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Significance of stroma in biology of oral squamous cell carcinoma

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ABSTRACT

The worldwide annual incidence of oral squamous cell carcinoma (OSCC) is over 300,000 cases with a mortality rate of 48%. This cancer type accounts for 90% of all oral cancers, with the highest incidence in men over 50 years of age. A significantly increased risk of developing OSCC exists among smokers and people who consume alcohol daily. OSCC is an aggressive cancer that metastasizes rapidly. Despite the development of new therapies in the treatment of OSCC, no significant increase in 5-year survival has been recorded in the past decades. The latest research suggests focus should be put on examining tumor stroma activation within OSCC, as the stroma may contain cells that can produce signal molecules and a microenvironment crucial for the development of metastases. The aim of this review is to provide an insight into the factors that activate OSCC stroma and hence faciliate neoplastic progression. It is based on the currently available data on the role and interaction between metalloproteinases, cytokines, growth factors, hypoxia factor and extracellular adhesion proteins in the stroma of OSCC and neoplastic cells. Their interplay is additionally presented using the Systems Biology Graphical Notation in order to sublimate the collected knowledge and enable the more efficient recognition of possible new biomarkers in the diagnostics and follow-up of OSCC or in finding new therapeutic targets.

Keywords: Biomarker, Cytokines, Estrogen receptor, OSCC, Stroma androgen receptor

Introduction

Oral cancer is the sixth most common cancer in the world, constituting 3%-5% of all malignant diseases in both sexes. Over 90% of oral cancers are oral squamous cell carcinomas (OSCC) (1-3), with an annual worldwide incidence of over 300,000 cases and a mortality rate of 48% due to its metastatic potential and limited treatment options (4). The incidence of OSCC has significantly increased during the past 2 decades, with a 0.7% and 1.8% increase per year in men and women, respectively (5).

OSCC primarily affects women after menopause (6), smokers, people who consume alcohol and red meat on a daily basis, and people infected with human papillomaviruses (7, 8). There is a significantly higher incidence of OSCC in men than women (9, 10), which may be the result of greater consumption of tobacco and alcohol by men (11). However, both alcohol and tobacco modify the levels of estrogen and testosterone within gender-specific frames (12), which yields possible additional xenohormonal mechanisms involved in the etiology of OSCC. Oral cancer in women occurs almost exclusively after menopause, which suggests that a decrease in estrogen levels may play an important role in the initiation of oral cancer. In female patients with oral cancer, there is a significantly higher prevalence of OSCC compared with control subjects among hysterectomized women and those whose menopause occurred at a significantly younger age (13).

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The OSCC microenvironment consists of tumor cells, different stromal cells, and the extracellular matrix (ECM). Based on the latest research, the activated tumor environment in OSCC has a significantly greater effect on metastases than had previously been assumed (14, 15). However, it is still unknown whether the mechanisms in OSCC-activated stromal cells that promote metastases are similar to those identified in the stroma of other cancers (16-18).

Cancer-associated fibroblasts (CAFs) are major promoters of metastasis progression in cancer tissue and the dominant cell population in tumor stroma (19, 20). They may have their origin in different cell types such as mesenchymal stem cells, bone marrow stem cells, endothelial cells and even cancer cells (21). CAFs produce growth factors, cytokines, chemokines and metalloproteinases that stimulate cancer growth (22). Reprogrammed CAFs resembling myofibroblasts that aggregate around neoplastic tissue have been reported to invade normal tissue (23). These spindle-shaped cells in the stroma of OSCC play an active role in producing ECM components. Their increased presence significantly correlates with stage, regional recurrence and distant metastasis (15).

It has been suggested that CAFs induced in stroma mimic a wound-healing microenvironment and stimulate the activation of multiple active molecules that promote angiogenesis, desmoplasia and paracrine activities (19). CAFs initiate vascularization of the tumor edge through the production of vascular endothelial growth factor (VEGF) (24). Together with CAFs, tumor-associated mast cells (TAMCs) also promote angiogenesis and discharge signal molecules such as tumor necrosis factor alpha (TNF-α) and hypoxia-inducing factor 1 alpha (HIF-1α) as well as proinflammatory cytokines such as interleukin 6 (IL-6) (25, 26), which orchestrate cancer growth and influence the dynamics of metastasis.

