Cellular Adaptations to Oxidative Phosphorylation Defects in Cancer

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Abstract Mitochondrial DNA (mtDNA) somatic mutations or mutations in nuclear genes encoding mitochondrial proteins important for the assembly, activity, or maintenance of the individual oxidative phosphorylation (OXPHOS) complexes have been observed in tumors. Although the functional consequence of such mutations is unclear at the moment, retrograde signaling in response to OXPHOS defects can activate various nuclear genes and signaling pathways that alter mitochondrial function, tumor invasion, metastasis, redox-sensitive pathways, programmed cell death pathways, calcium signaling pathways, and cellular pathways leading to global changes in cellular morphology and architecture. In addition, we have found that some cancer cell lines harboring deleterious mtDNA mutations upregulate the expression of members of the peroxisome-proliferator activated γ coactivator 1 family of coactivators, probably to sustain the necessary ATP production for cell proliferation. In this chapter, we describe such cellular adaptations and changes in response to OXPHOS defects that are associated with a variety of cancer cell types.

Keywords mtDNA · Retrograde signaling · OXPHOS · Cancer · Mitochondria

1 Mammalian Oxidative Phosphorylation

Mammalian mitochondrial oxidative phosphorylation (OXPHOS) occurs in five multimeric enzyme complexes (complexes I, II, III, IV, and V) using two electron transport carriers (ubiquinone or coenzyme Q10 and cytochrome c). Electrons from reduced equivalents (NADH and $FADH_2$) are transported along these complexes via ubiquinone and cytochrome c to molecular oxygen, producing water. At the same time, protons are pumped across the mitochondrial inner membrane (from the matrix to intermembrane space) by enzyme complexes I, III, and IV thereby generating a

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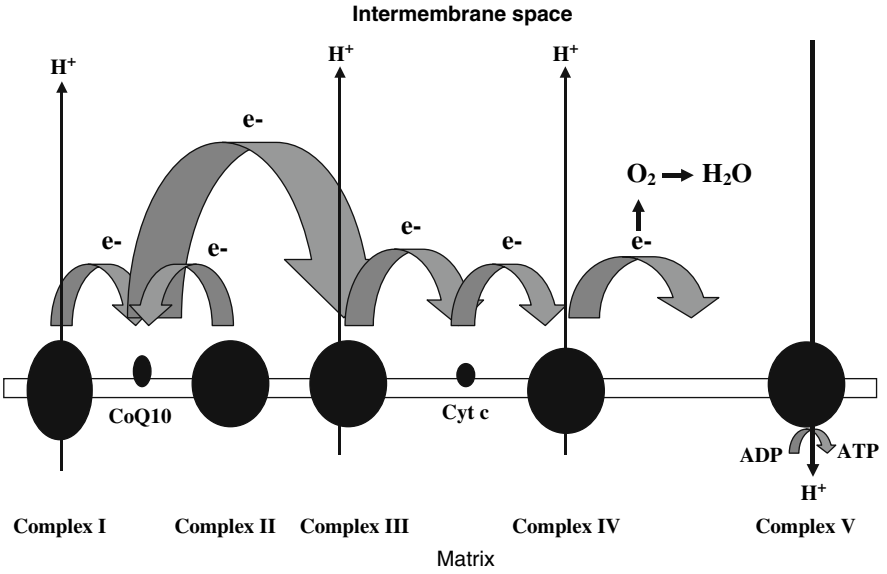

Fig. 1 Schematic representation of mammalian mitochondrial OXPHOS. Mammalian electron transport chain (ETC) consists of four multimeric complexes (complexes I, II, III, and IV). Electrons from the reduced equivalents, NADH and $FADH_2$, enter the ETC and reduce complex I and complex II, respectively. Coenzyme Q10 or ubiquinone is an electron carrier in the inner mitochondrial membrane that accepts an electron from either complex I or complex II and donates it to complex III. Cytochrome c, another electron carrier in the mitochondrial intermembrane space, accepts an electron from complex III and donates it to complex IV. Complex IV donates electrons to molecular O_2, which results in the formation of H_2O. During the electron flow, complexes I, III, and IV pump the protons from the matrix toward the intermembrane space thereby generating an electrochemical gradient across the mitochondrial inner membrane. The energy in the electrochemical gradient is harnessed by complex V to generate ATP from ADP (oxidative phosphorylation)

proton gradient or membrane potential ($\Delta\Psi m$). The energy in this gradient is harnessed by complex V (ATP synthase) to synthesize ATP from ADP, a phenomenon termed OXPHOS, during which protons flow back from the mitochondrial intermembrane space to the mitochondrial matrix (Fig. 1).

Mitochondria are also important for cytosolic Ca^{+2} buffering and are the predominant source of ROS production in most cell types. Superoxide anions are generated at the level of complex I and complex III in the inner mitochondrial membrane.

2 OXPHOS Defects in Cancer

OXPHOS defects in cancer cells were first reported more than 50 years ago by Warburg who stated that "*cancer cells are impaired in respiratory chain function and are very glycolytic*" [1]. Studies on various types of cancer cells have found

this hypothesis to be correct in several but not all tumor cells. Several independent groups have also reported that many cancer cells possess a full complement of respiratory chain enzymes and can couple electron transport chain to ATP production [2]. Isolated tumor mitochondria have also been found to respire on a variety of substrates. Specific changes that occur in the energy metabolism of tumor cells have not yet been established.

