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EVALUATION OF MIDKINE AS AN ANGIOGENTIC AND PROGNOSTIC MARKER IN ORAL SQUAMOUS CELL CARCINOMA

Maii Ibrahim Sholqamy* ; Mohamed Hosam El-Din Elmalahy**
and Safaa Marzouk Tohamy***

ABSTRACT

Midkine is a heparin-binding growth factor that promotes the proliferation, survival, migration and differentiation of various target cells. Midkine plays an important role in tumorigenesis and tumor progression, and is overexpressed in many human malignant tumors. The aims of this study were to evaluate the expression of von Willebrand Factor (vWF) and Midkine (MK) factor in oral squamous cell carcinoma (OSCC).And to correlate the expression of both (vWF) and (MK) with some clinicopathological data and lymph node metastasis. Immunohistochemical showed that MK protein expression was significantly higher in specimens of carcinomas with lymph node metastasis. Furthermore, vWF expression tended to be higher in cases that exhibited high expression of MK. These results suggest that MK may play important role in tumor's progression and angiogenesis.

INTRODUCTION

Oral squamous cell carcinoma (OSCC), a common head and neck cancer, account for approximately 90 % of all oral cancers. It is characterized by an aggressive growth pattern, a high degree of local invasiveness, and cervical lymph node metastasis. Despite improved therapeutic modalities, the survival of patients with oral cancer has remained unchanged over the last three decades. The patient survival depends on conventional prognostic factors used in clinical practice [(1)].

The most important factor affecting the outcome of this tumor is the clinical stage of the disease at first diagnosis. However, the presence of clinically positive lymph nodes is the single most important predictor of survival. Once regional metastasis have occurred, the 5-year survival rate of patients with OSCC decreases by one half that of patients with early-stage disease [(2)].In many cases, these factors are inadequate and are unable to discriminate between tumors in the same clinical stage that may have distinct clinical outcomes and respond differently to the same treatment [(1)].

* Demonstrator in oral pathology in Minia University,
** Assistant professor of oral pathology in Ain Shams University
*** Lecturer of Oral Pathology in Minia Unversity.

Angiogenesis is the formation of new blood vessels from the endothelium of the existing vasculature and is the result of a complex multistep process involving extracellular matrix remodeling, endothelial cell migration and proliferation , loop formation , capillary differentiation , anastomosis , and finally lumen development [3].

There is a need for biological prognostic markers that better reflect the biological diversity of oral cancers and more accurately predict clinical outcomes and responses to particular types of adjuvant therapy [1]. Studies have shown that vascularity increases from normal mucosa to moderate dysplasia to carcinoma [4]. The most common antibodies used for microvessel staining so far are against von Willebrand Factor (Factor VIII) [5], and recently Midkine [6]. von Willebrand factor (vWF) is a multimeric plasma glycoprotein that plays a prominent role in primary hemostasis [7]. And it represents a potential candidate to mediate platelet-tumor cells interactions [8].

As vWF in the tissues derives uniquely from vascular endothelial cells, this feature makes vWF particularly useful to detect activation of the endothelium, an early sign of angiogenesis, in tumors [9].

Midkine (MK), a heparin-binding growth factor, is expressed intensely during the midgestation period and its expression becomes generally weak in adults. MK which was initially found as a molecule expressed in embryonal carcinoma cells, has been shown to promote the growth, survival and migration of various cells, including endothelial cells, and has also been shown to be involved in the regulation of epithelial–mesenchymal interactions [10].

In addition, the expression of MK was found to be increased in various human tumors [11] and has also been suggested to promote or modulate angiogenesis [12]. MK expression in blood and cancer tissues is indicative of a strong relationship with malignant potential, and high MK expression suggests a bad prognosis [13].

MATERIALS AND METHODS

On the basis of archival pathology specimens and case-note reviews a total of 30 patients with primary OSCC treated between 2009 and 2011 in the National Cancer Institute, Cairo University. Seventeen patients were males and 13 patients were females, ranging from 30 to 72 years old (mean age 53.5 years) divided into two groups (above 53.5 and below 53.5) years.

The tissue samples were from the following sites: tongue (9), gum (6), cheek (5), floor of the mouth (7), retromolar area (2) and lip (1).All tumors were classified according to the international Union against Cancer tumors size nodal metastasis distant metastasis (TNM) classification. Histological grading was done according to World Health Organization classification (2005).

3 cases of nearly normal tissue were obtained from gingivectomy used as a control. Paraffin-embedded tissues 4-μm-thick sections were prepared, deparaffinized in xylene, and rehydrated in a graded series of alcohol. Endogenous peroxidase activity was blocked by immersing the section in 3% peroxidase, and antigens were retrieved by using a microwave (MK: 15min; vWF: 10min) with the sections in acetate buffer (pH 6). The slides were incubated at room temperature overnight with primary antibodies (MK and vWF). After being washed with phosphate buffered saline, the sections were labeled with streptavidin-biotin for 10 minutes at room temperature. The sections were visualized using diaminobenzidine hydrochrolide. Finally, the sections were counted stained with Mayer's hematoxylin.

