Rom J Morphol Embryol 2022, 63(2):407–411 ISSN (print) 1220–0522, ISSN (online) 2066–8279 doi: 10.47162/RJME.63.2.11

ORIGINAL PAPER

RJME Romanian Journal of Morphology & Embryology http://www.rjme.ro/

The mast cell reaction in premalignant and malignant lesions of the head and neck

RALUCA MIOARA COSOROABĂ[1)], NELA PUŞA GAJE[2)], AMALIA RALUCA CEAUŞU[2)], CRISTINA ŞTEFANIA DUMITRU[2)], LIANA TODOR[3)], RAMONA AMINA POPOVICI[1)], ANCA PORUMB[3)], DANIELA DOMOCOŞ[3)], MARIANA IOANA MIRON[4)]

[1)]*Discipline of Management, Legislation and Communication in Dental Medicine, 1st Department of Dentistry, Faculty of Dental Medicine, Victor Babeş University of Medicine and Pharmacy, Timişoara, Romania*

[2)]*Department of Microscopic Morphology/Histology, Angiogenesis Research Center Timişoara, Faculty of Medicine, Victor Babeş University of Medicine and Pharmacy, Timişoara, Romania*

[3)]*Department of Dental Medicine, Faculty of Medicine and Pharmacy, University of Oradea, Romania*

[4)]*Department 2, Faculty of Dental Medicine, Victor Babeş University of Medicine and Pharmacy, Timişoara, Romania*

Abstract

Head and neck squamous cell carcinoma (HNSCC) is one of the most frequent and aggressive neoplasms of this anatomical region. Many studies evaluated the neoplastic cells, but few works focused on the tumor microenvironment. In the present study, we investigated the distribution and mast cell density (MCD) in malignant and premalignant lesions of the oral cavity, tongue, pharynx, and larynx. There were analyzed 52 specimens of HNSCC, and 15 biopsies taken from patients with dysplasia. Results were compared with those found in a control group of 10 biopsies of oral mucosa from patients with inflammatory diseases. Slides stained with Hematoxylin–Eosin were used for the histopathological diagnosis and grade, and mast cells (MCs) were identified by immunohistochemistry, using anti-MC tryptase. MCs were counted using a method similar to that proposed for microvessel density. We found a significant increase in the number of MCs from the normal oral mucosa until overt carcinoma. Unlike normal tissues, in HNSCC, many MCs were found between tumor cells. We found no relationship between MCs and blood vessels in the tumor area. A significant statistical correlation was found between dysplastic and malignant tumors, but not between tumors with a different grade. Also, it was not found relationship between MCD and the anatomical location of the tumor. Based on these results, we believe that MCD evaluated by anti-MC tryptase is an independent factor of prognosis and reflects an unfavorable outcome.

Keywords: mast cell, premalignant, squamous cell carcinoma, prognosis, head and neck.

Introduction

Although head and neck squamous cell carcinoma (HNSCC) is among the most frequent malignant tumors of this anatomical region [1, 2]. HNSCCs are aggressive tumors that give rise relatively soon to lymph nodes and distant metastases. In the last decades, it was shown a significant increase in the number of new cases, and equally important, these tumors are characterized by aggressive behavior and limited survival. Remarkably, most of these tumors are squamous cell carcinomas (SCCs), and in many cases, it is suspected infection with human papillomaviruses [3, 4]. Such an infection of the head and neck tissues induces major changes not only in epithelial cells but also in the connective tissue compartment that is *lamina propria* and it seems to be the origin of the tumor microenvironment.

There is relatively few and lacunar data on the natural history of SCC. Precursor lesions are rarely diagnosed, and just a few cases were included in a rigorous follow-up to allow conclusions. Epithelial dysplasia, which ranges from mild to severe, based on pure morphological criteria, seems to be the true precursor of HNSCC [5]. Otherwise, there is a lack of data on the evolution of dysplasia, like the time necessary to give rise to a carcinoma or which are cases with dysplasia that can give rise to a carcinoma. In the current work, we showed that a cellular change of the microenvironment occurs even in the stage of dysplasia, and it is furthermore evident in overt carcinoma. The succession from dysplasia to carcinoma has been demonstrated in animal models, but there is a lack of data concerning the human counterpart.

