Annotation

for dissertation work of Assiya Maratovna Kukanova on the topic: "Suppression of KRAS mutation in colorectal cancer by oxidative stress in patients after surgical treatment", submitted for the degree of Philosophy Doctor (PhD) in the specialty 8D10102 - "Medicine"

Relevance of the study

According to WHO, colorectal cancer (CRC) ranks third in the structure of cancer incidence and second in cancer mortality worldwide and is one of the global health problems of the world. In 2020, the number of new cases of colorectal cancer was 1931590 cases, and the mortality rate was 935173 cases (9.4%) (GLOBOCAN 2020). According to KazNIIOR statistics for 2019-2020, the situation with colorectal cancer in Kazakhstan looks the same as in the rest of the world. CRC ranks 3rd in the structure of cancer pathology, both in terms of morbidity and mortality (Indicators of the Oncological Service of the Republic of Kazakhstan for23 2023, KazIOR).

The standard types of treatment for CRC are surgical treatment, mono - or multimode chemotherapy. In recent years, targeted drugs targeting target molecules in tumor cells have been used to treat disseminated forms. The use of targeted therapy made it possible to increase the survival rate of patients with metastatic CRC in some cases. FOLFIRI/FOLFOX or CAPIRI/CAPOX regimens are still the mainstay for CRC chemotherapy. Targeted drugs, such as inhibitors of the epidermal growth factor receptor and vascular endothelial growth factor, are included in combined treatment regimens based on chemotherapeutic regimens and seem promising in terms of effectiveness and safety (Andreev D. A., Zavyalov A. A., Kokushkin K. A., Davydovskaya M. V., 2018).

Determination of the status of mutations in the KRAS and NRAS genes is a necessary requirement in the treatment of patients with CRC. Patients with certain mutations in the KRAS and NRAS genes are resistant to therapy with anti-EGFR drugs and have a median survival rate lower than with WT (wild type) genotypes, which indicates a negative prognosis in the case of mutations. The presence of a mutant allele in one of these genes indicates an unfavorable prognosis for the patient and insensitivity to anti-EGFR therapy. Currently, there is no officially registered drug that inhibits GTPase Ras (Brovkina O. I., Nikitin A. G. M, 2020). In the course of experimental studies, it was noted that cultured human CRC cells containing KRAS or BRAF mutations selectively die when exposed to high levels of vitamin C. This effect is due to increased uptake of the oxidized form of vitamin C, dehydroascorbate (DHA), via the glucose transporter GLUT1. Increased DHA uptake causes oxidative stress, as intracellular DHA is reduced to vitamin C, depleting glutathione. Thus, reactive oxygen species accumulate and inactivate glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Inhibition of GAPDH in highly glycolytic KRAS or BRAF mutant cells leads to an energy crisis and cell death that is not observed in wild-type KRAS and BRAF cells (Jihye Yun et al., 2015).

The data of these studies allow us to justify further study of the effect of high doses of DHA on KRAS-mutant CRC cells in order to improve the therapeutic response.

Purpose of the study:

Development of a therapy for colorectal cancer treatment through a combination of oxidative drugs targeting kras mutant cancer cells.

Research object:

Patients with a diagnosis of colorectal cancer of stage I, II, III, and IV stages, with a confirmed KRAS mutation status.

Subject of the study:

Мутация KRAS mutation in patients with colorectal cancer after surgical treatment.

Research objectives:

1. To study the relationship between the types of mutations and the prevalence of the process.
2. To study the relationship between mutation types and treatment response.
3. Determination of the cytotoxic generation of ROS under the action of a combination of oxidizing drugs on KRAS mutant cancer cells.
4. Determination of colorectal cancer types that are sensitive to a combination of oxidative agents.
5. Development of recommendations for the use and combination of oxidative drugs according to the type of colorectal cancer.

Scientific novelty:

Prognostic factors that determine the right-sided localization of the KRAS mutation "wild type" and the left-sided localization of the KRAS mutation G12D, G13D, which affect the prevalence and aggressiveness of the disease, were determined.

For the first time, experimental biochemical studies of mitochondrial-dependent ROS production induced by a combination of oxidative drugs ATO/D-VC, D-VC, ATO/L-VC, and L-VC in KRAS mutant colorectal cancer cells were performed and the ROS detection method was regulated. The combination of ATO and D-VC was highly effective, causing the death of up to 80% of KRAS mutant cells after 48 hours and a significant increase in oxidative stress.

Provisions submitted for defense:

1. The combination of ATO and D-VC drugs acts by inducing mitochondrial ROS generation and cytotoxic oxidative stress of KRAS mutant cancer cells.
2. The combination of ATO/D-VC drugs is effective in suppressing the tumor growth of KRAS G12D mutant tumors.

Practical significance:

A combination of anti-oxidative drugs ATO/D-VC has been developed for suppression of KRAS-mutant colorectal cancer cells.

Our results point to the therapeutic potential for developing a new type of antitumor therapy and entering clinical trials.

Scientific project No. 0122RK00015 dated October 16, 2023 "Development of anti-cancer therapy by induced glucose-dependent cytotoxic oxidative stress". Phase I and II clinical trials based on KazNRIOR

Conclusions

1. The KRAS G12d mutation G12D is characteristic of right-sided colon cancer, more often than the" wild type " it has a low differentiation (χ^2=22.8, p <0.001), is characterized by a longer hospitalization period of 16 days [IQR, 14.00;32.00] (p= 0.007), and the average median survival is 411 days [IQR, 14.00; 32.00]. 324.00; 583.00] (p <0.001) and relapse-free 467 days [IQR, 301.00; 842.00] (p= 0.018). KRAS WTWhile KRAS WT was found in left-sided colon cancer and rectal cancer, characterized by moderate to high differentiation, the average median survival was 1004 days [IQR, 749.00; 1069.00] (p <0.001) and recurrence-free 627 days [IQR, 456.00; 1039.50] (p= 0.018).

