Mitochondrial Genome Mutations and Pathological Features of Prostate Cancer: an Update

Arnaldo A. Arbini[1] and Loredana Moro[2,*]

[1]*Department of Pathology, NYU Langone Medical Center, 550 First Avenue, 10016 New York (NY), USA*

[2]*National Research Council, Institute of Biomembranes and Bioenergetics, Via Amendola 165/A, 70126 Bari, Italy*

Abstract: Mitochondria are organelles involved in a variety of cellular functions that are central to the life and death of a cell. Oxidative phosphorylation (OXPHOS), the main energy provider of the cell, takes place inside mitochondria and is known to be altered in carcinogenesis and tumor progression contributing to the "metabolic reprogramming", one of the hallmarks of cancer cells. Due to the central role of energy metabolism in cancer cell pathogenesis, mutations in the mitochondrial genome (mtDNA), which encodes for essential components of the OXPHOS pathway, have been suggested to play a role in many cancers, including prostate cancer. Recent studies provide evidence for increased levels of mutant mtDNA in prostate cancer patients with higher Gleason grade and relapse, as well as in bone metastatic sites. In this review, we will provide an overview of recent studies investigating the presence of mtDNA mutations in prostate cancer cells and their significance in the context of clinical pathological features of prostate cancer.

Keywords: Mitochondria, Mitochondrial DNA mutations, Prostate cancer, Mitochondrial genome, Nuclear genome.

INTRODUCTION

Mitochondria are the powerhouse of the cells, being the main supply of energy as ATP. Besides energy production, mitochondria are main actors in the execution of apoptosis and regulate several anabolic pathways, including biosynthesis of aminoacids, nucleic acids, lipids, and substrates such as NADH and NADPH [1, 2]. Mitochondria are semi-autonomous organelles containing their own genome, a 16,569 bp double-stranded, circular DNA molecule (mtDNA) which can replicate independently and that encodes for 13 proteins essential for the mitochondrial respiratory chain [NADH dehydrogenase 1, 2, 3, 4, 4L, 5, 6 (ND1, 2, 3, 4, 4L, 5, 6); Cytochrome b, Cytochrome oxidase I, II, III, ATPase 6, 8], 22 transfer RNAs (tRNAs) and 2 ribosomal RNAs (12 S and 16S rRNAs) required for mitochondrial protein synthesis (Figure **1**). In addition, the mtDNA has a non-coding control region (D-loop) with regulatory regions for mtDNA replication. Mitochondria contain a variable number of mtDNA molecules, depending on the cell/tissue type, with an average of 5 molecules/mitochondrion. Within the same cell, some mitochondria may contain mtDNA mutations while others have wild-type mtDNA, a feature known as "heteroplasmy". "Homoplasmy" instead indicates when a cell or a cell subpopulation contains a uniformly normal or mutant mtDNA pool.

Although mitochondria contain their own genome, they are semi-autonomous because most of the proteins residing in mitochondria are encoded by the nuclear DNA. So far, a number of genetic and metabolic mitochondrial modifications have been described in cancer cells, affecting both the mitochondrial and the nuclear genome, and they have been implicated in metabolic reprogramming as well as in the response of cancer cells to chemotherapeutic drugs [3-6].
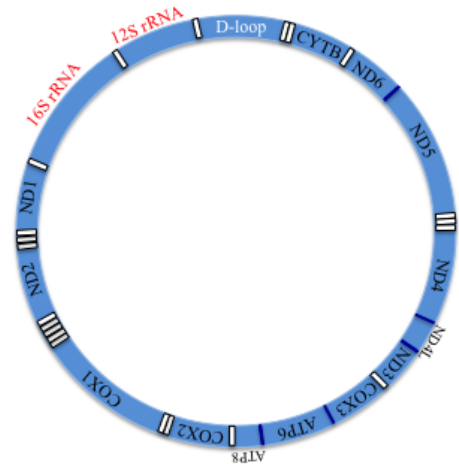
Figure 1: Human Mitochondrial DNA. The human mtDNA is a 16,569 bp circular molecule encoding for 13 proteins of the mitochondrial respiratory chain, 2 rRNA (12S and 16S rRNA) and 22 tRNAs (white rectangles). The D-loop sequence contains regulatory regions. ND, NADH dehydrogenase; COX, cytochrome oxidase; ATP, ATPase.

