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Interplay of epigenetics and epistasis drives oral submucous fibrosis

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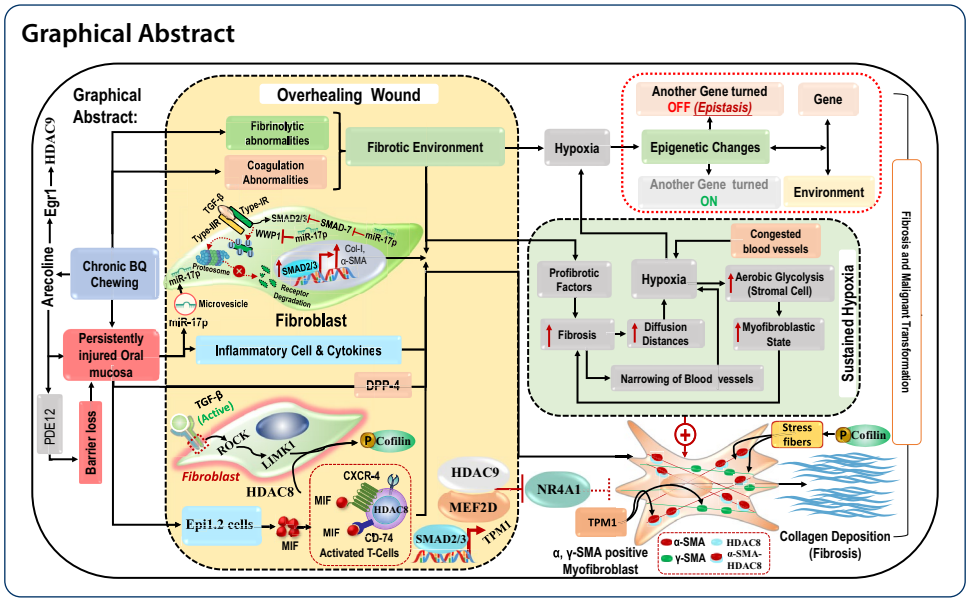
Abstract

Persistent injury to oral mucosa due to habitual quid chewing, resulting in the upregulation of inflammatory cytokines and consequential myofibroblastic persistence, emphasizes the role of epigenetic aberration in the pathogenesis of oral submucous fibrosis (OSF). However, there is a dearth of research on the role epistasis plays in the pathophysiology of OSF. Among the important epistatic interactions in the pathophysiology of OSF are those between Phosphatase and Tensin Homologue (PTEN) and Insulin-Like Growth Factor 1, Transforming Growth Factor-β (TGF-β), Cyclooxygenases, and lipoxygenases (LOX). Additionally, PTEN and Nuclear Factor Kappa B (NF-κB) have an epistatic relationship that is particularly mediated by the p65 subunit of NF-κB. Given the importance of epigenetic modification in the pathogenesis of OSF, the potential use of DNA methyltransferase and Histone deacetylase inhibitors as a therapeutic option holds promise. Another in vivo epigenetic therapeutic option for treating OSF is using stimulatory microRNAs against antifibrotic genes and inhibitory microRNAs against profibrotic genes. This review aims to connect numerous epigenetic and epistatic components to the mechanism of OSF. A better understanding of the disease process may provide OSF management with newer therapeutic options.

Article Highlights

- Epigenetic changes from chronic oral injury drive persistent scarring and raise cancer risk in oral submucous fibrosis.
- Unique gene–gene interactions amplify fibrosis, revealing new targets for future drug therapies.
- Understanding these molecular pathways opens the door to innovative, precision-based treatments for this serious oral disease.
- Cyclooxygenase (COX) and Lipoxygenase (LOX) enzymes that generate ROS and profibrotic mediators, contributing to PTEN inactivation and fibrosis.

Keywords Oral submucous fibrosis, Epigenomics, Epistasis, DNA methyltransferases, Histone deacetylase inhibitors



1 Introduction

Oral submucous fibrosis (OSF) is a chronic, progressive disease affecting the oral mucosa, causing reduced mouth opening, burning sensations, and facial deformities. It's a potentially malignant disorder, with 3%-10% of patients developing oral cancer [1]. Around 600 million people worldwide chew areca nut (AN), with approximately 5% of AN chewers being OSF patients, according to epidemiological statistics [2, 3]. Multiple studies across various countries have demonstrated a correlation between areca nut chewing and OSF [3, 4].

When compared with other oral potentially malignant disorders, oral leukoplakia (OLE), the 10-year malignant transformation rates (MTR) for OSF and OLE were 10.82 (± 1.73) and 9.58 (± 1.02), respectively. The difference in MTR between OLE and OSF was further magnified with increasing follow-up times. These findings are corroborated by log-rank test results for long-term (≥ 10 years) time-to-event analysis, indicating that OLE + OSF carries a higher risk of MTR than OLE and OSF alone ($p = 0.0086$). Furthermore, OSF has a higher MTR than OLE for follow-up periods exceeding 10 years. The 14-year MTR for OSF patients increased to 20%, nine years following the diagnosis of OLE, while the oral cancer rate stabilized at 10%. The study indicates that while oral cancer incidence in OLE plateaued after 10 years, it significantly increased in OSF patients, leading to the recommendation of a 10-year surveillance period for OLE, whereas for OSF, it should extend to 15 years [5, 6]. Yang et al. in a 2017 statewide cohort study in Taiwan, with a 13-year follow-up period, reported a 9.13% MTR (1.9 to 8.63% in previous studies) [7]. Chiang et al., in 2020, reported that in a 5-year follow-up at a single hospital in Taiwan, 18.5% of OSF + OLE transformed to cancer, compared to 4.6% of OSF patients alone [8].

According to these investigations, OSF is one of the most significant public health issues and may have the potential to develop into malignancy. The aetiology of OSF is currently thought to be complex, nevertheless. The start and progression of OSF are known to be influenced by metabolic disruption, hereditary factors, and chemical and physical irritants. Despite the superfluity of research in this field, a deeper

comprehension of molecular interactions, particularly epigenetics, is necessary to address this worldwide health issue.

2 Epigenetic and epistatic drivers of OSF: the overhealing wound paradigm

Epigenetics is the study of heritable changes in gene expression that are reversible without involving changes in the DNA sequence. Fibrosis is greatly influenced by the epigenetic changes caused by DNA hyper- and hypomethylation, histone acetylation and deacetylation, and microRNAs, among others [9–12]. Considering all these factors, epigenetic modifications may best explain the transition of fibroblasts into myofibroblasts and their persistence in any fibrotic disorder. These factors may also be important for the pathogenesis of OSF [11, 13], which is now regarded as an overhealing wound [14, 15]. Chronic injury to the oral mucosa caused by habitual quid chewing sustains a proinflammatory microenvironment characterized by persistent infiltration of immune cells and elevated cytokine levels. This ongoing inflammatory stimulus drives an aberrant wound-healing response, central to the pathogenesis of OSF. Dysregulation of coagulation and fibrinolysis pathways, coupled with the prolonged presence of activated myofibroblasts, perpetuates a hyper-reparative state. This pathological repair response not only contributes to progressive fibrosis but also creates a microenvironment conducive to malignant transformation [2, 15–19].

