ABSTRACT

of the dissertation for the degree of Doctor of Philosophy (PhD) in the specialty "6D060700-Biology"

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"**Mechanisms of modulation of mitochondria metabolism of prostate normal and cancer cells by naturally – derived polyphenols**"

General characteristics of the thesis. This thesis reviews the cellular mechanisms of modulation of natural polyphenols on healthy and cancer prostate cells *in vitro*.

Relevance of the topic. Prostate cancer is the second most common cancer in men. According to the experts, as of 2018, every ninth man will be diagnosed with prostate cancer during his lifetime. In Kazakhstan, prostate cancer was the third most common cancer in men in 2018 (Indicators of the Oncological Service of the Republic of Kazakhstan for 2018, ed. D.R. Kaidarova).

The study of mechanisms of action of natural polyphenols on mitochondrial metabolism of healthy and cancerous prostate cells allows developing therapeutic approaches when using natural polyphenols together with chemotherapy to reduce cytotoxicity and increase the treatment efficacy. The effectiveness of using polyphenols is directly dependent on their quantity and bioavailability in dietary products. Whereas, their action depends also on the chemical structure (polymerization, esterification, acetylation, methylation, and esterification), the food matrix, and the metabolism. In addition, the digestive tract absorption of polyphenols is different and ambiguous. This affects their influence on the signaling pathways that they modulate.

Polyphenols modulate key proteins in signaling cascades associated with the differentiation of cells in the body during their proliferation and metastasis, or apoptosis. Polyphenols are natural food compounds found in fruit, vegetables, and grains. To date, more than 8,000 compounds of polyphenolic origin have been identified in the human diet. Polyphenol molecules are identified as secondary metabolites of plants containing one or more hydroxyl (OH) groups, that is, they are polyhydroxylic conjugates. The most common polyphenols are classified into different groups depending on their chemical structure and orientation of the number of phenolic rings linked together. They are divided into four main subclasses: phenolic acids (30%), stilbenes, curcuminoids, and flavonoids (60%). One of the curcuminoids, Curcumin and its derivatives, has anti-inflammatory, antioxidant, and anti-carcinogenic properties. It inhibits cell signaling pathways, including growth factor (NFκB), tumor necrosis factor receptor (TNFR), enzymes (COX-2, MMP, мTOR), and protein kinases (C and EGFR). Therefore, Curcumin inhibits the viability of prostate cancer cells and induces cell apoptosis. Yu et al. report that Curcumin suppresses the expression in the prostate cancer cell line (PC3), which is a key signaling molecule in prostate cancer carcinogenesis and metastatic progression. Curcumin significantly inhibits the phosphorylation of extracellular signal-regulated kinases and vascular endothelial growth factor (VEGF) and modulates the osteopontin/integrin signaling pathway. It also induces

the MMP-9 activation associated with angiogenesis while regulating the secretion of VEGF and angiostatin in the prostate cancer cells metastasized in the bones (PC3). As an endoplasmic reticulum activity modulator, Curcumin effectively protects from prostate cancer metastasis.

The effects of carnosic acid were examined together with curcumin. Carnosic acid is effective against cancer and has a significant inhibitory and cytotoxic effect on prostate cancer cell lines metastasized to the brain (Du145) and PC3 metastases. The cells lose up to 13 and 20% of viability, respectively, when exposed to the concentration of 6.25 μg/ml for 48 hours. Carnazolic acid also prevents oxidation of low-density lipoproteins in endothelial cells of the human aorta and also the oxidative stress in Caco-2 cells mediated by lipid hydroperoxide. Carnosic acid inhibits lipid peroxidation in rat liver microsomes and brain phospholipids liposomes. The antioxidant activity of carnosic acid was comprehensively studied *in vitro*. Carnosic acid has a different effect on ROS and lipid radicals, which makes this diterpenoid tandem a specific and effective antioxidant system.

