

Mitochondria and Human Cancer

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Abstract: The better part of a century has passed since Otto Warburg first hypothesized that unique phenotypic characteristics of tumor cells might be associated with an impairment in the respiratory capacity of these cells. Since then a number of distinct differences between the mitochondria of normal cells and cancer cells have been observed at the genetic, molecular, and biochemical levels. This article begins with a general overview of mitochondrial structure and function, and then outlines more specifically the metabolic and molecular alterations in mitochondria associated with human cancer and their clinical implications. Special emphasis is placed on mtDNA mutations and their potential role in carcinogenesis. The potential use of mitochondria as biomarkers for early detection of cancer, or as unique cellular targets for novel and selective anti-cancer agents is also discussed.

I. GENERAL BACKGROUND

I.A: Mitochondrial Structure and Function

In electron micrographs of fixed tissue specimens, mitochondria are most commonly observed as oval particles, 1–2 μm in length and 0.5–1 μm in width. These dimensions approximate to those of the bacterium *Escherichia coli*. The organelle is bound by two membranes. The peripheral, or outer, membrane encloses the entire contents of the mitochondrion. The inner membrane has a much greater surface area and forms a series of folds or invaginations, called cristae, which project inward towards the interior space of the organelle. The total surface area of the inner membrane varies considerably depending upon the tissue and type of cell. Since the enzymes involved in oxidative phosphorylation are located on the inner mitochondrial membrane, its surface area and number of cristae are generally correlated with the degree of metabolic activity exhibited by a cell. The spatial arrangement of the outer and inner membranes creates two distinct internal compartments: the intermembrane space is located between the outer and inner membranes; and the matrix is the space enclosed by the inner mitochondrial membrane. By contrast to the static, 'cigar-shaped' organelles commonly observed in electron micrographs, living cells stained with the lipophilic cation rhodamine 123 (Rh123) and observed by fluorescence microscopy reveal mitochondria as a dynamic network of long filamentous structures, capable of profound changes in size, form and location [1]. These mitochondria can be seen extending, contracting, fragmenting and even fusing with one another as they move in three dimensions throughout the cytoplasm. Interestingly, the treatment of cells with microtubule-

depolymerising agents has been shown to result in an altered distribution of mitochondria [2,3]. This suggests that mitochondria are associated with and travel along a molecular 'highway' composed of a cytoplasmic microtubule network.

Mitochondria play a central role in oxidative metabolism in eukaryotes (reviewed in [4]). In the catabolism of carbohydrates (Fig. (1a)), this begins with the transport of pyruvate from the cytosol into the mitochondrion, and its subsequent oxidative decarboxylation to acetyl CoA by a soluble, multi-enzyme pyruvate dehydrogenase complex, which is located in the mitochondrial matrix. The oxidation of acetyl CoA is achieved by a cyclic process involving eight catalytic steps. This process is known as either the citric acid or the tricarboxylic acid (TCA) cycle. All but one of the TCA cycle enzymes are soluble proteins found in the inner mitochondrial matrix compartment. The single insoluble enzyme, succinate dehydrogenase, is tightly bound to the matrix side of the inner mitochondrial membrane. Each round of the TCA cycle results in the production of two molecules of CO_2 , three molecules of reduced nicotinamide adenine dinucleotide (NADH), one molecule of reduced flavin adenine dinucleotide (FADH_2), and one molecule of GTP (the energetic equivalent of ATP).

The next stage of aerobic metabolism is oxidative phosphorylation, an energy-generating process that couples the oxidation of respiratory substrates (such as the NADH and FADH_2 generated through the TCA cycle) to the synthesis of ATP. Substrate oxidation involves a series of respiratory enzyme complexes that are located on the inner mitochondrial membrane and are capable of accepting and donating electrons in a specific sequence based on their relative oxidation-reduction potentials and substrate specificity. Complex I (NADH-ubiquinone reductase) transfers electrons from NADH to the mobile electron carrier ubiquinone, or coenzyme Q. It is the largest and most labile of all the respiratory enzyme complexes.

