Original paper

Overexpression of ADAM10 in oral squamous cell carcinoma with metastases

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ADAMs (a disintegrin and metalloproteinase) are important mediators of cell signalling events, which play a role in the pathogenesis and progression of cancers. Immunohistochemical method was used to examine the immunoexpression of ADAM10 and microvessel density in 80 cases of oral squamous cell carcinoma (OSCC): without metastases – OSCC M(–) (n = 38), and with metastases – OSCC M(+) (n = 42), in 24 cases of oral leukoplakia (OLK), (15 cases with low-grade dysplasia – OLK-LG, and 9 cases with high-grade dysplasia – OLK-HG), and 19 controls. The immunoexpression of ADAM10 and the mean number of vessels were significantly increased in both groups of OSCC in comparison to both groups of OLK and controls. Moreover, the immunoexpression of ADAM10 and microvessel density were significantly increased in the OSCC M(+) group in comparison to the OSCC M(–) group. No statistically significant differences were found between immunoexpression of ADAM10 and microvessels density in the OLK-LG, OLK-HG, and control cases. In conclusion, the present study revealed overexpression of ADAM10 in OSCCs, especially in OSCC with metastasis. These findings suggest that ADAM10 could potentially contribute to metastases of oral cancer. Although, our findings suggest that ADAM10 may be involved in angiogenesis of OSCC, further studies are required to determine the role of ADAM10 in this process.

Key words: ADAM10, microvessels density, oral cancer, oral leukoplakia.

Introduction

Oral squamous cell carcinoma (OSCC) is the most common type of malignant tumour of the oral and maxillofacial region. Despite advances in diagnostics and treatment, OSCC is associated with a poor prognosis because of its high recurrence rate, frequent invasion, and metastasis [1, 2]. Oral leukoplakia (OLK) with features of dysplasia is a premalignant lesion of oral mucosa. Approximately 3-17% of OLKs transform into oral squamous cell carcinoma, and the risk of malignant transformation is usually proportional to the severity of dysplasia [3]. Therefore, investigation of the mechanism of development and progression is critical to treatment of OSCC.

The ADAMs (a disintegrin and metalloproteinase) are a zinc-dependent family of proteins that contain: an integrin-binding domain, an *N*-terminal signal sequence followed by a prodomain, a metalloproteinase domain, a disintegrin domain with cysteine-rich region, a transmembrane, and cytoplasmic tail [4, 5]. In the mammalian genome, approximately 40 ADAMs were identified and described [4]. Some ADAMs can be expressed in somatic cells and some are present on reproductive system cells (testis) suggesting tissue-specific function [4]. The importance of ADAMs is underscored by the fact that some of the most vital ADAM genes (ADAM10 and ADAM17) can be found in primitive chordates, *Drosophila melanogaster*, and in yeast, *Schizosaccharomyces pombe* [6, 7].

ADAMs can participate in both proteolytic cleavage and cellular adhesion of various cell surface molecules. Via the proteolytic cleavage, ADAMs are involved in regulating the activity of multiple membrane-bound proteins (receptors, cytokines, adhesion molecules, growth factors) [4, 5]. Therefore, ADAMs are important contributors to physiological and pathophysiological processes, and may be potential therapeutic targets in various diseases including cancer [4, 8].

Literature data shows that ADAM10, ADAM12, and ADAM17 may play a pivotal role in the pathogenesis and progression of cancers, including proliferation, migration, and invasion [5, 9, 10]. ADAM10 has potential implications for regulation of tumour growth and metastasis as well as for tumour angiogenesis through the rapid modulation of cell signalling pathways and cell adhesion [11]. Literature data demonstrates that ADAM10 contributes to cell proliferation by increasing the transcriptional activity of β-catenin and modulating its nuclear translocation [12]. Overexpression of ADAM10 has been described in a various types of cancer, including melanoma, hepatocellular and pancreatic carcinoma, lung, gastric, bladder, and breast cancer [13, 14, 15, 16, 17, 18, 19]. It has been demonstrated that downregulation of ADAM10 in HepG2 cell line significantly suppresses proliferation, migration, and invasion, and decreases chemotherapy drug resistance *in vitro* and tumour growth *in vivo* [20, 21].

