Original Article

Establishment of the role of myofibroblasts in invasive process of oral squamous cell carcinoma: A pilot study

Vaishali Gandhi[1], Anupama Mahajan[2], Yash Pal Kansal[3]

[1]Department of Human Anatomy, SGRD University of Health Sciences, Amritsar, Punjab, [2]Department of Human Anatomy, SGRD Medical College and Hospital, Amritsar, Punjab, [3]Department of General Pathology, Dasmesh Institute of Research and Dental Sciences, Faridkot, Punjab, India

Abstract

Aims: To establish the role of myofibroblasts in invasive process of oral squamous cell carcinoma (OSCC). **Materials and Methods:** Four study groups were formed as follows: group 1 consisting of 30 cases of well-differentiated OSCC, group 2 consisting of 30 cases of moderately differentiated OSCC, group 3 consisting of 30 cases of poorly differentiated OSCC, and group 4 consisting of controls. Staining of all of the specimens was done using α-SMA antibody through immunohistochemistry (IHC) procedure. Multiplication product of staining intensity (A) and percentage of α-SMA-stained immuno-positive cells (B) gave a final staining index (FSI). According to FSI, score zero was graded as index zero; score one and two were graded as index low; score three and score were graded as index moderate; and score six, seven, eight and nine were graded as index high. **Results:** Mean FSI among specimens of groups 1, 2, 3, and 4 was 7.93, 8.47, 8.8, and 0, respectively. Non-significant results were obtained while comparing the mean final staining index among specimens of groups 1, 2 and 3. However; while comparing between groups 1 and 4, groups 2 and 4, and groups 3 and 4, significant results were obtained. While comparing the FSI among overall OSCC group (groups 1, 2 and 3) and controls (group 4), significant results were obtained. **Conclusion:** Myofibroblasts are an integral component of processes associated with the creation of a permissive environment for cancer invasion process in patients with OSCC. **Clinical Significance:** Myofibroblasts are associated with the creation of progressive and invasive processes of oral squamous cell carcinoma. Hence, they might be employed as a part of future target for therapeutics in cancer therapy.

Keywords: Alpha smooth muscle actin, myofibroblasts, oral squamous cell carcinoma

Introduction

Carcinoma of head and neck regions is frequently encountered form of cancer.[1] The prevalence rate of head and neck carcinomas is differentially reported among different areas of the world.[2] In the Indian subcontinent, it is one of the most routinely encountered carcinoma among male patients while in western countries, it comprises of approximately 4% of all carcinomas.[3]

Address for correspondence: Dr. Vaishali Gandhi, PhD Research Scholar, Department of Human Anatomy, SGRD University of Health Sciences, Amritsar, Punjab, India.
E-mail: drvaishaligandhiphd@gmail.com

Received: 10-06-2021 **Revised:** 02-05-2022
Accepted: 04-05-2022 **Published:** 14-10-2022

Access this article online

Quick Response Code:

Website: www.jfmpc.com

DOI: 10.4103/jfmpc.jfmpc_1123_21

According to data reported at the end of 2018, lip, oral cavity, and oropharynx collectively accounted for approximately 2.5% of all cancer mortalities. In comparison to all other nations and countries, the Asian continent has the highest incidence and mortality rates of oral cavity and oropharynx carcinomas.[4]

In epithelial carcinoma cases, the tumor microenvironment comprises of heterogenous stromal cellular component. Dynamic equivalency between synthesis and degradation of extracellular matrix (ECM) maintains the structural niche in which the tumor propagates. Apart from providing scaffold to the tumor cells, ECM also acts as a reservoir for matrix. Fibroblast is a generic terminology which is often employed to denote collagen-producing populations of heterogeneous cells that are

How to cite this article: Gandhi V, Mahajan A, Kansal YP. Establishment of the role of myofibroblasts in invasive process of oral squamous cell carcinoma: A pilot study. J Family Med Prim Care 2022;11:5485-9.

