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Impact of C-Fos Overactivation on Laryngeal Squamous Cell Carcinoma Biological Parameters

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ABSTRACT

Objectives: *Deregulation of transcriptional factors is a severe genetic event during carcinogenetic process. Among them, C-Fos proto-oncogene (gene locus: 14q24.3) encodes for a nuclear phosphoprotein*

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that is involved in critical intracellular functions. Our aim was to explore the impact of C-Fos different expression patterns on a series of laryngeal squamous cell carcinoma (LSCC) cases.

Materials and methods: *A set of fifty-five (n=55) LSCC tissues were analyzed by implementing a combination of anti-C-Fos-based immunohistochemistry and digital image analysis assays, respectively.*

Results: *Overexpression of the molecule (high staining intensity levels) was confirmed in 34/55 (61.8%) of the selected cases, whereas the remaining 21/55 (38.2%) were characterized by low expression rates. C-Fos deregulation was marginally correlated with the stage of the examined malignancies (p=0.048), demonstrating a progressively increased protein overexpression. No further statistical correlations were established referring to gender, anatomic location, grade or tobacco and alcohol consumption, respectively.*

Conclusion: *C-Fos oncogene overactivation is a frequent event in subgroups of LSCC patients. C-Fos overexpression seems to lead to an aggressive phenotype increased metastatic potential (advanced stage). Identifying specific C-Fos genetic signatures that lead to its overactivation should be a significant step for an optimal oncological approach by targeting the gene.*

Keywords: Fos, oncogene, larynx, carcinoma, immunohistochemistry, image analysis.

INTRODUCTION

Among malignancies that are derived from the laryngeal (L) anatomic area, squamous cell carcinoma (SCC) is the most prominent demonstrating globally an increased incidence (1). Etiopathogenetic factors that are implicated in the pathogenesis of LSCC include chronic tobacco and alcohol consumption, chemical carcinogens (*i.e.*, asbestos, nickel fumes, chewing betel, sulfuric acid mist), persistent high risk types' viral infection [*i.e.*, human papilloma virus (HPV)] and also genetic predisposing (genetic syndromes such as Plummer-Vinson, Fanconi anemia) (2, 3). Additionally, the role of combined oncogene overactivation (*i.e.*, k-RAS) and suppressor gene silencing (*i.e.*, PTEN) is crucial, leading to a complete disorganization of the corresponding signaling transduction pathways inside the cellular micro-environment (4, 5). Concerning the alterations of specific genes, deregulation of transcription factors is involved in the onset and progression in a broad variety of solid malignancies, including LSCC (6).

Fos proto-oncogene (C-Fos/AP-1 transcription factor subunit) corresponds to a FOS superfamily molecule. FOSL2, FOSL1 and FOSB are the other members of it. C-Fos is a proto-oncogene similar to the human homolog of the retroviral oncogene v-fos (gene locus: 14q24.3) firstly isolated in rat fibroblasts. Finkel–Biskis–Jinkins murine osteogenic sarcoma virus was the source of the C-Fos human homologue gene (7). A 62 kDa protein (380 amino acids) is the product of the gene which strongly interacts with the C-Jun transcription factor that antagonizes the AP-1 (activator protein-1) complex formation (8). C-Fos aberrant expression is a relatively frequent event in head and neck squamous cell carcinomas (HNSCCs), including the LSCCs (9).

Our research aim was to implement a C-Fos protein expression analysis in a set of LSCC tissues based on a combination of immunohistochemistry and digital image analysis research algorithms.

MATERIALS AND METHODS

Patients and tissue samples

A pool of fifty-five (n=55) primary LSCC formalin-fixed paraffin-embedded tissues was obtained and experimentally analyzed. Approval of the Hippokration Hospital Ethics Committee was asked and obtained (Reference ID Research Protocol:2226/09.09.2018) for the current experimental research protocol, based on the World Medical Association Declaration of Helsinki guidelines (2008, revised 2014). The surgically obtained tissue sections, referred to 55 oncological cases between the years 2019 and 2024, were initially fixed in a 10% neutral-formalin buffer. Histopathological diagnoses were assessed by microscopically reviewing the corresponding Haematoxylin and eosin (H&E)-stained slides. Tumours were categorized according to the Head and Neck Malignant Tumours histological classification of the World Health Organization (WHO) (10). None of the analysed cases

