Review Article

The role of myofibroblasts in the progression of oral submucous fibrosis: A systematic review

Vijay Wadhwan[1], Arvind Venkatesh[2], Vandana Reddy[1], Sangeeta Malik[3]

[1]Department of Oral and Maxillofacial Pathology and Oral Microbiology, Subharti Dental College, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, [2]Department of Oral and Maxillofacial Pathology and Oral Microbiology, Smile Square Multispecialty Dental Centre, Karur, Tamil Nadu, [3]Department of Oral Medicine and Radiology, Subharti Dental College, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, India

Abstract

Oral Submucous Fibrosis (OSMF) is a chronic progressive scarring oral disease predominantly affecting people of South Asian origin. It is characterized by juxtaepithelial inflammatory cell infiltration followed by fibrosis in the lamina propria and submucosa of the oral mucosa. The pathogenesis of the disease is not well established and a number of mechanisms have been proposed regarding the pathogenesis. A renewed interest has been shown in myofibrobasts which have been implicated to play a significant role in the pathogenesis of OSMF. The myofibroblast were initially identified by means of electron microscopy in granulation tissue of healing wounds as a modulated fibroblast exhibiting features of smooth muscle cells, with prominent bundles of microfilaments, dense bodies scattered in between, and gap junctions. The presence of myofibroblasts has successively been described in practically all fibrotic situations characterized by tissue retraction and remodeling. This review paper is an attempt to identify all the studies involving myofibroblasts and explaining the pathogenesis in a simplified manner.

Keywords: Epithelial mesenchymal interaction, myofibroblasts, oral submucous fibrosis

Address for correspondence: Dr. Vijay Wadhwan, Department of Oral Pathology and Microbiology, Subharti Dental College, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, India. E-mail: pathogen@rediffmail.com

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INTRODUCTION

Oral submucous fibrosis (OSMF) is a chronic progressive scarring oral disease predominantly affecting people of South Asian origin. It is characterized by juxta-epithelial inflammatory cell infiltration followed by fibrosis in the lamina propria and submucosa of the oral mucosa.[1] The pathogenesis of the disease is not well established. The chewing of betel quid has been recognized as one of the most important risk factors for OSMF.[2] The microtrauma produced by the friction of coarse fibers of the areca nut also facilitates the diffusion of betel quid alkaloids and flavonoids into the subepithelial connective tissue.[3] OSMF has been included under potentially malignant disorders by the WHO Collaborating Centre for Oral Cancer and Precancer in 2008.[4] OSMF has a malignant transformation rate of around 7.6%.[5,6] The exact pathophysiology behind this malignant transformation of OSMF is still unclear, but the progression of carcinomas has conventionally been attributed to a stepwise accumulation of genetic changes within the target epithelium. Such molecular progression has been demonstrated in the oral mucosa where it is

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initially reflected in the appearance of precursor lesions with epithelial hyperplasia and dysplasia followed later by the development of frank carcinoma, changes paralleled by increases in genetic alterations in the epithelium.[6] Various new mechanisms on the pathogenesis and progression of OSMF proposes the possible role of the composition and structure of extracellular matrix (ECM) and the epithelial–mesenchymal transition (EMT) in the progression of the disease and its malignant transformation.[7] The myofibroblast was identified by electron microscopy and has successively been described in practically all fibrotic situations characterized by tissue retraction and remodeling.[8] Less generally appreciated is the notion that the transformation of fibroblast to myofibroblasts is a key, perhaps essential, event for the cells to perform these functions. Myofibroblasts are a unique group of cells phenotypically intermediate between smooth muscle cells and fibroblast. They can be identified by certain characteristic features of the cytoskeleton, particularly by the expression of alpha-smooth muscle actin (α SMA), and are believed to be primary producers of ECM after injury.[9]

In this review, we have dealt in detail about myofibroblasts and their possible role in the progression of OSMF and its malignant transformation.

