Evaluation of Role of Myofibroblasts in Oral Cancer: A Systematic Review

Harjeet K Sekhon, Keya Sircar, Gurbani Kaur, Muneet Marwah

ABSTRACT

Aim: To conduct a systematic review on the role of myofibroblasts in progression of oral cancer. The myofibroblast is essential for the integrity of the mammalian body by virtue of its role in wound healing, but it also plays a negative role due to their role in promoting tumor development.

Settings and design: Systematic review.

Materials and methods: Bibliographic searches were conducted in several electronic databases using all publications in PubMed, PubMed central, EMBASE, CancerLit, Google scholar, and Cochrane CCTR between 1990 and June 2015.

Results: The search of all publications from various electronic databases revealed 1,371 citations. The total number of studies considered for systematic review was 43. The total number of patients included in the studies was 990.

Conclusion: Myofibroblasts are a significant component in stroma of oral cancer cases, though not identified in all cases. This systematic review shows that clinical, pathological, and immunohistochemistry tests have correlated the presence of high myofibroblast count in oral cancer cell stroma.

Keywords: Myofibroblast, Oral cancer, Precancer.

Key Messages: Myofibroblasts play a significant role in oral cancer invasion and progression. Various studies have demonstrated their association with oral cancer. This review tends to highlight their role in the pathogenesis of oral cancer over the decade.

INTRODUCTION

The myofibroblasts are a type of fibroblasts, which constitute a family of paracrine cells that play an important role in the regulation of fundamental processes, such as cell motility, proliferation, differentiation, apoptosis, morphogenesis, tissue repair, inflammation, and the immune response. It has been identified as the cell which produces stress fibers, have alpha-smooth muscle expression and even help in the production of tension, collagen fibrils, and growth factors. Their presence has been described practically in all fibrotic situations characterized by tissue retraction and remodeling. In many organs like liver, lung, and kidney, they are primarily involved in fibrosis. In the wound tissue, it is implicated in wound strengthening by extracellular collagen fiber deposition and then wound contraction by intracellular contraction and concomitant alignment of the collagen fibers by integrin-mediated pulling onto the collagen bundles. Myofibroblast may be considered as a foe or friend due to their beneficial role in normal healing granulation tissue and its damaging effects when it occurs in hypertrophic scars, scleroderma, dupuytren disease, fibromatosis, fibrotic response to implants, lung fibrosis, heart fibrosis, kidney fibrosis, atheromatous plaque evolution, and chronic asthma.

The term “oral cancer” includes all the malignancies arising from lips, oral cavity, oropharynx, nasopharynx, hypopharynx, and other ill-defined sites within lip, oral cavity, and pharynx. In accordance to the study of diverse pathologic conditions in which the myofibroblast has been described, three fundamental processes were identified in 1980: Diverse responses to injury and repair phenomena, quasi-neoplastic proliferative conditions, the...
stromal response to certain forms of neoplasia. Many invasive and the metastatic carcinomas, especially those characterized by their hard consistency, retraction, and fixation to adjacent tissues, elicit a desmoplastic stromal reaction. The reaction that occurs in these carcinomas is brought about by stromal myofibroblasts. It was originally believed that the myofibroblastic stromal reaction represented a host response to cancer possibly to contain the invasive neoplasm. However, few authors have suggested that in invasive sarcomas, the stromal myofibroblast secretes stromal-degrading enzymes, which would favor cancer invasion. Myofibroblasts also produce a variety of factors that are involved in the pathogenesis of oral submucous fibrosis (OSMF). Myofibroblasts interact with epithelial cells and other connective tissue cells and may thus control phenomenon as tumor invasion and angiogenesis. Some studies have suggested secretion of enzymes that degrade the extracellular matrix, thereby facilitating tumor invasion by the myofibroblasts. The aim of this study was to systematically review the literature on the role of myofibroblasts in oral cancer development and progression.

