

Mitochondrial dysfunction and prostate cancer racial disparities among American men

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1. ABSTRACT

The gap between prostate cancer disparities among American men is narrowing, which is mostly due to increased screening of African American (AA) men. However, the biological reasons for prostate cancer disparities among American men still remain undefined. Mitochondrion, an organelle within cells, regulates both cell survival and cell death mechanisms. These cellular signaling pathways require various proteins localized to mitochondria, which are encoded by both nuclear DNA (nDNA) and mitochondrial DNA (mtDNA). Interestingly, prostate tissues from AA men harbor reduced mtDNA content compared to Caucasian American (CA) men. Therefore, changes in mitochondrial genes may have detrimental consequences in terms of cellular signaling regulated by mitochondria in AA men. This review describes the plausible underlying mechanism of mtDNA depletion and its impact in driving resistance to therapy leading to faster progression and poor prognosis in African American men with prostate cancer. Since defective cellular signaling is critical for prostate cancer cell survival, restoring mitochondrial function may provide strategies to alleviate prostate cancer disparities among American men.

2. INTRODUCTION

Prostate cancer is one of the most commonly diagnosed cancers among American men. It is now well established that higher incidence and mortality rate associate with African American (AA) men compared to Caucasian American (CA) men (1-3). This gap between prostate cancer disparities among American men is narrowing, which is mostly due to increased screening

of AA men (4, 5). Higher incidence and mortality rates in AA men with prostate cancer may occur due to various reasons, which may involve differences in molecular or genetics changes between AA and CA men with prostate cancer. Indeed, noteworthy molecular differences occur between AA and CA men with prostate cancer (6). Our findings suggest that mitochondrial DNA (mtDNA) depletion may play an important role in prostate cancer disparities among American men (7). MtDNA encodes 13 essential proteins required for proper functioning of the oxidative phosphorylation (OXPHOS) system. Therefore, reduced levels of mtDNA will contribute to reduced synthesis of mitochondrial-encoded OXPHOS proteins, causing a stoichiometric imbalance between nuclear- and mitochondrial-encoded subunits. The "mito-nuclear imbalance" causes defective OXPHOS complexes and leads to the development of an aggressive phenotype (8). Indeed, reduction of mtDNA content associates with acquisition of an androgen-independent phenotype and prostate cancer progression (9-11). In addition, reduced levels of mtDNA and mutations in mtDNA have been reported in multiple cancer types including prostate (12, 13) breast (14), renal (15), and liver (16). In contrast, increased variance in mtDNA copy number with paralleled increase in mtDNA mutations have also been reported in other types of cancer including prostate cancer (17-19). Mutations in mtDNA render respective protein products less active or dysfunctional and thus mimics the phenotype produced by mtDNA depletion. Thus, any imbalance of mtDNA may have deleterious consequences for prostate cancer patients. For example, depletion or reduction of mtDNA inhibits apoptosis, thereby contributing to resistance to

