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Review article

# The functional roles of microRNAs in the pathogenesis of oral submucous fibrosis

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**Abstract** Oral potentially malignant disorders (OPMD) are lesions that may precede the onset of cancers in the oral cavity, and oral submucosal fibrosis (OSF) is one of the OPMD that is usually found in the buccal mucosa. Considerable effort has been made to elucidate the pathogenesis of OSF, and emerging evidence has suggested that microRNAs may play significant roles in the development of OSF. Several studies demonstrated that aberrant expression of miRNAs is also observed in the fibrotic BMFs (fBMFs) derived from OSF tissues. For instance, it has been shown that miR-10b, miR-21, and miR-1246 are significantly elevated, and miR-29b, miR-200b, and miR-200c are reduced in fBMFs. This review systematically summarizes the current knowledge regarding the aberrant expression of microRNAs, molecular mechanisms underlying oral fibrogenesis by the dysregulated microRNAs, and how the interaction between microRNAs and long non-coding RNAs contributes to the progression of OSF. An overview of the modes of action by these microRNAs will provide a fundamental basis for clinical application.


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## Introduction

### Oral submucous fibrosis

Oral submucous fibrosis (OSF) is a precancerous disease of the oral cavity characterized by juxta-epithelial inflammation and progressive deposition of collagen in buccal mucosa, palate, retromolar region, or pharynx. Patients often suffer from burning sensation, ulceration, excessive salivation, gustatory dysfunction, and occasional dryness of the mouth. Moreover, a gradual loss of mobility and inability of mouth opening is also observed, which greatly affect the quality of life of patients with OSF. Besides, the malignant transformation rate of OSF is about 4—9%.[1,2] Several contributing factors of OSF have been discovered, such as genetic signatures, consumption of areca nut, chewing smokeless tobacco, nutritional deficiencies of iron, folate & vitamin B12, and ingestion of chilies and so on.[3] Among these factors, the habit of areca nut chewing has been considered as the major etiologic agent, which promotes OSF development via the activation of the TGF-β pathway.[4] It has been known that alkaloids from areca nut increase myofibroblast proliferation and collagen synthesis, and tannins from areca nut stabilize the collagen structure through enhancing the resistance to collagenases (see Review).[5]

Myofibroblasts are α-smooth muscle actin (SMA)-expressing contractile fibroblasts that play integral roles in tissue regeneration and development of pathological fibrosis.[6] The expression of α-SMA has been shown to initiate the transdifferentiation of myofibroblast and upregulate contractile activity.[7] The accumulation of α-SMA-expressing myofibroblasts has been observed in multiple fibrosis conditions, including OSF.[8,9] The existence of myofibroblasts is also correlated with the severity of OSF,[8] and it has been proven that arecoline, a major alkaloid in areca nut, can induce myofibroblast transdifferentiation from human primary buccal mucosal fibroblasts (BMFs).[9] Chang et al. showed that epithelial—mesenchymal transition (EMT) transcription factor zinc finger E-box binding homeobox 1 (ZEB1) mediates arecoline-induced α-SMA expression as they demonstrated that arecoline increased the binding of ZEB1 to the α-SMA promoter in BMFs. In addition, they demonstrated that ZEB1 mRNA expression is positively associated with the expression of various fibrogenic genes, and the arecoline-induced ZEB1 expression is mediated by the activation of insulin-like growth factor-1 receptor (IGF-1R).[9] Among various sources of myofibroblast precursors, cells that undergo EMT have been regarded to contribute to tissue fibroses.[10] As mentioned above, transcription factors involved in the activation of the EMT programme modulate the myofibroblast transdifferentiation. Apart from ZEB1, other EMT factors are shown to induce myofibroblast activation of BMFs as well, such as Snail,[11] Slug,[12] or Twist.[13] Furthermore, accumulating evidence has suggested that non-coding RNAs may serve as important regulators of these transcription factors.

### MicroRNAs

Non-coding RNAs (ncRNAs) are RNA molecules that do not encode proteins and can be divided into the following categories based on the length: (1) ncRNAs shorter than 200 nucleotides, such as microRNA (miRNA), piwi-interacting RNA (piRNA), and small nucleolar RNA (snoRNA); (2) ncRNAs longer than 200 nucleotides, such as long non-coding RNA (lncRNA) and ribosomal RNA (rRNA). Aside from these linear ncRNAs, circular RNA (circRNA) is a recently defined type of ncRNA that forms a covalently closed loop without 5′ end caps or 3′ Poly (A) tails.[14] Over the past decades, the biological roles of these ncRNAs in disease progression have become hotspots of scientific research. It has been known that miRNAs (~18—22 nucleotides) can post-transcriptionally regulate gene expression by base-pairing with the 3 untranslated regions (3′UTRs) of target mRNAs. Emerging evidence suggested that the interaction between miRNAs and lncRNAs forms a regulatory network to mediate various cellular processes and disease initiation. It has been considered that a lncRNA has multiple microRNA response elements (MREs), and these miRNA-binding sites can titrate miRNAs and thereby impair their capacity to repress target mRNAs.[15] We summarized how aberrantly expressed miRNAs are implicated in the pathogenesis of OSF in the subsequent section. The microRNAs-mediated targets in OSF are listed in Table 1.