In the past 10 years, studies on mitochondrial DNA (mtDNA) mutations that cause OXPHOS defects and their potential role in cancer have regained momentum. Mitochondrial DNA mutations have been increasingly identified in various types of cancer cells [3, 4]. Polyak et al. were first to report a detailed analysis of somatic mtDNA mutations in human colorectal cancer cells by sequencing their complete mitochondrial genome [3] (somatic mtDNA mutations are those present in the tumor tissue but absent from the adjacent normal tissue). After this initial finding, mtDNA from various tumor cell types was sequenced by different groups, and mtDNA mutations have now been reported in esophageal, ovarian, thyroid, head, neck, lung, bladder, and renal cancer cells [4–6]. The majority of the mtDNA mutations reported in different tumor cell types are transitions (G-to-A or T-to-C), a feature that is characteristic of reactive oxygen species (ROS)-derived mutations. Further, most of the somatic mtDNA mutations have been reported to be homoplasmic suggesting their dominance at intra- and intercellular levels.

2.1 Functional Significance of OXPHOS Defects in Cancer

Although in the past decade, the list of mtDNA mutations affecting OXPHOS in cancer cells has increased steadily, the functional relevance of these mutations in tumor promotion or formation process are yet obscure. Because the majority of the somatic mtDNA mutations found in tumor cells have been reported to be homoplasmic, one of the existing hypotheses is that the homoplasmic levels of these mutations were probably achieved through some kind of replication or growth advantage that the mutant mtDNA harbored over the wild type in the tumor progenitor cells. It has also been suggested that mtDNA mutations may cause a moderate increase in ROS production that in turn stimulates cell growth [3, 4]. Low levels of ROS have been shown to stimulate mitosis in various cell types [7, 8]. In contrast with this hypothesis, Coller and co-workershave demonstrated that there is a sufficient opportunity for a tumor progenitor cell to achieve homoplasmy through unbiased mtDNA replication and segregation during cell division [9]. Coller et al.constructed a theoretical model based on computer simulation studies and showed that there is a good correlation between the reported frequency of homoplasmic mutations in tumor cells and the predicted frequency of homoplasmy in tumor progenitor cells that can be attained in the absence of any selection [9]. The authors suggested that mtDNA mutations such as silent substitutions of amino acid coding regions or alterations in the length of polynucleotide tracts that are polymorphic in healthy individuals are the ones that are unlikely to confer any selective advantage to the mtDNA or the

host cell. However, the authors did not exclude the possibility that certain mtDNA point mutations that cause functional alterations (e.g., mutations affecting the conserved amino acid regions in the coding region of a protein) might be segregated in a nonrandom manner and may provide a selective growth advantage to the host cell [9].

mtDNA mutations leading to OXPHOS dysfunction have also been shown to increase tumorigenicity in certain tumor cell types. Mutations in the mtDNA COXI gene were shown to increase tumorigenicity in prostate cancer patients [10]. Petros et al. reported that ∼11% to 12% of prostate cancer patients harbor pathogenic COXI mtDNA mutations that alter the conserved amino acid regions in the protein and suggested that these mutations may play a role in the etiology of prostate cancer [10]. mtDNA ATP6 gene mutation (T8993G) has also been shown to increase tumorigenicity by two independent groups. The first study was from Petros and coworkers who showed that PC3 prostate cancer cybrid cells harboring T8993G ATP6 mutant mtDNA when introduced in nude mice formed tumors that were ∼7 times larger than cells harboring the wild-type mtDNA suggesting a role of mtDNA mutation in tumor promotion process [10]. They also showed that tumor cells harboring ATP6 mutant mtDNA generated significantly higher levels of ROS than did those harboring wild-type mtDNA, further suggesting that high levels of ROS promoted tumor cell growth. The second study was from Shidara and co-workers. They constructed HeLa transmitochondrial cybrids harboring homoplasmic T8993G ATP6 mtDNA mutation and showed that these cybrids grew much faster in culture as well as in nude mice forming tumors that were larger in size compared with the cybrids harboring the wild-type mtDNA suggesting that the mtDNA ATP6 mutation in these cells conferred a selective growth advantage [11]. Further, transfection of a wild-type nuclear version of the mitochondrial ATP6 gene in these HeLa cybrids harboring homoplasmic ATP6 mutant mtDNA followed by their transplantation in nude mice was shown to slow down the tumor cell growth. Conversely, the expression of a nuclear version of the mutant ATP6 gene in the wild-type cybrids was found to accelerate tumor cell growth thereby suggesting that the growth advantage in these tumor cells depended on the mitochondrial ATP6 function [11].

