Journal of Cranio-Maxillo-Facial Surgery 47 (2019) 1583–1588

Contents lists available at ScienceDirect

Journal of Cranio-Maxillo-Facial Surgery

journal homepage: www.jcmfs.com

Upregulation of ADAM10 in oral squamous cell carcinoma and its correlation with EGFR, neoangiogenesis and clinicopathologic factors

Shahroo Etemad-Moghadam, Mojgan Alaeddini*

Dental Research Center, Dentistry Research Institute, Tehran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Article history:
Paper received 26 January 2019
Accepted 14 July 2019
Available online 19 July 2019

Keywords:
ADAM10
EGFR
Neoangiogenesis
Squamous cell carcinoma

ABSTRACT

ADAM10 (a disintegrin and metalloproteinase-10) is a known sheddase for EGFR (epidermal growth factor receptor) ligands and has been suggested to modulate angiogenesis. We aimed to evaluate the expression of ADAM10 in patients with oral squamous cell carcinoma (OSCC) and to determine its correlation with EGFR, CD105 and clinicopathologic parameters. Fifty primary OSCCs with clinical data were graded according to the histologic risk assessment (HRA) model and subjected to immunohistochemical staining using antibodies against ADAM10, EGFR1 and CD105. ADAM10 was assessed in both epithelial and stromal components. The associations among all three proteins and clinicopathologic factors including tumor size, lymph node status and distant metastasis (TNM) were statistically analyzed ($P < 0.05$). Epithelial-ADAM10, stromal-ADAM10 and EGFR were overexpressed in 92%, 40% and 56% of the OSCCs, respectively. EGFR expression occurred in peripheral and diffuse patterns, which were also separately considered in our analyses. A significant correlation was found between ADAM10 and CD105 ($r = -0.455$; $P < 0.001$). Lymphocytic infiltration scores ($P = 0.04$) and tumor size ($P = 0.001$) showed significant differences between EGFR+ and EGFR− tumors, but none of the other variables had any relationships with either clinicopathologic factors or each other ($P > 0.05$). ADAM10 was upregulated in OSCC but had no correlation with survival-associated factors such as TNM or the HRA model. At the protein level, epithelial ADAM10 negatively regulated neoangiogenesis, but its interaction with EGFR was minimal. Reduction in host immunologic responses was associated with a decrease in EGFR. These findings, if corroborated, could be interesting in combination therapies used for cancer treatment.

1. Introduction

Patients with oral squamous cell carcinoma (OSCC), the sixth most common cancer worldwide, have a 5-year survival rate of only 50% (Gröbe et al., 2014). The main treatment for this cancer is surgery; however, factors such as inaccessibility, location, proximity to vital structures, and local aggressiveness negatively affect the outcome of therapy. Therefore, identification of pathogenic mechanisms in OSCC may provide additional options to supplement surgery and treatment of infiltrative and residual tumors. Generally, exploring new markers has been an ongoing effort in this context; however, evaluation of controversial existing ones is also essential, especially when pharmaceutical blockers have been developed against them and used in clinical trials.

ADAMs (a disintegrin and metalloproteinases) are a family of enzymes with adhesion and proteolytic functions leading to shedding of ligands with different outcomes including proliferation, migration and angiogenesis, which are considered hallmarks of cancer. These proteins are upregulated in the breast, lung, prostate and a number of other malignancies and are associated with prognosis in some but not others (Duffy et al., 2009, 2011). Epidermal growth factor receptor (EGFR), also known as ErbB1 or HER1, is a member of the ErbB family of receptor tyrosine-kinases on the plasma membrane. This protein is activated via a number of ligands including EGF and TGF-α, which, through downstream signaling molecules, result in the trigger of pathways involved in neoplastic progression and survival (Diniz-Freitas et al., 2007; Duffy et al., 2009, 2011; Gröbe et al., 2014). Neoangiogenesis is the newly formed network of abnormal tumor vasculature that is

* Corresponding author. Dentistry Research Institute, Dental Research Center, Ghods St, Enghelab Ave, P.O. Box: 14155-5583, 14174 Tehran, Iran. Fax: +98 21 88986688.
E-mail addresses: malaeddini@sina.tums.ac.ir, malaeddini@yahoo.com (M. Alaeddini).

https://doi.org/10.1016/j.jcms.2019.07.008
1010-5182/

essential for continued tumor growth and progression (Marioni et al., 2010).

ADAM10 is a proteolytically active member of the ADAM superfamily and serves as a sheddase for EGF release, which upon interaction with EGFR induces tumor-promoting features (Duffy et al., 2011). EGFR has been suggested to promote angiogenesis through various pathways such as Notch1, mTOR and VEGF (Lionello et al., 2014; Wang et al., 2015). In contrast to the limited number of studies on ADAM10 in OSCC patients (Jones et al., 2013; Ko et al., 2007; Stasikowska-Kanicka et al., 2018; Zepeda-Nuño et al., 2017), EGFR and angiogenesis have been separately evaluated in many studies with extremely conflicting results (Gröbe et al., 2014; Marioni et al., 2010). However, the relationship between ADAM10, EGFR and neoangiogenesis has not been investigated in OSCC.

