УДК 616.321-006-036.4-091.8:575.21 https://doi.org/10.26641/2307-0404.2024.2.307479

I.S. Shponka,
O.O. Bondarenko *,
O.V. Kovtunenko,
V.V. Rakhmanov

DELETION OF CYCLIN DEPENDENT KINASE INHIBITOR 2a GENE AS A MARKER OF OROPHARYNGEAL CARCINOMAS NON-ASSOCIATED WITH HUMAN PAPILLOMAVIRUS AND ITS PROGNOSTIC VALUE

Dnipro State Medical University
Volodymyra Vernadskoho str., 9, Dnipro, 49009, Ukraine
Дніпровський державний медичний університет
вул. Володимира Вернадського, 9, Дніпро, 49009, Україна
**e-mail: olex.o.bondarenko@gmail.com*

Цитування: ***Медичні перспективи. 2024. Т. 29, № 2. С. 56-61***
Cited: ***Medicni perspektivi. 2024;29(2):56-61***

Key words: *squamous cell carcinoma, oropharynx, p16, human papillomavirus, cyclin dependent kinase inhibitor 2a gene, hybridization in situ*
Ключові слова: *плоскоклітинний рак, ротоглотка, p16, вірус папіломи людини, ген інгібітор циклін-залежної кінази 2а, гібридизація in situ*

Abstract. Deletion of cyclin dependent kinase inhibitor 2a gene as a marker of oropharyngeal carcinomas non-associated with human papillomavirus and its prognostic value. Shponka I.S., Bondarenko O.O., Kovtunenko O.V., Rakhmanov V.V. *Patients with human papilloma virus associated oropharyngeal squamous cell carcinoma generally have better treatment outcomes and prognosis compared to those with non-papillomavirus-associated oropharyngeal squamous cell carcinoma. However, prognostic evaluation for non-papillomavirus-associated oropharyngeal squamous cell carcinoma remains a problem that could be solved through the molecular mechanisms of squamous cell carcinoma for the purpose of further development of target therapies. Detection of cyclin dependent kinase inhibitor 2a gene deletion in oropharyngeal squamous cell carcinomas can have clinical significance as it may serve as a prognostic marker and potentially guide treatment decisions. To investigate and analyze cyclin dependent kinase inhibitor 2a gene alterations in oropharyngeal squamous cell carcinoma comparing with clinical data (age of the patient, TNM stage), their histological features and occurrence of papillomavirus infection markers (p16 expression). Formalin-fixed and paraffin-embedded samples after transoral radical surgery of oropharyngeal tumors from 26 male patients with average age 57.35±10.33 years were studied. Histological, immunohistochemical analyses and fluorescent in-situ hybridization were performed to assess histological features, p16 expression, and cyclin dependent kinase inhibitor 2a gene gene abnormalities respectively. Homozygous deletion of cyclin dependent kinase inhibitor 2a gene was statistically analyzed and compared with p16 expression, age, and occurrence of nodal metastases in investigated patients. Our study demonstrated that the patients with non-papillomavirus-oropharyngeal squamous cell carcinoma with cyclin dependent kinase inhibitor 2a gene homozygous deletion had the highest risk of the nodal metastases development. Our findings suggest that not only detection of the loss of p16 expression, but also the evaluation of homozygous cyclin dependent kinase inhibitor 2a gene deletion might be predictive of worse outcome specifically in oropharyngeal squamous cell carcinomas.*

Реферат. Прогностичне значення делеції гена інгібітора циклін-залежної кінази 2а як маркера раків ротової частини глотки, неасоційованих з вірусом папіломи людини. Шпонька І.С., Бондаренко О.О., Ковтуненко О.В., Рахманов В.В. *Пацієнти з плоскоклітинними карциномами ротоглотки, асоційованими з вірусом папіломи людини, як правило, мають кращий прогноз та відповідь на лікування порівняно з пацієнтами з плоскоклітинними карциномами ротоглотки, які не асоційовані з вірусом папіломи людини. Однак оцінка прогнозу для плоскоклітинних карцином ротоглотки, неасоційованих з папіломавірусною інфекцією, залишається проблемою, яка може бути вирішена шляхом вивчення молекулярних механізмів розвитку цих злоякісних новоутворень з метою подальшої розробки таргетної терапії. Виявлення делеції гена інгібітора циклін-залежної кінази 2а в плоскоклітинних карциномах ротоглотки матиме клінічне значення, оскільки може слугувати прогностичним маркером і потенційно визначати стратегію лікування. Метою дослідження було дослідити та проаналізувати зміни гена інгібітора циклін-залежної кінази 2а при плоскоклітинних карциномах ротоглотки у порівнянні з клінічними даними (вік пацієнта, стадія за TNM), їх гістологічними особливостями та наявністю маркерів папіломавірусної інфекції (експресія p16). Були досліджені гістологічні зразки, отримані*