More than 70 years ago, Otto H. Warburg demonstrated that neoplastic cells have an altered

*Address correspondence to this author at the National Research Council, Institute of Biomembranes and Bioenergetics, Via Amendola 165/A, 70126 Bari, Italy; Tel: +39-080-5443316; Fax: +39-080-5443317; E-mail: l.moro@ibbe.cnr.it

energy metabolism: he observed that instead of using an oxidative metabolism, cancer cells convert glucose into lactate even in the presence of high oxygen tension. This "Warburg effect" is referred to as "metabolic reprogramming" and is a hallmark of cancer [7]. In his pioneering research, Warburg hypothesized that mitochondrial dysfunction and consequent cell inability to effectively oxidize glucose carbon to CO_2 was crucial for the metabolic shift to glycolysis. More recently, the metabolic shift has been shown to occur even in the presence of functional mitochondria [8-10]. The metabolic reprogramming would indeed provide a biosynthetic advantage to tumor cells diverting energy substrates into key anabolic reactions required for differentiation, proliferation and growth [8-11]. As such, carbon and nitrogen derived from glucose and glutamine would be used for amino acid, fatty acid and nucleic acid synthesis, thus supporting cancer growth and development. In turn, the increase in lactate production due to the metabolic switch to glucose fermentation would promote acidification of the micro-environment fostering local invasion and metastasis.

Prostate cancer is the most commonly diagnosed malignancy in men of the Western world and a major cause of cancer death. Elevated serum prostate specific antigen (PSA) and/or abnormal digital rectal examination are currently used as the rationale for needle biopsy, histopathological examination and Gleason scoring. The latter is a grading system based on the histological architectural pattern of the prostate glands and it is used for prognosis: low Gleason grade indicates a well differentiated tumor that will probably have a biological behavior close to normal, thus not very aggressive; a high Gleason grade instead predicts a more aggressive cancer. Lack of availability of prognostic biomarkers as well as the extreme heterogeneity of this disease make prostate cancer management complicated. Until recently, biomarker discovery has been focused on the nuclear genome with little attention paid to the mitochondrial genome. Recently, studies on somatic mtDNA mutations have become an important aspect in cancer research because mtDNA mutations might have pathological significance and/or might serve as biomarkers for tumor detection and progression. While it was evident that mtDNA mutations are acquired during prostate tumorigenesis and cancer progression [12-14], only recently the potential prognostic value of mtDNA mutations has been underscored. In this review we will provide an update of studies published in the last years (2010-2016) underlying the significance of mtDNA

mutations in the etiopathology and prognosis of prostate cancer.

METHODS

A comprehensive search was carried out using PubMed and Google Scholar databases to identify pertinent articles within the timeframe 2010-2016. The following terms and their combinations were used: "prostate cancer", "prostate carcinoma" and "mitochondrial DNA mutations". Inclusion criteria were studies reporting assessment of the somatic nature of tumor mutations in prostate cancer and correlations with clinical presentation of prostate cancer (staging, PSA levels, relapse).

The search resulted in a total of 38 potentially relevant articles. After examining their content, 32 articles were excluded according to the inclusion criteria, leaving the remaining 6 included in this review.

RESULTS AND DISCUSSION

MtDNA Mutations in Prostate Cancer

Prostate cancer is typically associated with aging. Because mtDNA depletion and deletion mutations accumulate with age in many tissues of the body, a potential link between prostate cancer and mtDNA mutations has been suggested [15]. Heteroplasmic large deletion mutant mtDNA is indeed very common in prostate cancer [16]. Acquired mtDNA mutations have been reported to accumulate in prostate carcinoma [13, 14] at a rate approximately 55-fold higher than nuclear DNA [17] and are more frequent in prostate cancer, after gastric and hepatic carcinomas, than any other human cancer [12].

