UDK: 616.31–006.6–097–037

EVALUATION OF CARCINOMA - ASSOCIATED FIBROBLASTS (CAFS) AND TUMOR - ASSOCIATED MACROPHAGES (TAMS) IN ORAL SQUAMOUS CELL CARCINOMA (OSCC): AN IMMUNOHISTOCHEMICAL STUDY - case report

ЕВАЛУАЦИЈА НА ФИБРОБЛАСТИ - АСОЦИРАНИ СО КАРЦИНОМ (CAFS) И МАКРОФАГИ - АСОЦИРАНИ СО ТУМОР (TAMS) ВО ОРАЛНИОТ ПЛАНОЦЕЛУЛАРЕН КАРЦИНОМ (OSCC): ИМУНОХИСТОХЕМИСКА СТУДИЈА - приказ на случаи

Idoska S[1*]., Popovik-Monevska D.[1], Petrusevska G.[2], Pancevski G.[1], Bozovic S.[1], Kirkov A[1], Iliev A[1].

[1]University Clinic for Maxillofacial Surgery, Ss. Cyril and Methodius University in Skopje, Faculty of Dentistry - Skopje, [2]University Institute for Pathology, "Ss. Cyril and Methodius" University, Skopje, * Author to whom correspondence should be addressed

Abstract

Stromal cells in the tumor microenvironment (TME) of oral squamous cell carcinoma (OSCC) interact closely with tumor cells and influence their behavior in many different ways by promoting tumor growth, cancer cell invasion, and metastasis, thus influencing the prognosis of the disease also. This study shows the presence of CAFs (cancer-associated fibroblasts) and TAMs (tumor-associated macrophages) in TME of three patients diagnosed with OSCC and discusses the association of the density of CAFs and TAMs with the clinicopathological parameters of presented OSCC. The presentation of these 3 cases is important because the density of CAFs and TAMs in the tissue samples correlate with the clinicopathological parameters of the disease and may be used as potential prognostic factors in OSCC.

Апстракт

Стромалните клетки од туморската микросредина кај оралниот планоцелуларен карцином комуницираат со канцерските клетки и влијаат на нивното однесување на различни начини промовирајќи го растот на туморот, инвазијата на канцерските клетким метастазирањена карциномот, и на тој начин влијаат и на прогнозата на пациентот со оваа болест. Овој труд преку прикажување на случаите на 3 пациенти со орален планоцелуларен карцином имунохистохемиски го потврдува присуството на фибробласти-асоцирани со карцином (CAFs) и тумор асоцирани макрофаги (TAMs) и ја дискутира корелацијата на нивната густина со клиничкопатолошките параметри. Кај прикажаните три случаи дензитетот на CAFs и TAMs корелира со клиничкопатолошките параметри и можеби би можеле да се искористат како потенцијални прогностички фактори кај оралниот планоцелуларен карцином.

Introduction

Oral squamous cell carcinoma (OSCC) is one of the six most common malignancies in the world with a very high incidence and mortality. Treatment of choice is surgery with adequate margins, and radiotherapy and / or chemotherapy, as well as targeted therapy are complementary to surgery. Despite great advances in modern radiotherapy, chemotherapy and targeted therapy as well, the prognosis is poor. Many prognostic factors affect the oncological outcome in the form of 5-year survival and overall survival.

Clinical and pathological parameters are crucial when planning treatment and determining the prognosis of patients with this type of malignancy. Significant clinicopathological parameters are TNM status, location of the primary tumor, depth of tumor invasion (DOI), margin status following tumor resection, lymphovascular,

perineural and bone invasion, number and location of positive lymph nodes for metastasis, extranodal extension of metastatic tumor from the lymph nodes into the surrounding tissues, and presence of distal metastases are accepted criteria. Taking the biological nature of cancer cells into account, all of these parameters are interrelated and cannot be independent prognostic predictors[1].

Recent studies focus on the effects of the components of tumor microenvironment (TME) on the initiation and progression of OSCC. The components of TME, including cancer-associated fibroblasts, vascular and lymphatic endothelial cells, the extracellular matrix (ECM) and inflammatory immune cells are the most important modulators of the primary OSCC behavior. Stromal cells in the tumor microenvironment (TME) interact closely with tumor cells and influence their behavior in different ways, for example, promoting tumor growth, invasion, and metastasis, as well as resistance to anticancer treatment. So far, the complex relationship between TME and the immune system is still unclear.