MATERIALS AND METHODS

The bibliographic searches were conducted in several electronic databases using all publications in PubMed, PubMed central, EMBASE, CancerLit, Google scholar, and Cochrane CCTR between 1990 and June 2015.

Criteria for considering studies:
- Studies were considered eligible for study when they fulfilled the following criteria:
  - Randomized controlled trials
  - Experimental studies conducted on human species
  - Observational studies with a comparison and control group.
  - Full-text review articles
  - Case reports

Exclusion criteria were as follows:
- Duplicate studies
- Unpublished data
- Studies involving animal species.

The primary outcome considered in this study was to evaluate the role of myofibroblasts in progression of oral cancer and analyze their prevalence in oral cancer cases. Other outcome included was prevalence in potentially premalignant oral lesions.

RESULTS

The search of all publications from various electronic databases revealed 1,371 citations (Flow Chart 1). Searches of EMBASE, Google scholar, CancerLit, Cochrane CCTR, and bibliographies of review articles did not reveal any further relevant studies that had not been identified by PubMed search. Similarly, hand searching in the identified journals did not identify any other studies.

However, after implementing the terms of the search-strategy inclusion criteria, the studies that could be considered for systematic review reduced to 43 (Table 2).

| Table 1: Keywords used in the literature search |
|-------------------------------|-----------------|-----------|
| Oral                          | Cancer          | Myofibroblast |
| Mouth                        | Mouth neoplasm  |            |
| Lip                          | Precancerous condition | |
| Gingiva                      | Tumor           |            |
| Tongue                       | Carcinoma       |            |
| Oropharynx                   | Malignant       | Dysplasia   |

Flow Chart 1: Study design

Studies identified from various electronic databases:
- Experimental – 924
- Randomized controlled trials – 109
- Observational studies – 57
- Narrative reviews – 236
- Case reports – 45

Total (excluding overlapping references) – 1371

Records screened for relevance, full text articles assessed for eligibility

Studies included in the systematic review – 43
Table 2: Characteristics of studies included in investigating prevalence of myofibroblasts in oral cancer