після трансоральної радикальної хірургії пухлин ротової частини глотки від 26 пацієнтів чоловічої статі, середній вік яких становив 57,35±10,33 року. Гістологічний, імуногістохімічний аналізи, а також флюоресцентна гібридізація in situ були проведені для оцінки гістологічних особливостей, експресії p16 та аномалій гена інгібітора циклін-залежної кінази 2a відповідно. Гомозиготна делеція цього гена була статистично проаналізована та порівняна з експресією p16, віком та наявністю метастазів у лімфатичні вузли в досліджуваних пацієнтів. Наше дослідження показало, що пацієнти з плоскоклітинними карциномами ротоглотки, неасоційованими з вірусом папіломи людини з гомозиготною делецією гена інгібітора циклін-залежної кінази 2a, мали найвищий ризик розвитку метастазів у лімфатичні вузли. Отримані дані свідчать про те, що не тільки виявлення втрати експресії p16, але й оцінка гомозиготної делеції гена інгібітора циклін-залежної кінази 2a може бути прогностичним маркером при оцінці орофарингеального плоскоклітинного раку.

Eventually the fact that the most common contributor in the development of oropharyngeal squamous cell carcinoma (OPSCC) is the human papillomavirus (HPV) offers the opportunities for preventive reduction of the SCC incidence in this localization [1, 2, 3]. Furthermore, patients with HPV-associated OPSCC (OPSCC-HPV) generally have better treatment outcomes and prognosis compared to those with non-HPV-associated OPSCC [2]. Nevertheless, predicting the course and treatment effectiveness for the developed carcinomas, especially those that are not HPV-associated, remains an unresolved issue, where our hope relies on deciphering the molecular mechanisms of SCC for the purpose of further development of target therapies.

To date it is well known that non-HPV-associated OPSCCs harbor a spectrum of genetic mutations, including alterations in tumor suppressor genes, oncogenes, and genes involved in DNA repair and cell cycle regulation. Common mutations observed in non-HPV-associated OPSCC include mutations in *TP53* (p53 gene), gene of cyclin-dependent kinase inhibitor 2A (*CDKN2A*, p16), notch receptor 1 gene *(NOTCH1)*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha gene *(PIK3CA)*, and epidermal growth factor receptor gene *(EGFR)* [3]. These mutations disrupt key cellular pathways involved in controlling cell proliferation, apoptosis, and differentiation, leading to uncontrolled growth and tumor formation [4]. It is important to notice that although the most mutated genes in non-HPV-associated OPSCC are *TP53* and *CDKN2A*, the frequency of these genes alterations varies between 1 and 23% from case to case [5]. Deletion of *CDKN2A* has been implicated in the early stages of tumorigenesis in various cancers, including OPSCCs, where it contributes to the initiation and development of malignant tumors.

Detection of *CDKN2A* deletion in OPSCCs can have clinical significance as it may serve as a prognostic marker and potentially guide treatment decisions [6, 7, 8, 9]. Additionally, therapies targeting the underlying molecular alterations associated with *CDKN2A* deletion, such as cyclin-dependent kinase (CDK) inhibitors or strategies to restore p53 function, are areas of active research and may hold promise for the treatment of OPSCC in the future [7].

Fluorescence in situ hybridization (FISH) analysis is a molecular technique used to detect and visualize specific DNA sequences within chromosomes. In the context of OPSCC, FISH analysis can be employed to assess certain genetic alterations in *CDKN2A* that may be observed in these tumors [8]. FISH probes specific to this gene can be used to assess copy number changes, such as deletions, which may have prognostic implications or therapeutic relevance [7, 8, 9, 10].

