https://doi.org/10.29013/ESR-21-7.8-14-18

Polatova Djamila,
Republican Specialized Scientific and Practical Medical Center of Oncology and Radiology, Tashkent State Dental Institute, DSc in Medicine, Head of Oncology and Medical Radiology Department
Navruzova Visola,
PhD in Medicine, post-doctoral student in Oncology and Medical Radiology Department Tashkent State Dental Institute

MOLECULAR GENETIC FEATURES OF VULVA SQUAMOUS CELL CARCINOMA DEPENDING ON HPV STATUS

Abstract. The study included materials from 76 operations (110 paraffin blocks), which did not undergo neoadjuvant treatment methods, 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 a high viral load of HPV in VC correlates with the presence of metastases in the lymph nodes, invasion of the stroma, the degree of differentiation, and lymphovascular invasion, while it is not associated with the stage of the disease. Expression of PD-L1 receptors was more frequently observed in HPV negative VC patients compared to HPV positive ones (7.8 vs 3.7 p=0.03), while STK11 mutation was more common in HPV positive patients. At the same time, the PIK3CA E545 mutation occurred with the same frequency between the two groups of VC patients.

Keywords: mutation, signaling pathway, epigene, apoptosis receptor, cell cycle, transcription.

According to WHO, over the past few decades, there has been a rejuvenation of VC, in the direction of its increase among young women. Due to the variety of symptoms of vulvar cancer and the wide range of benign diseases of this localization, it is still difficult to diagnose, especially in the early stages. In addition, most 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 main molecular mechanisms involved in the pathogenesis of VC, and the development of new combination and complex therapy regimens is relevant. Direction of research in modern oncology [1; 2; 3; 4].

With the discovery and identification of new predictive biomarkers, approaches to the treatment of vulvar cancer will change from standard radical resections to personalized approaches. Since the identification of new prognostic variables may lead to further individualization of the treatment of vulvar cancer, research in the search for new biomarkers is an important area of modern oncology [5; 6; 7].

Materials and methods. We examined the materials of 76 operations (110 paraffin blocks) that did not undergo neoadjuvant treatment methods, since preoperative exposure to tumors could significantly affect the results of the study, which is why these samples were not included in the study. Prior to 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.

Pathological anatomical diagnosis was confirmed on the basis of the study of histological sections stained with hematoxylin and eosin. The sections were coarsely dissected as needed to achieve >20% of the estimated percentage of the nucleus in each tumor sample. From 2210 formalin-fixed tumor samples from tissue paraffin blocks, 40 μm sections, ≥60 ng DNA were excised for genomic analysis. Materials were analyzed with CGP using adapter tethering, and using hybrid capture. All genomic alteration sequences were sequentially analyzed, including minor variant alterations, copy number alterations, and gene fusion and rearrangement.

Tumor mutation load (TBM; mutations/Mb) was determined on 0.8–1.1 megabase pairs of sequenced DNA. Microsatellite instability (MSI) was determined by 114 loci.

Mutation marks were evaluated for all tumor samples. When the tumor sample had at least 40% concordance with the mutation process, including overexpression of AROBEC, hypofunction of the tumor suppressor BRCA, and in the presence of a repair match defect, the mutation label was considered positive. Immunohistochemical study was performed with the determination of programmed cell death ligand 1 (PD-L1) without fail with CGP, for the selection of patients for immunotherapy. PD-L1 protein expression was determined on 5-micron tissue sections using a Dako PD-L1 IHC22C3 pharm Dx analyzer (Agilent, Santa Clara, CA) or Ventana (Oro Valley, AZ) according to each manufacturer's instructions. Expression of Ventana PD-L1 is expressed as a percentage of tumor area, positively stained tumor and immune cells, and Dako PD-L1 as an indicator of tumor proportion. Tissue staining of less than 1% was rated as negative, up to 49% as slightly positive, and 50% or more staining as positive [1; 6; 7; 8]. Total RNA was extracted from frozen tissue by macrodissection. RNA was extracted from tissues cut and lysed using the RNeasy Mini Kit (QIAGEN) and Precellys according to the manufacturer's instructions. DNA pellets were incubated for 18 hours from the start of tissue lysis at 55 °C using cell lysis solution (Gentra Puregene Blood Kit, QIAGEN). 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 evaluating the quantity and quality of DNA and RNA samples, further analysis was carried out (for a detailed description of isolation, molecular and IHC analysis, see the special literature).