located in lamina propria. Since precise molecular indicators are missing, the term "fibroblast" could be employed to designate the cellular components of a tissue that persist after all other cells have been classified as per their unique criteria. Myofibroblasts are one such significant cell that coordinates the assimilation of the multifarious vibrant biochemical signals present within tissues undergoing repair, with the target of reinstating tissue homeostasis. However, in connective tissues under normal physiological state, myofibroblasts are typically absent.[5]

In the past literature, numerous authors have emphasized on the role of myofibroblasts in cancer patients. In a previous study conducted on the liver, malignancies showed that in the process of tumor invasion, hepatic stellate cells proliferate and differentiate into myofibroblasts in reaction to paracrine indicators originating from tumor cells or cellular components within the tumor environment.[6–9] Myofibroblasts possess smooth muscle properties and are often recognized by the expression of alpha–smooth muscle actin (α-SMA). In oral squamous cell carcinoma (OSCC) patients, the stromal component is related with the emission of differential cytokines like transforming growth factor beta-1 from malignant cells that facilitates differentiation of fibroblasts into myofibroblasts, neo-angiogenesis, enhances the inflammatory cells and elevates the expression of mesenchymal markers. Myofibroblasts also are said to promote tumor growth by reorganization and degeneration of ECM.[10] Hence; the present study was undertaken for establishing the role of myofibroblasts in invasive process of oral squamous cell carcinoma.

Materials and Methods

The current pilot study was commenced after obtaining ethical approval from institutional ethical committee (SGRD Ref No: Patho 689/19) (approval received on 21/10/2019) with the target of exploring the expression of myofibroblasts in different histopathologic grades of OSCC: well differentiated OSCC, moderately differentiated OSCC, and poorly differentiated OSCC. Normal oral mucosa obtained during therapeutic removal of impacted teeth was taken as controls. A total of four study groups were formed: group 1, consisting of 30 cases of well-differentiated OSCC; group 2, consisting of 30 cases of moderately differentiated OSCC, group 3, consisting of 30 cases of poorly differentiated OSCC; and group 4 as controls.

Staining of all of the specimens was done using α-SMA antibody through immunohistochemistry (IHC). Histopathologic examination of hematoxylin and eosin (H&E)-stained slides of all of the tissues was done for confirming the diagnosis. Paraffin-embedded tissue blocks of all of the study groups were obtained from the departmental archives. 3-μm sections of all of the tissues were obtained using microtome (Leica Biosystems, New Delhi, India). IHC staining of all of the samples was done by employing α-SMA antibody (Leica Biosystems, New Delhi, India), which is a marker for myofibroblasts. Criteria defined by Etemad-Moghadam *et al.*[6] were used for assessment of IHC-stained slides. The criteria were based on the evaluation of α-SMA-positive cells. As per these staining criteria, final staining score was assessed based on the multiplication outcome of staining intensity (A) and percentage of α-SMA-stained immuno-positive cells (B). Grading of staining intensity was done as zero percent (absence of immuno-positive cells), one percent (immuno-positive staining observed at × 400 magnification only), two percent (immuno-positive staining observed at × 400 and × 100 magnification only), and three percent (immuno-positive staining observed at even magnification of × 40). Percentage of immuno-positive cells at the tumor invasive front in OSCC cases and at subepithelial connective tissue in cases of controls was graded as zero percent (absence of immuno-positive cells), one percent (1%–25% immuno-positive cells), two percent (26%–50% immuno-positive cells), and three percent (51%–100% immuno-positive cells). Multiplication product of staining intensity (A) and percentage of α-SMA-stained immuno-positive cells (B) gave a final staining index (FSI). According to FSI, score zero was graded as index zero; score one and two were graded as index low; score three and score were graded as index moderate; and score six, seven, eight, and nine were graded as index high. Examination of all of the sections was done twice, for avoiding intra-observer variability. Analysis of all of the results was carried out using Statistical Package for the Social Sciences version 19 (SPSS, version 19, IBM Analytics). Mann–Whitney *U* test was used for analysis of significance level. A *P* value of less than 0.05 was taken as significant.

