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Immunohistochemical expression of periostin in oral squamous cell carcinoma

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ABSTRACT

Periostin is a non- structural extracellular matrix protein with characteristic domains to modify cellular signals and connective tissue microenvironment. It is secreted in various developmental, physiological and pathological conditions. As its role in various cancers have been frequently reported recently, we have attempted to assess the expression of Periostin in Oral Squamous Cell Carcinoma using Immunohistochemistry. Histopathologically confirmed cases of Oral Squamous Cell Carcinoma and healthy oral mucosa were recruited for the study. Immunohistochemical analysis of these cases were performed using periostin antibody. The Periostin staining intensity and proportionality index was analysed and were scored by two individual observers.The data obtained were tabulated and analysed using SPSS software version 21. All of the OSCC cases(100%) compared with that of normal healthy oral mucosa cases (only 57.14%) expressed periostin. The Periostin staining in OSCC was predominantly seen in extracellular matrix, fibroblasts around the tumor islands and in areas of desmoplasia. 71.43% periostin expression was seen associated with high grading patterns (Pattern III and IV) thus correlating with enhanced invasiveness.Periostin secreted in the tumor microenvironment of OSCC can be used as marker of aggressive behaviour as it correlates with poor prognosis.

Keywords:
Periostin, Oral squamous cell carcinoma, Invasion, prognosis, tumor microenvironment.

Introduction

Oral squamous cell carcinoma (OSCC) is the malignant neoplasm of oral keratinocytes with high incidence in Southern Asian countries like India, Pakistan, Srilanka, Taiwan and China (Yamamoto & Shibahara, 2015). Over 80% of early stage OSCC can be cured by treatment, whereas more than 70% of the advanced stage cases cannot be rescued. The overall low 5year survival rate of 50% for oral cancer has not improved over the decades (C. Scully & Bagan, 2009).

OSCC arises from multiple genetic alterations caused by chronic exposure to carcinogens such as Alcohol, Smoking, Viral infections and Inflammation (Warnakulasuriya, 2009). Recent advances in molecular biology have elucidated the molecular mechanisms of carcinogenesis, tumor progression and metastasis (Kirita & Omura, 2015).Although recent advancements have led to the understanding of the pathology, many unsolved questions remains to be answered.

Periostin or osteoblast-specific factor 2 is an extracellular matrix protein, first isolated from the pre-osteoblast cell line using a subtraction library as 90kda cell-adhesion molecule (Kirita & Omura, 2015; Takeshita et al., 1993)). It has a multi-domain structure with one of the domain as Fasclin-like domain which interacts with cell surface integrins to effect various intracellular signalling and Other domains such as EMI domain that interacts with other extracellular matricellular protein to produce modifications in the extracellular matrix (Kirita & Omura, 2015; Takeshita et al., 1993).

Recently, Periostin was found to be overexpressed in various types of human cancers such as non-small-cell lung carcinoma, breast cancer, colon cancer, head and neck cancer, ovarian cancer and pancreatic ductal adenocarcinoma. Periostin expression was well correlated with their malignant behavior such as invasion, metastasis and poor survival (Ratajczak-Wielgomas & Dziegiel, 2015).

Thus this study aims to evaluate the expression of Periostin in oral squamous cell carcinoma and predict its role in tumor progression, prognosis and its possibility as a therapeutic target.

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Materials & methods

Sample selection

Approval from the institution standard review board was obtained before commencement of the study (App no: SRB/SDMDS/17/OMP/02). The study material consisted of a total of 14 samples categorized into two groups, namely Group I- Oral squamous cell carcinoma cases (OSCC) (n=7) and Group II- histopathologically normal oral mucosa (n=7). The subgroups of group I include well differentiated squamous cell carcinoma (WDSCC) (n=4) and moderately differentiated squamous cell carcinoma (MDSCC) (n=3). The software used to calculate the sample size of both the groups are GPower version 3.1.9.2.

The diagnosis of all the cases were histopathologically confirmed using Hematoxylin and eosin stained sections obtained from the formalin fixed paraffin embedded blocks of the cases. All the tumors (OSCC cases) were graded into pattern I,II,III and IV according to the classification described by Jacobsson et al (1973).

Immunohistochemical procedure

2-3µm sections from the formalin fixed paraffin embedded blocks of these patients were taken in positively charged slides. The sections were deparaffinized in xylene, dehydrated in alcohol and rinsed in distilled water. Heat induced epitope was performed for retrieval of antigen using pressure cooker method and citrate buffer (pH 6.0). After which, Endogenous peroxide was blocked for 5 minutes, and protein block for 5 minutes. Sections were incubated with the Mouse monoclonal periostin primary-antibody (Santacruz Biotechnology, United states), for overnight at 4°c.

