Original Article

Comparison of orosomucoid-1 immunoexpression and angiogenesis between oral squamous cell carcinoma cases with different histological grades

Celeste Sánchez-Romero[1,2], Ronell Bologna-Molina[1,2], Rogelio González-González[2], Sirced Salazar-Rodríguez[3,4], Nataly Barreiro Mendoza[5]

[1]Molecular Pathology Area, School of Dentistry, University of the Republic, Montevideo, Uruguay, [2]Department of Research, School of Dentistry, Juarez University of the State of Durango, Durango, México, [3]Department of Anatomical Pathology, National Institute of Oncology and Radiobiology, Havana, Cuba, [4]Department of Anatomical Pathology, Society to Fight Cancer, Núcleo Manabí, [5]Department of Oral Pathology Research, School of Dentistry, University of San Gregorio Portoviejo, Portoviejo, Manabí, Ecuador

Abstract

Background: Oral squamous cell carcinoma (OSCC) is the most common malignancy in this region, and thus, further elucidation of its tumoral mechanisms is important. One of the main roles of the acute-phase protein orosomucoid-1 (ORM1) is the promotion of angiogenesis, which is key for tumor nutrition and growth.

Aim: Our aim was to evaluate the immunohistochemical expression of ORM1 and the angiogenic activity indicated by microvascular density (MVD) in OSCC samples according to histological grade.

Materials and Methods: Formalin-fixed, paraffin-embedded sections from 45 OSCC cases were submitted to immunohistochemistry: 25 were well-differentiated OSCC, 18 were moderately differentiated OSCC and 2 were poorly differentiated OSCC. ORM1 staining was evaluated by a semiquantitative method, and CD34-positive blood vessels were quantified to calculate the MVD. The results were statically analyzed.

Results: All cases exhibited immunoexpression of ORM1 and CD34. However, no significant differences were found between the expression of both markers among the histological grades. In addition, the presence of ORM1 in inflammatory cells and in the extracellular matrix was detected in most cases.

Conclusion: These results suggest that the induction of angiogenesis is not the main role of ORM1 in OSCC and may be associated with the regulation of the immune/inflammatory response or the transport of protumoral molecules, such as sialyl-Lewis X or phorbol esters, which requires confirmation in future studies.

Keywords: Acute-phase proteins, angiogenesis, immunohistochemistry, oral cancer, orosomucoid-1 protein

Address for correspondence: Dr. Nataly Barreiro Mendoza, Department of Oral Pathology Research, School of Dentistry, University of San Gregorio Portoviejo, Av. Metropolitana 2005, 130101, Portoviejo, Manabí, Ecuador.
E-mail: genaty8@hotmail.com

Submitted: 03-Mar-2020, **Accepted:** 04-Sep-2020, **Published:** 31-Aug-2021

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is a malignant neoplasm with squamous differentiation that arises from the oral mucosa epithelium, comprising more than 90% of oral cancers, and represents the sixth most common type of cancer worldwide. This disease occurs mainly in the fifth or sixth decade of life, and to date, smoking is

Access this article online

Quick Response Code:

Website: www.jomfp.in

DOI: 10.4103/0973-029X.325243

How to cite this article: Sánchez-Romero C, Bologna-Molina R, González-González R, Salazar Rodríguez S, Barreiro Mendoza N. Comparison of orosomucoid-1 immunoexpression and angiogenesis between oral squamous cell carcinoma cases with different histological grades. J Oral Maxillofac Pathol 2021;25:368.

the most important etiological factor.[1] However, there are diverse genetic, metabolic, angiogenic and inflammatory protumoral mechanisms involved in the pathogenesis and tumor behavior of OSCC.[2-5]

Despite the advances in prevention and multimodal therapies for several types of cancer in recent decades, the prognosis of patients with OSCC remains unfavorable.[6] Therefore, further elucidation of the biological and cellular mechanisms that contribute to OSCC tumoral progression is urgently needed to improve our approach to this disease and identify new biomarkers with possible clinical and therapeutic implications.

