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Original Article

Resveratrol inhibits arecoline-induced fibrotic properties of buccal mucosal fibroblasts via miR-200a activation

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Abstract *Background/purpose:* Oral submucous fibrosis (OSF) is a precancerous lesion in the oral cavity, commonly results from the Areca nut chewing habit. Arecoline, the main component of Areca nut, is known to stimulate the activation of myofibroblasts, which can lead to abnormal collagen I deposition. Meanwhile, Resveratrol is a non-flavonoid phenolic substance that can be naturally obtained from various berries and foods. Given that resveratrol has significant anti-fibrosis traits in other organs, but little is known about its effect on OSF, this study aimed to investigate the therapeutic impact of resveratrol on OSF and its underlying mechanism.

Materials and methods: The cytotoxicity of resveratrol was tested using normal buccal mucosal fibroblasts (BMFs). Myofibroblast phenotypes such as collagen contractile, enhanced migration, and wound healing capacities in dose-dependently resveratrol-treated fBMFs were examined.

Results: Current results showed that arecoline induced cell migration and contractile activity in BMFs as well as upregulated the expressions of α-SMA, type I collagen, and ZEB1 markers.

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Resveratrol intervention, on the other hand, was shown to inhibit arecoline-induced myofibroblast activation and reduce myofibroblast hallmarks and EMT markers. Additionally, resveratrol was also demonstrated to restore the downregulated miR-200a in the arecoline-stimulated cells.

Conclusion: In a nutshell, these findings implicate that resveratrol may have an inhibitory influence on arecoline-induced fibrosis via the regulation of miR-200a. Hence, resveratrol may be used as a therapeutic strategy for OSF intervention.

Introduction

Oral submucous fibrosis (OSF) is a chronic inflammatory disease in the oral cavity, commonly arises from the habit of chewing betel nuts.[1,2] OSF has been identified as a premalignant oral lesion, as characterized by the presence of inflammation and abnormal collagen I deposition.[3] Various research have shown that individuals with OSF are at much higher risk (up to 19.1 times) of acquiring oral cancer than healthy people.[4–6] Oral cancer is considered a deadly cancer with a high metastasis and mortality rate.[7] Thus, it is crucial to understand the underlying mechanism of betel nut in the development of OSF in order to develop a novel treatment to target it.

The loss of balance in the synthesis and degradation of extracellular matrix (ECM) is one of the key reasons for the development of OSF. The activation of myofibroblasts primarily contributes to this, as activated myofibroblasts are responsible for abnormal ECM deposition.[8] The expression of α-smooth muscle actin (α-SMA), a marker of differentiated myofibroblasts,[9] in fact, has a positive association with OSF.[10]

Another key factor in the pathogenesis of OSF is the development of epithelial-to-mesenchymal transition (EMT) among epithelial cells, in which these cells might have the potential to become myofibroblasts.[11,12] EMT is defined as a process of the transformation of epithelial cells into migratory mesenchymal cells. In light of that, its expression has been shown to be highly correlated with OSF and metastasis.[13,14] Arecoline, the main component of the Areca nut, was observed to upregulate the expression of zinc finger E-box binding homeobox 1 (ZEB1) among the myofibroblasts.[15,16] ZEB1, along with other E-cadherin transcriptional repressors such as Snail, Slug, and Twist, have been implicated in the promotion of EMT.[17]

Meanwhile, ZEB1 expression was thought to be regulated by microRNAs,[13,18] which are short-stranded RNAs that modulate messenger RNA (mRNAs) expression by targeting the 3′ region.[19] For instance, miR-200a was able to regulate transforming growth factor beta 1 (TGF-β1)-induced epithelial-mesenchymal transition in peritoneal mesothelial cells via targeting ZEB1/2 expression.[20] miR-200a, a member of the miR-200 family, has been shown to regulate various fibrotic diseases in the cardiac, renal, and liver tissues.[21–23] In addition, in peritoneal fibrosis, miR-200a agomir has been revealed to reduce EMT and reduce subsequent myofibroblast differentiation and collagen accumulation.[24] Given that miR-200a has the ability to modulate both collagen accumulation and EMT, targeting this mRNA within the myofibroblasts could be a promising therapeutic approach in preventing the development of pre-cancerous OSF.

In the recent, increasing attention is directed to resveratrol in the attempt to target fibrotic diseases. Resveratrol is a non-flavonoid phenolic substance natural compound exist in various berries and food. Specifically, resveratrol has exhibited excellent anti-fibrotic effect in various tissues or organs such as hepatocytes, perivascular, pancreas.[25–34] Considering that resveratrol has significant anti-fibrosis trait in many tissues but little is known about its effect on OSF, this study aimed to investigate the therapeutic impact of resveratrol on OSF and whether or not the effects are attributed to the modulation of miR-200a.