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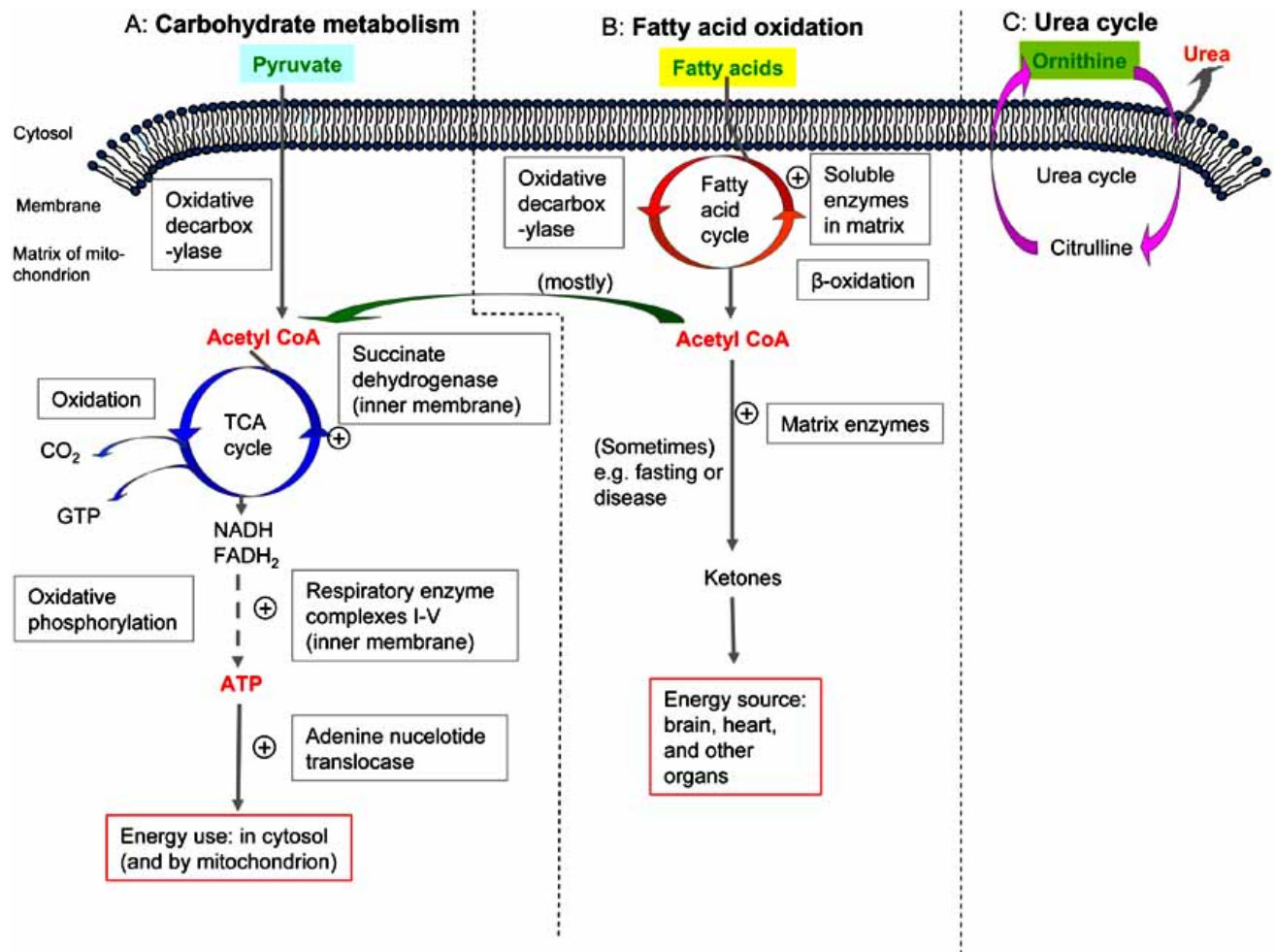


Fig. (1). Schematic of key mitochondrial metabolic pathways. (a) Carbohydrate metabolism. Pyruvate produced from glycolysis undergoes oxidative decarboxylation to acetyl CoA, which is then oxidized in an eight-step process known as the tricarboxylic acid (TCA) cycle. The respiratory substrates NADH and FADH₂ generated through the TCA cycle are next oxidized in a process coupled to ATP synthesis. Electrons are transferred from NADH and FADH₂ to oxygen via enzyme complexes located on the inner mitochondrial membrane. Three of the electron carriers (complexes I, III and IV) are proton pumps, and couple the energy released by electron transfer to the translocation of protons from the matrix side to the external side of the inner mitochondrial membrane. Energy stored in the resulting proton gradient (i.e. the proton-motive force) is used to drive the synthesis of ATP via the mitochondrial enzyme ATP synthetase (complex V). (b) Fatty acid oxidation. Fatty acids undergo oxidative decarboxylation in the mitochondrial matrix to give acetyl CoA, which is fed into the TCA cycle, and new acyl CoA molecules that are successively shortened with each round of the cycle. Under certain conditions (e.g. fasting), acetyl CoA molecules are converted into ketones for use as an alternative energy source. (c) Urea cycle. Amino acid degradation resulting in excretion of nitrogen as urea occurs partly in the mitochondrion. The mitochondrion is also essential for several other processes (not shown), including gluconeogenesis, regulation of cytosolic NAD⁺, intracellular homeostasis of inorganic ions, and apoptosis.

In bovine heart, for example, complex I comprises at least 41 different protein subunits. Complex II (succinate-ubiquinone reductase) transfers reducing equivalents from succinate to ubiquinone. It comprises four protein subunits, one of which is the FADH₂-linked TCA cycle enzyme succinate dehydrogenase. Complex III (ubiquinone-cytochrome c reductase) is an 11-subunit respiratory enzyme complex involved in the transfer of electrons from membrane-bound ubiquinone to oxidised cytochrome c, another mobile electron carrier located on the outer surface of the inner mitochondrial

membrane. Complex IV, or cytochrome c oxidase (COX), is the terminal electron acceptor. It comprises 13 different protein subunits and functions in the transfer of electrons from reduced cytochrome c to molecular oxygen, to form H₂O. The energy released by the exergonic transfer of electrons from respiratory substrate to oxygen is coupled to the translocation of protons from the matrix side to the external side of the inner mitochondrial membrane at three sites: respiratory enzyme complexes I, III and IV. In intact, well-coupled mitochondria, the inner membrane is relatively impermeable to the back flow

of these protons. According to the Chemiosmotic Hypothesis, which was first proposed by Peter Mitchell in 1961 (and for which he received the Nobel Prize in Chemistry in 1978), the energy stored in the resulting proton gradient (i.e. the proton-motive force) is used to drive the synthesis of ATP *via* complex V, the mitochondrial enzyme ATP synthetase [5]. The ATP that is produced by aerobic metabolism and not used by the mitochondrion is transported across the inner mitochondrial membrane in exchange for cytosolic ADP by the enzyme adenine nucleotide translocase (ANT). This exchange ensures not only the inavailability of mitochondrial ADP, which is the principal control molecule for the rate of oxidative phosphorylation, but also the availability of cytosolic ATP. Oxidative phosphorylation thus supplies a majority of the cellular energy produced under aerobic conditions and required to sustain cell viability and normal cell functions.

