REVIEW

Altered metabolism and mitochondrial genome in prostate cancer

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Mutations in mitochondrial DNA are frequent in cancer and the accompanying mitochondrial dysfunction and altered intermediary metabolism might contribute to, or signal, tumour pathogenesis. The metabolism of human prostate peripheral zone glandular epithelial cells is unique. Compared with many other soft tissues, these glandular epithelial cells accumulate high concentrations of zinc, which inhibits the activity of m-aconitase, an enzyme involved in citrate metabolism through Krebs cycle. This causes Krebs cycle truncation and accumulation of high concentrations of citrate to be secreted in prostatic fluid. The accumulation of zinc also inhibits terminal oxidation. Therefore, these cells exhibit inefficient energy production. In contrast, malignant transformation of the prostate is associated with an early metabolic switch, leading to decreased zinc accumulation and increased citrate oxidation. The efficient energy production in these transformed cells implies increased electron transport chain activity, increased oxygen consumption, and perhaps, excess reactive oxygen species (ROS) production compared with normal prostate epithelial cells. Because ROS have deleterious effects on DNA, proteins, and lipids, the altered intermediary metabolism may be linked with ROS production and accelerated mitochondrial DNA mutations in prostate cancer.

of diagnosis, prognostic stratification, and therapeutic intervention including pharmacogenomics will all culminate in reducing mortality as a result of prostate cancer.

“There is considerable evidence to suggest that oxidative stress contributes to the aetiology and/or pathogenesis of prostate cancer”

Although a role for the mitochondrion in tumour biology has been known for over seven decades,² it was not until relatively recently that the role of mitochondrial DNA (mtDNA) in carcinogenesis has attracted much attention.^{3–4} Indeed, the mitochondrion could be regarded as the “master of the orchestra”, controlling signalling pathways important in initiating and perpetuating the malignant phenotype.⁵ Interestingly, a recent study unravelled a new mitochondrial to cytosol signalling pathway that links mitochondrial dysfunction to tumour progression.⁶ It was shown in this study that downregulation of the Krebs cycle enzyme, succinate dehydrogenase, led to the accumulation of succinate, which inhibited hypoxia inducible factor 1 α (HIF1 α) prolyl hydroxylase in the cytosol, resulting in the stabilisation of HIF1 α , which then induced the expression of genes involved in tumour progression.⁶

Mitochondrial respiratory activity is associated with the generation of reactive oxygen species (ROS), and there is considerable evidence to suggest that oxidative stress contributes to the aetiology and/or pathogenesis of prostate cancer. For instance, inflammatory cells produce and secrete oxidants, and prostate inflammation is associated with increased incidence of prostate cancer.⁷ In addition, proliferative inflammatory atrophy is identified as a precursor of prostate cancer.⁸ Products of the gene encoding glutathione S-transferase π (*GSTP1*) protect cells against oxidative damage, and loss of *GSTP1* via hypermethylation of its promoter plays a role in the aetiology of many cancers, including prostate cancer.⁹ High plasma concentrations of antioxidants, such as the carotenoid lycopene, have been reported to reduce the risk of prostate cancer, and correlate with reduced DNA damage and low prostate specific antigen concentrations in prostate cells after prostatectomy.^{5–10–11} Given that the mitochondrion is a major site of ROS production,^{12–13} it would not be surprising for

Prostate cancer is one of the most frequently diagnosed tumours, and a major cause of death among men in the developed world; however, mortality is declining as a result of recent advances in screening and focused attention on people at increased risk of developing this disease. Environment, diet, lifestyle, sex hormones, and the genetic constitution of an individual are all contributing risk factors of prostate cancer. Recent advances in technology have considerably extended our knowledge of the molecular biology of prostate cancer. Mutations and altered gene expression have all been detected in prostate cancer samples (for a review see Nelson and colleagues¹); however, our ability to prevent or successfully treat prostate cancer lies in the complete understanding of all contributing factors and molecular mechanisms that are associated with this disease. For example, molecular approaches to risk factor determination, early detection and confirmation

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Abbreviations: HIF1 α , hypoxia inducible factor 1 α ; LCM, laser capture microdissection; mtDNA, mitochondrial DNA; np, nucleotides; PIN, prostate intraepithelial neoplasia; ROS, reactive oxygen species

altered mitochondrial bioenergetics and DNA to underlie the development of prostate cancer. In light of the special metabolism of the prostate gland, we propose a model for the accelerated mtDNA mutations in prostate cancer following alterations in intermediary metabolism of normal peripheral zone prostate cells.

