Original Article

Role of osteopontin in oral epithelial dysplasia, oral submucous fibrosis and oral squamous cell carcinoma

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Abstract

Background: Inflammatory cells and cytokines in the chronically injured mucosa promote fibrosis in the oral submucous fibrosis (OSF) fibrotic milieu. Osteopontin (OPN) is a wound-healing mediator that upregulates the inflammatory response and is involved in the malignancy and fibrosis of multiple organ systems.

Objectives: We investigated the expression of OPN in oral potentially malignant disorders (OPMDs) and oral squamous cell carcinomas (OSCCs) to determine its role in the malignant transformation and fibrosis of oral tissues. The expression of OPN in OPMDs and OSCCs was compared and correlated, and the role of OPN as a fibrotic mediator in OSF was explained.

Study Design: A total of 30 cases of normal mucosa and OPMDs (mild dysplasia, severe dysplasia, OSF and OSCCs) were studied by purposive sampling. In these groups, OPN immunoreactivity was examined and correlated with clinical findings.

Results: In mild dysplasia, OPN expression was restricted to the basal cell layer with moderate staining intensity. In severe dysplasia, it was extremely intense and extended throughout the epithelium. In the OSF, OPN expression was moderate in the perinuclear areas of the basal cell layer. The expression of OPN was very strong in OSCC. A flow diagram explaining the profibrotic role of OPN in OSF has been provided.

Conclusion: A positive role of OPN in both pathogenesis and malignant transformation of OPMDs and OSCC has been demonstrated.

Keywords: Angiotensin I, angiotensin II, angiotensinogen, mast cells, oral squamous cell carcinoma, oral submucous fibrosis, osteopontin

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INTRODUCTION

It is well established that fibrosis and cancer are biological consequences of overhealing wounds.[1] Inflammatory cells and cytokines in the chronically injured mucosa frequently prime the fibrotic milieu. Areca nut-induced persistent injury to oral mucosa due to habitual quid chewing implicates oral submucous fibrosis (OSF) as an overhealing wound. Abnormalities in coagulation, fibrinolytic systems and myofibroblast persistence confirm OSF as an overhealing wound.[2]

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Osteopontin (OPN) is a noncollagenous protein also known as bone sialoprotein I (BSP-1). It is a crucial inflammatory mediator that has frequently been demonstrated to upregulate the inflammatory response, and as a result, it is crucial for wound healing, fibrosis and malignancy of numerous organ systems.[3-6] Additionally, inhibiting OPN expression during wound healing delays the development of granulation tissue and scarring.[3] Recent studies have implicated OPN as a mediator of myofibroblast differentiation induced by transforming growth factor β_1 (TGF-β_1), and it is abundantly expressed in the fibrosis of several organs.[6] Hence, studying the role of OPN in the fibrotic process of OSF would be of great value and provides insights into therapeutic strategies. We investigated the expression of OPN in oral potentially malignant disorders (OPMDs), particularly OSF and oral squamous cell carcinomas (OSCCs), to determine its role in the genesis of oral cancer from OPMDS. To our knowledge, this is the first study of OPN expression that demonstrates the function of OPN in causing fibrosis in OSF in a schematic manner.

MATERIALS AND METHODS

Patient demographics

A total of 120 cases, including 30 each of mild dysplasia, severe dysplasia, OSF and OSCC, were selected by purposive sampling between January 1998 and January 2008 from the archives of the Department of Oral Pathology and Microbiology, MCODS, Manipal. Normal human oral mucosa tissue samples (n = 30) were received from tooth extraction sites. The OSCC samples were further graded into well-differentiated, moderately differentiated and poorly differentiated squamous cell carcinoma. The clinical data were retrieved from the medical records.