Fatty acid oxidation is another important metabolic activity located in the mitochondria (Fig. (1b)). The beta-oxidation pathway involves four separate enzymes that are soluble in the mitochondrial matrix and that function in a repetitive cycle. With each round of the cycle, a fatty acid undergoes oxidative decarboxylation to produce one molecule of acetyl CoA and one molecule of a new acyl CoA that is two carbons shorter than the starting fatty acid. The process continues until the original fatty acid molecule is completely degraded to acetyl CoA (for example, the 16-carbon palmitoyl CoA would undergo seven rounds of beta-oxidation to yield eight molecules of acetyl CoA). The acetyl CoA molecules thus generated normally enter into the

TCA cycle where they undergo oxidation to CO₂. However, during conditions of prolonged fasting and starvation, or in certain metabolic diseases (e.g. diabetes mellitus), the acetyl CoA molecules generated by fatty acid oxidation are converted into ketones (e.g., β-hydroxybutyrate, acetoacetate and acetone) by enzymes also located in the mitochondrial matrix. These molecules are then transported through the blood to other tissues, such as brain and heart, where they are used as an alternative energy source to glucose. In addition to its central role in oxidative metabolism, the mitochondrion is involved in a variety of other important cellular functions. For example, certain enzymes of the urea cycle (Fig. (1c)) and gluconeogenesis are located in the mitochondrial matrix. Mitochondria are involved in the regeneration of cytosolic NAD (required for the substrate-level phosphorylation step in glycolysis) and in the intracellular homeostasis of inorganic ions such as calcium and phosphate. A wealth of studies show that mitochondria also play an integral role in the cascade of intracellular events that lead to apoptosis, or programmed cell death [6, 7].

I.B: Mitochondrial Genome

Mammalian cells typically contain over 1000 mitochondria and up to 10,000 copies of mitochondrial DNA (mtDNA). The genome is a 16.6 kb closed-circular, double-helical molecule that encodes two rRNAs, 22 tRNAs and 13 polypeptides (Fig. (2)) [8]. Each of these polypeptides is a highly hydrophobic subunit of one of four respiratory enzyme complexes localized to the inner

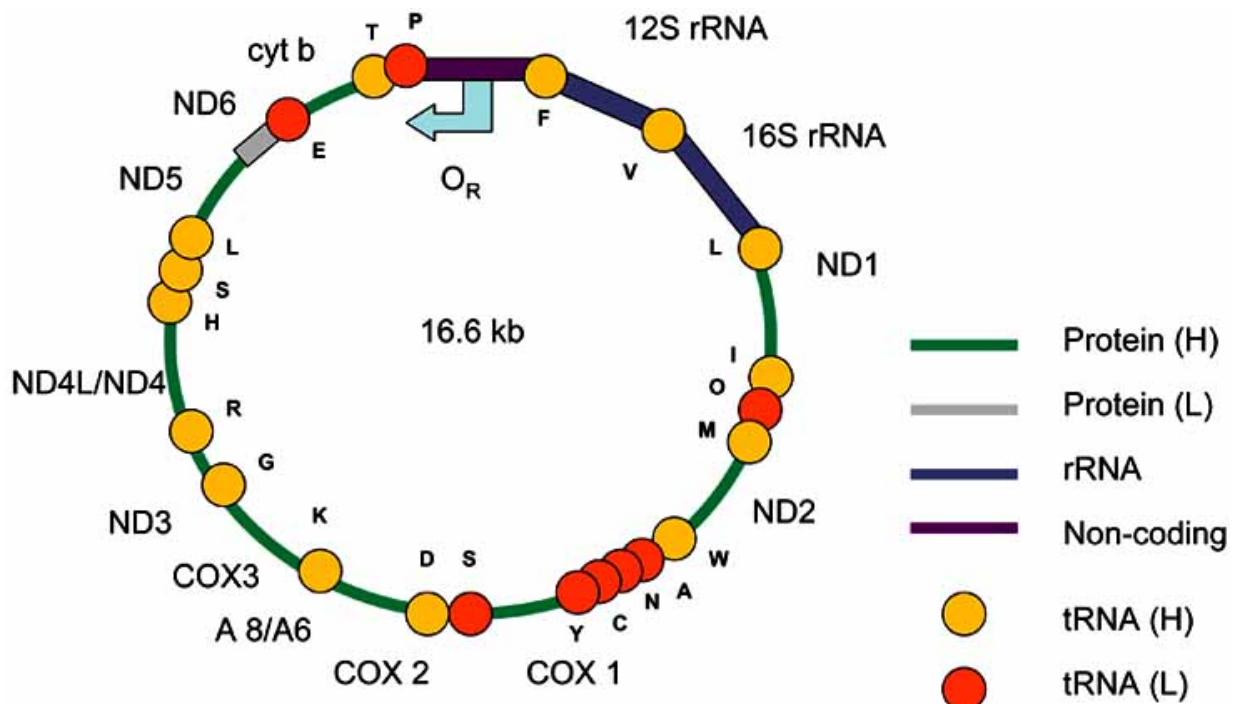


Fig. (2). Structure of mitochondrial genome.

mitochondrial membrane. They include seven subunits of respiratory enzyme complex I, one subunit of complex III, three subunits of complex IV, and two subunits of complex V. All other mitochondrial proteins, including those involved in the replication, transcription and translation of mtDNA, are encoded by nuclear genes and are targeted to the mitochondrion by a specific transport system [9]. In humans and other mammals, mitochondrial genes display a maternal inheritance (i.e. are inherited from the female parent). This is probably because the number of mtDNA copies in the egg is typically 10^3 -fold greater than that in the sperm [10]. Alternatively, paternal genomes and organelles might be preferentially degraded in the zygote [11, 12].

