ABSTRACT

of dissertation for the degree of philosophical doctor (PhD)
on specialty "6D070100 – Biotechnology"

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Mechanisms of modulation of prostate cancer cells energy metabolism by low temperature plasma

General characteristics of the work. The thesis is dedicated to the study of the mechanisms of changes in the energy metabolism of normal and cancer prostate cells under the influence of low temperature plasma.

Relevance of the research topic. Prostate cancer is the second greatest cause of death from cancer among men in the world after lung and bronchus cancer (*Siegel R.L., et al.*, 2018), and in Kazakhstan in third place after lung and stomach cancer (*Kaidarova D.R., et al*, 2017). It is a slow growing cancer, but as many other types of cancer, it is generally incurable once it reaches the metastatic stage (*Howlader N., et al.*, 2015). Existing chemotherapies have severe side effects and do not provide a cure for advanced stages of the disease. There is an urgent need for novel medical approaches for treating tumor types which tend to easily develop resistance to chemo- and radiation therapies (*Zhang W., et al.*, 2015). Low temperature atmospheric pressure plasma has been recently identified as a potent technology for modulating the function of both prokaryotic and eukaryotic cells. To realize the full potential of low temperature plasma treatment for cancer therapeutics, the exact mechanisms through which plasma causes cell death must be understood. It is also critical to study the side effects of low temperature plasma on healthy cells. The primary goal of this work is to explore the effects of indirect low temperature plasma generated by microsecond (pulse width) dielectric barrier discharge on mitochondria-mediated processes. The mitochondria orchestrate cell metabolism and signaling, and therefore, they are a promising target for cancer therapy (*Panngom K., et al.*, 2013). Yet, it has been demonstrated that high doses of plasma induce apoptosis in other cancers due to massive generation of intracellular reactive oxygen species (ROS) (*Kalghatgi S., et al.*, 2011; *Fridman A.*, 2008), and the mitochondria are one of the major intracellular sources of ROS (*Starkov A.A.*, 2008). These facts indicate that elucidating the mechanisms of low temperature plasma effects on mitochondria is critical for learning how we can advance proof-of-concept demonstrations into a clinically-relevant method for cancer treatment.

A new antitumor drug or therapeutic treatment targeted only to cancer cells without affecting normal ones it is one of the most pressing issues in cancer research. Achieving this kind of selectivity is very challenging which is why the side effects of chemo and radiotherapies remain a major problem. In this work, we compare the outcomes of low temperature plasma treatment for metabolically different normal and cancerous prostate cells. It is reasonable to hypothesize that both normal and

cancerous cells can be affected through the mitochondria-mediated mechanism to hopefully different extents.

Research goal and objectives. The goal of this research is to study the mechanisms of impact of low temperature plasma on the energy metabolism of prostate cancer and normal cells.

To achieve the above goal, the following main objectives were pursued:

1. To study the cytotoxic effects of low temperature plasma on prostate cancer and normal cells.
2. To identify the mechanism of apoptosis development of prostate cancer and normal cells when exposed to low temperature plasma.
3. To determine the effects of low temperature plasma on mitochondrial membrane potential and respiratory function of prostate cancer and normal cells.
4. To analyze the oxidative stress caused by low temperature plasma in prostate cancer and normal cells.
5. To investigate the effects of low temperature plasma on calcium levels in the cytosol of prostate cancer and normal cells.
6. To study the respiratory functions of prostate cancer cells in an acidic environment, and the role of dicarboxylic acid carriers in succinate transport across the cell membrane.

Objects of research. The object of the research are human metastatic prostate cancer cells DU145, as well as the primary normal prostate cells PrEC.

Methods of research. *In vitro* cell culturing, flow cytometry (*BD Accurri C6*), confocal microscopy (*Olympus FluoView*), high resolution respirometry (*Oroboros Oxygraph-2k*), fluorescence spectroscopy (*BioTek Synergy 4*), microscopy (*Leica MZ16F, Motic AE2000*), spectrophotometry (*NanoDrop*), plasma generation method (*Quinta*), Western blotting, PCR and statistical analysis method (*GraphPad Prism*).