Clinicopathological parameters		C-Fos expression DIA		p-value
		High DIA	Low DIA	
LSCC cases **(n=55)**	*n (%)*	*34/55 (61.8%)*	*21/55 (38.2%)*	
Sex				0.713
Male	52/55 (94.5%)	33/55 (60%)	19/55 (34.5.3%)	
Female	3/55 (5.5%)	1/55 (1.8%)	2/55 (3.6%)	
Anatomic location				0.233
Transglottic	51/55 (92.7%)	32/55 (58,1%)	19/55 (34.5%)	
Supraglottic	4/55 (7.3%)	2/55 (3.6%)	2/55 (3.6%)	
Grade				0.423
I	5/55 (9.1%)	3/55 (5.4%)	2/55 (3.6%)	
II	29/55 (52.7%)	16/55 (29%)	13/55 (23.6%)	
III	21/55 (38.2%)	15/55 (27.2%)	6/55 (11.8%)	
Stage				**0.048**
I	21/55 (38.2%)	11/55 (20%)	10/55 (18.2%)	
II	4/55 (7.3%)	2/55 (3.6%)	2/55 (3.6%)	
III	16/55 (29%)	10/55 (18.2%)	6/55 (11.8%)	
IV	14/55 (25.4%)	11/55 (20%)	3/55 (5.4%)	
Tobacco consumption				0.478
Yes	54/55 (98.2%)	34/55 (61.8%)	20/55 (36.4%)	
No	1/55 (1.8%)	0/55 (0%)	1/55 (1.8%)	
Alcohol consumption				0.617
Yes	45/55 (81.8%)	29/55 (52.7%)	16/55 (29%)	
No	10/55 (18.2%)	5/55 (9.1%)	5/55 (9.1%)	

TABLE 1. Clinicopathological features of the examined cases, C-Fos expression results and statistics

LSCC=laryngeal squamous cell carcinoma; DIA=digital image analysis assay (staining intensity values range: 0-255); High DIA: high staining intensity values (67-125); low DIA: low staining intensity values (138-172); statistically significant values are shown in Bold.

demonstrated HPV infection history. All clinic-pathological features are described in Table 1.

Antibodies and immunohistochemistry assay (IHC)

The mouse monoclonal anti-C-Fos (clone CF2, Novocastra, Leica Biosystems, Newcastle, UK; dilution 1:40) was applied in the corresponding LSCC slides. The selected IHC protocol used a soluble dextran-polymer system which prevented endogenous biotin reaction and also enhanced the quality of immunostaining procedure, according to the manufacturer's instructions. 3-3, diaminobenzidinetetrahydrochloride (DAB) was applied as chromogen substrate for visualizing the antigen-antibody conjunction. Nuclear and peri-nuclear/cytoplasmic staining pattern was considered positive for the marker (Figure 1).

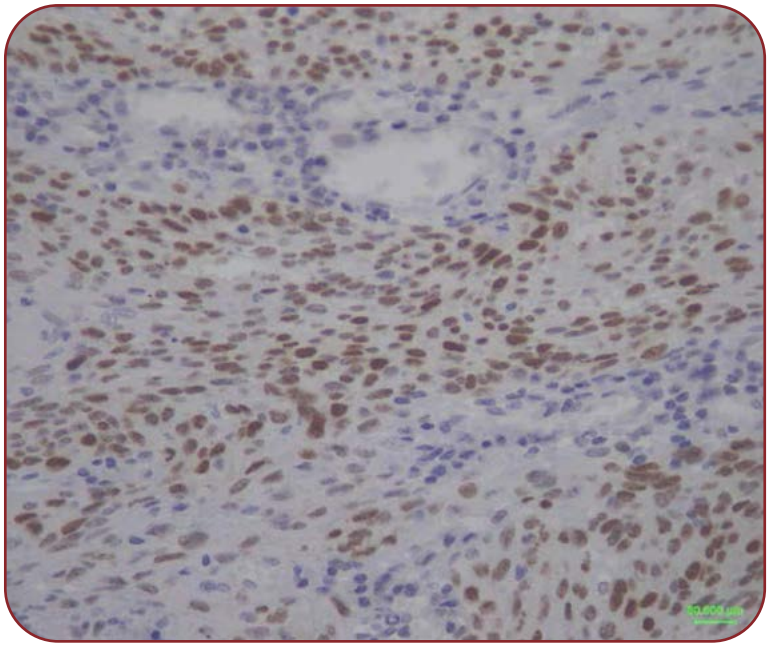

FIGURE 1. C-Fos overexpression (high staining intensity) in a LSCC case. Note the predominantly nuclear dark brown staining pattern (anti-C-Fos antibody, DAB staining substrate, original magnification 400x)