In tumors with EGFR overexpression, antibodies such as cetuximab have been developed for targeted therapy, but its response rate in OSCC was reported to be less than 20% (Kimura et al., 2016). Therefore, an appealing strategy to prevent the tumor-promoting effects of EGFR would be to inhibit the sheddase activity of ADAM10 through substances such as INCB3619 that are being currently investigated in other cancers (Duffy et al., 2011). Functional overlap between different factors involved in cancer necessitates the consideration of several crossing molecular pathways when developing anticancer strategies. Therefore, regarding the interesting molecular interaction between the above-mentioned proteins and their alteration in oral cancer, the aim of the present study was to determine the expression and relationship of ADAM10, EGFR and neoangiogenesis in patients with OSCC, and to correlate these factors with clinicopathologic parameters and the histologic risk assessment (HRA) model.

2. Materials and methods

2.1. Procedure

The protocol for this research was approved by the Ethics Committee of our University. All cases with a diagnosis of OSCC were retrieved from our patient files and demographic information along with clinical data including age, sex, tumor size (T), lymph node (N) status, and distant metastasis (M) were recorded. TNM was assessed according to the Seventh American Joint Committee on Cancer (AJCC) TNM system (American Joint Committee on Cancer, 2010). For confirmation of diagnoses, all hematoxylin and eosin-stained slides were collected and reviewed by two oral and maxillofacial pathologists following the 2017 World Health Organization guidelines. Primary tumors with complete excision, a diagnosis of conventional SCC, minimal necrosis, adequate amount of connective tissue beyond the tumor–host interface, and no history of chemo/radiotherapy or systemic diseases were selected and graded by both pathologists according to the histologic risk assessment model (Brandwein-Gensler et al., 2005).

For immunohistochemical staining, 3-μm sections were cut from representative paraffin blocks, deparaffinized in Xylene, rehydrated in alcohol, and rinsed in PBS for 3 min and 0.3% H_2O_2 for 10 min. This was followed by antigen retrieval in a microwave oven, using citrate buffer (pH = 6) for ADAM10 and enzyme digestion with proteinase K (10 min) for EGFR and CD105. All slides were incubated for 1 h with primary antibodies ADAM10 (sc-25578, Santa Cruz, Heidelberg, Germany), EGFR (H11, Dako, Golstrup Denmark) and CD105 (SN6h, Dako Cytomation, Denmark) in dilutions of 1:50, 1:50 and 1:100, respectively. After rinsing in PBS, the sections were covered with EnVision (Dako Cytomation) for 30 min at room temperature, washed and incubated with diaminobenzidine and counterstained with Mayer hematoxylin. Each batch of slides had its own positive and negative controls, with sections known to be positive for each marker serving as positive controls and replacement of primary antibodies with PBS constituting negative controls.

All immunostained sections were analyzed by the same pathologists and microscope. ADAM10 was calculated according to the method proposed by You et al. (2015) using a multiplication of intensity (0: negative, 1: weak, 2: strong) by percentage (0, 1, 2, 3, and 4 were equal to 0%, 1–25%, 26–50%, 51–75% and 76–100%, respectively). Final scores of less than 4 were considered as weak or negative, and those between 4 and 8 were regarded as overexpression. For EGFR, intensity was categorized as negative, weak, moderate or strong, corresponding to numerical values of 0, 1, 2 and 3, whereas staining extension was recorded as 0 for no staining, 1 where 1–30% of cells were positive, 2 in cases with 31–50% immunostaining and 3 for 51–100% expression. Addition of intensity and extent values reflected positivity when equal to 5–6 and negativity for scores below 5, as described previously (Shiraki et al., 2005). Neoangiogenesis was assessed by analysis of microvessel density (MVD) as follows: five hotspots consisting of the highest numbers of CD105-stained cells at low magnification (×40) were selected, and all immunoreactive cells regardless of lumen formation were counted under a magnification of ×200. Vessels containing muscular walls were not included in the final counts.

2.2. Statistical analysis

Comparisons between variables were performed using analysis of variance, Mann–Whitney, Kruskal–Wallis and independent-sample Student *t* tests. Spearman, Pearson and Phi coefficients were calculated for assessment of correlation between variables. $P < 0.05$ was considered significant.

