Review

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Androgen receptor signaling–mitochondrial DNA–oxidative phosphorylation: A critical triangle in early prostate cancer

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Abstract

Mitochondria are more than just the cellular powerhouse. They also play key roles in vital functions such as apoptosis, metabolism regulation, and other intracellular interactions. The mitochondrial DNA (mtDNA) encodes for 12 subunits of the oxidative phosphorylation (OXPHOS) system. Depletion of mtDNA in androgen-dependent prostate cancer (PCa) cell lines renders them androgen-independent and more aggressive. Paradoxically, pharmaceutical inhibition of OXPHOS is lethal for subsets of PCa cells, whereas others become dependent on androgen receptor (AR) signaling for survival. Given that the AR-mitochondria interaction is critical for early PCa, it is crucial to understand the details of this interaction. Technical hurdles have made mitochondria traditionally difficult to study, with many techniques used for isolation masking the properties of given individual mitochondria. Although the isolation of mitochondria enables us to study OXPHOS, we miss the context in which mitochondria interact with the rest of the cell. Both AR signaling and mtDNA affect apoptosis, metabolism regulation, cellular calcium storage and homeostasis, intracellular calcium signaling, and redox homeostasis. In this review, we will attempt to understand how the crosstalk between AR-mtDNA-OXPHOS is responsible for "life or death" decisions inside the cells. Our aim is to point toward potential vulnerabilities that can lead to the discovery of novel therapeutic targets.

Keywords: Androgen receptor; Mitochondria; Oxidative phosphorylation; Prostate cancer

1. Introduction

Mitochondria are mostly known for their role as the cellular powerhouses [1]. The mitochondrial oxidative phosphorylation (OXPHOS) system consists of 5 multisubunit complexes.[2] These complexes play a critical role in electron transfer and cellular energy production in the form of ATP. Mitochondria also regulate cellular metabolism, cellular calcium homeostasis, cell proliferation, and apoptosis.[1] They also play a role in steroid synthesis, hormonal signaling, immune signaling, heme synthesis, and so on.[1] Moreover, mitochondrial defects have been implicated in tumorigenesis and cancer aggressiveness.[3–5] The resulting oxygen accumulation has been linked to the expression and activation of protumorigenic signaling such as Ras, ERK, Akt, and nuclear factor κB.[6–9] Mitochondrial dysfunction also has a direct inhibitory effect on apoptosis and the function of tumor suppressors (eg, p53).[10–12] In addition, OXPHOS dysfunction favors a metabolic shift toward glycolysis, which is known as the Warburg effect, a well-established hallmark of cancer.[13]

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2. Literature review

2.1. Mitochondrial DNA

The vast majority of mammalian mitochondrial proteins (approximately 1200) are encoded and expressed by the nuclear genome, but a small subset of these proteins is encoded by mitochondrial DNA (mtDNA).[2,14] The protein components of complexes I, III, IV, and V are encoded both from nuclear DNA and mtDNA.[15,16] The subunits of complex II are encoded only by nuclear DNA.[16] The mtDNA encodes for 12 subunits of the OXPHOS system, 2 ribosomal RNAs, and 14 tRNAs.[17,18] It encodes for subunits 1, 2, 3, 4, 5, and 6 of complex I, cytochrome b of complex III; Subunits 1, 2, and 3 for complex IV; and F0 subunits 6 and 8 of complex V (ATP synthase).[19–22] Reduced levels or defective mtDNA can cause imbalances in the structure of the OXPHOS complexes and result in defective mitochondrial respiration. Although the main activity of these proteins is related to OXPHOS and energy production, they are not limited solely to their role as parts of the respiratory chain. For example, protein 1 of complex I is a substrate of caspase-3 and plays a critical role in the induction of apoptosis; complex IV has been observed in extra-mitochondrial locations, and so on.[23,24] It has also been proposed that mutations or deletions in mtDNA might play a causative role in tumorigenesis and cancer aggressiveness, but robust experimental evidence is still lacking.[25,26]