The acute-phase response (APR) occurs due to imbalances in homeostasis caused by infection, tissue damage, immunological disorders or neoplastic growth;[7] in this response, the concentration of a set of proteins in the plasma, called acute-phase proteins, is modified.[8] These proteins are mainly involved in protection against invading agents, prevention of excessive cell damage through immunomodulation, induction of repair and contribution to functional restoration.[9] Orosomucoid-1 (ORM1), also known as alpha-1-acid glycoprotein, is one of the proteins released in the APR that regulates the inflammatory response and angiogenesis in tissues.[10] The exact functions of ORM1 are still unclear; however, ORM1 is considered an immunomodulatory and anti-inflammatory agent with a predominant role in immunosuppression that helps prevent excessive tissue damage due to the acute inflammatory response.[9] High serological concentrations of these proteins have been reported in patients with OSCC[7] and other malignant neoplasms, such as lung cancer, mainly in the advanced stages.[11]

To the best of our knowledge, only one study has evaluated the expression of ORM1 in squamous cell carcinoma of the head and neck, including 15 cases of OSCC.[12] Therefore, our study, with a larger population, will help establish the detailed expression pattern of this protein in OSCC with different histological grades.

Blood supply and vascularization are necessary for tumor growth and eventual dissemination. Some types of cancer produce certain growth factors to induce angiogenesis; therefore, endothelial markers such as CD34 are used to quantify the microvascular density (MVD) in the tumor tissue.[13] Several studies have used MVD as a parameter to assess the angiogenic potential of diverse neoplasms, including OSCC, to find a correlation between angiogenic potential and histological grade.[14]

As the CD34 and ORM1 proteins are associated with tumor behavior, the objective of this study was to establish the expression patterns of ORM1 and MVD in OSCC cases with different histological grades. The findings of this study could suggest applications for ORM1 in the study of head-and-neck neoplasms.

MATERIALS AND METHODS

Sample selection

Forty-five formalin-fixed paraffin-embedded tissue samples of OSCC obtained from the National Institute of Oncology and Radiobiology of Cuba were included in the study. The cases were classified according to histological grade: well-differentiated carcinoma (WD-OSCC), moderately differentiated carcinoma (MD-OSCC) and poorly differentiated carcinoma (PD-OSCC).

Immunohistochemistry

Immunohistochemistry for ORM1 (2F9-1F10, Abcam, 1:100) and CD34 (QBEnd-10, Dako, 1:50) was performed according to previously described protocols.[15]

The evaluation of ORM-1 cytoplasmic and membrane immunoreactivity was semiquantitatively scored using a four-grade scoring scale as follows: 0 (no staining); 1 (+, low): 1%–10% positive cells; 2 (++, intermediate or moderate): 11%–50% positive cells and 3 (+++, high): >50% positive cells.

The MVD was determined using the method originally described by Weidner.[16] At low magnification, five "hot spots" with a high number of CD34-positive blood vessels were assessed. Subsequently, counting was performed in each area at ×200 field magnification. The MVD is expressed as the highest number of vessels within any ×200 field. The kappa coefficient (intraobserver concordance) was 0.886.

Statistical analysis

Central tendency measures were generated for both markers: means, medians and standard deviation. Subsequently, Student's *t*-test was used for determination of the significance of the immunoexpression of each protein between the groups.

RESULTS

After a histopathological review, the 45 OSCC cases were classified according to their histological grade as WD-OSCC (25), MD-OSCC (18) and PD-OSCC (2).

All cases showed intense and diffuse staining for ORM1, mainly cytoplasmic staining; however, in focal areas,

predominant membranous staining and/or intercellular bridges was observed. A common feature was an intense expression at certain tumor sites, while other sites, even those adjacent to stained cells, remained negative [Figure 1a-d]. Focal nuclear positivity was observed in 7 cases [Figure 1e]. In addition, another staining pattern with the appearance of secretion in the extracellular matrix (so-called "shedding") was observed in 30 cases, [Figure 1f] with a similar distribution between histological grades [Table 1]. Stromal cells, such as endothelial cells, inflammatory cells and some fibroblasts, presented immunoexpression of ORM-1. In addition, in foci of intravascular invasion, ORM1-positive tumoral emboli were found [Figure 1c and d].

