Evaluation of Midkine Expression in Oral Epithelial Dysplasia and Oral Squamous Cell Carcinoma: A Cross-Sectional Study

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Abstract

Introduction  Oral cancer accounts for 3% of all malignancies diagnosed worldwide annually. Midkine (MK) plays a role in oncogenesis and tumor progression, including cell migration, mitogenesis, antiapoptosis, and angiogenesis.

Aim and Objective  To study and compare MK expression in various grades of oral epithelial dysplasia (OED) and oral squamous cell carcinoma (OSCC).

Methodology  This is a cross-sectional study where 60 archival specimens of OSCC, OED, and control were taken for immunohistochemical examination. Antibody MK (A-9) SC-46701 mouse monoclonal (200µg/mL, Santacruze Biotechnology) was used for immunohistochemical staining. The stained sections were observed under the objective (100x) of research microscope (DM 1000 LED) with computer-assisted image analyzer (Leica Application Suit V-4.1). Quantitative analysis was done to count MK reactive cells in all groups. Statistical analysis was performed using Kruskal–Wallis H test with posthoc Bonferroni’s test for multiple group comparisons, and p-value < 0.05 was considered as statistically significant.

Results  MK expression was observed in OSCC and OED and was absent in control. The distribution of average percentage positivity was significantly higher in OED compared with OSCC and control. Statistically significant correlation was found between the size of the tumor and increased MK expression.

Conclusion  MK protein is overexpressed in OED and OSCC, and can be used as biological marker for early detection.

Keywords  ► oral squamous cell carcinoma  ► midkine  ► oral epithelial dysplasia
Introduction

Oral cancer is known to be preceded by potentially malignant disorders (PMDs). Diagnostic delay of potentially malignant lesions is due to lack of awareness in the general population and even health care providers, which results in onset of oral squamous cell carcinoma (OSCC), one of the most common malignancies.

Premalignant lesions of the upper aerodigestive tract run an increased risk of progressing to squamous cell carcinoma. They are known as altered epithelial lesions. The prognosis of individual case is different, and role of accurate prognostic marker is crucial. These potentially malignant lesions could be best studied microscopically for the architectural and cytological changes. OSCC is an aggressive carcinoma; 5-year survival rate is approximately 50%. Identification of new diagnostic and prognostic biological markers can be helpful in early diagnosis and improve the prognosis.

The Midkine (MK) is also known as MDK, FLJ27379, and NEGF2. It is a 13-kDa heparin-binding growth factor. It was found as a product of a retinoic acid gene, located at chromosome 11p11.2.

As per different studies, MK was found overexpressed in various tumors such as Wilms tumors, neuroblastomas, meningiomas as well as colon, gastric, esophagus, pancreatic, lung and renal cell carcinomas. Previous study results showed overexpression of MK in OSCC; however, its role in the development and progression of this cancer is still unclear.

Carcinogenesis involves transformation, proliferation and dedifferentiation of cells followed by uncontrolled growth of transformed cells. The role of MK has been documented in various oncogenic activities such as cell migration, angiogenesis, mitogenesis, and antiapoptosis.

Thus, it may be used as a molecular target for therapy in human carcinoma. In OSCC, diagnosis at an early stage is paramount for better prognosis, for which development of sensitive, specific, and noninvasive tumor marker is needed.

This study was undertaken with the aim to understand the role of MK in prognosis of oral potentially malignant disorders (OPMD) and OSCC.

Methodology

Study Population

The study population comprised 60 cases divided into group 1 (OSCC), group 2 (OED), and group 3 (normal mucosa). Group 1 constituted histologically confirmed cases of well (group A), moderate (group B), and poor (group C) grades of OSCC from archival specimens. Eighteen cases of well-differentiated OSCC, 3 cases of moderate OSCC, and 4 cases of poorly differentiated OSCC were selected (Fig. 1).

Group 2 constituted of histopathologically diagnosed cases of various grades of OED. In it, 10 mild OED (group A), 10 moderate OED (group B), and 10 severe OED (group C) cases of epithelial dysplasia each were selected (Fig. 2).

Group 3 constituted five oral normal mucosal (NM) tissues. Five renal/lung carcinoma tissues were used as positive control.

About 4-µm thick tissue sections of paraffin-embedded blocks from all the groups were cut and subjected to immunohistochemical staining with MK for microscopic examination. Antibody MK (A-9) SC-46701 mouse monoclonal (200 µg/mL, Santacruze Biotechnology) with antibody diluent into 1:50 ratio was used. The sections stained with MK antibody were observed under objective (100 ×) of research microscope (DM 1000 LED) with computer-assisted image analyzer (Leica Application Suit V-4.1) by two observers to study expression of MK in three grades of epithelial dysplasia (Figs. 3, 4, 5), OSCC (Figs. 6, 7, 8), and normal mucosa (Fig. 9).

Results

Quantitative analysis was done to count MK reactive cells in all groups. Statistical data analysis was performed to calculate p-values with the help of Kruskal–Wallis H test and
Fig. 3  Photomicrograph showing Midkine expression in mild epithelial dysplasia (40 ×).

Fig. 4  Photomicrograph showing Midkine expression in moderate epithelial dysplasia (40 ×).

Fig. 5  Photomicrograph showing Midkine expression in severe epithelial dysplasia (40 ×).