3. Results

A total of 50 OSCCs fulfilled the inclusion criteria and were subjected to immunohistochemical analysis and grading. According to the available clinical data, there were 28 men and 20 women. Our youngest patient was a 19-year-old female patient and our oldest was an 88-year-old male patient. The mean and median ages of the patients were 63.3 years and 64 years, respectively, with 17 patients younger and 30 patients older than 60 years. Information for 3 and 2 cases were missing for age and sex, respectively. Based on the HRA model, 12 of the cases were low-, 18 were intermediate- and 20 were high-risk tumors. According to the AJCC TNM system, 20, 14, 11 and 5 of the patients were classified as T1, T2, T3 and T4, respectively. Only one of the 5 T4 tumors was T4b and the rest were T4a. Lymph node status was recorded as N0 in 44, N1 in 2 and N2 in 4 (3N2 and 1N2b) patients. Only 2 of 50 individuals demonstrated metastasis to distant organs, both of which were to the lungs. Based on clinicopathologic findings including TNM status, perineural invasion, and other relevant factors, individualized treatment was planned by the oncologist and surgeon, which consisted of surgery with or without radiation with or without chemotherapy.

3.1. Epithelial ADAM10 expression

ADAM10 was observed in the epithelial component of all OSCCs with 46 cases (92%) demonstrating overexpression and only 4 showing weak immunostaining. Its localization was mostly cytoplasmic except for a few tumors that displayed both cytoplasmic and nuclear staining (Fig. 1). In the majority of tumors containing normal mucosa, the entire epithelial layer stained with this protein,

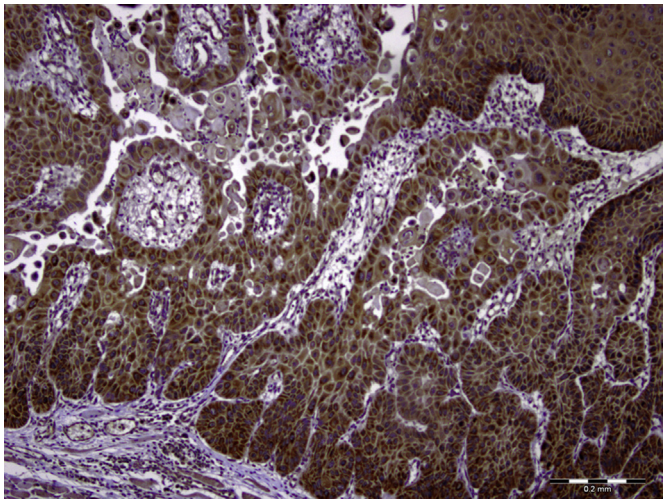

Fig. 1. Representative section of oral squamous cell carcinoma depicting ADAM10 (a disintegrin and metalloproteinase-10) immunoreactivity in both epithelial and stromal components. This specimen contained both nuclear and cytoplasmic staining in neoplastic cells (scale bar represents 0.2 mm).

exhibiting reduced intensity in the more superficial layers. We did not find a significant difference in any of the demographic or clinicopathologic factors between ADAM10^{+} and ADAM10^{-} tumors (Table 1).

3.2. Stromal ADAM10 expression

The stromal components of most OSCCs also expressed this marker in the fibroblasts, endothelial cells, neurons, muscles and scattered lymphocytes. In all, 21 tumors (42%) had strong expression, with only one sample (2%) indicating no immunostaining in this component. No significant difference in any of the clinicopathologic factors was found between stromal between ADAM10^{+} and ADAM10^{-} cases (Table 1). Three of the tumors with weak-expressing epithelial ADAM10 also had negative/weak expression in the stroma, and only one of the weak epithelial ADAM10 cases showed overexpression of this marker in the stroma. There was no significant relationship between epithelial and stromal ADAM10 expression (Phi = 0.102, $P = 0.63$).

3.3. EGFR expression

EGFR was predominantly detected in the cell membrane and occasionally in the cytoplasm and/or nucleus of the neoplastic cells. Its expression depicted two patterns (Fig. 2): peripheral, in which the outermost layer(s) of the tumor islands displayed increased intensity/expression; and diffuse, in which both central and peripheral cells of the neoplastic islands equally expressed this protein. Only two (4%) of our 22 weak-expressing cases were completely negative, whereas 28 (56%) showed EGFR overexpression. Among the clinical factors, only tumor size demonstrated significant difference between EGFR^{+} and EGFR^{-} tumors

Table 1
Immunohistochemical characteristics of patients with oral squamous cell carcinoma based on clinicopathological variables.[a]