Digital image analysis algorithme (DIA)

C-Fos protein expression staining intensity levels were quantitatively measured by applying a DIA software package (Windows XP/ImageProPlus v.6, MediaCybernetics, Rockville, MD, USA). Calculation of protein expression levels was managed

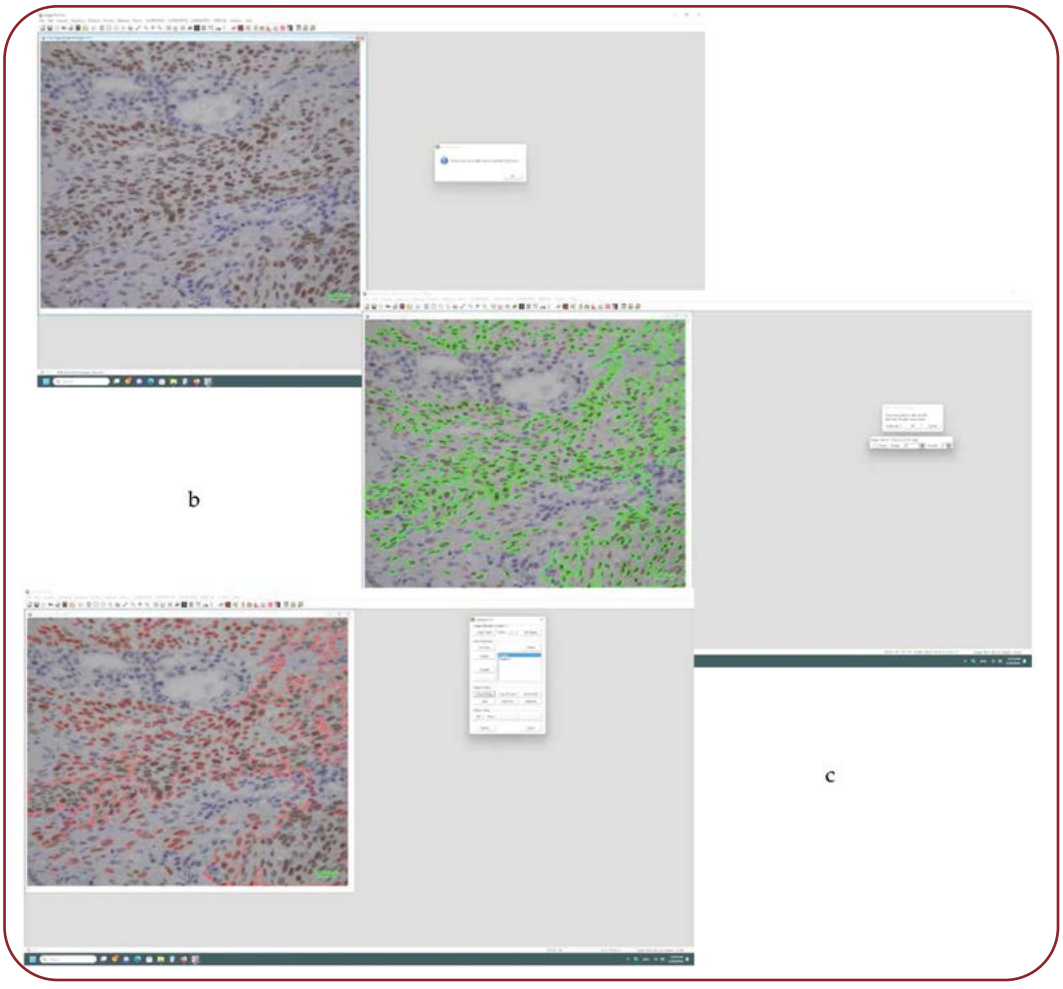

FIGURE 2 a-c. C-Fos digital image expression analysis in the previous referred LSCC case. The authentic microscopic slide field is transformed to a digitized image file. Then, all the objects of interest (stained nuclei) are detected (green contours) according to the constructed digital algorithm based on the C-Fos expression. In the final stage, the algorithm calculates the exact staining intensity levels of every object (red contours), original magnification 400x

by writing a unique macro for the research purposes (nuclear staining pattern for the tumour cells as required by manufacturer's guidelines). A specific algorithm was developed including normal (controls) and malignant oropharyngeal epithelia sections (controls) for the evaluation of C-Fos expression in them, respectively (Figure 2). Values that approach the 0 lead to a progressive overexpression of the molecule. In contrast, values increasing to 255 drive the marker to a multi-step loss of its staining intensity.

