ANNOTATION
dissertation work
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on the topic: "The role of oncoprotein H-Ras expression in the efficacy of neoadjuvant chemotherapy for breast cancer" degree of Doctor of Philosophy (PhD) specialty 6D110100 - Medicine

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The relevance of research

The results of treatment for breast cancer (BC) have improved thanks to an in-depth study of the biological properties of this tumor and the development of personalized medicine.

However, breast cancer is still the leading cause of death. Therefore, active research is being carried out on the molecular genetic characteristics of this tumor and protein structures [Leong AS, Zhuang Z., 2011] involved in ensuring the high viability of malignant cells in order to determine the prospects of using such proteins as diagnostic and prognostic biological markers and molecular targets for drug prescribing means of targeted action.

Proteins of the RAS family (H-Ras, K-Ras and N-Ras) are small G-proteins that are involved in signal transmission to the nucleus. Mutations in the KRAS gene are associated with the highest percentage of all cancers — 21.6%, followed by NRAS — 8.0% and HRAS — only 3.3% [Gloria M., Jorge A., 2016]. Activation of RAS oncogen mutations can lead to malignant transformation and is found in many human cancers, including 90% of pancreatic tumors, 50% of colon cancer, 50% of thyroid cancer, and 5% of breast cancer [Cree I. A., 2016]. The low percentage of RAS mutations in breast cancer has led to the fact that they were not significant in the pathogenesis of malignant neoplasms until recently [Pylayeva-Gupta Y., 2011].

H-Ras is the most studied gene in the RAS family and the mutation frequency is minimal, as a result of which the interest of researchers gradually decreased until Spandidos DA et. al. in their work they did not show for the first

time that malignant breast tumors have increased expression of the H-Ras oncogen [Spandidos D.A., Karaiossifidi H., Malliri A., 1992].

To perform its function, the H-Ras oncoprotein needs to acquire an appropriate structure and join the inner surface of the membrane using the farnesyl transferase enzyme [Kazi A., Xiang S., Yang H., 2019]. Without the farnelization process, the RAS loses its ability to phosphorylate and transmit signals from the receptor to the cell nucleus. From this point of view, drug therapy aimed at inhibiting farnesyl transferase is a promising area of modern oncology.

RAS oncoproteins are also activated through upstream mechanisms, as some growth factor receptors transmit their signals via RAS and can be expressed in breast cancer [Cox A.D., Der C.J., Philips M.R., 2015]. Growth factor receptors include the epidermal growth factor receptor EGFR and the Her / 2neu tyrosine kinase receptor, which overexpress from 20 to 50% of breast cancers [Ali R., Wendt M.K., 2017]. A number of studies have shown a significant decrease in EGFR expression after neoadjuvant chemotherapy [Haddad T.C., Goetz M.P., 2015], which has been clinically manifested by an improvement in immediate efficacy and an increase in overall and relapse-free breast cancer survival rates.

Thus, the study of the expression ability of H-Ras cancer proteins in breast cancer, as well as their ability, together with other molecular markers, to influence the prognosis of breast cancer is relevant.

Such studies will contribute to a more in-depth study of the pathogenetic mechanisms involved in the progression of this disease and will open up opportunities for the development and implementation of new targeted drugs in clinical practice.

The aim of this study to evaluate the effect of H-Ras expression of oncoproteins on the effectiveness of neoadjuvant therapy for breast cancer.

Research Objectives:

1. To determine the level of expression of H-Ras oncoproteins before treatment with breast cancer patients and to study the correlation between the expression of H-Ras oncoproteins with the main tissue markers (Her2neu, ER, PR, Ki 67) that determine the tumor phenotype.

2. To assess the effect of the expression of H-Ras oncoproteins on the immediate efficacy of neoadjuvant chemotherapy in patients with breast cancer.

3. To study the effect of the expression of H-Ras oncoproteins on the rates of relapse-free survival of patients who received neoadjuvant chemotherapy.

4. To develop a prognostic model of relapse-free survival of breast cancer, depending on immunohistochemical features.

Scientific novelty

1. For the first time, the expression of H-Ras cancer proteins in breast cancer patients was studied and its correlation with Her2neu positive breast cancer and a high proliferative activity index Ki 67 was determined (certificate of state registration of rights to a copyrighted object issued by the Committee of the Ministry of Justice of the Republic of Kazakhstan No. 3990 dated June 11, 2019 .).