Table 1: Presence of stromal secretion (shedding) of orosomucoid-1 by histological group

Histological grade	Absent, *n* (%)	Present, *n* (%)
WD-OSCC	9 (36.0)	16 (64.0)
MD-OSCC	6 (33.3)	12 (66.7)
PD-OSCC	0	2 (100)

WD: Well differentiated, MD: Moderately differentiated, PD: Poorly differentiated, OSCC: Oral squamous cell carcinoma

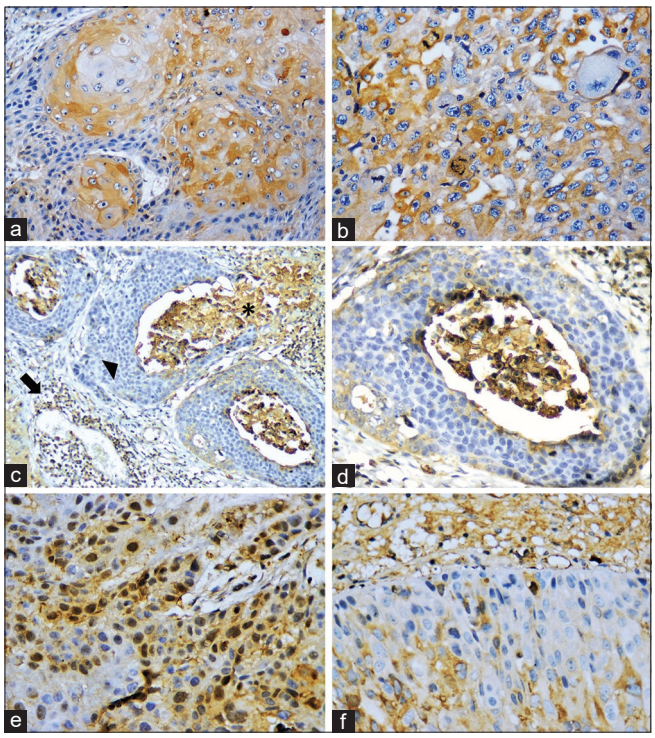

Figure 1: Immunostaining of orosomucoid-1 in well-differentiated (a), moderately differentiated (c and d) and poorly differentiated (b) oral squamous cell carcinoma. Note the presence of adjacent negative and positive cells exhibiting predominantly cytoplasmic staining (a-f). Perivascular invasion of negative tumor cells (arrowhead) was observed in the lumen of the affected vessels, and the presence of positive tumor emboli (asterisk) and positive endothelial and inflammatory cells was observed (arrow) (c and d). Few cases presented nuclear positivity (e). Presence of orosomucoid-1 with the appearance of secretion in the stroma (shedding) (f) (Immunohistochemistry, a, b, d, e and f: ×400, c: ×200)

CD34 showed positivity in the blood vessels of all cases, with a predominantly peritumoral or intratumoral arrangement [Figure 2]. The median values of MVD for each group are shown in Table 2.

The majority of the cases showed high immunostaining (++) of ORM1: 84% of the CBD, 55.6% of the CMD and 100% of the CPD (n = 2) [Figure 3]. The difference in the expression of CD34 was not significant between histological grades or in relation to ORM1 immunoexpression [Table 2].

DISCUSSION

The immunoexpression of ORM1 observed in all cases in this study suggests that this protein may have a role in the pathogenesis and tumoral mechanisms of OSCC and can be used as a biomarker for further studies. The differences in immunoexpression of ORM1 and MVD (CD34) between histological grades were not significant, suggesting

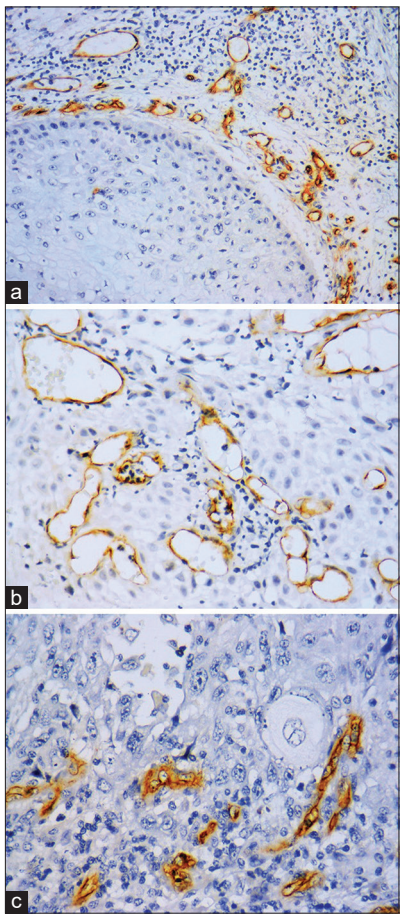

Figure 2: CD34 staining of blood vessels in well-differentiated (a), moderately differentiated (b) and poorly differentiated (c) oral squamous cell carcinoma. Note the peritumoral (a) and intratumoral (b and c) distribution of blood vessels (Immunohistochemistry, a: ×200, b and c: ×400)