MITOCHONDRIAL GENETICS

Mitochondria are highly specialised eukaryotic organelles that are necessary for the aerobic glycolysis required for efficient energy production. In addition to respiration, mitochondria perform many other cellular functions, including heme and lipid biosynthesis; metabolism of amino acids, iron, and nucleotides; calcium homeostasis; regulation of cytosolic signalling pathways; and apoptosis.^{6, 14–16} Although mitochondria are dependent largely on the import of nuclear proteins for their function, they are the only cytoplasmic organelles that possess their own functional DNA. Importantly, the mtDNA molecule differs from the nuclear genome in several respects. It has no chromosomal homologue and, in general, is maternally inherited. Human mtDNA is a 16.5 kb closed circular molecule that lacks protection from histone and non-histone proteins. Furthermore, the naked mtDNA molecule has no adequate proofreading and repair mechanisms, yet exists in an environment with high concentrations of ROS. Thus, this molecule mutates at a rate that is about 10–100 times higher than nuclear DNA.

Structurally, the mtDNA molecule is made up of a double stranded coding region and a triple stranded non-coding displacement loop (D-loop; nucleotides (np)16024–576). The D-loop region houses the cis regulatory elements required for replication and transcription of the molecule. Within this region are two highly polymorphic segments referred to as hypervariable regions 1 (HVS1; np16024–383) and 2 (HVS2; np57–372). The coding region is packed with “intronless” genes that encode 13 polypeptides, two ribosomal RNA molecules, and 22 transfer RNA molecules.

“The naked mitochondrial DNA molecule has no adequate proofreading and repair mechanisms, yet exists in an environment with high concentrations of reactive oxygen species”

Each mitochondrion contains three to 10 mtDNA genomes and over 1000 mitochondria exist in a cell. In general, the mtDNA sequences of healthy mitochondria are identical in a cell, a state referred to as homoplasmy. However, mutations may result in different mtDNA molecules in a cell or tissue, and this condition is referred to as heteroplasmy. Mitochondrial pathologies are associated with a minimal threshold of heteroplasmy, although there is no correlation between mutant mtDNA load and phenotypic presentation of mitochondrial diseases.¹⁷

MITOCHONDRIAL BIOENERGETICS AND ROS PRODUCTION

The respiratory function of the mitochondria provides the bulk of the energy requirements of a cell. The respiratory machinery, which resides in the inner mitochondrial membrane, is organised into five protein complexes (I–V). The mitochondria encode 13 of the more than 80 proteins in these complexes. Nuclear genes encode the remainder. Energy (ATP) production begins when electrons and reducing equivalents from succinate generated from intermediary metabolism (Krebs cycle) are received by complex I and II, respectively, and sequentially transported through redox groups to the final acceptor, complex IV, which transfers

the electrons to oxygen to form water. In the process, protons are pumped from the matrix to the intermembrane space by complexes I, II, III, and IV. Complex V then uses the energy from the electrochemical gradient established to pump the protons back into the mitochondrial matrix. This process is coupled with ATP synthesis.