Statistical analysis

The IBM SPSS v25 (SPSS Inc, Chicago, IL, USA) statistics software package was applied. Pearson χ^2 and spearman's rank tests was applied. Two-tailed values with $p \leq 0.05$ demonstrated statistical significance.

RESULTS

Concerning the image analysis-based results, all cases enrolled in the present study were characterized by different C-Fos expression levels. In details, C-Fos overexpression (high immunostaining intensity) was established in 34/55 (61.8%) of the examined cases, whereas the remaining 21/55 (38.2%) were characterized by low expression rates. Besides the core tumor area, C-Fos expression was sporadically observed in the corresponding stromal lymphocytes. C-Fos deregulation was marginally correlated with the stage of the examined malignancies

(p=0.048), demonstrating a progressively increased protein overexpression. No other statistically significant correlations were observed regarding the other clinic-pathological parameters (gender: p=0.713, anatomic location: p=0.233, grade: p=0.423, tobacco consumption: p=0.478, alcohol consumption: p=0.617). Complete DIA results and statistics (p-values) are described in Table 1.

DISCUSSION

Deregulation of C-Fos/C-Jun/AP-1 transcriptional factors is a very promising field of research regarding the onset and progression of HNSCCs generally and especially LSCCs, although there are controversial published data for their exact role (11). A molecular study analyzed comparatively carcinoma and adjacent microscopically normal mucosa at the mRNA level of those genetic markers, also including two members of the Fos family, the Fos-related antigens 1-2 (Fra 1-2). They detected Fra-1 high levels of expression in the malignant tissues compared to normal mucosa, whereas C-Fos expression was also increased in them, but without a significant statistical difference (12). Another study group focused on the co-analysis of C-FOS/C-JUN complex and other molecules including matrilysin, gelatinase and urokinase-type plasminogen activator. They reported a lack of C-Fos involvement in the regulation of the previous referred proteins (13). In contrast, C-Jun was found to be a direct regulator of matrilysin and gelatinase expression levels. Similarly, C-Jun activation *via* the collagenase-1 matrix metalloproteinase in stroma negatively influences the biological behavior of LSCCs and for this reason it is a target for inhibition. Interestingly, C-Fos seems to play a significant central role in the activation of HNSCC cancer stem-like cells. An experimental study based on malignant cells culture revealed a critical implication of C-Fos overactivation in HNSCC cancer stem cells by increasing the C-myc and cyclin D1 activity and enhancing the epithelia-mesenchymal transition (EMT) (14). Additionally, C-Fos aberrant expression seems to modify the immune microenvironment in HNSSC by increasing fibroblast and myeloid cell proliferation in regionally recurrent cases (15). For those reasons, C-Fos/FosL1/AP-1 is a target for chemotherapy. Based on this, a study suggested that specific experimental molecules – such as T-5224 – inhibit this complex and down regulate the activity of cancer stem cells (16). Interestingly, a study group showed that high risk HPV persistent infection leads to strong overexpression of C-Fos due to E6/E7/E5 viral transcripts overactivation (17). In the current experimental study, we analyzed the C-Fos protein expression in a series of LSCC tissues using a combination of IHC and DIA techniques. According to our results, a progressively strong C-Fos expression was observed in different stages of the examined specimens. In fact, C-Fos high-level overactivation was detected in advanced stages (mainly III/IV) in those cases. There are few published data regarding gene expression especially in LSCC. Another study based on its protein analysis revealed a strong relation between its overexpression not only in the core malignancy tissue, but also in the peri-intra tumor T (CD4/CD8) infiltrating lymphocytes (18). Interestingly, the study group showed that these C-Fos lymphocyte positive LSCC patients remarkably responded to radiation therapeutic regimens. Concerning the development of anti-C-Fos targeted therapeutic strategies, there are limited available data. An experimental study explored the impact of a specific agent that affects squamous cell epithelia, the synthetic retinoid 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naph-thalene carboxylic acid (CD437). They concluded that CD437 enhanced the apoptotic rate in cutaneous SCCs (19). The same factor seems to play a dual function in HNSCCs, including the LSCCs, by promoting apoptosis *via* C-Fos overexpression in two ways: dependently and independently of the nuclear retinoic acid receptors beta and gamma pathway (20). Concerning the implementation of a DIA protocol in our study, this is a modern sophisticated method leading to accurate and objective measurements of a broad spectrum of IHC-stained histopathological and cytological slides (21-23). Densitometric (depth of staining intensity) and geometrical (max diameters, aspect ratio, nuclear/cytoplasmic areas) measurements are available depending on the corresponding constructed algorithms.