All the steps performed for immunohistochemical evaluation were carried out using image analysis software. Phase analysis was calculated automatically to give the percentage of immunopositive area to

total area of the microscopic field. The mean area fraction for each case was then calculated and used for statistical analysis.

Statistical analysis

The data was stored and analysed by SPSS 20.0 for windows software. Chi-square test was used for unvariate analysis of categorical data. And Spearman's correlation test was used for non-parametric variables. Tests were considered significant when their P-values were < 0.05.

RESULTS

Regarding vWF expression, the normal oral tissue showed a vWF immunopositive reaction at the endothelial cell-lined microvessels. All cases of OSCC were immunopositive reaction (Fig: 1&2).

Regarding MK expression, the normal oral tissue showed MK immunonegative reaction (no cytoplasmic or nuclear staining of MK in normal epithelium), and all cases of OSCC revealed cytoplasmic expression of MK. MK expression was also found in keratin pearl of well differentiated SCC and in nucleus of moderately and poorly differentiated SCC (Fig: 3&4).

Expressions of (Mk and vWF) with clinicopathological data

MK and vWF had different immunoreactivity. Samples were considered as having a low level of MK expression if the area fraction was <11.002 % and high if ≥ 11.002 %. vWF expression was considered low if area fraction was < 20.797 % and high if ≥ 20.797%.

Statistical analysis of the present study regarding relationships between vWF expression and clinicopathological parameters revealed a non-significant relation with (age, sex and site) in OSCC. While, a statistically significant relation was found with tumor differentiation, lymph node metastasis and cancer stages (p=.03, p=.03 & p=.01), respectively.

With respect to the relationship between the expression of MK in OSCC and clinicopathological parameters, the current study revealed a non-significant relation with (age, sex, site and tumor differentiation). And a statistically significant relation with lymph node metastasis and cancer stages (p=.0001 & p=.0001).

An interesting aspect of the current research is that the correlation of MK expression with vWF expression. It is a moderate positive correlation (r=.695) which was found to be a highly Significant (P=.0001).

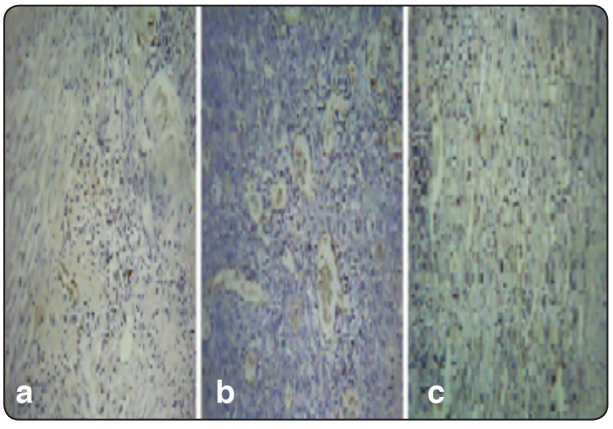

Fig. (1): a, b &c showed anti-vWF factor in well, moderate and poor differentiated OSCC having lymph node metastasis

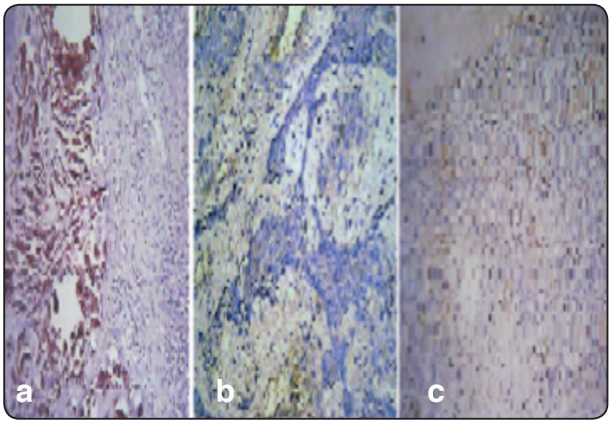

Fig. (2) a ,b &c showed anti-vWF in well, moderate and poor differentiated OSCC don't have lymph node metastasis.