Although mtDNA represents less than 1% of total cellular DNA, its gene products are essential for normal cell function. Unlike nuclear DNA, mammalian mtDNA contains no introns, has no protective histones and is exposed to deleterious reactive oxygen species generated by oxidative phosphorylation. In addition, replication of mtDNA is error prone [13-18]. The accumulation of mutations in mtDNA is approximately tenfold greater than that in nuclear DNA [19, 20].

In general, all mitochondria within a single cell are homoplasmic, i.e., they share identical DNA sequences. Differences in mtDNA sequences within the same cell, or heteroplasmy, can occur in response to de novo or "somatic" mutations. Mitochondrial pathologies usually arise only after a minimal threshold of heteroplasmy has been reached. That threshold is apparently different for different tissues and is dependent upon the energy status of a cell and its requirement for oxidative metabolism.

Although the mitochondrial and nuclear genomes are physically distinct, the high degree of functional interdependence between them is suggestive of a host-parasite relationship. The 'endosymbiont' theory proposes that early in the evolution of the eukaryote, a primitive proto-eukaryote cell that was incapable of aerobic respiration served as host to a eubacterium with the unique capacity for oxidative metabolism [21]. During the early stages of this endosymbiotic association, the eubacterium retained its genetic autonomy. In time, however, most of its genetic material was transferred to the nuclear genome of the host. The resulting mitochondrion retained only those few (i.e. 13) genes encoding polypeptides that are essential to aerobic ATP production yet have a hydrophobicity that precludes nuclear synthesis and cytoplasmic transport to mitochondria.

II. MITOCHONDRIA AND HUMAN CANCER

II.A: Differences at Metabolic Level

An association between mitochondrial dysfunction and cancer was made as early as 1930, when Otto

Warburg first hypothesized that the increased rates of aerobic glycolysis which he observed in a variety of tumor cell types might be due to an impaired respiratory capacity in these cells [22]. Subsequently, a number of additional metabolic alterations associated with mitochondrial function have been observed in cancer cells, including increased gluconeogenesis [23], reduced pyruvate oxidation and increased lactic acid production [24], increased glutaminolytic activity [25], and reduced fatty acid oxidation [26].

Metabolic aberrations specifically associated with mitochondrial bioenergetic function in cancer cells have also been observed. These include differences between normal and malignant cells with regard to preference for respiratory substrates, rates of electron and anion transport, and the capacity to accumulate and retain calcium (reviewed in [27]). The activities of certain enzymes integral to the process of oxidative phosphorylation are known to be decreased in cancer versus normal cells. For example, the measured maximal velocity for ATPase activity in mitochondria [28] and submitochondrial particles [29-31] isolated from hepatocellular carcinoma is considerably lower than that in normal liver. Mitochondrial cytochrome c oxidase activity in total cellular homogenate and the mitochondrial samples from cultured human carcinoma cell lines is also significantly lower than that measured in the control epithelial cell line [32]. Similar decreases in cytochrome c oxidase activity have been reported in biopsies of human colonic adenocarcinoma versus normal colon mucosa [33], and in cultured rat HC252 hepatoma cells versus non-neoplastic liver [34]. The adenine nucleotide exchange function of adenine nucleotide translocase (ANT) [35-37] and the sensitivity of this enzyme to bongkreikic acid [37-39] are also known to be decreased in certain hepatoma versus normal liver mitochondria. In addition, the mitochondrial membrane potential has been shown to be significantly higher in carcinoma cells than in normal epithelial cells [1, 40-42]. It is important to note that despite the large number of metabolic aberrations thus far identified, apparently none is common to all cancer cells.

Numerous differences in the molecular composition of the mitochondrial inner membrane between normal and cancer cells have also been reported. Analysis of the inner membrane lipid composition of various tumor mitochondria has indicated elevated levels of cholesterol, varying total phospholipid content, and/or changes in the amount of individual phospholipids relative to normal controls [43]. Polypeptide profiles of normal versus cancer cells reveal a number of differences in the appearance and/or relative abundance of several proteins as well. It has been suggested that alterations in F_1F_0 ATPase function in hepatocellular carcinoma might be associated with a decrease in immuno-detectable levels of the β subunit of the F_1 component of mitochondrial ATPase and/or with overexpression of the ATPase inhibitor protein (IF₁)

