ejpmr, 2019,6(4), 110-114

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

THE POSSIBILITIES OF MOLECULAR-BIOLOGICAL MARKERS IN SUPPRODUCTIVE IMMUNOTHERAPY IN PATIENTS WITH ONCINE-INTAL DISEASES

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Article Received on 03/02/2019 **Article Revised on 23/02/2019** **Article Accepted on 17/03/2019**

SUMMARY

The aim of the study was to study the expression of molecular biological tumor markers in patients with cervical cancer and ovarian cancer, and their effect on the course of accompanying immunotherapy. It was shown that the accompanying immunotherapy scheme, including EIPHT with plasmapheresis, had the greatest effect in increasing the 5-year survival of patients with cervical cancer and OC. The analysis of the correlation of the level of tumor markers and 5-year survival of patients suggests that a positive level of p53, VEGF and Ki-67 in patients with cervical cancer and OC may, along with the high proliferative activity of the tumor, be the basis for carrying out this category of accompanying patients immunotherapy with EIPHT, which can significantly increase the effectiveness of standard antitumor treatment regimens. With a positive level of these tumor markers, along with a high proliferative activity of the tumor, it is possible to recommend carrying out an accompanying immunotherapy with EIPHT with plasmapheresis.

KEYWORDS: Cervical cancer, ovarian cancer, molecular biological markers, immunotherapy, accompanying therapy.

Topicality

It is known that modern methods of treating and predicting the course of cancer, including oncologic and gynecological diseases are the study of molecular biologic markers in the tumor tissue and the mechanisms of their influence on the course of the oncological process.[2,4,5,9] Thus, it was found that the use of modern immunotherapy in the treatment of oncologic diseases using new opportunities to study molecular-biological markers can help obtain data on the prognosis of the disease, moreover, promote the appointment of rational and effective treatment, which determines the topicality of this study.[1,3,6,19]

The immune system is at the center of all attempts currently being made to improve the effectiveness of antitumor therapy, and the task of activating the antitumor potential of the immune system is most important in modern oncology. Over time, immunotherapy may be the most promising method for treating tumors, since it is a physiologically adequate method that restores the natural strength of the patient's body to combat the neoplastic process and infectious complications that often occur during treatment. Obtaining new unique immunomodulatory drugs has created a qualitatively new basis for the correction of immunity disorders, it became possible to act more selectively on individual components and links of this system.[3,5,10,11] On the other hand, there are prospects for methods that have a positive effect on the immune system as a whole - the use of adaptogens, plasma exchange methods, perfusion of blood through sorbents, treatment with various activators, etc. Numerous studies have shown that modern methods of immunotherapy in the treatment of malignant tumors can have a normalizing effect on the immune status of cancer patients, give an objective antitumor effect, and also contribute to the regression of tumor pleurisy and ascites with chemoresistant forms of cancer. A promising direction in the treatment of malignant tumors at the present stage of development of immunotherapy is a combination of methods for the activation of specific and nonspecific immunity.[13,14,15,18] Unlike conventional immunotherapy methods, when immunomodulatory drugs are taken in pill form or administered intramuscularly or intravenously, the use of methods of **extracorporeal immunopharmacotherapy** (EIPHT) allows selectively isolating the blood cells of the immune system directly from the blood. The isolated leukocytes are processed by special technologies with immunomodulating drugs and then, already activated, they are returned back into the vascular bed, after which

they are able to synthesize the factors that activate the immune system and activate other cells of the immune system. This direction of immunotherapy has great promise in oncology practice in connection with the ability to remove the effects of cancer and chemoradiation intoxication, as well as activate its own system of antitumor defense of the body.[10,11,12,17] However, in the literature there is very little information about the use of the EIPHT method in the treatment of cancer. Today, one of the most promising areas in the diagnosis of malignant tumors is the identification of tumor markers, which may provide additional information about the biological characteristics of the tumor. Much attention is paid to the study of markers characterizing apoptosis, cell proliferation, such as p53, Bcl-2, Ki-67 and EGFR, EGF proteins.[5,8,11,16] In addition, many methods of immunotherapy in the field of oncology, are still empirically used, there are no clear criteria for indications and contraindications in the treatment of malignant tumors of various localizations. Determining the optimal doses of drugs, the sequence of different effects on the immune system, their duration, as well as the effect of immunotherapy on the immediate and long-term results of antitumor therapy, require the efforts of many researchers.

