10.5005/jp-journals-10024-1944

ORIGINAL RESEARCH

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

[1]B Vikas Prasad, [2]Gauri S Kakatkar, [3]Preet Jain, [4]Meetu Jain, [5]Maulik Patel, [6]Javed Khan

ABSTRACT

Introduction: Oral squamous cell carcinoma (OSCC) is one of the most common types of malignancy affecting the orafacial 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.

How to cite this article: Prasad BV, Kakatkar GS, Jain P, Jain M, Patel M, Khan J. Expression of Myofibroblasts in Oral Squamous Cell Carcinoma: An Immunohistochemical Study. J Contemp Dent Pract 2016;17(10):857-860.

Source of support: Nil

Conflict of interest: None

[1]Department of Oral and Maxillofacial Pathology and Microbiology, Geetanjali Dental and Research Centre, Udaipur Rajasthan, India

[2]Department of Public Health Dentistry, Geetanjali Dental and Research Centre, Udaipur, Rajasthan, India

[3]Department of Prosthodontics, Buraydah Colleges, Burhaydah Al-Qassim, Kingdom of Saudi Arabia

[4]Department of Periodontics, Buraydah Colleges, Burhaydah Al-Qassim, Kingdom of Saudi Arabia

[5,6]Pacific Dental College and Hospital, Udaipur, Rajasthan, India

Corresponding Author: B Vikas Prasad, Department of Oral and Maxillofacial Pathology and Microbiology, Geetanjali Dental and Research Centre, Udaipur, Rajasthan, India, Phone: +917023283322, e-mail: drvikasprasad@yahoo.co.in

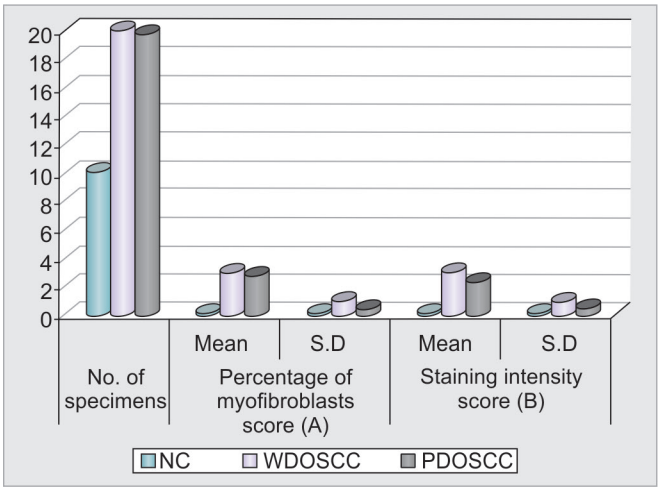
INTRODUCTION

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).[1] 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.[2,3] Tumor cells initiate the remodeling of stroma of the neoplastic tissue while these activated stromal cells are responsible for the process of stromagenesis.[2] One of the cells of the activated

stroma responsible for the tumor progression are the fibroblasts.[2,4] Numerous growth factors and mediators are secretes by the myofibroblasts that stimulate proliferation of epithelial cells.[5] 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 with monoclonal antihuman α-SMA antibody. The method used by Angadi PV et al[5] 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.



Graph 1: Percentages of myofibroblasts score (A) and staining intensity score; and (B) observed in normal control (NC), WDOSCC, and PDOSCC

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 PDOCC 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 (n = 40) and normal control (n = 10). Statistically significant results were obtained while comparing the final staining index score between the OSCC group and normal control group ($p < 0.05$). Table 2 highlights the final staining index score (A × B) comparing between WDSCC and POSCC. 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.[6-9] 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.[7,9,10] In most of the malignancies, including OSCC, these Stroma

Table 1: Final staining index score (A × B) comparing between OSCC and normal control (NC)

Groups	No. of specimens	Staining index score (I) I = A × B	
		t-value	p-value
OSCC *vs* NC	50	7.314	<0.05 S