While oral keratinocytes may indirectly contribute to the profibrotic effects of arecoline on buccal mucosal fibroblasts (BMFs) by altering their collagen metabolism [20], emerging evidence points to a more direct role of epithelial cells in initiating fibrosis. A recent study on OSF identified a distinct epithelial cell subset, termed Epi1.2, characterized by both profibrotic and proinflammatory phenotypes. These Epi1.2 cells initiated the fibrotic process in fibroblasts through interactions with T cells via receptor-ligand interactions between macrophage migration inhibitory factor (MIF)- Cluster of Differentiation 74 (CD74) and C-X-C chemokine receptor type 4 (CXCR4) [16]. The Dipeptidyl peptidase 4 (DPP-4) secretion by OSF epithelium drives the activation of fibroblasts, which also suggests an overhealing process. DPP-4 inhibitors seem to reverse the activation of alpha smooth muscle actin (α-SMA) in fibroblasts. Increased DPP-4 levels and collagen deposition correlate with excessive scarring and fibrosis [17]. Arecoline, a byproduct of AN chewing, through upregulation of phosphodiesterase 12 (PDE12) localized within the mitochondrial matrix of oral mucosa cells, contributes to oral mucosal barrier loss [18] and makes the mucosa conditioned for further injury. The concept of oral mucosa as an overhealing wound is further supported by an epigenetic mechanism involving the induction of miR-17p in oral epithelial cells exposed to arecoline. The miR-17p-laden exosomes secreted by oral epithelial cells fuse with the fibroblast cell membrane, and intracellular miR-17p then upregulates the profibrotic signaling in fibroblasts by two mechanisms. First, binding to the 3-UTR region of SMA Mothers Against Decapentaplegic-7 (SMAD-7) inhibits it and second, by inhibition of E3 ubiquitination ligase WW domain-containing E3 ubiquitin protein ligase 1 (WWP1), which targets Transforming Growth Factor-beta Receptor I (TGF-βR-I) for ubiquitin-mediated proteasomal lysis, both changes result in upregulation of Collagen-I and α-SMA through augmented SMAD2/3 signaling [19]. Epistasis is a well-known concept in genetics where one gene masks the effect of another gene. This review seeks to connect numerous epigenetic and epistatic elements in the mechanism of OSF considering the dismal forecast that OSF

cases will increase due to an increase in BQ use. This may provide a better understanding of the disease process, offering rational therapeutic options for managing OSF. The pathogenesis and malignant transformation of OSF caused by epigenetic and epistatic alterations are depicted in a graphical abstract (Fig. 1, Graphical Abstract).

3 Epigenetic mechanisms hardwired in the genesis of fibrosis

Epigenetic reprogramming orchestrates the conversion of temporary repair into permanent scarring in fibrosis, a maladaptive reaction to chronic damage. There is growing evidence that fibrotic development is hardwired across tissues by four interrelated epigenetic axes: histone acetylation, deacetylation, hypoxia-mediated remodeling, and a persistent inflammatory milieu.

3.1 Histone acetylation in activation of TGF-β

Recent research suggests that the development of fibrosis may include inbuilt epigenetic processes [21–23]. This is clearly illustrated by the fact that epigenetic machinery is used during the transcription of collagen by TGF-β [13, 24–27].

Egr-1 independently stimulated collagen 1A1 (Col1A1) expression in normal fibroblasts by directly binding to its promoter [26, 27]. There was synergistic induction of OSF by constitutive overexpression of the Egr-1 protein in BMFs and increased TGF-β signalling during areca nut chewing. TGF-β-induced collagen production, cell migration, and myofibroblast transdifferentiation were all reduced in Egr-1-deficient fibroblasts, despite intact Smad activation, indicating that the full complement of fibrotic response to TGF-β requires Egr-1 [27].

This can be explained by the following mechanism: 1. TGF-induced Egr-1 binds to the Egr-1 binding Sequence (EBS) in p300 promoter to upregulate p300 expression. Augmented p300 expression causes increased acetylation of the promoter chromatin of the Col1A1 gene, facilitating its transcriptional activation by Smad2/3 [24]. Additionally,

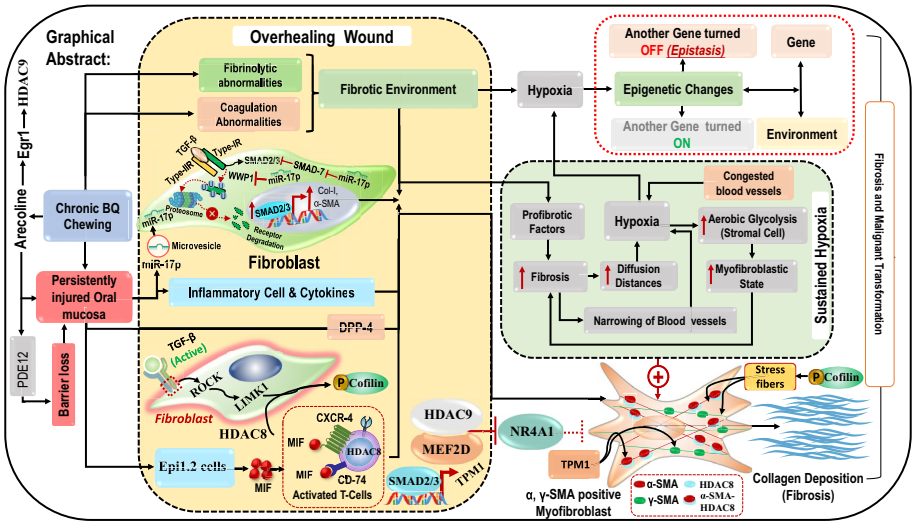


Fig. 1 Graphical Abstract: Oral submucous fibrosis is an overhealing wound caused by chronic chewing of betel quid, leading to barrier loss and a sustained inflammatory environment. This environment activates fibroblasts and epithelial cells, producing inflammatory cytokines and promoting differentiation into myofibroblasts. Key epigenetic regulators, such as HDAC8, HDAC9, and miR-17p, contribute to fibrosis and malignant transformation in OSF, perpetuating hypoxia and fibrotic remodeling

p300 increases the amount of plasminogen activator inhibitor I (PAI-1) that TGF-β1 induces by acetylating histone residues on the PAI-1 promoter [13]. TGF-β1 induced Transcription factors like Specificity Protein 1 (Sp1) also amplify the process [25]. PAI-1 is thought to accelerate fibrosis by facilitating the accumulation of fibrin [28, 29].

The current highly selective p300 inhibitors like CCS1477, A-485, B026, CREB-binding protein 30 (CBP30) and NEO2734 target both p300 and CREB-binding protein (CBP), leading to side effects [30–34]. BT-O2C is a recent development that selectively targets p300, sparing CBP. BT-O2C achieves greater selectivity by binding the histone acetyltransferase domain of p300 and an E3 ubiquitin ligase like cereblon and von Hippel–Lindau (VHL). This creates a ternary complex that tags p300 for proteasomal degradation, sparing CBP due to structural differences. BT-O2C acts catalytically, enabling the degradation of multiple p300 molecules per degrader, reducing the dose required and minimizing off-target effects [35, 36]. These findings suggest that p300 inhibition could mitigate fibrosis in OSF by reducing collagen synthesis and ECM deposition (Fig. 2A).

3.2 Histone deacetylation: the role of HDACs in the pathogenesis of OSF

Fibrotic disorders are linked to the modulation of cellular signaling by histone deacetylases (HDACs), enzymes that modify epigenetic changes by removing the acetyl group from histone's N-acetyl-L-lysine amino acid. The HDAC superfamily includes Zn-dependent and NAD-dependent classes. HDAC8, a member of class I HDACs, promotes the development of oral squamous cell carcinomas (OSCCs), while class I HDAC inhibition suppresses OSCC initiation and recurrence [37].

3.2.1 HDAC-8, 1 and 5

Immunocytochemistry findings indicate a cytoskeleton-like distribution of HDAC-8, and double-immunofluorescence staining and confocal microscopy showed HDAC-8 co-localized with α-SMA in stress fibre-like structures [38]. TGF-β through Rho-associated