2.2. Prostate cancer and mitochondrial DNA

Prostate cancer (PCa) is among the most frequently diagnosed cancers in the world. One of the most notable characteristics of the disease is the tumor cell dependence on the androgen receptor (AR) for activation of luminal differentiation, proliferation, and survival.[27] The role of mitochondria in PCa is also very important.[28]

Depletion of mtDNA prevents apoptosis and induces PCa progression.[29] It also induces epithelial-to-mesenchymal transition and promotes tumor cell motility by upregulating phosphatidylinositol-3-kinase/Akt2 signaling.[30] Reduction of mtDNA can also result in a more invasive phenotype via increased migration onto the basement membrane protein laminin-1 and decreased expression of poly (ADP-ribose) polymerase 1.[31] In vitro studies have shown that the depletion of mtDNA from androgen-dependent lymph node carcinoma of the prostate (LNCaP) cells results in androgen-dependence loss.[32,33] Strikingly, the depleted cells grow significantly faster than the parental cell line. Restoration of mtDNA results in restoration of androgen dependence. The androgen-independent cell line C4-2 established by inoculation of LNCaP cells in castrated mice has 8 times less amount of normal mtDNA than the parental LNCaP. Androgen-independent cell lines PC3 and DU125 exhibit less mtDNA content than LNCaP cells as well. These results indicate that mtDNA is connected to androgen dependence in PCa cells. Interestingly, mtDNA-depleted LNCaP cells in the absence of androgens grow faster than both LNCaP cells and C4-2 cells.[32,33] This is particularly surprising because most mtDNA-depleted cells grow slower than their parental cells, because of loss of energy generation via normal cellular respiration. In vivo studies in athymic nude mice were also in line with these findings, where mtDNA depletion was sufficient to render the cells androgen-independent and resistant to the effects of androgen deprivation.[32]

2.3. Oxidative phosphorylation inhibition in prostate cancer

It is well known that PCa metabolism relies more and more on glycolysis as it evolves toward the aggressive phenotype.[28–33] Based on the previous results, one would expect that the pharmaceutical OXPHOS inhibition in androgen-dependent cancer cells will result in a cellular reprogramming that renders them androgen-independent, more aggressive, and more dependent on aerobic glycolysis. What is interesting is that when we inhibit OXPHOS in LNCaP cells, we observe the exact opposite effect in vitro and in vivo. In the context of androgen deprivation and OXPHOS inhibition, almost all LNCaP cells eventually die, mostly through apoptosis.[34–37] The cells not only do not become androgen-independent when exposed to oligomycin (complex V inhibitor) or IACS-010729 (complex I inhibitor), but also the addition of androgens has a prosurvival effect. Oxidative phosphorylation inhibition in C4-2 and C4-2B cells (androgen-independent derivatives of LNCaP cells) poses a similar antitumor effect, albeit smaller. The addition of androgens also has a prosurvival effect in C4-2 and C4-2B cells under OXPHOS inhibition. Moreover, in vertebral cancer of the prostate (VCaP) cells, the combination of OXPHOS inhibition with oligomycin and androgen deprivation is lethal for all cells within 72 hours in vitro. When VCaP cells under oligomycin are exposed to androgens, not only do they escape death, but they also continue growing. VCaP cells harbor increased AR expression via AR gene amplification.

These results are interesting and suggest that OXPHOS inhibition might be used to synergize the antitumor effects of androgen deprivation in subsets of androgen-dependent PCa cells. One must be careful, though, because complex V inhibition does not equal complex I or II or III or IV inhibition. For example, in PCa, succinate anaplerosis has a tumor-promoting effect and restores cellular respiration through complex II.[38] This can overcome complex I inhibition. We also mentioned previously that subunit 1 of complex I is a substrate of caspase-3.[23] Although inhibition of complexes III, IV, or V can trigger proapoptotic signals, complex I inhibition can potentially sabotage apoptosis.[39] It would be preferable to target complex III, IV, or V, but so far, toxicity has been limiting our efforts. At this time, mostly complex I inhibitors are being studied in clinical trials.[40] Only time will tell if there is a therapeutic window to use various OXPHOS inhibitors in clinical practice for the treatment of PCa patients. Interestingly, reformulated niclosamide, a splice-variant AR-V7 inhibitor, has shown promising synergistic antitumor effects with androgen deprivation in PCa patients.[41] Niclosamide is also a potent mitochondrial respiratory chain uncoupler, suggesting that at least part of its activity might be due to OXPHOS inhibition.[42]