An inevitable byproduct of the respiratory chain and oxidative phosphorylation is the production of ROS (super-oxide anion, O_2^- ; hydrogen peroxide, H_2O_2 ; and the hydroxyl radical, $\cdot OH$). Efficient Krebs cycle activity implies increased production of electrons for the respiratory chain. In such circumstances, electrons can be donated directly to oxygen to begin the production of ROS. The direct acceptance of electrons by oxygen produces the first family member, O_2^- , which can be converted to H_2O_2 by manganese superoxide dismutase. The H_2O_2 generated is usually detoxified by the antioxidants, glutathione peroxidase and catalase. However, in the presence of reduced transition metals (for example, Fe^{2+}), H_2O_2 can be converted to $\cdot OH$. It can be surmised that ROS production will increase in conditions of excess electrons, such as may occur in states of excess calories and/or defective respiratory chain activity.¹²

MITOCHONDRIAL DNA MUTATIONS IN PROSTATE CANCER

Knowledge on the molecular genetics of prostate cancer would not be complete without a careful consideration of mitochondrial genomics and proteomics in the pathogenesis of this disease. For instance, a recent study of the mitochondrial proteome in normal, premalignant, and invasive prostate tumour tissues uncovered a significant shift in the relative concentrations of some nuclear encoded mitochondrial complex IV subunits (complexes IV, Vb, and VIc) when compared with mitochondrial encoded subunits I and II.¹⁸ More importantly, these protein changes were seen at the premalignant stage, attesting to the potential usefulness of such proteins as biomarkers for early prostate cancer detection, and as targets for therapeutic intervention. Over the past few years, five studies on mtDNA mutations in prostate cancer have been reported. We summarise the major findings from these studies (fig 1), and carefully examine issues of methodology and study design that may account for discrepant results. Finally, we provide guidelines that will allow data comparison between laboratories.

Figure 1 Mitochondrial DNA mutations in prostate cancer. The circular mitochondrial genome is depicted and genes are labelled accordingly. Solid lines between genes represent transfer RNAs (tRNAs). Numbers in circles indicate the number of mutations; the 38 mutations in the D-loop include four small insertions and deletions. rRNA, ribosomal RNA.

The first study of mtDNA alterations in prostate cancer was reported in 2001 by Jessie *et al.*¹⁹ They examined prostate cancer samples for mtDNA deletions from 34 individuals ranging in age from 41 to 81 years. They found that the number of deleted mtDNA fragments in prostate cancer correlated positively with advancing age. Although this is an interesting finding, the lack of normal tissue controls makes it difficult to dissociate deletions caused by the ageing process from those related to tumour biology. A study looking at peripheral blood, malignant, and normal glands from the same individual patients for these deletions will help uncover the importance of these mtDNA deletions in prostate cancer.

Jeronimo *et al* microdissected glands from prostate cancer lesions and paired prostate intraepithelial neoplasia (PIN) lesions from 16 prostatectomy samples, and studied the D-loop and adjacent 16S ribosomal RNA and NADH genes.²⁰ Homoplasmic mtDNA mutations were found in only three of the 16 individuals, one of whom had identical mutations in a PIN lesion. From this limited analysis, it was concluded that mtDNA mutations were uncommon in prostate cancer and, when present, occurred early in disease progression. In addition, paired urine and blood samples were examined for mtDNA mutations using a method that allowed the selective detection of specific nucleotide changes. Surprisingly, the homoplasmic mutations seen in the three individuals were also detected in their body fluids. It was suggested then that neoplastic mtDNA was shed from the prostate epithelium into these biofluids, and the high copy number of mutant mtDNA in the tumours offered a detection advantage. Although these findings are very promising for diagnosis, they may be useful in disease monitoring or detection of recurrent tumours, but of limited use in early cancer detection programmes. However, early detection of tumours confined to the prostate gland will provide the greatest benefit with respect to treatment. Nonetheless, this initial finding calls for more rigorous studies of the usefulness of mtDNA alterations as a biomarker for prostate cancer.