Results

Results of the current pilot study demonstrate that mean staining intensity value among specimens of groups 1, 2, 3, and 4 was 2.73, 2.86, 2.97, and 0, respectively, as shown in Table 1. It also showed that mean percentage of immuno-positive cell values among specimens of groups 1, 2, 3, and 4 was 2.90, 2.93, 2.97, and 0, respectively, as shown in Table 2. Table 3 shows the FSI score of all of the four study groups. Mean FSI among specimens

Table 1: Staining intensity values

Staining intensity value	Group 1	Group 2	Group 3	Group 4
Mean	2.73	2.86	2.97	0
SD	0.45	0.35	0.18	0

Table 2: Percentage of immuno-positive cells value

Percentage of immuno-positive cells intensity value	Group 1	Group 2	Group 3	Group 4
Mean	2.90	2.93	2.97	0
SD	0.31	0.25	0.18	0

Table 3: Final staining index (FSI)

Final staining index (FSI)	Group 1	Group 2	Group 3	Group 4
Mean	7.93	8.47	8.8	0
SD	1.57	1.43	0.76	0

of groups 1, 2, 3 and 4 was 7.93, 8.47, 8.8 and 0, respectively. As shown in Table 4, non-significant results were obtained while comparing the mean FSI among specimens of groups 1, 2 and 3. However, while comparing between groups 1 and 4, groups 2 and 4, and groups 3 and 4, significant results were obtained. This shows that the mean FSI was significantly higher among the well-differentiated OSCC group, moderately differentiated OSCC group, and poorly differentiated OSCC group in comparison to healthy controls [Table 5]. While comparing the FSI among overall OSCC group (groups 1, 2 3) and controls (group 4), significant results were obtained. Figure 1a shows the H&E-stained section of well-differentiated OSCC, and Figure 1b shows the IHC-stained section of well-differentiated OSCC. Figure 2a shows the H&E-stained section of moderately differentiated OSCC, and Figure 2b shows the IHC-stained section of moderately differentiated OSCC. Figure 3a shows the H&E-stained section of poorly differentiated OSCC, and Figure 3b shows the IHC-stained section of poorly differentiated OSCC. Figure 4a shows the H&E-stained section of controls, and Figure 4b shows the IHC-stained section of controls.

Discussion

Across the globe, head and neck carcinoma is the sixth most common cancer. In the Indian region also, OSCC is a significant health issue, accounting for a major proportion of morbidity and mortality. Also, OSCC leads to impairment of mastication functions along with speech and esthetic issues that further worsen the patient's quality of life.[11–13] Myofibroblasts are a significant component of tumor stroma along with other immunocompetent and inflammatory cells.[11] Myofibroblasts display phenotypic intermediate characteristics of both the fibroblasts and smooth muscle cells. Myofibroblasts originate chiefly from fibroblasts, smooth-muscle cells, and bone marrow. These cells modulate the ECM by their direct effect on cytokine leading to proliferation and growth of tumor cells. They also preserve the vascularity of the tumor micro-environment and therefore increase the structural integrity of stroma.

Table 4: Comparison of final staining index among different study groups

Group comparison	T-statistic	*P*
Group 1 vs Group 2	1.393	0.17
Group 1 vs Group 3	1.638	0.34
Group 1 vs Group 4	-	0.00 (Significant)
Group 2 vs Group 3	1.116	0.26
Group 2 vs Group 4	-	0.00 (Significant)
Group 3 vs Group 4	-	0.00 (Significant)

Table 5: Comparison of final staining index (FSI) among overall OSCC group (groups 1, 2 and 3) and controls (group 4)

Group comparison	T-statistic	*P*
OSCC group vs control group	-	0.00 (Significant)