Immunohistochemical staining

For immunohistochemical staining, 4-micron-thick tissue sections were obtained from formalin-fixed, paraffin-embedded tissue blocks. For antigen retrieval, deparaffinised sections were incubated in 0.01 M buffered citric acid and microwaved at 60°C for 10 min. To block endogenous peroxidase, sections were exposed to peroxide and protein-blocking agents for 5 min each and then washed with Tris-buffered saline (TBS) solution for 10 min to avoid nonspecific reactions. Mouse monoclonal anti-OPN antibody (IBL, Leica Microsystems) diluted 1:50 with 0.01 M phosphate-buffered saline (PBS) was applied as the primary antibody for 30 min at 37°C. After incubation with post-primary block and secondary polymer separately for 30 minutes and peroxidase-conjugated horseradish streptavidin, staining was visualised using 3,3'-diaminobenzidine (DAB) in 0.05 M Tris–HCl (pH 7.6) with hydrogen peroxide. The sections were then counterstained with haematoxylin and examined using a conventional light microscope. Staining was seen in 10 randomly selected high-power fields, corresponding to 2 mm^2 of tissue.

Evaluation of immunostaining

The immunostaining intensity was evaluated in accordance with the four-degree system, which categorises the staining into negative, weakly positive, moderately positive and strongly positive immunostaining. The staining was evaluated in epithelial layers in cases of OEDs and OSF and in tumour islands in cases of OSCC. The immunostaining distribution was considered positive in any one or all layers when more than 70% of the cells were positive for OPN.

Immunostaining was also evaluated in perimembranous and perinuclear areas in 10 random high-power fields. It was considered diffuse when more than 70% of the area showed both perinuclear staining and perimembranous staining. Staining was considered perimembranous or perinuclear when more than 70% of the field observed showed immunopositivity in either perimembranous or perinuclear areas, respectively.

Statistical analysis was performed by comparing the percentage of immunopositive specimens in each group utilising the modified Fisher's exact test and Pearson's Chi-square test.

RESULTS

Of 30 mild dysplasia sections, 12 had receded due to discontinuation of the habit, five had been treated surgically, five had not been reported back and eight had recurred. Eight severe dysplasia sections had not been reported back, seven had been treated surgically and fifteen had progressed to OSCCs. In the case of OSFs, there were four grade 1 lesions, six grade 2 lesions and twenty grade 3 lesions. All grades 1 and 2 were successfully treated with physiotherapy, hyaluronidase application and corticosteroid medication. Grade 3 lesions were subjected to various surgical treatments.

Two of the grade 3 lesions had progressed to squamous cell carcinoma (SCC) and were surgically treated. All cases of OSF had bilateral palpable fibrotic bands on the buccal mucosa. All ten well-differentiated OSCCs had been treated with surgery and radiotherapy, with no recurrences. Six of the ten moderately differentiated SCCs were successfully treated with chemoradiotherapy and surgery, two did not

return after surgery and two died within three years of surgical intervention. Three poorly differentiated SCCs died within two years after refusing surgical intervention for financial reasons, and seven had distant metastasis that was treated at the university's oncology department.

For immunohistochemical analysis, all the cases were reviewed by two experienced pathologists for OPN immunoreactivity. According to our findings, 20% of normal human oral mucosal specimens expressed OPN weakly in the spinous cell layers. As a positive control, normal human muscle tissue was used [Figure 1a]. Positive immunoreactivity was seen in 43.3% of the mild dysplastic lesions, 76.6% of the severe dysplastic lesions, 83.3% of the OSCCs and 73.3% of the OSF [Table 1].

Mild dysplasia samples with resolved lesions after cessation of habit showed moderate OPN expression in the perinuclear areas of spinous cell layers. Those cases with severe dysplasia expressed OPN with high intensity in all layers except the keratinised cell layers. Those lesions that recurred and were surgically treated expressed OPN with moderate intensity in perinuclear areas of the basal and parabasal cell layers [Figure 1b]. The severe dysplasia cases that had been successfully treated with early intervention showed moderate expression of OPN in perinuclear areas of the basal and parabasal cell layers. Lesions that had histopathologically progressed to microinvasive carcinoma and were treated with adjunctive radiotherapy showed strong OPN expression in all cell layers except keratinised cell layers, with diffuse immunolocalisation [Figure 1c].