S: Significant

Table 2: Final staining index score (A × B) comparing between WDOSCC and PDOSCC

Groups	No. of specimens	Staining index score (I) I = A × B	
		t-value	p-value
WDOSCC *vs* PDOSCC	20	0.475	>0.05 NS

NS: Nonsignificant

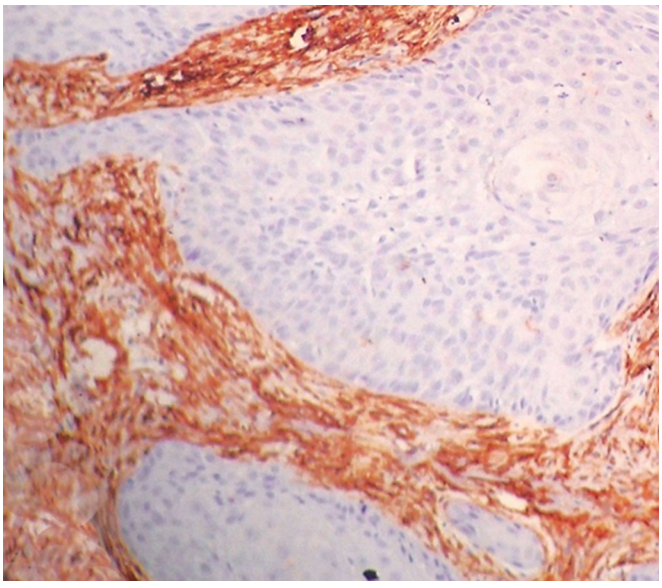


Fig. 1: Immunohistochemistry stained of WDOSCC showing intense staining of myofibroblast (10×)

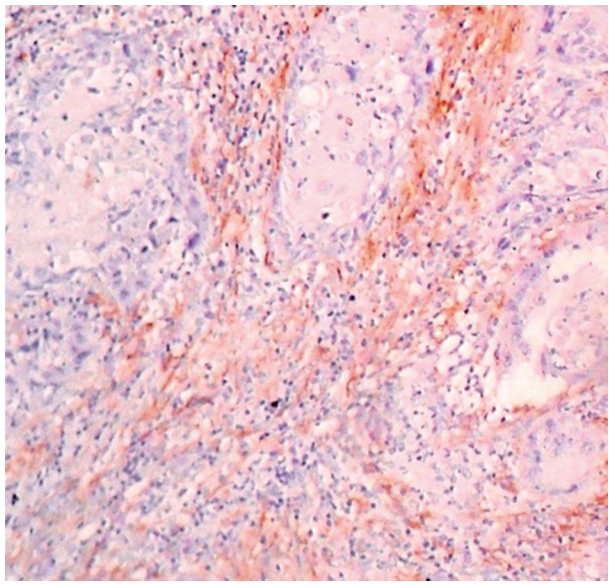


Fig. 2: Immunohistochemistry stained of PDOSCC showing moderate staining of myofibroblast (10×)

changes are observed.[11] With the characteristic of expressing α-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.[6,7,12,13] Recent data quote that significantly higher amount of myofibroblasts are associated with poorer prognosis of OSCC.[7,10,14] Hence, we evaluated the presence of myofibroblasts in OSCC, by IHC using α-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[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[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 α-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[16] analyzed the distribution of myofibroblasts in OL, oral submucous fibrosis (OSMF), and OSCC cases using α-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[17] evaluated myofibroblast's expression in OSMF and OSCC cases using α-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[15] assessed the staining pattern and intensity of myofibroblasts in oral epithelial dysplasia (OED) and OSCC specimens using α-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[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 α-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