Myofibroblasts

The simplest definition of myofibroblasts is that they are smooth muscle-like fibroblasts. Some investigators define them as activated smooth muscle cells; others call them lipocytes because of their propensity to store retinoids (Vitamin A). They are also known as stellate cells due to a shape change when they are transiently differentiated.[10,11] In both cell culture and in native tissues, myofibroblasts possess several distinguishing morphologic characteristics. They display prominent cytoplasmic actin microfilaments (stress fibers) and are connected to each other by adherens and gap junction.[12,13] Myofibroblasts exist in two distinct morphological states as follows: (1) Activated myofibroblast and (2) Stellate transferred myofibroblast, a transiently differentiated myofibroblast.[14]

Origin of myofibroblasts

Myofibroblasts of wound tissue and fibrosis have been assumed to originate from local recruitment of fibroblasts in the surrounding tissue. This is supported by the presence of many fibroblasts showing proliferation marker-positive nuclei at the periphery of the wound.[12] Another possible source of myofibroblasts is represented by pericytes or vascular smooth muscle cells around vessels.[15] During renal fibrogenesis, it has been shown that fibroblasts arise in large numbers by local EMT. In addition, fibroblasts may originate from fibrocytes, a subpopulation of bone marrow-derived leukocytes with fibroblast characteristics.[16]

Markers for myofibroblasts

Two of the three filament systems of eukaryotic cells, actin (a component of the microfilaments) and vimentin, desmin, laminin or glial fibrillary acidic proteins (members of the intermediate filament system) differentiate myofibroblasts from smooth muscle cells. Myofibroblasts have not been characterized with regard to tubulins (proteins of the microtubules). Beta and gamma actins are expressed by all cells, including myofibroblasts. Myofibroblasts stain negatively for α-cardiac and α-skeletal actin, but positively for α SMA.[10] Studies conducted to determine the origin of liver fibrogenic cells show that smoothelin can be used as a marker for myofibroblasts.[17] Faust *et al*., in 2013,[18] proposed human xylosyl transferase-I activity, in addition to α SMA expression, as a new biomarker for myofibroblast differentiation and fibrotic development based on their study conducted in skin fibrosis.

The mechanical feedback loop in myofibroblast development

Fibroblasts in intact tissue are stress-shielded by a functional ECM; they do not develop contractile features and cell-matrix adhesions. After injury, inflammatory signals activate fibroblasts to spread into the provisional wound matrix. Local cell remodeling activity leads to gradual increase in global matrix stiffness that counteracts cell traction forces. The resulting formation of small focal adhesions (FAs) and stress fibers that contain only cytoplasmic actins characterize the proto-myofibroblast. Transforming growth factor (TGF) β1 stimulates proto-myofibroblasts to express α SMA, which at first is not incorporated into stress fibers but organizes in cytoplasmic rod-like structures. Continuing ECM fiber alignment creates larger surfaces for adhesion formation; larger adhesions permit the development of stronger stress fibers and generation of higher contractile forces. When adhesion sites grow to the size of supermature FAs, intracellular tension reaches a critical level that allows incorporation of α SMA into pre-existing stress fibers. The force generated by α SMA-containing stress fiber is significantly higher than cytoplasmic actin stress fibers leading to further FA supermaturation and ECM contraction, thereby establishing a mechanical loop. Myofibroblasts may exit this cycle when the original structure of the ECM is reconstituted and again takes over the mechanical load; stress-released myofibroblasts eventually undergo apoptosis.[19]

ORAL SUBMUCOUS FIBROSIS

OSMF is a chronic debilitating and a premalignant condition affecting the oral cavity, pharynx and upper digestive tract of the oral cavity. The characteristic pathophysiology of the disease is submucosal fibrosis characterized by juxta-epithelial inflammatory reaction followed by chronic change in the fibro-elasticity of the lamina propria and associated with epithelial atrophy.[20] The etiology of OSMF is unknown. The various hypotheses proposed to suggest a multifactorial origin for this condition. There is also clinical and experimental evidence of the presence of circulating immune complexes, immunoglobulin contents and circulating auto-antibodies associated with specific HLA antigens in patient's sera and alteration in cellular and humoral responses suggesting an autoimmune etiology and genetic propensity. However, the existing scientific literature at present makes it apparent that areca nut is the major etiological factor.[3,21,22] The emerging paradigm is that inflammatory mediators that are produced in response to injury cause EMT, which can lead to fibrosis. The critical importance of keratinocyte inflammation to the process of fibrosis, together with the crucial role for EMT in fibrogenesis in other tissues, naturally raise the question of whether EMT contributes to the pathogenesis of fibrosis in the oral mucosa.[23] The likelihood of EMT in OSMF is further supported by the findings that many cytokines, nucleus proteins and signaling pathways involved in EMT had been expressed and activated in OSMF or in models *in vitro*.[24,25]