Table 2: Descriptive statistics and statistical significance between both markers by histological subtype

Histological grade	ORM1	*n* (%)	Mean±SD CD34 (MVD)	Mean CI (95%)		*P*
				Lower	Upper	
WD-OSCC (*n*=25)	+	4 (16)	12.35±4.47	5.25	19.46	0.845
	++	21 (84)	11.82±5.00	9.54	14.10	
MD-OSCC (*n*=18)	+	8 (44.4)	10.95±3.25	8.23	12.67	0.475
	++	10 (55.6)	9.53±4.62	6.23	12.84	
PD-OSCC (*n*=2)	++	2 (100)	9.7±0.99	*	*	*
OSCC-total (*n*=45)	+	12 (26.7)	11.42±3.56	9.16	13.68	0.784
	++	33 (73.3)	11±10.96	9.30	12.69	

*It was not possible to perform the statistical tests due to the small number of cases. WD: Well differentiated, MD: Moderately differentiated, PD: Poorly differentiated, OSCC: Oral squamous cell carcinoma, *n*: Number of cases, SD: Standard deviation, MVD: Microvascular density, CI: Confidence interval, ORM1: Orosomucoid-1

that angiogenesis may not be influenced by ORM1 in OSCC; these results were consistent with findings indicating the participation of nonangiogenic pathways for vascularization and nutrition of tumors.[13] We suggest that the role of ORM1 in OSCC could be associated with the regulation of the immune/inflammatory response rather than angiogenesis. However, this hypothesis requires further confirmation.

In normal conditions, ORM1 is secreted by endothelial cells, participates in the regulation of capillary permeability and therefore helps to maintain homeostasis.[17] Since angiogenically activated vessels have an increased permeability, and this modification is one of the main effects of ORM1 on endothelial cells, the increase in ORM1 is associated with angiogenic activation.[18] However, the angiogenic modulation of ORM1 is bimodal and depends on the context: it inhibits angiogenesis induced by injury or tumor necrosis factor-alpha and stimulates developmental or vascular endothelial growth factor (VEGF)-induced angiogenesis.[9] Irmak *et al.* confirmed the active role of ORM1 in angiogenesis in collaboration with VEGF-A through *in vitro* and *in vivo* tests.[10]

ORM1 has been suggested to be necessary for the capillary permeability that allows tumors to maintain an active angiogenic process; thus, the upregulation of ORM1 has been associated with the neovascularization of malignant processes as well as with clinical parameters. For example, Yildirim *et al.* found that serum ORM1 levels in patients with advanced-stage lung cancer were high.[11] Similarly, advanced stage bladder cancer has a high expression of ORM1.[18]

To the best of our knowledge, only Croce *et al.* (2001) evaluated and described the immunohistochemical expression of ORM1 in cases of head-and-neck cancer, including 15 OSCC cases, and similar to those in our study, most of the cases presented strong and diffuse staining in the cytoplasm. Similarly, the study described the positivity between intercellular bridges and focal staining in nuclear and cytoplasmic membranes, as well as the adjacency of positive and negative tumor areas.[12] Although this study did not report the expression of ORM1 in endothelial and inflammatory cells or extracellular matrix observed in our cases, this pattern of labeling has been described in odontogenic myxoma. These results suggested that ORM1 is an important component of the extracellular matrix of this odontogenic tumor, contributing to its characteristic viscosity, which has been associated with a high invasive capacity. In addition, the authors suggest an angiogenic regulatory role of ORM1 in conjunction with VEGFA, since both markers were expressed in endothelial cells.[15]

Blood supply is necessary for tumor growth and spread. An angiogenic mechanism has been described in some types of carcinomas, in which tumor cells secrete growth factors to produce new vessels from the existing circulation (angiogenesis).[13] Aggressiveness parameters have been related to the degree and pattern of angiogenesis in various malignant neoplasms.[19-21]

In the oral mucosa, a significant vascularity increase has been verified through CD34 and CD31 immunoexpression during the transition from normal mucosa to severe dysplasia and toward invasive carcinoma.[20] As in the present study, several authors have reported an absence of a correlation between MVD and angiogenesis with the histological grade of OSCC.[13,14,22] In the present study, we did not find an association between the presence of ORM1 and angiogenesis through MVD. The lack of a correlation between the levels of ORM1 and MVD suggests that the induction of angiogenesis is not the main role of ORM1 in OSCC; therefore, this molecule could be related to other tumor mechanisms, such as those associated with the regulation of the immune response, which requires confirmation in future studies.