Therefore the main objective of this study was to investigate and analyze *CDKN2A* alterations in oropharyngeal squamous cell carcinomas comparing with clinical data (age of the patient, TNM stage), their histological features and occurrence of HPV infection markers (p16 expression).

MATERIALS AND METHODS OF RESEARCH

The study examined biopsies and material after transoral radical surgery of oropharyngeal tumors from 26 patients (all males) admitted to Metchnikov Dnipro Regional Clinical Hospital. Age ranged from 41 to 77 years, with an average 57.35±10.33 years.

The study was conducted according to the legislated consent of the participants and in accordance with the principles of bioethics mentioned in the Helsinki Declaration for Ethical Principles for Medical Research Involving Human Subjects and the Universal Declaration of Bioethics and Human Rights (meeting minutes of the Biomedical Ethics Committee of Dnipro State Medical University No. 2 dated 26.10.2021).

All cases were grouped according to the occurrence of nodal metastases: OPSCC without metastases 9 (34.6%) and OPSCC with nodal metastases 17 (65.4%); no recurrent OPSCC or cases with distant metastases were observed within selected patients.

Histological method

Formalin-fixed and paraffin-embedded samples were taken from the biobank of the Dnipro Regional Pathological Bureau. Paraffin sections of 4 μm were obtained on a Microm HM-340 microtome and stained with hematoxylin and eosin according to the standard method [11]. Multilayered squamous epithelium without dysplastic changes was used as an

internal control. Microscopy was performed using a ZEISS "Axio Imager" light microscope (×10, ×20, ×40 objectives). Digital images were processed with the licensed software ZEN 2 blue edition.

Immunohistochemical method

Paraffin sections were applied to SuperFrost Plus adhesive slides. After deparaffinisation, rehydration, heat-induced antigen retrieval and inhibition of endogenous peroxidase activity, sections were incubated with primary antibodies in humid chamber at 4°C overnight. The primary monoclonal antibody to $p16^{INK4}$ (clone MX007, Master Diagnostica, Spain) and the UltraVision Quanto imaging system (LabVision) were used. To identify the reaction, a solution of chromogen 3-diaminobenzidine tetrachloride (Quanto, LabVision) was applied under the control of a microscope for 20 seconds to 3 minutes, with a brown colouration. The nuclei were additionally stained with Mayer's haematoxylin for 1-3 minutes. Following the recommendations of Ferreira et al. (2021), the expression of the p16 marker, which is approved as a surrogate marker of OPSCC-HPV, was considered positive only if it demonstrates strong diffuse nuclear-cytoplasmic staining in more than 75% of cells [12].

Fluorescent in situ hybridization

Fluorescence in situ hybridization (FISH) was performed for assessment of the *CDKN2A* gene (at 9p21) using ZytoLight SPEC CDKN2A/CEN 9 Dual Color Probe (ZytoVision, GmbH, Germany) consisting of polynucleotides (~10 ng/µl), which target sequences mapping in 9p21.3 (chr9:21,742,629-22,056,853) harboring the CDKN2A gene region labeled with ZyGreen (excitation 503 nm/emission 528 nm) and polynucleotides (~1.5 ng/µl), which target sequences mapping in 9q12 specific for the classical satellite III region D9Z3 of chromosome 9 labeled with ZyOrange (excitation 547 nm/emission 572 nm). A following counting strategy was used for assessment of the *CDKN2A* gene: a signal pattern of two red and two green signals indicated two intact *CDKN2A* loci on chromosome 9; one red and two green signals indicated heterozygous deletion of *CDKN2A*, and no red and two green signals indicated a homozygous deletion of *CDKN2A*. It was scored a total of 100 tumor cells for each specimen [13].

Statistical analysis

For statistical analysis OPSCCs were categorized by patients' age divided on groups of 10-year periods: 40-49, 50-59, 60-69, and 70-77 year old patients. Data were compared between groups (p16-positive versus p16-negative expression and homozygous deletion of *CDKN2A* versus other variants) by using. Age was compared with *Mann-Whitney* test. Patients with nodular metastases versus metastasis-free cases were also estimated with Fisher's exact tests and compared with p16 expression and FISH by using relative risk test (RR) with calculation of 95% confidence intervals (CI) using Koopman asymptotic score. *P*-values less than 0.05 were considered statistically significant [14]. All data analyses were performed in GraphPad Prism version 8.0.2 (263) for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com).