[29-31]. The decrease in specific activity of cytochrome c oxidase in cancer cells may also be due to alterations in the level of gene expression. The mean level of expression of the mtDNA encoded subunit COX III was found to be lower in human colonic biopsies of carcinoma versus normal mucosa samples [44]. Cultured HT29 colon carcinoma cells also exhibited low levels of the COX III transcript; however, expression of COX III returned to higher (normal) levels when the cells were induced to differentiate by exposure to sodium butyrate. By contrast, increased levels of RNA transcripts of the nuclear-encoded subunit COX IV and mitochondrially encoded subunits COX I and II have been observed in Zajdela hepatoma as compared with normal liver [45]. More recently, an increase in the expression of nuclear:mtDNA encoded cytochrome c oxidase subunits has been observed in human prostate carcinoma relative to normal control [46]. High transcript levels for ANT2, the gene encoding one of three isoforms of the translocase, have been observed in several dedifferentiated, proliferating, renal tumor cell types [47, 48]. Additional alterations in gene expression between normal and cancer cells include the anti-apoptotic oncogenes encoding Bcl-2 and Bcl-XL, and genes encoding the peripheral benzodiazepin receptor (PBR), the PBR-associated protein Prax-1, and mitochondrial creatine kinase [49-56]. The expression of BAX, a proapoptotic, inner mitochondrial membrane protein, is also reduced in some cancer cell lines [57, 58].

II.B: Differences at Genetic Level

II.B. 1: Mitochondrial DNA

Mutations in mtDNA have been reported in all cancers examined to date [99]. A summary of mtDNA mutations in various tumors is presented in Fig. (3). While these mutations are known to occur throughout the mitochondrial genome, the displacement loop (or D-loop) region has been shown to be a mutational "hot spot" in human cancer. The D-loop is a triple stranded non-coding region of mtDNA (np 16024-516) that houses cis regulatory elements required for replication and transcription of the molecule. Thus mtDNA mutations in this region might be expected to affect copy number and gene expression of the mitochondrial genome. Somatic mutations in the D-loop region have been observed in gastric cancer [59, 60], hepatocellular carcinoma [61], uterine serous carcinoma [62] ovarian cancer [63], breast cancer [64], colorectal cancer [65], and melanoma [66]. In a recent comprehensive study involving 54 hepatocellular carcinomas, 31 gastric, 31 lung, and 25 colorectal cancers, the incidence of somatic D-loop mutations in cancers of later stage was found to be higher than that of early stage cancers [67]. These findings suggest that instability in the D-loop region of mtDNA may be involved in carcinogenesis of human cancers.

In addition to the mtDNA mutations found in the D-loop region, deletions, point mutations, insertions

and duplications in other parts of the mitochondrial genome have been noted in a variety of human cancers including ovarian, thyroid, salivary, kidney, liver lung, colon, gastric brain, bladder, head and neck, prostate, and breast cancer, and leukemia [68-93]. For example, a 40 bp insertion localized in the COX I gene appears to be specific for renal cell oncocyoma [74], and a deletion mutation resulting in the loss of mtDNA within NADH dehydrogenase subunit III is associated with renal carcinoma [80]. In a recent population based study involving 260 prostate cancer patients of European and African-American descent and 54 benign controls without cancer, the frequency of COX I missense mutations was found to be significantly higher in prostate cancer patients compared to the no-cancer controls (12% vs. 1.9%, respectively). At least some of these COX I sequence variants were thought to represent germ line mutations [89]. The co-occurrence of somatic and germ-line mtDNA mutations in renal cell carcinoma has also been reported [92].

Tumor specific changes in mtDNA copy number have also been noted in human cancers. For example, the mtDNA content was found to be elevated in primary tumors of head and neck squamous cell carcinoma [94], and to increase with histopathologic grade in premalignant and malignant head and neck lesions [95]. In addition, mtDNA copy number was shown to increase in papillary thyroid carcinomas [96] and during endometrial cancer development [97]. Conversely, it was reported that mtDNA content is reduced in 80% of breast tumors relative to normal controls. Furthermore, in one recent study investigating mtDNA copy number in a variety of cancers, both increases and decreases in mtDNA content relative to non-malignant controls were observed for each cancer type [67]. Thus, the actual mtDNA copy number in certain cancers might depend upon the specific site of mutation associated with that cancer. For example, mutations in the D-loop region, which controls mtDNA replication, would be expected to result in a decrease in copy number. On the other hand, mtDNA mutations in genes encoding oxidative phosphorylation proteins might be expected to result in an increase in mtDNA copy number. It has been hypothesized that this might occur as a compensatory response to mitochondrial dysfunction [95].

A number of recent studies suggest the functional significance of mtDNA mutations and depletions in tumorigenesis and/or tumor progression. For example, the presence of somatic D-loop mutations appears to be a factor of poor prognosis in colorectal cancer patients, and may be a factor of resistance to fluorouracil based adjuvant chemotherapy in stage II cancers [65]. Somatic mtDNA mutations and mtDNA depletion occur in gastric cancer and may be involved in carcinogenesis and/or cancer progression of breast and gastric carcinoma [60, 138], while mtDNA mutations also appear to play a role in development but not progression of digestive tract cancer [90]. Additionally, there is epidemiological

Tissue	D-Loop	12 S RNA	16 S RNA	ND1	ND2	ND3	ND4	ND 4L	ND5	ND6	COX 1	COX 2	COX 3	Cyt b	ATP 6	ATP 8
Breast	↑	↑		↑	↑								↑	↑	↑	
Ovary	↑	↑												↑		
Bladder/ Esophagus/ Head/Neck /Hepatic/ Lung	↑															
Colon		↑	↑	↑				↑	↑		↑	↑	↑	↑		
Gastric	↑			↑				↑			↑					
Pancreas	↑	↑	↑	↑	↑		↑	↑	↑	↑	↑	↑	↑	↑	↑	↑
Thyroid	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑
Prostate	↑		↑				↑	↑								
ALL									↑			↑			↑	
MDS												↑	↑	↑		↑

Fig. (3). Summary of mtDNA mutations found in various tumors examined. Arrows indicate the coding and non-coding (D-loop) regions of mtDNA mutated in tumors.

evidence that the mtDNA 10398A polymorphism, which alters the structure of electron transport complex I, is associated with increased risk for breast cancer in African-American women [93]. It has been hypothesized also that the prevalence of germ-line COX I mutations may explain the increased incidence of prostate cancer in African American men [89].

