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Diving into the cellular puzzle: Exploring the connection between mitochondrial DNA depletion and prostate cancer development

Dilbeste DEMİR YEŞİLYURT *, Sercan ERGÜN, Neslihan TAŞKURT HEKİM, Sezgin GÜNEŞ

Department of Medical Biology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Türkiye

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Abstract

Mitochondria, essential components of eukaryotic cells, play a central role in generating cellular energy, regulating metabolism, and facilitating cellular interactions. A distinguishing characteristic of mitochondria is their unique circular double-stranded DNA, known as mitochondrial DNA (mtDNA), essential for energy synthesis and overall mitochondrial function. MtDNA depletion is a notable decrease in mtDNA levels and is affected by a combination of genetic and environmental elements. Genetic contributors include inherited mutations, nuclear DNA changes that impact mitochondrial activity, and mutations within the D-loop region of mtDNA. Environmental factors encompass exposure to specific medications, oxidative stress, and insufficient nutrient consumption. Prostate cancer, a primary contributor to male cancer fatalities, has been associated with anomalies in mtDNA structure and function. The involvement of mitochondria in prostate cancer is intricate, influencing energy metabolism, the stability of the genome, and the emergence of aggressive, androgen-independent cancer variants. Notably, mtDNA depletion is implicated in the shift from androgen-sensitive to androgen-resistant prostate cancer, emphasizing its crucial role in disease advancement. Additionally, mtDNA depletion correlates with the development of a cancer stem cell-like phenotype, marked by increased tumor aggressiveness and heightened resistance to therapeutic agents. Research indicates that changes in mtDNA quantity are linked to the progression and prognosis of prostate cancer. Elevated levels of POLRMT, a nuclear enzyme essential for mtDNA synthesis, have been associated with prostate cancer proliferation, while diminished mtDNA levels are linked to increased invasiveness and a shift towards a mesenchymal cell state. In summary, grasping the intricate relationship between mtDNA depletion and prostate cancer is essential for formulating targeted treatment approaches and enhancing patient outcomes. This review article emphasizes the critical role of mtDNA in the advancement of prostate cancer and underscores its potential as a therapeutic focal point in addressing this widespread cancer.

Keywords: prostate cancer, androgen dependence, cancer stem cell, mitochondrial DNA depletion, mitochondrial DNA copy number

1. Introduction

Mitochondria, known as the power plant of eukaryotic cells, play a role in critical cellular activities as well as ATP production by oxidative phosphorylation. Mitochondria are involved in cellular energy production (1), metabolic regulation (2), regulation of reactive oxygen species (ROS) (3), apoptosis and cell fate (4), calcium homeostasis (5), cellular communication (6), and mitochondrial inheritance (7). In particular, mitochondria form a unique structure with mtDNA located in their matrix. The complex connection between mitochondria and mitochondrial DNA (mtDNA) highlights the essential functions that these organelles fulfil in both cellular energy production and genetic information transfer.

Cells structurally have more than one mitochondrion, depending on the cell type and energy requirements. Mitochondria have circular double-stranded DNA about 5µm length. Each mitochondrion contains 2-10 mtDNAs (8). Since the number of mtDNA per cell shows variation in tissue and different developmental stages, thousands of mtDNAs are reported to be present in an organism. ATP demand, nucleotide availability and replication origin regulation are among the factors that affect the number of mtDNAs in cells (9). The mtDNA encodes genes required for energy production and mitochondrial function. The size of mtDNA varies between different organisms, but is usually several kilobases in length. In humans, for example, mitochondrial genomes are approximately 16.569 base pairs long, contain no introns and carry two non-coding regions. Therefore, the mitochondrion is comprised of almost entirely of coding regions (8). MtDNA has a higher mutation rate compared to nuclear DNA. This is thought to be related to the generation of high levels of ROS during the energy production process in mitochondria, which lacks a DNA repair system (10).

Similarly, mtDNA is highly susceptible to oxidative damage due to the lack of histone proteins. Unlike nuclear DNA, which is packaged with histone proteins to form chromatin structure, mtDNA lacks histone proteins (11).