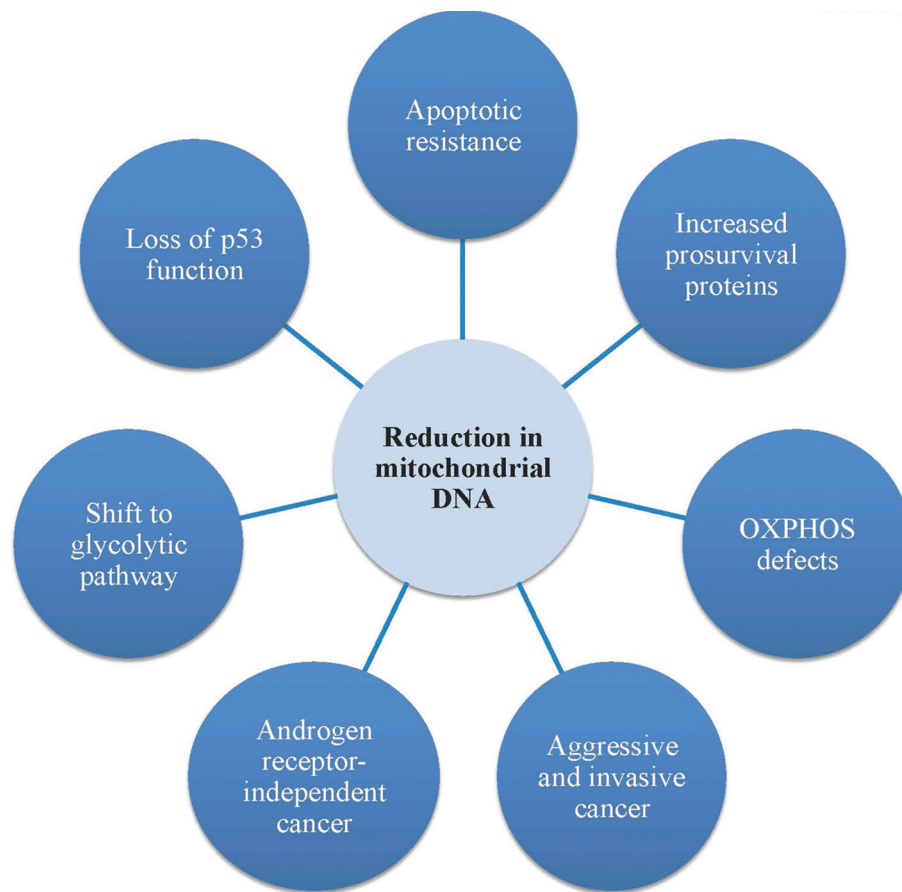


Figure 1. Impact of mitochondrial DNA depletion on various cellular functions in prostate cancer.

current available therapeutics (20-22), such as cisplatin and adriamycin (23). The present review highlights the importance of mtDNA reduction and mitochondrial dysfunction between AA in CA men with prostate cancer. We comprehensively highlight the significant impact of mtDNA depletion on many cellular functions in prostate cancer (Figure 1). Future studies may provide new avenues on how an imbalance of mtDNA and altered cellular signaling in cancer cells can predict therapy outcome and may allow for better personalized therapy for American men with prostate cancer.

3. REDUCED LEVEL OF mtDNA ASSOCIATES WITH OXPHOS DEFECTS AND PREDISPOSITION TO CANCER IN AFRICAN AMERICAN MEN

Although various factors contribute to tumorigenesis, a defective OXPHOS system is one of the hallmarks of cancer (24). The OXPHOS system consists of five multisubunit complexes including Complex I, II, III, IV and V. These complexes play critical roles during electron transfer and energy production in the form of ATP. The protein subunits of OXPHOS complexes are

encoded by both nuclear DNA (nDNA) and mtDNA. MtDNA encodes 7 subunits of complex I, 1 subunit of complex III, 3 subunits of complex IV, and 2 subunits of complex V (19, 25, 26). We observed reduced level of mtDNA in prostate epithelial cells of AA men (7). Thus it could be concluded that except for Complex II, reduced level of mtDNA may cause imbalance between protein synthesis encoded by mtDNA and nDNA, leading to defective OXPHOS complexes. These assumptions are supported by the notion that mutation, reduction or deletion of mtDNA lead to defective OXPHOS, enhanced reactive oxygen species (ROS) production, shift to the glycolytic pathway, as well as increased expression of prosurvival proteins (26, 27). These activities may ultimately predispose normal cells with reduced mtDNA to cancerous phenotypes leading to increased cancer cell proliferation and tumorigenicity (22, 28-30) in AA men compared to CA men.

Prostate cancer incidence in AA men is higher than in CA men and AA men with prostate cancer present with a more aggressive phenotype (1-3). Reduced level of mtDNA in prostate cancer cells was observed in AA men (7). Thus reduction in mtDNA may be considered one