Detection was performed using Dako envision kit detection system [DAKO Denmark A/S]. Sections were counterstained with Mayer's hematoxylin following which they were dehydrated and mounted. Negative and positive controls were used in each run. Normal human fallopian tube sections were used as a positive control. Negative controls were achieved by omission of primary antibody to the respective sections.

Evaluation of the slides

Presence of brown coloured end product at the antigen specific sites were taken as indication of positive reactivity. The slides were assessed and scored for staining intensity and proportionality index for Periostin-positivity by two individual expert pathologists who were blinded to the clinical information and diagnosis. The grade of staining intensity was recorded as 0 =negative staining , 1 = mild positivity (+) , 2= moderate positivity (++) , 3= strong positivity (+++). Proportionality index was scored 0= <5% positivity, 1= 5-25% positivity, 2= 26-50% positivity and 3= >50% positivity. The individual scores were added to give a total score.

Statistical analysis

All the data obtained were tabulated in Microsoft Excel. The Periostin expression was cross-tabulated against invasion patterns and chi-square test with fisher exact test was performed for significant association using SPSS software version 23.0.

Results

A total of 14 cases , comprising 7 cases of OSCC and 7 cases of Normal oral mucosa were evaluated immunohistochemically for the presence of Periostin. Among the 7 cases of OSCC, all the cases (100%) expressed periostin. The staining index was negative in none of the cases, low in 2 cases (28.57%), moderate in 3 cases (42.86%) and intense in 2 cases (28.57%). The staining index of Periostin according to the different histological grades of OSCC summarised in Table 1.The Periostin staining was seen predominantly seen in extracellular matrix and fibroblasts around the tumor islands and in areas of desmoplasia.

Periostin expression was compared with invasion patterns in all the 7 OSCC cases. Jacobsson's classification was used for assessment of invasion patterns (Jacobsson et al, 1973). It was evident that the 71.43% periostin expression was seen associated with high invasive patterns (Pattern III and IV) and 28.57% of periostin expression was seen among low invasive patterns (Pattern I and II), although not statistically significant (Figure 1).

With regard to normal oral mucosa - out of 7 cases, four cases had positive periostin expression (57.14%). The grade of staining intensity : three of the cases were negative (42.86%), one of the case showed mild (14.28%), two of the cases showed moderate (28.57%)and one of the case showed high (14.28%) staining index of periostin. The staining of Periostin in normal oral mucosa was predominantly near the basement membrane in the connective tissue subjacent to the

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epithelium. Some of the inflammatory cells also had taken the Periostin staining. The immunohistochemical staining index of normal oral mucosa has been summarised in Table 2.

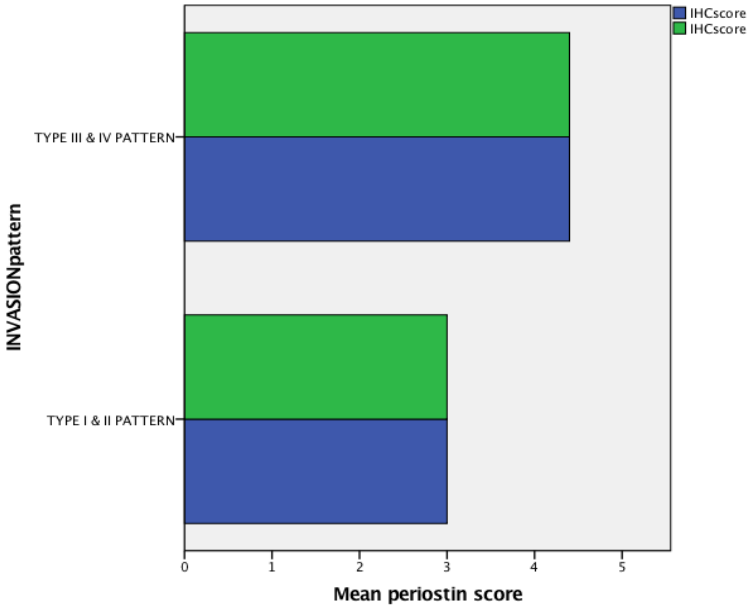

Figure 1: Correlation between Periostin expression and Invasion pattern in oral squamous cell carcinoma. 71.43% periostin expression was seen associated with high invasive patterns (Pattern III and IV) and 28.57% of periostin expression was seen among low invasive patterns (Pattern I and II). Thus Periostin expression was associated with the aggressive behaviour of the tumor.

Table 1: Immunohistochemical staining index for periostin in well and moderately differentiated grades of OSCC. Low intensity Periostin staining was observed in one case of WDSCC and one case of MDSCC. Moderate intensity Periostin staining was observed in two cases of WDSCC and one case of MDSCC. High intensity Periostin staining was seen in two cases of WDSCC and no case of MDSCC.