OXPHOS dysfunction caused by partial mtDNA depletion (genetic stress) or treatment with mitochondrial-specific inhibitors (metabolic stress) has been shown to induce cell invasion and tumor progression in otherwise noninvasive cells [12, 13]. A correlation between OXPHOS impairment and tumor aggression has also been reported in renal carcinomas [14]. Simonnet et al. studied the mitochondrial respiratory chain enzyme content in three different renal tumors and found that the OXPHOS impairment increased from the less aggressive to the most aggressive types of renal carcinomas [14]. They also found that renal oncocytomas (benign tumors) are exclusively deficient in mitochondrial complex I activity, whereas the activity of all other OXPHOS complexes are increased in these tumors [15]. These tumors further showed dense mitochondrial proliferation suggesting an increase in mitochondrial biogenesis as an attempt to compensate for the potential loss of OXPHOS function. Complex I deficiency was also found in normal cells adjacent to the tumor tissue leading the authors to suggest that the complex I deficiency could be

an early event in the formation of renal oncocytomas [15]. Similarly, OXPHOS dysfunction and mitochondrial proliferation have also been reported in thyroid oncocytomas [16].

The current studies on potential effects of mtDNA mutations and OXPHOS dysfunction in cancer cells therefore suggest their role in tumor promotion and/or progression. It is believed that nuclear DNA mutations and not mtDNA mutations control the tumor initiation process. Akimoto and co-workers have tested this hypothesis [17]. They showed that cells carrying nuclear DNA from tumor cells and mtDNA from normal cells form tumors in nude mice whereas those carrying nuclear DNA from normal cells and mtDNA from tumor cells do not form tumors [17]. However, mutations in the mitochondrial enzymes that are encoded by the nuclear genome have been associated with certain types of cancer. Germ-line mutations in the tricarboxylic acid (TCA) cycle enzyme fumarate hydratase have been shown to be associated with uterine fibroids, skin leiomyomata, and papillary renal cell cancer [18], and mutations in the succinate dehydrogenase (SDH) subunits B, C, and D have been shown to be associated with hereditary paragangliomas and pheochromocytomas [19–21]. The potential role of these mutations in the tumor formation process is yet unclear.

3 Cellular Adaptations to OXPHOS Defects in Cancer

The cross-talk between mitochondria and nucleus plays an important role in maintaining mitochondrial function and integrity. Mitochondrial dysfunction affects the mitochondrial-nuclear cross-talk leading to altered signaling cascades (Fig. 2). The nuclear-mitochondrial stress signaling, also called retrograde signaling, is a phenomenon that involves changes in the nuclear gene expression in response to

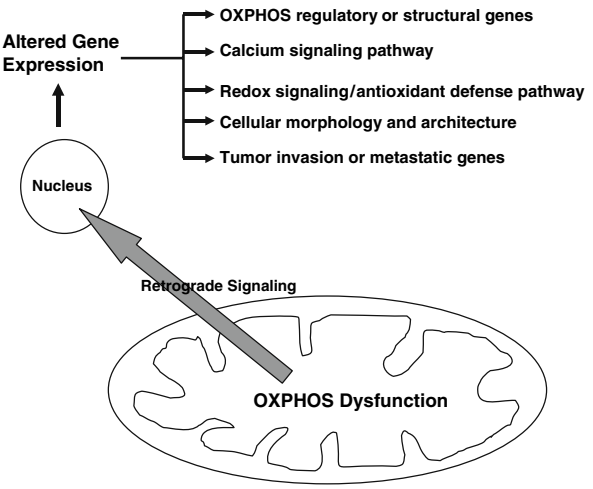

Fig. 2 Mitochondrial-nuclear intergenomic signaling in response to OXPHOS defects. Mitochondrial OXPHOS dysfunction in cancer cells can activate a retrograde signaling cascade. Altered retrograde signaling can affect the expression of various nuclear genes and cellular pathways that allow tumors cells to adapt to the environment and/or promote tumor cell growth or progression

OXPHOS dysfunction. These nuclear gene expression changes allow the tumor cells to adapt to the environment that may promote tumor cell growth and/or progression. Studies have shown that a variety of nuclear genes are altered in response to OXPHOS defects in different tumor cell types and have implicated their role in tumorigenesis.

3.1 Retrograde Signaling in Cancer Cells

Retrograde signaling has been implicated as an important mechanism in carcinogenesis although the precise mechanism(s) of this pathway is yet elusive in mammalian systems. Studies have shown that retrograde signaling in response to mitochondrial dysfunction can alter the expression of nuclear genes involved in energy metabolism, tumor invasion and metastasis, calcium signaling, redox signaling, anti-apoptotic genes, as well as genes involved in regulating cellular morphology and architecture.

3.1.1 Altered Expression of OXPHOS Regulatory Genes and Subunits

The mitochondrial OXPHOS complexes (I, III, IV, and V) comprise subunits encoded by both mtDNA and nuclear DNA. The expression of nuclear encoded OXPHOS subunits is under a tight regulation by regulatory genes, namely transcription factors and transcriptional coactivators. Nuclear respiratory factors 1 and 2 (NRF-1 and NRF-2) are the nuclear transcription factors, and the members of the peroxisome-proliferator activated γ coactivator 1 (PGC-1) gene family are the nuclear transcriptional coactivators that directly or indirectly modulate the expression of nuclear encoded OXPHOS genes, respectively. Besides regulating the expression of nuclear encoded OXPHOS genes, the NRFs also exert a regulatory control over the expression of mitochondrial transcription and replication machinery components, the protein import machinery components, and heme biosynthesis. A change in the expression levels or activity of NRFs and/or PGC-1 family of transcriptional coactivators would therefore influence the expression of OXPHOS subunits and mitochondrial biogenesis.