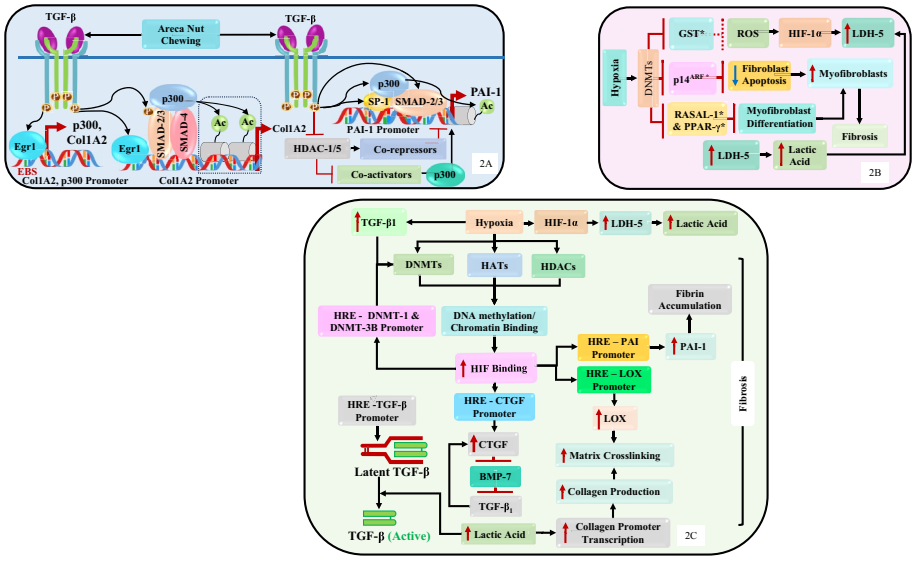


Fig. 2 **A** The epigenetic pathway is hardwired in fibrosis through activation of TGF-β/Early Growth Response protein 1 (Egr1)/p300/Col1A2, TGF-β/p300/SMAD/Col1A2 and TGF-β/p300/SMAD/PAI-1 (B) through hypoxia-induced hypermethylation (indicated by *) of RASAL-1 & PPAR-γ, p14ARF & GST and **C** Hypoxia-induced Regulation of Fibrotic Pathways via TGF-β1, HIF-1α, and Epigenetic Modifications

protein kinase (ROCK)/cytokine LIM kinase 1 (LIMK1) upregulates phospho-cofilin [39], with HDAC-8 aiding in the phosphorylation of Cofilin [40]. Only when α-SMA and γ-SMA are incorporated into stress fibers does the myofibroblast develop contractile capabilities, at which point it should be regarded as a functionally competent cell. Phospho-cofilin may help α-SMA and γ-SMA integrate into stress fibers by cutting the actin filaments. This process is also aided by trophomyosin-1 (TPM1) through TGF-β/SMAD2/3 pathway in OSF [39]. This indicates that it is a critical HDAC involved in the cytoskeletal modelling of Myofibroblasts, the knockout of HDAC-8 halts OSF progression, consistent with this role [37] (Fig. 1, Graphical Abstract).

Inspired by the garlic component ajoene, a new class of sulfur-based compounds has been created with a unique zinc-binding group. These substances exhibit strong HDAC8 selectivity over other HDAC isoforms [41]. Virtual screening using ligands and structures revealed new scaffolds for novel, non-hydroxamic acid inhibitors. Specifically, SD-01 and SD-02 showed excellent selectivity for HDAC8 and substantial nanomolar inhibition (IC_{50}: 9.0 nM and 2.7 nM, respectively) [42]. The established HDAC8 inhibitor PCI-34051 promotes replication stress and genome instability in cancer cells, which results in high synthetic lethality when paired with checkpoint kinase inhibitors. HDAC8i-1 is another potential inhibitor that was discovered in recent high-throughput epigenetic screens. Similar to PCI-34051, it is effective when combined with checkpoint kinase inhibitors in preclinical cancer models [43]. HDAC 1 & 5 in OSF seem to have an antifibrotic role as they activate the co-repressors of PAI-1 and inhibit co-activators of PAI-1 (Fig. 2A).

3.2.2 HDAC 9

HDAC9 is involved in the activation of fibroblasts, facilitating their transformation into myofibroblasts, which are characterized by increased expression of markers such as α-SMA and collagen type I. Arecoline-induced Egr-1 seems to drive HDAC9 upregulation. HDAC9 regulates the expression of pro-fibrogenic genes induced by TGF-β, thereby promoting the fibrotic response and ECM deposition. Knockdown of HDAC9 significantly inhibits the transformation process, reducing collagen gel contraction, cell migration, and wound-healing abilities of fibroblasts, highlighting its critical role in this transition [44].

The transcriptional complex formed by HDAC9 and myocyte enhancer factor 2D (MEF2D) prevented the production of Nuclear Receptor Subfamily 4 Group A Member 1 (NR4A1), a pro-apoptotic gene targeted by MEF2D. As a result, HDAC9 knockdown markedly reduced cell division and triggered cell death. This data suggested that the HDAC9/MEF2s axis may play a role in myofibroblasts' ability to avoid apoptosis in OSF tissue and may signal the possibility of malignant transformation [44] (Fig. 1, Graphical Abstract).

3.3 Hypoxia initiates fibrosis and propagates it through epigenetic mechanisms

3.3.1 Sustained hypoxia is obligatory for the initiation and maintenance of fibrosis

Hypoxia is critical for the maintenance of fibrosis, as demonstrated by a number of studies, including OSF [2, 15, 45–49], is sustained through several mechanisms:

1. As fibrosis progresses, oxygen diffusion distances from blood vessels to cells gradually increase, which worsens hypoxia [49].

2. Hypoxia also increases a number of profibrotic mediators like PAI-1, Platelet Derived Growth Factor (PDGF), TGF-β, connective tissue growth factor (CTGF), basic fibroblast growth factor (b-FGF), Transforming Growth Factor-α (TGF-α), Tissue Inhibitor of Matrix Metalloproteinase-1 (TIMP-1), Lysyl Oxidase (LOX), Endothelin-1 (ET-1), and Vascular Endothelial Growth Factor (VEGF), which further the fibrotic state [15, 29, 49–52].
3. The hypoxic state induces Glycolytic Switch, i.e. a shift to aerobic glycolysis in stromal cells, contributing to the persistence of myofibroblasts and ongoing fibrosis [15].
4. Chronic inflammation through constant AN chewing, contributes to OSF by disrupting normal tissue responses to ischemia, resulting in a hypoxic microenvironment and perpetuating inflammation, thereby aggravating tissue damage and fibrosis [2, 53, 54].
5. The constricted blood vessels due to fibrosis and congested blood vessels resulting from local hypercoagulability also add to the hypoxia profile [2, 15].

As a result, hypoxia and fibrosis create a continuous cycle that amplifies one another (Fig. 1, Graphical Abstract).

3.3.2 Hypoxia-induced gene methylation facilitates fibrosis

Hypoxia induced hypermethylation of antifibrotic genes (indicated by the * sign in figures), which is an important event in fibrosis induction. Hypermethylation of Glutathione-S-Transferase (GST) lowers antioxidant defenses and increases ROS production [55]. ROS scavenging nanomaterials can be mooted as newer therapeutic agents for the treatment of fibrosis [56]. Increased ROS triggers the overexpression of Hypoxia Inducible Factor-1α (HIF-1α), which in turn activates the anaerobic Lactic Dehydrogenase-5 (LDH-5) isoform, promoting anaerobic glycolysis and lactic acid generation [57]. Lactic acid increases enhanced myofibroblast activation through increased TGF-β activation [58]. The Gossypol, an LDH inhibitor, effectively inhibited TGF-β-induced myofibroblast differentiation in both healthy and fibrotic human lung fibroblasts. It also reduced the expression of collagen 1, collagen 3, and fibronectin, and reduced the generation of extracellular lactic acid and acidification [58]. However, Gossypol inhibits all isoforms of LDH [59], leading to undesirable side effects. The recent pyridazine derivative RS6212 has shown specificity to inhibit LDH-5, which might hold promise [60] (Fig. 2B).

p14 Alternate Reading Frame (p14ARF) hypermethylation, which inhibits apoptosis, allows the persistence of fibroblasts [12, 61]. 5-Azacytidine (5-AzaC) or 5-Aza-2'-Deoxycytidine (5-Aza-CdR), and HDAC inhibitors like trichostatin A (TSA) can epigenetically activate $p14^{ARF}$ [62]. Moreover, the hypermethylation of RAS Protein Activator Like 1 (RASAL1) and peroxisome proliferator-activated receptor-γ (PPAR-γ) prevents the dedifferentiation of myofibroblasts [12, 63, 64]. The ten-eleven translocation (TET) family enzyme TET3, which catalyzes DNA demethylation via hydroxymethylation, can be specifically induced by Bone Morphogenic Protein-7 (BMP-7). BMP-7 significantly reduces methylation of the RASAL1 promoter, thereby enhancing RASAL1 expression [65]. Administration of 5-Aza-CdR and glycyrrhizic acid (GA) demethylated PPARγ promoter, restored PPARγ loss and alleviated fibrotic lung pathologies, including structural alterations and adverse expression of fibrotic mediators and inflammatory cytokines [66] (Fig. 2B).