The scientific novelty of research:

- It was found for the first time that phosphate-saline buffer (PBS) treated with low temperature plasma (frequency 250 Hz, power 2.2 W and processing time 30 seconds) has a strong cytotoxic effect on prostate cancer cells.
- It is established that apoptosis caused by the action of low temperature plasma occurs as a result of the activation of caspases. That is, under the action of low temperature plasma, prostate cancer cells undergo apoptosis through internal (mitochondria associated) and external (supported by death receptor) pathways.
- For the first time, low temperature plasma induced changes in mitochondrial energy metabolism of prostate cancer cells were detected. The effect of selected plasma doses on mitochondrial membrane potential, the process of cell oxidative phosphorylation and oxidative stress was studied.
- It was first investigated the changes of energy metabolism (where special attention was paid to the oxidation of succinate and the role of carriers in the uptake succinate) prostate cancer cells under acidic environment.
- The expression profile of dicarboxylic acid transporters (NaDC1, NaDC3, and NaCT) in normal and cancer prostate cells was evaluated for the first time.

Theoretical significance of the work. The results presented in the thesis contribute to the understanding of the fundamental problems of prostate cancer, and

in particular, the cellular mechanisms of action of low temperature plasma and acidic environment. Investigation of metabolic parameters of prostate cancer and benign cells mitochondria will help to identify those bioenergetic characteristics that can be considered anti-cancer targets. The data obtained in this study contributed to the determination of low temperature plasma mediated mitochondria-dependent pro-apoptotic cascades in normal and cancer cells.

Practical significance of the work. So far there is very limited number of reports on bioenergetic features of prostate cancer although it is known that the entire cellular activity is energy-driven, and both life and death mechanisms are to great extend determined by mitochondria activity. The data obtained in this work will enable to understand those mechanisms which promote apoptotis resistance of prostate cancer cells. Secondly, evaluation of the effects of low temperature plasma on cell energetic system has potential to provide a basis for the development of effective anticancer treatment complimentary to surgery and chemotherapy.

The data presented in this work demonstrate that NaDC3 expression by prostate cells correlates with malignant transformation rather than being a universal mechanism gained by alterations in pH of the cellular microenvironment. In prostate cancer cells was demonstrated a succinate influx via NaDC3. It has potential to be used for molecular imaging-based diagnostics for non-glycolytic tumors in an acidic microenvironment similar to glucoseutilization based positron emission tomography. The use of labeled succinate could yield technology for determining the body succinate fluxes for prognosis of disease stages and chemotherapy responses. In addition, the dicarboxylate transporter-mediated succinate uptake could yield a novel prognostic biomarker and target for anti-cancer therapy with no effect on normal prostate cells.

Also, the received materials can be included in lectures on cell biology, oncology, physiology, pathophysiology, bioenergetics, biomedicine and biophysics for students, masters and doctoral students of higher educational institutions of medical biological profile.

The main statements for defense:

1. Low temperature plasma has a cytotoxic effect for two different types of human prostate cells (cancer DU145 and normal PrEC cells) depending on the intensity (dose) and duration of exposure.
2. Low temperature plasma treatment induces apoptosis in both DU145 cancer and in normal PrEC cells.
3. Low temperature plasma has a negative effect on the respiratory abilities of DU145 cancer and PrEC normal prostate cells. However, normal cells showed a tendency to restore the activity of oxidative phosphorylation within 24 hours compared with cancer cells, which had reduced bioenergetic activity.
4. Reactive oxygen species induced by low temperature plasma are of non-mitochondrial origin.
5. After treatment with low temperature plasma, the level of Ca^{2+} ions in the cytosol varies depending on the cell type.
6. In DU145 cancer cells, an active succinate oxidation occurs at an acidic pH. Succinate transport occurs through NaDC3 transporter.