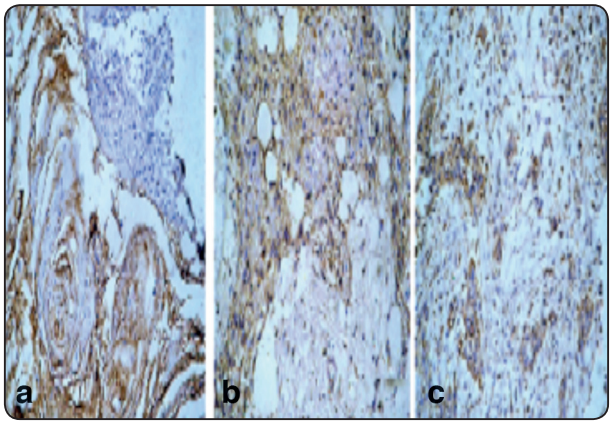

Fig. (3) a, b &c showed anti-MK factor in well, moderate and poor differentiated OSCC having lymph node metastasis.

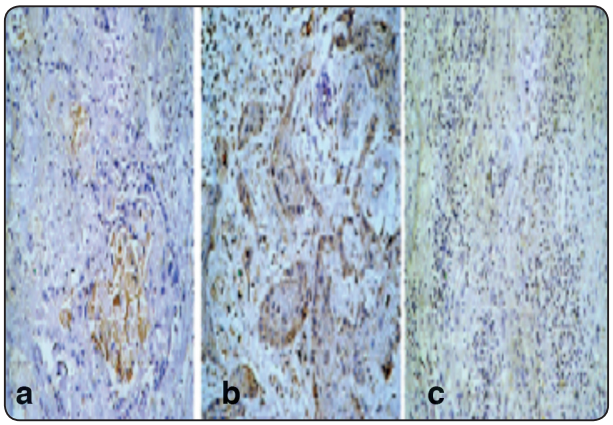

Fig. (4) a, b &c showed anti-MK in well, moderate and poor differentiated OSCC don't have lymph node metastasis.

DISCUSSION

The enhanced vascular supply reflects an increased malignant potential because greater number of tumor vessels increase the opportunity for tumor cells to enter the circulation [14] .Hence, the process of metastasis to a large extent angiogenesis dependent. Angiogenesis is quantified through the staining of blood vessels with various endothelial cell (EC) markers [15] like von Willebrand factor (vWF) [9]. Midkine (MK) exert cancer-related activities in the process of carcinogenesis, including transformation, fibrinolysis, cell migration, cell survival, anti-apoptotic and angiogenesis [16]. The angiogenic action of MK in tumors is strongly suggested by the observation that transfection of the breast carcinoma line MCF-7 with MK accelerates tumor growth and increases tumor vascularity after cell implantation in nude mice [17]. In the present study, immunorectivity for vWF protein was located in endothelial cell-lined microvessels in OSCC. These results were found to be in agreement of that reported by ***Li et al*** [18]. Regarding MK expression, in the present study the normal oral mucosa showed MK immunonegative reaction. These also revealed by ***Ruan et al*** [19]. MK expression was mainly distributed in the cytoplasm of the cancer cells these results supported by previous studies by ***Ruan et al*** [19] and ***Seki et al*** [20] . Cytoplasmic accumulation of MK may result from default which supported by ***Arnoys et al*** [21]. This study revealed that, MK expression was also distributed in some cancer cell nuclei. These results were found to be in agreement with that reported by ***Ota et al*** [22]. Expression of MK dominantly in nucleus of poor differentiated OSCC. ***Shibata et al*** [23] reported that full MK activity required nuclear targeting during promotion of cell survival. The current study showed that MK protein expression was correlated with tumor differentiation. In well differentiation OSCC of this study expression of MK distributed in keratinization of epithelial cell. These results were found to be in agreement with those of previous study by ***Ren and Zhang*** [24]. They found that MK may participate in keratinization of epithelial cell, because it is expressed more intensely in well differentiated than poorly differentiated squamous cell carcinoma in esophageal and vulvar region.

Regarding the lymph node metastasis, this study revealed a statistically significant (p=.03) relationship between the expression of vWF in OSCC and lymph node metastasis. A study by ***Ahmed & Mohamed*** [14] stated that the mean of surface expression of vWF was significantly associated with lymph node metastasis in 40 cases of colorectal adenocarcinoma. Concerning the

relationship of MK expression with lymph node metastasis, the statistical analysis of the present study showed a highly significant (p=.0001). These results in agreement with a study by ***Su et al*** [25] Contradictory, ***Ruan et al*** [19] stated that no significant differences were observed in the expression of MK between the cases with neck lymph-node metastasis and those without them.

Hence, vWF is particularly useful to detect activation of the endothelium, an early sign of angiogenesis, in tumors. That means the MK can be also useful to detect an early sign of angiogenesis. These results supported by Ruan et al [19], who reported that MK stimulate tumor growth in an autocrine manner: promotion of endothelial cell proliferation by paracrine secretion, induction of expression of vascular endothelium growth factors (VEGF) and some other active angiogenic stimulators, and thus enhancement of tumor metastasis resulting in poor prognosis.

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