Materials and methods

Cell culture and chemicals

Arecoline, resveratrol and collagen solution from bovine skin were purchased from Sigma-Aldrich (St. Louis, MO, USA). All procedures were carried out in accordance with the approved guidelines from the Institutional Review Board of Chung Shan Medical University Hospital and informed written consent was granted from each individual prior to commencing the study. As previously indicated, primary cultures of BMFs were grown, and the third and eighth passages were employed in the OSF investigation.[35] BMFs were treated with 20 μg/mL arecoline for 24 h and were furtherly treated with varied doses of resveratrol for 48 h for functional study.

Evaluation of cell proliferation

Cell proliferation/survival were determined using the MTT test for IC50 determination. 1×104 cells were grown per well for 48 h at 37 °C in control or varied doses of resveratrol -containing media. The MTT test was carried out in accordance with previously established guidelines.[36]

miR-200a overexpression or inhibition

miR200a mimic, and miR-200a inhibitor, and scramble (Scr) control were purchased from Applied Biosystems (Foster

City, CA, USA). LipofectamineTM 3000 transfection reagent (Invitrogen, Thermo Fisher Scientific Inc., Carlsbad, CA, USA) was used in this experiment according to the manufacturer's protocol.[37]

Quantitative real-time PCR (qRT-PCR)

Total RNA was isolated using the Trizol Reagent and reverse transcribed to cDNA using a High-Capacity cDNA reverse transcription kit as directed by the manufacturer's instructions (Invitrogen Life Technologies, Carlsbad, CA, USA). qRT-PCR analysis is carried out using a standard protocol.[35]

Western blot examination

All techniques in this assay will adhere to the protocols formerly published. Primary antibodies will be used against SMA, COL1A1, and ZEB1.[38]

Collagen gel contraction assay

After dispersion of cells in collagen gel solution (Sigma-Aldrich, St. Louis, MO, USA), the mixture was placed to a 24-well plate before being incubated at 37 °C for 2 h. Following polymerization, the gels were cultured in 0.5 ml media for 48 h. ImageJ software was used to determine the variation in collagen gel size (contraction index) (NIH, Bethesda, MD, USA).[39]

Transwell migratory assay

Cells along with serum-free media were added in the upper chamber of a transwell (Corning, Acton, MA). The transwell membranes were pre-coated with Matri-gel (2.5 mg/ml, Sigma) for the transwell invasion assay, prior to supplying the lower compartment with medium containing 10% FBS, and the cells were cultivated for 24 h. Cells adhering to the other end of the membrane was dyed with crystal violet, and five to ten random areas were recorded.[40]

Statistical analysis

Statistical Package of Social Sciences software (version 13.0) (SPSS, Inc., Chicago, IL) will be utilized for statistical analysis. Data from analyses performed in at least three replicates were presented as mean SD. The statistical significance of the differences between the experimental groups was assessed using the Student's *t*-test; *P* values less than 0.05 was regarded as statistically significant.

Results

To investigate the effect of resveratrol on the cell viability of buccal mucosal fibroblasts, cell proliferation was examined after resveratrol treatment by using MTT assay. From there, 50% inhibitory concentration (IC50) values were calculated to quantitatively evaluate the drug responses. IC50 value of BMFs were 185.9 μM (Fig. 1A). The present study revealed that resveratrol has low toxicity to BMFs, but at a high concentration of 100 μM, resveratrol exhibited a significant inhibitory effect on the cell proliferation of arecoline-induced BMFs (Fig. 1B). The main contributing factors in the progression of OSF are the activation of myofibroblasts and development of EMT. In regard to myofibroblast, myofibroblasts are defined as fibroblasts with a more contractile phenotype. Thus, it is crucial to explore the effect of resveratrol on the contractility of BMFs. It was demonstrated that arecoline stimuli increased the contractility in BMFs while resveratrol intervention significantly reversed this (Fig. 2). Besides that, arecoline was demonstrated to induce migration in BMFs whereas the addition of resveratrol inhibited the migration in arecoline-induced BMFs (Fig. 3).

As mentioned, myofibroblasts may be derived from cells who undergo EMT.[11] ZEB1, as an E-cadherin transcriptional