Table 1.– Gene Mutations Studied for Comprehensive Analysis of the Genetic Profile of Vulvar Tumors

№	Functional class of mutant genes	Mutations
1	*2*	*3*
1.	**Pi3K/AKT/mTOR pathway**	*PIK3CA* *PIK3CA T545K* *KMT2D* *PTEN* *STK11* *FBXW7SOX2amp* *PIK3R1* *AKT1* *MTOR*
2.	**Epigenetic regulator**	*EP300* *BAP1* *PBRM1*

1	*2*	*3*
2.	**Epigenetic regulator**	*KDM6A* *KMT2C* ARID1A
3.	**Cell cycle regulator**	RB1
4.	**Transcription regulator**	*CDK12 inactivating* *AR*
5.	**Tyrosine kinase receptor**	FGFR3
6.	**Cytokeratin**	CR
7.	**Programmed cell death receptor 1 (PD-1) ligand**	PD-L1
8.	**Mismatch repair gene (microsatellite instability)**	MSI-H/dMMR
9.	**Vascular Endothelial Growth Factor**	VEGF
10.	**Epidermal growth factor**	HER1/EGFR
11.		P53

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

We also conducted a correlation study of the relationship between human papillomavirus and the genetic profile of vulvar cancer. Staining 1 apoptosis ligand (PD-L1) of vulvar squamous cell carcinoma, negative for human papillomavirus, showed a higher frequency of this ligand, while the incidence of PD-L1 was significantly low in HPV positive (Table 2).

Table 2. – Gene Mutations Studied for Comprehensive Analysis of the Genetic Profile of Vulvar Tumors

№	**Genes**	**HPV+**	**HPV–**	**p**
1	***2***	***3***	***4***	***5***
1.	PIK3CA	32.6	14.7	0.005
	PIK3CA T545K	13.9	3.1	0.0005
	KMT2D	16.3	7.9	0.05
	PTEN	11.9	1.5	<0.0001
	STK11	11.8	1.5	<0.0001
	FBXW7	10.1	3.6	0.02
	SOX2amp	4.4	1.3	0.0218
	PIK3R1	3.1	0.8	0.18
	AKT1	2.1	0.8	0.61
	MTOR	2.1	0.9	0.59
2.	EP300	14.1	1.4	<0.0001
	BAP1	5.2	0.9	0.02
	PBRM1	5.4	1.5	0.07
	KDM6A	6.6	2.7	0.01
	KMT2C	6.6	3.4	0.40
	ARID1A	3.1	2.9	0.07
3.	RB1	5.5	1.3	0.09

1	*2*	*3*	*4*	*5*
4.	CDK12 inactivating AR	5.5 4.6	1.6 0.3	0.07 0.002
5.	FGFR3	4.7	0.1	0.003
6.	CR	4.2	0.3	0.09
7.	PD-L1	7.8	3.5	0.03
8.	MSI-H/dMMR	21.3	3.7	0.0001
9.	VEGF	27.3	41.1	0.005
10.	HER1/EGFR	11.2	18.2	0.0013
11	P53	15.9	39.7	0.0021

Although the median TBM for HPV+ vulvar squamous cell carcinoma was generally higher than the HPV- result (7.8 vs 3.7; p = 0.03), a complicating factor was the higher percentage of HPV- vulvar squamous cell carcinoma sequenced from the primary tumor.

STK11 in HPV+ was significantly higher than HPV test results.

When comparing mutation rates between HPV positive and HPV negative groups for vulvar squamous cell carcinoma (VSC), a difference in mutations between HPV+ and HPV– tumors was observed. Most CCND1-amplified VSC have shown amplification of other genes, such as in 11q13, including FGF3, FGF4, and FGF19. A major specific point mutation with a significant difference between HPV+ and HPV– tumors, which was saturated with the PIK3CA E545K activating mutation.

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 detected in TP53, TERTp, CDKN2A, CCND1, FAT1, NOTCH1, EGFR.