Although the immunoexpression of ADAM10 in various types of tumours has been extensively characterised, its precise role in oral carcinogenesis and association with microvessel density are not clear. Therefore, the aim of our study was to evaluate the immunoexpression of ADAM10 and microvessel density in oral leukoplakia (OLK with low-grade dysplasia – OLK-LG, OLK with high-grade dysplasia – OLK-HG), and oral squamous cell carcinomas with metastases (OSCC M(+)), and without metastases (OSCC M(–)). Another purpose was to find a possible association between immunoexpression of ADAM10 and the presence of the metastases and microvessel density.

Material and methods

Patients

The university review board approved the methods of the present retrospective study. The authors complied with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects. The study was conducted under the assumption that research findings would be kept anonymous.

Eighty cases of OSCC, 24 cases of OLK, and 19 controls (normal mucosa) were sourced from archival tissue blocks of the Department of Pathomorphology, Medical University of Lodz, Poland. Normal oral mucosa from the floor of the mouth (non-cancer affected patients), originated from the Department of Plastic and Reconstructive Surgery, Medical University of Lodz. All tissue sections taken from postoperative material were routinely fixed in formalin, processed, embedded in paraffin, and stained with haematoxylin and eosin. Histopathological diagnoses were established according to the current standards [22]. The main criteria used for the selection of cases was an anatomical placement of lesions (the floor of the mouth for OSCC and gingiva for OLK). The histopathological grade of cases of OLK was moderate (15 cases with low-grade dysplasia – OLK-LG), to severe (9 cases with high-grade dysplasia – OLK-HG). All cases of OSCC were divided into two groups: OSCC M(–) (without metastases, n = 38) and OSCC M(+) (with metastases to regional lymph nodes or/and with distant metastases, n = 42). Cases of OSCC were graded according to the WHO classification (for OSCC M(–): G1 n = 3, G2 n = 34, G3 n = 1, and for OSCC M(+): G1 n = 0, G2 n = 36, G3 n = 6). The age range for the OSCC M(–) group was from 28 to 75 years (the mean ±SD = 59.36 ±10.69), for the OSCC M(+) group it was from 40 to 84 years (the mean ±SD = 59.79 ±11.56), for OLK it was from 21 to 84 years (mean ±SD = 50.02 ±11.14), and for controls it was from 15 to 74 years (mean ± SD = 45.25 ±19.02).

Immunohistochemistry

Immunohistochemical staining was carried out according to a standard method. Tissue sections 3 μm in size were deparaffinised in xylene and rehydrated through a graded alcohol series. Heating in a microwave oven in a solution of TRS (Target Retrieval Solution, pH 9.0, Dako) for 30 minutes was carried out for antigen retrieval. Endogenous peroxidase was quenched with 0.3% hydrogen peroxide in methanol for 30 minutes. The sections were washed with TBS and incubated with monoclonal mouse primary antibodies against ADAM10 (Thermo Scientific, Rockford, USA, PA 5-28161, dilution 1 : 300) and CD31 (Dako, Glostrup, Denmark, clone JC70A, dilution 1:50). After washing, an adequate EnVision-HRP detection system (Dako, Carpinteria, CA, USA) was used. 3,3'-diaminobenzidine was used as the chromogen. After counterstaining with Mayer's haematoxylin, the slides were washed, dehydrated, cleared in xylene, and coverslipped. The negative controls involved the same procedure, with the primary antibodies replaced by antibody diluent.

In each specimen distribution and cytoplasmic staining intensity of ADAM 10 in epithelial cells were recorded semiquantitatively by two independent observers in 7-10 (depending on the specimen size) adjacent high-power fields and graded: 0 (staining not detectable), 1 (weak immunostaining), 2 (moderate

immunostaining intensity), or 3 (strong staining). The mean grade was calculated by averaging grades assigned by the two authors and approximating the arithmetical mean to the nearest unity.

Morphometry

CD31-positive vessels were evaluated using a computer image analysis system consisting of a PC equipped with a Pentagram graphic tablet, Indeo Fast card (frame grabber, true-colour, real-time), produced by Indeo (Taiwan), and colour TV camera Panasonic (Japan) coupled with a Carl Zeiss microscope (Germany). This system was programmed (MultiScan 18.03 software, produced by Computer Scanning Systems, Poland) to calculate the number of objects (semiautomatic function).