*Correspondence: dilbestedemir@gmail.com

Instead, it forms a nucleoid structure through the organization of a compact protein-DNA complex with the transcription factor TFAM (Mitochondrial transcription factor A) and proteins. The nucleoid structure regulates processes such as protection, replication and transcription of mtDNA. The nucleosome structure formed by histone proteins in nuclear DNA protects DNA against free radicals. At the same time, however, in the event of ROS-induced damage, nuclear DNA is compensated by various repair mechanisms and enzymes. However, since the nucleoid structure in mtDNA does not provide complete protection, it is usual for reactive oxygen species to reach mtDNA and cause damage. Furthermore, the limited DNA repair systems in the mitochondrial genome increase the sensitivity of mtDNA to ROS. This makes mtDNA vulnerable to oxidative damage and is associated with mitochondrial diseases (12).

Recently, mtDNA depletion, associated with nuclear DNA mutations, has attracted attention. MtDNA depletion is defined as a significant reduction or depletion of mtDNA content or copy number in mitochondria (13). This phenomenon is influenced by genetic and environmental factors. In terms of genetic factors, mtDNA depletion syndrome is associated with both the mitochondrial genome and the nuclear genome. Therefore, mtDNA depletion is associated with nuclear genes (14). In particular, mutations in nuclear genes involved in the mitochondrial nucleotide pool (TK2, SUCLA2, SUCLG1, RRM2B, DGUOK, MPV17, TYMP), mutations in nuclear genes involved in mtDNA replication (POLG, Twinkle, POLRTM, TFAM, TEFM) and mutations in the mitochondria-specific D-loop region explain the link between mtDNA depletion syndrome and nuclear genes (15-18). Not only are mitochondria critical for cellular function, but defects in mtDNA structure or function have been linked to many diseases, including cancer (19, 20).

Cancer and mtDNA depletion are associated in many aspects, such as energy metabolism, genomic instability, ROS production and tumor microenvironment (19). The study of mtDNA depletion is particularly relevant for prostate cancer, which is one of the leading causes of death after lung cancer in the male population and has limited therapeutic potential. Understanding the link between mtDNA depletion and prostate cancer will contribute to the development of new methods to investigate the complex molecular mechanisms behind the initiation and development of this common cancer.

2. Mitochondrial DNA depletion mechanisms

The leading causes of mitochondrial DNA depletion are genetic and environmental factors. Genetic factors are classified as hereditary, nuclear DNA mutations and *D-loop* region mutations (21-23). In addition, point mutations, large deletions or insertions, structural chromosomal changes in mtDNA can have critical consequences such as deletion, insertion or rearrangement of genes in mtDNA (24).

Individuals may inherit genetic mutations encoding enzymes required for mitochondrial DNA replication and repair. Thus, the appearance of defective proteins during mtDNA replication and repair may increase the susceptibility of individuals to mtDNA depletion (25). However, when mutations are evaluated in terms of nuclear genes, it is seen that the mitochondrial genome carries a gene anatomy different from but in touch with the nuclear genome. Therefore, mutations in nuclear DNA will affect mitochondrial function. The mitochondrial nucleotide mechanism requires a functional replication process and an adequate supply of the mitochondrial deoxyribonucleotides required for this process. In this context, defects in the mitochondrial nucleotide mechanism, related to nuclear genes will ultimately lead to diseases associated with mitochondrial depletion (22). *D-loop* mutations, which are highly prevalent in cancer cells, have a high mutation rate due to their triple helix structure and susceptibility to oxidative damage and constitute one of the main causes of mtDNA depletion. The *D-loop* region contains all the necessary materials for mitochondrial transcription and replication and is responsible for the regulation of this process. *D-loop* region mutations can alter the promoter sequences of mtDNA transcription modulators; therefore, due to the change in their protein binding affinity, faulty replication occurs, insufficient amounts of mtDNA are transferred into cells and mtDNA depletion results (23).