HPV status and type were determined in all samples of 186 patients. 86 of 186 patients had HPV, predominantly types 16 and 18. Mostly HPV+ result was found in younger patients than in the group of elderly patients. Eighty-six patients (46.2%) were infected with HPV, of which 23.3% had very little infection, 31.4% had a clinically significant lesion, 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 into the stroma, tumor gradation, lymphovascular and vascular invasion had a natural relationship with viral invasion. In the presence of metastases in 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 was observed in younger patients, while in elderly patients, vulvar cancer was the result of degenerative-dystrophic changes in the vulva.

Of the 86 HPV positive patients, 76 (88.4%) patients were under the age of 60 years. At the same time, among patients with vulvar cancer up to 40 years, about 82%, up to 50 to 93% of cases of infection with the human papillomavirus. Among the patients in the age group up to 60 years old, infected with the human papillomavirus, 32 patients were under 55 years old. Given this fact, 76.7% of patients with HPV were under the age of 55 years. As in the general group, squamous cell carcinoma (56.9%) and intraepithelial neoplasia (27.9%) were most frequently diagnosed. Although the median TBM for HPV+ vulvar squamous cell carcinoma was generally higher than the HPV- result (7.8 vs 3.7; p = 0.03),

a complicating factor was the higher percentage of HPV– vulvar squamous cell carcinoma sequenced from the primary tumor. STK11 in HPV+ was significantly higher than HPV test results.

When comparing mutation rates between HPV positive and HPV negative groups for vulvar squamous cell carcinoma (VSC), a difference in mutations between HPV+ and HPV- tumors was observed. Most CCND1-amplified VSC have shown amplification of other genes, such as in 11q13, including FGF3, FGF4, and FGF19. A major specific point mutation with a significant difference between HPV+ and HPV– tumors, which was saturated with the PIK3CA E545K activating mutation.

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 detected in TP53, TERTp, CDKN2A, CCND1, FAT1, NOTCH1, EGFR.

It was found that a high viral load of HPV in VC correlates with the presence of metastases in the lymph nodes, invasion of the stroma, the degree of differentiation, and lymphovascular invasion, while it is not associated with the stage of the disease. Expression of PD-L1 receptors was more frequently observed in HPV negative RV patients compared to HPV positive ones (7.8 vs 3.7 p=0.03), while STK11 mutation was more common in HPV positive patients. At the same time, the PIK3CA E545 mutation occurred with the same frequency between the two groups of VC patients.

References:

1. Allen D. G., Hutchins A. M., Hammet F., White D. J., Scurry J. P., Tabrizi S. N., et al. Genetic aberrations detected by comparative genomic hybridisation in vulvar cancers. Br J Cancer – 86. 2002.– P. 924–8.
2. Bustin S. A., Benes V., Garson J. A., Hellemans J., Huggett J., Kubista M., et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem – 55. 2009.– P. 611–22.
3. Hampl M., Sarajuuri H., Wentzensen N., Bender H. G., Kueppers V. Effect of human papillomavirus vaccines on vulvar, vaginal, and anal intraepithelial lesions and vulvar cancer. Obstet Gynecol.– 108. 2006.– P. 1361–1368.
4. Hansen B. T., Campbell S., Nygard M. Long- term incidence trends of HPV– related cancers, and cases preventable by HPV vaccination: A registry- based study in Norway. BMJ Open.– 8:2018.– e019005.
5. Lavorato-Rocha A. M., Anjos L. G., Cunha I. W., Vassallo J., Soares F. A., Rocha R. M. Immunohistochemical assessment of PTEN in vulvar cancer: best practices for tissue staining, evaluation, and clinical association. Methods – 77–8. 2015.– P. 20–4.
6. Lavorato-Rocha A. M., de Melo Maia B., Rodrigues I. S., Stiepcich M. M., Baiocchi, da Silva Cestari F. M., et al. Prognostication of vulvar cancer based on p14ARF status: molecular assessment of transcript and protein. Ann Surg Oncol – 20. 2013.– P. 31–9.
7. Lavorato-Rocha A. M., Rodrigues I. S., de Melo Maia B., Stiepcich M. M., Baiocchi G., Carvalho K. C., et al. Cell cycle suppressor proteins are not related to HPV status or clinical outcome in patients with vulvar carcinoma. Tumour Biol – 34. 2013.– P. 3713–20.