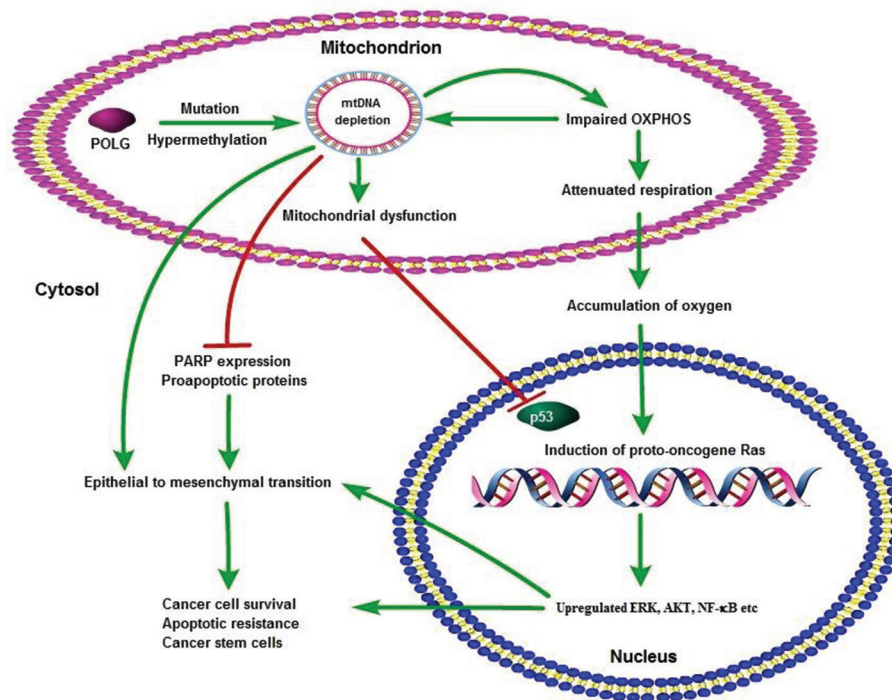


Figure 2. Mitochondrial DNA depletion modulates multiple cellular signaling leading to prostate cancer racial disparities among American men.

of the contributing factors for prostate cancer disparities among American men. As mentioned above, human mtDNA encodes 13 protein subunits of the mitochondrial respiratory chain, 22 transfer RNAs, and 2 ribosomal RNAs (25, 26). Thus, reduced mtDNA content may cause an imbalance in the OXPHOS complex assembly leading to the attenuation of mitochondrial respiration (25, 31), which will ultimately slow down oxygen consumption. This persistent reduction in mtDNA contributes to accumulation of oxygen, which induces a signaling cascade that leads to increased expression and activation of proto-oncogenic Ras in prostate epithelial cells (32). Ras activation constitutively triggers various signaling events including ERK, AKT, and NF-κB pathways. The combined effects of these survival signaling over a long period of time may contribute to other oncogenic changes and ultimately lead to initiation and progression of prostate cancer at greater extent in AA men compared to CA men (Figure 2). Together, the available evidence suggests a correlation of reduced mtDNA content with higher incidence of prostate cancer in AA men compared to CA men (1, 2). Since abrogation of OXPHOS function favors faster growth and invasiveness (33), reduced mtDNA level in already transformed prostate epithelial cells will promote more aggressive and invasive prostate cancer in AA men. Indeed, our recent published findings show that cancer cells derived from AA men with prostate cancer are highly invasive in nature compared to cancer cells derived from CA men (34). Animal models of mtDNA mutations further support our conclusion that mtDNA

mutations and reduced mtDNA content results in tumor development in mice (35).

4. P53 REGULATION OF mtDNA DEPLETION AND PROSTATE CANCER RACIAL DISPARITY

We have demonstrated earlier that prostate tumors as well as normal tissues from AA men harbor reduced mtDNA levels compared to CA men (7). Although causes and consequences of these observations require further investigation, decreased level of mtDNA in AA men with prostate cancer suggests a strong correlation of prostate cancer incidence and aggressive prostate cancer in AA men compared to CA men. Thus the identification of underlying mechanisms of mtDNA reduction may have future significance in understanding the disease process in African American men with prostate cancer. It has been shown previously that mutations in the D-loop region of mtDNA may contribute to depletion/reduction of mtDNA, as the D-loop is the critical site responsible for replication of the mitochondrial genome because it contains the leading-strand of the origin of replication and the major promoter elements required for transcription (26, 27). MtDNA replication and repair are maintained by polymerase γ (POLG), the only DNA polymerase in mitochondria (36). POLG is encoded by nDNA and is mutated in breast cancer and other mitochondria-associated diseases (37-39). It is interesting to note that POLG genes are also hypermethylated in