When mtDNA depletion is evaluated in terms of environmental factors, some chemotherapeutic agents or antiretroviral drugs, high production of ROS, inadequate intake of essential nutrients, ionizing radiation and environmental toxins can increase toxicity in mitochondria and results in mtDNA depletion (21). To understand the relationship between genetic and environmental factors, which are the leading causes of mtDNA depletion, a more comprehensive investigation is required.

3. The role of mitochondria in prostate cancer

According to the recent global cancer data conducted by the World Health Organization, prostate cancer has an increasing incidence in the male population and is the second leading cause of cancer-induced deaths after lung cancer. There are approximately 1.4 million new cases and approximately 10 million existing patients worldwide each year (26). Due to the heterogeneity of the disease and treatment response, asymptomatic or symptomatic, hormone naive or castration-resistant stages of prostate cancer, limited treatment modalities and drug resistance in the treatment of patients, it is necessary to investigate the cellular mechanisms underlying prostate cancer. In this respect, these findings will allow the development of new therapeutic targets for the treatment of prostate cancer (27-29).

Mitochondria, which contribute highly to cancer development are critical at the cellular level. In particular, defects in mtDNA, impaired electron transport system and increased ROS contribute to the carcinogenesis process. In

addition, as a result of mitochondrial dysfunction, epithelial-mesenchymal transition, an essential step in cancer progression, explains the critical role of mitochondria in cancer cells. In particular, dysfunction of mtDNA replication, repair or mito-dynamic processes as a result of nuclear DNA mutations or altered mitochondrial copy numbers results in impaired oxidative phosphorylation, increased production of ROS and metabolic reprogramming. As a result of mtDNA mutations that may occur due to aging are associated with the carcinogenesis process (30).

Loss of p53 function, changes in the glycolytic pathway, oxidative phosphorylation defects, increased survival proteins, and apoptotic resistance mechanisms that occur in the context of mitochondrial DNA depletion lead to androgen-independent, aggressive and invasive prostate cancer. A better understanding of these concepts will contribute to both the evaluation of prostate cancer in terms of mtDNA depletion and the development of new therapeutic targets in prostate cancer progression (31).

4. Mitochondrial DNA depletion affects androgen dependence in prostate cancer

The major regulator of prostate cancer is the androgen receptor. The process of carcinogenesis develops through increased activation of the androgen receptor-dependent signaling mechanism or through androgen-independent signaling (32, 33). Therefore, treatment is usually provided by androgen antagonists targeting the androgen receptor. The androgen receptor is targeted through medical or surgical castration. Although this method, called androgen deprivation therapy, has a positive effect on cancer patients at first, it limits the treatment due to the resistance that develops after 18-24 months. The most well-known agents in this part include various chemotherapeutic agents such as enzalutamide, abiraterone acetate, docetaxel, cabazitaxel, orteronel (34). Therefore, new combined treatment methods are being developed for androgen-dependent/independent prostate cancer (27, 28).

As it is known, mitochondria constitute the power plant of the cell. It plays an active role in apoptotic process, oxidative stress and intracellular signaling pathways. Therefore, any defect that may occur in mitochondria directly or indirectly affects intracellular systems. Mitochondria play an active role especially in cancer development. For example, free radicals, a by-product of oxidative phosphorylation, may contribute to cancer development due to DNA damage. In addition, impaired mitochondrial signaling may affect androgen-dependent/independent processes in prostate cancer. Apart from this, changes in the amount of mtDNA in cancer cells have been observed in studies (35-39).

Another constituent that can be targeted in this context is mitochondria, which have a critical role in the carcinogenesis process. The association of mtDNA depletion with prostate cancer as a result of the decrease in the number of copies of mitochondrial DNA has been stated in studies (40, 41).