In 2002, Chen *et al* reported on mtDNA mutations from a study of 16 prostatectomy specimens.²¹ Laser capture microdissection (LCM) was used to obtain pure populations of prostate cancer cells, benign epithelial gland cells, and PIN cells (where available) from each specimen. Similar to Jeronimo *et al*,²⁰ they restricted their analysis to the D-loop region of the mitochondrial genome. In 90% of the tumours and/or PIN samples, heteroplasmic and homoplasmic somatic mutations were detected in 34 nucleotide positions, 30 of which were substitutions and four of which were small insertions and deletions (indels). Two D-loop regions—a mononucleotide sequence repeat, and a dinucleotide microsatellite segment—were more susceptible to indels. Apart from one individual with a homoplasmic deletion in a dinucleotide repeat, they found no common mutations between prostate cancer and PIN samples. In addition the mutations they found did not correlate with tumour grade or age of the patients.

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In a follow up study reported a year later, Chen *et al* selected three individuals from the previous study who had at least five mutations in the D-loop for further study of the mechanisms by which mtDNA mutations occur in prostate cancer.²² Using LCM and mutant specific polymerase chain reaction methodology, they showed that mtDNA mutations in prostate lesions were linked on the same molecule, and

that the mutations were focally confined in the glands. Based on these findings, it was concluded that a burst of mtDNA mutations occurred simultaneously in the prostate, probably following oxidative stress.

Note that there is some discrepancy in the results from Jeronimo and colleagues²⁰ and Chen *et al*,²¹ who both studied the D-loop region known to be more susceptible to electrophile damage.²³ Whereas Jeronimo and colleagues²⁰ found homoplasmic mutations in only three of the 16 samples, Chen and colleagues²¹ reported both homoplasmic and heteroplasmic mutations in 14 of the 16 samples studied. The difference may be related to study design and/or sensitivity of the detection methods used by each group. For example, Jeronimo *et al* included peripheral blood as normal controls for germline polymorphisms,²⁰ but Chen *et al* did not.²¹ Rather, Chen *et al* used histological benign prostate glands from the same specimen that had the tumour as normal controls.²¹ Given that histological benign glands in the presence of a tumour have mutations (our observations, 2004), such tissue may not be suitable for normal controls. The difficulty of data comparison on mtDNA mutations in cancer is being realised. Indeed, a need for methodological standardisation for molecular oncology laboratories dealing with mtDNA instability in cancer has been raised by Alonso.²⁴ In view of these observations, we propose several guidelines to be followed to permit easy interpretation and comparison of data on mtDNA mutations in prostate cancer.

- (1) The study design should include appropriate control samples. Tissue samples from age matched patients with prostate cancer should be compared with benign prostate biopsies from a different group of individuals. A prostate specimen and normal peripheral lymphocytes from each person should be sampled for analysis. This experimental design will permit identification of somatic and germline mutations.
- (2) A certified pathologist should examine the slides and document the multicentric or focal nature of each tumour. The same pathologist should procure pure populations of PIN, adenocarcinoma, or benign epithelial cells by LCM for study. If necessary, immunological stains such as PIN cocktail (p63/p504S)²⁵⁻²⁶ should be used for the sampling of PIN cells.
- (3) All primers used for polymerase chain reaction amplification reactions must be tested on DNA extracts from mtDNA-less cells to ensure that they do not coamplify mitochondrial pseudogenes (RL Parr *et al*, unpublished data, 2004).²⁷
- (4) The method of sequencing or detection should be chosen to allow sensitive detection of mutations and heteroplasmy.

Given that heteroplasmy is a hallmark of early prostate cancer and other mitochondrial diseases, and that current methods of heteroplasmy detection are not very sensitive, technologies should be developed to provide high throughput ultrasensitive heteroplasmy detection devices.