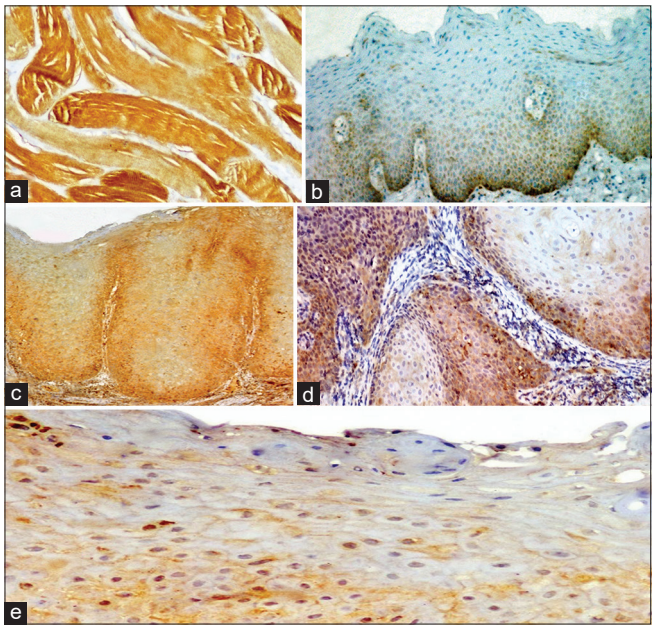

Figure 1: (a) OPN expression by muscle tissue of normal oral mucosa (positive control). (b) Expression of OPN in the basal and suprabasal layers in mild dysplasia. (c) Expression of OPN in the basal, suprabasal and upper layers in severe dysplasia. (d) Expression of OPN with moderate intensity in epithelial layers in OSF. (e) Expression of OPN in tumour islands with bright intensity in OSCC

OSF lesions in grades 1 and 2 showed weak OPN expression in basal layers localised in perinuclear areas. OSF grade 3 lesions exhibited moderate OPN expression in basal layers localised in both perinuclear and perimembranous areas [Figure 1d]. The majority of OSCC cases had strong positive staining in the cytoplasm of tumour islands and all layers of the overlying epithelium in both the perinuclear and perimembranous areas. Cases referred to oncology centres showed more intense staining in perinuclear areas [Figure 1e].

Table 2 summarises the patients analysed for OPN protein expression, including the number of cases, diagnosis, clinical type, age, sex and ethnicity as well as the number of OPN-positive lesions. Tables 3–5 show the overall expression of OPN in terms of intensity, tissue localisation and cellular distribution.

DISCUSSION

OPN is a secreted arginyl-glycyl-aspartic acid (RGD)-containing extracellular matrix protein that interacts with $\alpha_v\beta_1$, $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins.[7,8] This protein, which was discovered in bone, is also found in other tissues, including the epithelium of the gastrointestinal tract, exocrine glands and renal tubules. The expression of OPN as an extracellular matrix has been studied in relation to the carcinogenesis and metastasis of various malignant tumours, including lung, breast, oesophagus and salivary glands, and the results have suggested that OPN is a candidate biomarker of malignant tumours.[9,10]

OPN is capable of promoting cell attachment, chemotaxis and signal transduction in several cell lines as it acts as

Table 1: Clinical details of the cases included in the study

Diagnosis	Cases	Gender		Average age	Age range	Site					Habits	
		M	F			Lip	Cheek	Tongue	FOM	Ridge	Y	N
Mild dysplasia	30	20	10	55	19-72	5	10	3	4	8	22	8
Severe dysplasia	30	22	8	52	36-70	2	14	5	3	4	23	7
OSF	30	18	12	30	39-65	3	14	4	3	5	20	10
OSCC	30	16	15	54	25-57	4	16	4	2	4	25	5

a ligand for CD44 cell surface receptors and stimulates the chemotaxis of CD44-expressing cells.[11] Several extracellular matrix proteins bind OPN *in vitro*, including fibronectin (FN), osteocalcin and collagen types I, II, III, IV and V, and may modulate cell–extracellular matrix interactions *in vivo*.[12,13] Furthermore, by inducing proteinases, OPN promotes angiogenesis, increases the propensity of cells to metastasise and acts in conjunction with other growth factors to induce malignant properties.[7,8,14] OPN works as an early T lymphocyte activation protein 1 (Eta-1) and enhances the Th1 immune response but inhibits Th2 responses.[15] The mechanism by which OPN, a predominant Th1 cytokine (antifibrotic), demonstrates a profibrotic effect is not clear.[16] This protein was shown to be elevated in histological sections of several types of human precancer tissues compared with normal tissue.[17]

