MITOCHONDRIAL DEFECTS AND THEIR ROLE IN DEVELOPMENT OF PROSTATE CANCER

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ABSTRACT

Introduction and Objectives: One of the characteristic changes of tumor formation is accumulation of genetic disorders in mitochondrial and nuclear genome. Mitochondrial disorders, from its side, are responsible for failure of metabolism, apoptosis, cell growth, formation of reactive oxygen species, etc. Overproduction of reactive oxygen species (ROS) significantly impacts the respiration chain enzymes and entirely the antioxidant system of mitochondria. Finally this may become a favorable condition for normal cells transformation.

The purpose of the presented work was to study the mitochondrial defects and to establish their role in prostate cancer development.

Results: Experimental results demonstrate significant increase of the activity of mitochondrial succinate dehydrogenase (complex II) of the malignant epithelial cells of prostate, and slight changes in cytochrome oxidase (complex IV) activity. Also significant activation of the antioxidant system (glutathione-dependant system) of mitochondria in prostate malignant epithelial cells was revealed.

Conclusion: The above mentioned mitochondrial changes (II and IV complexes of respiration chain, activity of the antioxidant system) partially demonstrate the alterations in mitochondrial energy metabolism, which from its side, may indicate to resistance of prostate cancer cells and correspondingly to intensification of proliferation processes.

UDC CODE & KEYWORDS

■ UDC: 616-006 ■ Prostate ■ Cancer ■ Mitochondrial defects ■

INTRODUCTION

Investigation of the role of mitochondrial defects in prostate cancer development and progression is still popular. Most of these defects are associated with altered expression and activity of different subunits of the respiration chain and glycolytic enzymes (Chen J.Z., et al. 2003; Ripple, M.O., et al. 1999), inhibition of those subunits, which are connected with nicotinamide adenine dinucleotide (NADH), mutations of mitochondrial DNA (mtDNA), etc. (Higuchi M. et al, 2006).

Genesis of any type of cancer, and of the prostate cancer as well, is tightly associated with metabolic transformation (Khandrika L. et al, 2009).

Studying of the role of mitochondria in prostate cancer is especially urgent, since in epithelial cells of prostate peripheral zone (peripheral zone comprises 70% of the prostate gland, and 80% of malignant transformation occurs just on this segment; McNeal, J.E et al, 1988) energy transformation occurs specifically, diversely from other types of tumors. Usually these cells reveal partial activity of the Krebs cycle, low level of respiration and terminal oxidation. They are energetically ineffective and generate insufficient amount of electrons for transport (Dakubo GD, et al 2006). Small amounts of reactive oxygen species (ROS) are produced in epithelial cells of prostate, thus they are easily detoxified by cell mechanism of detoxification (Lim S.D. et al, 2005).

Malignant transformation of the prostate glandular epithelium is followed by the activation of Krebs cycle, diversely from other tumors. This presumably causes enhanced production of electrons for the mitochondrial electron transport chain. Under these conditions electrons may join directly the oxygen and, as a result, big amount of ROS may be formed (Khandrika L. et al, 2009; Nijtmans L.G.J. and Smeitink J.A.M., 2007). Accordingly, the sharp "outburst" of mutations in mitochondrial genome is expected, with further genomic changes up to the critical level (Chen J.Z. et al, 2003; Petros J.A. et al, 2005).

Materials and methods

The tumour tissue of the patients with benign hyperplasia of the prostate (BHP), Prostate benign hyperplasia with PIN_{G3-4} (Prostate Intraepithelial Neoplasia) regions and of prostate adenocarcinoma (CaP) served as the material for the studies. The patients in the age of 60-75 were studied at primary revealing of the tumor. The control group was consisted of apparently healthy males with the appropriate age. n=15 for each group. The research data were supplied and the clinical stage of disease was diagnosed by the Georgian National Institute of Urology, by means of rectal, histological, and echographic examination of the prostate gland.

Isolation and purification of mitochondria was performed by differential centrifugation method (14 000g) using sucrose density gradient. Ice-cold isolation buffer was used (0,001M EDTA (pH 7,4); 0,25M sucrose) during the isolation procedure (Martínez Federico et al., 1997).