cancer stem-like cells and hypermethylation leads to silencing of *POLG* protein leading to the reduction of mtDNA (40). Thus it is possible that *POLG* mutations result in loss of *POLG*, which may lead to reduction in mtDNA causing mitochondrial dysfunction. Mitochondrial dysfunction could then trigger *p53* mutations, which are rare in prostate adenocarcinomas. More likely, continued dysfunction of mitochondria may lead to loss of *p53* function, which is a hallmark of transdifferentiated neuroendocrine prostate cancer, and is highly resistant to radiotherapy and androgen deprivation therapy (ADT) (41-43). Because mtDNA integrity is maintained by *p53* protein (44) through its interaction with *POLG* (45), it is also possible that *POLG* mutations are due to non-functional *p53* or due to *p53* mutations in AA men with prostate cancer. These findings may have significance because higher tumor stage and grade, androgen-independence, and metastatic prostate cancer are associated with high levels of *p53* mutations (46-48). Mitochondrial dysfunction causes the suppression of *p53* activity (49) and *p53* positively regulates mitochondrial respiration (50, 51). Therefore, reduced mtDNA levels in normal prostate epithelial and cancer cells from AA men may be associated with defects in *POLG* and *p53* functions (Figure 2).

5. OXPHOS DEFECTS INHIBIT APOPTOSIS LEADING TO RESISTANCE TO THERAPY IN PROSTATE CANCER

Reduced mtDNA content in prostate cancer cells may suppress apoptosis either by directly targeting mitochondria and/or by depleting *p53* protein in cancer cells derived from AA men. Apoptosis is a programmed cell death that requires the permeabilization of mitochondrial membrane leading to the release of apoptogenic proteins such as cytochrome c, Smac, and AIF (52-54). The permeabilization of mitochondrial membrane is regulated by Bcl-2 family proteins, which are divided into two broad groups: proapoptotic and antiapoptotic Bcl-2 proteins based on their function in the apoptosis program (55). Antiapoptotic proteins Bcl2, Bcl-xL and Mcl-1 inhibit channel-forming proteins Bax and Bak. Proapoptotic BH3-only proteins such as Bim/Bid inhibit antiapoptotic proteins while activating Bax and Bak. The activation of BH3-only proteins triggers the permeabilization of the outer mitochondrial membrane leading to the release of apoptogenic proteins such as cytochrome c, which interacts with an adaptor protein Apaf-1 to form the apoptosome (56, 57). In the cytosol, the apoptosome initiates the caspase cascade leading to the execution of apoptosis. *P53* is also known to activate mitochondrial apoptosis both by active transcription of proapoptotic proteins including Apaf-1, Bak and by direct activation of Bax on the mitochondrial membrane (58).

Reduced mtDNA level in both normal prostate epithelial and prostate cancer cells of AA origin may

inhibit apoptosis both by regulating mitochondrial channel activity and by deactivating/suppressing *p53* protein. It is interesting to note that depletion of mtDNA leads to the increased expression of antiapoptotic Bcl-2 protein (59). Reduction of mtDNA or mitochondria function also suppresses *p53* protein in mammalian cells (49). As mentioned above, *p53* regulates apoptosis by targeting mitochondria or transcriptional activation of proapoptotic proteins Apaf-1 and Bak. In addition, *p53* also positively regulates mitochondrial respiration (60).

Mitochondrial respiration is required for apoptosis execution (61-63). Reduction or depletion of mtDNA in cancer cells further abrogates mitochondrial respiration leading to the inhibition of apoptosis induced by multiple anticancer agents (21, 63-66). Thus reduced mtDNA will make prostate epithelial and tumor cells refractory to anticancer agents at greater level in AA men with prostate cancer (7). Indeed, reduction of mtDNA content associates with acquisition of an androgen-independent phenotype and prostate cancer progression (9-11). Although higher mtDNA level and resistance to anticancer agent docetaxel have been reported (67), which may be due increased mtDNA replication in response to oxidative stress. The lack of mtDNA in cancer cells of AA origin creates mitochondrial dysfunction leading to development of therapy resistance. The relatively higher level of mtDNA in CA men with prostate cancer will maintain relatively better functional mitochondria and thus respiration, which predicts better response to therapy compared to AA men with prostate cancer. Additionally, increased level of mtDNA in tumors from CA prostate cancer patients may lead to mitochondrial biogenesis and relatively functional mitochondria, which promote apoptosis (68) in response to chemotherapy.