Therefore, the search for natural compounds that increase the resistance of the body to tumor development and reduce the potential tumor recurrence after radiation therapy or chemotherapy is of vital importance for the prevention and treatment of cancer diseases, and their mechanisms of action shall be studied. An effective approach could be an anti-tumor herbal therapy combining medicines with different mechanisms of action.

Purpose and tasks of the research. The purpose of this research was to investigate cellular mechanisms of action of natural polyphenols on mitochondrial metabolism of intact and cancerous prostate cells.

The following **tasks** were set to reach the purpose:

1) To determine the cytotoxicity and optimal exposure of healthy and cancerous prostate cells to natural polyphenols like curcumin and carnosic acid;

2) To study the mechanisms of action of curcumin and carnosic acid, individually and in combination, on the proliferation of prostate cell lines;

3) To determine the effect of polyphenols on the mitochondrial membrane potential and the specifics of mitochondria oxidative stress in cancer and epithelial prostate cells;

4) To study the respiratory function of cancerous prostate cells when exposed to a combination of natural polyphenols;

5) To show the specifics of a combined effect of curcumin and carnosic acid on the cell cycle of cancerous prostate cells.

Object of research. The human prostate cells with DU145 metastases (metastases to the brain) and PC3 metastases (more aggressive, metastases to the bones) were obtained from the American Type Culture Collection and used up to passage 70 (Manassas, USA). The cells were placed in the Roswell Park Memorial Institute 1640 (RPMI 1640) growth medium supplemented with 10% fetal bovine serum (FBS).

Prostate epithelial cells PrEC were placed in the recommended PrEGM medium with all necessary additives according to the manufacturer's protocol, except for gentamicin, and were used up to passage 5.

HCT116 cells were purchased from the American Type Culture Collection and were used up to passage 65 (Manassas, USA). 2-Amino-2- (hydroxymethyl) propane-1,3-diol (based on Trizma) was purchased from Sigma-Aldrich (Milwaukee, WI, USA).

Methods of research. *In vitro* cell cultivation, fluorescence spectroscopy (Platereader, Flowcytometer), flow cytometry BD Accuri C6, respirometry (OROBOROS Oxygraph-2K), microscopy (Leica MZ16F), confocal microscopy (Olympus FluoView), statistical data processing (GraphPad Prism).

Scientific novelty of research.

- The study included the first comparison of the effects of natural polyphenols of curcumin and carnosic acid on healthy and cancerous prostate cells.
- The combined antitumor effect of curcumin and carnosic acid 0n the mitochondrial metabolism of healthy and cancerous prostate cells (mitochondria membrane potential and oxidative stress) was first studied.
- The synergistic effect of curcumin and carnosic acid and the therapeutic activity of these polyphenols were established.
- The combinatorial effect of natural polyphenols, curcumin 7 μM, and carnosic acid 5 μM, was shown to increase the prostate cancer cell respiration.
- The specifics of cell cycles of prostate cancer cells under the combined exposure to natural polyphenols of Curcumin and carnosic acid were first revealed.

Theoretical significance of the research lies in establishing the role of natural polyphenols of curcumin and carnosic acid in their combined use with chemotherapy for the development of therapeutic approaches to reduce cytotoxicity and increase prostate cancer treatment effect.

Combined and individual effects of different concentrations of natural polyphenols on cellular mechanisms (the membrane potential, oxidative stress, cell respiration, and cell cycle) were revealed.

Cytotoxic and optimal concentrations of carnosic acid and curcumin, individually and in combination, were established.

The combination of Curcumin 7 μM and carnosic acid 5 μM was found to inhibit prostate cancer cell growth in the G1 phase.

The combined exposure to polyphenols was found to decrease the membrane potential of the cell mitochondria and stimulate the cell respiration.

Practical relevance. The practical relevance of the research lies in the implementation of new methods of using natural polyphenols and their action on prostate cancer cells in the Kazakh Institute of Oncology and Radiology (Reports of implementation No. 2 – 2017, No. 3 – 2019, No. 4 – 2019 – Appendix A). The form of implementation: a master class was held, which included a presentation and practical study in animals.