CD31 immunostains were evaluated in the vessels only (not in the individual cells), in the most vascular areas. The number of CD31-positive vessels (microvessel density) was estimated by counting all positive vessels in 7-10 high-power fields (HPF) (0.25 mm^2 each), marking immunopositive cells (semiautomatic function). The results are presented as the number of positive vessels per HPF.

Statistical methods

Differences between groups were tested using one-way ANOVA with post-hoc Tukey test. The Kruskal-Wallis test with post-hoc Dunn's test was used where appropriate. Correlation coefficients were calculated using Spearman's method. Results were considered statistically significant if $p < 0.05$.

Results

The immunoexpression of ADAM10 was localised in the epithelial cells, dysplastic and cancer cells, vascular endothelial cells, and infrequently in the infiltrating stromal lymphocytes. Our study revealed that the majority of the ADAM10 was localised in the cytoplasm, but extracellular immunoexpression was also noted.

The semiquantitative data of the immunoexpression of ADAM10 and the number of CD31+ vessels appear in Table I. The immunoexpression of ADAM10 in the OSCC M(+) group (Fig. 1A) was significantly increased in comparison to OSCC M(–) (Fig. 1B), both groups of OLK (Fig. 1C), and control groups (Fig. 1D). Moreover, in the OSCC M(–) group immunoexpression of ADAM10 was significantly increased in comparison to OLK-LG, OLK-HG, and controls. No statistically significant differences were found between immunoexpression of ADAM10 in both groups of OLK and control cases.

The mean number of the vessels was significantly increased in the OSCC M(+) group (Fig. 1E) in comparison to the OSCC M(–) (Fig. 1F), OLK-LG, OLK-HG, and control group. We found also significantly increased mean number of vessels in the OSCC M(–) group compared to both groups of OLK and the control group.

Table I. The immunoexpression of ADAM10 and the number of CD31-positive vessels in oral squamous cell carcinomas with metastases – OSCC M(+), without metastases – OSCC M(–), in oral leukoplakia with low-grade dysplasia – OLK-LG, and with high-grade dysplasia – OLK-HG, and controls

GROUPS	ADAM 10 (MEAN SCORE)	CD31+ VESSELS VESSELS/HPF
OSCC M(+) (n = 42)	2.42 ±1.85	69.1 ±30.9
OSCC M(–) (n = 38)	1.29 ±1.05	43.2 ±18.1
OLK-LG (n = 15)	0.69 ±0.49	30.2 ±14.4
OLK-HG (n = 9)	0.57 ±0.27	28.9 ±15.6
Controls (n = 19)	0.65 ±0.53	27.2 ±16.2
OSCC M(+) vs. OSCC M(–)	$p < 0.002$	$p < 0.001$
OSCC M(+) vs. OLK-LG	$p < 0.001$	$p < 0.001$
OSCC M(–) vs. OLK-LG	$p < 0.04$	$p < 0.02$
OSCC M(+) vs. OLK-HG	$p < 0.001$	$p < 0.001$
OSCC M(–) vs. OLK-HG	$p < 0.05$	$p < 0.04$
OSCCPP vs. control	$p < 0.001$	$p < 0.001$
OSCCBP vs. control	$p < 0.02$	$p < 0.002$
OLK-LG vs. controls	$p = 0.82$ (NS)	$p = 0.60$ (NS)
OLK-HG vs. controls	$p = 0.67$ (NS)	$p = 0.79$ (NS)

