

Abstract

research work presented for the degree of Doctor of Philosophy (PhD) on the theme: «Ultrastructural organization of hepatocytes and cells of hepatocarcinoma which resistant to the action of inducers cell death» by Dossymbekova Raushanon specialty «6D060700 Biology»

General characteristics of scientific work. In scientific work were investigated peculiarities of the ultrastructural organization of hepatocytes and hepatocarcinoma cells were studied in vitro conditions under the action of lithium carbonate

The relevance of the study. The search for options to increase the sensitivity of tumor cells, not only stem cells, but also other cells of the population to the effects of inducers of cell death.

One of the importance problems of modern biology and medicine is the problem of induction death cancer cell. It is known that tumors can have heterogeneity of cellular composition (Wang K., et al., 2018).

Recently, the term "cancer stem cells" has been used for tumor tissue, believing that these cells are capable of long-term survival under various therapeutic effects and that they should be the targets of drugs (Marjanovic N. D., et al., 2013).

Hepatocellular carcinoma-liver cancer is one of the most common types of cancer and the second leading cause of cancer mortality worldwide (Ladju R. B., et al., 2018).

It is believed that the development of hepatocarcinoma correlates with a violation of the regulation of programmed cell death (Degterev A., 2008).

It is believed that hepatocarcinoma cells can develop necrosis, apoptosis, and autophagy (Cui J., 2013). Necrosis often stimulates local and systemic inflammation. Apoptosis and autophagy do not provoke inflammation, and therefore they are considered therapeutic targets for cancer treatment (Zhang, C., 2016). It has been shown that lithium carbonate, acting by suppressing the activity of HSC-3 β and reducing the expression of cyclin E, can cause proliferation arrest due to the arrest of the cell cycle in the G2/M phase of tumor cells (Erdal E., et al., 2005, Tsui M. M., et al., 2012), as well as induce apoptosis of tumor cells (Li L., 2015). There is evidence that lithium modulates autophagy in cancer cells (O'Donovan T. R., et al., 2015).

At the same time, there is no data on the sensitivity of normal liver cells – hepatocytes to the effects of various doses of lithium carbonate used to affect malignant, transformed liver tumor cells. In addition, when exposed to lithium salts-inducers of cell death, a certain population of hepatocarcinoma cells always remains viable, giving new tumor growth. It is important to identify subcellular mechanisms of resistance of hepatocarcinoma cells that contribute to their survival under the influence of a damaging agent, and to study the structural organization of

normal, non – tumor liver cells-hepatocytes in these conditions, which is necessary for the development of effective antitumor therapy.

Object of research. In vitro experiments will use a culture of mouse hepatocytes and a cell line of mouse hepatocellular carcinoma-29 (GC-29)

Objectives of the study:

1. Using light microscopy and morphometry to determine the volume of hepatocytes, their nuclei and the nuclear-cytoplasmic ratio of isolated hepatocytes in the dynamics of cultivation in a standard culture medium and with the addition of lithium carbonate.

2. When using flow cytofluorometry, evaluate the distribution of hepatocytes by cell cycle phases when they are cultured in a standard culture medium and when lithium carbonate is added.

3. To Study the ultrastructural organization of hepatocytes in the dynamics of cultivation in a standard culture medium and with the addition of lithium carbonate.

4. Determine the cytotoxicity of lithium carbonate on hepatocytes and hepatocarcinoma-29 cells using the MTT test.

5. Using light microscopy and morphometry, determine the volume of hepatocarcinoma-29 cells, their nuclei, and the nuclear-cytoplasmic ratio in the dynamics of cultivation in a standard culture medium and with the addition of lithium carbonate.

6. Using flow cytofluorometry, evaluate the distribution of hepatocarcinoma-29 cells by cell cycle phases when they are cultured in a standard culture medium and lithium carbonate is added.

7. To study the ultrastructural organization of hepatocarcinoma-29 cells in the dynamics of cultivation in a standard culture medium and with the addition of lithium carbonate.

The object of the study. In the experiments will be used in vitro culture of murine hepatocytes and cell line of murine hepatocellular carcinoma-29 (CG-29).

Research methods: Cultivation of cells in vitro, MTT-test, flow cytometry CytoFlexS (Beckman Coulter, USA), light microscope LEICA DME” (Germany), transmission electron microscope (JEM 1010, Japan), morphometric analysis ImageJ (WayneRasband, USA) and statistical data processing, analysis of Statistica 6.0 (StatSoft, USA).

Scientific novelty of the study.

For the first time, the ultrastructural organization of isolated hepatocytes will be studied and the nature of their intracellular changes in the dynamics of their cultivation will be determined.

For the first time in vitro, the ultrastructural organization of hepatocytes under the influence of lithium carbonate will be studied in a comparative aspect. Promotes the development of autophagy and preservation of proliferative activity of hepatocytes.

For the first time, a heterogeneous population of GC-29 cells will be divided into types by size and nuclear-cytoplasmic ratio in the dynamics of their cultivation

in a nutrient medium. Ultrastructural features of cells in the process of their differentiation will be revealed.

The scientific significance of the work is to obtain previously unknown facts about the development of basal autophagy in hepatocarcinoma cells of stages IV and V of differentiation, which is a way of survival of tumor cells.

For the first time, the processes of apoptosis and autophagy in the studied cells will be revealed, which will determine the role of these processes in the survival of GC-29 cells and increase cell death in the population of hepatocarcinoma cells by exposing lithium carbonate to resistant cells.

For the first time, the effect of lithium carbonate on the state of non – tumor liver cells (hepatocytes) will be revealed when using a concentration of 5 mm of lithium carbonate, which causes destructive changes in hepatocarcinoma cells.