The obtained data was also included in the curriculum of undergraduate, Master and PhD students in Biology under the topic: “The mechanism of action of curcumin on mitochondrial metabolism of healthy and cancer prostate cells” (Report on the introduction of completed research results in 2018 curriculum – Appendix B).

Main statements to be defended:

Cytotoxic effects and optimal exposure to the natural polyphenols of curcumin and carnosic acid of intact and cancerous prostate cells depend on concentration and exposure time;

The combined exposure to curcumin and carnosic acid increases respiration in Du145 cancer cells and prostate epithelial cells (PrEC), but inhibits respiration in PC3 prostate cancer cells;

The combined exposure to curcumin and carnosic acid results in time- and concentration-dependent reduction of mitochondrial membrane potential;

The combined effect of polyphenols on cancerous and epithelial prostate cells causes time-dependent oxidative stress;

The combined effect of curcumin and carnosic acid on the cell cycle of prostate cancer cells depends on the polyphenol concentration.

Conclusions.

1. The cytotoxic concentrations of curcumin and carnosic acid and the optimal doses of curcumin (7 μM) and carnosic acid (5 μM) were determined.

2. The natural polyphenols such as Curcumin and carnosic acid were found to delay the proliferative ability of prostate cancer cells.

3. The combined polyphenol action was shown to reduce the mitochondrial membrane potential in prostate cancer cells.

4. The combined exposure to curcumin and carnosic acid was found to cause oxidative stress.

5. The combined action of curcumin and carnosic acid was found to stimulate the respiratory process in prostate cancer cells.

6. The polyphenol combination was found to inhibit the cell cycle in prostate cancer cells.

Connection to the key research action plan.

The research was supported by the Cornelius Beukenkamp foundation (2014-2015), the Louise foundation, and Bessie Stein Fellowship (2014-2015) in the framework of the prostate cancer study managed by Prof. Z.S. Orynbayeva at Drexel University. The studies were also conducted at the Biophysics and Biomedicine Department under the topic "Current challenges in biophysics and biomedicine" (2013-2018) managed by Prof. S.T. Tuleukhanov.

The research results and main statements of the thesis were presented and reported at international conferences:

1. Frontiers in Basic Cancer Research – October 23-26, Pennsylvania Convention Center, Philadelphia, the US, 2015;

2. XXIII International Scientific and Practical Conference "Progressive Processes of World Scientific Thought in XXI Century Research" (M-23). – No. 24, Section 7. – Kazan, 2015;

3. First Annual International Research Showcase. – May 26, Philadelphia, the US, 2016;

4. European Bioenergetics Conference. – July 2-7, Riva del Garda, Italy, 2016;

5. Students' and Young Scientists' Scientific Conference "Farabi elemi" (Farabi's world). – Almaty, Kazakhstan, 2017;

6. VI Congress of Oncologists and Radiologists of Kazakhstan with International Participation. – Almaty, 2017;

7. International Scientific and Practical Conference "Urgent issues in medicine." – May 2-3, Baku, Azerbaijan, 2018;

8. X Congress of Oncologists and Radiologists from the CIS and Eurasia. April 23-25, Sochi, the Russian Federation, 2018;

9. International Scientific and Practical Conference "Urgent issues in environmental genetics and experimental biology". – Almaty, 2018;

10. VII Congress of Oncologists and Radiologists from Kazakhstan with International Participation. – October 17-18, Nur-Sultan, Kazakhstan, 2019.

Publications. The research results were published in 16 scientific papers, including 1 article in a journal indexed in Thomson Reuters and Scopus, 3 articles in editions from the list of the Committee of the Republic of Kazakhstan for Control in Education and Science; 12 abstracts were published in international conference proceedings.

Thesis structure. The thesis consists of 120 pages and includes an introduction, literature review, research materials and methods, research results and discussion, conclusions, and the list of references of 304 entries. The results and experimental findings are presented in 26 figures and 1 table and 4 appendix.