It is interesting to mention that miR-21, which regulates cell proliferation, apoptosis and epithelial-to-mesenchymal transition during neoplastic progression, is increased in OSCC stroma and its stromal expression has a particularly negative prognostic value (27, 28), showing the potency of this finding for future investigation.

The aim of this review is to provide insight into the complex multifactorial biology of OSCC stroma and the tumor microenvironment based on the currently available data on the role and interaction between stromal metalloproteinases, cytokines, growth factors and extracellular stromal proteins, all related to the mechanisms involved in the biology of OSCC and especially its progression. Additionally, using the systems biology tool "Systems Biology Graphical Notation" (SBGN), a presentation of the interaction between stromal active factors and tumor mechanisms is provided so as to suggest new approaches in the investigation of new biomarkers for diagnostics and therapy targets.

Impact of stromal cytokines on the aggressiveness of OSCC

IL-6 is produced by a variety of cells, primarily monocytes, macrophages and tumor cells, and plays a central role in the regulation of inflammatory and immune responses. It has an important role in cancer progression via effects on proliferation, migration and angiogenesis in several cancers. Oral cancer cells induce CAFs to produce IL-6 (29-31). Additionally, IL-6 produced by stromal cells plays a role in the osteoclast formation induced by OSCC (32).

Interleukin-1 (IL-1) beta mediates the promotion of inflammatory cell migration from blood into inflamed tissue, and regulates the synthesis and decomposition of the ECM (33, 34). It can thus be used as a marker of disease progression (35). IL-1 alpha produced by OSCC cells has a dual effect on CAFs: promotion of CAF proliferation and upregulation of the secretion of cytokines in CAFs such as CCL7, CXCL1 and IL-8 (36).

Studies have suggested that TAMs produce IL-10, which points to a stromal contribution in immunosuppression mechanisms (37).

The influence of gender on the levels of stromal cytokines in the progression of OSCC has been poorly investigated, although cytokine levels are known to be estrogen and testosterone dependent (38, 39). Such information would be of significant value in designing personalized therapy, especially in OSCC, where significant gender differences in incidence and survival have been described.

OSCC stromal growth factors and hypoxia factor

The proliferation and spread of tumor cells depend on their angiogenesis. Tumors that grow quickly lead to hypoxia, which regulates tumor cell survival factors such as HIF-1α and VEGF, leading to even stronger tumor proliferation, angiogenesis and increased radioresistance (40-42). Thus, VEGF interacts with mast cells, especially in the early stages of OSCC carcinogenesis, by promoting epithelial growth and angiogenesis (43, 44). Over 90% of OSCCs have elevated levels of VEGF and HIF-1α (45), both being related to poorer survival (46). These findings support the hypothesis that tumor angiogenesis is closely related to – but not strictly dependent on – the hypoxic conditions of a tumor's microenvironment (47).

VEGF-A and VEGF-C protein expression is significantly higher in primary OSCC than in normal oral mucosa. Lymph node metastases in patients with oral cancer are closely correlated with positive VEGF and matrix metalloproteinase 9 (MMP-9) expression (25). Besides, VEGF has been shown to be a predictive marker of malignant potential, as increased levels were found in precancerous oral lesions and oral cancer in comparison with controls (48). VEGF acts as an activator of tumor lymphangiogenesis within metastases also via CD163-positive stromal tumor-associated macrophages (44, 49).

Transforming growth factor beta (TGF-β) family members show different expression in OSCC subtypes. CAFs from genetically unstable OSCC, which promote the malignant phenotype by weakening intercellular epithelial adhesion, have shown increased levels of active TGF-β1 and TGF-β2. (50). Additionally, TGF-β1 secreted by stromal fibroblasts induces expression of PDPN (the gene encoding podoplanin) in OSCC cells, which significantly correlates with invadopodia formation and lymph node metastasis (51).