Theoretical significance of the study:

The obtained results of the study will add known mechanisms of life support and death of hepatocellular carcinoma cells when exposed to autophagy inducers. The revealed processes of autophagy in hepatocarcinoma cells and isolated hepatocytes will expand the understanding of the mechanisms of macroautophagy in mammalian cells.

The effect of lithium on the ultrastructural organization of hepatocarcinoma cells and hepatocytes will reveal new mechanisms of the biological effects of lithium.

The obtained data on the development of autophagy in hepatocarcinoma cells under normal conditions and the effects of lithium carbonate can be used to compare the effectiveness of the developed autophagy inducers.

The data obtained indicate the contribution of autophagy to the survival of the primary hepatocyte culture and can be used as an indicator of the adequacy of cultivation conditions.

Practical significance of the study:

The practical significance of the results of the work is that based on the detected ultrastructural changes in hepatocarcinoma cells under the influence of lithium carbonate, it is possible to develop approaches to targeted therapy of this type of cancer.

The developed morphological criteria for dividing hepatocarcinoma cells into types depending on their size and nuclear-cytoplasmic ratio can be used to determine target cells and the effectiveness of various drugs being developed for antitumor therapy.

Ultrastructural features of autophagic structures in the cytoplasm of hepatocytes and hepatocarcinoma cells can be used in lectures on cell biology, cytology, and histology.

The obtained results have found practical application in the laboratory of physiology of the project system of Research Institute of Clinical and Experimental Lymphology, a branch of the Institute of Cytology and Genetics, Siberian Branch of the Russian Academy Sciences in Novosibirsk (Russia), in the laboratory of physiology of the lymphological system of the Institute of Human and Animal Physiology of the Science Committee of the Ministry of Education and

Science of the Republic of Kazakhstan in Almaty (Kazakhstan), embedded in course curriculum University. Abay in the disciplines "Cell biology" and "Biology of cells and tissues" for students of the specialty "5B060700-Biology". Received utility model patent No. 2020/0105.2, on the topic "Method for including lithium carbonate in the culture of isolated hepatocytes" (19.06.2020).

Key points for thesis scientific work and conclusions:

1. When isolated hepatocytes are cultured in a standard medium, cell volume decreases, the nuclear-cytoplasmic ratio increases, the cell cycle stops at the G0/G1 stage, and basal autophagy develops, with a predominance of glycophy and mitophagy.

2. Introduction to the culture of hepatocytes lithium carbonate at a concentration of 5mm in the primary culture of hepatocytes has no toxic effect on cells, does not block cell cycle in G0/G1 phase, no stimulates hepatocyte apoptosis and promotes autophagy and preservation of proliferative activity of hepatocytes.

3. During the cultivation of hepatocarcinoma -29 cells, the volume of cells and their nuclei increases, the nuclear-cytoplasmic ratio decreases, and basal autophagy develops, which is aimed at preserving the homeostasis of tumor cells.

4. The Introduction of lithium carbonate into the culture of hepatocarcinoma-29 cells at a concentration of 5 mm leads to a stop of the cell cycle in the G2/M phase, disruption of ultrastructural organization, induction of apoptosis and autophagy in GC-29 cells, which confirms the potential of lithium as a promising drug for the treatment of HCC.

Personal contribution of the author to the work: the analysis of the literature data on the subject under study, the definition of the goals and objectives of the study, the conduct of experimental research, statistical processing and analysis of the results, the writing of the dissertation and the design of the manuscript were carried out with the personal participation of the author.

The relationship of the work with the research program.

Scientific work refers to the author's individually completed work and is not related to funded scientific projects. The research work was carried out in the branch Scientific Research Institute of Clinical and Experimental Lymphology (SRIofCEL) - Cytology and Genetics of the Siberian Branch of the Russian Sciences Academy under the bilateral agreement No. 07-02-31/003 with the Kazakh National Pedagogical University named after Abai dated 02.11.2017.

Approbation of the research results:

The main research results were presented and discussed at international scientific conferences:

- at the XIV Eurasian Symposium, international scientific and practical conference «Cell heterogeneity and autophagy in the population of hepatocarcinoma-29» (Kyrgyzstan,2018);
- at the XIII International scientific and practical conference «Lymphangiogenesis and angiogenesis in experimental hepatocarcinoma-29» (Novosibirsk, Russia, 2018);

- at the III international morphological scientific and practical conference of students and young scientists «Structural organization of the nephron in conditions of distant tumor growth» (Novosibirsk, Russia, 2018);
- at International scientific and practical conference of young scientists «Structural and functional changes in hepatocarcinoma-29 cells under the influence of lithium» (Almaty, Kazakhstan, 2019);
- at Interuniversity International Congress Higher School: scientific research «Basal autophagy in the cytoplasm of isolated hepatocytes in the dynamics of cultivation» (Moscow, Russia, 2021);
- at XV Eurasian Symposium, international scientific and practical conference «Ultrastructural change in tumor growth and development of autophagy in the kidneys and liver of CBA mice» (Kyrgyzstan, 2021).

Publications: 11 articles were published on the topic of the dissertation, including 3 articles in journals recommended by Committee for control in education and science in the Ministry of Education and Science of RK, 2 articles in an international scientific publication with a non-zero impact factor on the Scopus information database, 6 articles in materials of international conferences.

The structure and content of work on the topic. The dissertation consists of introduction, four chapters, conclusion, list of references and application.

The total volume of the manuscript is 123 pages, including 9 tables and 49 figures. The list of references includes 348 sources and 4 applications.