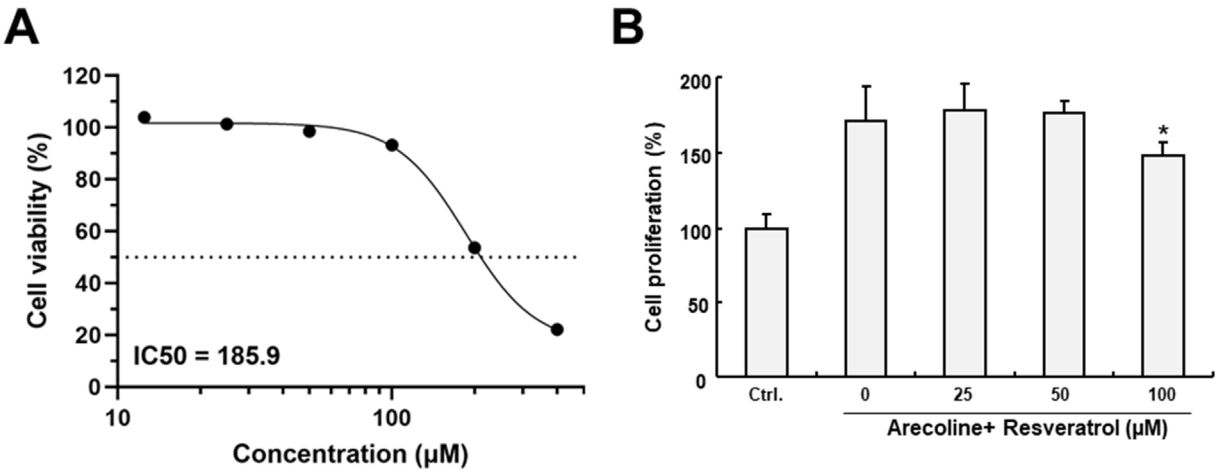

Figure 1 Suppressive effects of resveratrol on arecoline-induced BMFs. (A)The cytotoxic effect of resveratrol on the viability of BMFs. (B) Resveratrol ranging from 25 to 50 μg/ml did not significantly affect the cell proliferation rate in arecoline-induced BMFs. Resveratrol only had a significant inhibition on cell proliferation at concentration of 100 μg/ml. Data represent the mean ± SD. *$P < 0.05$ compared to arecoline-stimulated control group.

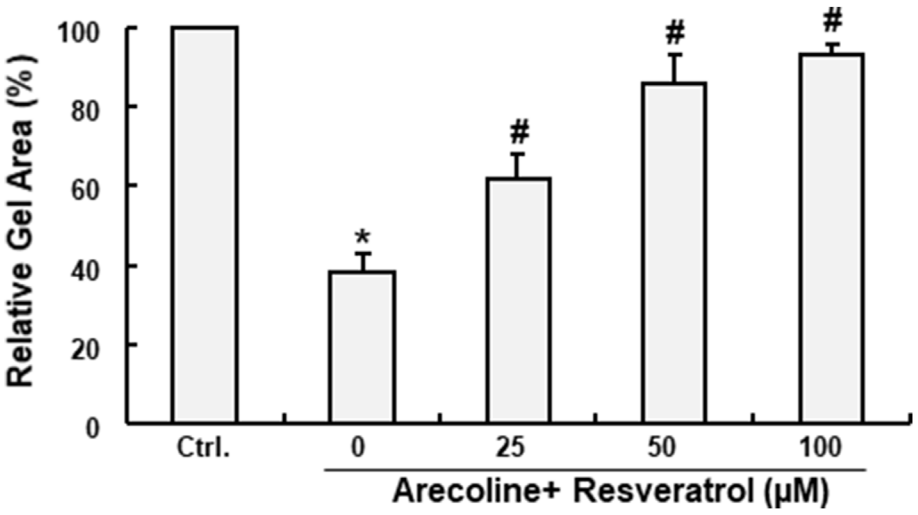

Figure 2 The arecoline-induced BMFs heightened contractile activity was dose-dependently reversed by resveratrol. Arecoline significantly increased the contractile activity in BMFs and this was restored with resveratrol intervention in a dose-dependent manner. *$P < 0.05$ compared to control group; #$P < 0.05$ compared to arecoline only group.