Recently, a comprehensive population based study of mtDNA mutations in prostate cancer was reported by Petros *et al*.²⁸ In this study, the entire mitochondrial genome of one individual tumour was first sequenced. In total, 38 substitution mutations were seen, 31 of which were reported polymorphisms and the rest were new mutations. Because of a missense mutation in the cytochrome c oxidase subunit I (*COI*) gene that created a stop codon, and the rarity of *COI* polymorphisms and pathogenic mutations in the general population, this gene was chosen for a large scale study. Of 260 patients with prostate cancer of European and African-American descent, and 54 benign controls without cancer,

COI mutations were found to be over-represented in the prostate cancer group compared with controls. They also analysed a population sample of 1019 European and African mtDNA sequences, and the frequency of *COI* mutations was found to be 7.8%, which was still much lower than the 12% observed in the prostate cancer cohort. Given that *COI* polymorphisms are more frequent in African mtDNA of macrohaplogroup L than in the rest of the world, they analysed data from Europeans only and still found more *COI* mutations in patients with prostate cancer (11%) than in controls without cancer (0%) and the general population (6.5%). The over-representation of *COI* mutations in patients with prostate cancer compared with those without cancer suggests that mutations in the *COI* gene might be a risk factor for developing prostate cancer. The major strength of this mutation analysis is the large sample size, which permitted an epidemiological study of the mtDNA mutations. Its main weakness is the limited part of the mitochondrial genome studied. Importantly, this study shows that the coding region of the mitochondrial genome is also susceptible to mutations in prostate cancer, and mutations in the *COI* gene in particular might be important for prostate cancer risk assessment.

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In addition to the epidemiological analysis, Petros *et al* studied the role of oxidative stress on tumour growth.²⁸ Given that low amounts of ROS are mitogenic,^{25–29} they reasoned that the compromised respiratory chain activity resulting from mtDNA mutations in prostate cancer cells will lead to overproduction of ROS, and that this might contribute to tumour growth. To investigate this possibility, they established cybrids that harboured a homoplasmic mtDNA mutation (*ATP6*; np 8993G) known to cause increased mitochondrial ROS production. When wild-type and mutant cells were injected into nude mice, it was found that after 110 days the tumour volume was more than seven times greater in those animals receiving mutant cells compared with wild-type cells. This finding demonstrates the potential in vivo relevance of ROS in prostate tumour growth.

FUTURE RESEARCH INTO MTDNA MUTATIONS IN PROSTATE CANCER

Mitochondrial polymorphisms in both the coding and non-coding regions have been associated with the risk of certain diseases such as Parkinson's.³⁰ In cancer, homoplasmic somatic mutations occur more frequently in the hypervariable regions, and the nucleotide changes are in most cases identical to those seen in germline polymorphisms.^{20–21} These findings suggest that the D-loop might be susceptible to mutations at specific nucleotide positions. Therefore, germline studies of polymorphic sites in the coding region, with special attention focused on analysing both synonymous (neutral amino acid) and non-synonymous (amino acid substitution) changes, in addition to the non-coding regions of patients with prostate cancer and controls, might reveal some associations between specific germline polymorphisms and the risk of developing prostate cancer, or malignant progression. For instance, in African-American men, the prevalence of germline *COI* polymorphisms is suggested to be a risk factor for prostate cancer.²⁸ Of even greater interest will be the study of somatic mutations at polymorphic loci and how they relate to tumour progression, recurrence, and response to treatment.

Almost all prostate cancers are slow growing in nature, but some can be very aggressive, requiring immediate and radical treatment. To date, there is no in depth study of the association between mtDNA mutations and tumour grade. Notably, only one study mentions a lack of association between mtDNA mutations and Gleason grade. Conceivably, most of the reported mtDNA alterations in prostate cancer involve individuals with Gleason grades 5–7, the most frequent clinical grade at presentation.³¹ Thus, many more studies are required to ascertain whether there is an association between specific mutations or mutation load and Gleason grade or tumour stage. In addition, most high grade PIN lesions develop in the peripheral zone of the prostate, and may be precursors to prostate adenocarcinoma.³² Jeronimo *et al* found identical mutations in PIN lesions and the resident adenocarcinoma in one specimen, suggesting that PIN was a precursor of the tumour.²⁰ Large scale mtDNA mutation studies are needed to clarify the relations between adenocarcinoma and high grade PIN.