The large number of immune cells that infiltrate tumor tissue show functional plasticity and at some point may acquire pro-tumor or anti-tumor activity. There is evidence that stromal cells, including fibroblasts, endothelial cells, and mesenchymal cells, play a key role in shaping the tumor immune environment. One of the most active cell types in tumor stroma are fibroblasts who have the potential for transdifferentiation into an activated-myofibroblast phenotype known as cancer-associated fibroblasts (CAFS). CAFs play an important role in growth and development of malignant epithelial tumors e.g. promoting tumorigenesis, invasion, and metastasis by stimulating angiogenesis and reconstituting the extracellular matrix thus preparing the territory for metastasis in the early stages of the disease[2]. Several studies have suggested that CAFs may be used as an important prognostic factor in a variety of tumors[3,4], although their prognostic role in OSCC is rarely published.

The tumor-fighting host organism sends a variety of immune cells to the tumor tissue, including dendritic cells and macrophages to induce and direct the anti-tumor immune response. Of these, macrophages are the most common type of tumor-infiltrating immune cells and are called tumor-associated macrophages (TAMs)[5]. There are two paradoxical types of TAMs: M1 and M2 macrophages. M1 macrophages are responsible for the anti-tumor response through tumoricidal activity and the production of pro-inflammatory cytokines[6]. In contrast, M2 macrophages have an immunosuppressive (pro-tumor) function by stimulating angiogenesis, and supporting tumor cell invasion. Numerous microenvironmental factors released by CAFs and tumor cells in the TME may be major regulators of TAM polarization[6].

Objectives

This study shows the presence of CAFs and TAMs in the oral squamous carcinoma tumor microenvironment by reporting 3 (three) characteristic cases; and confirms the association between the density of CAFs and TAMs with the clinicopathological parameters of OSCC. Additionally, we assessed the relation between the number of TAMs in TS (tumor stroma) and TN (tumor nest) with the OSCC parameters. We hope that this publication will give a contribution to the literature published so far.

Material and methods

Patients and tissue samples

Tissue samples were obtained from 3 patients with histopathologically confirmed OSCC, with no history of radiation or chemotherapy. They underwent surgery at the University Hospital for Maxillofacial surgery within the "Mother Teresa" University Clinical Center in Skopje, in 2020. Patient data were collected from hospital histories. The primary tumors were localized on the tongue and the sublingual region. As a control sample for the presence/absence of CAFs and TAMS, a tissue section with incisional surgical biopsy of a clinically healthy mucosa was taken from a patient with previously confirmed OSCC in the bilateral sublingual region.

The stage of the disease was classified according to the criteria of the AJCC TNM classification system of the OSCC. Tumor differentiation was classified into 4 levels: good, moderate, poorly differentiated, and undifferentiated (anaplastic) according to Broder's histological classification of tumor cell differentiation in oral squamous cell carcinomas (Broder's grading descriptive system)[7].

Immunohistochemistry (IHC) and IHC evaluation

Tissue samples were processed and analyzed at the Institute of Pathology, within the "Mother Teresa" University Clinical Center in Skopje. Immunohistochemistry was performed using Dako EnVision flex system.

The following primary mouse monoclonal antibodies were used: anti-α-SMA and CD68. Nikon

Patologist use Nikon 80 digital microscope in the analysis and for taking microphotographs.

CAFs and TAMs Assessment

CAFS were generated from the samples and evaluated by immunohistochemistry. Positive or negative fibroblasts were identified on the basis of α-SMA expression. This confirmed the identity of the CAFs for further research.

So, CAFs are defined as large spindle-shaped fibroblasts that express α-SMA. First, by selecting 4 typical visual field of tumor stroma in every slice by high-power magnification of the microscope, we counted the area of α-SMA-positive fibroblasts (CAFs) and calculated the CAFs density (dCAFs) = the positive staining area/tumor stromal area (%) of every visual field. CAFs density is the mean value of the 4 fields. The cut-off value for CAFs is 10%[2].