Altered expression of OXPHOS subunits and transcriptional regulatory genes has been reported in different tumor cell types [22, 23]. We studied a human colorectal tumor cell line (termed V425) harboring a homoplasmic nonsense mutation in the mitochondrial COXI gene. Despite the mtDNA mutation load, these cells were found to maintain a very high rate of mitochondrial respiration (∼75% of control cells) and a low level of COX activity (∼10% of control cells). Interestingly, osteosarcoma cybrids harboring the V425 mutant mtDNA showed a significant decline in respiration and COX activity [22]. Previous studies have shown that ∼10% COX activity cannot sustain a functional respiratory chain [24]. We found that the steady-state transcript levels of a transcriptional coactivator PGC-1α and its homolog PGC-1β were highly upregulated in V425 cells. In parallel, we also observed an increase in the steady-state levels of several mitochondrial proteins (e.g., SDH, COXIV, and cytochrome c) in these

cells. Further, the overexpression of PGC-1α in osteosarcoma cybrids harboring the V425 mutant mtDNA showed a significant improvement in mitochondrial respiration and an increase in the steady-state levels of several mitochondrial proteins suggesting that PGC-1α upregulation can improve mitochondrial respiration in a colorectal cancer cell line harboring COX deficiency [22]. It has also been shown that PGC-1α stimulates mitochondrial biogenesis and respiration by activating the expression of NRFs, augmenting their transcriptional activity or activating both the expression and transcriptional activity [25].

Altered expression of PGC-1 related coactivator (PRC), NRF-1, and mitochondrial transcription factor A (TFAM) have also been observed in thyroid oncocytomas harboring an OXPHOS defect [23]. Savagner et al. found that dense mitochondrial proliferation in 90% of the tested oncocytic tumors were associated with overexpression of the PRC compared with the controls suggesting that increased mitochondrial biogenesis might be a feedback mechanism to compensate for the presence of OXPHOS deficit in these tumors. They also found that the increase in PRC levels was associated with a 5-fold increase in NRF-1 transcripts, 10-fold increase in TFAM transcripts, and a 3-fold increase in COX activity further suggesting that the overexpression of PRC pathway is likely responsible for the increased mitochondrial proliferation in thyroid oncocytomas [23].

In contrast with the above studies, low levels of expression of peroxisome-proliferator activated receptor gamma (PPAR-γ) and PGC-1 have also been reported in breast cancer tissues [26]. Jiang et al. showed that the human metastatic breast cancer tissues have low levels of PPAR-γ and PGC-1 transcripts compared with those of the normal tissues. Further, lower levels of these molecules were found to be associated with poor clinical outcomes in breast cancer patients [26]. Interestingly, agonists of PPAR-γ have also been shown to inhibit growth and proliferation of cancer cells by inducing apoptosis [27].

3.1.2 Activation of Genes Involved in Tumor Invasion and Progression

OXPHOS dysfunction has been shown to modulate tumor invasive properties by transcriptional upregulation of genes coding for specific members of matrix remodeling pathway and activation of tumor-specific marker genes. van Waveren et al. showed that OXPHOS dysfunction can alter the expression of nuclear genes coding for factors involved in extracellular matrix remodeling in human osteosarcoma cells [28]. These genes included members of the matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMP) family, urokinase plasminogen activator and its inhibitor, plasminogen-activator inhibitor 1 (PAI-1), and CTGF and CYR61 (members of the cysteine-rich 61, connective tissue growth factor and nephroblastoma-overexpressed [CCN] gene family of growth regulators). Changes at the protein level for some of these factors have also been observed, though at a lesser magnitude. The study also showed that osteosarcoma cells harboring mtDNA mutations were associated with an increased matrigel invasion [28].

Activation of tumor-specific marker genes in response to an OXPHOS dysfunction was observed by Amuthan et al. [12, 13]. They found that partial mtDNA

depletion (genetic stress) or treatment of cells with mitochondrial-specific inhibitors (metabolic stress) activated Ca^{2+}-dependent kinases (PKC, ERK1, ERK2, and calcineurin) and signaling pathways that in turn activated the expression of nuclear genes involved in tumor invasion and progression. Among these genes, the extracellular matrix protease cathepsin L, transforming growth factor β (TGF-β), and mouse melanoma antigen (MMA) were found to be upregulated in both genetically and metabolically stressed rhabdomyosarcoma and pulmonary carcinoma cells. Using *in vitro* Matrigel invasion assays and *in vivo* rat tracheal xenotransplants in *Scid* mice, they further showed that these genetically and metabolically stressed cells were ∼4- to 6-fold more invasive than were their respective controls thereby implicating a role of OXPHOS dysfunction in inducing genes involved in tumor invasion and progression. The invasive behavior of mtDNA-depleted rhabdomyosarcoma and pulmonary carcinoma cells was reverted by the restoration of the mtDNA content [12, 13]. These findings clearly established a role of mitochondrial retrograde signaling in inducing phenotypic changes and tumor progression in osteosarcoma, rhabdomyosarcoma, and human pulmonary carcinoma cells. OXPHOS dysfunction was also shown to be associated with tumor aggressiveness in renal carcinoma cells [14]. Retrograde signaling in breast cancer has been reported by Delsite et al. They performed a comparative microarray analysis of the nuclear gene expression changes in a human breast cancer cell line (MDA-435) and its ρ^o derivative devoid of mtDNA. They found that the expression of several nuclear genes involved in cell signaling, cell growth, energy metabolism, cell architecture, cell differentiation, and apoptosis were altered in the ρ^o derivative of the breast cancer cell line [29]. Functional characterization of these genes is further required to gain a clear insight to the potential role of retrograde signaling in tumorigenesis.