<table>
<thead>
<tr>
<th>References</th>
<th>Year of publication</th>
<th>Journal of publication</th>
<th>Details of the study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zidar et al17</td>
<td>2002</td>
<td>Oncology</td>
<td>Sample – n₁ = 42 (resected larynx) n₂ = 40 (laryngeal biopsies of epithelial hyperplastic lesions and squamous carcinoma-myofibroblast found exclusively in squamous carcinoma)</td>
</tr>
<tr>
<td>Barth et al18</td>
<td>2004</td>
<td>Virchows Arch</td>
<td>Sample – n = 39 (OSCC and tumor-free oral mucosa) 31 carcinomas showed increased stromal α SMA positive myofibroblast Myofibroblastic differentiation is commonly seen in the stroma of OSCC (11), particularly at the invasive front of the tumor, no α SMA positive myofibroblast in the connective tissue of fibroepithelial hyperplasia</td>
</tr>
<tr>
<td>Lewis et al19</td>
<td>2004</td>
<td>Br J Cancer</td>
<td>Sample – n = 25 (15 OSCC and 10 fibre epithelial hyperplasia) Myofibroblastic differentiation is commonly seen in the stroma of OSCC (11), particularly at the invasive front of the tumor, no α SMA positive myofibroblast in the connective tissue of fibroepithelial hyperplasia</td>
</tr>
<tr>
<td>Vered et al20</td>
<td>2005</td>
<td>Oral Oncol</td>
<td>Sample – n = 53, high frequency of stromal myofibroblast in known aggressive odontogenic lesion, such as odontogenic keratocyst parakeratinized type and solid ameloblastoma implies that myofibroblast can contribute to the biological behavior of these odontogenic lesions</td>
</tr>
<tr>
<td>Kellermann et al21</td>
<td>2007</td>
<td>Histopathology</td>
<td>Sample – n = 117, myofibroblast in the stroma of the oral carcinoma are associated with poor prognosis</td>
</tr>
<tr>
<td>Kellermann et al22</td>
<td>2008</td>
<td>Oral Oncol</td>
<td>60% of the OSCC contain myofibroblast in the stroma of the tumor During tumor invasion OSCC-derived TGF-β 1 promote fibroblast myofibroblast transdifferentiation and tumor cell proliferation can be induced by factors released from myofibroblast favoring tumor growth</td>
</tr>
<tr>
<td>Verad et al23</td>
<td>2009</td>
<td>Cancer Microenviron</td>
<td>Sample – n = 19 (pair matched-oral tongue SCC and metastatic tumor to regional lymph node), expression of cancer-associated fibroblast is common to both primary and metastatic SCC</td>
</tr>
<tr>
<td>Franz et al24</td>
<td>2009</td>
<td>Histochem Cell Biol</td>
<td>Sample – n = 152, Snail-positive cell in the stroma of OSCC invasive front without statistically significant correlation, histological grade, or nodal metastasis.</td>
</tr>
<tr>
<td>Kawashiri et al25</td>
<td>2009</td>
<td>Head Neck</td>
<td>Sample – n = 84, high level of stromal collagen fibers in invasive tumors, myofibroblast appearance increased with increasing tumor invasiveness with frequent lymph node metastasis</td>
</tr>
<tr>
<td>Etemad-Moghadam et al26</td>
<td>2009</td>
<td>J Oral Pathol Med</td>
<td>Sample – n = 70, presence of myofibroblast in the stroma of OSCC but not in dysplasia and normal mucosa</td>
</tr>
<tr>
<td>Franz et al27</td>
<td>2010</td>
<td>J Oral Pathol Med</td>
<td>Sample – n = 5, mediated by myofibroblast OSCC development is associated with a stromal upregulation of laminin isoform possibly contributing to a migration promoting microenvironment</td>
</tr>
<tr>
<td>Seifi et al28</td>
<td>2010</td>
<td>Asian Pac J Cancer Prev</td>
<td>Sample – n = 54, increase in the number of myofibroblast and change in the distribution pattern occur during carcinogenesis signifying their role in tumor invasion characteristics</td>
</tr>
<tr>
<td>Sobral et al29</td>
<td>2011</td>
<td>Oral Oncol</td>
<td>Sample – n = 30, myofibroblast in the stroma of OSCC may influence proliferation and invasion</td>
</tr>
<tr>
<td>Angadi et al13</td>
<td>2011</td>
<td>J Oral Pathol Med</td>
<td>Sample – n = 85, statistically significant increase in the myofibroblast between early and advance stages was observed</td>
</tr>
<tr>
<td>Sridhara et al31</td>
<td>2013</td>
<td>J Oral Maxillofac Pathol</td>
<td>Sample – n = 10, α-SMA cases were more in the metastatic group than in the nonmetastatic tumor</td>
</tr>
<tr>
<td>Lúcio et al32</td>
<td>2013</td>
<td>Braz J Otorhinolaryngol</td>
<td>Myofibroblasts are important components of the stroma for SCC</td>
</tr>
<tr>
<td>Angadi et al33</td>
<td>2014</td>
<td>Ada Odontol Scand</td>
<td>Sample – n = 65, (50-OSCC and histologically normal mucosa adjacent to OSCC, 15-control) significant co-relation was established for the presence of myofibroblast in the stroma of OSCC and HNMAOSCC. Myofibroblasts are early stromal change in the HNMAOSCC that highlights the possible role of myofibroblast as likely mediator for field cancerization</td>
</tr>
<tr>
<td>Routray et al34</td>
<td>2014</td>
<td>Oral Dis</td>
<td>Myofibroblast can arise locally from endothelial mesenchymal transformation at the invasive edge of the cancer leading to development of high-grade malignancies and poor prognosis</td>
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DISCUSSION

In neoplasia, proliferation of myofibroblasts was as a host stromal response to invasive carcinomas characterized by desmoplasia. The persistence of myofibroblast in a fibrotic lesion leads to scarring along with the functional impairment of the affected organ. The sustained myofibroblast activation stimulates the dysfunction repair mechanisms, leading to accumulation of fibrotic extracellular matrix, i.e., rich in collagens that bind to form fibrous bundles that are resistant to degradation. The fibrotic extracellular matrix disrupts cell polarity and stimulates cell proliferation, which creates an environment for the cancer formation and progression. Myofibroblast-induced inflammation and angiogenesis facilitate tumor growth and progression.