Platelet-derived growth factor receptor beta is exclusively expressed in CAFs of OSCC but not in tumor cells, which gives this biomarker particular significance in diagnostics (52).
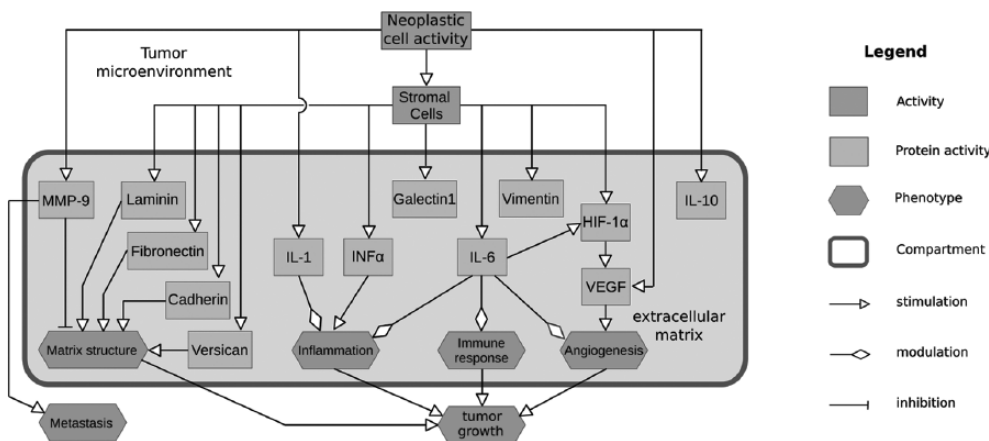
Role of stroma in the formation of ECM and desmoplasia

Cancer cells degrade the ECM, thus facilitating migration and metastasis by mechanisms similar to wound healing. As described previously, the OSCC stroma secretes several growth factors and inflammatory mediators that play a crucial role in cancer progression. On the other hand, cancer stroma also expresses altered levels of collagens, elastin, hyaluronic acid, fibronectin, proteoglycans and glycoproteins, leading to the loss of tissue architecture (53).

ECM degradation is caused by proteinases. Matrix metalloproteinases (MMPs) are involved in the initiation, invasion and metastasis of cancer. In oral cancers, MMPs, especially MMP-9, are associated with ECM degradation, allowing the cancer to spread (54).

Disruption in the expression of MMP-1 promotes OSCC progression via increased levels of hyaluronan synthase 2, which is associated with alpha smooth muscle actin (α-SMA)-positive myofibroblasts in the stroma (55). It has been shown that a stromal environment that promotes cancer cell migration is accomplished via stimulated expression of MMPs. This type of expression is the responsibility of thrombospondin-1 produced by stromal cells (56).

In OSCC, retrodifferentiation is demonstrated by the expression of oncofetal fibronectins in the stroma. Both tumor cells and recruited stromal myofibroblasts take part in the formation of the fetal ECM milieu. As shown in one study (57), the oncofetal type III extra-domain (ED-B) fibronectin matrix is produced by recruited stromal cells, which enables the remodeling of the carcinoma invasion front.

A migration-promoting microenvironment in OSCC is also mediated by myofibroblast progression with stromal upregulation of laminin isoforms, which leads to vascular basement membrane reorganization (alpha-3- and gamma-2-chain laminins) (58). Myofibroblasts are α-SMA and vimentin positive, by which they also contribute to OSCC invasion (59). The Gas 6/Axl axis, which takes part in cadherin and vimentin expression and promotes cell invasion, migration and the triggering of NF-κB signaling, has been reported to be active in OSCC and to induce cancer to become apoptosis resistant (37). Additionally, the OSCC stroma seems to have a desmoplastic response that has been described in highly invasive tumors (60).

Versican is overexpressed in different cancer types. It plays a significant role in tumor growth by repressing cell adhesion, stimulating cell proliferation and migration, and regulating angiogenesis. OSCC has strong stromal versican expression, which has been shown to be an independent predictor of unfavorable prognosis (61). Invasion in OSCC is associated with the increased deposition of laminin in stroma, beneath the invading carcinoma cells. Increased expression of laminin-5 has been shown to occur in the areas of direct tumor-stroma interaction (57, 62).

Galectin 1 increases CAF-conditioned-medium-induced tumor cell migration and invasion, possibly by production of monocyte chemotactic protein-1 (MCP-1/CCL2) (63), which has been reported to be associated with decreased disease-free survival in OSCC (64).

A significant inverse correlation has been reported between the Ki-67 level and the stroma/tumor proportion and degree of keratinization (65).