In particular, it has been proven that the androgen-dependent stage of prostate cancer is the result of mutation-induced deletion or depletion of mtDNA in the transition to the androgen-independent stage. Loss of mtDNA causes LNCaP cells to grow faster, while pharmaceutical OXPHOS inhibition leads these cells to undergo apoptosis or necrosis. This suggests that some mitochondrial function protects LNCaP cells, maintaining ATP production and preventing diversion to glycolysis. The androgen receptor (AR) is involved in this process; AR reduces OXPHOS by crossing into mitochondria. Mitochondrial disruption increases the expression of AR, which in turn enhances its localization in mitochondria. Thus, cancer cells develop survival mechanisms by activating OXPHOS or AR signalling pathways. Therefore, the role of mtDNA depletion in the transition to the more aggressive androgen-independent stage of prostate cancer is inevitable (42-44).

In another proof of concept, the role of mtDNA depletion on mitochondrial function was evaluated by comparing mtDNA depletion in minimally invasive androgen-dependent and highly invasive androgen-independent cells. In androgen-independent cells, a higher decrease in *ND-6 (NADH-ubiquinone oxidoreductase chain 6 protein), D-Loop, Cox-I (cytochrome c oxidase subunit I), Cox-III (cytochrome oxidase III)* and mitochondrial membrane potential was observed compared to androgen-dependent cells. Furthermore, the study was elaborated in the context of migration rate and drug sensitivity. As predicted, the migration rate in androgen-independent cells and cell viability due to chemotherapeutic agent application to the cells were higher in androgen-independent cells. In this context, the effect on cell death was also examined and reported that when the *PARP-I(Poly [ADP-ribose] polymerase I)* gene was evaluated in terms of chemotherapeutic agent +/-, potential cell death was less in androgen-independent cells compared to androgen-dependent cells. This is attributed to the more invasive phenotype of androgen-independent cells (45).

5. Mitochondrial DNA depletion provides cancer stem cell phenotype

As is known, cancer stem cell phenotype emerges when normal stem cell proliferation and differentiation pathways are compromised. In this context, reduced mtDNA copy number is associated with cancer cell stemness through reprogrammed metabolism (46).

In a study, mtDNA depletion was directly induced in PC-3 cells, an aggressive and castration-resistant form of prostate cancer, by EtBr intervention. In these cells, decreased levels of *ND-4 (NADH-ubiquinone oxidoreductase chain 4), D-Loop, Cox-I (cytochrome c oxidase subunit I) and Cox-III (cytochrome oxidase III)* levels and immature mitochondrial morphology were observed. Accordingly, mitochondrial membrane potential and mito-dynamic induced gene levels

were decreased. Simultaneously, ATP production decreased in the cells, but the cells expressed high levels of Warburg effect-related genes. Likewise, cancer stem cell markers (Cd44, Aldh1a1 (Aldehyde Dehydrogenase 1 Family Member A1), α2β1 integrin, Met (*MET* Proto-Oncogene, Receptor Tyrosine Kinase), Cxcr4 (C-X-C chemokine receptor type 4), Cxcr8 (C-X-C Motif Chemokine Ligand 8), Mmp1 (Matrix metalloproteinase-1) and Dpp4 (Dipeptidyl peptidase-4) were expressed at high levels. However, the cells exhibited increased hypoxia and oxygen tension due to high invasiveness and metastatic potential. PC-3 cells with mtDNA depletion develop higher levels of resistance to docetaxel, an additional anti-mitotic cytotoxic chemotherapeutic agent, compared to wild-type PC-3 cells (46).