Search strategy for identification of studies

The search strategy was in accordance with the Cochrane guidelines for systematic reviews. Articles were searched and selected using PubMed, MEDLINE, PubMed CENTRAL till the year 2015. In addition, Google Scholar and the Cochrane Library were also used to obtain the relevant articles of our interest. Due to the scarcity of studies on this topic, we wished to exhaust all the possible articles; accordingly, no timeline was included in the search. The article search included only those published in the English literature. The title of the articles and abstracts were reviewed. The articles were reviewed, and data were tabulated by the following PRISMA-P protocol (2015) of recommended items to be addressed in a systematic review.

Search methodology

The search methodology through PubMed was done using the following keywords:

(((((“myofibroblasts”[MeSH Terms] OR “myofibroblasts”[All Fields] OR “myofibroblast”[All Fields]) OR s100a4;[All Fields]) OR fsp1[All Fields]) OR (alpha[All Fields] AND (“muscle, smooth”[MeSH Terms] OR (“muscle”[All Fields] AND “smooth”[All Fields]) OR “smooth muscle”[All Fields] OR (“smooth”[All Fields] AND “muscle”[All Fields])) AND (“actins”[MeSH Terms] OR “actins”[All Fields] OR “actin”[All Fields]))) OR (“vimentin”[MeSH Terms] OR “vimentin”[All Fields])) AND (“oral submucous fibrosis”[MeSH Terms] OR (“oral”[All Fields] AND “submucous”[All Fields] AND “fibrosis”[All Fields]) OR “oral submucous fibrosis”[All Fields]).

In addition, an Internet search was also done through Google Scholar using the keywords “oral submucous fibrosis” and “myofibroblasts.” Similar keywords were employed for searching relevant literature in the Cochrane Library within the same stipulated timeline. Cross references of articles included in the review were also searched to include all possible publications in the field of the study and falling under the inclusion criteria laid down.

Selection criteria

Inclusion criteria

i. Studies that demonstrated the presence of myofibroblasts in OSMF
ii. Studies that analyzed the expression pattern and intensity of markers of myofibroblasts in OSMF
iii. Studies that compared the expression pattern of markers for myofibroblasts between OSMF and other pathologies
iv. Studies conducted on the expression of factors which influence the production or expression of myofibroblasts in OSMF.

Exclusion criteria

i. Studies conducted on the etiopathogenesis of OSMF not involving myofibroblasts
ii. Studies evaluating myofibroblasts in pathologies other than OSMF
iii. Review articles
iv. Case reports.

Data extraction and analysis

Once the potentially relevant articles for systematic review were obtained, data extracted from each article was tabulated and was later cross checked.

Studies included

Based on the search criteria, a total of 12 articles were selected to be included in the review. Of these, five studies were both *in vitro* and *ex vivo* and the other eight were only *in vitro* studies. The parameters measured were different

from study to study and also the methods used varied in between the studies. Immunohistochemistry (IHC) was a common investigation to demonstrate myofibroblasts which was employed in all the studies, whereas few studies employed immunoblotting, reverse transcription polymerase chain reaction (RT-PCR) and the western blot additional to IHC. The parameters evaluated mainly included intensity, percentage and pattern of staining. The studies and the relevant results obtained are tabulated and discussed in Table 1.