Biology of OSCC presented by SBGN

Systems biology has become an inevitable part of data interpretation and presentation in research based on complexity. In the near future, it will undoubtedly become a potent tool in communicating the achievements of clinical research and will probably very soon take part in decision-making processes regarding personalized medicine. In this paper, the collected data on the interactions between stromal biomarkers associated with OSCC progression are presented using SBGN, whose main advantages are its clarity and wide applicability (66). This set of 3 languages was developed a few years ago and is accompanied by detailed technical specifications and a software application that makes it simple to use. This paper uses the Activity Flow language of SBGN, the simplest of the 3 and perfectly suited to the "bird view" description of disease mechanisms. Figure 1 shows OSCC in its microenvironment as well as the interaction of the molecules described in this paper, known to play a significant role in the etiology and progression of this cancer type. This is the first time that SBGN has been applied for clinical purposes and we believe it has shown a unique capacity not only to summarize data but also to examine biological pathways in the contexts of the living environment and gender on the cellular, stromal and matrix level.

Conclusion

Understanding the interaction between neoplastic cells and their microenvironmental niche opens up new possibilities in OSCC diagnosis, prognosis and therapy. Although OSCC incidence and survival are gender related, the cross-reaction between estrogen and androgen receptors, estrogen and testosterone levels, and relevant stromal factors and proteins has so far not been investigated in OSCC. Similarly, following the knowledge gained from research into other cancer types, the induction of cytokines, MMPs, ECM and growth factors should be integrated with other factors relevant for early OSCC profiling and success of therapy. The level of stromal activation could become a significant factor for histopathological diagnostics as well as for decisions on the treatment and monitoring of OSCC patients. Stromal secretome profiling will contribute to a better future exploitation of the tumor microenvironment as a novel therapeutic target.

Abbreviations

CAF   cancer-associated fibroblast
ECM   extracellular matrix
HIF   hypoxia-inducing factor
IL   interleukin
MMP   matrix metalloproteinase
OSCC   oral squamous cell carcinoma
SBGN   Systems Biology Graphical Notation
SMA   smooth muscle actin
TAMC   tumor-associated mast cell
TGF   transforming growth factor
TNF   tumor necrosis factor
VEGF   vascular endothelial growth factor

Disclosures

Conflict of interest: The authors have no conflicts of interest related to this article.

References

1. Forastiere AA, Ang K, Brizel D, et al. National Comprehensive Cancer Network. Head and neck cancers. J Natl Compr Canc Netw. 2005;3(3):316-391.
2. Hunter KD, Parkinson EK, Harrison PR. Profiling early head and neck cancer. Nat Rev Cancer. 2005;5(2):127-135.
3. Casiglia J, Woo SB. A comprehensive review of oral cancer. Gen Dent. 2001;49(1):72-82.
4. Torre LA, Siegel RL, Ward EM, Jemal A. Global cancer incidence and mortality rates and trends--an update. Cancer Epidemiol Biomarkers Prev. 2016;25(1):16-27.
5. Braakhuis BJM, Leemans CR, Visser O. Incidence and survival trends of head and neck squamous cell carcinoma in the Netherlands between 1989 and 2011. Oral Oncol. 2014;50(7): 670-675.
6. van Zyl A, Bunn BK. Clinical features of oral cancer. SADJ. 2012;67(10):566-569.
7. Chaturvedi AK, Engels EA, Anderson WF, Gillison ML. Incidence trends for human papillomavirus-related and -unrelated oral