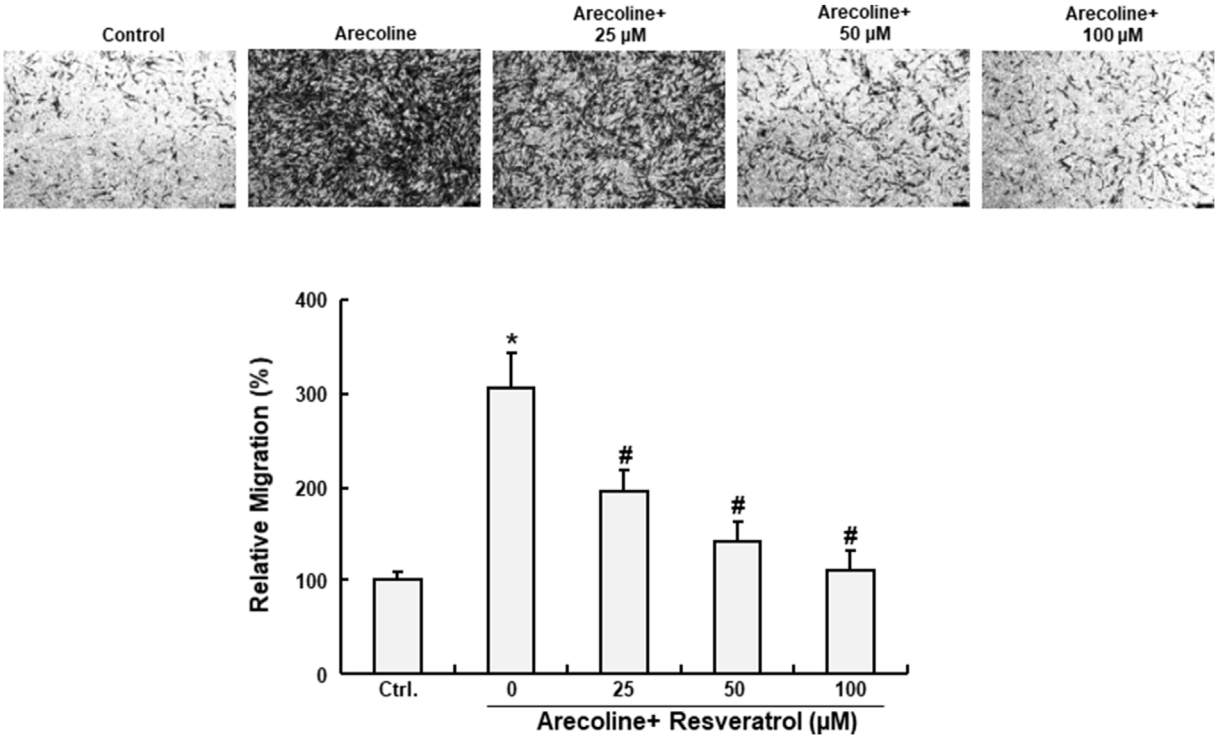

Figure 3 Resveratrol inhibited the migration ability in the arecoline-induced BMFs. (A) Representative images and (B) quantification data demonstrated that resveratrol treatment inhibited the increased migration in the arecoline-stimulated BMFs. *$P < 0.05$ compared to control group; #$P < 0.05$ compared to arecoline only group.

repressors, has been correlated to EMT upregulation. Hence, to elucidate the impact of this compound on EMT and myofibroblast expression, we investigated the influence of resveratrol on the gene expression of ZEB1 and the protein expression of myofibroblast markers. Our results demonstrated that arecoline increased ZEB1's gene expression, while this upregulated expression was suppressed when resveratrol was given as intervention (Fig. 4A). As for the protein expression of myofibroblast markers, it was observed that all the markers- ZEB1, COL1A1 and α-SMA were increased in arecoline-induced cells, and this phenomenon was reversed by resveratrol (Fig. 4B).

Lastly, we would like to investigate if the inhibitory effects of resveratrol on arecoline-induced BMFs is due to the modulation of miR-200a. Therefore, we investigated the

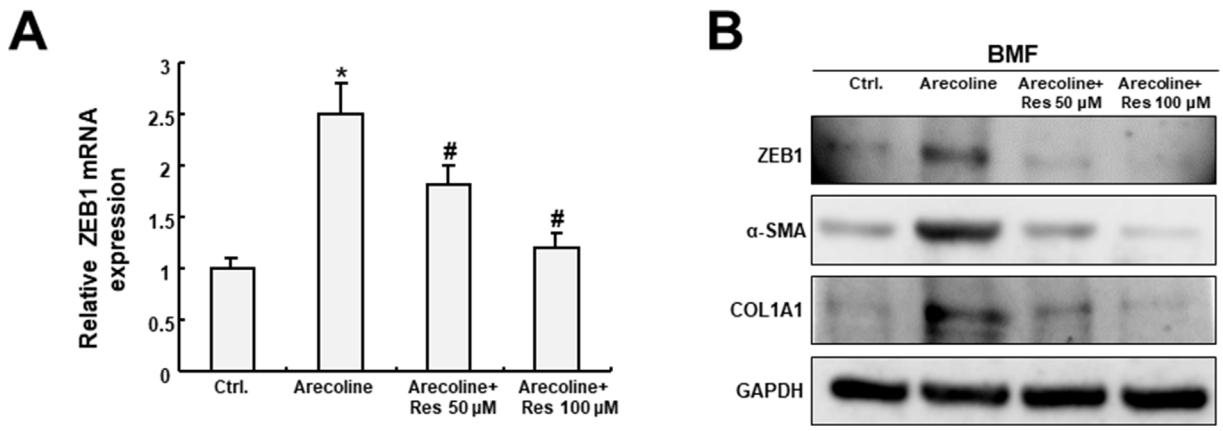

Figure 4 Resveratrol reversed the expression of EMT and collagen markers in arecoline-stimulated BMFs. (A) The mRNA level of ZEB1 and (B) protein expression levels of ZEB1, α-SMA and type I collagen A1 were found heightened in arecoline-induced BMFs and they were downregulated by resveratrol. *$P < 0.05$ compared to no treatment group. #$P < 0.05$ compared to arecoline only group.

expression of miR-200a in the arecoline-induced BMFs and resveratrol-treated cells. As shown in Fig. 5, the expression of miR-200a was found reduced in arecoline-induced cells and was upregulated in resveratrol group. To clarify the roles of miR-200a in BMFs, we upregulated miR-200a expression in BMFs and assessed its cell migration and myofibroblast markers. The results showed that both the cell migration (Fig. 6A) and protein levels of all myofibroblast markers (Fig. 6B) were reduced.

