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

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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 four 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. 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. The prevalence rate of head and neck carcinomas is differentially reported among different areas of the world. 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. 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.

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...
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.[9]

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 process of oral squamous cell carcinoma.

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 | Group 1 | Group 2 | Group 3 | Group 4 |
|---------------------------------|---------|---------|---------|---------|
| cells intensity value | 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.\cite{11,12} Myofibroblasts are a significant component of tumor stroma along with other immunocompetent and inflammatory cells.\cite{13} 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.

| 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   | -           | 0.00 (Significant) |

Myofibroblasts generate a physical obstacle between malignant cells and the body’s immune system against cancer.\cite{12} Enhanced expression of myofibroblasts might be beneficial for predicting the prognosis of OSCC patients.\cite{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 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.\cite{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.\cite{15} in 2004, authors assessed the expression of myofibroblasts.
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.,[14] 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.[15] 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.[10]

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.

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**Conflicts of interest**

There are no conflicts of interest.

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