MOLECULAR AND GENETIC FEATURES OF SQUAMOUS CELL CARCINOMA OF VULVAR CANCER DEPENDING ON HPV STATUS

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Abstract. *The study included materials from 76 operations (110 paraffin blocks) that were not treated with neoadjuvant treatments, since preoperative exposure to tumors could significantly affect the results of the study, which is why these samples were not included in the study. It was found that the high viral load of HPV in RV correlates with the presence of metastases to the lymph nodes, invasion of the stroma, the degree of differentiation, as well as lymphovascular invasion, while it is in no way related to the stage of the disease. PD-L1 receptor expression is more often observed in HPV negative RV patients compared to HPV positive ones (7.8 vs. 3.7 p=0.03), while HPV positive patients were more likely to have STK11 mutation. At the same time, the PIK3CAE545 mutation occurred with the same frequency between the two groups of RV patients.*
Keywords: *mutation, signaling pathway, epigene, apoptosis receptor, cell cycle, transcription.*

Introduction. According to WHO, over the past few decades, there has been a rejuvenation of the Russian Federation, in the direction of its increase among young women. Due to the variety of symptoms of vulvar cancer and a wide range of benign diseases of this localization, it is still difficult to diagnose, especially in the early stages.In addition, most of the research in the field of vulvar cancer is currently focused on innovative treatment regimens, including biological agents and immunotherapy, which require a deep understanding of the basic molecular mechanisms involved in the pathogenesis of RV, and the issues of developing new schemes of combined and complex therapy are an urgent area of research in modern oncology [1,2,3,4]. With the discovery and identification of new prognostic biomarkers, approaches to the treatment of vulvar cancer will change from standard radical resections to personalized approaches. Since the identification of new prognostic variables can lead to further individualization of vulvar cancer treatment, research in the search for new biomarkers is an urgent area of modern oncology[5,6,7].

Materials and methods. We examined the materials of 76 operations (110 paraffin blocks) for which neoadjuvant treatments were not performed, since preoperative exposure to tumors could significantly affect the results of the study, which is why these samples were not included in the study. Before the molecular and IHC examination, all tissue samples were reviewed by experienced pathologists. Clinical data were obtained from patients and their medical records, after approval by the ethics committee. The pathoanatomic diagnosis was confirmed on the basis of a study of histological sections stained with hematoxylin and eosin. The sections were subjected to major dissection as needed to achieve >20% of the calculated percentage of the nucleus in each tumor sample. From 2210 formalin-fixed tumor samples from tissue paraffin blocks, 40 microns sections, ≥60 ng of DNA were excised for genomic analysis. The materials were analyzed using CGP using adapter binding, and using hybrid capture. All sequences of genomic change were

sequentially analyzed, including small variants of changes, changes in the number of copies, as well as fusion and rearrangement of genes.

The mutational load of the tumor (TVM; mutations/Mb) was determined on 0.8 – 1.1 megabase pairs of sequenced DNA. Microsatellite instability (MSI) was determined by 114 loci. Mutation tags were evaluated for all tumor samples. When the tumor sample had at least 40% compliance with the mutation process, including overexpression of ARES, hypofunction of the BRCA tumor suppressor and in the presence of a defect of repair compliance, the mutation label was considered positive.

An immunohistochemical study was performed with the determination of ligand 1 of programmed cell death (PD-L1) mandatory with CGP, for the selection of patients for immunotherapy. PD-L1 protein expression was determined on 5–micron tissue slices using a DakoPD-L1 IHC22C3 pharmDx analyzer (Agilent, Santa Clara, California) or Ventana (Oro Valley, Arizona) in accordance with the instructions of each manufacturer. Ventana PD-L1 expression is expressed as a percentage of the tumor area positively stained by tumor and immune cells, and DakoPD-L1 as an indicator of the tumor fraction. Staining of tissue samples of less than 1% was evaluated as a negative result, up to 49% as weakly positive and 50% or more percent staining as a positive result [1,6,7,8]. Total RNA was extracted from frozen tissue by microdissection. RNA was extracted from the cut and lysed tissues using the RNeasy Mini Kit (QIAGEN) and Precellys according to the manufacturer's instructions. DNA precipitates were incubated for 18 hours from the beginning of tissue lysis at a temperature of 550C using a cell lysis solution. The resulting mass was centrifuged and phenol – chloroform – isoamyl alcohol was added, which was alternated with glycogen 20 mg/ml. The alcohol was removed, the samples were dried after washing in distilled water. After assessing the quantity and quality of DNA and RNA samples, further analysis was carried out (a detailed description of isolation, molecular and IHC analysis can be found in special literature).