Lastly, to investigate the relationship of miR-200a and ZEB1 expression on myofibroblast differentiation, we first inhibited miR-200a and then inhibited both miR-200a and ZEB1 and then measured the relative migration. The results showed that when miR-200a expression was inhibited, the cell migration increased tremendously. However, when accompanied by the inhibition of ZEB1, the migration ability in cells was suppressed (Fig. 7A), marking that miR-200a has the ability to negatively regulate the expression of ZEB1. Overall, resveratrol inhibits arecoline-induced OSF via the regulation of miR-200a/ZEB1 axis (Fig. 7B).

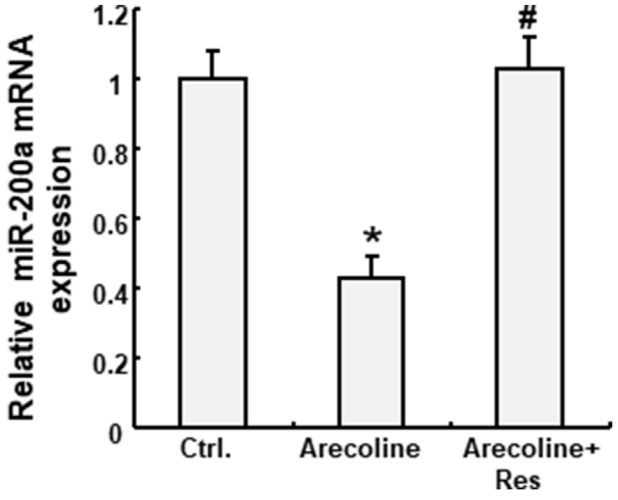

Figure 5 Resveratrol restored the downregulated expression of miR-200a in arecoline-stimulated BMFs. The expression of MiR-200a in BMFs cultured with arecoline was downregulated, while treatment with resveratrol restored it. *$P < 0.05$ compared to control group; #$P < 0.05$ compared to arecoline only group.

Discussion

In the current study, we investigated the potential of resveratrol in the treatment of pre-cancerous OSF. In order to simulate OSF, we first cultured the BMFs with arecoline, which is the main component of the Areca nut. We then investigated resveratrol's effect on their cell viability, and it was shown that resveratrol has rather low toxicity towards the cells. Resveratrol only had a significant inhibition on cell proliferation at the concentration of 100 μg/ml.

As previously mentioned, the differentiation of BMFs to myofibroblasts and the development of EMT are the two most important reasons for the progression of OSF. Myofibroblasts conduct wound healing, but their continuous existence in tissues is the core of accelerating fibrosis.[41] A myofibroblast differs from BMF in the sense that a myofibroblast possesses a much superior contractile nature[42] and cell migration than BMF.[43] In agreement with the prior studies, arecoline was shown to enhance the cells contractility and migratory skills in this study while intervention with resveratrol reversed these phenomena in a dose-dependent manner. The high contraction and migratory capability in a myofibroblast is due to the presence of cytoskeletal proteins that are normally found in smooth-muscle cells — in particular, α-smooth muscle actin.[42,44] As expected, in this study, the expression of markers of myofibroblasts, including α-SMA and type I collagen A1 was found to be heightened in arecoline-induced BMFs. Interestingly, when resveratrol was added as a treatment, all the myofibroblast markers were suppressed.

Another important aspect of the pathophysiology of OSF is the emergence of epithelial-to-mesenchymal transition (EMT).[11,12] EMT is defined as a process of the transformation of epithelial cells into migratory mesenchymal cells, and its expression has been shown to be highly correlated with OSF and metastasis.[13,14] In line with previous studies,[15,16] the current study demonstrated that the