RESULTS AND DISCUSSION

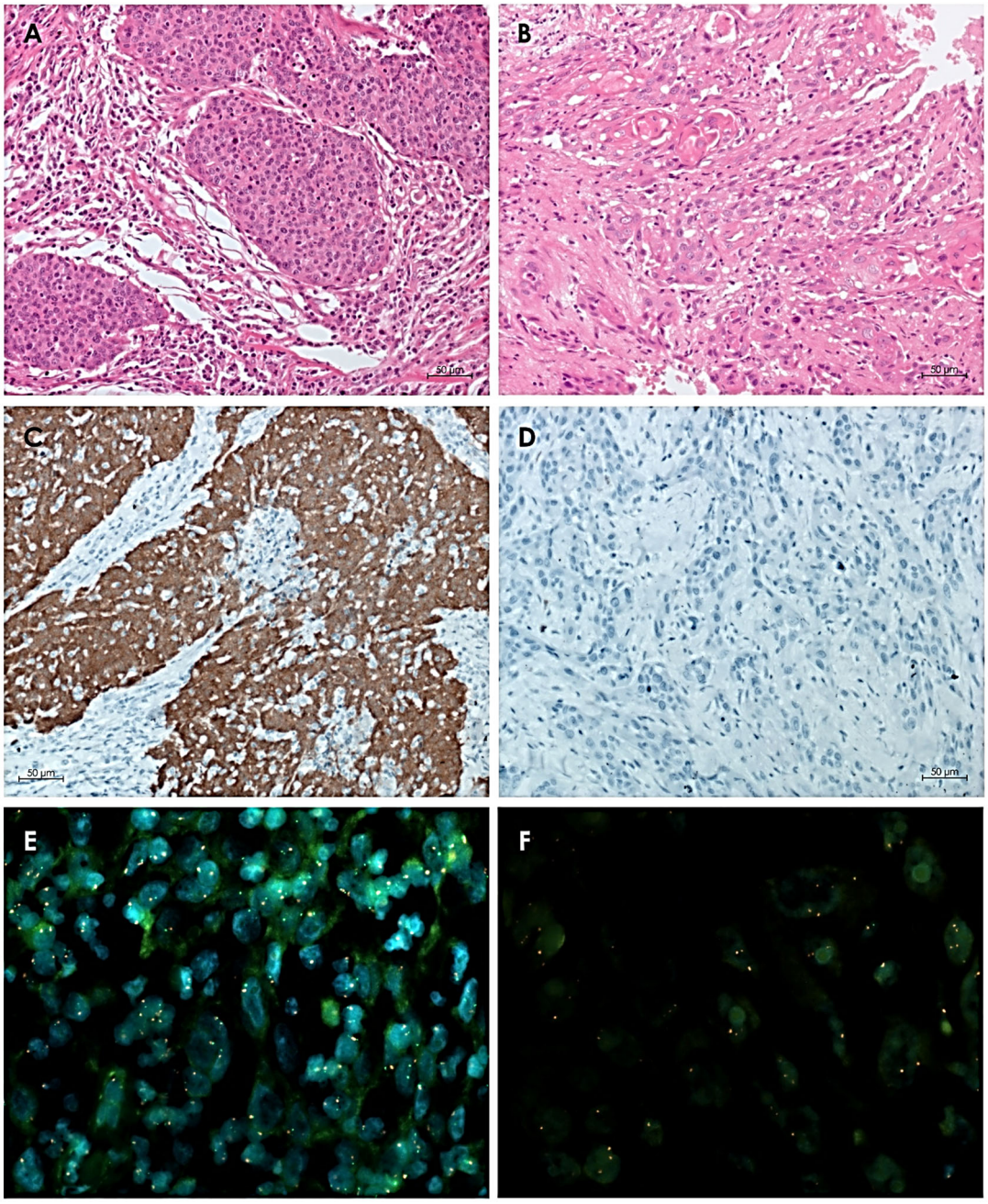
Histological examination demonstrated the presence of moderately differentiated SCCs with keratinization in sixteen cases, the remaining cases appeared as moderately differentiated SCC without keratinization (Fig., A-B). Immunohistochemically in eleven cases p16 expression was revealed as the positive diffuse nuclear-cytoplasmic staining in more than 75% of cells. The other fifteen cases were evaluated as negative (Fig., C-D). Six out of eleven p16-positive SCCs (54.5%) were non-keratinized and predominately had nested arrangements of tumor cells. Respectively, in five cases of p16-positive SCCs the presence of keratinization was found. In contrast, a keratinized/non-keratinized tumor ratio comprised 11/4 in p16-negative cohort. The patients with p16-positive SCCs were overall younger than the patients with p16-negative tumors (mean age 55.3 and 58.9 respectively), though it was not statistically meaningful (p=0.3916). Furthermore, five patients with p16-positive OPSCC clearly revealed the presence of nodal metastases.