The aim of the research was to study the expression of molecular biological tumor markers in patients with cervical cancer and ovarian cancer (oncogynecological patients), and their effect on the course of accompanying immunotherapy.

MATERIAL AND RESEARCH METHODS

Molecular biological studies of the tumor were performed in patients with cervical cancer (CC) and ovarian cancer (OC) $T_{2-3}N_{0-1}M_0$ stages (stage II-III), which were hospitalized in the oncologic and chemotherapy departments in 2010-2015. Immunohistochemical methods of research were carried out on histological specimens of surgical biopsy material of the primary tumor of patients with cervical cancer and OC. Histological preparations were stained with conventional methods and immunohistochemical studies were performed. Paraffin sections were deparaffinized and rehydrated according to standard procedures. For visualization of the immunohistochemical reaction, the DAB + system [DakoCytomation, Denmark] was used. Evaluation of staining results was performed using a Leica light microscope (Germany).

Immunotherapy in the complex of standard polychemotherapy was carried out on three groups of patients: 1 group - 22 patients with cervical cancer and OC without using immunotherapy (control group); Group 2 - 19 patients with cervical cancer and OC in combination with extracorporeal immunopharmacotherapy (EIPHT); Group 3 - 24 patients with cervical cancer and OC in combination with EIPHT + plasmapheresis (EIPHT + PPh).

THE RESULTS AND DISCUSSION

The analysis showed that the highest 5-year survival rates were observed in the group of patients with cervical cancer who underwent EIPHT with plasmapheresis (PPh). Thus, in the control group of patients, the survival rate with a negative level of p53 was 64.4 ± 6.7%, whereas in the groups with EIPHT and EIPHT + PPh, these indicators were 73.2 ± 7.7 ($p > 0.05$) and 77, 4 ± 8.4% ($p > 0.05$), respectively. With a positive value of this tumor marker, 5-year survival was 46.1 ± 5.1% for the control group without immunotherapy and 55.6 ± 4.9 ($p < 0.05$) and 60.5 ± 6.4% ($p < 0.05$) for groups using immunotherapy methods, respectively. The p53 suppressor gene encodes a nuclear protein that modulates gene expression, which is responsible for DNA repair, cell division and apoptosis. To date, there is no consensus in the literature regarding the dynamics of p53 expression during cervical cancer progression. According to the data of a number of authors, it can both increase and decrease with this disease. The mut-p53 accumulation rate increases with an increase in the malignancy of tumors, while with benign tumors mut-mutant accumulation does not occur, and in malignant tumors the accumulation rate increases to 46%.[6,9,18,19] Bcl-2 protein has an important role in the regulation of apoptosis. It was shown that a high degree of Bid tumor cell expression (a protein from the Bcl family, which plays an important role in the regulation of apoptosis and integrating signals for mitochondria) correlates with an unfavorable cervical cancer forecast. Bcl-2 can completely delay apoptosis caused by p53 and other stimulants, including cytotoxic drugs, but does not stop apoptosis caused by cytotoxic T-lymphocytes.[12,17,19] In various studies, it was shown a sharp and significant increase in the expression of Bcl-2 in localized forms of cervical cancer compared with the initial stages and the subsequent decrease in expression in the locally advanced process.[15,18,19] Indicators of 5-year survival in the control group of patients with cervical cancer when considering the Bcl-2 marker in the control group were 57.2 ± 5.0% with its negative level and 49.5 ± 5.6% with its positive value. Accordingly, in the groups with immunotherapy, this indicator was 73.7 ± 7.2% ($p < 0.05$) and 76.4 ± 7.0% ($p > 0.05$) with a negative level and 57.5 ± 5, 4% ($p > 0.05$) and 66.2 ± 7.4% ($p > 0.05$) - with its positive value. The epidermal growth factor EGFR receptor is a transmembrane glycoprotein located on chromosome 7p12. EGFR functions through dimerization, activating tyrosine kinase, participating in the regulation of normal and neoplastic cell proliferation. During cell transformation, there is an increase in the synthesis of these proteins and overexpression of receptors on the cell surface. The EGFR is present in many normal tissues and pronounced expression is observed in solid tumors, including cervical cancer. According to literature data, EGFR expression is observed in approximately 40% of malignant tumors of the gastrointestinal tract, lung, ovary, uterus. At the same time, the role of EGFR as a prognostic marker in cervical cancer is not definitively defined.[9,13,17,19] When