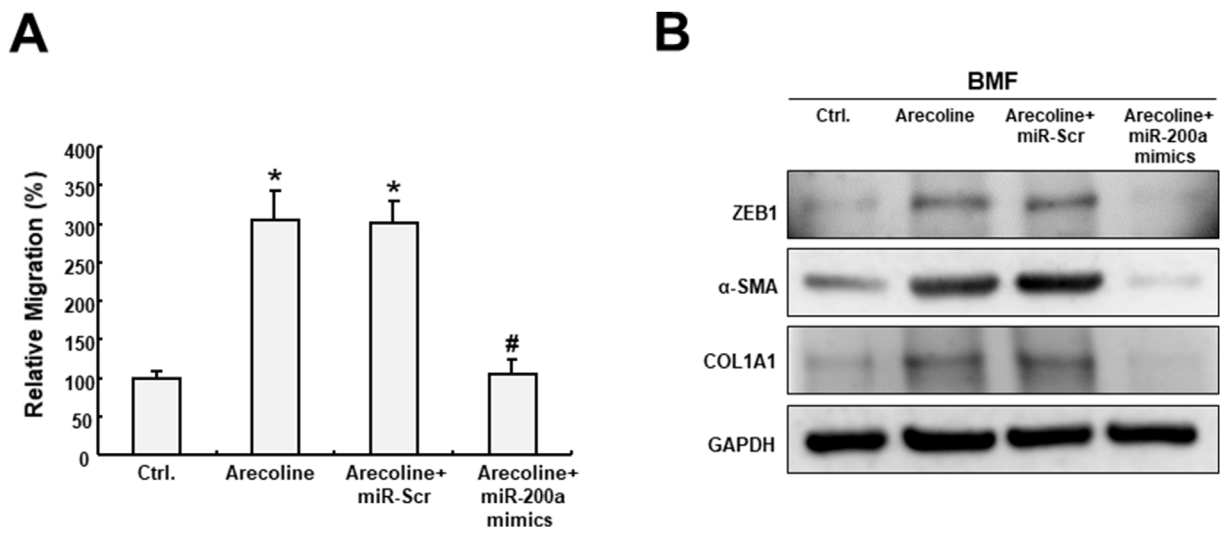

Figure 6 miR-200a mimics inhibited cell migration ability, EMT and myofibroblast differentiation. When miR-200a mimics was given to BMFs, the cell migration **(A)** and protein levels of EMT and myofibroblast markers **(B)** were reduced. *$P < 0.05$ compared to control group; #$P < 0.05$ compared to arecoline only group.

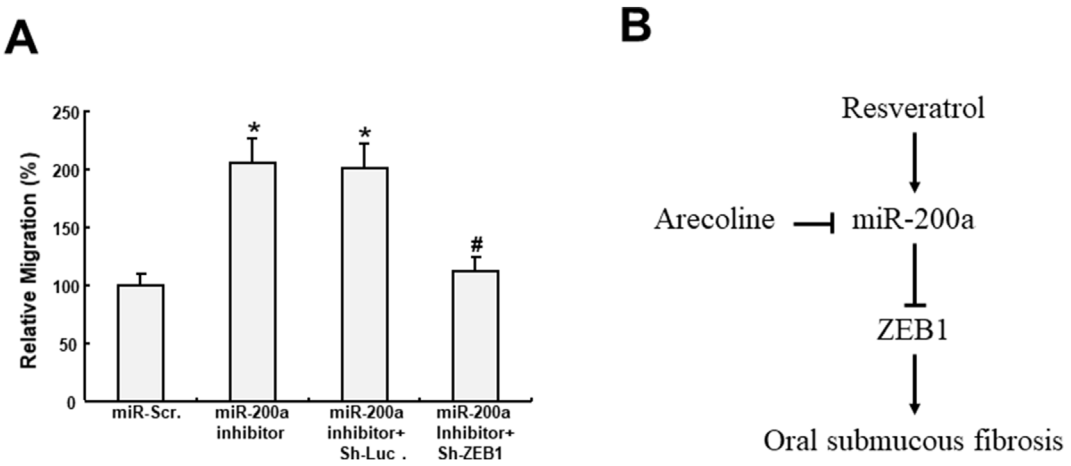

Figure 7 The inverse association of miR-200a and ZEB1 in cell migration in BMFs. (A) The enhanced cell migration ability in miR-200a-knockdown BMFs was inhibited by the knockdown of ZEB1 and miR-200a. *$P < 0.05$ compared to control group; #$P < 0.05$ compared to miR-200a inhibition. (B) Schematic representation of the study.

expression of ZEB1 was markedly increased in arecoline-induced BMFs while resveratrol reversed its expression. ZEB1, along with other E-cadherin transcriptional repressors such as Snail, Slug, and Twist, have been associated with the establishment of EMT.[17]

Since resveratrol was able to suppress ZEB1 expression in cells, it was crucial to investigate the underlying molecular mechanism to have a better understanding of the pathophysiology of this oral disease. We targeted miR-200a in our study as it has been revealed to be able to target ZEB1/2 expression and reversed the epithelial-mesenchymal transition,[20] as well as regulate various fibrotic diseases in the cardiac, renal, and liver tissues.[21—23] The present study revealed that the expression of miR-200a was down-regulated in arecoline-induced BMFs, while treatment with resveratrol restored it. When miR-200a-mimic was given to the cells, the cell migratory ability and protein levels of EMT and myofibroblast markers were found to be considerably reduced. These propose that the lowered miR-200a in arecoline-stimulated BMFs is responsible for promoting EMT and subsequent myofibroblast activation and collagen accumulation.[24] Moving on, to explore the relationship between miR-200a and ZEB1 in BMFs/myofibroblasts, miR-200a was first inhibited, followed by the measurement if the relative migration. The results showed that when miR-200a expression was inhibited, cell migration increased tremendously. However, when both miR-200a and ZEB1 were knocked down, the initially enhanced cell migration in miR-200a-knockdown BMFs was suppressed. This may signify that miR-200a can inversely regulate the expression of ZEB1 in BMFs/myofibroblasts.