CDKN2A analysis was conducted successfully by FISH in all selected patients. Ten out of 26 tumors (38.5%) exhibited homozygous deletion of *CDKN2A* (Fig., E-F). Eleven out of 26 cases (42.3%) harbored normal (two) copies of *CDKN2A*. Five cases (19.2%) revealed a single copy deletion of *CDKN2A*.

Loss of p16 protein expression correlated with homozygous *CDKN2A* deletion (p<0.00012). In all ten cases with homozygous *CDKN2A* deletion, p16 expression was absent (Table). The remaining five of the fifteen p16-negative OSCCs (33.3%) had two copies in three cases or a single copy deletion of *CDKN2A* in two remaining cases. No homozygous deletion of *CDKN2A* was detected in a tumor which expressed p16, however two cases revealed single *CDKN2A* copy deletion.

Further we evaluated p16 expression and *CDKN2A* gene status of OPSCC separately regarding the age of the patient and occurrence of nodal metastases. Patients with OPSCC lacking p16 expression were older at time of surgery than patients with tumors expressing p16 though it was not statistically significant (median age 60 versus 52 years, p=0.3491). Similarly, patients with non-

HPV OPSCC showing homozygous *CDKN2A* deletion (n=10) were overall older at time of surgery than patients with five non-HPV-associated tumors not harboring *CDKN2A* loss (median age 65.5 versus 49 years, p=0.0263). In entire cohort of patients with OPSCC, loss of p16 expression was associated with higher risk of nodal metastases when compared with patients with p16 expression though it was not statistically significant (RR for p16-positive versus p16-negative patients = 0.682, 95% CI: 0.34–1.17, p=0.218). Within p16-negative OPSCC group patients with homozygous deletion of *CDKN2A* showed highest risk of nodal metastasizing in comparison with patients without *CDKN2A* homozygous deletion (RR for *CDKN2A* loss versus patients with at least one copy of *CDKN2A*=2.5, 95% CI: 2.18–2.56, p=0.022).



Comparison of histological and histogenetic features of HPV and non-HPV-associated OPSCC. A, C, E – HPV-associated SCC with non-keratinizing nested appearance, p16-positivity and normal number of *CDKN2A* gene copies; B, D, F – keratinizing non-HPV-associated SCC demonstrates a lack of p16 expression and homozygous deletion of *CDKN2A* gene. A,B – H&E staining (×200); C,D – p16 expression (×200); E, F – CDKN2A/CEN9 FISH, orange – chromosome 9 labelling, green – *CDKN2A* labeling: homozygous deletion is notable due to lack of green labels (×1000)

Distribution of *CDKN2A* deletion in patients with OPSCC, n

Group/Feature	n	p16+	p16–	*CDKN2A* loss
Age (years) 40-49	**8**	**4**	**4**	**1**
50-59	**6**	**3**	**3**	**2**
60-69	**8**	**3**	**5**	**4**
70-80	**4**	**1**	**3**	**3**
With nodal metastases	**17**	**5**	**12**	**10**
Without metastases	**9**	**6**	**3**	**0**
TOTAL	**26**	**11**	**15**	**10**

Notes: n – number of patients; "p16+" – cases with positive p16-immunostaining; "p16–" – cases with negative p16-immunostaining; "*CDKN2A* loss" – homozygous *CDKN2A* deletion.