2.4. Androgen receptor effects in mitochondria

We previously mentioned that mtDNA mutation or depletion contributes to PCa tumorigenesis, induces androgen independence, and enhances proliferation. However, pharmaceutical OXPHOS inhibition is detrimental for androgen-dependent cells, whereas androgens have a positive survival effect. What is the cause of this paradox?

Complete depletion of mtDNA makes LNCaP cells proliferate faster, but pharmaceutical OXPHOS inhibition makes them undergo apoptosis or necrosis. Thus, the presence of some mitochondrial activity causes LNCaP cells to undergo apoptosis under pharmaceutical OXPHOS inhibition and/or prevents a metabolic shift toward glycolysis to generate ATP to avoid necrosis. And this activity is (at least partly) inhibited by AR. It is well known that AR activity and mitochondria are connected.[43,44] Upon activation, AR is imported into the cells and localized to the mitochondria. There it plays multiple roles in regulating multiple mitochondrial processes. The import of AR in the mitochondria is dependent on a 36-amino-acid-long mitochondrial localization sequence. Increased expression of AR decreases OXPHOS. Mitochondrial impairment increases AR expression and in turn increases its localization inside the mitochondria.[44] This creates a loop that enables cancer cells to survive AR ablation or OXPHOS inhibition by upregulating OXPHOS or AR signaling, respectively.

2.5. Apoptosis

It is well known that AR inactivation or inhibition has proapoptotic effects in PCa cell lines.[45] The 2 most understood apoptotic mechanisms include the intrinsic pathway and the extrinsic pathway. The intrinsic pathway is also known as the mitochondrial pathway. Intracellular signals generated from cellular stress, such as energy depletion (eg, from OXPHOS inhibition), hypoxia, heat, radiation, nutrient deprivation, infection, or increased intracellular calcium concentration activate the intrinsic pathway of apoptosis.[45–48] A multitude of Bax/Bak homodimers and heterodimers are then inserted into the outer mitochondrial membrane, which results in the release of cytochrome c from the mitochondria. Cytochrome c binds with apoptotic protease activating factor-1 and ATP to create the apoptosome. The apoptosome cleaves procaspases to create caspase-3 and caspase-9. Mitochondria also release proteins known as second mitochondria-derived activator of caspases, which bind to cytosolic factors, which inhibit apoptosis, thereby inactivating them and further promoting apoptosis.[49] Hence, the presence of functional mitochondria is critical for the execution of apoptosis after the energy depletion that OXPHOS inhibition causes. Several studies demonstrated that mtDNA depletion prevents apoptosis and contributes to tumor progression and metastasis.[50–54] Subunit 1 of complex I is a substrate of caspase-3. Caspase-3, apart from its essential role in the apoptotic body formation, also functions before or at the stage when commitment to loss of cell viability is made.[23,55] Androgen withdrawal triggers the programmed cell death in androgen-dependent PCa cells.[56] Hence, both AR signaling and mtDNA depletion have prosurvival effects. However, the interplay between AR and apoptosis is complex.[57] For example, the

apoptosis-inducing activity of tumor suppressor protein retinoblastoma is AR-dependent.[58] Androgen-independent PCa cells do not initiate apoptosis during androgen deprivation, but they retain the ability to do so when sufficiently damaged by exogenous factors.[59]