The main results of the research and conclusions:

1. It was established that PBS treated with low temperature plasma has a cytotoxic effect on prostate cancer cells. The cytotoxicity level of the plasma-treated PBS solution was 20% lower in normal PrEC cells than in DU145 cancer cells, and depended on plasma intensity (dose), duration of exposure, and cell type.
2. It was determined that low temperature plasma treatment induces apoptosis in both metabolically active DU145 cancer and metabolically passive PrEC normal cells. At the same time, the process of apoptosis in cancer cells occurs through the internal mitochondria and external death receptors, whereas in normal cells, this process occurs only through external death receptors.
3. It was shown that exposure to cancer and normal cells with the solution of PBS treated with low temperature plasma causes a decrease in the mitochondrial membrane potential by up to 30%. After plasma treatment in 24 hours normal PrEC cells were able to restore the mitochondrial membrane potential of mitochondria up to 70%, while the DU145 cancer cells only up to 40%.
4. It was established that PBS treated with low temperature plasma negatively affects the respiratory functions of DU145 cancer and normal PrEC cells. However, after 24 hours, normal cells as compared to cancer cells with reduced bioenergetic activity are able to restore the activity of oxidative phosphorylation.
5. It was found that low temperature plasma leads to oxidative stress of cancer DU145 and PrEC normal cells of prostate. It was established that under the influence of low temperature plasma there is the appearance of reactive oxygen species outside the mitochondria.
6. It was shown that as a result of low temperature plasma treatment of DU145 cancer cells, the level of Ca^{2+} in these cells did not change. Calcium ion fluctuations occurred when ATP was added to DU145 cancer cells that were not treated with low temperature plasma. A sharp increase in the level of cytosolic calcium was observed when ATP was added to PrEC normal cells incubated in a PBS solution treated with low temperature plasma, and stable growth was observed in DU145 cancer cells.
7. Succinate oxidation was detected in prostate cancer DU145 cells under conditions of acid microenvironment. It is shown that the oxidation process is carried out under conditions of pH 6.8 of the medium. In addition, it has been established that the transport of succinate occurs through NaDC3 transporter.

The personal contribution of the author. The author independently conducted the analysis of literary data on the topic of research, experimental studies, statistical processing and analysis of results of the research, writing and design of the thesis manuscript.

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Approbation of the work. Materials of the thesis are reported and discussed on the following conferences and symposiums:

- International Scientific Conference of Students and Young Scientists "Farabi Alemi" (2013, 2014, 2017, 2019, Almaty, Kazakhstan);
- International scientific conference «Research Day 2014» (2014, Philadelphia, USA);
- «Gordon Research Conferences, Bioelectrochemistry» (2014, Biddeford, USA);
- «The 1st International Workshop on Plasma for Cancer Treatment» (2014, Washington, D.C., USA);
- «International Symposium on molecular medicine and infectious disease. Cancer biology and neoplastic disease» (2014, Philadelphia, USA);
- International Farabi Readings (2015, 2017, Almaty, Kazakhstan);
- «Fourth AACR International Conference on Frontiers in Basic Cancer Research» (2015, Philadelphia, USA).

Publications. The main content of the thesis is reflected in 19 printed works, including 2 articles and 1 abstract in the international journals with the Impact Factor quoted in the database of *Thomson Reuters* and *Scopus*; 4 articles in the republican scientific journals, recommended by the Committee on Control in Education and Science of the Republic of Kazakhstan; 1 article in the republican journal; 4 abstracts in materials of international conferences and symposium; 7 abstracts in materials of international conferences of the Republic of Kazakhstan.

The structure of the dissertation. The thesis is set out on 131 pages of computer text and consists of normative references, notations and abbreviations, introduction, review of literature, materials and methods, results and discussion, conclusion, list of used sources from 320 titles, contains 36 figures, 4 tables and 2 attachments.