REVIEW OF LITERATURE

Chang *et al*., in 2002,[26] conducted an *in vitro* study using cell cultures from samples obtained from OSMF patients. The cytotoxicity assay on these cells showed severity proportional to arecoline concentration. It was found that anti-vimentin antibody was found to efficiently hybridize the elevated protein detected in arecoline-treated cell extracts by immunoblotting. Homogeneous and intensive staining for vimentin was noted subepithelially and in the deeper layers of the connective tissue stroma in moderately advanced and advanced cases of OSMF by IHC. These results revealed that arecoline activates vimentin expression in the buccal mucosal fibroblasts (BMFs). This could, however, be the result of the presence of a subtype of fibroblast which is more susceptible to external stimulation or gene modulation.

Angadi *et al*., in 2011,[9] conducted a study to evaluate the presence of myofibroblasts in various histological stages of OSMF. The number of α SMA-stained myofibroblasts in OSMF was significantly increased when compared to that of the normal controls. In addition, a statistically significant increase in the myofibroblasts population between early and advanced stages was observed. The results showed the possibility that OSMF actually represents an abnormal healing process in response to chronic mechanical and chemical irritation because of areca nut chewing as demonstrated by the increased incidence of myofibroblasts in this disease.

Moutasim *et al*., in 2010,[27] conducted a study to detect the role of αvβ6 integrin in promoting OSMF. IHC revealed that αvβ6, which is implicated in pathological fibrosis of various organs was upregulated in OSMF. Several cell functions like the activation of TGF-β1 are mediated by αvβ6. The results of this study confirmed that arecoline-dependent up-regulation of αvβ6 promoted the transdifferentiation of oral fibroblasts into myofibroblasts. The authors proposed possible pathogenesis of OSMF mediated by TGF-β1 resulting in the pathological fibrosis of several epithelial organs.

A study was conducted by Sawant *et al*., in 2013,[28] to investigate the clinical significance of vimentin expression at early and late events of areca nut associated oral tumorigenesis. IHC using vimentin as primary antibody showed aberrant vimentin expression in hyperplastic, dysplastic and fibrotic tissues. The results of the analysis were confirmed using immunofluorescence staining on methanol fixed cryostat sections, western blotting and RT-PCR wherever fresh and adequate tissue was available. The results suggested a possible role of vimentin in early events of areca nut associated oral tumorigenesis which may prove useful to predict the malignant potential of high-risk oral lesions.

Nayak *et al*., in 2013,[29] conducted a study to compare the expression of vimentin in various histological grades of OSMF. The study sample included histologically confirmed cases of OSMF, which were split into two groups: mild cases of OSMF and severe cases of OSMF, respectively. Significant difference was noted in the fibroblasts staining between the scores of normal and OSMF cases. The authors suggested that this difference could be the result of the presence of a subtype of fibroblast which is more susceptible to external stimulation or gene modulation.

To determine the role of S100A4 expression in the pathogenesis of OSMF both *in vitro* and *in vivo*, Yu *et al*., in 2013,[30] conducted a study in which OSMF samples were analyzed using IHC for S100A4 expression. S100A4 expression was higher in areca quid chewing-associated OSF specimens than normal buccal mucosa specimens. The study concluded that arecoline, a major areca nut alkaloid, leads to dose- and time-dependent elevation of S100A4 expression in normal buccal mucosa fibroblasts.

Rao *et al*., in 2014,[31] evaluated myofibroblasts by studying the expression of the marker α SMA for fibrosis dysplasia and carcinomas. The results obtained concluded that myofibroblasts play a role in fibrosis and also concluded that activated myofibroblasts secrete proteolytic enzymes and cause matrix degeneration which is instrumental in cancer cell invasion and metastasis.

Chang *et al*., in 2014,[32] conducted a study to investigate the expression of zinc finger E-box-binding homeobox 1 (ZEB 1), which is a well-known transcriptional factor in EMT, in OSMF tissues and its role in arecoline-induced myofibroblast transdifferentiation from BMFs. The expression of ZEB 1 and α SMA was significantly increased in OSMF patients. Long-term exposure of BMF to arecoline induced the expression of fibrogenic genes and ZEB 1. Silencing of ZEB 1 in fibrotic BMFs from an OSMF

Table 1: Description of included studies in the chronological order of publication