In conclusion, our results have demonstrated that resveratrol has a protective effect against arecoline-induced BMFs in terms of myofibroblast activation, EMT development, and collagen production. It was proposed that resveratrol exerts its anti-fibrotic effect in OSF via the

upregulation of miR-200a. Hence, resveratrol may be a potent therapeutic approach with great potential as an OSF intervention in the future.

Declaration of competing interest

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

Acknowledgements

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References

1. Chung CH, Yang YH, Wang TY, Shieh TY, Warnakulasuriya S. Oral precancerous disorders associated with areca quid chewing, smoking, and alcohol drinking in southern taiwan. *J Oral Pathol Med* 2005;34:460—6.
2. Prabhu RV, Prabhu V, Chatra L, Shenai P, Suvarna N, Dandekeri S. Areca nut and its role in oral submucous fibrosis. *J Clin Exp Dent* 2014;6:e569.
3. Shih YH, Wang TH, Shieh TM, Tseng YH. Oral submucous fibrosis: a review on etiopathogenesis, diagnosis, and therapy. *Int J Mol Med* 2019;20:2940.
4. Sadat SA, Shaikh MH, Tajin F, Akter R, Shuvo SA, Ahmed H. Oral submucous fibrosis associated oral squamous cell carcinoma: a case report with review of literature. *Oral Maxillofac Surg Cases* 2022;8:100246.
5. Ray JG, Ranganathan K, Chattopadhyay A. Malignant transformation of oral submucous fibrosis: overview of histopathological aspects. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2016;122:200—9.
6. Merchant A, Husain SS, Hosain M, et al. Paan without tobacco: an independent risk factor for oral cancer. *Int J Cancer* 2000;86:128—31.
7. Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. *Oral Oncol* 2009;45:309—16.
8. Gayathri K, Malathi N, Gayathri V, Adtani PN, Ranganathan K. Molecular pathways of oral submucous fibrosis and its progression to malignancy. *Arch Oral Biol* 2023;148:105644.
9. Sousa AM, Liu T, Guevara O, et al. Smooth muscle α-actin expression and myofibroblast differentiation by tgfβ are dependent upon mk2. *J Cell Biochem* 2007;100:1581—92.
10. 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.
11. Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. *Cell* 2009;139:871—90.
12. Wadhwan V, Venkatesh A, Reddy V, Malik S. The role of myofibroblasts in the progression of oral submucous fibrosis: a systematic review. *J Oral Maxillofac Pathol* 2019;23:257.
13. Panda A, Mishra P, Mohanty A, Sundaragiri KS, Singh A, Jha K. Is epithelial-mesenchymal transition a new roadway in the pathogenesis of oral submucous fibrosis: a comprehensive review. *Cureus* 2022;14:e29636.
14. Heerboth S, Housman G, Leary M, et al. Emt and tumor metastasis. *Clin Transl Med* 2015;4:1—13.
15. 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.
16. Chang YC, Lin CW, Yu CC, et al. Resveratrol suppresses myofibroblast activity of human buccal mucosal fibroblasts through the epigenetic inhibition of zeb1 expression. *Oncotarget* 2016;7:12137.
17. Peinado H, Portillo F, Cano A. Transcriptional regulation of cadherins during development and carcinogenesis. *Int J Dev Biol* 2004;48:365—75.
18. Harazono Y, Muramatsu T, Endo H, et al. Mir-655 is an emt-suppressive microrna targeting zeb1 and tgfbr2. *PLoS One* 2013;8:e62757.
19. Dexheimer PJ, Cochella L. Micrornas: from mechanism to organism. *Front Cell Dev Biol* 2020;8:409.
20. Guo R, Hao G, Bao Y, et al. Mir-200a negatively regulates tgf-β1-induced epithelial-mesenchymal transition of peritoneal mesothelial cells by targeting zeb1/2 expression. *Am J Physiol Ren Physiol* 2018;314:F1087—95.
21. Yang S, Banerjee S, de Freitas A, et al. Participation of mir-200 in pulmonary fibrosis. *Am J Pathol* 2012;180:484—93.
22. Jiang XP, Ai WB, Wan LY, Zhang YQ, Wu JF. The roles of microrna families in hepatic fibrosis. *Cell Biosci* 2017;7:1—9.
23. Zhang H, Hu J, Liu L. Mir-200a modulates tgf-β1-induced endothelial-to-mesenchymal shift via suppression of grb2 in haecs. *Biomed Pharmacother* 2017;95:215—22.
24. Wei X, Bao Y, Zhan X, et al. Mir-200a ameliorates peritoneal fibrosis and functional deterioration in a rat model of peritoneal dialysis. *Int Urol Nephrol* 2019;51:889—96.
25. Abd-Elgawad H, Abu-Elsaad N, El-Karef A, Ibrahim T. Piceatannol increases the expression of hepatocyte growth factor and il-10 thereby protecting hepatocytes in thioacetamide-induced liver fibrosis. *Can J Physiol Pharmacol* 2016;94:779—87.
26. Abdu SB, Al-Bogami FM. Influence of resveratrol on liver fibrosis induced by dimethylnitrosamine in male rats. *Saudi J Biol Sci* 2019;26:201—9.
27. Chen HY, Lin TC, Chiang CY, et al. Antifibrotic effect of bletilla striata polysaccharide-resveratrol-impregnated dual-layer carboxymethyl cellulose-based sponge for the prevention of epidural fibrosis after laminectomy. *Polymers* 2021;13:2129.
28. Hong SW, Jung KH, Zheng HM, et al. The protective effect of resveratrol on dimethylnitrosamine-induced liver fibrosis in rats. *Arch Pharm Res (Seoul)* 2010;33:601—9.
29. Inanaga K, Ichiki T, Matsuura H, et al. Resveratrol attenuates angiotensin ii-induced interleukin-6 expression and perivascular fibrosis. *Hypertens Res* 2009;32:466—71.
30. Lee ES, Shin MO, Yoon S, Moon JO. Resveratrol inhibits dimethylnitrosamine-induced hepatic fibrosis in rats. *Arch Pharm Res (Seoul)* 2010;33:925—32.
31. Natalin HM, Garcia AF, Ramalho LN, Restini CB. Resveratrol improves vasoprotective effects of captopril on aortic remodeling and fibrosis triggered by renovascular hypertension. *Cardiovasc Pathol* 2016;25:116—9.
32. Ramakrishnan P, Loh WM, Gopinath SCB, et al. Selective phytochemicals targeting pancreatic stellate cells as new antifibrotic agents for chronic pancreatitis and pancreatic cancer. *Acta Pharm Sin B* 2020;10:399—413.
33. Wang S, Shi K, Lu J, et al. Microsphere-embedded hydrogel sustained-release system to inhibit postoperative epidural fibrosis. *ACS Appl Bio Mater* 2021;4:5122—31.
34. Xia Y, Xiao HT, Liu K, Zhang HJ, Tsang SW. Resveratrol ameliorates the severity of fibrogenesis in mice with experimental chronic pancreatitis. *Mol Nutr Food Res* 2018;62:e1700561.