3.3.3 Hypoxia-induced upregulation of DNA methylation enzymes

The key enzymes mediating epigenetic modification influencing fibrosis through hypoxia are DNA methyltransferase (DNMT), histone acetyltransferase (HAT), and HDACs. These mediators of epigenetic regulation increase HIF binding to HIF-responsive elements (HRE) of DNMT-1 & DNMT-3B promoters [67]. Additionally, HRE of promoters of CTGF [68], TGF-β [69], PAI-1 [70] and LOX [71] increase the profibrotic signaling upon HIF binding. Through the upregulation of TGF-β and the promotion of fibrosis, BMP-7 is downregulated by CTGF, and it even appears to be necessary for TGF-β's profibrotic effect [72] (Fig. 2C). The culmination of all these hypoxia-induced modifications results in fibrosis and constitutively activated myofibroblasts.

3.4 Inflammation initiates fibrosis and propagates it through epigenetic mechanisms

Areca nut chewing induced inflammation upregulates the inflammatory cytokine Interleukin-6 (IL-6). IL-6 acts through its receptor IL-6 receptor (IL-6R), which comprises of the ligand-binding chain (IL-6Rα) and the signal-transducing chain (gp130) subunits. Upon binding to IL-6R, the Janus kinase (JAK)/Signal Transducer and Activator of Transcription-3 (STAT-3) pathway signalling is initiated, which then downregulates SMAD-7. Since SMAD-7 downregulates TGF-β, SMAD7 inhibition leads to upregulation of TGF-β signaling [73].

IL-6 employs miR-148a and miR-152 to epigenetically upregulate the expression of DNMT-1 [74]. As a result, IL-6 increases its own activity by inhibiting its antagonist (p53-wt) via DNMT-1 induced hypermethylation. Also inhibits RASAL1, p14ARF, Phosphatase and Tensin Homologue (PTEN), PPARγ by the same mechanism [74, 75]. The increased IL-6 signaling changes the caveolin receptor to the non-raft fraction, which results in a reduction in the caveolin-mediated internalization of TGF-β and an amplification of TGF-β signaling [76].

The inflammation-induced DNMT-1 upregulation also promotes fibrosis through hypermethylation of the PTEN promoter and upregulation of Akt, Erk and FAK pathways [74, 75, 77, 78]. IL-6 is increased in BMFs, and studies have indicated that this enhances OSF [79, 80]. According to Tsai et al. (2004), the depletion of intracellular glutathione in OSF fibroblasts leads to an increase in IL-6, with arecoline acting as a dose-dependent mediator of this effect [79].

IL-6 through STAT-3 upregulates miR-21 [81], which then inhibits SMAD-7 [82].TGF-β and Arecoline upregulate NADPH Oxidase-4 (NOX-4), which then augments ROS [83, 84], and subsequent miR-21 upregulation [85]. Given the persistent inflammation that exists in the microenvironment of OSF, the increased levels of cytokines that are linked with inflammation, such as IL-6, may channel epigenetic mechanisms to propagate OSF (Fig. 3A).

Current clinical trials examining IL-6 inhibitors as antifibrotic agents are predominantly in phases 2 and 3. Tocilizumab, an IL-6 receptor inhibitor, has undergone phase 2 and phase 3 trials for systemic sclerosis-associated interstitial lung disease. Phase 3 results indicate a potential benefit in preserving lung function, although the primary endpoint related to skin fibrosis was not achieved. Moreover, new inhibitors targeting the IL-6/JAK/STAT3 pathway are currently undergoing early-phase clinical trials for fibrotic diseases [86]. DNMT inhibitors like 5-AzaC and 5-Aza-CdR are widely used for

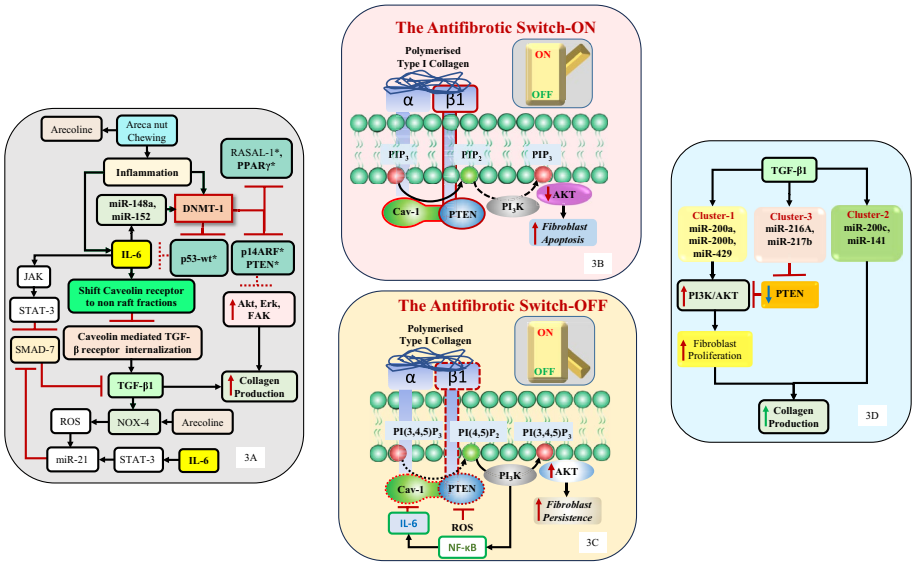


Fig. 3 **A** Schematic representation of molecular mechanisms involved in AN-induced fibrotic signalling pathways leading to collagen production. AN chewing introduces arecoline, which triggers inflammation and activates interleukin-6 (IL-6) production. IL-6 further upregulates miR-148a and miR-152, influencing DNMT-1 expression. DNMT-1 downregulates tumor suppressor genes such as p53-wt, p14ARF, and PTEN, promoting activation of Akt, Extracellular signal-regulated kinase (Erk), and Focal adhesion Kinase (FAK) pathways, which contribute to enhanced collagen production. Inflammatory responses also suppress RASAL-1 and PPARγ, further exacerbating fibrotic signaling. Arecoline stimulates NOX-4 expression, leading to increased Reactive Oxygen Species (ROS) and miR-21, which enhance TGF-β1 signaling. TGF-β1 expression is influenced by STAT-3 activation and further modulated by caveolin-mediated TGF-β receptor internalization. JAK/STAT-3 and SMAD-7 pathways interact with IL-6 and TGF-β1 to regulate caveolin receptor localization, affecting receptor internalization and signaling. **B** The Antifibrotic Switch-ON: An Epistatic Pathway Regulating Fibroblast Apoptosis. **C** The Antifibrotic Switch epistatically switched "OFF" through the ROS/PTEN pathway and the PI3K/NF-κB/IL-6/Caveolin-1 (Cav-1) pathway. **D** Schematic representation of the regulatory network involving TGF-β1 and miRNA clusters in fibroblast proliferation and collagen production. TGF-β1 activates three distinct miRNA clusters: Cluster-1 (miR-200a, miR-200b, miR-429), Cluster-2 (miR-200c, miR-141), and Cluster-3 (miR-216A, miR-217b). Cluster-1 promotes PI3K/AKT signaling, enhancing fibroblast proliferation. Cluster-3 downregulates PTEN, further activating the PI3K/AKT pathway. Both mechanisms synergistically lead to increased collagen production. Cluster-2 also contributes to collagen production through a separate pathway. Red lines indicate inhibitory interactions, and arrows indicate activation

haematological malignancies, with nearly 70 in development. However, fibrosis inhibitors are in preclinical or early clinical research [87–89].

4 Epigenetic and epistatic interaction in OSF

The PTEN gene is a tumor suppressor gene that acts as a negative regulator of the Akt/PKB signaling pathway. PTEN dephosphorylates FAK, inhibits AKT activation [90], downregulates adhesive signaling and inhibits fibrosis. PTEN loss is seen in a number of cancers and fibrotic disorders, including OSF, and this loss signals the onset of TGF-β induced myofibroblast differentiation [77, 78]. Loss of epithelial PTEN thereby disrupts the process of epithelial repair, exacerbates epithelial damage, and unfavorably advances to fibrosis [77, 91], which is aided by the phosphorylation of FAK and PI3K/Akt pathways [91] (Fig. 3A).