	Epithelial ADAM		*P*-value	Stromal ADAM		*P*-value	EGFR		*P*-value	EGFR pattern[b]		*P*-value	Mean (SD) CD105	*P*-value
	−	+		−	+		−	+		d	p			
Age, yr														
<60	0	17	0.28	8	9	0.54	10	7	0.22	8	9	0.76	88.12 (48.57)	0.43
≥60	4	26		18	12		11	19		11	17		101.77 (59.84)	
Sex														
Male	3	25	0.63	19	9	0.08	12	16	0.88	12	15	0.87	101.71 (56.65)	0.43
Female	1	19		8	12		9	11		8	11		88.85 (54.07)	
T stage														
T1/T2	4	30	0.29	17	17	0.13	9	25	0.001	15	18	0.76	96.47 (61.44)	0.83
T3/T4	0	16		12	4		13	3		6	9		92.88 (37.20)	
N stage														
N0	3	41	0.41	25	19	0.69	21	23	0.21	17	25	0.38	94.20 (56.51)	0.70
N1–3	1	5		4	2		1	5		4	2		103.50 (39.22)	
M stage														
M0	3	45	0.16	28	20	1.00	22	26	0.50	20	26	1.00	92.40 (48.55)	0.63
M1	1	1		1	1		0	2		1	1		165.50 (156.27)	
Risk score														
Low	1	11	0.65	5	7	0.24	4	8	0.09	4	7	0.64	99.17 (59.64)	0.88
Intermediate	2	16		11	7		6	12		10	7		90.28 (44.55)	
High	1	19		13	7		12	8		7	13		97.55 (61.48)	
PNI														
0	2	27	0.29	15	14	0.97	11	18	0.27	12	15	0.27	102.79 (48.19)	0.28
1	2	15		12	5		8	9		7	10		78.47 (62.33)	
3	0	4		2	2		3	1		2	2		112.75 (59.42)	
LI														
0	3	16	0.28	9	10	0.09	6	13	0.04[c]	6	11	0.62	104.37 (52.53)	0.54
1	0	17		9	8		6	11		9	8		84.12 (48.68)	
3	1	13		11	3		10	4		6	8		96.64 (64.53)	
WPOI														
0	1	26	0.53	16	11	0.74	14	13	0.20	13	13	0.37	98.44 (55.25)	0.70
1	3	18		11	10		7	14		8	12		89.10 (56.53)	
3	0	2		2	0		1	1		0	2		118.50 (2.12)	

ADAM, a disintegrin and metalloproteinase; EGFR, epidermal growth factor receptor; d, diffuse pattern; p, peripheral pattern; PNI, perineural invasion; LI, lymphocytic infiltration; WPOI, worst pattern of invasion; SD, standard deviation.

[a] Information for three and two cases were missing for age and sex, respectively.

[b] The two cases with no EGFR expression could not be classified as either diffuse (d) or peripheral (p).

[c] Significant.

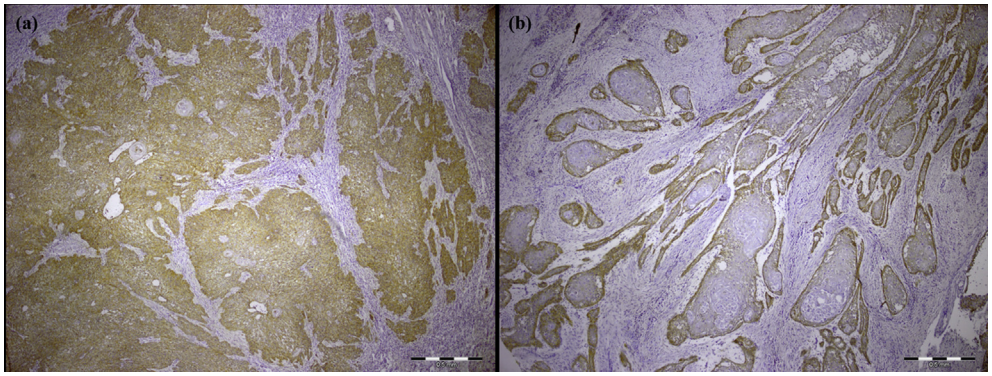

Fig. 2. EGFR (epidermal growth factor receptor) immunostaining in oral squamous cell carcinoma with diffuse (a) and peripheral (b) patterns (scale bar represents 0.5 mm).

($P = 0.001$), with smaller neoplasms showing stronger expression. Additionally, according to the Kruskal–Wallis test used to evaluate histopathologic grading (HRA), a significant difference in EGFR was found among the three lymphocytic infiltration (LI) scores ($P = 0.04$). Two-by-two comparisons using the Mann–Whitney test showed a significantly larger number of $EGFR^+$ cases in LI scores of 0 compared to LI scores of 3 ($P = 0.03$) (Table 1).

3.4. CD105 expression

Both $CD105^+$ single cells and variable-sized cell clusters with and without lumen formation were recognizable in the examined OSCCs (Fig. 3). MVD ranged from 16 to 276 with a mean of 95.32. Using analysis of variance, neither demographic/clinical factors nor HRA grading showed significant relationships with neoangiogenesis ($P > 0.05$) (Table 1).