To understand the role of somatic mtDNA mutations in prostate cancer, Kloss-Brandstatter A. *et al.* [18] have analyzed frequency and nature of somatic mtDNA mutations and their suitability for monitoring malignant transformation, tumor progression, and metastasis in 30 prostate cancer patients (Table **1**). The authors sequenced the entire mitochondrial genome in macro-dissected prostate cancer tissue with varying Gleason scores as well as distant benign prostate cells by using a high-quality sequencing strategy, able to detect low levels of heteroplasmy. Out of 30 patients, 22 showed mtDNA point or length heteroplasmies on 39 nucleotide positions for a total of 41 somatic mutations. To exclude a germline origin of the mutations, blood samples from 10 patients with somatic tumor mutations

were collected and mtDNA from white blood cells was sequenced: they all showed a mtDNA profile identical to benign prostate cells and were free of somatic mtDNA tumor mutations. The control region (D-loop) showed a low degree of somatic mutations, with only three point mutations detected. Seven mutations were instead detected within the two rRNA genes. Intriguingly, none of the mutations identified in 16S rRNA were previously observed in the human phylogeny overseeing data from 5140 individuals, suggesting a potentially pathological role of these mutations in prostate cancer. In particular, the somatic deletion at position m.2150_2151delTA is located in the GTPase-associated center, which is involved in binding EFG, the elongation factor that catalyzes the translocation oftRNAs on the ribosome. This mutation might thus impair mitochondrial protein synthesis. Five somatic mutations were mapped within tRNA genes; among these, m. T10436Y was located within the anticodon of mitochondrial (MT)-TR and changed the arginine anticodon to a glutamine anticodon, likely resulting in a misincorporation of arginine at glutamine codons; m.G1623R was located in the D-stem of MT-TV and could disrupt a Watson-Crick base pair with a possible impact on the tRNA D-loop, thus affecting the conformation of the tRNA. Finally, in the context of protein-coding mtDNA regions, the mutation m. G8184R in MT-Cytochrome oxidase II was found in the benign tissue as well as in the tumor tissue from the prostate gland and the seminal vesicle from a patient: the amount of the mutated nucleotide increased from benign tissue (15%) to prostate tumor (30%) to seminal vesicle tumor (40%). A similar trend was found for the MT-ATP synthase 6 mutation m.C870Y: the benign tissue exhibited 40% of the mutated nucleotide, but in the cancerous tissue the mutated mtDNA was predominant, supporting the hypothesis that the proportion of mutated mtDNA increases with malignancy [18].

Arbini A. *et al.* [19] screened 12 benign prostate hyperplasias and 24 prostate carcinoma specimens for the presence of large mtDNA deletions. Large mtDNA deletions, absent or present in low number ($\leq$2) in benign prostates, were significantly increased in prostate cancer tissues but not in the corresponding mtDNA from peripheral blood lymphocytes, confirming their somatic origin and further supporting the hypothesis that prostate cancer cells with a high mutant mtDNA load may be positively selected during tumorigenesis.

Arnold R.S. *et al.* [20] sequenced the mtDNA from autopsy specimens of 10 prostate cancer patients with late stage disease and bone metastases. For 9 out of 10 patients, mtDNA from four different tissues was sequenced: primary prostate cancer, a soft tissue metastasis, a bone metastasis and a normal tissue as a healthy control. In the 10th patient, primary prostate was not available, but both soft and bone metastatic sites were sequenced as well as skin as the uninvolved tissue. Some mutations were identified in the primary tumor and in the two metastatic sites but at different heteroplasmic levels. For example, in one patient the G9820A somatic mutation was heteroplasmic in the primary prostate tissue and lymphnode metastasis but reached homoplasmy in the bone metastatic site. Overall, significantly increased number of mtDNA somatic mutations was detected in the bone metastatic sites compared to the primary tumor. Intriguingly, one mtDNA nucleotide position was mutated in 77% of bone samples: A10398G (Thr in Ala) in the ND3 gene of the respiratory Complex I. The effect of this single mutation on cancer growth was previously tested in a breast cancer model: a cybrid cell line with the A10398G mutation displayed cell cycle delay, increased ROS levels, decreased mitochondrial membrane potential, increased resistance to apoptosis and increased metastatic potential in mice [21]. The results reported by Arnold *et al.* [20] suggest that this mutation may enable prostate cancer to metastasize to the bone, indicating a selective pressure exerted by the bone microenvironment for metastatic cells that had acquired the G10398A mtDNA mutation.