The mechanism underlying the loss of PTEN expression in OSF appears to be epigenetic, which was previously unknown. Significantly, ROS mediates fibrosis in OSF by oxidizing the nucleophilic cysteine residue of the PTEN protein, leading to loss of PTEN function [92]. Furthermore, ROS have been proven to stimulate TGF-β, creating a vicious loop [93, 94].

Low membrane expression of PTEN often correlates with low membrane expression of Cav-1 [91]. Overexpression of Cav-1 restores PTEN levels, inhibits AKT phosphorylation and fibroblast proliferation, indicating the role of Cav-1 as a determinant of membrane PTEN levels. Normally, fibroblast interaction with polymerized type I collagen results in the formation of Cav-1-PTEN-β_1 integrin complex in the fibroblast membrane, positioning PTEN in a precise location to inhibit PI3K/AKT signal generated by integrin β_1-matrix interaction. PTEN is a lipid phosphatase and is epistatic to Phosphoinositide 3-kinase (PI3K)/AKT/mTOR by dephosphorylating PI3K product Phosphatidylinositol (3,4,5)-trisphosphate (PIP-3) into phosphatidylinositol 4,5-bisphosphate (PI (PIP-2). PIP-3 dephosphorylation into PIP-2 helps to downregulate the PI3K/AKT/mTOR signaling pathway, leading to fibroblast Apoptosis, the antifibrotic switch is turned "ON" [95–97] (Fig. 3B). However, loss of Cav-1 or any of the three members of this complex leads to reduced membrane accumulation of PTEN-Cav-1-β1 integrin complex, turning the antifibrotic switch "OFF". This restricts the ability of PTEN to inactivate AKT phosphorylation [96], thereby facilitating fibrosis (Fig. 3C).

4.1 Role of microRNA in OSF

TGF-β also regulates three microRNA clusters via miR-192. Cluster 1 consists of miR-200a, miR-200b, and miR-429, and it drives fibroblast proliferation via the PI3K-Akt pathway and upregulates collagen [98]. Cluster 2 includes miR-200c and miR-141, while cluster 3 includes miR-216a and miR-217, both of which have been demonstrated to mediate fibroblast proliferation through the PI3K-Akt pathway [98]. TGF-β has also been demonstrated to inhibit PTEN through miR-216a, miR-217 and miR-21 [99, 100]. TGF-β-mediated increases in miR-21 block inhibitory SMAD-7, thereby promoting fibrosis [101–103] (Fig. 3D).

No FDA-approved drugs directly target miR-216a and miR-217, but preclinical studies suggest PI3K/Akt inhibitors (e.g., LY294002) or TGF-β pathway blockers (e.g., galunisertib) could counteract their effects [104, 105]. The expression of miR-21 can be suppressed by increasing methylation at the promoter region. According to studies, using miR-21 inhibitors (such locked nucleic acid (LNA) anti-miR-21) raises the expression of DNMT1, DNMT3A, which lowers miR-21 levels and boosts promoter methylation [106]. Preclinical models have employed synthetic miR-200a/b mimics to decrease fibrosis and restore function [107–109].

4.2 TGF-β is epistatic to PPAR-γ

Areca nut mediated epithelial injury may lead to fibrosis by upregulating CCL-2/MCP-1 [14], which in turn, downregulates prostaglandin E2 (PGE2) and PPAR-γ, both of which are positive regulators of PTEN stimulating its expression [110]. It has been demonstrated that PPAR-γ is epistatic to PAI-1 and suppresses endothelial and platelet activation [111]. The combined effects of these processes make up the antifibrotic action of PPAR-γ. TGF-β is epistatic to PPAR-γ [112]. Thus, PPAR-γ and TGF-β_1 demonstrate reciprocal epistatic effects [112, 113]. This coercive interaction shifts to a more fibrotic phenotype through IL-6, IL-13, Wingless-related integration site (Wnt), lysophosphatidic acid (LPA), CTGF, hypoxia, epigenetic modifications along with CCL-2/MCP-1, all of which inhibit PPAR-γ [14, 113]. Arecaidine, another Areca nut byproduct has also been shown to be epistatic to PPAR-γ [20] (Fig. 4A).