2. For the first time it was found that the positive expression of H-Ras proteins did not affect the indices of the immediate effectiveness of neoadjuvant therapy according to the schemes: AS, AC + arglabin, arglabin in breast cancer patients.

3. For the first time, it was shown that neoadjuvant therapy with the inclusion of the drug Arglabin improved the rates of relapse-free survival of breast cancer patients with positive expression of H-Ras proteins.

4. For the first time, a prognostic model of relapse-free survival of patients with breast cancer was developed taking into account the expression status of H-Ras oncoproteins.

The practical significance of the work

Determination of the expression of H-Ras oncoproteins in patients with a positive status of the Her2neu tissue marker and a high level of Ki-67 proliferative activity index indicates a more aggressive type of breast cancer and requires a special approach to treatment.

The type of combined neoadjuvant therapy of breast cancer was determined, which is most effective in the positive expression of H-Ras oncoproteins. The combination of the standard AS regimen (adriablastin + cyclophosphamide) with the farnesitransferase inhibitor arglabin statistically significantly increases the period of relapse-free survival in patients with breast cancer.

Determining the period of relapse-free survival of patients with breast cancer based on a prognostic model depending on the expression of immunohistochemical parameters (Her2neu, Ki-67, H-Ras) allows to determine the occurrence of relapse individually for each patient.

The main provisions to be defended:

1. The expression of H-Ras cancer proteins depends on the presence of Her2neu tissue marker in the tumor and the high level of Ki-67 proliferative activity index.

2. The positive expression of H-Ras oncoproteins has a negative effect on the results of the immediate effectiveness of neoadjuvant drug therapy with the inclusion of Arglabin in patients with breast cancer.

3. Relapse-free survival rates differ significantly depending on the expression status of H-Ras oncoproteins in patients with breast cancer: with positive expression of H-Ras, the period of relapse-free survival is higher than with

negative expression with Arglabin monotherapy and in combination with standard therapy with Arglabin.

4. The constructed prognostic model of the period of relapse-free survival of patients with breast cancer is an additional diagnostic tool for predicting the occurrence of relapse.

Practical implementation

Based on the dissertation materials, 1 certificate of registration of rights to the copyright object No. 3930 dated June 11, 2019 “Immunohistochemical Features of H-Ras Cancer Proteins in Breast Cancer after Combined Treatment” was obtained. There are acts of introducing the results of scientific research into practical activities.

Work approbation

The main provisions and results of the dissertation were reported and discussed at the XIV All-Russian Scientific and Practical Conference with international participation named after A.Yu. Baryshnikova “Domestic antitumor drugs”, Moscow March 16-17, 2017; VI Congress of Oncologists and Radiologists of the Republic of Kazakhstan, Almaty, April 27-28, 2017; International Scientific Symposium "Astana Biotech-2018", Astana, June 12-13, 2018; The conference of young scientists "Science and Health" dedicated to the 70th anniversary of the corresponding member of the NAEN of the Republic of Kazakhstan, Professor A. Dyusupov and Associate Professor B. Dyusupova, Semey, October 5, 2018; IV International scientific-practical conference "Global science and innovation 2019: Central Asia", Astana, January 21, 2019; II Russian Winter School of young scientists and doctors in pharmacogenetics, pharmacogenomics and personalized therapy, Moscow, February 12-15, 2019; XVI All-Russian Scientific and Practical Conference with International Participation named after A.Yu. Baryshnikova “Domestic antitumor drugs”, Yekaterinburg March 10-14, 2019; Oncology chair meeting of 06/26/2019, protocol No. 2.

List of scientific papers published on the topic of the dissertation

Based on the materials of the dissertation, 13 scientific papers were published, of which: 3 in scientific publications recommended by the Committee for Control in the Field of Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan; 2 publications in the international scientific publication included in the Scopus information base - “Georgian Medical News”, “Open Access Macedonian Journal of Medical Sciences”; 5 publications in the materials of international conferences; 3 publications in materials of foreign conferences.

Scope and structure of the dissertation: The dissertation is presented on 108 pages of a computer text consisting of introduction, literature review, materials

and methods, research results, discussions, conclusions and applications. The dissertation is illustrated by 20 tables and 37 figures. The list of references includes 218 sources of domestic, Russian-language and English-language literature.