6. Mitochondrial DNA copy number is associated with prostate cancer

The mitochondrial genome contains a limited number of gene regions. In order to maintain its own replication and transcription cycle flawlessly, the relevant enzymes must be encoded from the nuclear genome. For example, *POLRMT (RNA polymerase mitochondrial)*, which plays a major role in mitochondrial replication, acts as a mitochondrial RNA polymerase and is encoded by the nuclear genome. Therefore, any defects in this gene will also affect mitochondrial function. For this purpose, tumor cells were obtained from individuals with castration-resistant prostate cancer and normal prostate epithelial tissues were also obtained from the patients to examine the correlation between these individuals. *POLRMT* expression level was found to be higher in tumor tissues compared to normal prostate epithelial tissues which was associated with poor survival. Likewise, *POLRMT* levels were found to be higher in the reference cell lines compared to primary prostate epithelial cells. When *POLRMT* was silenced by lentiviral *POLRMT* shRNAs, the cells became *POLRMT*-depleted. The cells showed mtDNA depletion for *POLRMT* encoded from the nuclear genome. In terms of mitochondrial function, this resulted in mitochondrial depolarization, oxidative stress, inhibition of mitochondrial complex-I and ATP depletion. Interestingly, *POLRMT* depletion inhibited prostate cancer cells proliferation, migration and survival while inducing apoptosis. Similarly, *in vivo* xenograft models resulted in a reduction in tumor volume and weight. The same results were verified by confirming the study with *POLRMT* inhibitor. Correspondingly, high-level expression of *POLRMT* increased pro-cancer activity in primary prostate cancer cells. The findings reveal that POLRMT contributes not only to mitochondrial functions but also to broader metabolic processes in prostate cancer cells. Understanding the interplay between mitochondrial health and cancer cell metabolism may aid in the development of novel therapeutic strategies. The fact that POLRMT is associated with poor prognosis offers the possibility to evaluate it as a biomarker for aggressive prostate cancer, which may benefit treatment selection. Furthermore, studying the molecular mechanisms by which POLRMT promotes cancer cell survival and proliferation will provide a deeper understanding of prostate cancer biology. Ultimately, the potential of POLRMT as a therapeutic target, its applicability in clinical applications, and the effect of combining POLRMT inhibitors with existing treatment modalities have the potential to improve treatment efficacy for castration-resistant prostate cancer (CRPC) patients (47).

In another research corroborating this subject, altered mitochondrial genome content was associated found associated with poor pathology and prognosis of prostate cancer. Data from 115 prostate cancer tissues and normal adjacent prostate tissues were analyzed to determine the ratio of genomic to mitochondrial DNA content in the two tissues and to evaluate this ratio in terms of pathologic features and disease outcomes in prostate cancer tissues. Simultaneously, it was found that the amount of mtDNA was less in prostate cancer tissues compared to the genomic DNA ratio. However, high levels of mtDNA were detected in advanced cancer tissues. It was concluded that this situation was heterogeneous in terms of prostate cancer. The findings of this study reveal the complex relationship between mitochondrial DNA (mtDNA) content and prostate cancer pathology. Through comparative analysis of 115 prostate cancer and normal tissues, the study showed that mtDNA levels were lower in cancer tissues than in genetic DNA, but mtDNA levels increased in advanced cancers. This highlights the heterogeneous nature of prostate cancer and suggests that mtDNA changes may be linked to tumor aggressiveness. Changes in mtDNA levels have the potential to be used as a biomarker for disease prognosis. Furthermore, these findings highlight the importance of future research to better understand the role of mitochondrial dynamics in cancer development and progression. Overall, the results of the study suggest the need to customize treatment strategies based on metabolic profiles of prostate cancer (48).

The stage of prostate cancer is mainly determined by the Gleason score (49). In this study, depending on this score, tumor grade and mitochondrial genome content and related cancer survival pathways were investigated (50). In a study, in which mitochondrial DNA copy number was evaluated, especially in terms of *ND-1* gene, a lower copy number was detected in tumors with a high grade Gleason score compared to tumors with a low grade (50). The study was simultaneously confirmed in prostate cancer cell lines, and a lower copy number of the *ND-1* gene was detected in C4-2, PC-3 and DU-145 cells compared to androgen-dependent LNCaP cells. The effect of mitochondrial genome content on prostate cancer progression was examined and the mitochondrial genome was knocked out in LNCaP cells. By the FISH method, a lower mitochondrial genome content was detected in knockout cells compared to parental LNCaP cells. In addition, Erk subunits and Akt protein levels, which play a major role in cancer survival pathways, were evaluated and found to be activated in knockout LNCp0-8 cells. At the same time, the reduction of

mitochondrial genome content increased GTP-bound Ras protein levels (51).