For more than a hundred years, research in cancer was focused on malignant cell morphology and behavior. Less attention was given to the tumor microenvironment, which is a very complex structure and includes various cells, fibers, and a particular ground substance. In the last years, much data was accumulated, and support the crucial role of the microenvironment in the evolution of tumor cells [6]. In experimental model and *in vitro*, it was shown that different types of the microenvironment induce different behavior of the tumor [7, 8]. The components of the tumor microenvironment are extremely diverse. *In vivo*, between tumor cells, there were identified fibroblasts, lymphocytes, macrophages, mast cells (MCs), and many others, included in a more or less dense fibrillar network. Although there are a lot of data concerning the tumor-associated macrophages

or lymphocytes, few and controversial data are known about the MCs.

MCs are usually found in the connective tissue, particularly around small blood vessels, and are characterized by the presence of specific granules in the cytoplasm, containing biologically active substances. For many years, the MCs were in the shadow of basic sciences, as they are identified only with some specific staining methods. Although discovered more than a hundred years ago, it is not yet very clear if MCs represent an individual cell or if many subtypes are included in this group, based on some common proprieties [9]. It is remarkable the high number of different substances contained in the specific granules and released after immunological stimulation, like heparin, histamine, serotonin, chemotactic factors, derivates of the arachidonic acid, and others. MCs express a high number of immunoglobulin E (IgE) receptor on the membrane, and maybe this is the reason why it was investigated more like an immune cell and less like a component of the tumor microenvironment. Some authors noticed a significant accumulation of MCs in some human tumors, and their reaction to skin carcinogenesis was demonstrated in an experimental model. Besides conventional mediators found in MC granules, MCs can secrete, in a given condition, some specific substances, like vascular endothelial growth factor (VEGF) [10, 11]. It was supposed that MCs transfer VEGF to tumor cells or directly stimulate the formation of new blood vessels by a process that is largely known as tumor angiogenesis. This is the main reason why we investigated the MC reaction in HNSCC because until now there are many controversial results on the role of MCs in this group of tumors.

Moreover, MCs contain two constitutional enzymes, which are unique in the human body, namely MC tryptase and MC chymase. They are similar but not identical to pancreatic enzymes, and they are highly specific to detect MCs [12]. MC tryptase is expressed as a basic marker by all MCs, and MC chymase is expressed only by a subclass of MCs [13]. This aspect confirms once again the heterogeneity of this cell population, but it is the support to identify them with a highly specific method [14].

The MCs have been identified in tumors of the oral cavity and head and neck a long time ago. Results regarding both their number of functional type content of specific granules and mainly the clinical significance are still a matter of debate and results are controversial. The increase in the number of MCs in malignant tumors has been reported by many authors [15]. Located close to the tumor or even in the tumor area, usually, MCs do not show degranulation and they are found in the proximity of tumor cells. These aspects are not observed in non-malignant tissues, and it is suspected that at least some of these cells change dramatically their local function, depending on the pathological conditions. However, the nature of the MCs' response to tumor proliferation is virtually unknown.

Aim

In the current work, we have investigated the distribution and incidence of MCs in HNSCC, premalignant lesions, and normal oral mucosa. We found that the number of MCs significantly increases from the normal mucosa to the invasive HNSCC.