NS – not significant

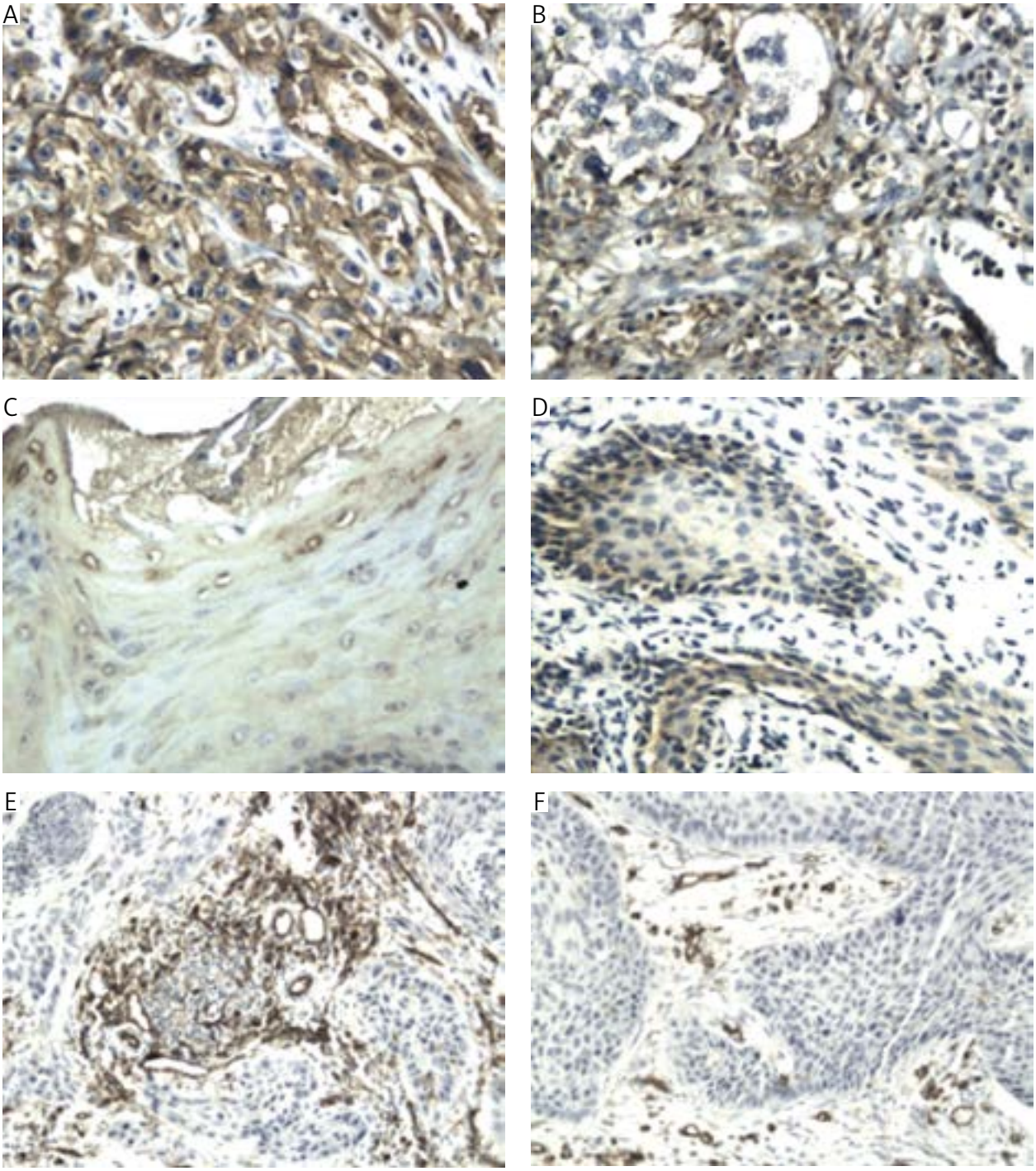

Fig. 1. A) Immunoexpression of ADAM10 in oral squamous cell carcinomas with metastases – OSCC M(+). Immunohistochemistry. Total magnification 200×. B) Immunoexpression of ADAM10 in oral squamous cell carcinomas without metastases – OSCC M(-). Immunohistochemistry. Total magnification 200×. C) Immunoexpression of ADAM10 in oral leukoplakia (OKL). Immunohistochemistry. Total magnification x 200. D) Immunoexpression of ADAM10 in control. Immunohistochemistry. Total magnification 200×. E) Immunoexpression of CD31 in oral squamous cell carcinomas with metastases – OSCC M(+). Immunohistochemistry. Total magnification 200×. F) Immunoexpression of CD31 in oral squamous cell carcinomas without metastases – OSCC M(–). Immunohistochemistry. Total magnification 200×

In OSCC M(+) and OSCC M(–) groups there were positive significant correlations between the immunoexpression of ADAM10 and the microvessel density ($r = 0.42$, $p < 0.007$; $r = 0.35$, $p < 0.04$, respectively), whereas this correlation was not statistically significant in OLK -LG ($r = 0.25$, $p = 0.36$ [NS]) and OLK-HG ($r = 0.31$, $p = 0.41$ [NS]).