35. Yu CH, Hsieh PL, Chao SC, Chen SH, Liao YW, Yu CC. Xist/let-7i/hmga1 axis maintains myofibroblasts activities in oral submucous fibrosis. *Int J Biol Macromol* 2023;232:123400.
36. Ho DCY, Chen SH, Fang CY, et al. Paeonol inhibits profibrotic signaling and hotair expression in fibrotic buccal mucosal fibroblasts. *J Formos Med Assoc* 2022;121:930—5.
37. Liao YW, Tsai LL, Lee YH, Hsieh PL, Yu CC, Lu MY. Mir-21 promotes the fibrotic properties in oral mucosa through targeting pdcd4. *J Dent Sci* 2022;17:677—82.
38. Chen PY, Ho DCY, Liao YW, et al. Honokiol inhibits arecoline-induced oral fibrogenesis through transforming growth factor-β/smad2/3 signaling inhibition. *J Formos Med Assoc* 2021;120:1988—93.
39. Lee YH, Hsieh PL, Chao SC, Liao YW, Liu CM, Yu CC. A-mangostin inhibits the activation of myofibroblasts via down-regulation of linc-ror-mediated tgfb1/smad signaling. *Nutrients* 2023;15:1321.
40. Yang HW, Sun YH, Fang CY, et al. Downregulation of histone deacetylase 8 (hdac8) alleviated the progression of oral submucous fibrosis. *J Dent Sci* 2023;18:652—8.
41. Tai Y, Woods EL, Dally J, et al. Myofibroblasts: function, formation, and scope of molecular therapies for skin fibrosis. *Biomolecules* 2021;11:1095.
42. 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.
43. Saito S, Kitabatake M, Ouji-Sageshima N, et al. Angiopoietin-like 4 is a critical regulator of fibroblasts during pulmonary fibrosis development. *Am J Respir Cell Mol Biol* 2023; Online ahead of print.
44. 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.