Materials and Methods

There were investigated 52 cases of HNCSS and 15 cases with premalignant conditions, namely epithelial dysplasia. The age of patients ranged between 52 and 79 years, and biopsies were taken from tumors of the oral mucosa, including lesions of the tongue and lips (n=24), tongue (n=9), pharynx (n=6), and larynx (n=13). Biopsies from patients without tumors were taken from the gingiva (n=10) from patients with periodontal disease to serve as external control. Diagnostic biopsy was performed in all cases, and specimens were processed according to the standard histological technique. Briefly, after fixation in 10% neutral buffered formalin (pH 7.2), specimens were dehydrated in alcohols, clarified on xylene, and then included in paraffin. Three μm thick sections were performed from each paraffin block. To establish the histopathological (HP) diagnosis and the degree of differentiation, slides were stained with the conventional Hematoxylin–Eosin (HE) staining method. Additional sections were immunostained for anti-MC tryptase antibody, using the fully automated procedure. Briefly, slides were dewaxed and hydrated, and then submitted to antigen retrieval. All steps of the immunohistochemical (IHC) technique were performed using Leica Bond-Max (Leica Microsystems GmbH, Wetzlar, Germany). Antigen retrieval was performed in buffer citrate solution pH 7.2 for 5 minutes. Endogenous peroxidase was blocked with Dako REAL™ Peroxidase-Blocking Solution for five minutes. Incubation with the primary antibody, anti-MC tryptase (clone AA1), last for 30 minutes. The working system was labeled Streptavidin–Biotin 2 (LSAB2), and visualization was performed with 3,3'-Diaminobenzidine (DAB) dihydrochloride. The final product of the reaction was stained in brown. Nuclei were stained with modified Mayer's Hematoxylin (HMM500, ScyTek Laboratories, Inc., Logan, Utah, USA). Slides were then dehydrated, clarified, and mounted with Leica CV Mount (Leica Biosystems).

Mast cell density (MCD) was evaluated using a system similar to the method proposed by Weidner to count blood vessels. At the low power examination, we have chosen three fields with the maximum MCD. After that we counted MCs at ×400 magnification, the arithmetical mean was the final result for each case. Results were evaluated in accord with the HP form and grade, applying Student's t-test, where $p<0.05$ was considered to be statistically significant.

Results

The microscopic examination of HE-stained slides showed non-tumoral gingiva in 10 cases, used as external control. In these cases, we found mild and focal inflammatory infiltrate with low cell density. We noticed in these cases many small blood vessels that form a plexus close to the junction with the epithelium. Dysplastic lesions were diagnosed in 15 cases (nine with moderate, and six with severe dysplasia). The diagnostic criteria for dysplasia followed the recommendation of the *World Health Organization* (WHO), based on changes in the architecture and location of atypical cells. Main atypical aspects found in the current study were nuclear changes, like enlarged nuclei, multiple nucleoli, the irregular contour of the nucleus, and changes in the nuclear/cytoplasmic ratio.

We evaluated 52 consecutive HNSCCs, which were characterized by the proliferation of tumor cells as cords and diffuse and compact areas. Keratin pearls were found only in well and moderately differentiated carcinoma. Malignant cells showed a heterogeneous aspect, with atypical features significantly different from one case to another and even in the same case. Necrosis was found particularly in less differentiated carcinoma, usually accompanied by a moderate inflammatory infiltrate, primarily composed of lymphocytes and macrophages. Based on the aspect of malignant cells, including atypical mitoses, the architecture of the tumor, necrosis, and the presence/absence of keratin pearls, we found 18 cases with G1, 23 cases with G2, and 11 cases with G3.

MCs were identified in deep brown in all the cases included in the present study. In the control cases, the oral mucosa showed numerous MCs stained in brown, preferentially located in the perivascular space (Figure 1A). No significant accumulation of MCs was found close to the covering epithelium.

The HP examination revealed the existence of six moderate and nine severe dysplasia of the oral mucosa. Dysplastic lesions were characterized by a significant change in the architecture of the covering epithelium, associated with asynchronous maturation. Enlarged, intensely stained and irregular nuclei with prominent nucleoli were the main criteria for the diagnosis of dysplasia. Atypical aspects were more evident in severe dysplasia, which was associated with a significant increase in the number of MCs in comparison with the normal oral mucosa. In cases with dysplasia, MCs were significantly concentrated at the border between the epithelium and microenvironment (Figure 1B). We found no significant differences in the number of MCs between control cases and cases with dysplasia. On the other hand, their distribution was significantly different, in dysplastic lesions, MCs were concentrated at the border between epithelium and *lamina propria*. MCD was not significantly higher than in control cases, but their distribution and concentration close to the basement membrane was different (Table 1). One particular aspect found in cases with dysplasia was the presence of MC tryptase-positive cells within the covering epithelium (Figure 1C). We did not observe similar aspects in the control cases.