Analysis of Succinate dehydrogenase activity was performed spectrophotometrically in mitochondrial suspension according to the method described by Brusque A. M. et al., 2002. Activity of the enzyme was evaluated by the change of optical

density at 600 nm (succinate consumed per mg protein in 1 min) and was shown as nmol/min per mg of mitochondrial protein.

Activity of Cytochrome oxidase was measured spectrophotometrically at 510nm in mitochondrial suspension according to the method described by Alejnikova T.L. 1988. Activity of the enzyme was evaluated by the quantity of oxidized dimethyl-*p*-phenylenediamine and was shown as nmol/min per mg of mitochondrial protein.

GSH-Px activity was determined spectrophotometrically according to the method described by Kanbagli Öznur et al., 2002. Extinction of the oxidized glutathione (GSSG) was measured at 260 nm wavelength. Activity of the enzyme was expressed in micromols/min per mg protein.

GR activity was measured also spectrophotometrically at 340 nm according to the method described by Kanbagli Öznur et al., 2002. Enzyme activity was expressed in micromols per 1g hemoglobin per minute.

Ellman's reagent was used for the determination of reduced glutathione (GSH), according to the method described by the Kanbagli Öznur et al., 2002. The amount of GSH was measured spectrophotometrically at 412 nm. Content of GSH was shown in $\mu\text{M/L}$.

Results and discussion

Investigation of the activity of mitochondrial succinate dehydrogenase (SDH) in tumor tissue of patients with prostate tumors has revealed activation of the enzyme both in patients with benign hyperplasia with PING₍₃₋₄₎ regions and prostate cancer (CaP), compared with benign prostate hyperplasia (BHP) (table 1).

Activity of SDH in patients with BHP with PING₍₃₋₄₎ regions was higher (~1.4times), compared with BHP group. While in tumor tissues of CaP males activity of SDH was 2-fold higher, compared with benign tumor tissue (table 1).

Table 1: The activities of Succinate dehydrogenase and Cytochrome oxidase in patients with prostate tumors

Object	Activity of Succinate dehydrogenase (SDH) (nmol/min)	Activity of Cytochrome oxidase (COX) (nmol/min)
B Benign Prostate Hyperplasia (BHP)	11,1 $\pm$ 0,48	119,4 $\pm$ 5,2
BHP with PING ₍₃₋₄₎ regions	15,4 $\pm$ 0,37	130,2 $\pm$ 4,66
Prostate Cancer (CaP)	21,8 $\pm$ 1,21	135,7 $\pm$ 6,4

Source: Authors

Increase of the activity of SDH in BHP with PING₍₃₋₄₎ regions and CaP patients, as of the Krebs' cycle enzyme, on the one hand, and as of the enzyme of the II complex of electron transport chain, on the other hand may be conditioned by strengthening of succinate flow towards the mitochondrial respiratory chain (on the background of Krebs' cycle activation) in prostate tumor tissue. Our results are in accordance with mentioned data. Sharp increase of SDH activity in case of prostate cancer (table 1) indicates that intensive oxidation of succinate (as a result of complete functioning of Krebs cycle) and transfer of electrons from reduced FADH₂ to ubiquinone takes places in prostate malignant tissue.

Studies of the activity of another enzyme of electron transport chain – cytochrome oxidase (COX) has revealed minor changes of the enzyme activity in tumor tissue of the men with prostate tumors (table 1). Negligible changes of COX activity, on the background of activation of Krebs cycle and SDH, in case of malignant transformation of prostate gland, indicates to the failure of enzyme activity, which is stipulated by several factors. Those may be: oxidative stress developed in mitochondria and overproduction of ROS, affecting negatively on COX activity (semireduced ubiquinone donates electrons directly to oxygen, thus causing generation of super oxide. This from its side causes reduction of the electron flow towards the IV complex of the respiration chain – COX). This was clearly reflected on our research results, and was expressed in insignificant changes of the enzyme activity. More over, it is known that the main source of super oxide (O₂⁻) and peroxide (H₂O₂) in respiration chain is the section of semiubiquinone-cytochrome-b (complex III) (Daniel G Clark, 2000), which is situated closely to COX. This fact also may have a negative effect on the enzyme's activity (Fig. 1).