Mitochondrial dysfunction resulting from changes in mtDNA invokes mitochondria-to-nucleus retrograde response in human cells [144,148]. To identify proteins involved in retrograde response and their potential role in tumorigenesis, Kulawiec *et al.* carried out a comparative proteomic analysis using a parental cell line, a rho⁰ cell line in which the mitochondrial genome was completely depleted (and the cells were therefore lacking all mtDNA-encoded protein subunits), and a cybrid cell line in which mtDNA was restored [144]. This comparative proteomic approach revealed marked changes in the cellular proteome including quantitative changes in expression of several proteins in breast and ovarian tumors, which suggest that retrograde responsive genes may potentially function as tumor suppressor or oncogenes.

Trans-mitochondrial hybrid (cybrid) studies also suggest that mtDNA plays a key role in establishing and/or maintaining the tumorigenic phenotype. In a recent study, Singh *et al.* demonstrated that a rho⁰ derivative of human osteosarcoma cells display increased tumorigenic phenotype as evidenced by increased anchorage independent growth compared to the parental cells [142]. Interestingly, the parental phenotype, displaying reduced anchorage independent growth, was restored by transfer of wild type mitochondrial DNA to rho⁰ cells. These studies suggest that intergenomic cross talk between mitochondria and cancer plays an important role in tumorigenesis and that retrograde mitochondria-to-nucleus signaling may be an important factor in restoration of the non-tumorigenic phenotype. Indeed, additional studies by Singh's group demonstrate that retrograde mitochondria-to-nucleus signaling is also important in regulation of NADPH oxidase (Nox1), and that this enzyme is over expressed in a majority of breast and ovarian tumors [143]. The family of Nox enzymes comprise seven structurally related homologues, Nox 1-5 and dual oxidase 1 and 2 [143, 146], and nuclear DNA encoded Nox 1 is a major source of endogenous

ROS in the cell [143]. Until recently, these enzymes were known only to function as phagocytic respiratory burst oxidases which catalyze the NADPH-dependent reduction of molecular oxygen to superoxide, hydrogen peroxide and other ROS, and to participate in host defense by killing or damaging invading microbes [145-147].

Transmitochondrial cybrid studies also suggest the functional significance of mtDNA mutations. Cybrids harboring the ATP6T8993G mtDNA mutation in prostate cancer (PC3) cells were found to generate tumors that were 7 times larger than wild type cybrids, which barely grew in mice [89]. Additionally, cybrids constructed using a common HeLa nucleus and mitochondria containing a point mutation in ATP synthase subunit 6 were conferred a growth advantage in early tumor stages after transplantation into nude mice. It was suggested that this growth advantage might possibly occur *via* prevention of apoptosis [98]. These studies support the hypothesis that mtDNA mutations might directly promote tumor growth *in vivo*.

II.C. Nuclear DNA Encoded Mitochondrial Proteins

Recent evidence suggests that certain nDNA encoded mitochondrial proteins act as tumor suppressors, and that germ line and/or somatic mutations in genes encoding these proteins may be involved in the tumorigenesis of both benign and malignant lesions [104]. The proteins involved include three subunits of the succinate dehydrogenase (SDH) enzyme complex, and the enzyme fumarate hydratase (FH). Both enzymes catalyze reactions in the tricarboxylic acid cycle, whereas SDH is also a component (complex II) of the mitochondrial electron transport chain. Missense

mutations in SDH genes were shown to underlie hereditary paraganglioma syndrome [105], and mutations in FH underlie genetic predisposition to hereditary leiomyomatosis and renal-cell cancer syndrome [106]. It is suggested that loss of function of SDH or FH induces a hypoxic response, mediated by hypoxia-inducible transcription factor (HIF), under normoxic conditions. HIF promotes glycolysis and stimulates blood vessel growth [107, 108] two important determinants for tumor growth and survival. Interestingly, a reduction in SDH activity is also associated with an increase in ROS production, which may in turn cause further nDNA or mtDNA damage that contributes to tumorigenesis. It has been suggested also that ROS might be the link between mitochondrial dysfunction and HIF induction associated with SDH loss of function mutations [109].