Myofibroblasts generate a physical obstacle between malignant cells and the body's immune system against cancer.[12] Enhanced expression of myofibroblasts might be beneficial for predicting the prognosis of OSCC patients.[13] Hence, the present study was undertaken to establish the role of myofibroblasts in the invasive process of oral squamous cell carcinoma. In the present study, non-significant results were obtained while comparing the mean FSI among specimens of groups 1, 2 and 3. However, while comparing between groups 1 and 4, groups 2 and 4, and groups 3 and 4, significant results were obtained. This shows that the mean FSI was significantly higher among the well-differentiated OSCC group, moderately differentiated OSCC group, and poorly differentiated OSCC group in comparison to healthy controls. While comparing the FSI among the overall OSCC groups (groups 1, 2 and 3) and controls (group 4), significant results were obtained. Our results were in accordance with the results obtained by Gandhi P *et al.*, Etemad *et al.*, de-Assis EM *et al.* and Barth *et al.,* all of whom also reported significantly higher expression of myofibroblasts in OSCC patients in comparison to healthy controls.[6,10,14,15] These results advocate the hypothesis that myofibroblasts are almost indispensible for invasive process of epithelial cells. In a previous research carried out by Barth *et al.*[15] in 2004, authors assessed the expression of myofibroblasts

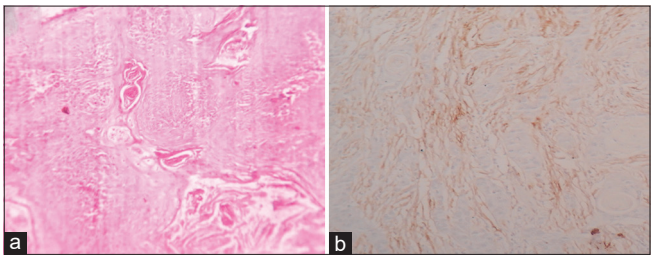

Figure 1: a) H&E-stained section of well-differentiated OSCC, b) IHC-stained section of well-differentiated OSCC

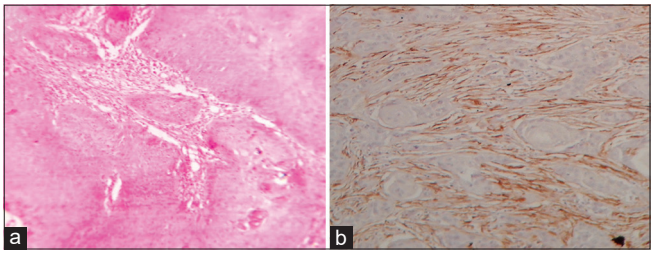

Figure 2: a) H&E-stained section of moderately differentiated OSCC, b) IHC-stained section of moderately differentiated OSCC

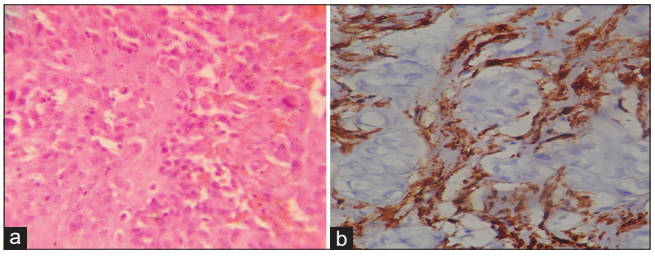

Figure 3: a) H&E-stained section of poorly differentiated OSCC, b) IHC-stained section of poorly differentiated OSCC

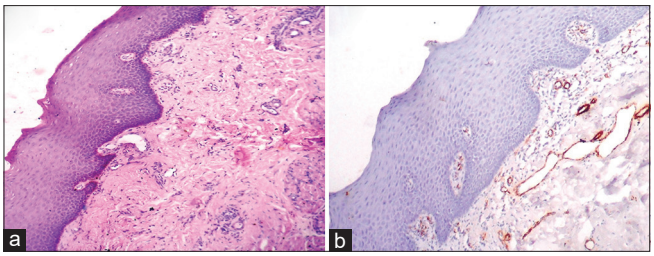

Figure 4: a) H&E-stained section of controls, b) IHC-stained section of controls

in OSCC cases and demonstrated its positive expression in OSCC cases and negative expression in tumor-free mucosa. In another corresponding research conducted by Kellermann *et al.*,[16] authors also demonstrated significant positive expression of myofibroblasts in tumor invasive front and its negative expression in tumor-free oral mucosa.