3.5. Correlation among markers

Comparisons among ADAM10, EGFR and CD105 MVD (Table 2) showed no significant correlation among the markers, except for epithelial ADAM10 and CD105, which, according to the Pearson coefficient, had a negative association ($r = -0.455$; $P < 0.001$). The mean CD105 value for negative and positive EGFR was 87.23 ± 50.34 and 101.68 ± 57.63 ($P = 0.36$), and for diffuse and peripheral patterns, 86.19 ± 48.77 and 96.81 ± 54.03 ($P = 0.48$), respectively.

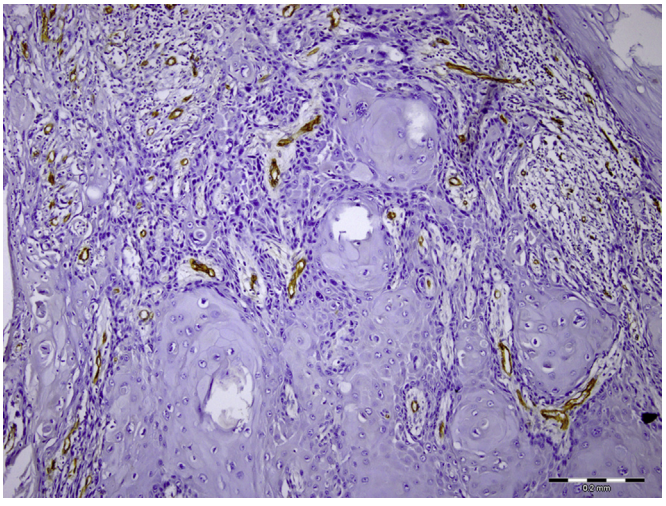

Fig. 3. Neoangiogenesis in oral squamous cell carcinoma as shown by CD105 immunoexpression (scale bar represents 0.2 mm).

4. Discussion

The ADAM family participates in major events such as extracellular matrix remodeling, cell proliferation and angiogenesis (Arribas et al., 2006; Duffy et al., 2009, 2011). According to our findings, ADAM10 was up-regulated in the epithelial component of 92% of the OSCCs, indicating its possible involvement in the oncogenesis of this cancer, similar to malignancies of the stomach, nasopharynx, colon and uterus/ovary (Arribas et al., 2006; You et al., 2015). The number of studies on ADAM10 in OSCC patients is extremely limited, reporting an overexpression of 41.2–100%, which includes the percentage obtained here (Jones et al., 2013; Ko et al., 2007; Stasikowska-Kanicka et al., 2018; Zepeda-Nuño et al., 2017).

We found a significant negative association between epithelial ADAM10 and CD105, which was in line with the results reported by Caolo et al. (2015), who found increased vascular density following ADAM10 blockage, indicating a suppressive effect of this marker on angiogenesis. In the course of angiogenesis, cells that lead the sprouting process, *i.e.* tip-cells, express the Notch-ligand DLL4 (delta-like 4), which is cleaved by ADAM10. Activation of the Notch pathway through its binding with DLL4, results in inhibition of tip-cell sprouting during neoplastic angiogenesis. Therefore, blocking DLL-Notch signaling can lead to elevated vascular density. Interestingly, in neoplastic tissues, these elevated vessels have been shown to be abnormal, leaky and nonfunctional, ultimately causing tumor growth inhibition (Caolo et al., 2015; Kuhnert et al., 2011). In contrast to our findings, another study (Stasikowska-Kanicka et al., 2018) using CD31, found a significant positive correlation between ADAM10 and MVD in OSCC. To explain this difference, it should be noted that CD31is a pan-endothelial antigen that stains both new vessels and stable pre-existing ones, whereas CD105, used in the present study, stains newly formed tumor-specific vessels and is more specific for these structures than pan-endothelial markers (Marioni et al., 2010) and therefore demonstrates a more reliable association with factors involved in tip-cell events and tumor angiogenesis. It may be postulated that the reaction of ADAM10 is different with pre-existing vessels from that of neoplastic new vasculature.

A new measure that was taken in the current investigation was the scoring of ADAM10 in the stroma of OSCC, following the suggestion of Zepeda-Nuño et al. (2017). No significant difference in ADAM10 immunopositivity was found between the epithelium and stroma of the studied OSCCs, which supports the idea that the presence of this protein in stromal elements, similar to the

Table 2
Immunoreactivity of ADAM10 in relation to EGFR and CD105 MVD counts in surgical specimens of OSCC patients.