squamous cell carcinomas in the United States. J Clin Oncol. 2008;26(4):612-619.
8. Chuang SC, Jenab M, Heck JE, et al. Diet and the risk of head and neck cancer: a pooled analysis in the INHANCE consortium. Cancer Causes Control. 2012;23(1):69-88.
9. Gervásio OL, Dutra RA, Tartaglia SM, Vasconcellos WA, Barbosa AA, Aguiar MC. Oral squamous cell carcinoma: a retrospective study of 740 cases in a Brazilian population. Braz Dent J. 2001;12(1):57-61.
10. Sharma P, Saxena S, Aggarwal P. Trends in the epidemiology of oral squamous cell carcinoma in Western UP: an institutional study. Indian J Dent Res. 2010;21(3):316-319.
11. Pires FR, Ramos AB, Oliveira JB, Tavares AS, Luz PS, Santos TC. Oral squamous cell carcinoma: clinicopathological features from 346 cases from a single oral pathology service during an 8-year period. J Appl Oral Sci. 2013;21(5):460-467.
12. Fucic A, Gamulin M, Ferencic Z, et al. Environmental exposure to xenoestrogens and oestrogen related cancers: reproductive system, breast, lung, kidney, pancreas, and brain. Environ Health. 2012;11(Suppl 1):S8.
13. Suba Z, Maksa G, Mihályi S, Takács D. [Role of hormonal risk factors in oral cancer development]. Orv Hetil. 2009;150(17): 791-799. [Article in Hungarian]
14. Rivera C, Venegas B. Histological and molecular aspects of oral squamous cell carcinoma. Oncol Lett. 2014;8(1):7-11.
15. Luksic I, Suton P, Manojlovic S, Virag M, Petrovecki M, Macan D. Significance of myofibroblast appearance in squamous cell carcinoma of the oral cavity on the occurrence of occult regional metastases, distant metastases, and survival. Int J Oral Maxillofac Surg. 2015;44(9):1075-1080.
16. Liu YL, Chi SM, Zhu YL, et al. Expression of estrogen receptors alpha and beta in human tongue squamous cancer cell and influence of beta-estradiol on the proliferation of tongue cancer cell. Zhongguo Ying Yong Sheng Li Xue Za Zhi 2003;19(1):65-69. [Article in Chinese].
17. Kenny HA, Chiang CY, White EA, et al. Mesothelial cells promote early ovarian cancer metastasis through fibronectin secretion. J Clin Invest. 2014;124(10):4614-4628.
18. Papatheodorou H, Papanastasiou AD, Sirinian C, et al. Expression patterns of SDF1/CXCR4 in human invasive breast carcinoma and adjacent normal stroma: correlation with tumor clinicopathological parameters and patient survival. Pathol Res Pract. 2014;210(10):662-667.
19. Karagiannis GS, Poutahidis T, Erdman SE, Kirsch R, Riddell RH, Diamandis EP. Cancer-associated fibroblasts drive the progression of metastasis through both paracrine and mechanical pressure on cancer tissue. Mol Cancer Res. 2012;10(11):1403-1418.
20. Fu H, Yang H, Zhang X, Xu W. The emerging roles of exosomes in tumor-stroma interaction. J Cancer Res Clin Oncol. 2016;142(9): 1897-1907.
21. Chang HY, Chi JT, Dudoit S, et al. Diversity, topographic differentiation, and positional memory in human fibroblasts. Proc Natl Acad Sci USA. 2002;99(20):12877-12882.
22. Ha SY, Yeo SY, Xuan YH, Kim SH. The prognostic significance of cancer-associated fibroblasts in esophageal squamous cell carcinoma. PLoS ONE. 2014;9(6):e99955.
23. Kalluri R, Zeisberg M. Fibroblasts in cancer. Nat Rev Cancer. 2006;6(5):392-401.
24. Watnick RS. The role of the tumor microenvironment in regulating angiogenesis. Cold Spring Harb Perspect Med. 2012;2(12): a006676.
25. Li M, Wang Z, Xing Y, et al. A multicenter study on expressions of vascular endothelial growth factor, matrix metallopeptidase-9 and tissue inhibitor of metalloproteinase-2 in oral and maxillofacial squamous cell carcinoma. Iran Red Crescent Med J. 2014;16(3):e13185.

26. Hugo HJ, Lebret S, Tomaskovic-Crook E, et al. Contribution of fibroblast and mast cell (afferent) and tumor (efferent) IL-6 effects within the tumor microenvironment. Cancer Microenviron. 2012;5(1):83-93.

27. Hedbäck N, Jensen DH, Specht L, et al. MiR-21 expression in the tumor stroma of oral squamous cell carcinoma: an independent biomarker of disease free survival. PLoS ONE. 2014;9(4):e95193.

28. Yu EH, Tu HF, Wu CH, Yang CC, Chang KW. MicroRNA-21 promotes perineural invasion and impacts survival in patients with oral carcinoma. J Chin Med Assoc. 2017;80(6):383-388.

29. Nagasaki T, Hara M, Nakanishi H, Takahashi H, Sato M, Takeyama H. Interleukin-6 released by colon cancer-associated fibroblasts is critical for tumour angiogenesis: anti-interleukin-6 receptor antibody suppressed angiogenesis and inhibited tumour-stroma interaction. Br J Cancer. 2014; 110(2):469-478.

30. Shiga K, Hara M, Nagasaki T, Sato T, Takahashi H, Takeyama H. Cancer-associated fibroblasts: their characteristics and their roles in tumor growth. Cancers (Basel). 2015;7(4): 2443-2458.