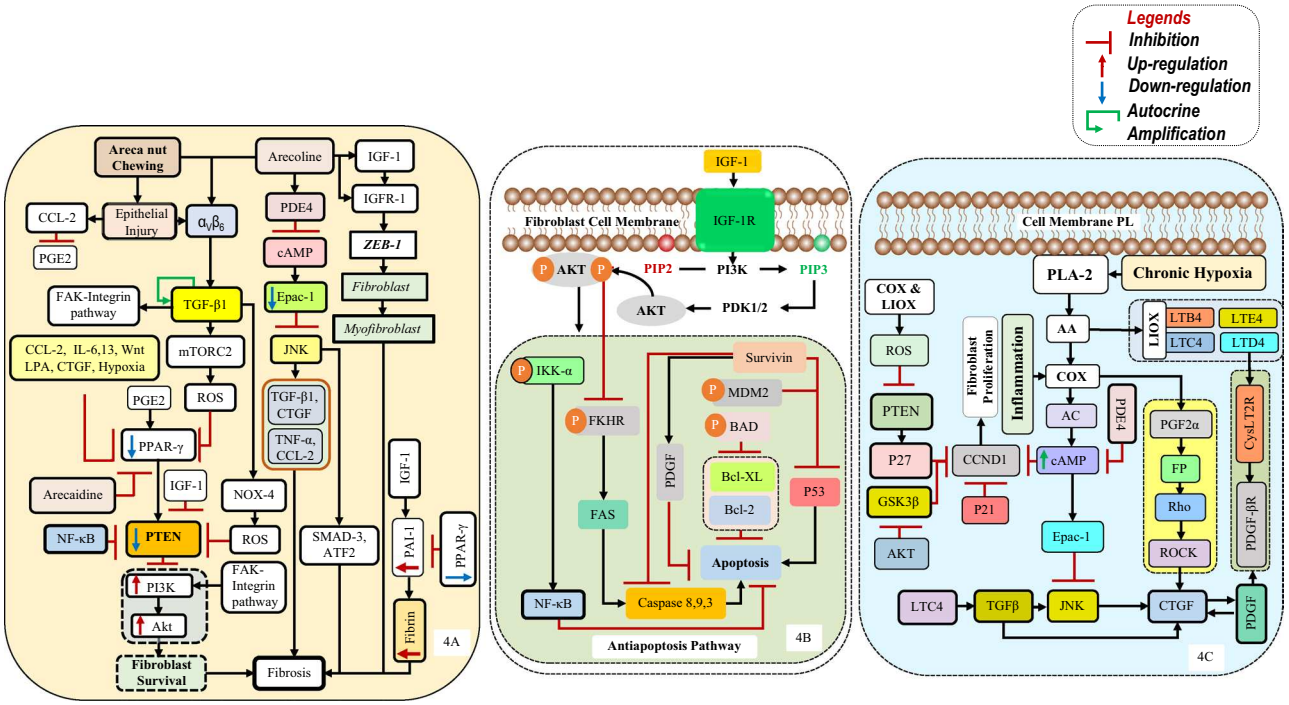


Fig. 4 **A** Schematic of the molecular mechanisms by which areca nut chewing contributes to OSF. The process initiates with areca nut exposure, leading to epithelial injury and activation of the αvβ6 integrin, which stimulates TGF-β1 signaling—a central pro-fibrotic cytokine. TGF-β1 activation is further enhanced by multiple stimuli such as C motif chemokine 2 (CCL-2)/Monocyte Chemoattractant Protein-1 (MCP-1), IL-6/13, Wnt, LPA, CTGF, and hypoxia, driving downstream pathways including mTORC2, ROS generation, and FAK-integrin signaling, ultimately promoting fibrosis. Arecoline, a major alkaloid in areca nut, inhibits PDE4, reducing cAMP levels and Epac-1, which leads to JNK activation and further induction of pro-fibrotic mediators like TGF-β1, CTGF, TNF-α, and CCL-2. Additionally, arecoline upregulates IGF-1, which, through IGFR-1 and Zinc finger E-box-binding homeobox 1 (ZEB-1), facilitates fibroblast-to-myofibroblast differentiation. These myofibroblasts, along with NOX-4 mediated ROS and SMAD-3/ATF2 transcription factors, exacerbate fibrosis. Negative feedback mechanisms, such as PGE2, PPAR-γ, and PTEN, attempt to counterbalance fibrosis by inhibiting the PI3K/Akt pathway, fibroblast survival, and pro-inflammatory responses mediated by NF-κB. However, suppression of these anti-fibrotic regulators enhances PI3K/Akt activation, promoting cell survival and sustaining fibrotic responses. Finally, the figure also highlights PAI-1 and fibrin as downstream elements contributing to fibrosis. Arrows indicate activation, while red bars represent inhibitory actions, illustrating the dynamic interplay between pro- and anti-fibrotic signaling in the pathogenesis of OSF. **B** IGF-1 associated Epistatic Pathway in OSF. **C** Chronic hypoxia, Chronic inflammation, COX and LIOX mediated epistatic pathways in OSF

4.3 *TGF-β_1 is epistatic to PTEN*

TGF-β_1 inhibits PTEN via the TGF-β1-mTOR complex 2 (mTORC2)-ROS pathway, which downregulates PPAR-γ [83, 114–116]. Additionally, it is epistatic to the PTEN gene via NOX-4, which increases ROS generation and thus oxidizes the nucleophilic cysteine residues of the PTEN protein, inhibiting PTEN's phosphatase activity [83, 92]. The NOX-4 gene has been found to be overregulated in OSF through hypomethylation [12, 83]. Although compounds such as GKT-136901 and Setanaxib are commonly referred to as NOX-4-selective inhibitors, they also inhibit NOX-1. The dual NOX-1/4 inhibition may promote early vascular ageing, causing increased perivascular fibrosis and inflammation [117–119]. This suggests the need for next-generation therapeutics that selectively inhibit NOX-4 while sparing NOX-1, achieving antifibrotic efficacy without adverse vascular effects, in OSF. As a result of elevated CTGF around the blood vessels, loss of PTEN expression around the blood vessels may be the cause of the perivascular fibrosis seen in OSF [91, 120]. As CTGF is undetectable in normal tissues and only upregulated in fibrosis [51], making it an attractive molecular target of drugs like Pamrevlumab, which are Peptides targeting CTGF (Phase II clinical trials) [121, 122].

In BMFs, arecoline increases phosphodiesterase-4 (PDE-4), which blocks the activity of Exchange Protein Directly Activated by cAMP 1 (EPac1) by inhibiting cAMP [123]. Although it has been demonstrated that both protein kinase A (PKA) and EPac1 inhibit JNK [124], the PKA pathway is inactive in BMFs [123]. JNK is unable to be inhibited by low EPac1 levels, and as a result, profibrotic factors such as TGF-β1, CTGF, TNFα, and CCL-2 are upregulated by JNK in conjunction with SMAD-3 and Activating Transcription Factor 2 (ATF2) [125] (Fig. 4A).

Insulin-like growth factor-1 (IGF-1) is markedly increased in a variety of fibrotic disorders, including OSF [126–129]. IGF-1 overexpression, however, has a synergistic effect on fibrosis when expressed just before TGF- β_1 but does not cause fibrosis on its own [126]. The *in-vitro* animal models of fibrosis have confirmed this sequential pattern of expression [126]. Furthermore, α-SMA, a marker of myofibroblast increases following the co-expression of IGF-1 and TGF-β_1, confirming their role in myofibroblastic proliferation [126]. While IGF-1 may induce myofibroblastic differentiation via ZEB-1 (Fig. 3B), neither TGF-β_1 inhibitors nor IGF-1 inhibitors reverse fibrosis [126], implying that the signaling activity downstream is important. TGF-β_1 is required for the initiation of fibrosis, while CTGF is required for the maintenance of fibrosis. CTGF, however, remains constitutively active in areas of fibrosis after its initial induction and escapes regulation by TGF-β_1 [51, 130, 131]. IGF-1, along with ROS, may directly promote fibrosis by suppressing PTEN protein (Fig. 4B).

IGF-1, through its receptor on the fibroblast membrane, activates PI3K, which then converts PIP-2 to PIP-3. PIP-3 then activates phosphoinositide-dependent kinase-1/2 (PDK-1/2), which then phosphorylates Akt/Protein Kinase B (Akt/PKB). Akt/PKB, with its recruitment on the cell membrane, inhibits fibroblast apoptosis through a molecular circuit involving Bcl-2-associated death promoter (BAD), B-cell lymphoma 2 (BCL-2), B-cell lymphoma extra-large (Bcl-XL), PDGF, Forkhead Transcription factor (FKHR), First Apoptotic Signal (FAS), Nuclear Factor Kappa-B (NF-κB) and Inhibitor of Nuclear Factor Kappa-B Kinase-α (IKK-α). IGF-1 receptor inhibition lowers fibrosis by facilitating apoptosis of the fibroblasts [126, 132] (Fig. 4B).

It is interesting to note that IGF-1 (as well as TGF-β_1 and NF-κB) inhibits PTEN by decreasing its phosphorylation or indirectly through the upregulation of NF-κB phosphorylation (Fig. 4A). The fibroblast proliferates because of the suppression of P21, P27 and Glycogen Synthase Kinase-3 (GSK-3), which increases Cyclin D1 (CCND-1) expression (Fig. 4C).

Additionally, the cyclooxygenase (COX) and lipoxygenase (LIOX) are epistatic on the PTEN gene, as they generate ROS by metabolism of arachidonic acid (AA), leading to inactivation of the PTEN protein through oxidation [92, 133]. LIOX produces profibrotic leukotrienes—LTB-4, LTC-4, LTD-4, LTE-4 by its effect on AA metabolites. The latter three leukotrienes, through cysteinyl leukotriene 2 receptor (CysLT2R) may transactivate PDGF-βR, which then causes fibrosis via PDGF-CTGF signaling [134]. In addition, LTC-4 may also directly upregulate TGF-β_1 [134]. Inflammatory environment upregulates COX, which also has profibrotic action via PGF-2α/Prostaglandin F Receptor (FP)/Rho/ROCK/CTGF pathway [28]. Furthermore, phospholipase A-2 (PLA-2) via its action on cell membrane phospholipids may bestow additional risk for fibrosis by providing a steady state AA level [135]. Cytosolic PLA-2 has been shown to be activated in the chronic hypoxic environment, which is commonly found in OSF [136–138]. Cyclooxygenase (COX) and Lipoxygenase (LOX) enzymes that generate ROS and profibrotic mediators, contributing to PTEN inactivation and fibrosis (Fig. 4C). Table 1 summarizes the epistatic relationships and interactions relevant to the role of TGF-β, PPAR-γ, PTEN, and other factors in the context of fibrosis, particularly in OSF.