		N	EGFR expression		*P*-value	EGFR pattern[a]		*P*-value	Mean (SD) CD105	*P*-value
			−	+		d	p			
Epithelial ADAM	−	4	1	3	0.621	2	1	0.574	178.50 (98.42)	0.001[b]
	+	46	21	25		19	26		88.09 (43.79)	
Stromal ADAM	−	29	15	14	0.254	10	17	0.382	101.00 (59.01)	0.39
	+	21	7	14		11	10		87.48 (47.80)	

ADAM, a disintegrin and metalloproteinase; EGFR, epidermal growth factor receptor; d, diffuse pattern; p, peripheral pattern.
[a] The two cases with no EGFR expression could not be classified as either diffuse (d) or peripheral (p).
[b] Significant, $r = -0.455$.

epithelium, is critical and complements its role in tumor development and invasion. Others have also observed ADAM10 in stromal cells of OSCC (Ko et al., 2007; Stasikowska-Kanicka et al., 2018).

Components of the TNM system, *i.e.* tumor size, lymph node status and distant metastasis, are known to be strong indicators of prognosis in cancer patients. In our specimens, there was no significant relationship between any of these parameters and ADAM10 expression. The association of ADAM10 with the TNM prognostic factors in OSCC is a complicated issue, owing to the limited and contradicting reported results, which are based on evaluating one or a combination of these factors. Stasikowska-Kanicka et al. (2018) found a positive correlation with N-status; Jones et al. (2013) observed a negative relationship with metastasis; Zepeda-Nuño et al. (2017) reported T3–T4 tumors to show significantly lower ADAM10 levels compared to T1–T2 neoplasms but indicated no association between this protein and lymph node metastasis; and finally Ko et al. (2007), in their evaluation of ADAM10 mRNA, did not find a significant relationship with either lymph node metastasis or TNM.

A possible explanation for our findings regarding the lack of correlation between ADAM10 and T, N or M status may be that after the basement membrane is cleaved by ADAM10 in the initial stages of tumor invasion, other more dominant or supplementary elements become involved to promote progression and metastasis (Ko et al., 2007). The degradation of collagen IV, a major basement membrane component, by ADAM10 (Millichip et al., 1998; Oh et al., 2009) supports this theory. Additionally, the HRA risk score has been shown to be associated with survival (Brandwein-Gensler et al., 2005). Our results demonstrated no relationship between this grading system and ADAM10, also supporting our TNM results. It seems that ADAM10 functions mainly as an initiating factor in OSCC and has an auxiliary impact on the later stages of cancer, including progression and metastasis.

A major function of ADAM10 is the release or shedding of EGFR ligands, leading to activation of downstream signaling pathways and resulting in cell growth, survival and migration (Duffy et al., 2011). In our study, we did not find an association between ADAM10 and EGFR. To explain this finding, it should be noted that there are seven EGFR ligands that can bind and activate this receptor (Tanida et al., 2004), whereas ADAM10 releases only two of them: namely, EGF and betacellulin (Blobel, 2005; Duffy et al., 2009, 2011; Huang et al., 2014). Other ADAMs may proteolyze the rest of the EGFR ligands such as TGFα and ultimately activate this receptor. In head and neck SCC cells, inhibition of ADAM17, but not ADAM10, has been shown to decrease EGFR phosphorylation, indicating a larger contribution of ADAM17 in this matter (Huang et al., 2014). Furthermore, maybe other factors unrelated to ADAMs have a larger effect on EGFR in OSCC. In corroboration, interleukin (IL)-1β (promoter of neoplastic transformation and aggressiveness in OSCC) (Lee et al., 2015), has been shown to activate EGFR, independent of ADAM10 (Tanida et al., 2004). Additionally, juxtacrine EGFR signaling can also exist and may be able to act independent of sheddases like ADAMs (Blobel, 2005). In contrast to our findings, Shao et al. (2015) suggested a possible modulating function of ADAM10 on EGFR in TCA8113 cells. The reason for our conflicting results may be that we assessed these markers in patient tissues including those from different intraoral sites, whereas their study was on one cell line of the tongue.

As evidenced by conflicting reports, the expression of EGFR and its role in OSCC is very complicated (Diniz-Freitas et al., 2007; Gröbe et al., 2014; Kimura et al., 2016; Solomon et al., 2016). A relatively high overexpression rate of this protein has been reported in OSCCs in some studies (Diniz-Freitas et al., 2007; Gröbe et al., 2014; Szentkúti et al., 2015), with lower measures appearing in investigations from the Asia-Pacific region that are closer to our findings (Putti et al., 2002; Solomon et al., 2016), suggesting a possible impact of environmental factors on this protein. Our results showed a significant increase of EGFR in tumors with dense band-like lymphocytic infiltration, which corroborates findings from previous studies that reported a positive relationship between chronic inflammation and EGFR (Jacobs et al., 2017; Putti et al., 2002; Tanida et al., 2004).