CD68 expression was determined by counting the number of CD68 positive macrophages (TAMs). Macrophages were defined as stromal cells larger than 10 μm in diameter which express CD68; and the same were counted in the invasive regions of the specimens. Each section was displayed on a low power magnification (low-power field with 100x magnification) to identify the areas with the highest macrophage density, then the macrophages were counted in three fields on high power magnification (high-power field on 400x magnification) and the average number of macrophages was obtained[8]. Additionally, we assessed the number of CD68+ macrophages in TS (tumor stroma) and TN (tumor nest).

Case reports

Clinico-histological features with results (Table 1)

Case No. 1. A patient with a primary OSCC in the tongue, 58 years old. The pathology findings confirm a moderate degree of tumor differentiation (G2); AJCC TNM classification pTNM = pT3, pN2b, pM0, pL1, pV0 and IVA-stage disease. α-SMA staining shows a high percentage of CAFs (> 50% of the mean value) (Figure 1). CD68 marks high density of TAMs in the tumor stroma counting 100-150 TAMs and high density in tumor nests (TN) counting 30-50 TAMs (Figure 2).

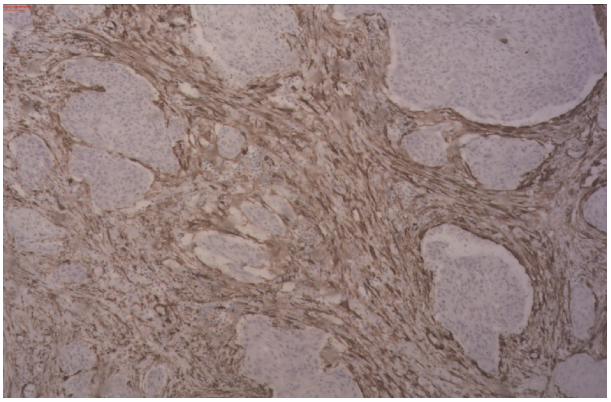

Figure 1. *Immunohistochemical staining of CAFs. CAFs are α-SMA-positive (original 100x magnification). A typical "network" pattern that forms when there is an abundance of CAFs that occupy almost the entire tumor stroma.*

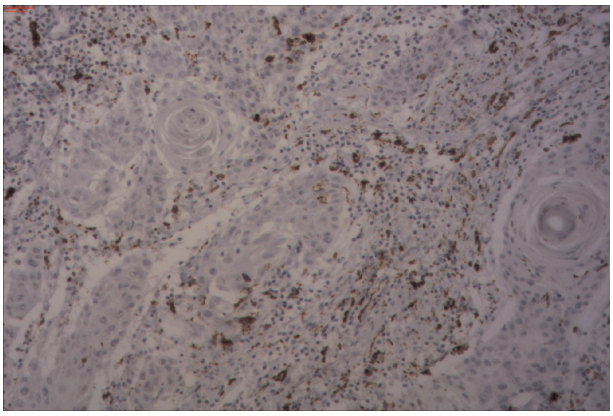

Figure 2. *Immunohistochemical staining of CD68-positive TAMs. Representative example of positive expression of CD68 in TAMs in tumor nests (TN) and tumor stroma (TS) of OSCC (original 100X magnification). CD68 cytoplasmic staining in cells identified as TAMs.*

Table 1.Clinicopathological parameters and results of immunostaining. OSCC, oral squamous cell carcinoma; T, tumor stage; N, node stage; G, Broder's grading system (G1(well-), G2 (moderately-), G3 (poor differentiation) and G4 (undifferentiated, anaplastic). L,V, lymphatic /or vascular infiltration; α-SMA-alpha smooth muscle actin. CD68+ TAMs, CD68-positive tumor-associated macrophages; TN, tumor nest; TS, tumor stroma.

OSCC	Case 1	Case 2	Case 3
T	T3	T1	Normal mucosa
N	N2b	N0	N0
Clinical stage (I-IV)	IVA	I	I
G (1,2,3,4)	G2	G1	Carcinoma was not identified
L,V	L1	L0	L0
α-SMA (%)	>50	>10	>1
CD68+ TAMs (TN)	30-50	9-10	No detected
CD68+TAMs (TS)	100-150	30-40	20-30

Case No. 2. Patient with OSSC in the oral sublingual region, aged 60 years. The finding is consistent with well-differentiated oral squamous cell carcinoma (G1). AJCC classification corresponds to first clinical stage of disease pTNM = pT1, Nx, pMx, pL0, pV0.