Deletion or inactivation of the *CDKN2A* gene is a common genetic alteration observed in various types of cancers, including OPSCC [5-9]. The *CDKN2A* gene, located on chromosome 9p21, encodes two important tumor suppressor proteins, $p16^{INK4a}$ and $p14^{ARF}$ [5]. In the context of OPSCCs, deletion of the *CDKN2A* gene can lead to loss of function of both $p16^{INK4a}$ and $p14^{ARF}$ proteins, contributing to further tumorigenesis [7]. $p16^{INK4a}$ functions as a negative regulator of the cell cycle by inhibiting the activity of CDKs, specifically CDK4 and CDK6. Inactivation of $p16^{INK4a}$ due to *CDKN2A* deletion results in dysregulated CDK activity, leading to unchecked cell cycle progression and increased cell proliferation [5, 7]. $p14^{ARF}$ is another product of the *CDKN2A* gene and plays a crucial role in stabilizing the tumor suppressor protein p53 [6]. Activation of p53 by $p14^{ARF}$ leads to cell cycle arrest, apoptosis, and senescence in response to various cellular stresses [5, 6]. Deletion of *CDKN2A* can disrupt the $p14^{ARF}$-p53 pathway, impairing the cell's ability to respond to DNA damage and leading to genomic instability [5, 6, 7, 8, 9].

This study confirmed the involvement of *CDKN2A* homozygous deletion as a common pathway of carcinogenesis in non-HPV-associated OPSCC. Our study shows that loss of p16 expression is not significantly associated with higher risk of nodal metastases development in oropharyngeal squamous cell carcinomas, at least within the represented cohort of the patients; however, larger cohorts of investigated patients reveal that this parameter does not worsen the general outcome of these patients [10]. Furthermore, the patients with non-HPV-OPSCCs that harbored *CDKN2A* homozygous deletion had the highest risk of the nodal metastases development. In addition, loss of p16 expression was associated with older age of patients with OPSCC; homozygous *CDKN2A* deletion in OPSCCs also tended to occur in older patients. Our findings suggest that not only detection of the loss of p16 expression, but also the evaluation of homozygous *CDKN2A* deletion might be predictive of worse outcome specifically in oropharyngeal SCC that was confirmed in other studies [9]. Moreover *CDKN2A* evaluation could be used as an independent of known prognostic parameters such as staging and complete resection, and is generally associated with older age of the patients with OPSCC [9, 15]. Certain amount of non-HPV-associated p16-negative OPSCC does not reveal *CDKN2A* deletion therefore it should be suggested and investigated another mechanism (e.g. frameshift mutation, methylation etc) for p16 inactivation that was described in previous studies [7, 8, 9].

CONCLUSION

In conclusion, homozygous deletion of cyclin dependent kinase inhibitor 2a gene could be suggested as a promising prognostic biomarker for non-papillomavirus-associated oropharyngeal squamous cell carcinomas in terms of increased risk of nodal metastasis development. Besides that, loss of cyclin dependent kinase inhibitor 2a gene is associated with older age of patients. Furthermore, on the basis of our findings, certain amount of non-papillomavirus-associated p16-negative oropharyngeal squamous cell carcinomas does not reveal cyclin dependent kinase inhibitor 2a gene deletion therefore it should be expected another mechanisms for p16 inactivation, however further molecular-genetic studies are needed to better understand these effects on oropharyngeal squamous cell carcinomas pathogenesis.

Contributors:

Shponka I.S. – project administration, supervision, resources, data curation, validation, writing – review & editing;

Bondarenko O.O. – methodology, investigation, formal analysis, visualization, validation;

Kovtunenko O.V. – project administration, supervision, resources, data curation, validation, writing – review & editing;

Rakhmanov V.V. – conceptualization, methodology, funding acquisition, resources, writing – original draft.

Funding. This research received no external funding.

Conflict of interests. The authors declare no conflict of interest.

REFERENCES

1. Berdugo J, Rooper LM, Chiosea SI. RB1, p16, and Human Papillomavirus in Oropharyngeal Squamous Cell Carcinoma. Head Neck Pathol. 2021;15(4):1109-18. doi: https://doi.org/10.1007/s12105-021-01317-5

2. Preissner SH, Nahles S, Preissner S, Heiland M, Koerdt S. Influence of sex on survival rates of HPV-positive oropharyngeal cancers. Front Oncol. 2022;12:917890. doi: https://doi.org/10.3389/fonc.2022.917890

