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).10 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 microRNA-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 microRNA-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.19,20 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.21 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-124624 are significantly elevated, and miR-29b,25 miR-200b,19 and miR-200c26 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 has also 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.27 Although the detailed molecular mechanism underlying
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. They showed miR-200b directly downregulated ZEB2 by binding to its 3’UTR and led to suppression of α-SMA and vimentin, an intermediate filament that modulates the cell motility and adhesion during EMT. Likewise, overexpression of miR-200c has been shown to mitigate the arecoline-stimulated collagen gel contraction, migration, invasion, and wound healing capacities of BMFs. Their results revealed that ZEB1 is a direct target of miR-200c and elevation of miR-200c results in a reduction of α-SMA due to ZEB1 suppression. Besides, it has been revealed a number of EMT-associated factors, such as Twist or ZEB1, 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 or associated with elevation of Twist. 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. They showed downregulation of miR-10b inhibited the expression of α-SMA and myofibroblasts activation. Besides, administration of miR-10b inhibitor ameliorated the collagen gel contractility in the Twist-overexpressing BMFs, 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. In OSF, it has been shown that the arecoline-stimulated TGF-β pathway upregulates the expression of lncRNA H19 in BMFs. 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 α-SMA, type I collagen, and fibronectin in fBMFs. One of the recent studies showed that circRNA circEPSTI1 is sequentially increased from normal buccal mucosa to OSF to OSCC, and it modulates EMT by binding...
to miR-942-5p and upregulating the expression of latent transforming growth factor-beta binding protein 2 (LTBP2). Their work suggested that the circEPST1/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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References
1. Yang PY, Chen YT, Wang YH, Su NY, Yu HC, Chang YC. Malignant transformation of oral submucous fibrosis in Taiwan: a nationwide population-based retrospective cohort study. J Oral Pathol Med 2017;46:1040–5.
2. Kujan O, Mello FW, Warnakulasuriya S. Malignant transformation of oral submucous fibrosis: a systematic review and meta-analysis. Oral Dis 2020. Online ahead of print.
3. Ray JG, Chatterjee R, Chaudhuri K. Oral submucous fibrosis: a global challenge. Rising incidence, risk factors, management, and research priorities. Periodontology 2000;80:200–12. 2019.
4. Khan I, Kumar N, Pant I, Narra S, Kondaiah P. Activation of TGF-β pathway by areca nut constituents: a possible cause of oral submucous fibrosis. PLoS One 2012;7:e51806.
5. Tilakaratne WM, Klinikowski MF, Saku T, Peters TJ, Warnakulasuriya S. Oral submucous fibrosis: review on aetiology and pathogenesis. Oral Oncol 2006;42:561–8.
6. Darby I, Skalli O, Gabbiani G. Alpha-smooth muscle actin is transiently expressed by myofibroblasts during experimental wound healing. Lab Invest 1990;63:21–9.
7. Hinz B, Celetta G, Tomasek JJ, Gabbiani G, Chaponnier C. Alpha-smooth muscle actin expression upregulates fibroblast contractile activity. Mol Biol Cell 2001;12:2730–41.
8. Angadi PV, Kale AD, Hallikerimath S. Evaluation of myofibroblasts in oral submucous fibrosis: correlation with disease severity. J Oral Pathol Med 2011;40:208–13.
9. Chang YC, Tsai CH, Lai YL, et al. Arecoline-induced myofibroblast transdifferentiation from human buccal mucosal fibroblasts is mediated by ZEB1. J Cell Mol Med 2014;18:698–708.
10. Hinz B, Phan SH, Thannickal VJ, Galli A, Bochaton-Piallat ML, Gabbiani G. The myofibroblast: one function, multiple origins. Am J Pathol 2007;170:1807–16.
11. Peng CY, Liao YW, Lu MY, Yang CM, Hsieh PL, Yu CC. Positive feedback loop of SNAIL-IL-6 mediates myofibroblastic differentiation activity in precancerous oral submucous fibrosis. Cancers 2020;12:1611.
12. Fang CY, Hsia SM, Hsieh PL, et al. Slug mediates myofibroblastic differentiation to promote fibrogenesis in buccal mucosa. J Cell Physiol 2019;234:6721–30.
13. Lee YH, Yang LC, Hu FW, Peng CY, Yu CH, Yu CC. Elevation of Twist expression by arecoline contributes to the pathogenesis of oral submucous fibrosis. J Formos Med Assoc 2016;115:311–7.
14. Liang D, Wilusz JE. Short intronic repeat sequences facilitate circular RNA production. Genes Dev 2014;28:2233–47.
15. Seitz H. Redefining microRNA targets. Curr Biol 2009;19:870–3.
16. Hou YY, Lee JH, Chen HC, et al. The association between miR-499a polymorphism and oral squamous cell carcinoma progression. Oral Dis 2015;21:195–206.
17. Das RK, Anura A, Pal M, et al. Epithelio-mesenchymal transition attributes in oral sub-mucous fibrosis. Exp Mol Pathol 2013;95:259–69.
18. Chickooree D, Zhu K, Ram V, Wu HJ, He ZJ, Zhang S. A preliminary microarray assay of the miRNA expression signatures in buccal mucosa of oral submucous fibrosis patients. J Oral Pathol Med 2016;45:691–7.
19. Liao YW, Yu CC, Hsieh PL, Chang YC. miR-200b ameliorates myofibroblast transdifferentiation in precancerous oral submucous fibrosis through targeting ZEB2. J Cell Mol Med 2018;22:4130–8.
20. Lu MY, Yu CC, Chen PY, et al. miR-200c inhibits the arecoline-associated myofibroblastic transdifferentiation in buccal mucosal fibroblasts. J Formos Med Assoc 2018;117:791–7.
21. Liu CM, Liao YW, Hsieh PL, et al. miR-1246 as a therapeutic target in oral submucosa fibrosis pathogenesis. J Formos Med Assoc 2019;118:1093–9.
22. Fang CY, Yu CC, Liao YW, et al. miR-10b regulated by Twist maintains myofibroblasts activities in oral submucous fibrosis. J Formos Med Assoc 2020;119:1167–73.
23. Yang HW, Yu CC, Hsieh PL, et al. Arecoline enhances miR-21 to promote buccal mucosal fibroblasts activation. J Formos Med Assoc 2021;120:1108–13.
24. Yu CC, Liao YW, Hsieh PL, Chang YC. Targeting lncRNA H19/miR-29b/COL1A1 axis impedes myofibroblast activities of precancerous oral submucous fibrosis. Int J Mol Sci 2021;22:22216.
25. Chattopadhyay E, Singh R, Ray A, et al. Expression deregulation of mir 31 and CXCL12 in two types of oral precancers and cancer: importance in progression of precancer and cancer. Sci Rep 2016;6:32735.
26. Yuan Y, Li N, Zeng L, Shen Z, Jiang C. Pathogenesis investigation of miR-199-5p in oral submucous fibrosis based on bioinformatics analysis. Oral Dis 2019;25:456–65.
27. Zheng L, Jian X, Guo F, et al. miR-203 inhibits arecoline-induced epithelial-mesenchymal transition by regulating secreted frizzled-related protein 4 and transmembrane-4 L six family member 1 in oral submucous fibrosis. Oncol Rep 2015;33:2753–60.
28. Singh P, Srivastava AN, Sharma R, et al. Circulating microRNA-21 expression as a novel serum biomarker for oral sub-mucous fibrosis and oral squamous cell carcinoma. Asian Pac J Cancer Prev APJCP 2018;19:1053–7.
29. Liu G, Friggeri A, Yang Y, et al. miR-21 mediates fibrogenic activation of pulmonary fibroblasts and lung fibrosis. *J Exp Med* 2010;207:1589–97.