### MicroRNAs and OSF

A growing number of studies suggested that various miRNAs are differentially expressed in OSF and the dysregulated miRNA may implicate the development of this fibrosis condition. It has been reported that a lower miR-499a-5p production genotype (T/C + C/C) was related to an increased risk of areca nut-associated OSF.[16] In the buccal mucosal tissues, it has been revealed that miR-455-3p, miR-455-5p, and miR-623 are overexpressed, while miR-205, miR-509-3-5p, miR-610, miR-760, miR-921, miR-1290, miR-3180-3p, miR-4792, miR-5189 are underexpressed using microarray analysis.[17,18] The expression of miR-10b, miR-21, miR-30c, miR-31, miR-199-5p, and miR-1246 are markedly upregulated, and miR-29b, miR-200b, miR-200c, miR-203, and miR-204 are down-regulated in OSF tissues using qRT-PCR.[19—27] In the serum of OSF patients, miR-21 has been found to be increased compared to healthy control. Moreover, they showed serum miR-21 expression is higher in OSCC patients than OSF patients and may be a prognostic marker.[28] Several studies demonstrated that aberrant expression of miRNAs is also observed in the fibrotic BMFs (fBMFs) derived from OSF tissues. For instance, it has been shown that miR-10b,[22] miR-21,[23] and miR-1246[21] are significantly elevated, and miR-29b,[24] miR-200b,[19] and miR-200c[20] are reduced in fBMFs.

Aside from genetic polymorphism, most of the existing literature focused on the effects of areca nut on the alteration of miRNAs.[19,20,23,24] For example, miR-21 has been known to be induced by arecoline treatment in BMFs, which was mediated by activation of TGF-β signaling.[23] Our recent study has shown that miR-21 may contribute to the myofibroblast activation through suppression of programmed cell death 4 (unpublished work). MiR-21 also has been known to target an inhibitory smad, Smad 7 to mitigate the severity of bleomycin-induced lung fibrosis by blocking the positive feedback loop of TGF-β signaling.[29] Although the detailed molecular mechanism underlying

**Table 1** The differential expression of microRNAs and their targets in OSF.

| MicroRNA | Expression | Molecular target (s) | Reference |
|---|---|---|---|
| miR-455a-9p | Downregulation | N/A | 16 |
| miR-455-3p | Upregulation | BMP7, BMPR, CDH10, MAPK14, MAPK11, IFG1, TIMP2 | 18 |
| miR-455-5p | Upregulation | BMP6, DSC1, MMP12, MMP19, PARD6G, TGM3, HSF1 | 18 |
| miR-623 | Upregulation | MAPK1, MAPK11, MAPK4, MMP1, MMP8, TIMP2, IL10 | 18 |
| miR-205 | Downregulation | N/A | 17 |
| miR-509-3-5p | Downregulation | BMPR2, CDH6, HAS3, PARD6B, TIMP3, THBS1, THBS2 | 18 |
| miR-610 | Downregulation | CDH1, DSC2, KRAS, MMP19, MAPK1, TIMP3, MMP24 | 18 |
| miR-760 | Downregulation | CDH4, COX10, IL6, IL6R, IGF1R, TIMP2, TGM2 | 18 |
| miR-921 | Downregulation | N/A | 18 |
| miR-1290 | Downregulation | N/A | 18 |
| miR-3180-3p | Downregulation | N/A | 18 |
| miR-4792 | Downregulation | N/A | 18 |
| miR-5189 | Downregulation | N/A | 18 |
| miR-10b | Upregulation | N/A | 22 |
| miR-21 | Upregulation | N/A | 23, 28 |
| miR-31 | Upregulation | DMD, CXCL12, WASF3 | 25 |
| miR-199-5p | Upregulation | N/A | 26 |
| miR-1246 | Upregulation | N/A | 21 |
| miR-29b | Downregulation | COL1A1 | 24 |
| miR-200b | Downregulation | ZEB2 | 19 |
| miR-200c | Downregulation | ZEB1 | 20 |
| miR-203 | Downregulation | N/A | 27 |