CONCLUSIONS

In conclusion, C-Fos overexpression is a relatively frequent event in LSCCs. This overactiva-

tion triggers a cataract of intracellular reactions disorganizing the C-Fos/C-Jun/AP-1 complex and seems to correlate with a progressively aggressive phenotype (advanced stage). Because the molecule is involved in critical pathways and functions participating in both gene transcription and apoptotic mechanism, it is considered a significant target for inhibition not only in LSCCs, but also in other HNSCCs (24). Understanding the molecular mechanisms that lead to its altered expression is the most important approach for developing novel anti-C-Fos inhibitors (25). ❑

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References

1. **El-Bayoumy K, Christensen N, Broach J, et al.** Current Challenges and Potential Opportunities for Interception and Prevention of Head and Neck Cancer. *Carcinogenesis* 2025;18:25-32. doi: 10.1093/carcin/bgaf025
2. **Jin Y, Jiang H, Sun Q, et al.** A retrospective review of treatment approach and outcomes for locally advanced head and neck squamous cell cancer. *J Cancer Res Ther* 2025;21:381-388. doi: 10.4103/jcrt.jcrt_1332_24.
3. **de Pablo García-Cuenca A, Rofín-Fontanet P, Lorente-Guerrero J, et al.** Head and Neck Cancer in Fanconi Anemia: Report of 11 Cases and a Systematic Review. *Cancers* (Basel) 2025;17:349-356. doi: 10.3390/cancers17030349.
4. **Papanikolaou V, Chrysovergis A, Mastronikolis S, et al.** Impact of K-Ras Over-expression in Laryngeal Squamous Cell Carcinoma. *In Vivo* 2021;35:1611-1615. doi: 10.21873/invivo.12418.
5. **Mastronikolis NS, Tsiambas E, Papadas TA, et al.** Deregulation of PTEN Expression in Laryngeal Squamous Cell Carcinoma Based on Tissue Microarray Digital Analysis. *Anticancer Res* 2017;37:5521-5524. doi: 10.21873/anticanres.11983.
6. **Camacho M, Bagué S, Valero C, et al.** Transcriptional Expression of SLC2A3 and SDHA Predicts the Risk of Local Tumor Recurrence in Patients with Head and Neck Squamous Cell Carcinomas Treated Primarily with Radiotherapy or Chemoradiotherapy. *Int J Mol Sci* 2025;26:2451-2457. doi: 10.3390/ijms26062451.
7. **Curran T, Peters G, Van Beveren C, et al.** FBJ murine osteosarcoma virus: identification and molecular cloning of biologically active proviral DNA. *J Virol* 1982;44:674-682. doi: 10.1128/JVI.44.2.674-682.1982.
8. **Jing Y, Zheng W, Zhou Z, et al.** Recent research advances of c-fos in regulating cell senescence. *Arch Biochem Biophys* 2025;769:110423-110429. doi: 10.1016/j.abb.2025.110423.
9. **Muhammad N, Bhattacharya S, Steele R, et al.** Involvement of c-Fos in the Promotion of Cancer Stem-like Cell Properties in Head and Neck Squamous Cell Carcinoma. *Clin Cancer Res* 2017;23:3120-3128. doi: 10.1158/1078-0432.CCR-16-2811.
10. **Silveira FM, Schuch LF, Bologna-Molina R.** Classificatory updates in verrucous and cuniculatum carcinomas: Insights from the 5th edition of WHO-IARC head and neck tumor classification. *World J Clin Oncol* 2024;15:464-467. doi: 10.5306/wjco.v15.i4.464.
11. **Mastronikolis N, Chrysovergis A, Papanikolaou V, et al.** C-Jun Transcription Factor Oncogenic Activation in Oral Carcinoma. *Maedica* (Bucur) 2024;19:350-354. doi: 10.26574/maedica.2024.19.2.350.
12. **Mangone FR, Brentani MM, Nonogaki S, et al.** Overexpression of Fos-related antigen-1 in head and neck squamous cell carcinoma. *Int J Exp Pathol* 2005;86:205-212. doi: 10.1111/j.0959-9673.2005.00423.x.
13. **Pacheco MM, Kowalski LP, Nishimoto IN, Brentani MM.** Differential expression of c-jun and c-fos mRNAs in squamous cell carcinoma of the head and neck: associations with uPA, gelatinase B, and matrilysin mRNAs. *Head Neck* 2002;24:24-32. doi: 10.1002/hed.10009.
14. **Westermarck J, Li S, Jaakkola P, et al.** Activation of fibroblast collagenase-1 expression by tumor cells of squamous cell carcinomas is mediated by p38 mitogen-activated protein kinase and c-Jun NH2-terminal kinase-2. *Cancer Res* 2000;60:7156-62.
15. **Liu S, Han B, Wang R, Fang J.** Elucidating the role of FOS in modulating the immune microenvironment through fibroblast and myeloid cell regulation in locoregional recurrent HNSCC. *Environ Toxicol* 2024;39:4531-4546. doi: 10.1002/tox.24262.
16. **Zaman SU, Pagare PP, Huang B, et al.** Novel PROTAC probes targeting FOSL1 degradation to eliminate head and neck squamous cell carcinoma cancer stem cells. *Bioorg Chem* 2024;151:107613-107619. doi: 10.1016/j.bioorg.2024.107613.
17. **Masago K, Ishihara H, Kuroda H, et al.** Biological landscape of human papilloma virus-related head and neck cancer according to virus genotype. *Cancer Genet* 2025;294-295:27-31. doi: 10.1016/j.cancergen.2025.02.010.
18. **Ogawa Y, Nishioka A, Hamada N, et al.** Immunohistochemical study of c-fos-positive lymphocytes infiltrated into human squamous cell carcinomas of the head and neck during radiation therapy and its clinical significance. *Clin Cancer Res* 1997;3:2301-2307.
19. **Hail N, Lotan R.** Synthetic retinoid CD437 promotes rapid apoptosis in