Most prostate cancers express the androgen receptor and respond to androgens, which facilitate their growth. Although most of these tumours will subsequently become hormone refractory upon hormonal treatment, they develop other means of maintaining androgen receptor activation, such as ligand independent crossactivation of the receptor.³³ Interestingly, physiological stimulation of the androgen receptor has been shown to increase ROS production.³⁴ Thus, will the levels of androgen stimulation in a tumour correlate with mtDNA mutations?

METABOLISM OF PROSTATE SECRETORY CELLS

Normal prostate epithelial cells

Before examining the altered metabolism of transforming prostate epithelial cells and how this might contribute to the accelerated mtDNA mutations and malignant process, we would first like to describe the metabolism of normal prostate glandular epithelial cells. The intermediary metabolism of the human prostate gland is not homogeneous in all glandular epithelial cells, so we first describe the parts of the prostate gland according to the model of McNeal *et al*.³⁵ Anatomically, the prostate gland is divided into three zones. The part that surrounds the proximal urethra is called the transition zone, and 10% of all prostate cancers are found in this area. The central zone surrounds the transition zone and extends to the angle of the urethra and base of the bladder; 5% of prostate cancers develop here. The peripheral zone composes the bulk (70%) of the gland and is the site of about 80% of prostate malignancies. The cells in the peripheral zone are metabolically different from those in the central zone of the prostate. For the purpose of this discussion, we will briefly summarise the metabolism of the peripheral zone glandular epithelial cells (for a detailed description of prostate metabolism see Costello and colleagues^{36–37}). The epithelial cells in the peripheral zone are highly specialised secretory cells. In contrast to cells in the central zone and the rest of the body, peripheral zone cells accumulate substantial amounts of zinc (3–10 fold higher), mainly as a result of the activity of the zinc uptake transporter in these cells. The presence of high zinc concentrations in the mitochondria of the peripheral zone cells inhibits the activity of the enzyme m-aconitase, which prevents the oxidation of citrate through the Krebs cycle. The accumulated citrate is secreted into the semen. High zinc accumulation also inhibits mitochondrial respiration and terminal electron transport. Thus, normal prostate glandular epithelial cells have a truncated Krebs cycle, low respiration, and low terminal oxidation, are energy inefficient, and presumably generate less ROS. These epithelial cells probably possess alternative metabolic pathways for energy production without coupled ROS production, as

occurs in other cells. Such low mitochondrial ROS production may be associated with downregulation of antioxidant gene expression (for example, *GPXI*), as occurs in mtDNA-less cells.³⁸ Therefore, it is possible that normal peripheral zone cells have less tolerance for ROS than other cell types.

Transforming prostate epithelial cells

Apart from the prostate gland, normal tissues from which tumours are derived exhibit Krebs cycle activity that leads to complete oxidation of glucose. In contrast, most tumour cells demonstrate inefficient aerobic glycolysis with impaired Krebs cycle activity. Therefore, tumorigenesis in general is associated with metabolic transformation from energy efficient benign cells to energy inefficient tumour cells. Interestingly, however, early on during malignant transformation, peripheral zone prostate glandular epithelial cells switch from energy inefficient benign cells to energy efficient tumour cells.³⁶ An early marker of this metabolic alteration is the downregulation of the zinc uptake transporter by the transforming glandular epithelial cells, which is followed by greatly reduced zinc concentrations (70–80% lower than normal peripheral zone cells).³⁷ In addition, prostate cancer in African-American men is associated with downregulation of the zinc transporter.³⁹ The low zinc values in the mitochondria of these cells activate m-aconitase, thus allowing complete metabolism of citrate through the Krebs cycle.^{37 40 41} This metabolic pathway generates 38 ATP/glucose molecule metabolised, which is more than twice that of normal peripheral zone cells (14 ATP/glucose molecule). Thus, malignant prostate cells completely oxidize citrate and are bioenergetically more efficient than their benign counterparts. Indeed, citrate concentrations in transforming cells are significantly lower than in benign cells.³⁶ Undoubtedly, the efficient energy production will facilitate the malignant process. Of note, part of the citrate in these transforming cells is used for the biosynthesis of lipid, which is a necessary requirement of rapidly dividing cells.³⁶