Immunohistochemistry showed the following results: low density of CAFs was found around arterial blood vessels (<10%). 9-10 TAMs were detected in tumor nests, and 30-40 cells in the tumor stroma.

The Case No. 3. presented with histopathologic identification of small focus of Human Papilloma Virus (HPV) positive oral squamous cell carcinoma, moderately differentiated (G2) and localized in the sublingual region billateraly. AJCC TNM classification was: pT1, N0, M0, L0, V0; and the patient was in the first clinical stage of the disease. Lymphovascular invasion was not detected. As a control sample, an extra material of a clinically healthy oral mucosa close to the primary tumor was additionally taken. Microscopic analysis of the control sample showed the presence of a regular oral mucosa. Immunohistochemistry was undertaken only on the control sample. α-SMA accounts for less than 1% of myofibroblasts. (Figure 3), and 20-30 CD68-positive macrophages (Figure 4).

Clinico-histological features with results are shown in Table 1.

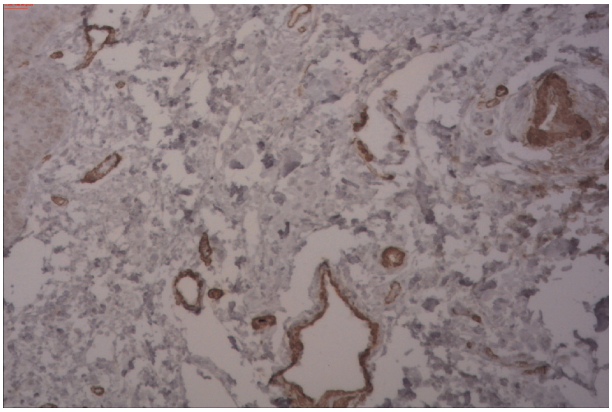

Figure 3. *Immunohistochemical staining of CAFs. Low density of α-SMA positive myofibroblasts (original 100x magnification*)

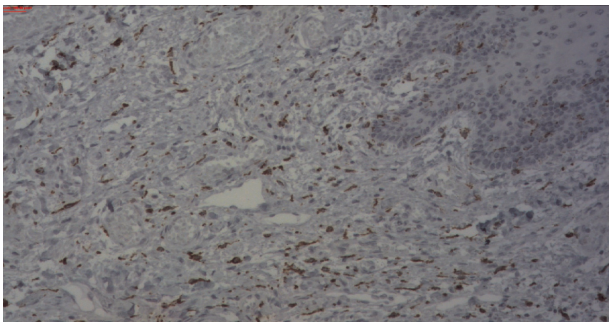

Figure 4. *Immunohistochemical staining of CD68 + TAMs. Low density of CD68 positive tissue macrophages (original 100x magnification).*

Discussion

Significance of infiltration with CAFs and TAMs

The molecular mechanism leading to the expression of immune antigens by the tumor cells is still poorly understood. Several immunohistochemical markers are available for evaluation of TAMs. CD68 is known as a pan-macrophage marker and facilitates the identification of both types of polarized macrophages (M1 and M2)[9]. This cytochemical marker is used for immunostaining of monocytes / macrophages in histochemical analysis of inflamed tissue, tumor tissue, and other immunohistopathological uses because macrophages and other mononuclear phagocytes exhibit strong CD68 expression.

CD68+ expression in TN and TS was found in the first two patients, but a very small number of CD68+ macrophages were found in the third patient who had no cancer in the control sample. The high CD68+ count in patient number one is associated with a higher stage of disease, a larger tumor diameter, poor tumor differentiation, lymphatic invasion, and lymph node metastasis. According to these clinical parameters, poor prognosis for the patient is expected. In the Jeong et al. study for tumor-associated macrophages as potential prognostic biomarkers in invasive breast cancer, the high number of CD68+ macrophages in TN and TS was associated with higher histological grade, larger tumor diameter and metastases. In breast cancer, the high number of CD68+ infiltration is associated with a poorer prognosis, also[8].

CD68 alone, or in combination with other cellular markers, is widely used as a cancer-associated diagnostic and prognostic marker[9]. Tumor cells used to express CD68 also, because metastatic tumor cells express immune markers to avoid macrophage-mediated phagocytosis and cell damage by cytotoxic CD8 + T-cells during invasion of normal, non-tumor tissue. Excessive expression of macrophage antigens in tumor tissue may indicate a pro-metastatic condition and may be associated with a poor prognosis[10], which is consistent with the prognosis of case number one, according to the clinico-histopathological prognostic parameters[7,11].

