Expression of Myofibroblasts in Oral Squamous Cell Carcinoma: An Immunohistochemical Study

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ABSTRACT

Introduction: Oral squamous cell carcinoma (OSCC) is one of the most common types of malignancy affecting the orofacial region and with a high mortality rate. The fact that stroma of the tumor modulates and facilitates the progression and metastasis of the malignancy has been shown in the past studies. The cells of the activated stroma that are responsible for the progression and metastasis of the tumor are the fibroblasts having smooth muscle properties. These myofibroblasts are said to secrete numerous inflammatory mediators and factors which are said to play a crucial role in tumor progression. Therefore, we evaluated the presence of myofibroblasts in OSCC, by immunohistochemistry using alpha smooth muscle actin (α-SMA) antibody.

Materials and methods: We evaluated a total of 50 biopsy specimens from the archives of the oral pathology, where 20 specimens out of 50 were of well-differentiated OSCC (WDOSCC), 20 were of poorly differentiated OSCC (PDOSCC), and 10 were of normal healthy controls. All the specimens were stained by immunohistochemically using with monoclonal antihuman α-SMA. Etemad-Moghadam et al method was used for assessing the myofibroblast distribution. Staining index was evaluated for the groups and compared. All the results were analyzed by Statistical Package for the Social Sciences (SPSS) software.

Results: The mean percentage of myofibroblasts score for WDOSCC and PDOSCC were 2.88 and 2.92 respectively. The mean staining intensity score in WDOSCC and PDOSCC were 2.88 and 2.55 respectively. Statistically significant results were obtained while comparing the final staining index score between the OSCC group and normal control group. No significant correlation could be obtained while comparing the mean staining index score in between WDOSCC and PDOSCC.

Conclusion: Malignant epithelium might induce the adjacent stromal tissue to produce myofibroblasts. These specialized cells may be utilized as therapeutic targets for the treatment of OSCC.

Clinical significance: Proliferation of myofibroblasts may be used as a stromal marker of premalignancy and malignancy.

Keywords: Alpha smooth muscle actin, Myofibroblast, Oral squamous cell carcinoma.

INTRODUCTION

One of the most common types of malignancy affecting the oral cavity with high mortality and low survival rate is oral squamous cell carcinoma (OSCC). Tumor progression and metastasis is modulated by the stroma of the malignant tissues which also has been shown by the results of various studies in the recent past. In coordination with the transformation of a noncancerous epithelial tissue to form a carcinoma, similar changes occur in the stroma leading to activation and conversion of nonneoplastic cells into the tumor associated cells. Tumor cells initiate the remodeling of stroma of the neoplastic tissue while these activated stromal cells are responsible for the process of stromagenesis. One of the cells of the activated
stroma responsible for the tumor progression are the fibroblasts. Numerous growth factors and mediators are secreted by the myofibroblasts that stimulate proliferation of epithelial cells. Therefore, we evaluated the presence of myofibroblasts in OSCC, by immunohistochemistry (IHC) using alpha smooth muscle actin (α-SMA) antibody.

MATERIALS AND METHODS

A total of 50 biopsy specimens from the archives of the department of oral pathology and microbiology of the dental institution were included for the present study. Out of 50 specimens, 20 were of well-differentiated OSCC (WDOSCC), 20 were of poorly differentiated OSCC (PDOSCC), and 10 were of normal oral mucosal tissue taken as control. Two sections of each tissue were taken. One was stained with hematoxylin and eosin (H&E) stain and the other was stained immunohistochemically using monoclonal antihuman α-SMA antibody. The method used by Angadi PV et al was used for the evaluation of staining intensity and percentage of α-SMA-positive cells in the immunohistochemically stained sections. At the tumor invasive front of the OSCC tissues, the percentage of immunopositive cells in four high-power fields (hpf) were calculated by recording average staining positive cells per hpf as follows: 0% = no positive cells, 1% = 1 to 25% positive cells, 2% = 26 to 50% positive cells, and 3% = 51 to 100% positive cells. Evaluation of staining intensity was evaluated as follows: 0% = when there was no staining; 1% = in parts where positivity was observed only at a magnification of 400 ×; 2% = in cases where the staining was obvious at 100 ×, but not at 40 ×; and 3% = in fields where immunopositive cells were seen even at 40 ×. Final staining index (I) was calculated by multiplying of the percentage and intensity scores. Classification of this index was done as Zero = 0; Low = 1, 2; Moderate = 3, 4; and High = 6 to 9. Calculation of each staining index for each group was done and compared with others. All the results were analyzed by Statistical Package for the Social Sciences (SPSS) software. Independent unpaired t test and chi-square test were used for assessment of level of significance.