Authors	Type of study	Investigation type	Antibody used to demonstrate myfibroblast	Sample used	Relevant parameters evaluated	Relevant results
Chang *et al*., 2002	*In vitro*	Immunoblotting, IHC	Vimentin	Cell culture and immunoblotting 10 cases - normal mucosa 10 cases – OSMF IHC 4 cases – normal 15 cases – OSMF	Immunoblotting: Protein expression pattern and intensity of expression IHC: Intensity of staining in the superficial and deep zone	Immunoblotting: Increased expression of 57 kDa insoluble protein (vimentin) in dose dependent manner in OSMF group IHC: Homogeneous subepithelial vimentin positivity in OSMF group
Angadi and Kale, 2011[9]	*In vitro*	IHC	α SMA	70 cases of OSMF 35 cases of early OSMF 35 cases of advanced OSMF 15 normal mucosa	Staining intensity and percentage of α SMA positive cells	IHC index for myofibroblasts Group 1a Zero expression-2 Low expression-33 Group 1b Low expression-7 Moderate expression-8 High expression-20 Group 2 Zero expression – 13 cases Low expression – 2 cases
Moutasim *et al*., 2010	*In vitro* *Ex vivo*	IHC Flowcytometry, TGF-1 bioassay RT PCR, collagen gel contraction assay, organotypic assay	αvβ6 integrin α SMA phosphor Smad 2, Smad 4	41 cases of OSMF 14 cases of fibroepithelial hyperplasia	αvβ6 expression, α SMA positivity	αvβ6 expression OSMF group Negative – 9 Low – 10 High – 22 Fibroepithelial hyperplasia: Negative – 3 Low – 11 High – 0 Increased expression of αvβ6, α SMA, pSmad 2, Smad 4 in OSMF group
Yu *et al*., 2013	*In vivo* *Ex vivo*	IHC, PCR	S100A4	30 cases of OSMF 10 normal mucosa	IHC - Intensity of staining Western blot – S100A4 protein expression RT-PCR: Intensity of s100a4 expression	IHC Group 1 Weak positivity – 5 cases Strong positivity – 25 cases Group 2 Weak positivity – 8 cases Strong positivity – 2 cases RT-PCR Group 1: stronger s100a4 staining Group 2: faint s100a4 staining Western blot Arecoline was found to upregulate S100A4 protein expression in a dose AND time dependent manner in BMFs
Nayak *et al*., 2013	*In vitro*	IHC	Vimentin	40 cases of OSMF 10 normal mucosa	Intensity of staining in fibroblasts	Group 1 Intense – 30% cases Very intense – 60% cases

Contd...

Table 1: Contd...

Authors	Type of study	Investigation type	Antibody used to demonstrate myfibroblast	Sample used	Relevant parameters evaluated	Relevant results
						Group 2 Intense – 50% cases Very intense – 10% cases
Sawant *et al*., 2013	*In vitro* *Ex vivo*	IHC, Immunofluorescence, western blot, RT-PCR	Vimentin	10 samples of normal oral mucosa 19 samples of inflammatory lesions 52 samples of leukoplakia 71 samples of OSMF 227 samples of tumours	Vimentin expression pattern	Vimentin positivity Vimentin expression in OSMF group Mild fibrosis – 3/20 cases Moderate fibrosis – 8/22 cases Severe fibrosis – 11/52 cases
Chang *et al*., 2014	*In vitro*	IHC	α SMA	30 cases of OSMF 10 normal mucosa	Intensity of staining	Group 1 Weak positivity – 20% of cases Strong positivity – 80% of cases Group 2 Weak positivity – 70% of cases Strong positivity – 30% of cases
Rao *et al*., 2014	*In vitro*; cross sectional	IHC	α SMA	25 cases of OSMF 10 cases of OSMF with dysplasia	Percentage of immunopositive cells seen among the stromal cells of the subepithelial connective tissue	Group 1 Zero staining – 14.6% cases 1%–25% - 46.3% cases 26%–50%- 39% cases Group 2 51%–75% staining – 100% cases
Philip *et al*., 2014	*In vitro*	IHC	α SMA	15 cases of early OSMF 5 cases of moderately advanced OSMF 5 cases of advanced OSMF	Semi quantitative analysis: Grades based on percentage of immunopositive cells seen among the stromal cells of the subepithelial connective tissue	Significant increase in the myofibroblasts population between early and advanced stage
Jayaraj *et al*. 2015	*In vitro*	IHC	α SMA	42 cases of OSCC 32 cases of pre- malignant disorders (including 16 cases of OSMF)	Semi- quantitative analysis: Stromal spindle cells that were positive for α SMA were regarded as myofibroblasts. Immunostaining was assessed by the evaluation of the staining intensity and percentage of α SMA positive cells	2/16 cases of OSMF showed presence of myofibroblasts
Lee *et al*. 2015	*In vitro* *Ex vivo*	IHC, western blot, RT-PCR, collagen contraction assay, cell migration assay, wound healing assay	Twist	Arecoline + fibroblast culture	Collagen gel contraction and migration capability	Treatment of arecoline dose-dependently increased Twist expression transcript and protein levels in BMFs.