In cases 1 and 2, CD68 marks a lower density of TAM in the tumor nest (TN) and a higher density in tumor stroma (TS). According to the results of the literature, in endometrial cancer, infiltration with CD68 + TAMs in TN shows a positive correlation with reduced recurrence, while in invasive breast cancer suggests an unfavorable prognosis. If these results are taken into account, recommendations are given to examine TAMs localization in both TN and TS of the malignant tumors[8].

α-SMA reflects the expression of CAFs in the tumor mesenchyme and is the most common marker of CAFs[12].

Normal mesenchymal fibroblasts do not express α-SMA. Our results showed a very high density of SMA+ fibroblasts (CAFs) in case number one, with moderately differentiated squamous cell carcinoma (G2), large tumor size, tumor progression through lymphatic permeation and metastasis, and high degree of malignancy. According to the clinico-pathological parameters, the patient has unfortunately a very poor prognosis[7,11]. A small percentage of CAFs is seen in a patient number two, with a smaller tumor size, absence of lymphovascular permeation and metastases, good tumor differentiation (G1) and the same was in the earlier stage of disease. We found almost no CAFs in healthy oral mucosa in case number three, but their minimal presence may be attributed to a pre-existing cancer in this patient. Identical to our result, the results of the study by Chen et al. indicate a statistically significant difference in the density of CAFs in tissue with nasopharyngeal carcinoma (NPC), normal nasopharyngeal mucosa, and NPC metastases, indicating that CAFs are an important component of tumor stroma that play a role in the growth and development of malignant epithelial tumors and participate in the early stages of tumor preparations for future metastases[2]. This study suggests that the occurrence of α-SMA+ myofibroblasts precedes the onset of invasion and contributes to tumor growth and progression[2,12]. Ibrahim O. Bello's[13] research on tongue cancer has shown that the density of CAFs is associated with the degree of tumor malignancy, tumor growth and progression[13]. Similarly, some studies have shown that stromal CAFs are associated with a higher risk of recurrence and poor prognosis in colorectal and breast cancer[14]. Kellerman's findings are similar, confirming that the higher degree of infiltration with CAFs is associated with more advanced TNM stage and lymph node metastasis[15]. Also, the study by Takahashi et al.[16] reveals that the high rate of infiltration with CAFs correlates with lympho-vascular invasion[16].

CAFs were hardly found in the normal mucosa sample in patient number three, which is consistent with the finding of Fujii et al.[17], where α - SMA+ cells were not found in the stroma of normal oral mucosa specimens or premalignant lesions, except in the smooth muscles of the vessel wall that were used as internal positive control. Findings from his study also show that approximately 60% of OSCCs contain a significant proportion of myofibroblasts and many of them contain myofibroblasts in the deep invasive front of the tumor. Maybe the absence of α - SMA + myofibroblasts in the stroma of the normal oral mucosa in our study is giving us the long-expected answer suggesting that close contact with cancer cells is required to induce myofibroblast transdifferentiation in the invasive tumor front. Most importantly, the study demonstrates that an abundance of myofibroblasts leads to a more aggressive type of SCC including increased proliferative potential[17], which is consistent with our finding in case number one.

The abundant presence of myofibroblasts, especially in the invasive tumor front, is significantly associated with shorter overall survival, so that increased myofibroblast counts may be useful in predicting the prognosis of oral SCC. The study by Sobral et al.[18] demonstrates that myofibroblasts in OSCC stroma are associated with increased tumor aggressiveness, and thus a shorter survival time. CAFs directly facilitate tumor invasion by producing proteases that digest the extracellular matrix (ECM) and produce a variety of pro-invasive molecules. These findings suggest that CAFs promote cancer invasion, resulting in a poor prognosis of OSCC patients. These claims cannot be convincingly refuted in our report because a large number of cases and their follow-ups are required to prove these suggestions wrong or false.