3.1.3 Activation of Calcium Signaling Pathway

Changes in intracellular calcium (from the approximate 100 nM at rest) mediate a number of processes that affect tumorigenesis, including angiogenesis, motility, cell cycle, differentiation, transcription, telomerase activity, and apoptosis [30]. Mitochondria take up Ca^{2+} in a process dependent on a $\Delta\Psi m$ [31]. Ca^{2+} uptake by mitochondria controls organelle metabolic activity, as pyruvate, α-ketoglutarate, and isocitrate dehydrogenases are activated by Ca^{2+}. In addition, some metabolite transporters have been shown to be regulated by Ca^{2+} as well and to enhance aerobic metabolism. Mitochondria, by buffering local [Ca^{2+}] (generated by Ca^{2+} channels on the plasma membrane or the ER/SR), can also modulate intracellular Ca^{2+} [31]. Therefore, Ca^{2+} can regulate cell growth and survival by modulating OXPHOS function. Likewise, a defective OXPHOS impairs Ca^{2+} buffering. As described above, impaired Ca^{2+}buffering capacity by defective mitochondria can activate proteins involved in tumor invasion and progression, including PKC, ERK1, ERK2, and calcineurin.

OXPHOS dysfunction has been shown to activate calcium-dependent signaling events in several tumor cell types. Avadhani and co-workers investigated the nuclear gene expression changes in C2C12 rhabdomyosarcoma and human A549 pulmonary carcinoma cells in response to OXPHOS dysfunction and found that

calcium-dependent signaling events were activated in these cells [12, 13, 32]. OXPHOS defects associated with decreased mitochondrial membrane potential ($\Delta\Psi m$) and ATP synthesis caused a sustained increase in intracellular calcium $[Ca^{2+}]_i$ levels in these cells. They found that the expression of ryanodine receptor-1 (RyR-1) and ryanodine recptor-2 (RyR-2) genes was upregulated in C2C12 rhabdomyosarcoma and A549 pulmonary carcinoma cells, respectively. Increased RyR gene expression correlated with high levels of Ca^{2+}release from ER to the cytosol in response to caffeine stimulation (RyR agonist). Avadhani and co-workers suggested that the defect in $\Delta\Psi m$ was associated with the observed increase in $[Ca^{2+}]_i$ levels as mitochondria are key players in sequestering cytosolic Ca^{2+} and a defect in $\Delta\Psi m$ would affect mitochondrial Ca^{2+}uptake ability. Restoration of the $\Delta\Psi m$ in both C2C12 rhabdomyosarcoma and A549 pulmonary carcinoma cells was found to revert the ryanodine receptor gene expression and $[Ca^{2+}]_i$ levels to near normal levels suggesting a direct link between OXPHOS dysfunction and elevated $[Ca^{2+}]_i$ in these cancer cells. Ca^{2+} responsive factors such as calcineurin, calcineurin-dependent NFATc (cytosolic counterpart of activated T-cell–specific nuclear factor), and JNK (c-Jun N-terminal kinase)-dependent ATF2 (activated transcription factor 2) were also found to be elevated in both C2C12 rhabdomyosarcoma and pulmonary carcinoma cells. Further, Ca^{2+}-dependent PKC (protein kinase C) and MAPK (mitogen activated protein kinase) genes were activated in C2C12 rhabdomyosarcoma and lung carcinoma cells, respectively. Another study by Avadhani and co-workers has shown that calcineurin that is activated in response to elevated cytosolic Ca^{2+} inactivates I-κBβ thereby leading to an activation of the NF-κB/Rel family of transcription factors in the OXPHOS-deficient C2C12 rhabdomyosarcoma cells [33]. They suggested that the NF-κB/Rel family of transcription factors might be involved in the activation of nuclear genes in response to OXPHOS dysfunction in these cells [33]. Rise in cytosolic Ca^{2+} in response to OXPHOS dysfunction has also been observed in rat pheochromocytoma PC12 cells. Luo et al. showed that the treatment of PC12 cells with mitochondrial uncoupler FCCP releases $Ca^{2+}$from internal stores leading to a rise in cytosolic Ca^{2+}, which in turn activates MAPK also suggesting a potential link between OXPHOS dysfunction and Ca^{2+}signaling in these cells [34].