2.6. Calcium cell storage and homeostasis

Mitochondria regulate calcium cell homeostasis and calcium storage.[60,61] Calcium ions regulate every aspect of cell function, including gene expression, movement, secretion, proliferation, metabolism, and so on. Calcium signals have to be tightly regulated to prevent cell injury.[62] Prostate cancer cells are characterized by calcium signals that are different from those in normal cells with regard to subcellular localization, amplitude or kinetics.[63,64] Calcium-dependent modifications in PCa cells rely on altered calcium homeostasis, which is caused by alterations in plasma membrane and endoplasmic reticulum channels, as well as gap junctions.[65] These changes result in influx/efflux ratio changes and altered calcium storage and sequestration.[64,66] It is known that mtDNA mutations/polymorphisms result in defective mitochondrial calcium regulation.[67,68] Increased levels of cytosolic calcium might overactivate calcium-binding proteins involved in tumor cell progression. One example is calcium/calmodulin-dependent kinase II (CAMKKII), which seems to play an important role in the ability of PCa cells to evade apoptosis (inhibits caspase-7 and caspase-8) and to progress into an androgen-independent state.[69] Androgen receptor signaling also promotes CAMKKII signaling. Calcium signaling is involved in the activity of AR on PCa proliferation.[69,70] Moreover, in LNCaP cells, androgen-dependent increases in intracellular calcium concentrations have been observed.[71] Upregulation of T-type calcium channels increases several proliferative signals such as Akt kinase, mTOR, CDK4, and others.[72,73] Androgen-dependent activation of CAMKKII signaling also promotes the glucose transporter GLUT12 trafficking to the plasma membrane and shifts cellular metabolism toward glycolysis.[74] In addition, CAMKKII overexpression promotes PCa growth via de novo lipogenesis.[75]

2.7. Metabolism regulation

Normal prostate epithelial cells halt tricarboxylic acid cycle to increase the secretion of citrate, which enhances sperm viability. In order to cover their energy needs, they use a relatively glycolytic metabolism.[76,77] On the other hand, early-stage prostate adenocarcinoma reprograms metabolism to enable tumor progression, by consuming citrate to power OXPHOS and to fuel lipogenesis. Despite the strict definition of the Warburg effect as aerobic glycolysis, early PCa displays both increased glycolytic and mitochondrial activity.[78] Androgen receptor signaling directly or indirectly regulates the expression and activity of several metabolism-related enzymes, such as fatty acid synthase, α-methylacyl-CoA-racemase or hexokinase 1/2. It also regulates the axis CAMKK2-AMPK-phosphofructokinase, Myc, SLC2A1 (facilitated glucose transporter), and so on. Hence, AR activity is a major regulator of metabolism in PCa and promotes both glycolysis and OXPHOS.[79] However, metabolism regulation in PCa cells is very complex and does not rely solely on AR. For example, c-Myc contributes to metabolic adaptations favoring glycolysis and glutaminolysis, often with the cooperation of hypoxia-inducible factor α and/or a mutated p53.[78,80,81] It is known that androgen-dependent PCa cells use a metabolic switch to survive androgen ablation.[36] Respiration-deficient LNCaP cells due to mtDNA depletion begin to grow in an androgen-independent manner.[28,29] It is evident that androgen-independent mechanisms rewire cellular metabolism to promote tumor cell survival and proliferation. However, potent OXPHOS inhibition with oligomycin makes subsets of PCa cells dependent on AR for survival.[34] This suggests that, in these cells, the mechanism that enables cells to switch their metabolism and become aggressive is likely still present, but these cells appear (for reasons not yet fully elucidated) to "choose" to die, unless AR signaling prevents them.