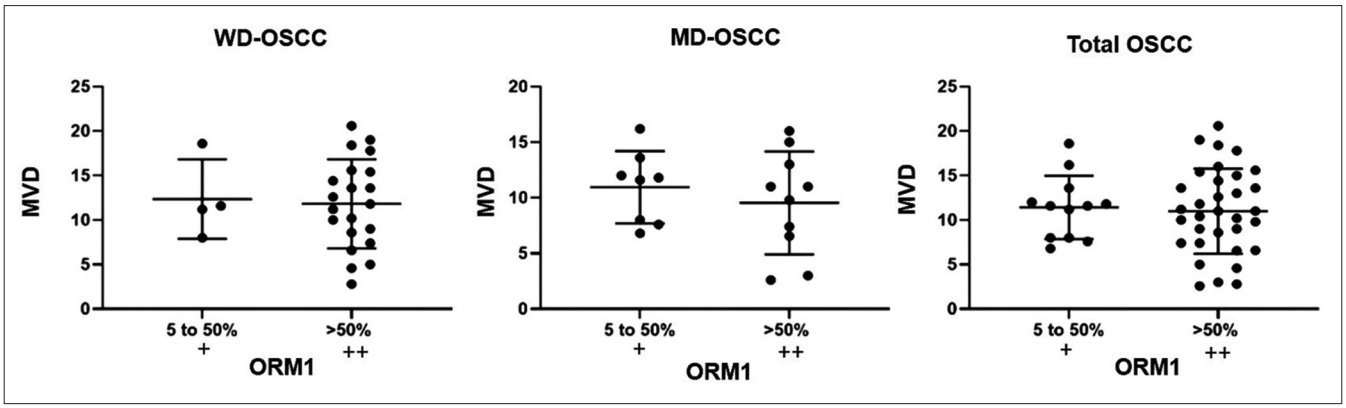

Figure 3: Distribution of cases according to microvascular density and orosomucoid-1 positivity. WD: well differentiated, MD: moderately differentiated, OSCC: oral squamous cell carcinoma

In some types of cancer, the relationship between tumor angiogenesis and clinicopathological parameters is controversial.[23,24] In the case of OSCC, tumor angiogenesis has been related to metastasis, tumor progression and prognosis.[25] However, this phenomenon was not associated with tumor size, degree of differentiation, invasion, metastasis, recurrence, prognosis or survival.[22] Therefore, nonangiogenic or less angiogenesis-dependent mechanisms for OSCC cell nutrition have been suggested, which may participate in tumor progression.[13]

There is evidence of the immunosuppressive activity of ORM1 during inflammation; however, its specific immunological or biological functions are still unclear.[12] ORM1 may be produced by some tumors to protect themselves through immunosuppression.[26] The immunomodulatory function of ORM1 could explain the positivity in the inflammatory infiltrate in the present study.

Studies on ORM1 immunoexpression in head-and-neck tumors are lacking, and Croce *et al.* (2001), who analyzed the association between ORM1 and head-and-neck cancer, is the only one that includes OSCC cases.[12] The authors subsequently (2005) performed an immunohistochemical study of ORM1 as a possible carrier of the sialyl-Lewis X antigen in colorectal carcinoma.[27] ORM1 and other glycoproteins have been identified as carriers of sialyl-Lewis X, whose expression is frequent in neoplastic cells, and this molecule has been strongly related to the migration of tumor cells and metastasis.[27] Interestingly, we found areas of intravascular invasion, indicating that the clusters of tumor cells within the blood vessels showed a strong expression of ORM1, unlike those with a perivascular invasion pattern, which could be associated with the aforementioned mechanisms of cell migration, tumor spread and even with the increase of vascular permeability.