CAFs play a very important role in recruiting and polarizing TAMs[16]. Many studies that have examined the relationship between CAFs and TAMs in the OSCC have found that CAFs primarily induce the pro-tumoral and immunosuppressive phenotype of macrophages[16]. Moreover, the infiltration of CAFs into tumor tissue correlates not only with the number of CD68 + macrophages but also with CD163 + macrophages, indicating CAFs tilt toward the M2 macrophages in the TME. The results of the study provide new insights of the role of CAFs in the immunosuppressive microenvironment of these tumors. CAFs promote immunosuppressive microenvironment by induction and accumulation of pro-tumoral macrophages[16]. In order to increase the effectiveness of immunotherapy, therapeutic strategies that will alter CAFs-mediated immunosuppressive microenvironment should be considered. TAMs that promote key processes in tumor progression such as angiogenesis, immunosuppression, invasion, and metastasis, may potentiate or antagonize the efficiency of anti-tumor cytotoxic chemotherapy, target-antibodies against cancer cells, and immunotherapies. TAMs are also responsible for the reparative mechanisms in the tumor after radiotherapy or treatment with agents that target vascularization[5]. Some studies discuss the biological significance and clinical implications of these findings and emphasize the need of novel therapeutical approaches that effectively target TAMs and improve the outcome[5].

Conclusion

With this report, we determined the presence of CAFs and CD68-positive TAMs in the early and advanced stages of OSCC and determined their absence in the regular oral mucosa. We also pointed out the inter-

connectedness of the CAFs and CD68 + TAMs density with the clinicopathological features of OSCC.

It is becoming increasingly clear that researching the components in TME will help in better understanding of the different responses to antitumor therapy and thus in more precise defining of the target cells for anticancer therapy.

So, as we realize that CAFs and TAMs play a key role in shaping the tumor immunosuppressive microenvironment, and in order to increase the effectiveness of conventional therapies as well as immunotherapies, we suggest that novel cancer therapies in which CAFs and TAMs would be potential targets, should be considered.