31. Chuang JY, Huang YL, Yen WL, Chiang IP, Tsai MH, Tang CH. Syk/JNK/AP-1 signaling pathway mediates interleukin-6-promoted cell migration in oral squamous cell carcinoma. Int J Mol Sci. 2014;15(1):545-559.

32. Kayamori K, Sakamoto K, Nakashima T, et al. Roles of interleukin-6 and parathyroid hormone-related peptide in osteoclast formation associated with oral cancers: significance of interleukin-6 synthesized by stromal cells in response to cancer cells. Am J Pathol. 2010;176(2):968-980.

33. Brailo V, Vucicevic-Boras V, Lukac J, et al. Salivary and serum interleukin 1 beta, interleukin 6 and tumor necrosis factor alpha in patients with leukoplakia and oral cancer. Med Oral Patol Oral Cir Bucal. 2012;17(1):e10-e15.

34. Arantes DA, Costa NL, Mendonça EF, Silva TA, Batista AC. Overexpression of immunosuppressive cytokines is associated with poorer clinical stage of oral squamous cell carcinoma. Arch Oral Biol. 2016;61:28-35.

35. Tsai CC, Chen CC, Lin CC, Chen CH, Lin TS, Shieh TY. Interleukin-1 beta in oral submucous fibrosis, verrucous hyperplasia and squamous cell carcinoma tissues. Kaohsiung J Med Sci. 1999;15(9):513-519.

36. Bae JY, Kim EK, Yang DH, et al. Reciprocal interaction between carcinoma-associated fibroblasts and squamous carcinoma cells through interleukin-1α induces cancer progression. Neoplasia. 2014;16(11):928-938.

37. Petruzzi MNM, Cherubini K, Salum FG, de Figueiredo MA. Role of tumour-associated macrophages in oral squamous cells carcinoma progression: an update on current knowledge. Diagn Pathol. 2017;12(1):32.

38. Janele D, Lang T, Capellino S, Cutolo M, Da Silva JA, Straub RH. Effects of testosterone, 17beta-estradiol, and downstream estrogens on cytokine secretion from human leukocytes in the presence and absence of cortisol. Ann N Y Acad Sci. 2006; 1069(1):168-182.

39. Kramer PR, Kramer SF, Guan G. 17 beta-estradiol regulates cytokine release through modulation of CD16 expression in monocytes and monocyte-derived macrophages. Arthritis Rheum. 2004;50(6):1967-1975.

40. Eckert AW, Kappler M, Schubert J, Taubert H. Correlation of expression of hypoxia-related proteins with prognosis in oral squamous cell carcinoma patients. Oral Maxillofac Surg. 2012;16(2):189-196.

41. DE Lima PO, Jorge CC, Oliveira DT, Pereira MC. Hypoxic condition and prognosis in oral squamous cell carcinoma. Anticancer Res. 2014;34(2):605-612.

42. Kang FW, Que L, Wu M, Wang ZL, Sun J. Effects of trichostatin A on HIF-1α and VEGF expression in human tongue squamous cell carcinoma cells in vitro. Oncol Rep. 2012;28(1):193-199.

43. Michailidou EZ, Markopoulos AK, Antoniades DZ. VEGF expression from human dysplastic or malignant oral epithelium may be related to mast cell density and the subsequent angiogenetic phenomena. Int J Oral Maxillofac Surg. 2012;41(12): 1467-1473.

44. Kudo Y, Iizuka S, Yoshida M, et al. Periostin directly and indirectly promotes tumor lymphangiogenesis of head and neck cancer. PLoS ONE. 2012;7(8):e44488.

45. Hsu HW, Wall NR, Hsueh CT, et al. Combination antiangiogenic therapy and radiation in head and neck cancers. Oral Oncol. 2014;50(1):19-26.

46. Qian J, Wenguang X, Zhiyong W, Yuntao Z, Wei H. Hypoxia inducible factor: a potential prognostic biomarker in oral squamous cell carcinoma. Tumour Biol. 2016;37(8):10815-10820.

47. Kyzas PA, Stefanou D, Batistatou A, Agnantis NJ. Hypoxia-induced tumor angiogenic pathway in head and neck cancer: an in vivo study. Cancer Lett. 2005;225(2):297-304.

48. Nayak S, Goel MM, Chandra S, et al. VEGF-A immunohistochemical and mRNA expression in tissues and its serum levels in potentially malignant oral lesions and oral squamous cell carcinomas. Oral Oncol. 2012;48(3):233-239.