3.1.4 Activation of Redox Signaling Pathway

Changes in nuclear gene expression in response to oxidative stress have been observed in a variety of tumor cells. mtDNA mutations or OXPHOS dysfunction predispose mitochondria to the generation of ROS leading to oxidative stress. Cellular oxidative stress has been implicated in initiation, promotion, and progression of carcinogenesis [35]. Low levels of ROS generation have been shown to be mitogenic in a variety of human and mouse cell types [36, 37]. For example, under cell culture conditions, 10 nM to 1 μM concentrations of both $O_2^{\bullet-}$and H_2O_2 have been shown to stimulate the growth of hamster and rat fibroblasts [37]. Low levels of ROS were also shown to stimulate the growth of mouse epidermal cells, human fibroblasts, and human astrocytoma cells [38, 39]. The increased tumorigenesis of

NARP cells with a pathogenic mtDNA mutation described above [10] could also be related to increased ROS, as the mutation in ATPase 6 was reported to significantly increase ROS production [40]. Further, many chemical carcinogens initiate carcinogenesis by stimulating ROS production, which in turn activates the expression of early growth related genes such as c-fos, c-myc, and c-jun [41].

Although the molecular mechanism(s) of ROS-mediated cell growth are yet unclear, cellular ROS have been shown to activate the expression of redox-sensitive nuclear genes and signaling pathways. It has been shown that ROS activate the expression of nuclear factor kappa B (NF-κB) and activator protein (AP-1) transcription factors [42, 43]. Both NF-κB and AP-1 are redox-sensitive transcription factors. NF-κB in its active form consists of two subunits, p50 and p65. In the absence of a stimulus, it is present in the cytoplasm in association with its inhibitory subunit I-κB. In response to a stimulus that leads to the I-κB subunit phosphorylation, NF-κB dissociates from the I-κB inhibitory complex and translocates to the nucleus where it activates the expression of specific target genes [44]. AP-1 is a heterodimer of transcription factors composed of Jun, Fos, or activating transcription factor (ATF) subunits and binds the AP-1 sites in the promoter region to activate the target gene expression [45].

Constitutive activation of NF-κB and Ap-1 genes in response to oxidative stress has been observed in a variety of cancer cell types. Shi et al. demonstrated that OH radicals activated the expression of NF-κB in Jurkat cells, macrophages, and mouse epidermal cells, and antioxidants that scavenge OH radicals inhibited its activation [46]. High levels of ROS were also shown to cause a constitutive elevation of NF-κB and AP-1 transcription factors in transformed mouse keratinocyte cells [7]. Constitutive elevation of AP-1 activity has also been shown to be associated with the conversion of benign papillomas to malignant carcinomas in mouse epidermal cells suggesting that the target genes induced by activated AP-1 were involved in cell growth and metastasis [47]. Inhibition of Ap-1 activity by a dominant negative c-Jun mutant was shown to suppress the tumorigenic phenotype of the malignant mouse epidermal cells thereby blocking the tumor formation in nude mice [48]. Constitutive activation of NF-κB has also been shown to induce genes involved in tumor progression and metastasis [49]. Further, treatment with an antioxidant, *N*-acetylcysteine (NAC), has been shown to inhibit the elevated expression of NF-κB and AP-1 levels in tumor cells implicating the role of ROS in the activation of redox-sensitive signaling pathways [7].

The activities of NF-κB and AP-1 transcription factors are modulated by phosphorylation. Although the precise signaling events that activate the phosphorylation of these transcription factors in response to increase in cellular ROS levels are yet not fully understood, protein kinases are central in their activation. It has been observed that MAPK pathways are involved in signaling the activation of both NF-κB and Ap-1 transcription factors. There are three subtypes of MAPKs; the extracellular signal regulated kinases (ERKs), the c-jun N-terminal kinases (JNKs), and the p38 MAPKS. Gupta et al. showed that the NF-κB activation in malignantly progressed mouse keratinocytes was associated with an increase in ERK-1/2 and p38 MAPK activities and that NAC treatment rapidly abolished the increase in MAPK activities [7]. Direct activation of I-κB by MEKK1 (MAPK/ERK kinase kinase-1)

has also been demonstrated *in vitro* [50]. Activation of AP-1 by MAPK in response to various stimuli has been demonstrated [51]. There is also evidence that antioxidants can attenuate MAPK activation suggesting that MAPK signaling cascades are activated in response to cellular oxidative stress conditions [7].

3.1.5 Altered Antioxidant Defense Pathway

The antioxidant defense enzymes have been found to be altered in several human tumors. Superoxide dismutases (SODs) are the first line of cellular defense against increase in intracellular ROS. There are two forms of intracellular SODs: a manganese-containing SOD (MnSOD) that localizes to the mitochondria and a copper-zinc–containing SOD that localizes to the cytosol. Most human tumor cell types have been found to be deficient in their antioxidant defense function. Antioxidant enzymes including catalase and glutathione peroxidase have also been shown to be lowered in cancer cells [52, 53] suggesting that tumor cells have a deficient antioxidant function.