Contd...

Table 1: Contd...

Authors	Type of study	Investigation type	Antibody used to demonstrate myfibroblast	Sample used	Relevant parameters evaluated	Relevant results
						The myofibroblast activity including collagen gel contraction and migration capability also induced by arecoline, while knockdown of Twist reversed these phenomena.
Gupta *et al*. 2015	*In vitro*	IHC	α SMA	14 cases of oral leukoplakia 11 cases of OSMF 25 cases of OSCC	Semi - quantitative analysis	Score 1 (1%–20%): 81.8% cases of OSMF Score 2 (21%–40%): 18.2% cases of OSMF

IHC: Immunohistochemistry, OSMF: Oral submucous fibrosis, α SMA: Alpha smooth muscle actin, RT-PCR: Reverse transcription polymerase chain reaction, OSCC: Oral squamous cell carcinoma, TGF: Transforming growth factor, BMFs: Buccal mucosal fibroblasts

patient also suppressed the expression of α SMA and simultaneously myofibroblast activity. These data suggested that ZEB 1 may participate in the pathogenesis of areca quid associated OSMF by activating the α SMA promoter and inducing myofibroblast transdifferentiation of BMFs.

In 2014, Philip *et al*.[33] conducted a study to evaluate and compare the myofibroblasts in various histological grades of OSMF. Fifteen cases of OSMF, which were further categorized histologically into early (5 cases), moderately advanced (5 cases) and advanced (5 cases), were subjected to immunohistochemical evaluation using α SMA antibody for the detection of myofibroblasts. The results of this study showed that expression of myofibroblasts within the OSMF group showed a progressive increase from the early OSMF through moderate OSMF and the advanced OSMF group indicating that myofibroblasts could serve as effective prognostic marker for disease progression in OSMF.

A study conducted by Jayaraj *et al*., in 2015,[34] investigated the presence of myofibroblasts in healthy oral mucosa, potentially malignant disorders and squamous cell carcinomas (SCCs). The study material consisted of a total of 106 samples categorized into three groups, namely, oral SCC (OSCC) (n = 42), PMDs (n = 32) and oral healthy mucosa (n = 32) subjected to immunohistochemical analysis using α SMA. The results showed that there was a significant difference in the myofibroblasts expression between the groups. These findings justify myofibroblast as one among the key stromal element in tumor progression.