In the present study, we didn't observe any significant difference in the expression of myofibroblasts while comparing among different grades of OSCC cases. Our results were in concordance with the results obtained by Gandhi P *et al.*[10] who also didn't observe any significant difference while comparing the expression of myofibroblasts among well-differentiated, moderately differentiated and poorly differentiated OSCC patients. In a recent study conducted by Shete MV *et al.*[17] in 2020, authors tried to relate the role of myofibroblasts in oral premalignant disorders, OSCC cases and healthy controls. They concluded that α-SMA expression in the mesenchyme component of OSCC patients was significantly enhanced in comparison to its expression in epithelial dysplasia and normal oral mucosa. Kellermann described a copiousness of myofibroblasts to be associated with N-stage.[16]

Results of therapeutic trials of role of stromal fibroblasts in cancer therapy have shown promising results in the recent past. Stromal cells offer the additional advantage of being genetically more stable in comparison to tumor cells and might have common qualities across multiple tumor variants. Perhaps, treatment protocols intended to aim for pro-tumorigenic mechanisms facilitated by stromal cells might synergistically increase the effectiveness of treatments targeted against the tumor cells themselves. These statistics thus sustain the notion of using stromal tumor cells as an effective treatment therapy to manage epithelial-derived solid neoplasms.[18]

In another previous study conducted by Mahajan A *et al.*,[19] authors correlated the progressive enhancement in the IHC expression of myofibroblasts in normal oral mucosa, epithelial dysplasia, and OSCC. They evaluated 49 tissue blocks: 7 normal oral mucosa, 21 epithelial dysplasia cases, and 21 OSCC cases. The specimens were subjected to α-SMA IHC staining followed by the calculation of the staining index. They observed that statistically significant staining index was obtained by α-SMA and vimentin between normal oral mucosa, epithelial dysplasia, and OSCC. They stressed on the role of myofibroblasts during initial tumorigenesis only. These results were similar to results obtained in our study. Dodani *et al.*[20] concluded that myofibroblasts generate a lenient environment for facilitating tumor invasion in carcinoma patients, and therefore, the presence of myofibroblasts could be employed as a prognostic marker and it can help in treatment as well, by assessing their expression in the stroma.

These myofibroblasts or cancer-associated fibroblasts are also the most eminent non-immune cells within the tumor framework (Bienkowska KJ *et al.*).[21] Since myofibroblasts are the crucial indicators in ECM remodeling, the substantial role of myofibroblasts in tumor progression, invasion and metastasis has recently been documented. Targeting myofibroblasts has obtained incredible consideration in order to limit the myofibroblast-induced tumor progression and metastasis.[22] Pinisetti S *et al.*, in another previous research, hypothesized that myofibroblasts play a vital role in OSCC invasion and progression. Hence, therapeutic strategies aiming the myofibroblasts might prove to be beneficial in OSCC patients.[23]

Conclusion

In view of the findings obtained in the current pilot study, it can be suggested that myofibroblasts are an integral component of processes associated with the creation of a permissive environment for cancer invasion process in patients with OSCC. However, further research with a larger sample size is recommended.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Capparuccia L, Tamagnone L. Semaphorin signaling in cancer cells and in cells of the tumor microenvironment--two sides of a coin. J Cell Sci 2009;122:1723–36.
2. Joshi P, Dutta S, Chaturvedi P, Nair S. Head and neck cancers in developing countries. Rambam Maimonides Med J 2014;5:e0009.
3. Al-Jaber A, Al-Nasser L, El-Metwally A. Epidemiology of oral cancer in Arab countries. Saudi Med J 2016;37:249–55.
4. Bugshan A, Farooq I. Oral squamous cell carcinoma: Metastasis, potentially associated malignant disorders, etiology and recent advancements in diagnosis. F1000Res 2020;9:229.
5. Hinz B, Lagares D. Evasion of apoptosis by myofibroblasts: A hallmark of fibrotic diseases. Nat Rev Rheumatol 2020;16:11-31.
6. 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;38:639–43.
7. Luksic I, Suton P, Manojlovic S, Virag M, Petrovecki M, Macan D. Significance of myofibroblast appearance in

squamous cell carcinoma of the oral cavity on the occurrence of occult regional metastases, distant metastases, and survival. Int J Oral Maxillofac Surg 2015;44:1075–80.