The fact that COX is encoded by mitochondrial genes (COI, COII, COIII), diversely from SDH, must be also taken into account. The latest investigations indicate to sharp increase of mutations in one of the genes (COI) of COX in mitochondrial genome, in case of prostate adenocarcinoma. Nowadays this is considered as one of the risk-factors for prostate cancer formation (Dakubo GD, 2006). Mutation of COX gene in malignant prostate tissue may be responsible for synthesis of abnormal forms of the enzyme, which presumably appears to be one more unfavorable factor for normal functioning of the enzyme.

Thus, sharp increase of the activity of SDH (complex II) and insignificant changes of COX (complex IV) activity in epithelial cells of prostate malignant tissue indicates to activation of Krebs cycle in mitochondria and increase of electrons flow in respiration chain on the one hand, and to impairment of the terminal oxidation of oxygen, on the other.

On the next step of our investigations the following components of mitochondrial glutathione-redox system has been studied in epithelial cells of tumor tissue of the men with prostate tumors: glutathione peroxidase (GSH-Px), glutathione reductase (GR) and reduced glutathione (GSH).

According to obtained results sharp activation of GSH-Px in mitochondria of patients with BHP with PING₍₃₋₄₎ regions was revealed (table 2). In males with CaP the activity of GSH-Px was also very high. It prevailed results of BHP tissue about 3 times and was 2 times higher than in BHP with PING₍₃₋₄₎ regions (table 2).

Activity of glutathione reductase (GR) – another enzyme of antioxidant system of mitochondria, was studied in epithelial cells of prostate tumors (table 2). Investigations have demonstrated activation of the enzyme in mitochondria of BHP with PING₍₃₋₄₎ regions (about 2 times) and in CaP patients (about 4 times), compared with BHP patients (table 2).

Studying of the content of GSH, as the other element of mitochondrial antioxidant system, has shown that in case of BHP with PING₍₃₋₄₎ regions amount of GSH is increased 2 times, while in case of CaP - 4-times (table 2).

Table 2: The changes in Mitochondrial Glutathione-redox system in epithelial cells of tumor tissue of the men with prostate tumors

Object	The activity of Glutathione Peroxidase (GSH-Px) (mM/min/mg/protein)	The activity of Glutathione Reductase (GR) (mM/min/1g/Hg)	Reduced glutathione (GSH) (mM/l)
B Benign Prostate Hyperplasia (BHP)	0,16±0,01	0,008±0,002	0,184±0,02
BHP with PING ₍₃₋₄₎ regions	0,32±0,05	0,019±0,001	0,336±0,05
Prostate Cancer (CaP)	0,67±0,03	0,031±0,002	0,736±0,07

Source: Authors

Thus, according to experimental results, sharp activation of mitochondrial antioxidant system, (GSH-Px, GR) revealed in BHP with PING₍₃₋₄₎ regions and malignant tumor epithelial cells, indicates to intensification of defensive abilities (to withstand switching of the mitochondrial way of apoptosis, induced by free radicals) of tumor cells (Della Rovere F, 2000).

Activation of Krebs cycle is characteristic for epithelial cells of prostate malignant tissue (diversely from normal epithelial cells of prostate gland and other cancers), as it was mentioned above, (Khandrika L. et al, 2009; Dakubo GD, et al, 2006). It is known that activation of Krebs cycle in mitochondria of prostate malignant tissue cells is caused by sharp decrease of zinc content and consequent activation of m-aconitase (Singh Keshav K, et al, 2006). Krebs cycle enzyme - m-aconitase catalyses the oxidation of citrate to isocitrate and is responsible for activation of other enzyme, isocitrate dehydrogenase. The last, from its side, oxidizes isocitrate and produces NADP(H).