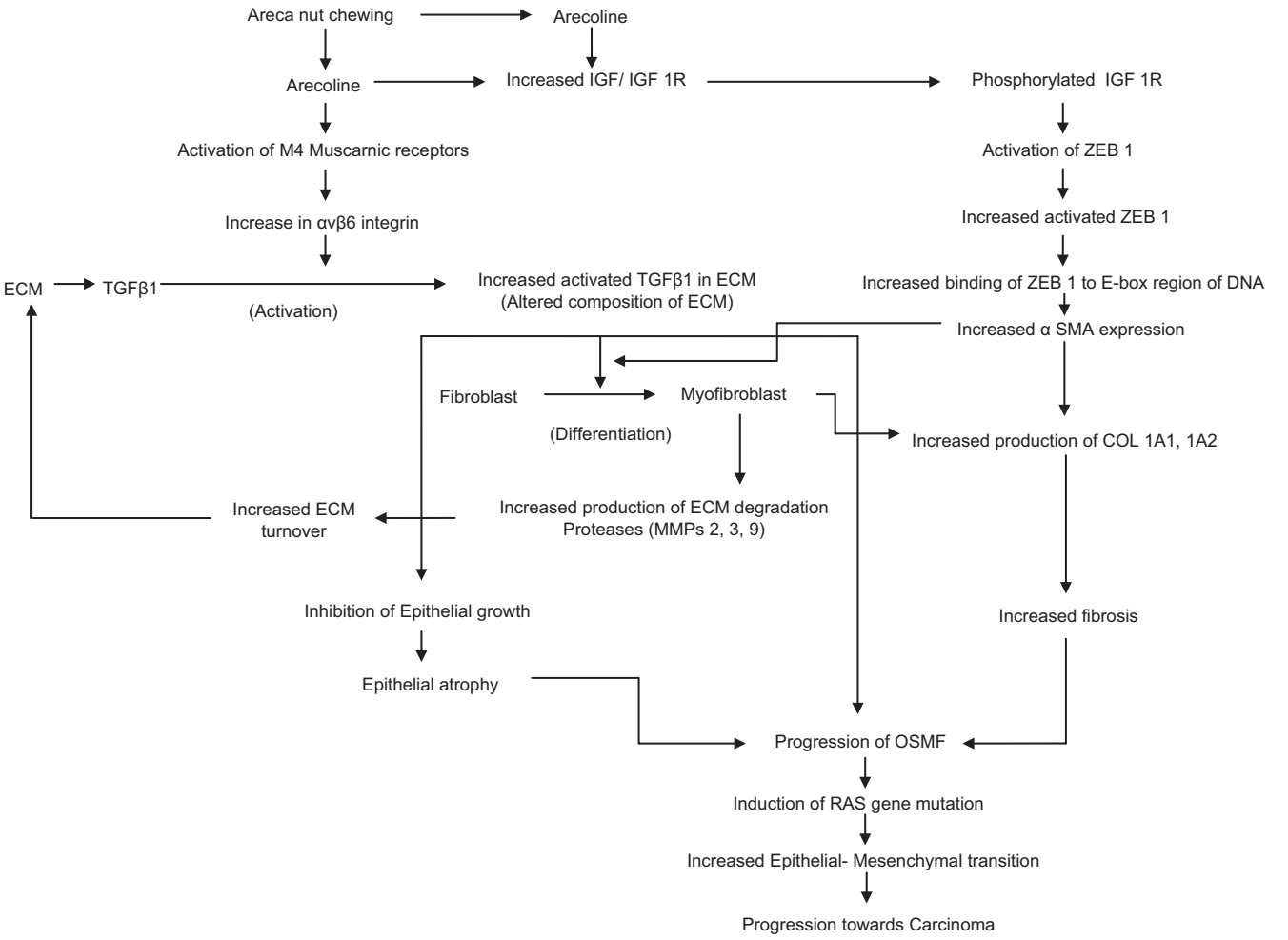
Lee *et al*., in 2015,[35] investigated the functional role of Twist, an EMT transcriptional factor, in myofibroblastic differentiation activity of OSMF. Arecoline, a major areca nut alkaloid, was used to explore whether expression of Twist could be changed dose-dependently in human primary BMFs. Collagen gel contraction and migration capability in arecoline-stimulated BMFs and primary OSMF-derived fibroblasts with Twist knockdown was presented. It was observed that the treatment of arecoline dose-dependently increased Twist expression transcript and protein levels in BMFs. The myofibroblast activity including collagen gel contraction and migration capability also induced by arecoline, while knockdown of Twist reversed these phenomena. Furthermore, Twist transcript and protein expression were higher in areca quid chewing-associated OSMF tissues than in normal oral mucosa tissues. These results suggested that upregulation of Twist might be involved in the pathogenesis of areca quid-associated OSMF through dysregulation of myofibroblast activity.