HNSCCs were stratified based on the differentiation grade, according to the requirements of the *WHO*, as we previously mentioned. Opposite to the control cases and premalignant conditions, in HNSCC, many MCs were located in the tumor area, usually in small groups of individual cells, close to the tumor cell. In many cases, MCs were not located close to or around blood vessels, like they are in the control.

The value for MCD was significantly higher in carcinoma in comparison to dysplastic lesions and control cases. We found lower values in G1 cases in comparison with G2 and G3, but we found no statistical correlation between these two groups (Figure 1, D–F). Looking for the anatomical location of the tumor, there were not found significant differences between tumors of the tongue, pharynx, and larynx, and higher for the cases with carcinoma of the lips. Notably, for all cancer locations, the values for MCD were higher in carcinoma than in dysplasia and control cases.

The values of MCD in each anatomical location of the tumor are given in Table 1.

Table 1 – ***MCD (×400) in tumors of the head and neck***

Anatomical location	Minimum	Maximum	Average	*p*
Tongue (*n*=9)	18	35	29.4	<0.027
Lips (*n*=24)	23	47	38.5	<0.0001
Pharynx (*n*=6)	15	36	28.1	<0.011
Larynx (*n*=13)	12	29	25.7	<0.023
Dysplastic lesions (*n*=15)	12	18	16.4	NS
Control cases (*n*=10)	10	19	16.8	–

MCD: Mast cell density; *n*: No. of cases; NS: Not significant. Note: *p* was rated to the control cases.

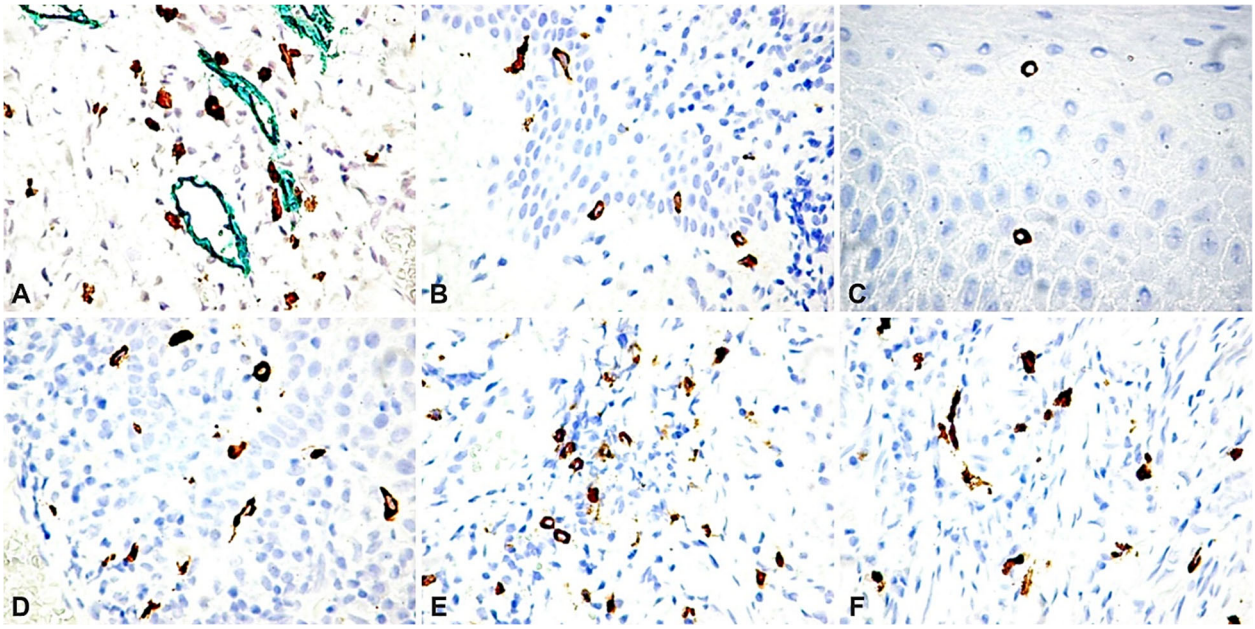

Figure 1 – ***Human gingiva: (A) External control with MCs located in the perivascular space without significant accumulation close to the epithelium – the endothelium of blood vessels was stained with Methyl Green; (B) Moderate dysplasia with accumulation of MCs under the basement membrane; (C) Moderate dysplasia with intraepithelial MCs; (D) Well-differentiated SCC with a relatively low number of MCs; (E) Moderate and less differentiated (F) SCC with a high number of MCs in the tumor area. Anti-MC tryptase antibody immunomarking: (A–F) ×400. MC: Mast cell; SCC: Squamous cell carcinoma.***