Many studies have shown that inflammatory mediators and growth factors, such as TNF-1, IL-1 and PDGF, play a role in the upregulation of OPN in OPMDs.[6,9,12] The observation of perinuclear expression of OPN with very weak intensity in the middle layers of normal oral epithelium in this study could be attributed to early inflammatory changes in the oral mucosa.

For many years, it has been recognised that OPN is associated with cellular transformation and tumorigenesis.[18] Devoll *et al*.[17] found positive OPN expression in hyperplastic, dysplastic and carcinoma *in situ* lesions of the oral epithelium. In our study, a heterogeneous pattern of OPN immunolocalisation, perinuclear and perimembranous staining and higher OPN expression found in cases of severe dysplasia indicated an early event in malignant progression.[10] OPN expression has been linked to patient survival in breast and lung carcinomas, implying that OPN may be a marker of disease progression.[19] The perimembranous and perinuclear immunostaining was observed in tumour nests, invasive tumour islands and surface dysplastic epithelium of OSCCs. This was explained in 2003 by Zhu *et al*.,[20] who discovered that under hypoxia, OPN expression is upregulated via a Ras-activated enhancer. Furthermore, *in vitro* studies indicate that OPN secretion into the local tumour microenvironment may promote tumour cell adhesion and migration by binding to cell surface receptors, such as β3 integrins and CD44. According to Zohar *et al*.[21] (2000), perimembranous OPN binds to CD44 receptors, which then bind to extracellular

Table 2: Immunoprofiling of OPN expression in all the cases analysed

Diagnosis	Total no. of cases	Total positive cases	Percentage positive cases (%)
Mild dysplasia	30	13	43.3
Severe dysplasia	30	23	76.6
OSF	30	22	73.3
OSCC	30	25	83.3

Table 3: Comparison in the intensity of OPN expression in the cases studied

Diagnosis	Total no. of cases	Negative staining	Positive staining		
			Weak	Moderate	Strong
Normal	30	24 (80%)	04 (13.3%)	02 (6.6%)	00 (0%)
Mild dysplasia	30	17 (56.6%)	04 (13.3%)	07 (23.33%)	02 (6.66%)
Severe dysplasia	30	07 (23.3%)	03 (10%)	06 (20%)	14 (46.66%)
OSF	30	08 (26.6%)	04 (13.3%))	14 (46.66%)	04 (13.3%)
OSCC	30	05 (16.6%)	03 (10%)	04 (13.3%)	18 (60%)

Table 4: Comparison in OPN expression in all the cases studied based on its localisation

Diagnosis	Cases (No.)	Negative staining	Localisation		
			Basal layer	Middle layer	Throughout
Normal	30	24 (80%)	01 (3.33%)	04 (13.3%))	01 (3.33%)
Mild dysplasia	30	17 (56.6%)	09 (30%)	02 (6.66%)	02 (6.66%)
Severe dysplasia	30	07 (23.3%)	04 (13.3%)	02 (6.66%)	17 (56.6%)
OSF	30	08 (26.6%)	12 (40%)	04 (13.3%))	06 (20%))
OSCC	30	05 (16.6%)	13 (43.33%)	03 (10%)	09 (30%)

Table 5: Comparison in the expression of OPN based on its cellular distribution

Diagnosis	Total no. of cases	Negative staining	Distribution		
			Perinuclear	Perimembranous	Diffuse
Normal	30	24 (80%)	1 (3.33%)	4 (13.3%)	1 (3.33%)
Mild dysplasia	30	17 (56.6%)	6 (20%)	3 (10%)	4 (13.3%)
Severe dysplasia	30	7 (23.3%)	5 (16.6%)	5 (16.6%)	13 (43.33%)
OSF	30	8 (26.6%)	12 (40%)	6 (20%)	4 (13.3%)
OSCC	30	5 (16.6%)	6 (20%)	5 (16.6%)	14 (46.66%)

matrix proteins, such as FN, osteocalcin and collagen types I, II, III, IV and V.