No statistically significant differences were found between immunoexpression of ADAM10 and the

mean number of vessels in the OLK-LG and OLK-HG groups (data not shown).

In the control group this correlation was weak and not significant (data not shown).

Discussion

Many studies have shown that members of the ADAM family mediate fundamental biological processes from cellular adhesion to migration. ADAM activity seems to be related to the control of several signalling pathways activated in cancer [4, 5]. For this reason, ADAM family members could be targets for antitumour therapy [23].

Increased expression of ADAM10 has been found in many cancers, playing a pivotal role in the cancer progression and affecting patient prognosis. Our study indicated that ADAM10 immunoexpression was significantly higher in both groups of OSCCs compared with that in controls. Moreover, our study revealed increased ADAM10 immunoexpression in OSCC with metastasis than in the group of cancers without metastases. In uveal melanoma, Caltabiano *et al*. also demonstrated significantly higher expression of ADAM10 in patients with metastases in comparison to patients without metastases, suggesting that ADAM10 expression is associated with a more rapid metastatic progression [24]. Zhang *et al.* revealed that overexpression of ADAM10 in hepatocellular carcinoma was significantly associated with tumour metastasis, as well as tumour grade, differentiation, and tumour size [13]. Our results also seem to be in agreement with experimental *in vitro* findings. Mullooly *et al.* reported that knockdown of ADAM10 or treatment with the selective inhibitor of ADAM10 decreased cell migration and invasion in a breast cancer cell line [25].

To the best of our knowledge, this is the first study to examine ADAM10 immunoexpression in premalignant lesions such as OLK. In our study, the immunoexpression of ADAM10 in both groups of OLK was significantly decreased in comparison to OSCC groups. Moreover, there were no statistically significant differences between immunoexpression of ADAM10 in OLK and control cases. Similar to our results, Kauttu *et al*. demonstrated higher expression of ADAM10 in oesophageal adenocarcinoma than in normal control tissues, but ADAM10 expression in premalignant dysplastic epithelium (Barrett's dysplasia) did not differ from normal oesophageal squamous epithelium [26]. A possible explanation for our results can be the small number of cases, but it is also possible that our findings reflect the situation that the biological action of ADAM10 decreased cell migration, and invasion influenced the advance stages of oral carcinogenesis but had little impact on the early stages of oral carcinogenesis (OLK).

To the best of our knowledge, this is also the first study considering the tumour immunoexpression of ADAM10 and microvessel density. Literature data shows that increased ADAM10-mediated proteolysis correlates with decreased vascular integrity, increased production of cytokines such as TNF-α, and increased transmigration of leukocytes [27]. ADAM10 was also identified as a binding partner of VEGFR2. In addition, VEGF induced ADAM10-mediated cleavage of VE-cadherin, which could increase vascular permeability and facilitate migration [28]. Moreover, it was demonstrated that ADAM10 expressed in human atherosclerotic lesions is associated with neovascularisation [27]. In this context, statistically significant correlation between the ADAM10 overexpression and the process of angiogenesis, defined as the increase in microvessel density in both tested groups of cancer, seems be in agreement with previous findings. On the other hand, Caolo et al. documented in mouse retinas that inhibition of ADAM10 induced vascular sprouting and density [29]. Differences concerning the association between ADAM10 and microvessel density in various studies may indicate that the precise role and mechanisms of action of ADAM10 in the process of angiogenesis are still not fully determined.

Conclusions

In conclusion, the present study revealed overexpression of ADAM10 in OSCC, especially in OSCC with metastases. These findings may indicate that ADAM10 could potentially contribute to metastases of oral cancer and suggest that increased angiogenesis may be involved in this process. However, further research is required, to determine the underlying mechanisms by which ADAM10 might play a role in angiogenesis of the oral region.

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The authors declare no conflict of interest.

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