considering the EGFR marker, it was shown that with its negative value in patients with cervical cancer in the group without immunotherapy, 5-year survival was 55.4 ± 5.3, and with positive - 54.8 ± 5.8%. In the group with EIPHT, the corresponding indicators were 66.8 ± 7.0 (p <0.05) and 64.3 ± 6.9% (p> 0.05), and in the group with EIPHT + PPh - 69.5 ± 6.3 (p> 0.05) and 67.4 ± 6.8% (p> 0.05). The main activator of angiogenesis is considered the vascular endothelial growth factor (VEGF), responsible for the proliferation and migration of endothelial cells, as well as directly related to invasion and metastasis of the tumor. Accumulated evidence of the involvement of VEGF and EGFR in the construction of the vascular bed, the growth and progression of malignant tumors. Moreover, the interaction of these ligands with transmembrane tyrosine kinase receptors is considered as the most important autocrine pathway for tumor promotion.[5,9] With a negative value of VEGF level in the control group, 5-year patient survival was 64.3 ± 7.2 and with a positive one - 53.0 ± 6.0%. In groups of patients in which accompanying immunotherapy was applied, the corresponding values for negative VEGF values were 73.4 ± 6.8 (p> 0.05) and 75.2 ± 7.4% (p> 0.05), with positive the level of this tumor marker is 60.7 ± 5.6 (p <0.05) and 67.4 ± 6.8% (p <0.05). The Ki-67 antigen is a nuclear protein whose expression is noted in the active phase of the cell cycle, including mitosis. According to the literature, the expression of Ki-67 increases with the defeat of the cervix. The proliferative index Ki-67 is considered as an independent prognostic indicator of the occurrence of relapse, general and relapse-free survival, a predictive factor for determining sensitivity to chemotherapy (CT) and RT. The Ki-67 index allows to assess the degree of malignancy of the tumor and predict the course of the disease in conjunction with other factors. It is shown that a high level of the Ki-67 index is associated with an unfavorable prognosis. In particular, with a high level of Ki-67, there is a deterioration in the rates of relapse-free and overall survival of patients with breast cancer, ovary, colon, bladder, soft tissue sarcomas, etc.[15,17] The Ki-67 tumor marker was not detected in tumors of 7 patients with cervical cancer, in whom 5-year survival in the control group was 65.2 ± 5.8%. With its positive level in the group without immunotherapy, this indicator was 44.2 ± 6.1%. In the groups with EIFI and EIPHT + PPh with a negative Ki-67, the survival rates were 76.4 ± 7.9 (p> 0.05) and 77.8 ± 6.7% (p> 0.05), respectively and with a positive - 57.8 ± 5.9 (p <0.05) and 63.3 ± 7.4% (p <0.05). With a low level of PA index, which was calculated by Ki-67, 5-year survival in patients with cervical cancer in the group without immunotherapy was 62.6 ± 5.8, with a high level - 44.8 ± 5.1%. In groups using immunotherapy methods, 5-year survival with a low PA index was 73.7 ± 8.2 (p> 0.05) and 78.1 ± 7.7% (p> 0.05), with a high index - 54.5 ± 5.4 (p> 0.05) and 62.2 ± 6.4% (p <0.05). When studying the expression of molecular biological tumor markers in patients with OC, it was shown that a negative p53 level was noted in 5 (16.6%) patients, and a positive one - in 25 (83.3%). The