1. Neville BW, Day TA. Oral cancer and precancerous lesions. CA Cancer J Clin 2002 Jul-Aug;52(4):195-215.
2. Beacham DA, Cukierman E. Stromagenesis: the changing face of fibroblastic microenvironments during tumor progression. Semin Cancer Biol 2005 Oct;15(5):329-341.
3. Amatangelo MD, Bassi DE, Klein-Szanto AJ, Cukierman E. Stroma-derived three-dimensional matrices are necessary and sufficient to promote desmoplastic differentiation of normal fibroblasts. Am J Pathol 2005 Aug;167(2):475-488.
4. Liotta LA, Kohn EC. The microenvironment of the tumour-host interface. Nature 2001 May 17;411(6835):375-379.
5. Angadi PV, Kale AD, Hallikerimath S. Evaluation of myofibroblasts in oral submucous fibrosis: correlation with disease severity. J Oral Pathol Med 2011 Mar;40(3):208-213.
6. Powell DW, Mifflin RC, Valentich JD, Crowe SE, Saada JI, West AB. Myofibroblasts. I. Paracrine cells important in health and disease. Am J Physiol 1999 Jul;277(1 Pt 1):C1-C9.
7. Desmoulière A, Guyot C, Gabbiani G. The stroma reaction myofibroblast: a key player in the control of tumor cell behavior. Int J Dev Biol 2004 48(5-6):509-517.
8. Kellermann MG, Sobral LM, da Silva SD, Zecchin KG, Graner E, Lopes MA, Nishimoto I, Kowalski LP, Coletta RD. Myofibroblasts in the stroma of oral squamous cell carcinoma are associated with poor prognosis. Histopathology 2007 Dec;51(6):849-853.
9. De Wever O, Demetter P, Mareel M, Bracke M. Stromal myofibroblasts are drivers of invasive cancer growth. Int J Cancer 2008 Nov 15;123(10):2229-2238.
10. Sobral LM, Bufalino A, Lopes MA, Graner E, Salo T, Coletta RD. Myofibroblasts in the stroma of oral cancer promote tumorigenesis via secretion of activin A. Oral Oncol 2011 Sep;47(9):840-846.
11. Lewis MP, Lygoe KA, Nystrom ML, Anderson WP, Speight PM, Marshall JF, Thomas GJ. Tumour-derived TGF-β1 modulates myofibroblast differentiation and promotes HGF/SF-dependent invasion of squamous carcinoma cells. Br J Cancer 2004 Feb 23;90(4):822-832.
12. Orimo A, Weinberg RA. Stromal fibroblasts in cancer: a novel tumor-promoting cell type. Cell Cycle 2006 Aug;5(15):1597-1601.
13. Kellermann MG, Sobral LM, da Silva SD, Zecchin KG, Graner E, Lopes MA, Kowalski LP, Coletta RD. Mutual paracrine effects of oral squamous cell carcinoma cells and normal oral fibroblasts: induction of fibroblast to myofibroblast transdifferentiation and modulation of tumor cell proliferation. Oral Oncol 2008 May;44(5):509-517.
14. de-Assis EM, Pimenta LG, Costa-e-Silva E, Souza PE, Horta MC. Stromal myofibroblasts in oral leukoplakia and oral squamous cell carcinoma. Med Oral Patol Oral Cir Bucal 2012 Sep 1;17(5):e733-e738.
15. Etemad-Moghadam S, Khalili M, Tirgary F, Alaeddini M. Evaluation of myofibroblasts in oral epithelial dysplasia and squamous cell carcinoma. J Oral Pathol Med 2009 Sep;38(8):639-643.
16. Gupta K, Metgud R, Gupta J. Evaluation of stromal myofibroblasts in oral leukoplakia, oral submucous fibrosis, and oral squamous cell carcinoma – an immunohistochemical study. J Cancer Res Ther 2015 Oct-Dec;11(4):893-898.
17. Rao KB, Malathi N, Narashiman S, Rajan ST. Evaluation of myofibroblasts by expression of alpha smooth muscle actin: a marker in fibrosis, dysplasia and carcinoma. J Clin Diagn Res 2014 Apr;8(4):ZC14-ZC17.
18. Seifi S, Shafaei S, Shafigh E, Sahabi SM, Ghasemi H. Myofibroblast stromal presence and distribution in squamous epithelial carcinomas, oral dysplasia and hyperkeratosis. Asian Pac J Cancer Prev 2010;11(2):359-364.