Materials and research methods

The collection of clinical material was carried out in accordance with the rules adopted by the Ethics Commission of the Karaganda State Medical University.

The study included 100 patients aged 29 to 78 years with an established diagnosis of breast cancer stage II and stage III disease (T2-4N0-2M0) who received complex therapy at the Karaganda Regional Oncology Center from 2012 to 2014.

In all patients, breast cancer was immunohistochemically and morphologically verified.

The study of morphological material for determining the expression of H-Ras was carried out on the basis of the Regional Oncology Center, Karagada, from September 2017 to October 2018, as part of a grant project (No. AP 05130956) of the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan "Pharmacogenetic study of terpenoid molecules and molecular mechanisms of their action" in the conditions of JSC "International Scientific and Production Holding" Phytochemistry ", Karaganda.

Before neoadjuvant drug therapy, the expression of H-Ras oncoproteins was determined and, depending on its level, patients were randomized into 2 treatment groups: a comparison group (without H-Ras expression) and a test group (with positive expression depending on H-Ras).

The comparison group included 55 patients with negative expression of H-Ras cancer proteins.

The study group included 45 patients with negative expression of H-Ras cancer proteins.

Neoadjuvant therapy was carried out with an assessment of its immediate effectiveness and the level of H-Ras of cancer proteins was determined after treatment.

At the final stage, an assessment of the long-term results of treatment was carried out and a prognostic model of an unfavorable prognosis of breast cancer was constructed.

Statistical processing of data

When processing the results, the following were used: the method of variation statistics with the assessment of reliable results by the Student criterion, the method of differences between the two students, the Spearman and Cramer

rank correlation method (rs), the Chi-square Pearson method (for binary and nominal indicators).

Statistical data processing was performed using application packages Statistica 10, Excel. To describe the quantitative indicators, the mean value and standard deviation in the format "M ± m" were used.

To calculate and compile statistical material, we used a personal computer and the SPSS Statistica 10 application software package, and the Excel processor from the Microsoft Office 2010 office software package. The relapse-free survival forecast was calculated in the Rstatistic program.

The level of statistical significance was fixed at the level of probability of error $p < 0.05$.

Conclusions:

1. Expression of H-Ras detected before the start of neoadjuvant drug therapy in 45 (45.0 ± 5.0%) breast cancer patients was more common in patients with Her2neu positive cancer and a high level of Ki-67 proliferative activity, which was demonstrated by the presence of a strong statistically significant communication at Her2neu ($Rs = 0.89$, $p < 0.05$), and at Ki-67 ($Rs = 0.70$, $p = 0.001$).

2. Expression of H-Ras adversely affected the immediate effectiveness of neoadjuvant drug therapy. In patients with negative expression, the frequency of the overall effect was statistically significant by 24.1% ($p < 0.05$), disease progression was 22.2% lower than in patients in the study group with positive expression of H-Ras ($p < 0, 05$). In the study group with positive H-Ras expression, 32.9% more often showed mild pathomorphosis of I - II degree ($p<0.05$), and in the control group with negative H-Ras expression, 32.9% more often - pronounced pathomorphism III - IV degrees ($p<0.05$).

3. Expression of H-Ras adversely affected relapse-free survival rates. In the control group of patients with negative expression of H-Ras, this indicator was 33.0 ± 1.1 months, in the study group with positive expression, respectively, –16.0 ± 1.0 months.

4. In patients with positive H-Ras expression who received Arglabin, a statistically significant increase in disease-free survival was observed up to 16.5 ± 1.1 months compared with the standard AS regimen (13.5 ± 1.1 months) ($p<0, 05$), the addition of Arglabin to the standard AS regimen also increased this indicator to 16.4 ± 1.2 months ($p<0.05$).

5. With a positive Her2neu and a high level of H-Ras (9-12 points), the period of relapse-free survival was 16.98 months, with a moderate severity of H-Ras (6-8 points) - 21.33 months, with a low degree of H- Ras - 25.68 months. The prognostic period of disease-free survival with negative expression of H-Ras oncoproteins (0-3 points) and positive Her2neu receptors in patients with breast

cancer was 32.00 months. Negative expression in both cases was accompanied by a shorter period of disease-free survival according to the prognostic model, which was 30.00 months.

Perfomed: Zhumakaeva A.M.