corresponding indicators in the study of HER-2 / neu were found in 24 (80.0%) and 6 (20.0%) patients, in the study of EGFR - in 21 (70.0%) and 9 (30.0%), VEGF - in 4 (13.3%) and 26 (86.7%) and in the study of Ki-67 - in 6 (20.0%) and 24 (80.0%) patients. Thus, in the majority of patients with OC, the level of tumor markers studied was positive, with the exception of HER-2 / neu and EGFR, which were negative in 80.0 and 70.0% of patients, respectively. As in the cervical cancer groups, with OC, the highest rates of 5-year survival were observed in patients undergoing EIPHT with plasmapheresis. Thus, in the control group of patients, the survival rate with a negative level of p53 was 62.6 ± 6.8%, whereas in the groups with EIPHT and EIPHT + PPh, these indicators were 74.1 ± 6.7 (p> 0.05) and 78, 1 ± 7.7% (p> 0.05), respectively. With a positive value of this tumor marker, 5-year survival was 43.8 ± 5.8% for the control group without immunotherapy and 58.6 ± 6.7 (p <0.05) and 60.4 ± 6.3% (p > 0.05) for groups using immunotherapy methods, respectively. The p53 protein, being a product of the tumor suppressor gene TP53, is expressed in all cells of the body. In the absence of damage to the genetic apparatus, the p53 protein is in an inactive state, and when a lesion appears, DNA is activated. The result of p53 activation is cell cycle arrest and DNA replication; with a strong stress signal - the launch of apoptosis. Disruptions in the development of apoptosis can occur when the key gene of this process, p53, loses its function. This may occur as a result of the mutation of the p53 gene with the formation of the mutant oncoprotein - mut-p53, which is observed in the conditions of pathology or as a result of the blockade of p53 by other proteins, which, first of all, include Bcl-2. An increase in the expression of mutated p53 in a tumor is accompanied by its greater aggressiveness, since the number of tumor cells undergoing apoptosis decreases. In OC, according to different researchers, mutant p53 is found in more than half of the patients already in the early stages of the disease.[3,6] In some works devoted to OC, it has been shown that HER-2 / neu amplification, occurring at 10–50%, indicates an unfavorable prognosis of the course of the disease.[2,5] However, there are opposing data, so the practical significance of HER-2 / neu testing remains disputable today.[7,11] Indicators of 5-year survival in the control group of patients when considering the HER-2 / neu marker in the control group were 62.7 ± 4.6% with its negative level and 55.2 ± 6.4% with its positive value. Accordingly, in the groups with immunotherapy, this indicator was 68.2 ± 7.2 (p <0.05) and 74.3 ± 6.7% (p <0.05) with a negative level and 69.8 ± 6.5 (p> 0.05) and 77.1 ± 6.7% (p> 0.05) - with its positive value. The epidermal growth factor receptor EGFR is a transmembrane glycoprotein located on chromosome 7p12. EGFR functions through dimerization, activating tyrosine kinase, participating in the regulation of normal and neoplastic cell proliferation. The EGFR receptor family consists of 4 members: EGFR / ErB1 / HER1, ErbB2 / Neu / HER2, ErB3 / HER3 and ErB4 / HER4. Under normal physiological conditions, the activation of HER receptors is controlled by the