We found a significant correlation between EGFR and tumor size, with smaller neoplasms showing stronger expression, similar to the results reported by Eriksen et al. (2004). However, neither lymph node nor distant metastases differed between $EGFR^+$ and $EGFR^-$ tumors. Studies have reported conflicting results regarding the association between EGFR and TNM, with some describing significant associations (positive or negative) and others indicating no relationship (Diniz-Freitas et al., 2007; Eriksen et al., 2004; Gröbe et al., 2014; O-charoenrat et al., 2002; Shiraki et al., 2005; Szentkúti et al., 2015). Also, there was no relationship between EGFR and neoangiogeness in our specimens, in line with the findings of Lionello et al. (2014), who also used CD105, but in contrast to another study that used CD31 (Wang et al., 2015). Different reactions to old and new vessels could also be applied to explain these different findings.

5. Conclusion

We suggest a possible role for ADAM10 in the tumorigenesis of OSCC. The exact mechanisms by which it contributes to this process remain to be clarified; however, our findings indicate its involvement in the negative regulation of neoangiogenesis, and show that its interaction with EGFR is limited. Additionally, according to our observations, EGFR expression appears to be affected by host immunity, as evidenced by its increase in tumors with increased lymphocytic infiltration. These could be critical findings to consider for patient treatment when using combination therapies in cancer.

Conflicts of interest

None declared.

Source of funding

This work was supported by Dental Research Center, Dentistry Research Institute, Tehran University of Medical Sciences and Health Services (grant number 132.198). Our funding source had no role in the study design, collection, analysis and interpretation of data, in the writing of the report, or in the decision to submit the manuscript for publication.

Contributors

Dr. S. Etemad-Moghadam and Dr. M. Alaeddini contributed equally to all aspects of this work. Both authors have approved the final article.

References

American Joint Committee on Cancer: Cancer staging manual, 7th ed. New York, NY: Springer, 2010

Arribas J, Bech-Serra JJ, Santiago-Josefat B: ADAMs, cell migration and cancer. Cancer Metastasis Rev 25: 57–68, 2006

Blobel CP: ADAMs: key components in EGFR signalling and development. Nat Rev Mol Cell Biol 6: 32–43, 2005

Brandwein-Gensler M, Teixeira MS, Lewis CM, Lee B, Rolnitzky L, Hille JJ, et al: Oral squamous cell carcinoma: histologic risk assessment, but not margin status, is strongly predictive of local disease-free and overall survival. Am J Surg Pathol 29: 167–178, 2005

Caolo V, Swennen G, Chalaris A, Wagenaar A, Verbruggen S, Rose-John S, et al: ADAM10 and ADAM17 have opposite roles during sprouting angiogenesis. Angiogenesis 18: 13–22, 2015

Diniz-Freitas M, García-Caballero T, Antúnez-López J, Gándara-Rey JM, García-García A: Pharmacodiagnostic evaluation of EGFR expression in oral squamous cell carcinoma. Oral Dis 13: 285–290, 2007

Duffy MJ, McKiernan E, O'Donovan N, McGowan PM: Role of ADAMs in cancer formation and progression. Clin Cancer Res 15: 1140–1144, 2009

Duffy MJ, Mullooly M, O'Donovan N, Sukor S, Crown J, Pierce A, et al: The ADAMs family of proteases: new biomarkers and therapeutic targets for cancer? Clin Proteomics 8(9), 2011

Eriksen JG, Steiniche T, Askaa J, Alsner J, Overgaard J: The prognostic value of epidermal growth factor receptor is related to tumor differentiation and the overall treatment time of radiotherapy in squamous cell carcinomas of the head and neck. Int J Radiat Oncol Biol Phys 58: 561–566, 2004

Gröbe A, Eichhorn W, Fraederich M, Kluwe L, Vashist Y, Wikner J, et al: Immunohistochemical and FISH analysis of EGFR and its prognostic value in patients with oral squamous cell carcinoma. Oral Pathol Med 43: 205–210, 2014

Huang Y, Benaich N, Tape C, Kwok HF, Murphy G: Targeting the sheddase activity of ADAM17 by an anti-ADAM17 antibody D1(A12) inhibits head and neck squamous cell carcinoma cell proliferation and motility via blockage of bradykinin induced HERs transactivation. Int J Biol Sci 10: 702–714, 2014

Jacobs JM, Traeger L, Eusebio J, Simon NM, Sequist LV, Greer JA, et al: Depression, inflammation, and epidermal growth factor receptor (EGFR) status in metastatic non-small cell lung cancer: a pilot study. J Psychosom Res 99: 28–33, 2017

Jones AV, Lambert DW, Speight PM, Whawell SA: ADAM 10 is over expressed in oral squamous cell carcinoma and contributes to invasive behaviour through a functional association with αvβ6 integrin. FEBS Lett 587: 3529–3534, 2013

Kimura I, Kitahara H, Ooi K, Kato K, Noguchi N, Yoshizawa K, et al: Loss of epidermal growth factor receptor expression in oral squamous cell carcinoma is associated with invasiveness and epithelial–mesenchymal transition. Oncol Lett 11: 201–207, 2016