Reference

1. Jadhav KB, Gupta N. Clinicopathological prognostic implicators of oral squamous cell carcinoma: need to understand and revise. N Am J Med Sci. 2013 Dec;5(12):671-9. doi: 10.4103/1947-2714.123239. PMID: 24404549; PMCID: PMC3877528.
2. Chen J, Yang P, Xiao Y, Zhang Y, Liu J, Xie D, Cai M, Zhang X. Overexpression of α-sma-positive fibroblasts (CAFs) in Nasopharyngeal Carcinoma Predicts Poor Prognosis. J Cancer. 2017 Oct 23;8(18):3897-3902. doi: 10.7150/jca.20324. PMID: 29151978; PMCID: PMC5688944.
3. Fuyuhiro Y, Yashiro M, Noda S, Kashiwagi S, Matsuoka J, Doi Y, Kato Y, Muguruma K, Sawada T, Hirakawa K. Myofibroblasts are associated with the progression of scirrhous gastric carcinoma. Exp Ther Med. 2010 Jul;1(4):547-551. doi: 10.3892/etm_00000086. Epub 2010 Jul 1.
4. Yamashita M, Ogawa T, Zhang X, Hanamura N, Kashikura Y, Takamura M, Yoneda M, Shiraishi T. Role of stromal myofibroblasts in invasive breast cancer: stromal expression of alpha-smooth muscle actin correlates with worse clinical outcome. Breast Cancer. 2012 Apr;19(2):170-6.doi: 10.1007/s12282-010-0234-5. Epub 2010 Oct 27.
5. De Palma M, Lewis CE. Macrophage regulation of tumor responses to anticancer therapies. Cancer Cell. 2013 Mar 18;23(3):277-86. doi: 10.1016/j.ccr.2013.02.013. PMID: 23518347.
6. Barros MH, Hauck F, Dreyer JH, Kempkes B, Niedobitek G. Macrophage polarisation: an immunohistochemical approach for identifying M1 and M2 macrophages. PLoS One. 2013 Nov 15;8(11):e80908. doi: 10.1371/journal.pone.0080908. PMID: 24260507; PMCID: PMC3829941.
7. M Akhter, S Hossain, Quazi B Rahman, Motiur R Molla. A study on histological grading of oral squamous cell carcinoma and its co-relationship with regional metastasis. J Oral Maxillofac Pathol. 2011 May-Aug; 15(2): 168–176. doi: 10.4103/0973-029X.84485.
8. Jeong H, Hwang I, Kang SH, Shin HC, Kwon SY. Tumor-Associated Macrophages as Potential Prognostic Biomarkers of Invasive Breast Cancer. J Breast Cancer. 2019;22(1):38-51. Published 2019 Jan 2. doi:10.4048/jbc.2019.22.e5
9. Chistiakov, D., Killingsworth, M., Myasoedova, V. et al. CD68/macrosialin: not just a histochemical marker. Lab Invest 97, 4–13 (2017).
10. Jakovic LR, Mihaljevic BS, Perunicic Jovanovic MD, Bogdanovic AD, Andjelic BM, Bumbasirevic VZ. The prognostic relevance of tumor associated macrophages in advanced stage classical Hodgkin lymphoma. Leuk Lymphoma. 2011 Oct;52(10):1913-9. doi: 10.3109/10428194.2011.580026. Epub 2011 Jun 12. PMID: 21663512.
11. de Araújo RF Jr, Barboza CA, Clebis NK, de Moura SA, Lopes Costa Ade L. Prognostic significance of the anatomical location and TNM clinical classification in oral squamous cell carcinoma. Med Oral Patol Oral Cir Bucal. 2008 Jun 1;13(6):E344-7. PMID: 18521052.
12. Galiè M, Sorrentino C, Montani M, Micossi L, Di Carlo E, D'Antuono T, Calderan L, Marzola P, Benati D, Merigo F, Orlando F, Smorlesi A, Marchini C, Amici A, Sbarbati A. Mammary carcinoma provides highly tumorigenic and invasive reactive stromal cells. Carcinogenesis. 2005 Nov;26(11):1868-78. doi: 10.1093/carcin/bgi158. Epub 2005 Jun 23. PMID: 1597596.
13. Bello IO, Vered M, Dayan D, Dobriyan A, Yahalom R, Alanen K, Nieminen P, Kantola S, Läärä E, Salo T. Cancer-associated fibroblasts, a parameter of the tumor microenvironment, overcomes carcinoma-associated parameters in the prognosis of patients with mobile tongue cancer. Oral Oncol. 2011 Jan;47(1):33-8. doi: 10.1016/j.oraloncology.2010.10.013. Epub 2010 Nov 26. PMID: 21112238.
14. Tsujino T, Seshimo I, Yamamoto H, Ngan CY, Ezumi K, Takemasa I, Ikeda M, Sekimoto M, Matsuura N, Monden M. Stromal myofibroblasts predict disease recurrence for colorectal cancer. Clin Cancer Res. 2007 Apr 1;13(7):2082-90. doi: 10.1158/1078-0432.CCR-06-2191. PMID: 17404090.
15. Kellermann MG, Sobral LM, da Silva SD, Zecchin KG, Graner E, Lopes MA, Kowalski LP, Coletta RD. Mutual paracrine effects of oral squamous cell carcinoma cells and normal oral fibroblasts: induction of fibroblast to myofibroblast transdifferentiation and modulation of tumor cell proliferation. Oral Oncol. 2008 May;44(5):509-17. doi: 10.1016/j.oraloncology.2007.07.001. Epub 2007 Sep 7. PMID: 17826300.
16. Takahashi H, Sakakura K., Kudo T., Kyoichi M., Oyama T., Chikamatsu K. Cancer-associated fibroblasts promote an immunosuppressive microenvironment through the induction and accumulation of protumoral macrophages). Oncotarget. 2017 Jan 31; 8(5): 8633–8647.
17. Fujii N, Shomori K, Shiomi T, Nakabayashi M, Takeda C, Ryoke K, Ito H. Cancer-associated fibroblasts and CD163-positive macrophages in oral squamous cell carcinoma: their clinicopathological and prognostic significance. J Oral Pathol Med. 2012 Jul;41(6):444-51. doi: 10.1111/j.1600-0714.2012.01127.x. Epub 2012 Feb 2. PMID: 22296275.
18. Sobral LM, Zecchin KG, Nascimento de Aquino S, Lopes MA, Graner E, Coletta RD. Isolation and characterization of myofibroblast cell lines from oral squamous cell carcinoma. Oncol Rep. 2011 Apr;25(4):1013-20. doi: 10.3892/or.2011.1161. Epub 2011 Jan 25. PMID: 21271223.