Discussions

As we mentioned in the introduction, the number of new cases with HNSCC continues to increase, and tumors seem to be more aggressive than half a century ago. In a recent publication, it was shown that the incidence of SCC increased in almost all anatomical locations of the head and neck [16]. Moreover, it was noticed that the incidence of HNCSS significantly and dramatically increased in some countries in Central and Eastern Europe. Until now, there are no convincing arguments to explain this increase.

The increase in the number of MCs in both normal and pathological conditions is still a matter of debate. Many authors suggest that high MCD is the result of an active process of precursor formation in the bone marrow. The precursors migrate to the target, which in this case is the tumor [17–19]. Unfortunately, this hypothesis was not yet demonstrated step-by-step, but for sure, as we showed, the MCD values increase from the normal tissue to the carcinoma. We noticed the accumulation of MCs at the border between epithelium and *lamina propria* in cases with dysplasia. It is not known nowadays is this layer of MCs forms a barrier against tumor cells or furnishes growth factors to tumor cells.

A MC layer between epithelium and *lamina propria* has been reported many years ago [20, 21]. At that time, it was believed that MCs form layers of glycosaminoglycans and form a barrier that limits the invasion of malignant cells. Although the involvement of MCs has been demonstrated in an experimental model in mice more than 50 years ago [22], their role in human carcinogenesis is still unclear. MCs were reported in a large variety of human malignant tumors, like gastric cancer, urothelial carcinoma, and many others [23]. All authors are in accord that in many tumors may be found a high number of MCs, but their real significance remains obscure.

The method of staining we used in the current work is highly specific and sensitive. On the other hand, this method reflects the presence of only MC tryptase and does not inform about other biological mediators usually released by MCs. The problem of the MC system which may include many functional subtypes is still largely unknown. Further studies are necessary to characterize biochemical differences between MCs associated or not to the tumor microenvironment.

MCs are tissue-resident immune cells that are usually activated on a large variety of pathological conditions, such as inflammation and cancer. A lot of authors observed tumor infiltration with MCs [24], as we did in HNSCC. We reported many MCs in the tumor area but if they promote or inhibit tumor growth, this is vet difficult and almost impossible to say based on a morphological study. Therefore, if MCs are pro- or anti-tumoral, this is a not yet solved problem, but for sure it has a multifaceted role [25, 26]. It is not clear if there are some tumor-derived chemotactic factors to attract MCs within the tumor and after that, they induce new blood vessels formation.

Complex therapy of HNSCC should include not only agents against malignant cells but also against the tumor microenvironment [27]. Maybe the best example is represented by the anti-angiogenic and anti-vascular therapy, which if efficient, consistently reduces the dimensions of the tumor. Changes induced by MC degranulation *versus* inhibitor drugs of degranulation in both experimental models and medical practice are largely unexplored [28].

Numerous plant extracts, such as the total leaf extract of *Melissa officinalis*, have multiple benefits (antioxidant, antimicrobial, antiangiogenic and cytotoxic effects) as a potentiating agent in chemoprevention in oral cancers [29].

It is very difficult to say at this moment that tumor-associated MCs could be a target for therapy, based on the model of MC activation syndrome [30]. On one hand, we still do not know if there are significant differences in comparison with so-called normal MCs. On the other hand, inhibition of MCs would restrict angiogenesis in the tumor area, but such a therapeutic approach was not yet introduced in clinical oncology. In the near future is expected for a better characterization of tumor-associated MCs, and a therapeutical strategy should exploit these differences.

Conclusions

We have investigated the location and number of MCs using the IHC method for MC tryptase. We found that MCD was significantly higher in HNSCC than in control and dysplastic tissues. The value of MC increases with grade and therefore we consider a higher MCD as an unfavorable element of prognosis in the HNSCC.