Ko SY, Lin SC, Wong YK, Liu CJ, Chang KW, Liu TY: Increase of disintergin metalloprotease 10 (ADAM10) expression in oral squamous cell carcinoma. Cancer Lett 245: 33–43, 2007

Kuhnert F, Kirshner JR, Thurston G: Dll4-Notch signaling as a therapeutic target in tumor angiogenesis. Vasc Cell 3: 20, 2011

Lee CH, Chang JS, Syu SH, Wong TS, Chan JY, Tang PH, et al: IL-1β promotes malignant transformation and tumor aggressiveness in oral cancer. J Cell Physiol 230: 875–884, 2015

Lionello M, Lovato A, Staffieri A, Blandamura S, Turato C, Giacomelli L, et al: The EGFR–mTOR pathway and laryngeal cancer angiogenesis. Eur Arch Otorhinolaryngol 271: 757–764, 2014

Marioni G, D'Alessandro E, Giacomelli L, Staffieri A: CD105 is a marker of tumour vasculature and a potential target for the treatment of head and neck squamous cell carcinoma. J Oral Pathol Med 39: 361–367, 2010

Millichip MI, Dallas DJ, Wu E, Dale S, McKie N: The metallo-disintegrin ADAM10 (MADM) from bovine kidney has type IV collagenase activity in vitro. Biochem Biophys Res Commun 245: 594–598, 1998

O-charoenrat P, Rhys-Evans PH, Modjtahedi H, Eccles SA: The role of c-erbB receptors and ligands in head and neck squamous cell carcinoma. Oral Oncol 38: 627–640, 2002

Oh ST, Schramme A, Stark A, Tilgen W, Gutwein P, Reichrath J: The disintegrin-metalloproteinases ADAM 10, 12 and 17 are upregulated in invading peripheral tumor cells of basal cell carcinomas. J Cutan Pathol 36: 395–401, 2009

Putti TC, To KF, Hsu HC, Chan AT, Lai GM, Tse G, et al: Expression of epidermal growth factor receptor in head and neck cancers correlates with clinical progression: a multicentre immunohistochemical study in the Asia-Pacific region. Histopathology 41: 144–151, 2002

Shao Y, Sha XY, Bai YX, Quan F, Wu SL: Effect of a disintegrin and metalloproteinase 10 gene silencing on the proliferation, invasion and migration of the human tongue squamous cell carcinoma cell line TCA8113. Mol Med Rep 11: 212–218, 2015

Shiraki M, Odajima T, Ikeda T, Sasaki A, Satoh M, Yamaguchi A, et al: Combined expression of p53, cyclin D1 and epidermal growth factor receptor improves estimation of prognosis in curatively resected oral cancer. Mod Pathol 18: 1482–1489, 2005

Solomon MC, Vidyasagar MS, Fernandes D, Guddattu V, Mathew M, Shergill AK, et al: The prognostic implication of the expression of EGFR, p53, cyclin D1, Bcl-2 and p16 in primary locally advanced oral squamous cell carcinoma cases: a tissue microarray study. Med Oncol 33: 138, 2016

Stasikowska-Kanicka O, Wągrowska-Danilewicz M, Kulicka P, Danilewicz M: Overexpression of ADAM10 in oral squamous cell carcinoma with metastases. Pol J Pathol 69: 67–72, 2018

Szentkúti G, Dános K, Brauswetter D, Kiszner G, Krenács T, Csákó L, et al: Correlations between prognosis and regional biomarker profiles in head and neck squamous cell carcinomas. Pathol Oncol Res 21: 643–650, 2015

Tanida S, Joh T, Itoh K, Kataoka H, Sasaki M, Ohara H, et al: The mechanism of cleavage of EGFR ligands induced by inflammatory cytokines in gastric cancer cells. Gastroenterology 127: 559–569, 2004

Wang WM, Zhao ZL, Ma SR, Yu GT, Liu B, Zhang L, et al: Epidermal growth factor receptor inhibition reduces angiogenesis via hypoxia-inducible factor-1α and Notch1 in head neck squamous cell carcinoma. PLoS One 10, 2015 e0119723

You B, Shan Y, Shi S, Li X, You Y: Effects of ADAM10 upregulation on progression, migration, and prognosis of nasopharyngeal carcinoma. Cancer Sci 106: 1506–1514, 2015

Zepeda-Nuño JS, Guerrero-Velázquez C, Del Toro-Arreola S, Vega-Magaña N, Ángeles-Sánchez J, Haramati J, et al: Expression of ADAM10, Fas, FasL and soluble FasL in patients with oral squamous cell carcinoma (OSCC) and their association with clinical-pathological parameters. Pathol Oncol Res 23: 345–353, 2017