Table 1 Summary of epistatic interactions in pathogenesis of OSF

Factor 1	Factor 2	Epistatic Relationship	Mechanism	Effect on Fibrosis	Reference
CCL-2	PGE2 & PPAR-γ	CCL-2 is epistatic to PGE2 & PPAR-γ	Downregulates PGE2 & PPAR-γ, positive regulators of PTEN	Suppresses PTEN, initiates myofibroblast differentiation	[14, 110]
TGF-β1	PPAR-γ	TGF-β1 is epistatic to PPAR-γ	Downregulates PPAR-γ via mTORC2-PKCα, IL-6, IL-13, Wnt, LPA, CTGF	Fibrotic phenotype; PPAR-γ inhibition removes antifibrotic brake	[112, 113]
Arecaidine	PPAR-γ	Arecaidine is epistatic to PPAR-γ	Inhibits PPAR-γ transcriptional activity in oral mucosa	Blocks antifibrotic response and promotes fibrosis	[20]
PPAR-γ	PAI-1	PPAR-γ is epistatic to PAI-1	Suppresses endothelial/platelet activation, inhibiting PAI-1 expression	Reduces pro-fibrotic signaling	[111]
TGF-β1	PTEN	TGF-β1 is epistatic to PTEN	Inhibits PTEN via mTORC2/PKCα and NOX-4-mediated ROS	PTEN loss promotes perivascular fibrosis	[92, 114]
IGF-1	PTEN	IGF-1 is epistatic to PTEN	Inhibits PTEN via PI3K/AKT and NF-κB phosphorylation	Promotes fibroblast survival and proliferation	[126, 132]
NF-κB	PTEN	NF-κB is epistatic to PTEN	Activates via IKKβ, suppresses PTEN	Promotes fibrogenic signaling	[132, 133]
COX/LIOX	PTEN	COX and LIOX are epistatic to PTEN	ROS from arachidonic acid metabolism oxidizes/inactivates PTEN	Enhances fibrotic response	[92, 133, 134]
TGF-β1	miR-21	TGF-β1 is epistatic via miR-21	Inhibits SMAD7, upregulates TGF-β loop	Sustains fibrotic signaling	[101, 102]
miR-216a/217	PTEN	miR-216a/217 inhibit PTEN	Post-transcriptional suppression of PTEN	Activates PI3K/AKT, fibroblast proliferation	[99, 100]

5 Potential biomarker is OSF

Based above, we suggest the following biomarkers

1. TGF-β is a central profibrotic cytokine, upregulated in OSF, that drives collagen transcription and myofibroblast activation.
2. CTGF maintains fibrosis after initial TGF-β induction; serum CTGF levels correlate with clinical staging and histopathological grading, suggesting utility as a serological biomarker.
3. PTEN loss by epigenetic silencing or ROS-mediated oxidation is associated with myofibroblast persistence and fibrosis in OSF.
4. NF-κB -Upregulated and epistatically interacts with PTEN, contributing to OSF pathogenesis.
5. IGF-1-Markedly increased in OSF; works synergistically with TGF-β1 to promote myofibroblast differentiation and fibrosis.
6. HIF-1α -Overexpressed in response to hypoxia and ROS; activates anaerobic glycolysis and promotes myofibroblast activation.
7. GST hypermethylation leads to reduced antioxidant capacity and increased ROS, contributing to OSF.
8. α-SMA & γ-SMA are marker of myofibroblast differentiation; increases with co-expression of IGF-1 and TGF-β1.
9. Cav-1 Low membrane expression correlates with low PTEN and increased fibrosis; overexpression restores PTEN and inhibits fibroblast proliferation.
10. MicroRNAs such miR-21, miR-192, miR-200 family, miR-216a, miR-217, miR-141, miR-148a, miR-152 regulate TGF-β signaling, PTEN inhibition, and fibroblast proliferation in OSF.

6 Future perspectives and therapeutic targeting of OSF through epigenetic mechanisms

Based on the above discussion, we have identified several epigenetic targets that might be utilized in the treatment of OSF in future studies. These targets are mainly based on the reactivation of the classical Vitamin A pathway by retinoic acid (RA) with concomitant use of epigenetic modifiers and the Calcitriol pathway.

Vitamin A is taken up by the cell, crosses the cell membrane, and is acted upon by the enzymes responsible for RA synthesis, and converted into RA [139]. Within the Cytosol, RA binds to the cellular retinoic acid-binding protein (*CRABP*), which transports it to the nucleus [140]. Once within the nucleus, it binds to the heterodimer of Retinoic acid receptor-*α* (*RAR-α*) and *retinoid X receptor* (*RXR*). *RAR-α-RXR heterodimer then binds to* Retinoic acid response elements (RARE), causing the transcription of Retinoic acid receptor-*β* (*RAR-β*) and downregulation of TERT [140–142]. Transcription of RAR-*β* is facilitated by an unmethylated promoter & acetylated Histones. RAR-*β transcription* promotes differentiation, arrest, and eventual apoptosis [140]. The above pathway is active at the physiological levels of RA [140] (Fig. 5A).

All trans-retinoic acid (ATRA) upregulates of CBP/p300, which then acetylates lysine 373 of p53 [143, 144]. This leads to p53 dissociation from E3-ubiquitin ligases like Human Double Minute2 (HDM2) and Tripartite motif-containing 24 (TRIM24), thereby stabilizing p53 [144] (Fig. 5B). The activated p53 binds to tumour necrosis factor

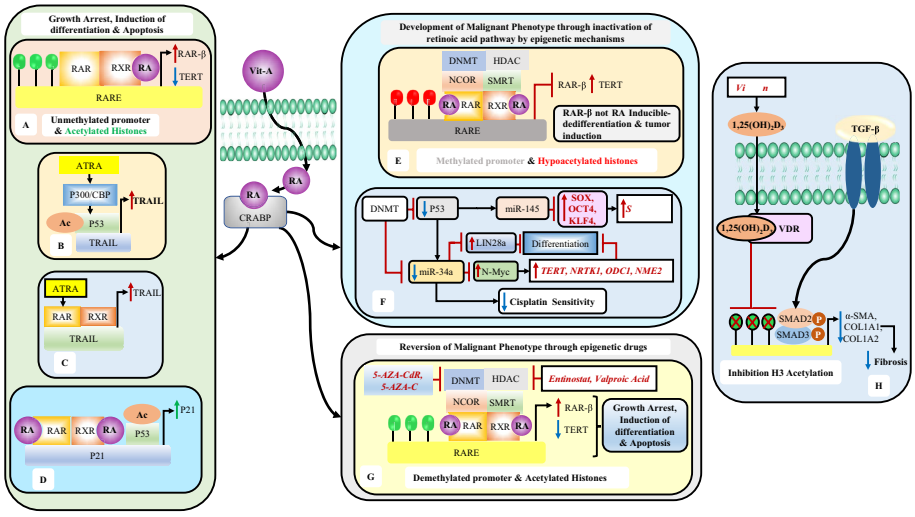


Fig. 5 Epigenetic targeting OSF and other OPMDs (**A**) Role of normal RAR/RXR pathway in the induction of growth arrest and differentiation induction by vitamin A. **B** Activation of apoptosis through ATRA via an epigenetic mechanism. **C** Activation of apoptosis through ATRA via TRAIL through RAR-RXR heterodimer binding to the TRAIL promoter. **D** Induction of growth arrest by binding of RXR-RXR homodimer or RXR-RAR heterodimer to p21 promoter or through activated p53 binding to p21 promoter. **E** Induction of methyltransferases like DNMT cause promoter methylation, and induction of deacetylases like HDAC leads to hypoacetylated histones, which render the pathway inoperative. This leads to dedifferentiation and tumor induction. **F** DNMT induction also leads to downregulation of tumor suppressor p53, and leading to upregulation of stem cell factors SRY (sex-determining region Y)-box 2 (SOX2), Octamer Transcription Factor 4 (OCT4), Krüppel-like factor 4 (KLF4), and Lin-28 Homolog A (LIN28A), while downregulation of miR-34-a leads to an augmentation of Telomerase Reverse Transcriptase (TERT), neurotrophic receptor tyrosine kinase 1 (NRTK-1), NME/NM23 Nucleoside Diphosphate Kinase 2 (NME2), Ornithine Decarboxylase 1 (ODC 1) through n-Myc. **G** Use of HDAC inhibitors like Entinostat & Valproic Acid and DNMT inhibitors like 5-AzaC or 5-Aza-CdR, along with ATRA, can inhibit tumors. H Vitamin D induced inhibition of myofibroblasts differentiation from fibroblasts and reduced profibrotic gene expression like COL1A1, COL1A2 and α-SMA

(TNF)-related *apoptosis*-inducing ligand (*TRAIL*) promoter activating it [144]. Another mechanism by ATRA that can be used to upregulate *TRAIL* in cancer cells and induce apoptosis is by binding as a ligand in RXR-RAR heterodimer in TRAIL promoter [144] (Fig. 5C). While activated p53 can bind to the p21 promoter upregulating it [143, 145]. Retinoids through RXR-RXR homodimer or RAR-RXR Heterodimer by binds to P21 promoter can cause its upregulation leading to p21-mediated cell growth arrest [145] (Fig. 5D).