temporary low expression of their ligands. During cell transformation, overexpression of these proteins and an increase in the number of receptors on the cell surface is observed. EGFR is present in many normal tissues and pronounced expression is observed in solid tumors. According to the literary data, the EGFR expression is observed in approximately 40% of malignant tumors of the gastrointestinal tract, lung, ovary, uterus.[1,4,7] When considering the EGFR marker, it was shown that with its negative value in the group without immunotherapy, 5-year survival was 64.4 ± 5.8%, and with a positive one - 57.2 ± 6.0%. In the group with EIPHT, the corresponding indicators were 72.4 ± 6.9 ($p > 0.05$) and 65.7 ± 5.6% ($p > 0.05$), and in the group with EIPHT + PPh - 74.8 ± 7.4 ($p < 0.05$) and 68.0 ± 6.8% ($p > 0.05$).

One of the most studied indicators of tumor growth aggressiveness is cell proliferation, which can be assessed using the mitotic index and the Ki-67 index. The Ki-67 antigen is expressed in almost all phases of the mitotic cycle, and in accordance with this reflects the proliferative pool of the tumor. The proliferative index Ki-67 is considered as an independent prognostic indicator of the occurrence of relapse, general and relapse-free survival, a predictive factor for determining sensitivity to chemotherapy (CT) and radiation therapy (RT). The Ki-67 index allows us to estimate the degree of malignancy of the tumor and predict the course of the disease in conjunction with other factors. It is shown that a high level of the Ki-67 index is associated with an unfavorable prognosis. In particular, with a high level of Ki-67, there is a deterioration in the rates of relapse-free and overall survival of patients with breast cancer, ovary, colon, bladder, soft tissue sarcomas, etc. The Ki-67 tumor marker was not detected in tumors of 6 patients in whom the 5-year survival group was 68.4 ± 6.9%. With its positive level in the group without immunotherapy, this indicator was 41.8 ± 3.8%. In groups with EIFI and EIPHT + PPh with negative Ki-67, the survival rates were 78.1 ± 6.5 ($p > 0.05$) and 76.8 ± 7.9% ($p > 0.05$), respectively and with a positive - 58.6 ± 6.3 ($p < 0.05$) and 60.4 ± 6.5% ($p < 0.05$). At a low level of the PA index, which was calculated by Ki-67, the 5-year survival rate in the group without immunotherapy was 68.3 ± 7.7, with a high level of it - 49.4 ± 5.4%. In groups using immunotherapy methods, 5-year survival with a low PA index was 76.1 ± 7.8 ($p > 0.05$) and 79.2 ± 8.0% ($p > 0.05$), with a high index - 64.2 ± 6.8 ($p < 0.05$) and 69.8 ± 6.7% ($p > 0.05$). Thus, in the majority of patients with cervical cancer (73.3, 80.0 and 76.7%, respectively), molecular biological markers p53 and Ki-67 were present. At the same time, markers of Bcl-2 and EGFR were detected in 36.7 and 30.0% of patients, respectively. A comparative assessment of the level of tumor markers and 5-year survival of patients with cervical cancer has shown that molecular biological markers p53, VEGF and Ki-67, as well as the level of proliferative activity (PA) of the tumor, have the greatest predictive value in terms of the effectiveness of treatment. The majority of patients with OC (83.3, 86.7, and 80.0%, respectively) had molecular biological markers p53, VEGF, and Ki-67. At the same time, the markers HER-2 / neu and EGFR were detected in 20.0 and 30.0% of patients, respectively. It was shown that a positive level of p53, VEGF and Ki-67 markers had a significant negative impact on the 5-year survival rates of patients with OC, in the case of HER-2 / neu and EGFR, this effect was not so pronounced, which indicates that they are less prognostic significance in this disease. The greatest effect in increasing the 5-year survival of patients with cervical cancer and OC, was provided by the accompanying immunotherapy scheme, including EIPHT with plasmapheresis: this effect was manifested at both positive and negative levels of tumor markers considered. The analysis of the correlation of the level of tumor markers and 5-year survival of patients suggests that a positive level of p53, VEGF and Ki-67 in patients with cervical cancer and OC may, along with the high proliferative activity of the tumor, be the basis for carrying out this category of accompanying patients immunotherapy with EIPHT, which can significantly increase the effectiveness of standard antitumor treatment regimens. With a positive level of these markers, along with a high proliferative activity of the tumor, it is possible to recommend carrying out an accompanying immunotherapy with EIPHT with plasmapheresis.

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