A LINK BETWEEN METABOLIC ALTERATIONS AND mtDNA MUTATIONS IN PROSTATE CANCER: A HYPOTHESIS

In the presence of a tumour, mtDNA mutations and altered intermediary metabolism are seen in histologically benign prostate epithelial cells (our observation, 2004). At the moment, however, it is unclear whether the mtDNA mutations precede the decreased expression of the zinc uptake transporter. Irrespective of whichever occurs first, malignant transformation of the prostate gland is unlikely to be initiated by mtDNA mutations. The initial events are probably genetic and epigenetic alterations in the nucleus that affect signalling pathways and/or regulatory systems, which may lead to downregulation of genes such as the zinc uptake transporter.

“The accelerated mutations in mitochondrial DNA could compromise oxidative phosphorylation, leading to further reactive oxygen species production, and thus setting in motion a vicious cycle of oxidative stress in some cells”

Based on our knowledge of the altered metabolism and mtDNA mutations in apparently histologically benign glands in the presence of a tumour, we propose a “metabolic” model of accelerated mtDNA mutations in prostate cancer, and its possible contribution to tumour development and progression (fig 2). The metabolic switch in prostate cancer cells described above may play a key role in the initiation of prostate cancer. An early event in malignant transformation of prostate epithelial cells is the downregulation of the zinc uptake transporter in premalignant lesions, resulting in

activation of m-aconitase and complete metabolism of citrate. The efficient Krebs cycle function predicts an increased generation of electrons for oxidative phosphorylation in transforming prostate cells compared with normal peripheral zone prostate cells. Although the regulation of ROS production is a complex phenomenon, the increased respiratory chain activity should result in increased ROS production in the transforming peripheral zone cells, which hitherto had not been used to such high ROS levels. Consistent with this assumption, a recent study demonstrated higher concentrations of H₂O₂ in prostate cancer cells compared with normal prostate cells.⁴² ROS have damaging effects on DNA, proteins, and lipids, and at low concentrations can stimulate cellular proliferation (see below).²⁹ Conceivably, different epithelial cells in the prostate will have different concentrations of ROS. Thus, depending on the amounts of ROS generated in specific epithelial cells in the prostate, it is plausible that a sudden burst of mutations in the mitochondrial genome is triggered at certain critical values. Such mutational events are consistent with the findings of Chen *et al*,²² who concluded that the locally confined multiple mtDNA mutations on the same molecule that they observed in prostate cancer might be simultaneously generated in a unique process of mitochondrial hypermutagenesis. The accelerated mutations in mtDNA could compromise oxidative phosphorylation, leading to further ROS production, and thus setting in motion a vicious cycle of oxidative stress in some cells. Despite the efficiency of nucleotide incorporation by the mitochondrial repair enzyme, polymerase γ , the damaging effects of ROS on this enzyme can compromise its fidelity, which may lead to increased nucleotide incorporation errors.⁴³ Presumably, the ROS generated and the adaptive mechanisms evoked will differ in different clonally expanded cells. Given the deleterious effects of ROS on mitochondrial membranes, this could lead to the release of cytochrome c oxidase into the cytosol, which will trigger apoptosis and the removal of some clones. It can then be envisaged that the cells that remain and survive the initial catastrophe might be in favourable “survival equilibrium” with their mitochondrial and nuclear DNA mutations, altered intermediary metabolism, ROS production, and signalling pathways evoked. Such selected clones could be homoplasmic at certain “useful” nucleotide positions, as is seen in some prostate cancers.^{44 45} Nevertheless, heteroplasmy is expected at the neoplastic stage, given the simultaneous burst of mutations in the mtDNA genome (our observation, 2004).^{21 28} If this “metabolic” hypothesis is indeed correct, then the mtDNA mutation patterns of prostate cancer from the central zone of the prostate gland that is metabolically different should differ from those found in the peripheral zone, and indeed resemble mtDNA mutations in cancers from other soft tissues, such as breast.