III. MECHANISM OF MITOCHONDRIA MEDIATED CARCINOGENESIS

Mitochondrial respiratory activity is associated with the generation of reactive oxygen species (ROS). Under physiological conditions, a small fraction of reducing equivalents from complex I or complex III of the mitochondrial electron transport chain may be transferred directly to molecular oxygen, generating the superoxide anion $O_2^{\cdot-}$. This ROS is converted to H_2O_2 by mitochondrial manganese superoxide dismutase. H_2O_2 can be converted to water by glutathione peroxidase or catalase, or acquire an additional electron from a reduced transition metal to generate the highly reactive hydroxyl radical $\cdot OH$. ROS generation has been shown to increase in mitochondria under conditions of excess electrons (e.g., increased caloric intake) or as a result of

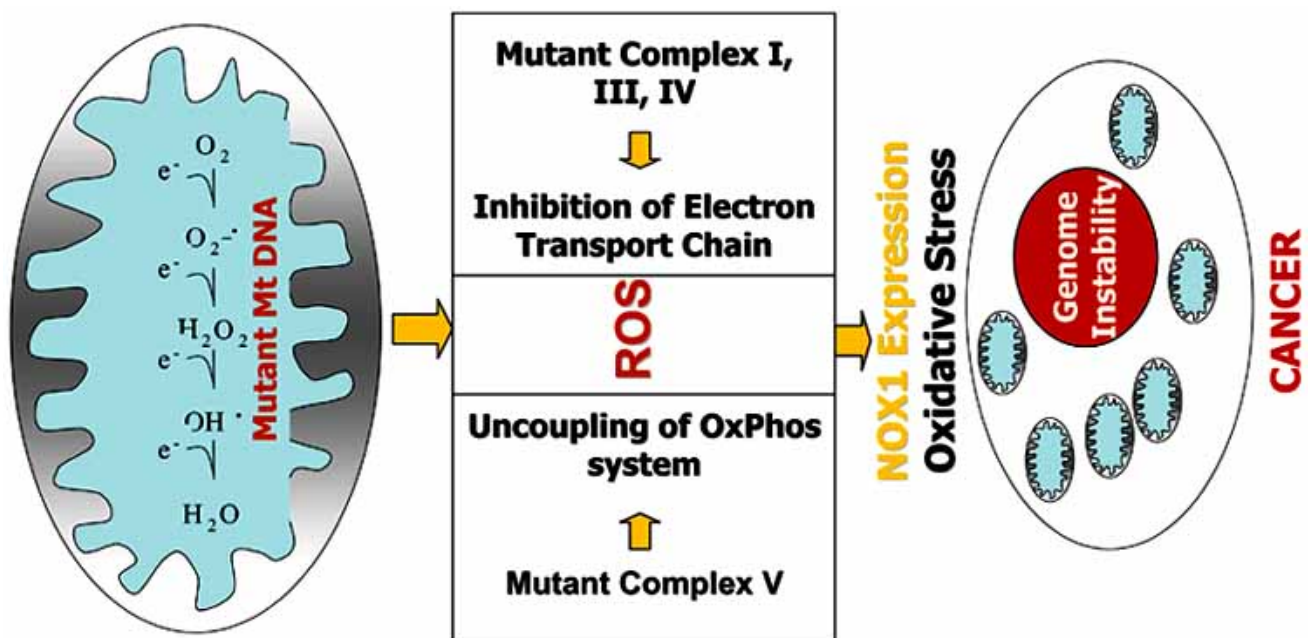


Fig. (4). Mechanisms of mitochondria-mediated mutagenesis and development of cancer (see text for details).

respiratory enzyme complex inhibition. High levels of ROS, or oxidative stress, can induce mutations in both mtDNA and nuclear (nDNA), and damage intracellular protein and lipid components. The mitochondrial genome is especially susceptible to ROS damage due to its proximity to the site of ROS production (i.e., the electron transport chain), its "intronless" DNA, and its limited mtDNA repair capabilities. Oxidative stress is therefore mutagenic to mtDNA and can thus impair mitochondrial function (Fig. (4)). It is generally accepted that oxidative stress in mitochondria throughout the human life-span is likely an important factor in the aging process.

Oxidative stress and resultant mtDNA mutations have also been suggested to underlie the development and/or maintenance of the malignant phenotype (Fig. (4)). It has been hypothesized that chronic inflammation may be linked with oxidative stress and carcinogenesis. For example, one recent study investigated the occurrence of mtDNA mutations in inflammatory colorectal mucosa of individuals with ulcerative colitis and colorectal cancer [101]. The data show a higher frequency of mtDNA mutations in ulcerative colitis than in control subjects and suggest that chronic inflammation associated with this disease causes accumulation of mtDNA mutations, including 8-hydroxy-2,deoxyguanosine, a DNA lesion typically associated with ROS production. In the same study, specimens from patients with colorectal cancer also contained a significantly higher number of mtDNA mutations, suggesting that analysis of mtDNA could provide a new criterion for therapeutic evaluation and may be useful for the prediction of risk of carcinogenesis. Interestingly, there is evidence that oxidative stress associated with chronic inflammation may also be involved in the pathogenesis of prostate cancer [102, 103].

MtDNA mutations causing defects in mitochondrial respiratory enzyme complexes are thought to increase ROS production (Fig. (4)). In support of this hypothesis it has recently been demonstrated that defects in mitochondrial respiratory chain activity lead to: 1) over expression of superoxide producing NADPH oxidase (NOX1) in the majority of breast and ovarian tumors examined [143]; 2) increased damage and hypermutagenesis in the nuclear DNA [142, 150-152]; and 3) resistance to apoptosis [148]. These transformations in cellular phenotype due to mitochondrial dysfunction can contribute to development of cancer (Fig. (4)).

An alternative "metabolic" model of accelerated mtDNA mutations has been proposed to play a role in the development and progression of prostate cancer [149]. In this model, a metabolic switch resulting in an increase in respiratory chain activity is the initial event that occurs early in the malignant transformation of peripheral zone prostate cells. The increased respiratory activity is thought to then induce a cascade of events including increased ROS production, increased mtDNA mutations,

compromised respiratory activity, and further ROS production, thus setting in motion a cycle of oxidative stress that may contribute to the etiology and pathogenesis of prostate cancer.