3. Mylly M, Nissi L, Huusko T, et al. Epidemiological Study of p16 Incidence in Head and Neck Squamous Cell Carcinoma 2005-2015 in a Representative Northern European Population. Cancers (Basel). 2022;14(22):5717. doi: https://doi.org/10.3390/cancers14225717

4. Lewis JS, Chernock RD, Ma XJ, Flanagan JJ, Luo Y, Gao G, et al. Partial p16 staining in oropharyngeal squamous cell carcinoma: extent and pattern correlate with human papillomavirus RNA status. Mod Pathol. 2012;25(9):1212-20.
doi: https://doi.org/10.1038/modpathol.2012.79

5. Vazquez-Guillen JM, Palacios-Saucedo GC, Alanis-Valdez AY, et al. p16INK4a and pRb expression in laryngeal squamous cell carcinoma with and without infection by EBV or different genotypes of HPV: a retrospective study. Infect Agents Cancer. 2023 Jul 11;18(1):43.
doi: https://doi.org/10.1186/s13027-023-00514-x

6. Sivarajah S, Kostiuk M, Lindsay C, et al. EGFR as a biomarker of smoking status and survival in oropharyngeal squamous cell carcinoma. J of Otolaryngol - Head Neck Surg. 2019;48:1.
doi: https://doi.org/10.1186/s40463-018-0323-6

7. Gadhikar MA, Zhang J, Shen L, et al. CDKN2A/p16 Deletion in Head and Neck Cancer Cells Is Associated with CDK2 Activation, Replication Stress, and Vulnerability to CHK1 Inhibition. Cancer Res. 2018;78(3):781-97. doi: https://doi.org/10.1158/0008-5472.CAN-17-2802

8. Aesif SW, Aubry MC, Yi ES, et al. Loss of p16INK4A Expression and Homozygous CDKN2A Deletion Are Associated with Worse Outcome and Younger Age in Thymic Carcinomas. J Thorac Oncol. 2017;12(5):860-71. doi: https://doi.org/10.1016/j.jtho.2017.01.028

9. Chen WS, Bindra RS, Mo A, et al. CDKN2A Copy Number Loss Is an Independent Prognostic Factor in HPV-Negative Head and Neck Squamous Cell Carcinoma. Front Oncol. 2018;8:95.
doi: https://doi.org/10.3389/fonc.2018.00095

10. Buckley L, Jackett L, Clark J, Gupta R. HPV-related Oropharyngeal Carcinoma: A Review of Clinical and Pathologic Features With Emphasis on Updates in Clinical and Pathologic Staging. Adv Anat Pathol. 2018;25(3):180-8.
doi: https://doi.org/10.1097/PAP.0000000000000179

11. Bancroft JD, Layton C. The hematoxylins and eosin. In: Suvarna SK, Layton C, Bancroft JD, editors. Bancroft's Theory and Practice of Histological Techniques. 8th ed. Elsevier; 2019. p. 126-138.
doi: https://doi.org/10.1016/B978-0-7020-6864-5.00010-4

12. de Ferreira C, Dufloth R, de Carvalho AC, et al. Correlation of p16 immunohistochemistry with clinical and epidemiological features in oropharyngeal squamous-cell carcinoma. PLoS One. 2021;16(6):e0253418. doi: https://doi.org/10.1371/journal.pone.0253418

13. Pessôa IA, Amorim CK, Ferreira WAS, et al. Detection and Correlation of Single and Concomitant TP53, PTEN, and CDKN2A Alterations in Gliomas. Int J Mol Sci. 2019;20(11):2658.
doi: https://doi.org/10.3390/ijms20112658

14. Grzechowiak I, Graś J, Szymańska D, et al. The Oncogenic Roles of PTTG1 and PTTG2 Genes and Pseudogene PTTG3P in Head and Neck Squamous Cell Carcinomas. Diagnostics (Basel). 2020;10(8):606. doi: https://doi.org/10.3390/diagnostics10080606

15. Moeckelmann N, Ebrahimi A, Tou YK, et al. Prognostic implications of the 8th edition American Joint Committee on Cancer (AJCC) staging system in oral cavity squamous cell carcinoma. Oral Oncol. 2018;85:82-6. doi: https://doi.org/10.1016/j.oraloncology.2018.08.013

Стаття надійшла до редакції 10.04.2024;
затверджена до публікації 31.05.2024