Many epithelial tumors are characterized by the local accumulation of connective tissue cells and extracellular material; this phenomenon is known as the stromal reaction. The interaction of myofibroblast with epithelial cells and other connective tissue cells may control such phenomenon as tumor invasion and angiogenesis.

In the recent history some studies have suggested secretion of enzymes that degrade the extracellular matrix, thereby facilitating tumor invasion and angiogenesis. Transdifferentiation of the fibroblasts to the myofibroblasts is a crucial and early event in tumorigenesis, which is mediated by the growth factors and cytokines, such as transforming growth factor-beta (TGF-beta) expressed by the tumor cells.

Squamous carcinoma cells may induce a myofibroblast phenotype in primary fibroblasts through the secretion of TGF-beta. Transforming growth factor-beta causes cancer progression through paracrine and autocrine effects. Paracrine effects of TGF-beta include the stimulation of angiogenesis, escape from immuno-surveillance and recruitment of myofibroblasts, while the autocrine effects of TGF-beta in cancer cells with a functional TGF-beta receptor complex may be caused by a convergence between TGF-beta signaling and betacatenin or activating Ras mutations.

The myofibroblasts along with immune cells support blood vessel formation, breakdown of basement membrane barriers, and facilitate tumor invasion and metastasis. They significantly upregulate the secretion of hepatocyte growth factor, which promotes invasion of squamous cell carcinoma.

Various experimental and clinical observations indicate the production of pro-invasive signals by the myofibroblasts which are implicated in cancer pain. N-Cadherin, expressed by myofibroblasts, promotes matrix invasion, perineural invasion, muscular invasion, and transendothelial migration.

Epithelial cell proliferation is mediated by growth factors and inflammatory mediators secreted by myofibroblasts. The role of myofibroblasts in promoting invasion has been shown in numerous aggressive and malignant neoplasms. Zidar et al demonstrated that myofibroblasts were positive for vimentin and smooth muscle actin. This indicated that invasion beyond the basement membrane is necessary for the occurrence of the myofibroblastic stromal reaction. Two patterns of stromal reaction were observed in squamous carcinomas:

1. Characterized by a marked proliferation of myofibroblasts and desmoplasia, with scarce lymphocytic infiltration. This pattern was associated with well- or moderately differentiated squamous carcinoma.
2. Characterized by few myofibroblasts, weak desmoplasia, and dense lymphocytic infiltration. This pattern

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<tr>
<td>Pinisetti et al35</td>
<td>2014</td>
<td>J Oral Maxillofac Pathol</td>
<td>Myofibroblast in focal epithelial dysplasia and SCC revealed a higher number of myofibroblast in OSCC</td>
</tr>
<tr>
<td>Rao et al36</td>
<td>2014</td>
<td>J Clin Diagn Res</td>
<td>Sample n = 62 (41 – OSMF, 10 – OSMF with dysplasia and 11 – OSCC). Presence of myofibroblasts was significantly higher in OSCC</td>
</tr>
<tr>
<td>Luksic et al49</td>
<td>2015</td>
<td>Int J Oral Maxillofac Surg</td>
<td>Sample n = 152, myofibroblast proliferation was suggested to facilitate tumor invasion and distant metastasis</td>
</tr>
<tr>
<td>Guan et al50</td>
<td>2015</td>
<td>Histopathology</td>
<td>Immunohistochemically, significant difference was observed in α-SMA expression in between normal controls and adenoid cystic carcinoma. This study demonstrated presence of myofibroblasts in adenoid cystic carcinoma.</td>
</tr>
<tr>
<td>Jensen et al51</td>
<td>2015</td>
<td>J Oral Pathol Med</td>
<td>In this study, budding tumor cells had decreased expression of E-cadherin. Thus, it is suggested that budding tumor cells in OSCC is not dependent upon either myofibroblast or complete epithelial–mesenchymal transition.</td>
</tr>
</tbody>
</table>