Studies have shown that OPN production in the stroma is likely at all stages of OSCC progression and is expressed in tumour cells only after malignant transformation.[22] The localisation of OPN during OSCC progression suggests that tumour-derived OPN may influence phenotypic features specific to later stages of progression. However, OPN appears to have antagonistic functions in both the host and the tumour. OPN derived from the host initially acts as a macrophage chemoattractant, whereas OPN-producing tumour cells keep macrophages inactive. Furthermore, tumour cell OPN expression correlated with an increase in metastases, which has been explained by differential and molecular changes that occur during tumour progression.[23]

Perimembranous staining was found in invasive neoplastic islands, which is consistent with the findings of Zohar *et al*., who proposed that intercellular/perimembranous OPN binds to CD44 and the ezrin–radixin–moesin (ERM) protein complex inside tumour cell plasma membranes.[21] It has been proposed that ERM proteins mediate the interaction between plasma membrane and actin filaments, regulating the formation of surface structures, such as microvilli, filopodia and membrane ruffles for cell motility and migration. In our study, a few sections of well-differentiated OSCC lacked perimembranous staining of OPN. This is explained by the fact that nonmigrating cells express cytoplasmic OPN but are unable to localise to perimembranous regions due to the lack of CD44 receptors.[12] The authors further state that the CD44 receptor is required for OPN to form the perimembranous OPN/CD44 ERM complex for cell motility.

The presence of OPN in tumour cells is thought to represent the secretory pathway and their ability to migrate and invade. Although it is unclear whether OPN has any functional differences, some studies suggest that posttranslational modification of OPN may alter protein-to-protein interactions or receptor binding on cells.[24,25] Furthermore, OPN expression varied within the histological grades of OSCC in the current study; thus, it is possible that different forms of OPN were secreted by tumour cells, which may use different receptors in transmitting unique signals during tumour progression.

However, the role of OPN as a profibrotic mediator in OSF has received less attention. A thorough review of the literature reveals that OPN not only initiates but also promotes several stages of fibrosis, as discussed in the following paragraphs. In our study, we found that OSF had higher OPN expression than mild dysplasia. OPN expression in OSF is consistent with its genesis as an overhealing wound due to chronic injury to oral mucosa, and this expression furthers the fibrotic process.[2,4,26-28]

Arecoline, a by-product of areca nut chewing, is cytotoxic to endothelial cells and causes endothelial damage.[29] Damaged endothelial cells release thrombin,[30] which can split full-length osteopontin (OPN-FL)/ osteopontin-A (OPN-A) into OPN-R.[31] OPN-R then binds to $\alpha_4\beta_1$, $\alpha_9\beta_1$ and $\alpha_1\beta_4$ integrin receptors[32,33] on inflammatory cells, such as mast cells, T cells, neutrophils and macrophages,[34-36] leading to their recruitment to the injured sites. Skin wound healing exhibits an overlapping expression of MMP-3 and OPN.[37] MMP-3 was found to be 10 times more abundant in OSF when compared to normal tissues,[38] and MMP-3 and MMP-7 then cleave OPN into OPN-R, which activates inflammatory cytokines as described[37] [Figure 2].

In a novel mechanism, the endocytosis of neutrophil-secreted elastase by fibroblasts, independent of the PI3K/ Akt pathway, causes myofibroblast differentiation. The collection of mast cells, neutrophils, T cells and macrophages and the repertoire of cytokines released contribute to the chronic inflammatory environment,[39-42] which promotes fibrosis.

OPN is produced by myofibroblasts and mast cells, and it contributes to the self-recruitment mechanisms of profibrotic inflammatory and immune cells to areas of fibrosis [Figure 2].[43,44] OPN expression was found in only 35% of OSF cases with atrophied epithelium, according to Routray *et al*. (2013).[45] However, Khan *et al*.[38] (2011) found a higher expression of OPN, which was consistent with OSF as an inflammatory lesion.