McCrow J.P. *et al.* [22] performed deep sequencing analysis of mtDNA in prostate tissue specimens and matched blood samples from 87 South African men, and identified 144 somatic mtDNA single nucleotide variants, of which 80 were present in 39 men with aggressive prostate cancer. The mutations identified occurred along the entire mtDNA. In an independent study, Kalsbeek A.M.F. *et al.* [23] have recently confirmed that total acquired genomic burden, rather than specific mtDNA mutations, is increased in prostate cancer progression and has diagnostic significance. They used next-generation sequencing to analyze the mtDNA from prostate tissue and matched blood samples of 115 men who underwent radical prostatectomy. In the study, 74 prostate somatic mtDNA variants were identified in 50 patients, distributed across the entire genome.

It should be pointed out that a recent study does not support a role for genetic variations of the

Table 1:   Characteristics of the Included Studies

Study	# Samples	Study Design	Results
Kloss-Brandstatter A. *et al.* [18]	30	Sequencing of mtDNA in prostate cancer tissue and distant benign prostate cells and matched blood cells by using a high-quality sequencing strategy, able to detect low levels of heteroplasmy.	22 specimens had mtDNA point or length heteroplasmies on 39 nucleotide positions for a total of 41 somatic mutations. Tumors with somatic mutation in mtDNA-encoded rRNA or tRNA had higher Gleason grade.
Arbini A. *et al.* [19]	36	Long-PCR analysis of mtDNA from 12 benign prostatic hyperplasias and 24 prostate cancer specimens and matched blood samples for detection of somatic large mtDNA deletions.	Somatic large mtDNA deletions were significantly increased in prostate cancer tissues.
Arnold R.S. *et al.* [20]	10	Sequencing of mtDNA from four different tissues (primary prostate cancer, a soft tissue metastasis, a bone metastasis and a normal tissue as a healthy control) derived from autopsy specimens of prostate cancer patients with late stage disease and bone metastases.	Increased number of mtDNA somatic mutations was detected in the bone metastatic sites compared to the primary tumor. Mutation A10398G in the ND3 gene of the respiratory Complex I was mutated in 77% of bone metastatic samples.
McCrow J.P. *et al.* [22]	87	Sequencing of mtDNA in prostate tissue specimens and matched blood samples from 87 South African men.	144 somatic mtDNA single nucleotide variants were identified: 80 of them occurred in 39 men with aggressive prostate cancer. Number and frequency of somatic mtDNA mutations were associated with higher pathological stage and elevated PSA levels.
Kalsbeek A.M.F. *et al.* [23]	115	Next-generation sequencing of mtDNA from prostate tissue and matched blood samples of 115 men who underwent radical prostatectomy.	74 prostate somatic mtDNA variants were identified in 50 patients, distributed across the entire genome. Significant correlation was found between the total amount of acquired mtDNA variants and elevated Gleason score at diagnosis and biochemical relapse.
Giorgi E.E. *et al.* [24]	7.784	350 Single Nucleotide Polymorphisms (SNPs) were tested in 4.086 prostate cancer cases and 3.698 controls in 5 ethnic populations: Europeans, Africans, Native Hawaiians, Latinos and Asian Americans.	SNPs in mtDNA-associated OXPHOS genes were not associated with increased prostate cancer risk.

mitochondrial genome in the risk of prostate cancer [24]. In this study, the potential role of the mitochondrial genome and the risk of prostate cancer were examined in 4.086 prostate cancer cases and 3.698 controls from the Multiethnic Cohort, by testing 350 Single Nucleotide Polymorphisms (SNPs) in 5 different ethnic populations: Europeans, Africans, Native Hawaiians, Latinos and Asian Americans. The mtDNA-associated OXPHOS pathway and genes were not associated with prostate cancer risk.