2.8. Reactive oxygen species formation

Mitochondrial respiration is a leading source of reactive oxygen species (ROS) that can cause considerable cell damage and even trigger cell death if levels become too high. Cancer cells have higher ROS than their noncancerous cells of origin.[82] The presence of basal levels of ROS is essential for protumorigenic signaling, important for cell survival, differentiation, and proliferation.[83] In PCa, chronically increased intracellular ROS (mainly via 5-lipoxygenase activation) results in ligand-independent Src-mediated activation of prosurvival epidermal growth factor receptor signaling.[84] The proliferative activity of LNCaP cells increases when exposed to low H_2O_2.[85] On the other hand, excessive amounts of ROS can trigger oxidative damage and cellular death.[83] Cancer cells counterbalance the detrimental effects of ROS by upregulating the production of antioxidant molecules, such as reduced glutathione and thioredoxin, which rely on the reducing power of nicotinamide adenine dinucleotide phosphate to maintain their antioxidative activities.[86] Mutations or deletions in mtDNA may result in defective respiratory chains and increased ROS production.[87] On the other hand, mtDNA depletion results in decreased ROS levels. This can affect cell proliferation and survival in variable ways, depending on the cell type.[82,83] Pharmacological inhibition of OXPHOS can also have a variable effect in ROS production. This depends on the specific site of the respiratory chain that the drug acts on. However, the most widely used OXPHOS inhibitors, such as rotenone, antimycin-C, or oligomycin, impede proton flow and promote ROS production.[88] Increased AR signaling has been shown to increase basal ROS levels in PCa cells.[89] Moreover, AR signaling enhances tumor cell proliferation, which in turn increases ROS levels.[90] The presence of ROS is required for androgen stimulation in androgen-dependent cells.[91] Increased ROS levels upregulate AR, whereas decreased ROS levels downregulate AR.[92,93] Moreover, AR signaling contributes to the redox balance by upregulating 6-phosphogluconate dehydrogenase, thus activating the pentose phosphate pathway and the production of nicotinamide adenine dinucleotide phosphate.[94] Androgen receptor also regulates glutathione S-transferases and attenuates oxidative stress in a redox environment.[95]

2.9. Role of tumor suppressors

Various PCa cell lines have different metabolism regulation mechanisms and different metabolic dependencies. The tumor suppressor status is a well-known determinant of how cells respond to mitochondrial damage and metabolic stress.[11,12] *PTEN*, *retinoblastoma*, and *TP53* are the most recurrently altered tumor suppressor genes in treatment-resistant PCa.[96] Cooperative loss of 2 or more of them is frequently a sign that the disease has progressed into an aggressive variant.[97] PTEN loss is found in more than 40% of metastatic castrate-resistant PCs.[98] PTEN protein is a well-known metabolism regulator.[98] PTEN loss can promote tumor growth via increased metabolic flux of glycolysis, glutaminolysis, and fatty acid metabolism.[99–101] Interestingly, mitochondrial complex I inhibition with deguelin was found to be selectively toxic for PTEN-deficient PCa cells.[102] This is in line with experiments showing that LNCaP cells do not survive pharmacologic complex I or complex V inhibition. LNCaP cells harbor one mutated *PTEN* allele and one deleted allele and do not express PTEN protein.[103] *TP53* is not only the

guardian of the genome that protects against ROS formation, but also an important metabolic regulator.[104,105] Aberrations in *TP53* can result in increased aerobic glycolysis. *TP53* protects against mtDNA mutations or deletions, and loss of *TP53* results in mtDNA depletion.[106] This further promotes an energy flux toward aerobic glycolysis. VCaP cells harbor mutated *TP53*.[107] In the presence of AR signaling, VCaP cells not only survive ATP synthase inhibition with oligomycin, but they also continue growing. *Retinoblastoma* deletion in PCa also increases tumorigenic growth by reprogramming lipid and amino acid metabolism.[108] It also protects against ROS formation through increased glutathione synthesis.[108] Other tumor suppressors such as *ATM*, *BRCA1/2*, and so on, can also play a role in the metabolism regulation of PCa cells.[109–111] Sometimes, the activity of tumor suppressor genes can be affected by the status of other suppressor genes or other regulatory genes.[112,113] In this case, the combination of genetic aberrations becomes more important than the status of individual genes. Given that cancer cells usually harbor numerous mutations, it is evident that every cancer carries a unique genetic signature. This implies that the tumor models that we usually use in research laboratories have limitations as they are only approximations of subsets of real-life cancers.

3. Conclusions

Technical hurdles have made mitochondria traditionally difficult to study, with many techniques used for isolation masking the properties of given individual mitochondria. Given that AR-mitochondria interaction in early PCa cells is responsible for life or death decisions, it is crucial to understand the details of this interaction. This will enable us to identify mechanisms that can be targeted alone or in combination with antiandrogen therapy. The selective toxicity of antiandrogen therapy to PCa cells can be exploited either via the discovery of a synergistic mechanism or by reducing the dose of other drugs to levels nontoxic to normal cells.

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Statement of ethics

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