guanidine (Kuin et al. 1998) and F-16 (Fantin et al. 2002). Compounds that are mitochondrial potential dependent in their localization are also used as photosensitizing agents in photodynamic therapy. Photosensitizing agents absorb light at their target site, i.e. cancer mitochondria, leading to the generation of ROS and induce apoptosis in cancer cell. Triaryl methane has gained prominence as a photosensitizer for anticancer therapy (Lewis & Indig 2002). Disadvantage with these mitochondrial potential dependent cations is that normal cells with high $\Delta\Psi_m$ may also get affected. Moreover, in certain cancers the mitochondrial $\Delta\Psi_m$ is not high as compared to the normal cells hence targeting in these cases will not be helpful.

Mitochondriotropics as drug carrier

With the advent of the concept of targeted selective mitochondrial drugs for a variety of diseases the need arises for the targeted drug carriers to the mitochondria. The use of vitamin E-TPPB conjugate was tested successfully for imparting an antioxidant protection system. Even peptide-nucleic acid synthesized to arrest and revert diseases are conjugated with triphenyl phosphonium. Dequalinium assembles on sonication yields vesicle like aggregates termed DQAsomes. DQAsomes are not only effective mitochondria-specific DNA delivery system but are also competent carriers of anticancer drugs like paclitaxel. DQAsomes efficiently bind and protect DNA and DQAsome/DNA complexes are selectively released at the inner and the outer mitochondrial membrane. Based on the intrinsic property of DQAsomes to preferentially accumulate in mitochondria and release DNA at mitochondria-like membranes, DQAsomes are proposed as the first mitochondria-specific vector to deliver DNA to mitochondria in living cells (Weissig & Torchilin 2001; Weissig et al. 2006). Targeting therapeutic agent to mitochondria will continue to be an active area of investigation, hopefully yielding newer therapies.

Mitochondria-targeted peptides and antisense poisons

Proapoptotic peptides may be tagged with organelle specific sequences and delivered to specific molecular targets. 'KLAKLAKKLAKLAK' sequence is fused with tumor-homing peptides using phage display that selectively bind to tumor mitochondrial membrane and induce apoptosis (Ellerby et al. 1999). Though in its infancy, this may develop into a major therapeutic modality of the future.

G3139 is an antisense Bcl-2 construct and has shown promise in Non-Hodgkins lymphoma, APL and AML (Marcucci et al. 2003). The high antiapoptotic Bcl-2 level is the hallmark of many cancers and hence antisense therapy may open up a new chapter in mitochondria-specific therapy.

Conclusions

Mitochondria hold great promise as targets for therapeutic intervention for cancer therapy. However, only a fraction of this potential has so far been exploited. Proper understanding of the fundamental difference in the phenotype, genotype and bioenergetic status of different cancers is required to provide a cancer type-selective treatment option. Studies related to the expression of the anti- and pro-apoptotic proteins, the ROS generation efficiency, status of antioxidant defense, ATP synthesis efficiency, substrate chanelling through complex I or II of the ETC, MTP sensitivity, mitochondrial $\Delta\Psi_m$ collapse and cytochrome c release are important for developing mitochondria-targeting therapeutics. Identification of mitochondrial drug targets in combination with the development of methods for selectively delivering mito-toxic molecules to the site of mitochondria will eventually launch a multitude of new therapies for the treatment of cancer. The combinatorial approach towards targeting the mitochondria and any other important target may lead to hybrid drug system as a new inclusion in the arsenal for the war against different cancers.

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