S.No	Cases	Periostin Staining Index				Total
		Zero	Low	Moderate	High	
1	WDSCC	0	1	2	2	5
2	MDSCC	0	1	1	0	2

Table 2: Immunohistochemical staining index for periostin in normal oral mucosa and Oral squamous cell carcinoma (OSCC). All the cases of OSCC group expressed Periostin.Two cases of OSCC showed low intensity, three cases showed moderate intensity and two cases showed high intensity of Periostin staining. In the normal oral mucosa group, periostin was not expressed in three cases, low intensity staining was seen in one case, moderate intensity in two cases aand high intensity in one case. Thus the expression of Periostin was higher in OSCC tissues compared to normal mucosa.

S.No	Cases	Periostin Staining Index				Total
		Zero	Low	Moderate	High	

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1	OSCC	0	2	3	2	7
2	Oral healthy mucosa	3	1	2	1	7

Discussion

Oral squamous cell carcinoma is an aggressive neoplasm with propensity for local invasion and early lymph node metastasis (Ratajczak-Wielgomas & Dziegiel, 2015).Tumorigenesis is a complex and dynamic process, consisting of three stages: initiation, progression and metastasis. ECM has complementary effects on development and metastasis of tumors in diverse ways: through extracellular secretion, altering phenotype of stromal or tumor cells, getting away with immune surveillance and providing a hypoxic environment (Crispian Scully & Bagan, 2009).

Periostin is a secreted extracellular matrix protein that was originally identified in cells from the mesenchymal lineage (osteoblasts, osteoblast-derived cells, the periodontal ligament, and periosteum). periostin binds to integrins on cancer cells, activating the Akt/PKB- and FAK-mediated signaling pathways. This leads to increased cell survival, invasion, angiogenesis, metastasis, and the epithelial-mesenchymal transition.

The present study was aimed to determine the expression of the periostin in oral squamous cell carcinoma in comparison to that of normal healthy oral mucosa. The present investigation showed that Periostin was expressed in 100% of OSCC cases (n=7). Whereas, previous studies (Siriwardena et al., 2006)(Kudo et al., 2006)(Siriwardena et al., 2006)(Choi et al., 2008)(Siriwardena et al., 2006)(Kudo et al., 2012)(Siriwardena et al., 2006)(Qin et al., 2016)(Choi et al., 2008) have shown a mean expression of 71.84% (with highest expression of 80.4% in a study (Wang et al., 2017)(Kudo et al., 2012)(Wang et al., 2017). This discrepancy may be predicted due to a small sample size and further extensive study using a large sample size can be followed.

Tumors are encircled by extracellular matrix (ECM) and stromal cells and the physiological state of the tumor environment (TME) is closely connected to every step of tumorigenesis(Wang et al., 2017). As the tumor cells invade the basement membrane causing a breach, they lie in close proximity with the juxtaposition stromal components. These tumor cells require a active stromal support and interactions for the progression

As the tumor cells interact with the stroma, the resident quiescent fibroblasts are converted into CAFs. The cancer associated fibroblasts plays a promoting role in tumor progression. The chemokines and cytokines, secreted them stimulate the tyrosine kinase signaling and EMT changes in tumor mass (Qin et al., 2016)(Choi et al., 2008) . The EMT process of cancer is responsible for the dedifferentiation program that leads to increased malignant behavior of the tumor [15]. TGF-b acts as a key regulator of POSTN expression in tumor microenvironment. And induces EMT in cancer progression(Qin et al., 2016)(Choi et al., 2008). More importantly, Periostin is proven to be involved in epithelial- mesenchymal transition of carcinoma cells (Jakobsson et al., 1973).

Periostin secreted in the extracellular matrix by cancer-associated fibroblasts can be invasion promoting factor .The correlation between periostin expression and invasion pattern was shown in the study as 71.43% of Periostin expression was seen in high invasive patterns (Jakobsson et al., 1973). This was comparable to results obtained by siriwardena et al (Siriwardena et al., 2006), where all the cases expressing Periostin was associated with pattern IV and those of Kudo Y et al (Qin et al., 2016)(Kudo et al., 2006)(Qin et al., 2016), in which 86.25% of showed cases with high invasive grading and 59% of low invasive grading showed Periostin expression. Some of the studies have also shown correlation of Periostin expression with angiogenesis (Siriwardena et al., 2006) and lymphangiogenesis (Siriwardena et al., 2006)(Kudo et al., 2012)(Siriwardena et al., 2006). High (Deeply) invasive patterns have higher metastatic rate and thus Periostin expression was correlated with enhanced invasiveness in OSCC (Jakobsson et al., 1973; Morra & Moch, 2011; Qin et al., 2016).

Conclusion

Periostin secreted in the tumor microenvironment of OSCC may be responsible for invasion-supporting environment conducive to the tumor cells. It can be used as a marker of aggressive behaviour as it correlates with poor prognosis. It might prove to be an important therapeutic target in antimetastatic therapy of OSCC patients in order to improve the survival rate.

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