Mast cell degranulation amplifies the chronic inflammatory environment further. The mast cell chymase (MC_C) and mast cell tryptase (MC_T) released by mast cell degranulation induce fibroblast to myofibroblast transition.[46] However, mast cells are only relevant to this process in the very early and early stages of OSF; in later stages, they either do not survive in the fibrotic and hyalinised environment or have already degranulated.[47] As a result, it could be argued that mast cells initiate but do not maintain fibrosis. Furthermore, PDGF produced by macrophages aids in the transition from fibroblast to myofibroblast.[48]

Areca nut chewing leads to the production of reactive oxygen species (ROS), and ROS lead to the activation

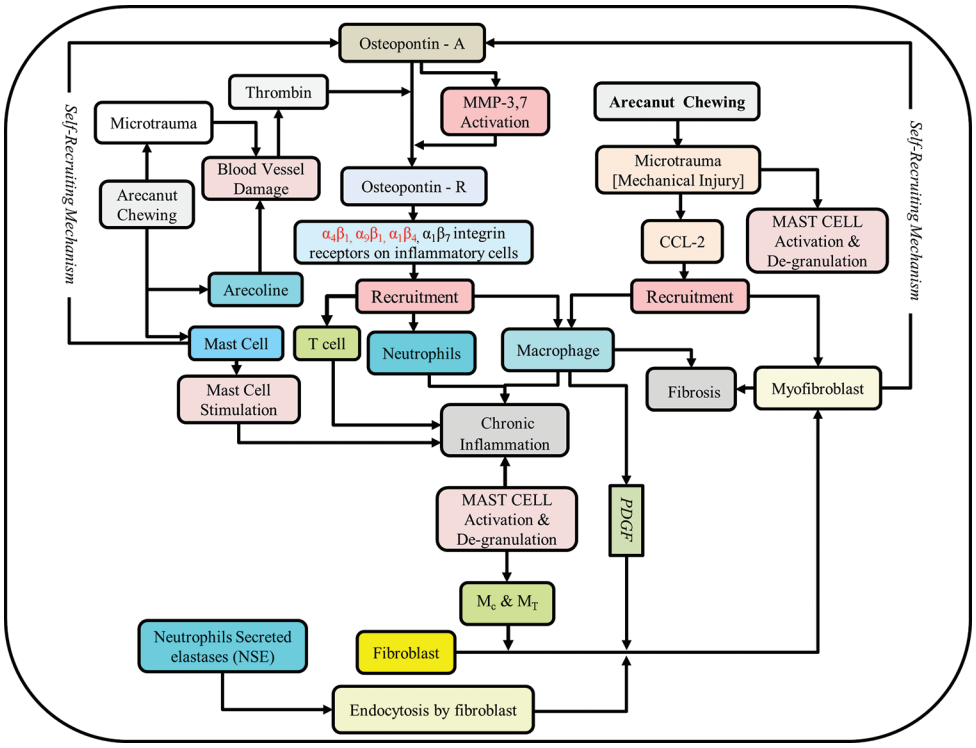

Figure 2: Schematic diagram illustrating the role of OPN in inflammatory and immune cell recruitment in areas of chronic injury and playing a central role in fibrosis

of renin, which converts angiotensinogen (AngN) into angiotensin I (AT-I).[49,50] Arecoline released as a by-product of areca nut chewing activates AT-1 into angiotensin II (AT-II) through angiotensin-converting enzyme (ACE). Mast cell degranulation leads to the release of Mc, which can independently convert AT-I to AT-II.[51] AT-II acts on AT-1 receptor to cause vasoconstriction, inflammation, oxidative stress and proproliferative and profibrotic effects.[52] AT-II acts on AT1R to promote OPN through the RAS/ERK/ETS-1 pathway.[52] AT-II may activate the antioxidant enzyme haem oxygenase-1 (HO-1).[50] HO-1 is upregulated in the areca nut chewers with OSF and OSSC,[53,54] and it has been linked to LNM.[54] HO-1 is dose-dependently upregulated by arecoline in OSF through the arecoline/ACE/AT-II/HO-1 pathway.[53,54]