Conflict of interests

None to declare.

Acknowledgments

The authors are grateful to Ciprian Emanuel Onica for his excellent job in processing slides and stainings. The authors thank their colleagues from the Angiogenesis Research Center Timişoara for their support and help.

References

[1] Ahani E, Fereydouni M, Motaghed M, Kepley CL. Identification and characterization of tunneling nanotubes involved in human mast cell FcεRI-mediated apoptosis of cancer cells. Cancers (Basel), 2022, 14(12):2944. https://doi.org/10.3390/cancers14122944 PMID: 35740607 PMCID: PMC9220880

[2] Almangush A, Alabi RO, Troiano G, Coletta RD, Salo T, Pirinen M, Mäkitie AA, Leivo I. Clinical significance of tumor–stroma ratio in head and neck cancer: a systematic review and meta-analysis. BMC Cancer, 2021, 21(1):480. https://doi.org/10.1186/s12885-021-08222-8 PMID: 33931044 PMCID: PMC8086072

[3] Balica N, Raica M, Cotulbea S, Doros C. Mast cell reaction in malignant laryngeal neoplasm. Rom J Morphol Embryol, 2007, 48(4):395–401. PMID: 18060190

[4] Cai Z, Tang B, Chen L, Lei W. Mast cell marker gene signature in head and neck squamous cell carcinoma. BMC Cancer, 2022, 22(1):577. https://doi.org/10.1186/s12885-022-09673-3 PMID: 35610596 PMCID: PMC9128261

[5] Ch'ng S, Sullivan M, Yuan L, Davis P, Tan ST. Mast cells dysregulate apoptotic and cell cycle genes in mucosal squamous cell carcinoma. Cancer Cell Int, 2006, 6:28. https://doi.org/10.1186/1475-2867-6-28 PMID: 17177999 PMCID: PMC1769399

[6] Donaldson G, McCadden L, Napier S, Tan TJ. Laryngeal dysplasia: a 10-year review of rates of progression to invasive carcinoma and treatment-specific outcomes in a regional ENT department in Northern Ireland. J Laryngol Otol, 2022, 136(6):547–553. https://doi.org/10.1017/S0022215122000366 PMID: 35177154

[7] Gaje PN, Ceausu RA, Jitariu A, Stratul SI, Rusu LC, Popovici RA, Raica M. Mast cells: key players in the shadow in oral inflammation and in squamous cell carcinoma of the oral cavity. Biomed