At pharmacological doses of retinoic acid, the recruitment of epigenetic modifiers such as nuclear receptor corepressor (NCOR) and silencing mediator of retinoic acid and thyroid hormone receptor (SMRT) still enables the RAR-β transcription [140]. But continued recruitment of epigenetic silencers like DNMT and HDACs renders classical RA pathway inoperative [140]. The inhibition of RAR-β causes the upregulation of TERT, which in turn promotes progression and the dedifferentiation of tumor (Fig. 5E).

While DNMT inhibits p53 by methylating it, p53 activates miR-34a and miR145 [143]. Additionally, miR-34a, a tumor suppressor microRNA that blocks n-Myc, is inhibited by DNMT. These microRNA suppress the stemness associated factors like SOX2, OCT4, KLF4, and Lin-28 Homolog A (LIN28A) [143]. n-Myc in turn promotes TERT, NRTK-1, NME, and ODC1. LIN28A downregulation and upregulation of TERT, NRTK-1, NME2, and ODC1 [146]. The net result of these epigenetic changes is the suppression of differentiation (Fig. 5F).

The inhibitors of DNMT (5-AzaC and 5-Aza-CdR) and HDAC (*Entinostat, Valproic Acid)* in concert with all-trans retinoic acid (ATRA) could be utilized to reactivate the classical RA pathway leading to growth inhibition & induction of differentiation & apoptosis of cancer cells [140] and downregulation of TERT (Fig. 5G).

Calcitriol (1,25(OH)2D3), also known as the active form of vitamin D binds to the active vitamin D receptor (VDR) and exhibits antifibrotic actions via suppressing histone 3 (H3) (lys-9) deacetylation without altering the SMAD2/3 phosphorylation. As a result, the transformation of fibroblasts into myofibroblasts is slowed down, and profibrotic molecules such as collagen 1A1 (COL1A1), collagen 1A2 (COL1A2), α-SMA, and fibronectin are present at reduced levels [83] (Fig. 5H).

7 Prevention strategy for OSF

Vitamin E (α-tocopherol) demonstrates efficacy in alleviating clinical symptoms such as restricted mouth opening and reduced tongue protrusion in OSF patients. It also reduces oxidative stress markers. Vitamin C (ascorbic acid) offers protective effects against lipid peroxidation, with lower levels observed in OSF patients compared to healthy controls. β-carotene supplementation has been associated with symptomatic improvement through reduction of malondialdehyde levels and enhancement of antioxidant status [141]. Lycopene acts by mitigating inflammation in OSF through the reduction of ROS and the modulation of gene expression involved in collagen deposition and degradation [142].

Meta-analyses indicate that curcumin treatment does not significantly improve mouth opening, burning sensation, or tongue protrusion in OSF patients. However, it significantly alleviates burning sensation over six months, suggesting benefits related to bioavailability [143].

Comparative studies reveal spirulina as more promising for clinical improvements, such as mouth opening and ulcer healing. Conversely, lycopene has demonstrated greater efficacy in similar assessments [144, 145].

8 Conclusion

The pathogenesis of OSF could be likened to a "molecular baton race", in which molecular mediators emerge sequentially as fibrosis progresses, with TGF-β as the initiator of fibrosis, HIF-α as a transitional amplifier and mediator and CTGF as an endpoint profibrotic agent. We would like to summarize some of the key points in our manuscript that have not previously been highlighted in the pathogenesis of fibrotic lesions, particularly OSF.

1. Epigenetic & non-epigenetic loss of PTEN function in OSF is a precursor to TGF-β induced myofibroblast differentiation.
2. Epigenetic and or Direct or indirect epistasis effects of IGF-1 and TGF-β_1 on PTEN or the epistasis of COX and LIOX on PTEN are relevant to the pathogenesis of OSF.
3. IGF-1 mediates its epistatic effects on PTEN either directly by decreasing phosphorylation or indirectly by increasing NF-κB phosphorylation.
4. TGF-β1 is epistatic to PTEN, via TGF-β1-mTORC2-PKCα pathway, resulting in downregulation of PPAR-γ. TGF-β1 is also epistatic to the PTEN gene through NOX-4 via enhanced production of ROS. Even COX and LIOX are epistatic to the PTEN gene through increased ROS.

5. NF-κB is epistatic to PTEN genes via the inhibitor of nuclear factor kappa-B Kinase-β (IKK-β) pathway.

Thus, restoring PTEN activity is an exciting treatment strategy for reducing fibrosis in OSF. Epigenetic intervention thus bears promise in the therapy of OSF, given that both surgical and therapeutic techniques to treat OSF have largely been unsatisfactory, with the former even accompanied by rebound fibrosis.

Abbreviations

Akt	Protein Kinase B
AN	Areca nut
ATRA	All-trans retinoic acid
BMF	Buccal mucosal fibroblast
Cav-1	Caveolin-1
CBP	CREB binding protein
CCL	Chemokine (C–C motif) ligand
COX	Cyclooxygenase
CTGF	Connective tissue growth factor
DNMT	DNA methyltransferase
ECM	Extracellular matrix
Epac-1	Exchange Protein Activated by cAMP-1
Egr-1	Early Growth Response Protein 1
ET-1	Endothelin-1
FAK	Focal adhesion kinase
GST	Glutathione-S-transferase
HDAC	Histone deacetylase
HIF-1α	Hypoxia-inducible factor 1-alpha
IGF-1	Insulin-like Growth Factor 1
IGFR	Insulin-like Growth Factor Receptor
IL	Interleukin
JAK	Janus kinase
JNK	C-Jun N-terminal kinase
KLF4	Kruppel-like factor 4
LDH	Lactate dehydrogenase
LIMK	LIM kinase
LIN28A	Lin-28 Homolog A
LOX	Lysyl oxidase
LPA	Lysophosphatidic acid
MEF2D	Myocyte enhancer factor 2D
miR	MicroRNA
mTORC2	Mammalian Target of Rapamycin Complex 2
NF-κB	Nuclear factor kappa B
NMDK1	Nucleoside Diphosphate Kinase 1
NOX-4	NADPH Oxidase 4
ODC1	Ornithine Decarboxylase 1
OCT4	Octamer-binding transcription factor 4
OSF	Oral submucous fibrosis
OPMD	Oral potentially malignant disorder
PAI-1	Plasminogen activator inhibitor 1
PDE	Phosphodiesterase
PDGF	Platelet-Derived Growth Factor
PI3K	Phosphoinositide 3-kinase
PPAR-γ	Peroxisome proliferator-activated receptor-gamma
PTEN	Phosphatase and Tensin Homolog
RAR	Retinoic acid receptor
RASAL-1	RAS Protein Activator Like 1
RAR/RXR	Retinoic acid receptor/Retinoid X receptor
ROCK	Rho-associated, coiled-coil-containing protein kinase
ROS	Reactive oxygen species
RXR	Retinoid X receptor
SD	Standard Deviation
SMAD	Mothers Against Decapentaplegic Homolog
SMA	Smooth muscle actin
SOX	SRY-Box Transcription Factor
STAT3	Signal Transducer and Activator of Transcription 3
TERT	Telomerase Reverse Transcriptase
TET	Ten-Eleven Translocation Enzymes
TGF-β	Transforming growth factor beta
TIMP-1	Tissue Inhibitor of Metalloproteinase-1

TNF-α	Tumor Necrosis Factor-alpha
TRAIL	TNF-related apoptosis-inducing ligand
TPM1	Tropomyosin 1
VEGF	Vascular Endothelial Growth Factor
ZEB-1	Zinc finger E-box-binding homeobox 1

Author contributions
MS: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Writing-original draft; Writing-review & editing. SSS: Data curation; Formal analysis; Methodology; Validation; Writing-review & editing. MS: Conceptualization; Formal analysis; Methodology; Validation; Writing-review & editing. FA: Data curation; Investigation; Resources; Methodology; RR: Conceptualization; Project administration; Resources; Supervision; Funding acquisition; Validation; Writing-review & editing.

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