ROS, such as H_2O_2 produced during areca nut chewing,[55] directly upregulate OPN via the eukaryotic translation initiation factor 4e (eIF4E), nuclear factor kappa B (NFκB) and activator protein 1 (AP-1).[56] OPN can act on $\alpha_v\beta_6$ integrin, which is highly expressed upon injury to epithelial cells, leading to TGF-β activation,[57] which then activates the renin–angiotensin circuit through ROS.[49,58] OPN upregulates itself through a positive feedback loop through downstream $\alpha_v\beta_3$ integrin/PI3K/Akt/NF-κB/HIF-1α/PAI-1.[6,31,59]

miR-21 is a profibrotic miRNA that promotes OPN expression through the previously mentioned positive feedback loop by suppressing PTEN.[60] Chronic OPN expression can lead to muscle fibrosis and contribute to limited mouth opening (LMO), while acute OPN expression contributes to muscle regeneration[28] [Figure 3a].

Several profibrotic factors, such as AT-II, TNF-α, IL-1, IL-6, S1P, PDGF and TGF-β, upregulate OPN.[6,12] AT-II acting on fibroblasts leads to the activation of the TGF-β/SMAD pathway, which leads to the accumulation of OPN, and OPN is essential for high mobility group box protein 1 (HMGB1) accumulation at focal adhesions (FAs). HMGB1 at FA through ERK leads to the upregulation of CTGF, and TGF-β through SMAD can also upregulate CTGF.[61] Thus, enhanced CTGF expression is central to the transdifferentiation of fibroblasts into myofibroblasts.[61] Several authors have shown CTGF to play an important role in the pathogenesis and malignant transformation of OSF.[2,38,62,63] However, the observation that α-SMA expression is absent in TGF-β-treated cardiac fibroblasts in OPN null mice[61] highlights the importance of OPN in the orchestration of fibrosis. OPN, through the CD44/JAK/STAT-3 pathway, produces OPN, forming a positive feedback circuit.[64] AT-II acting on AP-1 leads to the upregulation of miR-21, which through the SMAD-7/ERK pathway leads to the upregulation of CTGF and myofibroblast.[60] CTGF through chaperone action