Another type of molecule carried by ORM1 are phorbol esters, which act as tumor promoters through the activation of protein kinase C,[28,29] which has been linked to cutaneous squamous cell carcinoma caused by repeated exposures to ultraviolet radiation.[30] Within this group, 12-O-tetradecanoylphorbol-13-acetate has been described as a potent tumor promoter, by activating the transcription of genes involved in cell proliferation, transformation and apoptosis.[31] This finding suggests a possible additional role of ORM1 (in addition to immunomodulation) in OSCC pathogenesis.

One of the main limitations of the study was the absence of immunohistochemical tests for ORM1 in human normal oral epithelium, as there are no specific publications on this issue. Elucidation of the immunoexpression of ORM1 in healthy oral mucosa is important for comparisons with that presented in neoplastic tissues. The limited number of samples from the PD-OSCC cases ($n = 2$) did not allow to include this histological grade in the statistical tests.

CONCLUSION

The immunoexpression of ORM1 observed in all cases in this study suggests that ORM1 can be used as a biomarker for OSCC study. The lack of a significant relationship between the expression of ORM1 and MVD among the histological grades could indicate the participation of nonangiogenic pathways, as well as a role of ORM1 in other tumor mechanisms during OSCC progression, which is probably related to immunomodulation, vascular permeability or transport of protumoral molecules such as sialyl-Lewis X or phorbol esters. This hypothesis requires additional research for confirmation.