Our recent findings demonstrate that prostate cancer cells from CA origin are sensitive to cell death in response to taxol upon restoration of mitochondria function by dichloroacetate (DCA) (34), a small molecule that restores mitochondria function. It is interesting to note that anticancer agent-induced cell death does not require caspase activation, which suggests that DCA restores mitochondria function and suppresses survival pathways leading to increased cell death without an increase in caspase activation. These findings also suggest the involvement of either caspase-independent cell death or non-apoptotic cell death in CA prostate cancer cells (69). Forcing cancer cells to switch from a glycolytic state (pro-cancerous) to an OXPHOS (anti-cancerous) state is a new approach in cancer management/treatment (70) and may provide a therapeutic advantage to sensitize prostate cancer cells in both populations. However, such an effect was not observed in prostate cancer cells of AA origin, which may be due to various reasons. For example, AA cells possess very low basal levels of ROS and have decreased mitochondrial membrane potential (mtMP) compared to CA cells, thus DCA-induced increase in

mitochondrial ROS and mtMP in AA cells may not be sufficient to induce apoptosis. Thus severe mitochondrial dysfunction is an important factor contributing to the development of resistance to conventional chemotherapies. Indeed, due to the presence of severe mitochondrial dysfunction characterized by highly reduced ROS levels, reduced mitochondrial biomass, and reduced mtMP, AA subjects respond poorly to first line chemotherapy (20, 71-74). Anticancer agents that restore mitochondria function will have therapeutic advantages for AA men with prostate cancer. We observed that doxorubicin increases mitochondrial biomass and function, with a subsequent increase in apoptotic cell death (75). Importantly, doxorubicin shows higher toxicity to prostate cancer cells of AA origin in the presence of DCA. Thus doxorubicin-induced cell death in prostate cancer of AA origin may be associated with restoration of some mitochondrial function to show higher toxic effects in AA cancer cells. However, due to the presence of severe mitochondrial dysfunction in prostate cancer cells of AA men, doxorubicin alone and/or in combination with DCA is unable to restore mitochondria function above the threshold level to induce efficient cell death in prostate cancer cells of AA origin.

6. OXPHOS DEFECTS CONTRIBUTE TO AGGRESSIVE NATURE OF PROSTATE CANCER IN AFRICAN AMERICAN MEN

Depletion of mtDNA induces prostate cancer progression (9, 30, 76), prevents apoptosis, and promotes cell motility via upregulation of phosphatidylinositol 3-kinase (PI3K)/Akt2 signaling (77). Depletion of mtDNA also induces epithelial-mesenchymal transition (EMT) (10) and reduces PARP-1 levels (76), which indirectly inhibits apoptosis while promoting a more aggressive phenotype of the disease. For example, the androgen-independent derivative of LNCaP, LNCaP C42, expresses less mtDNA and consumes less oxygen than the parental LNCaP line (9). Strikingly, mtDNA-depleted LNCaP (LNp0) cells do not respond to androgens and grow significantly faster than the parental LNCaP cell line, which directly showcases the transformative property of mtDNA on prostate cancer cells (9). Furthermore, LNp0 cells are more motile and have increased expression of mesenchymal markers than the parental line, which demonstrates how mtDNA depletion may induce metastasis (10). Therefore, mtDNA reduction in prostate cancer cells is likely to inhibit apoptosis and promote invasiveness to a greater extent in AA men with prostate cancer. Based on these discussions, we suggest that reduced mtDNA content in AA men may be associated with more aggressive prostate cancers.

Mutated mtDNA also induces defective OXPHOS systems. Specific mutations in mtDNA have been shown to cause tumorigenesis and increased growth of the primary tumor. For example, a specific

mutation in the gene *ATP6* causes an increase in ROS formation and promotes growth of PC3 prostate cancer cells *in vivo* (28). Furthermore, PC3 hybrids containing the same *ATP6* mutation grow more readily in a bone environment than wild-type PC3 cells, which indicates that mtDNA mutations augment bone metastatic events (11). Additionally, germline mutations in the cytochrome c oxidase subunit 1 gene (*COI*) are associated with prostate cancer in CA men (28). However, of the 15 *COI* SNPs identified, 9 were specific to AA men (78). Interestingly, SNP T7389C is significantly associated with AA men with prostate cancer (78). However, SNP T7389C is part of the African-specific mitochondrial haplogroup (L), which is used as a marker for African ancestry (79). Thus, more studies are required in order to ascertain the importance of this SNP in prostate cancer progression in AA men.