30. Ford CE, Jary E, Ma SS, Nixdorf S, Heinzelmann-Schwarz VA, Ward RL. The Wnt gatekeeper SFRP4 modulates EMT, cell migration and downstream Wnt signalling in serous ovarian cancer cells. *PLoS One* 2013;8:e54362.

31. Tang Q, Chen J, Di Z, et al. TM4SF1 promotes EMT and cancer stemness via the Wnt/β-catenin/SOX2 pathway in colorectal cancer. *J Exp Clin Canc Res* 2020;39:232.

32. Mendez MG, Kojima S, Goldman RD. Vimentin induces changes in cell shape, motility, and adhesion during the epithelial to mesenchymal transition. *Faseb J* 2010;24:1838–51.

33. Ma L, Teruya-Feldstein J, Weinberg RA. Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. *Nature* 2007;449:682–8.

34. Guo Y, Lang X, Lu Z, et al. MiR-10b directly targets ZEB1 and PIK3CA to curb adenomyotic epithelial cell invasiveness via upregulation of E-Cadherin and inhibition of Akt phosphorylation. *Cell Physiol Biochem* 2015;35:2169–80.

35. Li C, Wang Z, Zhang J, et al. Crosstalk of mRNA, miRNA, lncRNA, and circRNA and their regulatory pattern in pulmonary fibrosis. *Mol Ther Nucleic Acids* 2019;18:204–18.

36. Sun F, Zhuang Y, Zhu H, et al. LncRNA PCFL promotes cardiac fibrosis via miR-378/GRB2 pathway following myocardial infarction. *J Mol Cell Cardiol* 2019;133:188–98.

37. Wang J, Jiang C, Li N, et al. The circEPSTI1/mir-942-5p/LTBP2 axis regulates the progression of OSCC in the background of OSF via EMT and the PI3K/Akt/mTOR pathway. *Cell Death Dis* 2020;11:682.