Financial support and sponsorship

This study was financially supported by Consejo Nacional de Ciencia y Tecnología, Sistema Nacional de Investigadores (Conacyt-SNI), México.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. El-Naggar A, Chan JK, Grandis JR, Takata T, Slootweg PJ. WHO Classification of Head and Neck Tumours. 4th ed. Lyon: IARC Press; 2017.
2. India Project Team of the International Cancer Genome Consortium. Mutational landscape of gingivo-buccal oral squamous cell carcinoma reveals new recurrently-mutated genes and molecular subgroups. Nat Commun 2013;4:2873.
3. Agostini M, Almeida LY, Bastos DC, Ortega RM, Moreira FS, Seguin F, *et al.* The fatty acid synthase inhibitor orlistat reduces the growth and metastasis of orthotopic tongue oral squamous cell carcinomas. Mol Cancer Ther 2014;13:585-95.
4. Nasry WHS, Rodriguez-Lecompte JC, Martin CK. Role of COX-2/PGE2 Mediated Inflammation in Oral Squamous Cell Carcinoma. Cancers (Basel) 2018;10:348.
5. Carnielli CM, Macedo CCS, De Rossi T, Granato DC, Rivera C, Domingues RR, *et al.* Combining discovery and targeted proteomics reveals a prognostic signature in oral cancer. Nat Commun 2018;9:3598.
6. Rivera C, Oliveira AK, Costa RAP, De Rossi T, Paes Leme AF. Prognostic biomarkers in oral squamous cell carcinoma: A systematic review. Oral Oncol 2017;72:38-47.
7. Gallo O, Gori AM, Attanasio M, Martini F, Giusti B, Brunelli T, *et al.* Interleukin-6 and acute-phase proteins in head and neck cancer. Eur Arch Otorhinolaryngol 1995;252:159-62.
8. Salazar Soler A, Pinto Sala X, Maña Rey J, Pujol Farriols R. Inflammatory response, cholesterol metabolism, and arteriosclerosis. An Med Interna 2001;18:100-4.
9. Ligresti G, Aplin AC, Dunn BE, Morishita A, Nicosia RF. The acute phase reactant orosomucoid-1 is a bimodal regulator of angiogenesis with time-and context-dependent inhibitory and stimulatory properties. PLoS One 2012;7:e41387.
10. Irmak S, Oliveira-Ferrer L, Singer BB, Ergün S, Tilki D. Pro-angiogenic properties of orosomucoid (ORM). Exp Cell Res 2009;315:3201-9.
11. Yildirim A, Meral M, Kaynar H, Polat H, Ucar EY. Relationship between serum levels of some acute-phase proteins and stage of disease and performance status in patients with lung cancer. Med Sci Monit 2007;13:CR195-200.
12. Croce MV, Price MR, Segal-Eiras A. Association of α1 acidic glycoprotein and squamous cell carcinoma of the head and neck. Pathol Oncol Res 2001;7:111-7.
13. Shieh YS, Lee HS, Shiah SG, Chu YW, Wu CW, Chang LC. Role of angiogenic and non-angiogenic mechanisms in oral squamous cell carcinoma: Correlation with histologic differentiation and tumor progression. J Oral Pathol Med 2004;33:601-6.
14. Cantín López M, Suazo Galdames I, Venegas Rojas B, Zavando Matamala D. Lower lip squamous cell carcinoma: Relationship between angiogenesis grade, histological grading and invasion tumoral front. Int J Morphol 2008;26:77-83.
15. Bologna-Molina R, Mosqueda-Taylor A, Domínguez-Malagón H, Salazar-Rodríguez S, Tapia G, Gónzalez-Gónzalez R, *et al.* Immunolocalization of VEGF-A and orosomucoid-1 in odontogenic myxoma. Rom J Morphol Embryol 2015;56:465-73.
16. Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis-correlation in invasive breast carcinoma. N Engl J Med 1991;324:1-8.
17. Sörensson J, Matejka GL, Ohlson M, Haraldsson B. Human endothelial cells produce orosomucoid, an important component of the capillary barrier. Am J Physiol 1999;276:H530-4.
18. Irmak S, Tilki D, Heukeshoven J, Oliveira-Ferrer L, Friedrich M, Huland H, *et al.* Stage-dependent increase of orosomucoid and zinc-alpha2-glycoprotein in urinary bladder cancer. Proteomics 2005;5:4296-304.
19. Hasina R, Lingen MW. Angiogenesis in oral cancer. J Dent Educ 2001;65:1282-90.
20. Siar CH, Oo VP, Nagatsuka H, Nakano K, Ng KH, Kawakami T. Angiogenic squamous dysplasia-like phenomenon in oral epithelial precursor lesions. Eur J Med Res 2009;14:315-9.
21. López JI, Erramuzpe A, Guarch R, Cortés JM, Pulido R, Llarena R, *et al.* CD34 immunostaining enhances a distinct pattern of intratumor angiogenesis with prognostic implications in clear cell renal cell carcinoma. APMIS 2017;125:128-33.
22. Moriyama M, Kumagai S, Kawashiri S, Kojima K, Kakihara K, Yamamoto E. Immunohistochemical study of tumour angiogenesis in oral squamous cell carcinoma. Oral Oncol 1997;33:369-74.
23. Kashani-Sabet M, Sagebiel RW, Ferreira CM, Nosrati M, Miller JR 3rd. Vascular involvement in the prognosis of primary cutaneous melanoma. Arch Dermatol 2001;137:1169-73.
24. Barnhill RL, Busam KJ, Berwick M, Blessing K, Cochran AJ, Elder DE, *et al.* Tumour vascularity is not a prognostic factor for cutaneous melanoma. Lancet 1994;344:1237-8.
25. Elpek GO, Gelen T, Aksoy NH, Erdoğan A, Dertsiz L, Demircan A, *et al.* The prognostic relevance of angiogenesis and mast cells in squamous cell carcinoma of the oesophagus. J Clin Pathol 2001;54:940-4.
26. Yuki M, MacHida N, Sawano T, Itoh H. Investigation of serum concentrations and immunohistochemical localization of α1-acid glycoprotein in tumor dogs. Vet Res Commun 2011;35:1-11.
27. Croce MV, Sálice VC, Lacunza E, Segal-Eiras A. Alpha 1-acid glycoprotein (AGP): A possible carrier of sialyl lewis X (slewis X) antigen in colorectal carcinoma. Histol Histopathol 2005;20:91-7.
28. Blumberg PM. Protein kinase C as the receptor for the phorbol ester tumor promoters: Sixth Rhoads memorial award lecture. Cancer Res 1988;48:1-8.
29. Fournier T, Medjoubi-N N, Porquet D. Alpha-1-acid glycoprotein. Biochim Biophys Acta 2000;1482:157-71.
30. Verma AK, Wheeler DL, Aziz MH, Manoharan H. Protein kinase Cepsilon and development of squamous cell carcinoma, the nonmelanoma human skin cancer. Mol Carcinog 2006;45:381-8.
31. Chao DC, Lin LJ, Hsiang CY, Li CC, Lo HY, Liang JA, *et al.* Evodiamine inhibits12-O-tetradecanoylphorbol-13-acetate-inducedactivator protein 1 transactivation and cell transformation in human hepatocytes. Phytother Res 2011;25:1018-23.