Accordingly, activation of Krebs cycle in epithelial cells of prostate malignant tissue presumably is responsible for switching on a cascade of reactions, activating isocitrate dehydrogenase, and correspondingly big amount of NADP(H) is accumulated. The latter presumably has a great influence on GSH-dependent system functioning (figure 1). Activation of isocitrate dehydrogenase induces activation of Krebs cycle and succinate dehydrogenase as well - following enzyme in the Krebs cycle. This was proved by our experiments too (table 1, fig. 1). We suppose that activation of succinate dehydrogenase in respiration chain is responsible for increased electron flow towards the III complex of the chain (this is the important place of free radicals generation). In case of normal mitochondria, electrons are transported to cytochrome oxidase (IV complex), and finally the metabolic water is formed (Daniel G Clark, 2000). Though, our results indicate that in prostate tumor tissue (BHP with PING₍₃₋₄₎ regions and CaP) this process has another mechanism. In particular, corresponding activation of COX did not take place (COX activity is increased insignificantly in cases of BHP with PING₍₃₋₄₎ regions and CaP), on the background of a clear activation of SDH (table 1).

As it was mentioned above, one of the reasons for diminishing of COX activity may be the synthesis and accumulation of super oxide (O_2^-), and hydrogen peroxide (H_2O_2) just nearby the IV complex (Daniel G Clark, 2000) (Fig. 1). If we assume that accumulation of H_2O_2 takes place in epithelial cells of prostate tumor tissue (BHP with PING₍₃₋₄₎ regions and CaP), than the activation of antioxidant enzyme – glutathione peroxidase must also take place, on the background of isocitrate dehydrogenase activation. This was proved in our investigations (table 2, fig. 1).

Enhanced biosynthesis of GSH in mitochondria may be the reply on the malignant cells request to accumulate as much amount of reduced glutathione as possible (for tumor cell GSH is a necessary regulatory unit for apoptosis and proliferation processes), and to reduce the amount of oxidized glutathione (GSSG) (Balendiran G.K. et al, 2004). Since increase of GSSG may result in interruption of the proliferation and cell death (apoptosis), both, on the expense of induction of p53 protein synthesis and by affecting the phosphoprotein kinase cascade of the Ras-signaling pathway (Balendiran G.K. et al, 2004). All above mentioned once again indicates to strengthening of the protective mechanisms of mitochondria of the tumor cells and totally of the prostate malignant cells.

Thus, stimulation of the activity of SDH and retention of COX activity in epithelial cells of prostate malignant tissue may be responsible for sharp activation of isocitrate dehydrogenase and correspondingly, for significant accumulation of NADP(H). The last may stipulate a sharp activation of the glutathione-dependent system, which was proved by our investigations. Activation of the GSH-dependent system (GSH-Px, GR) presumably would be responsible for resistance of cancer cells against the oxidative stress, on the one hand, and may support the ability of malignant cancer cells to confront the up-regulation of the mitochondrial way of apoptosis (GSH), provoked by free radicals, on the other.

Changes in the activity of enzymes of the II and IV complexes of mitochondrial respiration chain and antioxidant system, in case of prostate malignant transformation, are reflection of metabolic changes in mitochondria. This, from its side, indicates to resistance of prostate malignant cells and correspondingly, to intensification of proliferation processes.

Conclusion

According to the obtained results it may be concluded, that the sharp activation of succinate dehydrogenase (complex II) and slight changes in the activity of cytochrome oxidase (complex IV) in mitochondria of epithelial cells of prostate tumors (BHP with PING₍₃₋₄₎ regions and CaP) indicates to activation of the Krebs cycle and intensification of the electron flow to the respiration chain on the one hand, and on impaired terminal oxidation, on the other.

Figure 1. General scheme of energy metabolism alterations in mitochondria of epithelial cells of prostate malignant tissue (BHP with PIN G₃₋₄ regions, CaP).

Source: Authors

Activation of the antioxidant system (glutathione-dependent system) of mitochondria in prostate malignant cells (BHP with PIN_{G(3-4)} regions and CaP) may be a manifestation of increased resistance of these cells (to confront the up-regulation of the mitochondrial way of apoptosis (GSH), provoked by free radicals) under the oxidative stress.

Changes in mitochondria of the epithelial cells of prostate malignant tumor (BHP with PIN_{G(3-4)} regions and CaP) (II and IV complexes of the respiration chain and changes of the activity of antioxidant system) clearly demonstrate alterations of mitochondrial energy metabolism (switching to an energy effective system from an ineffective one), which from its side, points to enhanced resistance of malignant prostate cancer cells and intensification of a proliferation process.

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