Studies have shown that antioxidant treatment prevents the malignant transformation of cancer cells. Increased expression of MnSOD has been shown to suppress cancer phenotypes in several murine and human cancer cells. St Clair et al. showed that overexpression of human MnSOD in mouse cells significantly reduced the frequency of radiation-induced neoplastic transformation implicating a direct link between mitochondrial antioxidants and neoplastic transformation [54]. Zhao et al. demonstrated that in response to tumor promoters (phorbol esters), transgenic mice expressing human MnSOD in the skin showed a significant reduction in papilloma formation compared with that of the non-transgenic controls [55]. They further found that the decreased papilloma formation was associated with a delay and reduction in AP-1 transcription factor binding activity suggesting that the MnSOD overexpression suppressed the tumor formation and Ap-1 activation in these transgenic animals [55]. Overexpression of human MnSOD has also been shown to suppress the malignant phenotype of human pancreatic and breast cancer cells. Li et al. demonstrated that MnSOD-overexpressing MCF-7 breast cancer cells showed a marked inhibition in growth rate *in vitro* under culture conditions and *in vivo* upon inoculation in nude mice when compared with the wild-type MCF-7 cells [56]. In another study, they demonstrated that the transcriptional and DNA binding ability of NF-κB and AP-1 were reduced by ∼50% in MnSOD-overexpressing MCF-7 cells [57]. They further showed that the expression of NF-κB and AP-1 responsive genes was downregulated in these cells when compared with that of the wild-type MCF-7 cells suggesting that the tumor-suppressive effects of MnSOD were associated with the inhibition of NF-κB and Ap-1 activities that in turn downregulated the genes involved in malignant progression [57].

3.1.6 Altered Mitochondrial Morphology, Cell Surface, and Architecture

Mitochondrial fission and fusion are the central events that regulate mitochondrial morphology. Changes in mitochondrial morphology appear to be a key event during retrograde signaling. Studies have implicated a role of mitochondrial morphology in

modulating respiratory activity. For example, downregulation of mitofusin 2 (Mfn 2) decreases mitochondrial respiration and increases the cellular glucose uptake levels for glycolysis. Further, ectopic upregulation of Mfn 2 increases the expression of OXPHOS subunits, glucose oxidation, and $\Delta\Psi_m$ [58]. Optic Atrophy 1 (Opa 1) is an important player in the formation of mitochondrial cristae. Downregulation of Opa 1 has been shown to induce the loss of mitochondrial respiration and increased mitochondrial fragmentation [59].

Changes in mitochondrial morphology and ultrastructure were reported in different tumor cell types over several decades. Tumor cell mitochondria were found to contain abnormal size, shape (dumbbell or cup), cristae organization, or inclusions. For example, rapidly growing hepatomas were found to contain small-size mitochondria with fewer cristae, whereas slowly growing hepatomas were found to contain large-size mitochondria with densely packed cristae [60]. Changes in the mitochondrial morphology in tumor cells could therefore affect their respiratory activity. Conversely, it is also possible that in response to altered respiratory activity or OXPHOS dysfunction, the retrograde signaling events in tumor cells affect the expression of nuclear genes involved in the regulation of mitochondrial morphology and ultrastructure.

Changes in cell morphology and architecture are a characteristic feature of many cancer cells. These changes occur in cell surface charge, glycoprotein composition, membrane organization, and/or the ability of cells to agglutinate on lectin. The initial evidence of a potential role of OXPHOS dysfunction in inducing cell surface changes was obtained in yeast. In 1980, Evans and colleagues showed that mitochondrial *petite* mutations in yeast that cause respiratory deficiency lead to drastic changes in cell surface charge, cell wall organization, and lectin agglutinability in contrast with the wild-type yeast cells suggesting that the OXPHOS deficiency in yeast altered the retrograde signaling pathway leading to gene expression changes and hence altered cell surface properties [61]. Preliminary evidence in literature has also suggested a link between OXPHOS dysfunction and cell surface properties in mammalian cells. For example, Soslau et al. showed that baby hamster kidney cells when treated with ethidium bromide (Etbr) show an altered glycoprotein composition in the plasma membrane [62]. Further, they also showed that the glycopeptide elution profile of these Etbr-treated cells was similar to that of the cells transformed with Rous sarcoma virus. Low concentrations of Etbr are known to intercalate to mtDNA and abrogate mtDNA replication. Exponentially growing cells when treated with Etbr (25 ng/mL to 2 μg/mL) undergo mtDNA loss leading to either partial or complete loss of mtDNA molecules, generating an OXPHOS defect. These findings therefore suggest that altered retrograde signaling events during OXPHOS dysfunction could activate nuclear genes or pathways that cause global changes in cell surface properties.

3.1.7 Activation of Antiapoptotic Genes

Activation of antiapoptotic genes is a common occurrence in most human cancers. However, retrograde signaling pathway has also been shown to activate

antiapoptotic genes (e.g., Bcl-x_L and Bcl-2) in certain cancer cell types [12, 63]. Increased expression of the antiapoptotic genes may also contribute to metabolic homeostasis. It has been shown that members of the Bcl-2 antiapoptotic family of proteins regulate ATP/ADP exchange across the mitochondrial membranes and can prevent the loss of mitochondrial respiration during apoptosis [64]. Manfredi and colleagues showed that Bcl-2/Bcl-x_L can improve OXPHOS function in cells harboring pathogenic mtDNA mutations. The effect of Bcl-2 overexpression in mutated cells was found to be independent from apoptosis and was suggested to modulate the adenine nucleotide exchange between mitochondria and cytosol. This study therefore provided evidence that when OXPHOS function is reduced, Bcl-2 expression can improve it by regulating the levels of adenine nucleotides inside the mitochondria [64].