Results. As in the general group, squamous cell carcinoma (56.9%) and intraepithelial neoplasia (27.9%) were most often diagnosed.

We also conducted a correlation study of the relationship between human papillomavirus and the genetic profile of vulvar cancer. Staining of ligand 1 of apoptosis (PD-L1) of squamous cell carcinoma of the vulva, with a negative result for human papillomavirus, showed a higher incidence of this ligand, whereas with positive HPV, the occurrence of PD-L1 was significantly low.

Although the median TVM for HPV+ in squamous cell vulvar cancer was generally higher than the HPV result (7.8 vs. 3.7; p=0.03), a complicating factor was a higher percentage of HPV- squamous cell vulvar cancer sequenced from the primary tumor. STK11 at HPV+ was significantly higher than HPV-test results. When comparing the mutation frequency between groups with positive and negative HPV for squamous cell vulvar cancer (PRV), a difference in mutations between HPV+ and HPV tumors was observed. Most CCND1-amplified PRVs have demonstrated amplification of other genes, such as, in 11q13, including FGF3, FGF4 and FGF19. The main specific point mutation with a significant difference between HPV+ and HPV-tumors, which was saturated with the activating mutation PIK3CAE545K. Thus, this study showed that the presence or absence of human papillomavirus dramatically affects tumor differentiation. With a positive test for human papillomavirus, mutations in the PI3K/mTOR pathway increased, on the contrary, with a negative test, GA was more often determined in TP53, TERTp, CDKN2A, CCND1, FAT1,

NOTCH1, EGFR.

The status and type of HPV were determined in all samples of 186 patients. 86 patients out of 186 had HPV, mainly type 16 and type 18. The HPV+ result was mainly found in patients of younger age than in the group of elderly patients. 86 patients (46.2%) were infected with HPV, of which 23.3% had an extremely small infection, 31.4% had a clinically significant lesion in the material, and 45.3% had a high viral load. The viral load does not depend on the stage of the disease, but such indicators as the presence of metastases in the lymph nodes, invasion of the stroma, tumor gradation, lymphovascular and vascular invasion had a natural connection with viral invasion. In the presence of metastases to the lymph nodes, HPV infection was detected in all 61 cases (100%), in the presence of distant metastases in 94.1%, with G3 – 87.5%, lymphovascular and vascular invasion 94.4 and 92.6%, respectively (p= 0.95).

In our study, among patients with vulvar cancer, the greatest number of viral lesions were observed in younger patients, whereas, in elderly patients, vulvar cancer was the result of degenerative – dystrophic changes in the vulva area.

Of the 86 patients with positive HPV, 76 (88.4%) patients were under the age of 60. At the same time, among patients with vulvar cancer under 40 years of age, about 82%, up to 50 to 93% of cases of infection with human papillomavirus. Among the patients in the age group up to 60 years, 32 patients infected with the human papillomavirus were under 55 years old. If this fact is taken into account, 76.7% of patients with HPV were under the age of 55. As in the general group, squamous cell carcinoma (56.9%) and intraepithelial neoplasia (27.9%) were most often diagnosed. Although the median TVM for HPV+ in squamous cell vulvar cancer was generally higher than the HPV result (7.8 vs. 3.7; p=0.03), a complicating factor was a higher percentage of HPV-squamous cell vulvar cancer sequenced from the primary tumor. STK11 at HPV+ was significantly higher than HPV-test results. When comparing the mutation frequency between groups with positive and negative HPV for squamous cell vulvar cancer (PRV), a difference in mutations between HPV+ and HPV tumors was observed. Most CCND1-amplified PRVs have demonstrated amplification of other genes, such as, in 11q13, including FGF3, FGF4 and FGF19. The main specific point mutation with a significant difference between HPV+ and HPV-tumors, which was saturated with the activating mutation PIK3CAE545K.

Thus, this study showed that the presence or absence of human papillomavirus dramatically affects tumor differentiation. With a positive test for human papillomavirus, mutations in the PI3K/mTOR pathway increased, on the contrary, with a negative test, GA was more often determined in TP53, TERTp, CDKN2A, CCND1, FAT1, NOTCH1, EGFR.

It was found that the high viral load of HPV in RV correlates with the presence of metastases to the lymph nodes, invasion of the stroma, the degree of differentiation, as well as lymphovascular invasion, while it is in no way related to the stage of the disease. PD-L1 receptor expression is more often observed in HPV negative RV patients compared to HPV positive ones (7.8 vs. 3.7 p=0.03), while HPV positive patients were more likely to have STK11 mutation. At the same time, the PIK3CAE545 mutation occurred with the same frequency between the two groups of RV patients.

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