OSCC: Oral squamous cell carcinoma; HNMAOSCC: Histologically normal mucosa adjacent to oral squamous cell carcinoma; IHC: Immunohistochemistry; SMA: Smooth muscle actin; OSMF: Oral submucous fibrosis
was associated with moderately or poorly differentiated squamous carcinoma.

The degree of myofibroblast proliferation was inversely related to the density of lymphocytic infiltration. In invasive oral squamous cell carcinoma, an increase in smooth muscle actin-positive myofibroblasts has been observed. Etemad-Moghadam et al.26 conducted a study that demonstrated an increased number of myofibroblasts in oral squamous cell carcinomas compared to normal and dysplastic epithelium.

Kellermann et al.21 studied the prognostic significance of myofibroblasts in squamous cell carcinoma of tongue, preleukoplakia with histological dysplasia, and in normal tongue mucosa. No myofibroblasts were detected in the stroma of the normal mucosa or epithelial dysplasia which is in agreement with the results obtained by Etemad-Moghadam et al. Lewis et al.19 demonstrated the presence of myofibroblast in the vicinity of invasive squamous cell carcinoma but not in the mucosal polyps. Kellermann et al.21 and Vered et al.23 have described the presence of the myofibroblast in the stroma of most human oral squamous cell carcinoma. Two dominant patterns discovered were:

1. Spindle
2. Network

In the “network” pattern, myofibroblasts are exceptionally abundant and occupy almost the entire tumor stroma.

The “spindle” pattern is characterized by stromal myofibroblast that have spindle-shaped morphology and are located at the periphery of carcinomas as one to three concentric layers, a pattern that can also be found adjacent to a few or many tumor islands/nests.21,23

The presence of myofibroblasts in squamous cell carcinoma is considered to be inductive phenomena. The epithelial–stromal interactions, different growth factors released by malignant epithelial cells induce, have been considered as source for the myofibroblasts.26

In a study to assess the frequency of stromal myofibroblast in the different odontogenic cysts and tumors, Vered et al.20 found that the number of smooth muscle-actin positive stromal cells or myofibroblasts was significantly higher in odontogenic keratocysts as compared to dentigerous cysts. The myofibroblast counts in the parakeratinized odontogenic cyst and solid ameloblastoma were not significantly different from that in the squamous cell carcinoma. The number of myofibroblasts in the unicystic ameloblastoma and the ameloblastic fibroma was comparatively lower than that in the more aggressive odontogenic tumors and cysts. Thus, it was suggested that the myofibroblast has the potential to facilitate progression of epithelial lesions, and this can contribute to the biological behavior of these odontogenic lesions.

Based on semi-quantitative histological studies, myofibroblasts have been associated with tumor progression in various ways. Kellermann found an abundance of myofibroblast to be associated with the N-stage but not with tumor size (T stage).21,41-48 Most studies show increased quantities of myofibroblast to be associated with poor prognosis.

CONCLUSION

Myofibroblasts are significant components in the stroma of oral cancer lesions, though they may not be identified in all cases. A review of the literature indicates that myofibroblasts play an important role in facilitating invasion by oral squamous cell carcinoma by expression of growth factors, cytokines, extracellular components,23 and various proteolytic enzymes. This systematic review shows that clinical, pathological, and immunohistochemistry tests have correlated the presence of high myofibroblast count in oral cancer cell stroma. Most of these observations have been made in the last few years from 1990 to 2015. Thus there is a need for further research to understand the molecular mechanisms by which myofibroblasts impact the biological behavior of oral squamous cell carcinoma.

REFERENCES

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