3.2 Mechanism of Retrograde Signaling

The mechanism of retrograde signaling was first identified in yeast and it is still poorly understood in other systems. Three retrograde regulatory genes (RTG1, RTG2, and RTG3) play a central role in retrograde signaling in yeast [65] (Fig. 3). Rtg1p and Rtg3p are transcription factors, whereas Rtg2p is mitochondrial function sensor. During normal mitochondrial function, Rtg1p and Rtg3p form heterodimers

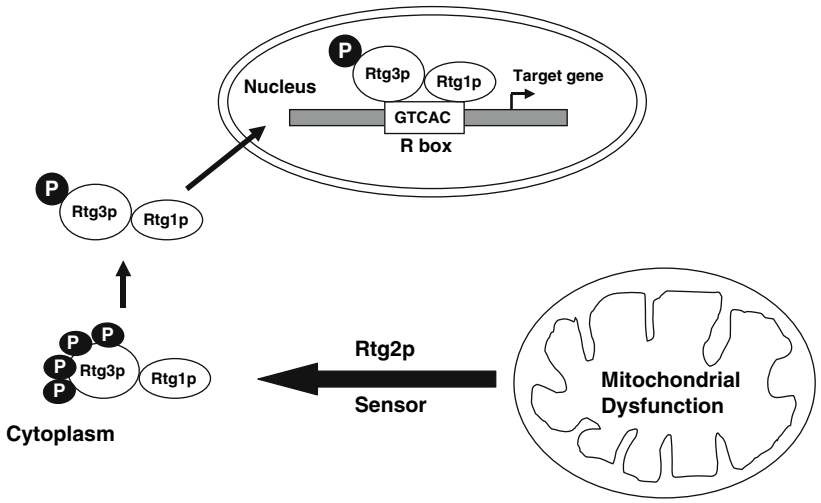

Fig. 3 Schematic model of retrograde signaling in yeast. RTG genes play a central role in retrograde signaling. Rtg1p and Rtg3p are sequestered in cytoplasm during normal mitochondrial function. Rtg3p is highly phosphorylated during this state. In response to mitochondrial dysfunction (sensed by Rtg2p), the Rtg3p undergoes partial dephosphorylation. Both Rtg3p and Rtg1p translocate to the nucleus and activate target gene expression leading to changes in nuclear genes and/or signaling pathways that in turn can alter cellular function and homeostasis

and are sequestered in the cytoplasm. Rtg3p is phosphorylated at multiple sites during this state. In response to mitochondrial dysfunction (sensed by Rtg2p), a retrograde signaling pathway gets activated. The Rtg3p undergoes partial dephosphorylation and translocates to the nucleus. Rtg1p also follows and translocates to the nucleus. Both Rtg1p and Rtg3p activate transcription at the target genes. In the absence of Rtg2p, the Rtg1p/Rtg3p complex remains in the cytoplasm during active retrograde signaling pathway implicating its role as mitochondrial function sensor and in signal relay to the Rtg1p/Rtg3p complex [65].

The precise mechanism of retrograde signaling in mammalian systems is yet not known. The mammalian homologs of RTG genes have yet not been identified. However, human MYC protein has been found to share a significant homology with yeast Rtg3p, and it is involved in the regulation of many cellular functions such as regulation of glycolysis, cellular stress response cell cycle progression, and apoptosis [66, 67].

4 Conclusion

Mitochondrial dysfunction has been reported in a variety of human cancers. Mitochondrial-nuclear intergenomic signaling is an important phenomenon in regulating mitochondrial and cellular function and homeostasis. Although the role of mtDNA mutations or OXPHOS defects in cancer progression is poorly understood, a considerable body of evidence has now shown that mitochondrial function has an important role in different aspects of tumorigenesis. Processes such as cell survival and cell invasion can be directly influenced by OXPHOS function. Less clear is the association between cell cycle progression and an OXPHOS defect. However, specific examples, such as SDH mutations in paragangliomas and pheochromocytomas, provide proof of principle that OXPHOS defects can somehow stimulate cell division.

It appears likely that some properties related to OXPHOS dysfunction can have an advantageous consequence for tumor development. However, this feature could impair cell performance at a different level. Therefore, tumor cells with an OXPHOS defect, in some cases, have compensatory mechanisms, such as an increase in mitochondrial biogenesis via overexpression of PGC-1 family members. Studies on the interplay between these metabolic and signaling pathways will help us better understand the basic mechanisms of tumor progression.

Note

A recent study by Ishikawa et al. (Ishikawa K, Takenaga K, Akimoto M, et al. ROS-generating mitochondrial DNA mutations can regulate tumor cell metastasis.

Science. 2008;320(5876):661–664) showed that specific mtDNA changes in complex I subunit can increase the metastatic properties of tumor cells.

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