2. In right-sided colon cancer, there is a decrease in the Garkavy index to 0.24 (p=0.003), which indicates an inferior immune response in the inflammatory process. And the Index of immunoreactivity was reduced in all localizations of colorectal cancer (p = 0.003), the agranulocyte index and ESR were also reduced in right-sided colon cancer to 0.72 (p = 0.002). While in left-sided colon and rectal cancer the Ostrovsky leukocyte intoxication index was reduced to 0.3 and 0.34, respectively (p <0.001).

3. The combination of 5 μm ATO and 1 mM D-VC caused the death of 70% of KRAS mutant cancer cells AK 192 at 24 h and 80% at 48 h. 70% and 90% of apoptotic cells were observed after combined treatment with 5 μm ATO and 1 mM D-VC at 24 and 48 h, respectively. ATO/D-VC caused a 7.3-and 7-fold increase in mitochondrial ROS (mtROS) after 24 and 48 hours, respectively, compared to the control group. This indicates that the combination of ATO/D-VC is more effective in inducing cell apoptosis.

4. KRAS-mutant adenocarcinoma cells have been experimentally proven to have different sensitivity to the ATO/D-VC combination. The most aggressive tumor cell lines AK 192 with the KRAS G12D mutation showed a pronounced sensitivity to this combination, which confirms the possibility of using oxidative stress in the treatment of specific tumor subtypes.

5. The Eurasian patent (Appendix K) describes a combination of ATO and D-VC for the treatment of malignant tumors with KRAS mutations, causing cytotoxic oxidative stress. D-VC is effective for inducing stress in cells with mutant KRAS, and in combination with триоксидомarsenic trioxide (ATO) significantly enhances its effect. The study showed that the combination of ATO/D-VC is more effective in suppressing tumor growth. This combination causes severe oxidative stress and cell death with the KRAS mutation in vivo. The mouse xenograft model with KRAS AK192 (G12D) and HCT116 cell lines showed a positive response and efficacy to treatment (the average tumor weight was 3.57 and 3.4 times lower compared to the control group, respectively). The invention has prospects for application in medical practice.

Approbation of the dissertation

The main results of the research and the thesis provisions were reported and discussed at:

– XIIIth Congress of Oncologists and Radiologists of the CIS and Eurasia, April 27-29, 2022, online.

- International Conference of the Center for Life Sciences "Modern perspectives for biomedical sciences", October 20-21, 2022, Astana, Kazakhstan.

- International Scientific and Practical Conference «Онкологияға инновациялық технологияларды енгізудің тәжірибесі мен болашағы», October 27-28, 2022, Astana, Kazakhstan.

- International Scientific and Practical Conference of Young Scientists and Students "Science and Youth: Discoveries and Prospects", April 12-13, 2023, Astana, Kazakhstan.

- International Scientific and Practical Conference «Замануи онкологияның инновациялары және жетістіктері, өзекті мәселелері», November 23-24, 2023, Astana, Kazakhstan.

- XIVth Congress of Oncologists and Radiologists of the CIS and Eurasia countries, dedicated to the 30th anniversary of ADIOR of the CIS and Eurasia, April 25-27, 2024, Dushanbe, Tajikistan.

- Extended meeting of the Department of Oncology of NJSC "Astana Medical University", June 26, 2024.

Publications on the topic of the dissertation

Based on the results of the dissertation research, 15 papers were published, including 1 in periodicals recommended by the Committee for Quality Assurance in Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan, 2 articles in peer-reviewed international journals indexed in international databases (Scopus), 10 publications in materials of international and national conferences (Kazakhstan, Russia, Tajikistan), 1 article in the Russian journal of Oncology and Surgery, 1 article in the Eurasian Journal of Applied Biotechnology.

2 certificates of entering information into the state register of rights to copyrighted objects were received (Appendix A,B), 3 acts of implementation in the "Multidisciplinary Medical Center" of Astana (Appendix C, D, E), and 1 act of implementation in the educational process in the discipline "Oncology "and" Oncology in hospital " NAO "Astana Medical University" (Appendix E).

Dissertation's personal contribution

The work was carried out in accordance with the direction of development of science in the field of "Life and HealthSciences" approved by the Higher Scientific and Technical Commission under the Government of the Republic of Kazakhstan.

The doctoral candidate independently collected clinical material, took informed consent to participate in the project, collected and analyzed medical records, and dynamically monitored patients with colorectal cancer.

The doctoral candidate independently collected postoperative material, transported the material, participated in the development of xenograft models, injected the studied mice, and performed autopsies, blood sampling, and analysis of mice.

The dissertation candidate independently analyzed and summarized the results of the study, carried out statistical data processing.

Scope and structure of the dissertation

The dissertation is written in Russian, presented on 132 pages of printed computer text, including the title page, content, normative references, definitions, notations and abbreviations, introduction, main part, conclusion, conclusions, practical recommendations and a list of references. The thesis is illustrated with 47 figures and 29 tables. The list of references consists of 90 sources.