Res Int, 2016, 2016:9235080. https://doi.org/10.1155/2016/9235080 PMID: 27847826 PMCID: PMC5101369
[8] Gruber BL, Marchese MJ, Kew R. Angiogenic factors stimulate mast-cell migration. Blood, 1995, 86(7):2488–2493. PMID: 7545457
[9] Guran K, Buzatu R, Pinzaru I, Boruga M, Marcovici I, Coricovac D, Avram S, Poenaru M, Susan M, Susan R, Radu D, Dehelean CA. *In vitro* pharmaco-toxicological characterization of *Melissa officinalis* total extract using oral, pharynx and colorectal carcinoma cell lines. Processes, 2021, 9(5):850. https://doi.org/10.3390/pr9050850 https://www.mdpi.com/2227-9717/9/5/850
[10] Hadler-Olsen E, Wirsing AM. Tissue-infiltrating immune cells as prognostic markers in oral squamous cell carcinoma: a systematic review and meta-analysis. Br J Cancer, 2019, 120(7):714–727. https://doi.org/10.1038/s41416-019-0409-6 PMID: 30808992 PMCID: PMC6461751
[11] Hayashi C, Sonoda T, Kitamura Y. Bone marrow origin of mast cell precursors in mesenteric lymph nodes of mice. Exp Hematol, 1983, 11(8):772–778. PMID: 6354738
[12] Iuliano C, Absmaier-Kijak M, Sinnberg T, Hoffard N, Hils M, Köberle M, Wölbing F, Shumilina E, Heise N, Fehrenbacher B, Schaller M, Lang F, Kaesler S, Biedermann T. Fetal tissue-derived mast cells (MC) as experimental surrogate for *in vivo* connective tissue MC. Cells, 2022, 11(6):928. https://doi.org/10.3390/cells11060928 PMID: 35326379 PMCID: PMC8946182
[13] Jamur MC, Moreno AN, Mello LF, Souza Júnior DA, Campos MRC, Pastor MVD, Grodzki ACG, Silva DC, Oliver C. Mast cell repopulation of the peritoneal cavity: contribution of mast cell progenitors *versus* bone marrow derived committed mast cell precursors. BMC Immunol, 2010, 11:32. https://doi.org/10.1186/1471-2172-11-32 PMID: 20576124 PMCID: PMC2912243
[14] Li J, Tang LL, Ma J. Survival-related indicators *ALOX12B* and *SPRR1A* are associated with DNA damage repair and tumor microenvironment status in HPV 16-negative head and neck squamous cell carcinoma patients. BMC Cancer, 2022, 22(1):714. https://doi.org/10.1186/s12885-022-09722-x PMID: 35768785 PMCID: PMC9241267
[15] Louredo BVR, Prado-Ribeiro AC, Brandão TB, Epstein JB, Migliorati CA, Piña AR, Kowalski LP, Vargas PA, Lopes MA, Santos-Silva AR. State-of-the-science concepts of HPV-related oropharyngeal squamous cell carcinoma: a comprehensive review. Oral Surg Oral Med Oral Pathol Oral Radiol, 2022, 134(2):190–205. https://doi.org/10.1016/j.oooo.2022.03.016 PMID: 35725962
[16] Mariz BALA, Kowalski LP, William WN Jr, de Castro G Jr, Chaves ALF, Santos M, de Oliveira TB, Araújo ALD, Normando AGC, Ribeiro ACP, Brandão TB, Vargas PA, Lopes MA, Santos-Silva AR; Latin American Cooperative Oncology Group – Brazilian Group of Head and Neck Cancer. Global prevalence of human papillomavirus-driven oropharyngeal squamous cell carcinoma following the ASCO guidelines: a systematic review and meta-analysis. Crit Rev Oncol Hematol, 2020, 156:103116. https://doi.org/10.1016/j.critrevonc.2020.103116 PMID: 33115701
[17] Niculescu Talpoş IC, Rumel RC, Scurtu AD, Dinu Ş, Miron MI, Preduţ AD, Cosoroabă RM, Popovici RA, Raica M. Oral squamous cell carcinomas: a histopathological review of multiple cases from Western Romania. Rom J Morphol Embryol, 2021, 62(4):929–937. https://doi.org/10.47162/RJME.62.4.05 PMID: 35673812 PMCID: PMC9289717
[18] Oliva C, Carrillo-Beltrán D, Boettiger P, Gallegos I, Aguayo F. Human papillomavirus detected in oropharyngeal cancers from Chilean subjects. Viruses, 2022, 14(6):1212. https://doi.org/10.3390/v14061212 PMID: 35746684 PMCID: PMC9229111
[19] Park JC, Krishnakumar HN, Saladi SV. Current and future biomarkers for immune checkpoint inhibitors in head and neck squamous cell carcinoma. Curr Oncol, 2022, 29(6):4185–4198. https://doi.org/10.3390/curroncol29060334 PMID: 35735443 PMCID: PMC9221564