Fig. 6  Photomicrograph showing Midkine expression in well-differentiated oral squamous cell carcinoma (OSCC) (40 ×).

Fig. 7  Photomicrograph showing Midkine expression in moderately differentiated oral squamous cell carcinoma (OSCC) (40 ×).

Fig. 8  Photomicrograph showing Midkine expression in poorly differentiated oral squamous cell carcinoma (OSCC) (40 ×).
posthoc Bonferroni’s test for multiple group comparisons. A p-value less than 0.05 is considered to be statistically significant.

In group 1, the distribution of average percentage positivity did not differ significantly between groups A and B (p-value > 0.05), groups A and C (p-value > 0.05), and groups B and C (p-value > 0.05).

Expression of MK was marginally increased with increase in grades of OSCC but without any statistical significance.

In group 2, the distribution of average percentage positivity did not differ significantly between groups A and B (p-value > 0.05), groups A and C (p-value > 0.05), and groups B and C (p-value > 0.05). The distribution of average percentage positivity was significantly higher in group 2 compared with group 1 and group 3 (p-value < 0.001 for both).

Expression of MK in severe dysplasia was highest, followed by mild and moderate epithelial dysplasia but without any statistical significance. The distribution of average percentage positivity is significantly higher in OSCC compared with control (p-value < 0.001). MK expression was absent in healthy control (Table 1).

The average (median) % positivity of MK expression did not differ significantly between lymph node (LN)-positive and LN-negative group of samples (p-value > 0.05) (Table 2).

### Discussion

Cancer is one of the leading causes of death after heart and diarrheal diseases. It is commonly preceded by PMD. Kado-matsu et al reported overexpression of MK in early stages of embryonal carcinoma. MK, which is multifunctional peptide along with pleiotrophin (PTN), forms a family of heparin-binding growth factors.

Overexpression of MK at mRNA and protein levels was reported in various human malignancies such as oral, gastrointestinal, hepatobiliary, lung, thyroid, bladder, cervical, ovarian, and prostate. MK expression has also been found in neuroblastomas, astrocytomas, pancreatic head carcinomas, or gastrointestinal stromal tumors with poor prognosis. Enzyme-linked immunoassay (EIA) showed increased MK levels in blood of patients with malignant tumors, including hepatocellular, gastric, and lung carcinoma.

Although overexpression of MK was reported in various malignancies, its role in initiation and progress of OSCC is unclear. Thus, we tried to study expression of MK in OSCC and PMD lesions which usually precede the malignancy.

In our study, we have found higher MK expression in OED, which may indicate the early stage in carcinogenesis. This was similar to the report by Ye et al.

Multivariate analysis done by Shimada et al reported on esophageal squamous cell carcinoma and that MK can be used as an independent prognostic marker. Overexpression of MK in this study was associated with poor prognosis. Ruan et al in their study evaluated the expression of MK in OSCC and its correlation with tumor angiogenesis.

### Table 1 Comparison of average percentage positivity of Midkine expression

<table>
<thead>
<tr>
<th>% Positivity</th>
<th>Group 1 (OSCC) (n = 25)</th>
<th>Group 2 (OED) (n = 30)</th>
<th>Group 3 (NM) (n = 5)</th>
<th>p-Values (Intergroup)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>51.92</td>
<td>70.89</td>
<td>0.00</td>
<td>Group 1 vs. group 2</td>
</tr>
<tr>
<td>Min–max</td>
<td>6.06–74.42</td>
<td>14.65–93.33</td>
<td>0.00–0.00</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

Abbreviations: NM; normal mucosal cell; OSCC, oral squamous cell carcinoma; OED, oral epithelial dysplasia.

*p-value < 0.05, **p-value < 0.01, * suggest significant p-value.

### Table 2 Association between positivity of LN and the percentage positivity of Midkine expression

<table>
<thead>
<tr>
<th>% Positivity</th>
<th>LN-positive (n = 8)</th>
<th>LN-negative (n = 9)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>55.51</td>
<td>52.78</td>
<td>0.920 NS</td>
</tr>
<tr>
<td>Min–max</td>
<td>31.58–63.04</td>
<td>23.81–74.42</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: LN, lymph node; NS, nonsignificant statistically.
results showed overexpression of MK in OSCC, and levels of MK expression were found to be significantly correlated with tumor size. Similar results were found in our study.

Jham et al found increased expression of MK in OSCC when compared with leukoplakia and normal mucosa, where leukoplakia and normal mucosa did not show any expression at all. On the contrary, our study results showed the expression of MK in both OSCC as well as OED, with significant overexpression in OED as compared with OSCC. Normal mucosa did not show any expression of MK. Significantly increased expression of MK was found in OED compared with OSCC cases. Intragroup comparison between various grades of OED and OSCC did not show statistically significant differences of expression. Percentage positivity of MK expression did not differ between LN-positive and LN-negative group of samples. The possible reason behind this could be due to the role of MK in early carcinogenesis.

Conclusion
Depending upon the results obtained in our study, we can conclude that MK protein is overexpressed in OED and OSCC and can be used as biological marker for early diagnosis. To prove this as a prognostic marker, long-term follow-up of the cases should be done. More studies with larger sample size and clinical follow-up should be done to understand MK as a prognostic tumor marker

Limitation of the Study
Small and uneven sample size.

Conflict of Interest
None declared.

References