Taken together, these studies imply that, while the presence of germline mtDNA variants does not increase the risk of prostate cancer *per se'*, newly acquired somatic mtDNA variants may promote prostate cancer progression to an aggressive disease.

MtDNA Mutations and PSA Levels, Gleason Grade and Relapse

Acquired mtDNA mutations are associated with biochemical indicators of aggressive prostate

carcinoma and may enhance prostate cancer bone metastasis [20].

Kloss-Brandstatter A. *et al.* [18] observed a tendency to higher Gleason scores in tumors with a somatic mutation in a mtDNA-encoded rRNA. In addition, patients with a somatic tRNA mutation had a significantly higher PSA value at diagnosis than patients without it (average 14.25 ng/ml *versus* 7.15 ng/ml; p = 0.004). Mutations in mtDNA-encoded tRNAs and rRNAs may negatively affect the mitochondrial function because they are essential for mitochondrial protein synthesis, thus resulting in a reduction or inhibition of oxidative phosphorylation.

McCrow J.P. *et al.* [22] found that both the number and frequency of somatic mtDNA single nucleotide variations were associated with higher pathological stage and extremely elevated serum PSA levels in men with African ancestry. The authors provide evidence that prostate cancer progression is likely characterized by accumulation of mtDNA mutations in the entire

genome rather than in "hot-spots". Consistently with this hypothesis, Kalsbeek A.M.F. *et al.* [23] have reported that the number and cumulative frequency of single nucleotide variants in the mtDNA may independently predict prostate cancer presentation, with significant diagnostic potential for aggressive cancer. In particular, a significant positive correlation was found between the total amount of acquired mtDNA variants and elevated Gleason score at diagnosis and biochemical relapse. The total somatic mtDNA variant burden provided a diagnostic and prognostic correlation, improving relapse prediction in combination with Gleason score.

Further studies aimed at determining and cataloging the spectrum and combination of prostate cancer-associated mtDNA variants as well as their pathological significance in populations of different ethnicity and genetic backgrounds may provide significant clinical potential in terms of improving diagnosis and prognosis.

CONCLUSIONS

Prostate cancer is diagnosed in about 80% of men at age 80 (*www.webmd.com/prostate-cancer*) and it represents the fifth leading cause of cancer-related deaths in men worldwide. Novel therapeutic strategies have improved the survival of men with prostate cancer; however, a subset of prostate cancer patients experience disease relapse and development of metastases invariably resistant to current therapies. Consequently, understanding the molecular mechanisms and genetic alterations that promote prostate cancer cell progression towards this lethal stage of disease is essential to develop new therapeutics that will improve the clinical outcome of this disease. As described in this review, recent studies provide evidence for increased levels of mutant mtDNA in prostate cancer patients with higher Gleason grade and relapse, as well as in diagnosed bone metastatic sites. Further studies aimed at assessing the spectrum of prostate cancer-associated mtDNA variants and their pathological significance in the progression of prostate cancer to lethal metastatic disease may provide new tools to achieve more accurate diagnosis and prognosis.

It is also worth mentioning recent studies that, though in early preclinical experimentation, have raised the possibility of editing the mitochondrial genome by using mitochondria-targeted nucleases (TALENs and ZFNs) [25-28]. These approaches have been able to reduce the load of mutant mtDNA in affected tissues, with important clinical implications for the future treatment of patients affected by "mitochondrial" diseases, including metastatic prostate cancer.

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