An alternative pathway of oxidative stress in prostate cancer pathogenesis without an initial metabolic switch is possible in certain situations, such as inflammation and proliferative inflammatory atrophy, which are risk factors of PIN and prostate cancer.^{8 46 47} In such tissues, microbial oxidants elaborated by inflammatory cells may cause genomic damage, probably in the absence of an initial metabolic switch. Nonetheless, the general findings of low zinc and citrate concentrations in all prostate cancers suggest that these cells eventually adopt the metabolic phenotype of prostate cancer.

ROLE OF ROS IN TUMOUR PROGRESSION

The ROS generated as a consequence of increased intermediary metabolism (our hypothesis) and compromised respiratory chain activity from mtDNA damage in transforming prostate cells could have a positive effect on tumour

Figure 2 The hypothetical “metabolic” model of accelerated mitochondrial DNA (mtDNA) mutations in prostate cancer. ROS, reactive oxygen species.

growth and invasiveness. Low concentrations of ROS, as demonstrated by Petros *et al.*,²⁸ may enhance prostate tumour growth. Given that many prostate cancers grow slowly, could high levels of ROS (as predicted by our hypothesis) be one of the growth inhibitory factors? However, some prostate tumours grow very rapidly, and could these tumours have low ROS values? Studies in this area are required to explain this intriguing possibility.

ROS induced mitochondrial dysfunction (mtDNA mutations and disruption of mitochondrial membrane potential) can activate nuclear genes and signalling pathways involved in tumour initiation and progression. For instance, ROS can induce pathways involved in increased HIF1 α expression,

which in turn can activate genes involved in angiogenesis and tumour metastasis.⁴⁸ In addition, ROS mediated disruption of mitochondrial functions has been shown to activate the calcium dependent protein kinase C pathway, which activates cathepsin L and other downstream genes involved in tumour invasiveness.⁴⁹ Although not all nuclear gene targets impinged upon by mitochondrial dysfunctions in cancer are known, the contribution of mitochondria to nuclear stress signalling in tumour progression may hold a clue to identifying subtypes of tumours that require pathway target disruption for effective treatment. Thus, mtDNA alterations in metastatic and confined prostate tumours deserve an in depth study.

Take home message

- Peripheral zone secretory cells of the human prostate gland accumulate high concentrations of zinc, which inhibits mitochondrial aconitase involved in citrate metabolism
- Inhibition of the terminal oxidation of citrate leads to abbreviated Krebs cycle activity and inefficient energy production via the electron transport chain in peripheral zone secretory cells. The accumulated citrate is excreted into the semen
- Prostate carcinogenesis is associated with loss of zinc accumulation, with activation of aconitase leading to increased terminal oxidation of citrate
- The efficient energy production in transforming peripheral zone prostate epithelial cells implies increased oxidative phosphorylation, and possibly increased mitochondrial reactive oxygen species (ROS) production
- If not scavenged, the “naked” mitochondrial genome that is in close proximity to the ROS, sustains substantial damage, as has been shown by several recent studies
- Although the metabolic and mitochondrial genome alterations might contribute to the pathology of prostate cancer, they could also serve as biosensors for the diagnosis and monitoring of prostate malignancy

CONCLUSION

Normal peripheral zone secretory cells of the human prostate gland have incomplete Krebs cycle activity, low respiration, and low terminal oxidation, are energy inefficient, and therefore should generate negligible electrons for the electron transport chain, and presumably produce very little ROS that can be detoxified by normal cellular mechanisms. Malignant transformation of the prostate glandular epithelial cells is preceded by increased Krebs cycle activity, suggesting increased production of electrons for the electron transport chain, with a possible production of high amounts of ROS. Could an increase in ROS in a cell that is perhaps physiologically unadjusted to high levels of ROS lead to the observed accelerated mutations in the mitochondrial genome (our observation, 2004),²² and can these mutations be used as a biomarker for detection and monitoring of prostate malignancy?

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