the role of miRNAs in the development of OSF remains largely unknown, several studies have investigated how these differentially expressed miRNAs affect collagen metabolism, cell proliferation, apoptosis, or myofibroblast transdifferentiation. For instance, it has been shown that overexpression of miR-200b elicits apoptosis in fBMFs.[19] Another study revealed that arecoline induces the expression of secreted frizzled-related protein 4 (SFRP4) and suppresses the expression of miR-203 and transmembrane-4 L six family member 1 (TM4SF1) in HaCaT cells. SFRP4[30] and TM4SF1[31] both have been known to be associated with the expression of EMT transcription factors. They demonstrated that miR-203 negatively regulates SFRP4 and positively modulates TM4SF1 using a luciferase reporter assay. Moreover, miR-203 mimics is able to inhibit the arecoline-stimulated cell proliferation and EMT.[27] Nevertheless, not all of the upregulated miRNAs in OSF are prone to enhance cell proliferation. It has been shown that overexpression of miR-199-5p decreased cell proliferation and elicited apoptosis in BMFs. However, transfection with a miR-199-5p mimic resulted in upregulation of collagen I and III, which may contribute to the oral fibrogenesis.[26] Besides, miR-1246 also has been proven to be associated with the expression of type I collagen, and downregulation of miR-1246 exhibited an anti-fibrosis effect.[21]

The majority of current research suggested that these aberrantly expressed miRNAs orchestrate myofibroblast transdifferentiation by affecting EMT factors. It has been revealed that the arecoline-induced myofibroblast activities and expression of $\alpha$-SMA were abrogated by forced expression of miR-200b in BMFs.[19] They showed miR-200b directly downregulated ZEB2 by binding to its 3′UTR and led to suppression of $\alpha$-SMA and vimentin, an intermediate filament that modulates the cell motility and adhesion during EMT.[32] Likewise, overexpression of miR-200c has been shown to mitigate the arecoline-stimulated collagen gel contraction, migration, invasion, and wound healing capacities of BMFs.[20] Their results revealed that ZEB1 is a direct target of miR-200c and elevation of miR-200c results in a reduction of $\alpha$-SMA due to ZEB1 suppression.[20] Besides, it has been revealed a number of EMT-associated factors, such as Twist[33] or ZEB1,[34] were direct targets of miR-10b in other types of cells. Our previous work has demonstrated that arecoline-induced myofibroblast transdifferentiation was mediated by ZEB1[9] or associated with elevation of Twist.[13] EMT transcription factors not only are under the regulation of miRNAs but also modulate certain miRNAs expression and their function. For instance, it has been demonstrated that silencing of Twist renders a reduction of miR-10b in fBMFs and arecoline-stimulated miR-10b.[22] They showed downregulation of miR-10b inhibited the expression of $\alpha$-SMA and myofibroblasts activation. Besides, administration of miR-10b inhibiter ameliorated the collagen gel contractility in the Twist-overexpressing BMFs,[22] indicating that Twist mediates myofibroblast activation through regulation of miR-10b.

Accumulating evidence reveals that the development and progression of various types of fibrosis may be attributed to the interaction between miRNAs and lncRNAs.[35,36] In OSF, it has been shown that the arecoline-stimulated TGF-$\beta$ pathway upregulates the expression of lncRNA H19 in BMFs.[24] Yu et al. demonstrated that H19 acts as a molecular sponge of miR-29b, which results in the reduced direct binding of miR-29b to the 3′-UTR of type I collagen. Most importantly, they showed H19 may contribute to fibrogenesis by interfering with the anti-fibrotic effects of miR-29b, such as the decreased myofibroblast phenotypes and expression of $\alpha$-SMA, type I collagen, and fibronectin in fBMFs.[24] One of the recent studies showed that circRNA circEPSTI1 is sequentially increased from normal buccal mucosa to OSF to OSCC,[37] and it modulates EMT by binding

to miR-942-5p and upregulating the expression of latent transforming growth factor-beta binding protein 2 (LTBP2).[37] Their work suggested that the circEPSTI1/miR-942-5p/LTBP2 axis may mediate the progression of OSCC in the background of OSF.

## Conclusion

To date, knowledge regarding the role of miRNAs in the development of OSF is still limited. It has been shown that the aberrant expression of miRNAs may be associated with the activation of the TGF-β pathway or upregulation of certain EMT factors, such as Twist. Moreover, various miRNAs have been known to contribute to oral fibrogenesis through regulating EMT factors. Most importantly, an increasing number of studies revealed the interplay between lncRNAs and miRNAs modulate the progression of OSF. It appears that their modes of action will be diverse and a better understanding of the biological functions will help us to generate novel therapies.

## Declaration of competing interest

All authors have no conflicts of interest relevant to this article.

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