malignant human epidermal keratinocytes and G1 arrest in their normal counterparts.
J Cell Physiol 2001;186:24-34.
doi: 10.1002/1097-4652(200101)186:1<24::AID-JCP1006>3.0.CO;2-S.

20. **Sun SY, Yue P, Chandraratna RA, et al.** Dual mechanisms of action of the retinoid CD437: nuclear retinoic acid receptor-mediated suppression of squamous differentiation and receptor-independent induction of apoptosis in UMSCC22B human head and neck squamous cell carcinoma cells.
Mol Pharmacol 2000;58:508-514.
doi: 10.1124/mol.58.3.508.
21. **Tsantoulas A, Roukas D, Mastronikoli S, et al.** Connexin-43 Protein Expression Pattern Analysis in Myocardial Infarction Tissues.
In Vivo 2025;39:1370-1377.
doi: 10.21873/invivo.13940.
22. **Piatriková L, Tobiášová K, Štefák A, et al.** Modelling the Ki67 Index in Synthetic HE-Stained Images Using Conditional StyleGAN Model.
Bioengineering (Basel) 2025;12:476-483.
doi: 10.3390/bioengineering12050476.
23. **Athanasopoulos M, Tsiambas E, Papanastasiou G, et al.** Caspase 3/8/9 Apoptotic Pathway Deregulation Analysis in Laryngeal Squamous Cell Carcinoma.
Maedica (Bucur) 2025;20:729-735.
doi: 10.26574/maedica.2025.20.4.729.
24. **Heroiu Cataloiu AD, Danciu CE, Popescu CR.** Multiple cancers of the head and neck.
Maedica (Bucur) 2013;8:80-85.
25. **Sun S, Liu S, Duan SZ, et al.** Targeting the c-Met/FZD8 signaling axis eliminates patient-derived cancer stem-like cells in head and neck squamous carcinomas.
Cancer Res 2014;74:7546-7559.
doi: 10.1158/0008-5472.CAN-14-0826.