Gupta *et al*., in 2015,[36] conducted a study to evaluate and inter compare the presence and distribution of α SMA-positive myofibroblasts in oral leukoplakia, OSMF and different histopathological grades of OSCC. Sections were subjected to IHC using α SMA as the primary antibody for the detection of myofibroblasts. The results showed a statistically significant increase in myofibroblast expression in OSCCs compared to oral leukoplakias and OSMF. These findings are suggestive of the role of myofibroblasts with the creation of a permissive environment for tumor invasion in OSCC.

DISCUSSION

Based on these studies, we hereby propose possible pathogenesis for the progression of OSMF and its malignant transformation [Table 2]. Studies have suggested that areca nut chewing is the main etiological factor for OSMF. Arecoline, which is the chief constituent of areca nut is responsible for the pathogenic effects of areca nut chewing. The arecoline released by chewing areca nut is known to be involved in two different pathways, which

Table 2: Flow chart showing progression of oral submucous fibrosis to oral sqquamous cell carcinoma



results in the progression of OSMF. The major alkaloid of areca nut up-regulates keratinocyte αvβ6 expression. This is modulated through the M4 muscarinic acetylcholine receptor.[27] Latent TGF β1, which is a cytokine, is concentrated at high levels within the ECM. Activation rather than increased production often regulates its function.[37] Integrin αvβ6 may activate TGF β1[27] which results in increased quantity of activated TGF β1 in the ECM which causes alteration in the normal composition of ECM. Studies have shown that fibroblast activation could be achieved by an altered ECM composition. This might result in the differentiation of ECM fibroblasts into myofibroblasts. In addition, activated fibroblasts also secrete increased levels of ECM-degrading proteases such as matrix MMP2, MMP3 and MMP9, facilitating increased ECM turnover and altered ECM composition,[38] which leads to elevated differentiation of fibroblasts. On the other hand, Chang *et al.* revealed that arecoline treatment up-regulated the transcription of insulin-like growth factor-1 receptor (IGF-1R) mRNA and also induced phosphorylation of IGF-1R which induced ZEB 1 activation. They also demonstrated that ZEB 1 could bind to α SMA promoter site in the E-box region.[32] This results in increased expression of α SMA. Studies also show that increased α SMA alone is sufficient to enhance fibroblast contractile activity.[19] That is, increased α SMA promotes the differentiation of fibroblasts into myofibroblasts. Myofibroblasts, in turn, promote fibrosis which leads to the progression of OSMF. In addition, TGF β1 is also known to inhibit epithelial growth which results in epithelial atrophy, which is a characteristic feature of advanced OSMF.[27]

TGF β1 and Ras may modulate EMT, a process that contributes to tumor cell invasion. OSCCs in OSMF patients have a higher incidence of Ras mutations,[39] which might be the reason for OSCCs as a sequlae of OSMF.

CONCLUSION

Numerous models have been proposed for the pathogenesis of OSMF related to areca nut and its components. It

affects the connective tissue compartment where the toxic substances released from areca nut chewing precipitate a change in gene expression in the mesenchymal cells. The increased presence of myofibroblasts is proportional to the progression of the disease in all the studies included. The tissue culture and PCR analysis conducted on the samples have confirmed the results. These imply significantly, the role of myofibroblasts toward the progression of OSMF. However, the molecular mechanisms involved in this progression and the agents which act in the downstream process of arecoline-induced fibrosis in OSMF is still unclear.

We acknowledge the limitations faced during this review, due to the limited number of studies available on this aspect of OSMF and the authenticity and specificity of the antibodies employed to study myofibroblasts.

Studies have to be conducted in this front, with greater sample size combined with molecular and proteomic analysis. Other antibodies which are specific to myofibroblasts should be simultaneously employed to authenticate and verify the results obtained. This will help in obtaining the exact pathophysiology of OSMF which might help in the development of molecular targeted treatment protocols and methodologies in the treatment of OSMF.

Limitations

We acknowledge the presence of publication bias in this review. The employed parameters for evaluation in all the studies are not homogeneous; therefore, the review proceeded as a heterogeneous study. If methods and modes of evaluating could be more standardized with minimal data set, it would help by providing homogeneous data for systematic reviews in future.

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Conflicts of interest

There are no conflicts of interest.

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