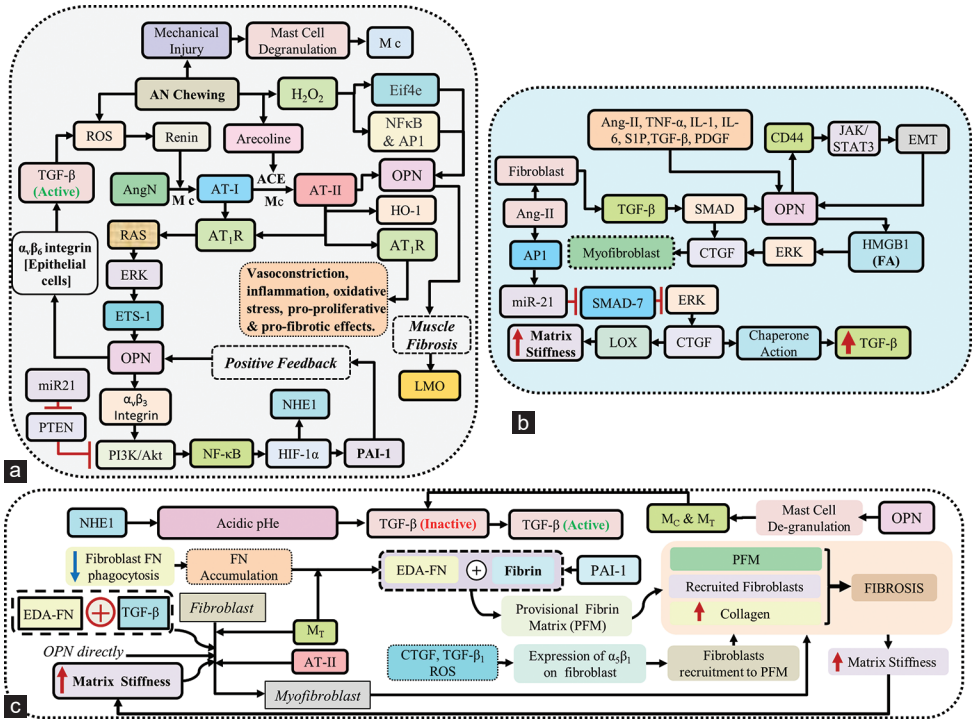

Figure 3: (a) OPN and its positive feedback mechanisms in the pathogenesis of OSF and muscle fibrosis leading to LMO. (b) Crosstalk between OPN and profibrotic mediators and the essentiality of OPN in myofibroblast differentiation. (c) Central role of OPN in fibroblast–myofibroblast transition and TGF-β activation through stimulation of mast cell granulation

facilitates TGF-β interaction with its receptors, and TGF-β in turn amplifies CTGF production, forming a positive feedback loop.[65] CTGF contributes to matrix stiffness through LOX-induced matrix crosslinking[66] [Figure 3b].

Chewing areca nut can lead to mechanical injury, which leads to the degranulation of the mast cell release of MC_C and MC_T release in the stroma [Figure 3a]. MC_C released from degranulated mast cells activate TGF-β. The hypoxic environment in OSF, mediated by HIF-1, increases NHE1 expression, resulting in extracellular pH (pHe) acidification.[67] Acidic pHe resulting from activated NHE1 may further activate TGF-β[68] [Figure 3a and 3c]. Reduced FN phagocytosis results in FN accumulation,[2] and FN is acted on by MC_T to produce EDA-FN. EDA-FN, together with fibrin (produced by PAI-1) from the provisional fibrin matrix (PFM), forms a scaffold for myofibroblasts to orchestrate fibrosis.[2] EDA-FN is required for myofibroblast activation and precedes it, and EDA-FN–fibroblasts exhibit decreased responsiveness to active TGF-1 and fail to activate latent TGF-1 for unknown reasons.[69] The CTGF, TGF-β_1 and ROS enhance the expression of $\alpha_5\beta_1$ on fibroblasts, which aids in their recruitment to the PFM.[2]

AT-II and OPN can independently induce the transition of fibroblasts to myofibroblasts.[6,46] Increasing matrix stiffness,[70] MC_C and MC_T,[46] TGF-β,[2] CTGF[2] and EDA-FN[69] all work together to promote the transition of fibroblasts to myofibroblasts. OPN is also secreted by mast cells and promotes their degranulation,[43] leading to the release of MC_C and MC_T in the matrix and the activation of TGF-β [Figure 3c].

In a murine dermal fibrosis model, it has been shown that OPN null mice develop less fibrosis than wild-type mice. OPN null mice show fewer Mac3+ macrophages and reduced inflammatory mediators along with reduced TGF-β1 and its downstream signalling.[5] Since OPN serves as a relay in TGF-β1 and CTGF-induced myofibroblast differentiation,[4,61] its reduced level leads to impaired myofibroblast differentiation in response to exogenous TGF-β1.[61] However, some fibroblasts manage to be converted into myofibroblasts by other mechanisms.[6,46,70]

CONCLUSION

The higher levels of expression of OPN in severe dysplasia compared with mild dysplasia indicate the role of OPN in predicting the higher propensity for malignant transformation. Additionally, high OPN expression in grade 3 OSF proves it to be a useful marker to identify cases with high malignant potential. OPN expression in tumour cells and invasive islands, with perimembranous staining in invasive islands, confirms its prognostic value. OPN may

be an important mediator of fibrosis and malignancy in oral lesions. Studying the expression of OPN in patients with OSCC, both pre- and posttreatment, can be useful in monitoring disease progression. Several pathways highlighting the profibrotic role of OPN in OSF have been proposed. While some of these fit in the proposed schema illustrating the role of OPN and its positive feedback mechanisms in the pathogenesis of OSF, others require further research and validation.

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Conflicts of interest

There are no conflicts of interest.

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