IV. MITOCHONDRIA AS CLINICAL MARKERS FOR CANCER

The abundance and homoplasmic nature of mitochondria make mtDNA an attractive molecular marker of cancer [110-112]. Indeed, mutant mtDNA in tumor cells has been reported to be 220 times as abundant as a mutated nuclear marker [113]. Mutant mtDNA is readily detectable in urine, blood and saliva samples from patients with bladder, head and neck, and lung cancers [113, 94]. Mutations in mtDNA have been used as clonal markers in hepatocellular carcinoma [114] and breast cancer [115]. More recently, rapid and high throughput sequencing protocols have been developed to detect mtDNA sequence variants in patient tumor and blood samples [116]. It is estimated that 1,000 different proteins comprise mitochondria. Advances in proteomic technologies have made possible the quantitative analysis of protein expression in mitochondria, and a mitochondrial proteomic database has recently been established by the National Institutes of Standards and Technology [117]. Research efforts to obtain mitochondrial protein profiles in normal and cancer cells will undoubtedly lead to identification of markers for clinical detection of cancer, and contribute to an understanding of how differential protein expression might influence the development of the disease.

V. MITOCHONDRIA AS THERAPEUTIC TARGET

The many distinct differences in mtDNA structure and function between normal cells and cancer cells offer the potential for clinical use of mitochondria not only as markers for the detection of cancer, but also as targets for novel and site-specific anti-cancer agents [118, 119]. One chemotherapeutic strategy utilizes delocalized lipophilic cations (DLCs) that accumulate selectively in carcinoma cells in response to increased mitochondrial membrane potential. Several of these compounds have exhibited at least some degree of efficacy in carcinoma cell killing *in vitro* and *in vivo* [120-127]. It is of interest to note that the mechanism of mitochondrial toxicity exhibited by these compounds is quite varied. For example, among the DLCs that display a concentration-dependent toxicity to mitochondria, Rh123 and AA-1 inhibit mitochondrial ATP synthesis at the level of F0F1-ATPase activity [128, 124], whereas DECA and certain DLC thiocarbonyanines interfere with NADH-ubiquinone reductase activity [120, 122]. In addition, the selective cytotoxicity to carcinoma cells exhibited by MKT-077 *in vitro* and *in vivo* has been attributed to a selective inhibition of mitochondrial respiration in cancer cells, most

probably as a result of a general perturbation of mitochondrial membranes and consequent inhibition of the activity of membrane-bound enzymes [126]. The selective cytotoxicity might also be a consequence of intercalation with and/or a mild to moderate degradative effect on mtDNA, but not nuclear DNA, of various carcinoma cell types [126, 127]. Attempts have also been made to enhance the selective tumor cell killing of DLCs by combination with other anti-cancer agents, including AZT [128].

Certain DLCs have been employed in photochemotherapy (PCT), an investigational cancer treatment involving light activation of a photoreactive drug, or photosensitizer, that is selectively taken up or retained by malignant cells [129-132]. There has been considerable interest in PCT as a form of treatment for neoplasms of the skin, lung, breast, bladder, brain or any other tissue accessible to light transmitted either through the body surface or internally *via* fiber optic endoscopes. Cationic photosensitizers are particularly promising as potential PCT agents. Like other DLCs, these compounds are concentrated by cells into mitochondria in response to negative-inside transmembrane potentials, and are thus selectively accumulated in the mitochondria of carcinoma cells. In response to localized photoirradiation, the photosensitizer can be converted to a more reactive and highly toxic species, thus enhancing the selective toxicity to carcinoma cells and providing a means of highly specific tumor cell killing without injury to normal cells.

An alternative strategy employs mitochondrial protein-import machinery to deliver macromolecules to mitochondria. For example, a mitochondrial signal sequence has been used to direct green fluorescent protein to mitochondria, which allows the visualization of mitochondria within living cells [133]. Certain short peptides comprising two functional domains (such as a "homing" motif that targets specific cell types and a pro-apoptotic sequence) readily penetrate the mitochondrial membrane and become toxic when internalized into the targeted cells by disruption of mitochondrial membranes [134]. Another chemotherapeutic strategy targets specific mitochondrial membrane proteins to induce membrane permeabilization and, ultimately, apoptosis [135].

Attempts are being made also to develop mitochondriotropic drug and DNA delivery systems. Recent data demonstrates that conventional liposomes can be rendered mitochondria-specific *via* the attachment of known mitochondriotropic residues to the liposomal surface [136]. Furthermore, DQAsomes prepared from derivatives of the self-assembling mitochondriotropic bola-amphiphile dequalinium chloride, have been shown to bind and transport oligonucleotides as well as plasmid DNA conjugated to a mitochondrial leader sequence (MLS) to mitochondria in living mammalian cells and

release DNA on contact with mitochondrial membranes [137]. The long-term therapeutic goal of this type of research is to someday create mitochondria-specific vehicles that will effectively deliver drugs or mtDNA into the organelle to destroy dysfunctional mitochondria or replenish mitochondria with healthy copies of the genome.

ACKNOWLEDGEMENTS:

Studies in our laboratory were supported by NIH grants R01 CA121904 and 113655 and a grant from Breast Cancer Coalition of Rochester (to KKS).

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