[20] Popovici RA, Ceausu RA, Cimpean AM, Talpos S, Raica M, Gaje PN. Mast cells as key players in periodontal disease. Arch Biol Sci, 2014, 66(2):801–809. https://doi.org/10.2298/ABS1402801P http://www.doiserbia.nb.rs/Article.aspx?ID=0354-46641402801P#.Y0EyhHZBxjs
[21] Rad HS, Shiravand Y, Radfar P, Ladwa R, Perry C, Han X, Warkiani ME, Adams MN, Hughes BG, O'Byrne K, Kulasinghe A. Understanding the tumor microenvironment in head and neck squamous cell carcinoma. Clin Transl Immunology, 2022, 11(6):e1397. https://doi.org/10.1002/cti2.1397 PMID: 35686027 PMCID: PMC9170522
[22] Raica M, Stamoran M, Rugea P. The mast cell reaction in gastric pathology. Morphol Embryol (Bucur), 1988, 34(1):39–43. PMID: 2452346
[23] Ribatti D, Annese T, Tamma R. Controversial role of mast cells in breast cancer tumor progression and angiogenesis. Clin Breast Cancer, 2021, 21(6):486–491. https://doi.org/10.1016/j.clbc.2021.08.010 PMID: 34580034
[24] Riley JF. Mast cells and cancer in the skin of mice. Lancet, 1966, 2(7479):1457–1459. https://doi.org/10.1016/s0140-6736(66)90610-6 PMID: 4163454
[25] Riley JF. Mast cells, co-carcinogenesis and anti-carcinogenesis in the skin of mice. Experientia, 1968, 24(12):1237–1238. https://doi.org/10.1007/BF02146643 PMID: 5703026
[26] Sawatsubashi M, Yamada T, Fukushima N, Mizokami H, Tokunaga O, Shin T. Association of vascular endothelial growth factor and mast cells with angiogenesis in laryngeal squamous cell carcinoma. Virchows Arch, 2000, 436(3):243–248. https://doi.org/10.1007/s004280050037 PMID: 10782883
[27] Segura-Villalobos D, Ramírez-Moreno IG, Martínez-Aguilar M, Ibarra-Sánchez A, Muñoz-Bello JO, Anaya-Rubio I, Padilla A, Macías-Silva M, Lizano M, González-Espinosa C. Mast cell–tumor interactions: molecular mechanisms of recruitment, intratumoral communication and potential therapeutic targets for tumor growth. Cells, 2022, 11(3):349. https://doi.org/10.3390/cells11030349 PMID: 35159157 PMCID: PMC8834237
[28] Sobiepanek A, Kuryk Ł, Garofalo M, Kumar S, Baran J, Musolf P, Siebenhaar F, Fluhr JW, Kobiela T, Plasenzotti R, Kuchler K, Staniszewska M. The multifaceted roles of mast cells in immune homeostasis, infections and cancers. Int J Mol Sci, 2022, 23(4):2249. https://doi.org/10.3390/ijms23042249 PMID: 35216365 PMCID: PMC8875910
[29] Stepan KO, Mazul AL, Larson J, Shah P, Jackson RS, Pipkorn P, Kang SY, Puram SV. Changing epidemiology of oral cavity cancer in the United States. Otolaryngol Head Neck Surg, 2022 May 3, 1945998221098011. https://doi.org/10.1177/01945998221098011 PMID: 35503657
[30] Valent P, Hartmann K, Bonadonna P, Gülen T, Brockow K, Alvarez-Twose I, Hermine O, Niedoszytko M, Carter MC, Hoermann G, Butterfield JH, Lyons JJ, Sperr WR, Greiner G, Sotlar K, Kluin-Nelemans HC, Schwaab J, Lange M, George TI, Siebenhaar F, Broesby-Olsen S, Jawhar M, Nedoszytko B, Castells M, Orfao A, Gotlib J, Reiter A, Horny HP, Triggiani M, Arock M, Metcalfe DD, Akin C. Global classification of mast cell activation disorders: an ICD-10-CM-adjusted proposal of the ECNM-AIM Consortium. J Allergy Clin Immunol Pract, 2022, 10(8):1941–1950. https://doi.org/10.1016/j.jaip.2022.05.007 PMID: 35623575

Corresponding authors

Liana Todor, Associate Professor, DMD, PhD, Department of Dental Medicine, Faculty of Medicine and Pharmacy, University of Oradea, 10 1 Decembrie Square, 410073 Oradea, Bihor County, Romania; Phone +40723–517 100, e-mail: liana.todor@gmail.com

Ramona Amina Popovici, Habilitated Professor, MD, PhD, Discipline of Management, Legislation and Communication in Dental Medicine, 1st Department of Dentistry, Faculty of Dental Medicine, Victor Babeş University of Medicine and Pharmacy, 2 Eftimie Murgu Square, 300041 Timişoara, Timiş County, Romania; Phone +40729–130 487, e-mail: ramona.popovici@umft.ro, www.angiotm.com

Received: April 30, 2022 *Accepted: October 28, 2022*