Mutated mtDNA has also been shown to directly affect metastatic potential. Alterations in the *ND6* gene cause respiratory dysfunction of Complex I, induce ROS formation, and increase metastatic potential (29). Intriguingly, transferring the mutated *MT-ND6* (mitochondrially encoded NADH: ubiquinone oxidoreductase core subunit 6) gene from a highly metastatic tumor cell line into a line with poor metastatic potential greatly heightened metastatic activity of the recipient cell line, thus illustrating the importance of mtDNA in inducing malignant phenotypes in cancer. Prostate cancer metastasis to the bone is a hallmark characteristic of advanced prostate cancer and is typically lethal. Indeed, the frequency of mtDNA mutations correlates to aggressiveness of the disease. For example, mtDNA mutations were detected in primary prostate tumors from a cohort of patients, but at a higher frequency in their visceral metastases and a higher frequency still in their bone metastases (80). Interestingly, a single missense mutation within the *ND3* gene of complex I was detected in 77% of all bone metastases (80).

7. DIFFERENTIAL EFFECT OF CANCER STEM-LIKE CELLS (CSCs) KILLING IN PROSTATE CANCER

CSCs represent a small subpopulation of cells are responsible for tumor progression, metastasis, drug resistance, tumor relapse, and are associated with poor response to conventional interventions (81). CSCs isolated from prostate cancer are critical for prostate cancer development and metastatic progression (81). Our recent findings demonstrate that DCA treatment induces higher enrichment of CD44⁺ and CD44⁺CD133⁺ cell populations in AA cells compared to CA cells, which may be associated with higher metastatic potential (34). Mitochondrial dysfunction in CSCs may confer higher metastatic features in AA cells compared to CA prostate cancer cells. Indeed, prostate cancer cells that undergo mtDNA depletion also become more stem-like. PC3

and DU145 cells significantly overexpressed stemness markers, such as Oct3/4, Nanog, and CD44 upon treatment with ethidium bromide (82). Similar observations were made in ovarian cancer cells, whereby depleting mtDNA induced expression of stem cell markers (83). Conversely, mammospheres were found to have greater than 60 mitochondrial-related genes upregulated compared to cells cultured as monolayer, which illustrates a requirement for mitochondrial biogenesis in cancer stem cells (84). Indeed, PC3 prostate cancer spheres were greatly reduced in number when treated with various classes of antibiotics that inhibit mitochondrial biogenesis or OXPHOS (85). However, most studies reviewed here utilized AR-independent cell lines, which limit observation-based conclusions to address end-stage, ADT-resistant prostate cancer. Therefore it is still uncertain how CSC-derived mitochondria may play a role in tumorigenesis.

8. CONCLUSIONS AND FUTURE PERSPECTIVES

Based on available evidence, we conclude that defective mitochondria are a leading cause of racial disparities among American men with prostate cancer. Mitochondrial dysfunction is caused by mtDNA depletion, which induces a mito-nuclear imbalance and subsequent defective assembly of OXPHOS complexes (8). The impaired OXPHOS complex function increases ROS production, which further mutates mtDNA, and is detrimental to anti-cancer proteins, such as p53. Dysfunctional mitochondria increase metastatic potential and stemness of prostate cancer cells and are associated with radioresistant disease (29, 80, 82, 83, 86). The insufficiency of mtDNA in AA men may render them more susceptible to developing prostate cancer, and helps to explain the higher incidence rate of prostate cancer within this group as well as why AA men typically have a more aggressive phenotype than CA men (Figure 1 and 2). Future studies focusing on the role of mtDNA copy number and/or mtDNA mutations and mitochondria-based cellular signaling may provide new strategies to differentially target metastatic diversity among American men for prostate cancer therapy and define novel biomarkers for prostate cancer aggressiveness.

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