RESULTS

Graph 1 shows percentages of myofibroblasts score (A) and staining intensity score (B) observed in normal control, WDOSCC, and PDOSCC. The mean percentage of myofibroblasts score for WDOSCC and PDOSCC were 2.88 and 2.92 respectively. The mean staining intensity score in WDOSCC and PDOSCC were 2.88 and 2.55 respectively. Table 1 shows the p value for the final staining index score (A × B) comparing between OSCC group and normal control group (p < 0.05). Table 2 highlights the final staining index score (A × B) comparing between WDOSCC and PDOSCC. No significant correlation could be obtained while comparing the mean staining index score in between WDOSCC and PDOSCC. Figure 1 shows the IHC stained of WDOSCC showing intense staining of myofibroblast (10 ×). Figure 2 highlights the IHC stained of PDOSCC showing moderate staining of myofibroblast (10 ×).

DISCUSSION

Tumors cells admixed with blood capillaries, fibroblasts, extracellular matrix (ECM), inflammatory cells, and, occasionally, myofibroblasts pooled in the tumor stroma comprise the neoplastic tissue. Previous concept stresses that stroma plays only the supportive role in the spread of neoplasm. However, recent studies quote that it plays a significant role in promoting malignant phenotypes. In most of the malignancies, including OSCC, these Stroma
changes are observed.\textsuperscript{11} With the characteristic of expressing $\alpha$-SMA and producing ECM proteins and various types of growth factors and inflammatory mediators, they are said to be of hybrid phenotype having qualities of both the fibroblasts and smooth muscle tissues.\textsuperscript{6,7,12,13} Recent data quote that significantly higher amount of myofibroblasts are associated with poorer prognosis of OSCC.\textsuperscript{7,10,14} Hence, we evaluated the presence of myofibroblasts in OSCC, by IHC using $\alpha$-SMA antibody.

We observed that OSCC sections had significantly higher staining index in comparison with the normal controls ($p < 0.05$) (Table 1). While comparing the staining index in between the different grade of OSCC, we observed that in-between cases of WDOSCC and PDOSCC, no significant difference of distribution or staining index could be obtained ($p > 0.05$) (Table 2). Similar results have been observed in the past by Etemad-Moghadam et al\textsuperscript{15} who also could not appreciate any significant difference in the distribution of myofibroblasts in between different grades of OSCC. These findings might indicate that increase in progressive grade and severity of OSCC does not alter the distribution of myofibroblasts. de-Assis et al\textsuperscript{14} assessed the amount of stromal myofibroblasts in patients with oral leukoplakia (OL) and OSCC. They evaluated a total of 30 OL cases and 41 OSCC cases and immunohistochemically stained them with $\alpha$-SMA. They observed the absence of myofibroblasts in normal healthy control specimens and in OL cases. They also observed significantly higher number of myofibroblasts in highly invasive OSCC cases in comparison with low-invasive OSCC cases. From the results, they concluded that for the invasion of OSCC, myofibroblasts might provide a permissive environment. Gupta et al\textsuperscript{16} analyzed the distribution of myofibroblasts in OL, oral submucous fibrosis (OSMF), and OSCC cases using $\alpha$-SMA antibody. They analyzed a total of 60 cases of normal healthy controls and pathologic specimens and observed that amount of myofibroblasts increased significantly in OSMF and OSCC cases. From the results, they concluded that myofibroblasts create a favorable environment for the metastasis and invasion of OSCC. Rao et al\textsuperscript{17} evaluated myofibroblast’s expression in OSMF and OSCC cases using $\alpha$-SMA antibody. They evaluated the 40 cases of OSMF, OSMF, dysplasia, and OSCC specimens and observed that in OSCC specimens, the amount myofibroblasts were statistically higher in number in comparison with the OSMF cases. From the results, they concluded that cancer cell invasion might be facilitated by the proteolytic enzymes secreted by activated myofibroblasts. Etemad-Moghadam et al\textsuperscript{15} assessed the staining pattern and intensity of myofibroblasts in oral epithelial dysplasia (OED) and OSCC specimens using $\alpha$-SMA antibody, vimentin, and desmin. They evaluated 70 cases of normal controls, OSCC, and OED specimens and observed significant difference in the amount of myofibroblasts staining in OSCC cases in comparison with healthy controls and OED specimens. From the results, they concluded that myofibroblasts might play a significant role in the pathogenesis of OSCC. Seifi et al\textsuperscript{18} evaluated the frequency and pattern of distribution of myofibroblasts in OSCC cases and compared them with OED. They evaluated tissue specimens of hyperkeratosis, OED, and OSCC cases and staining them through IHC using $\alpha$-SMA antibody. They observed that network and spindle pattern dominated in the OSCC specimens. From the results, they concluded that myofibroblast pattern might play a significant role in invasive process of the OSCC cases.
CONCLUSION

Authors concluded that malignant epithelium might have an inducing impact on the adjacent stromal tissue to produce myofibroblasts. In the future, myofibroblasts might give a ray of light for being used as therapeutic targets in the treatment of OSCC.

REFERENCES