49. Yamagata Y, Tomioka H, Sakamoto K, et al. CD163-positive macrophages within the tumor stroma are associated with lymphangiogenesis and lymph node metastasis in oral squamous cell carcinoma. J Oral Maxillofac Surg. 2017 Mar 18 [Epub ahead of print].

50. Cirillo N, Hassona Y, Celentano A, et al. Cancer-associated fibroblasts regulate keratinocyte cell-cell adhesion via TGF-β-dependent pathways in genotype-specific oral cancer. Carcinogenesis. 2017;38(1):76-85.

51. Hwang YS, Xianglan Z, Park KK, Chung WY. Functional invadopodia formation through stabilization of the PDPN transcript by IMP-3 and cancer-stromal crosstalk for PDPN expression. Carcinogenesis. 2012;33(11):2135-2146.

52. Kartha VK, Stawski L, Han R, et al. PDGFRβ is a novel marker of stromal activation in oral squamous cell carcinomas. PLoS ONE. 2016;11(4):e0154645.

53. Gupta K, Metgud R, Gupta J. Evaluation of stromal myofibroblasts in oral leukoplakia, oral submucous fibrosis, and oral squamous cell carcinomaan immunohistochemical study. J Cancer Res Ther. 2015;11(4):893-898.

54. Khot K, Deshmukh SB, Alex S. Comparative analysis of the immunohistochemical expression of vascular endothelial growth factor and matrix metalloproteinase-9 in keratocystic odontogenic tumor, dentigerous cyst and radicular cyst. J Cancer Res Ther. 2015;11(3):635-640.

55. Zhang Z, Tao D, Zhang P, et al. Hyaluronan synthase 2 expressed by cancer-associated fibroblasts promotes oral cancer invasion. J Exp Clin Cancer Res. 2016;35(1):181.

56. Pal SK, Nguyen CT, Morita KI, et al. THBS1 is induced by TGFB1 in the cancer stroma and promotes invasion of oral squamous cell carcinoma. J Oral Pathol Med. 2016;45(10): 730-739.

57. Kosmehl H, Berndt A, Strassburger S, et al. Distribution of laminin and fibronectin isoforms in oral mucosa and oral squamous cell carcinoma. Br J Cancer. 1999;81(6):1071-1079.

58. Franz M, Wolheim A, Richter P, et al. Stromal laminin chain distribution in normal, hyperplastic and malignant oral mucosa: relation to myofibroblast occurrence and vessel formation. J Oral Pathol Med. 2010;39(4):290-298.

59. 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;25(4):1013-1020.

60. Kawashiri S, Tanaka A, Noguchi N, et al. Significance of stromal desmoplasia and myofibroblast appearance at the invasive front in squamous cell carcinoma of the oral cavity. Head Neck. 2009;31(10):1346-1353.

61. Pukkila M, Kosunen A, Ropponen K, et al. High stromal versican expression predicts unfavourable outcome in oral squamous cell carcinoma. J Clin Pathol. 2007;60(3):267-272.

62. Zargaran M, Eshghyar N, Vaziri PB, Mortazavi H. Immunohistochemical evaluation of type IV collagen and laminin-332 γ2 chain expression in well-differentiated oral squamous cell carcinoma and oral verrucous carcinoma: a new recommended cut-off. J Oral Pathol Med. 2011;40(2):167-173.

63. Wu MH, Hong HC, Hong TM, Chiang WF, Jin YT, Chen YL. Targeting galectin-1 in carcinoma-associated fibroblasts inhibits oral squamous cell carcinoma metastasis by downregulating MCP-1/CCL2 expression. Clin Cancer Res. 2011;17(6): 1306-1316.

64. Chiang WF, Liu SY, Fang LY, et al. Overexpression of galectin-1 at the tumor invasion front is associated with poor prognosis in early-stage oral squamous cell carcinoma. Oral Oncol. 2008;44(4):325-334.

65. Bettendorf O, Herrmann G. Prognostic relevance of Ki-67 antigen expression in 329 cases of oral squamous cell carcinoma. ORL J Otorhinolaryngol Relat Spec. 2002;64(3):200-205.

66. Le Novère N, Hucka M, Mi H, et al. The Systems Biology Graphical Notation. Nat Biotechnol. 2009;27(8):735-741.