7. Role of circulating mtDNA in prostate cancer

Circulating mtDNA is defined as 50-200 bp fragmented molecules released from the inter-mitochondrial space into the cytosol or bloodstream from damaged or apoptotic cells as a result of altered mitochondrial cycle, uncontrolled mitophagy (52). These molecules can be detected in blood samples and their levels can be determined. Circulating mtDNAs have biomarker potential. It is also used for prognostic purposes (53). Intracellular mtDNA can be released into circulation under various stress conditions such as cancer (54-56).

In recent studies, when prostate cancer patients treated with docetaxel were compared before and after treatment, it was found that mtDNA concentrations in serum were at high levels after treatment in the same individual (57). In the diagnosis of prostate cancer, one of the urogenital malignancies of circulating mtDNAs for diagnostic purposes, 79 bp and 220 bp fragments of mitochondria-specific 16S RNA were determined and detected at high levels in individuals with cancer (58, 59). This proves that circulating mtDNA is a biomarker. In addition, studies have shown that circulating mtDNA is not only specific to prostate cancer, but also associated with ovarian, breast, kidney and most other cancer types (59-61). When circulating mtDNA is evaluated from a prognostic point of view, in a study proving this context, out of 75 prostate cancer patients (14 individuals with benign prostatic hyperplasia), it was determined that individuals with poor prognosis had a high level of circulating mtDNA in plasma compared to individuals with benign prostatic hyperplasia (62).

Studies have shown that circulating mtDNAs are associated with other cancer types in terms of biomarker, diagnostic and prognostic aspects, as well as being directly linked to prostate cancer.

8. Conclusion

All in all, the intricate interplay between mtDNA depletion and prostate cancer development underscores the significance of mitochondrial function in disease progression. Genetic and environmental factors contribute to mtDNA depletion, impacting critical cellular processes involved in energy metabolism, genomic stability, and tumor microenvironment regulation. Prostate cancer, a leading cause of male cancer fatalities, exhibits alterations in mtDNA structure and function, influencing the transition from androgen-sensitive to androgen-resistant stages, as well as promoting aggressive cancer variants. Moreover, mtDNA depletion correlates with the emergence of a cancer stem cell-like phenotype, characterized by increased tumor aggressiveness and resistance to therapy. Understanding the role of mtDNA depletion in prostate cancer pathogenesis is pivotal for the development of targeted therapeutic strategies and improving patient outcomes. This comprehensive review highlights the essential role of mtDNA in prostate cancer progression and underscores its potential as a therapeutic target in combating this prevalent malignancy.

9. Future Perspectives

Future perspectives for this review entail multifaceted approaches spanning basic research, clinical translation, and therapeutic innovation. Firstly, a deeper understanding of the molecular mechanisms underlying mtDNA depletion in prostate cancer progression is imperative, requiring continued exploration of genetic, epigenetic, and environmental factors contributing to mitochondrial dysfunction. Integrating cutting-edge technologies such as single-cell sequencing and spatial transcriptomics will unveil the spatial and temporal dynamics of mtDNA alterations within the tumor microenvironment. Moreover, leveraging large-scale multi-omics datasets and artificial intelligence methodologies will facilitate the identification of novel biomarkers for early detection, risk stratification, and treatment response prediction in prostate cancer patients with mitochondrial aberrations. Translating these discoveries into clinical practice demands the development of non-invasive diagnostic assays and targeted therapeutic modalities tailored to individual patients based on their mitochondrial profiles. Furthermore, combination therapies synergizing mitochondrial-targeting agents with conventional treatments, immunotherapies, or hormone therapies hold promise for overcoming treatment resistance and improving long-term outcomes in prostate cancer. By embracing a multidisciplinary and collaborative approach, the integration of mitochondrial biology into prostate cancer research and clinical management will pave the way for precision medicine paradigms that optimize patient care and ultimately reduce the burden of this prevalent malignancy.

Conflict of interest

The authors declare that they have no conflict of interest.

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