8. Guan H, Tan J, Zhang F, Gao L, Bai L, Qi D, *et al*. Myofibroblasts from salivary gland adenoid cystic carcinomas promote cancer invasion by expressing MMP2 and CXCL12. Histopathology 2015;66:781–90.
9. Jensen DH, Reibel J, Mackenzie IC, Dabelsteen E. Single cell migration in oral squamous cell carcinoma-possible evidence of epithelial-mesenchymal transition in vivo. J Oral Pathol Med 2015;44:674–9.
10. Gandhi P, Prasad UC. Evaluation of myofibroblasts in oral submucous fibrosis and oral squamous cell carcinoma: The pathogenesis and correlation. Dent Res J (Isfahan) 2017;14:314-20.
11. Sasahira T, Bosserhoff AK, Kirita T. The importance of melanoma inhibitory activity gene family in the tumor progression of oral cancer. Pathol Int 2018;68:278–86.
12. Mehanna H, Paleri V, West CM, Nutting C. Head and neck cancer-part 1: Epidemiology, presentation, and preservation. Clin Otolaryngol 2011;36:65–8.
13. Perry BJ, Zammit AP, Lewandowski AW, Bashford JJ, Dragovic AS, Perry EJ. Sites of origin of oral cavity cancer in nonsmokers vs. smokers: Possible evidence of dental trauma carcinogenesis and its importance compared with human papillomavirus. JAMA Otolaryngol Head Neck Surg 2015;141:5–11.
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;17:e733–8.
15. Barth PJ, Schenck zu Schweinsberg T, Ramaswamy A, Moll R. CD34+ fibrocytes, alpha-smooth muscle antigen-positive myofibroblasts, and CD117 expression in the stroma of invasive squamous cell carcinomas of the oral cavity, pharynx, and larynx. Virchows Arch 2004;444:231–4.
16. Kellermann MG, Sobral LM, da Silva SD, Zecchin KG, Graner E, Lopes MA, *et al*. Myofibroblasts in the stroma of oral squamous cell carcinoma are associated with poor prognosis. Histopathology 2007;51:849–53.
17. Shete MV, Deshmukh RS, Kulkarni T, Shete AV, Karande P, Hande P. Myofibroblasts as important diagnostic and prognostic indicators of oral squamous cell carcinoma: An immunohistochemical study in normal oral mucosa, epithelial dysplasia, and oral squamous cell carcinoma. J Carcinog 2020;19:1.
18. Santos AM, Jung J, Aziz N, Kissil JL, Pure E. Targeting fibroblast activation protein inhibits tumor stromagenesis and growth in mice. J Clin Invest 2009;119:3613–25.
19. Mahajan A, Manhas A, Aneja T, Parhar S, Kumari P, Anubhuti, *et al*. Comparison of expression of myofibroblasts in normal oral mucosa, oral epithelial dysplasia, and oral squamous cell carcinoma using α-SMA and vimentin: An immunohistochemical study. J Family Med Prim Care 2020;9:4368-74.
20. Dodani A, Siadati S, Salehinejad S, Hajian-Tilaki KO, Abbaszadeh-Bidokhty H. Comparative evaluation of the frequency of myofibroblasts between oral and cutaneous squamous cell carcinomas. Caspian J Dent Res 2016;5:24–9.
21. Bienkowska KJ, Hanley CJ, Thomas GJ. Cancer-associated fibroblasts in oral cancer: A current perspective on function and potential for therapeutic targeting. Front Oral Health 2021;2:686337.
22. Yazdani S, Bansal R, Prakash J. Drug targeting to myofibroblasts: Implications for fibrosis and cancer. Adv Drug Deliv Rev 2017;121:101-16.
23. Pinisetti S, Tadi D, Manyam R, Alla R. Immunohistochemical evaluation of myofibroblasts in oral epithelial dysplasia and oral squamous cell carcinoma. J Oral Maxillofac Pathol 2021;25:494-8.