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Original Article

Osthole mitigates the myofibroblast properties in oral submucous fibrosis by suppressing the TGF- β /smad2 signaling pathway and NCK-AS1 expression

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Abstract *Background/purpose:* Natural products are gaining increasing recognition as an alternative source for alleviating fibrosis as they can regulate various mediators or pathways against fibrosis by targeting non-coding RNAs. In the current study, we aimed to investigate the therapeutic effects of osthole in oral submucous fibrosis (OSF), a precancerous condition of the oral cavity.

Materials and methods: The cytotoxicity of osthole to normal and fibrotic buccal mucosal fibroblasts (fBMFs) derived from OSF tissues was assessed using MTT assay. Collagen gel contraction and transwell migration assays were conducted to examine the myofibroblast activities. Besides, the expression of TGF- β /Smad2 signaling as well as α -SMA and type I collagen were measured. Additionally, RNA sequencing was used to identify a potential target involved in the anti-fibrotic effect of osthole.

Results: Osthole exhibited a higher cytotoxic effect on fBMFs compared to normal BMFs and dose-dependently reduced several myofibroblast activities, including collagen gel contractility

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and transwell migration ability. In addition, the expression of the TGF- β /Smad2 pathway was inhibited along with a lower expression of α -SMA and type I collagen in osthole-receiving cells. Moreover, the administration of osthole downregulated the expression of NCK1-AS1 in fBMFs, which was proven to mediate the anti-fibrosis property of osthole.

Conclusion: Our results indicate that osthole may be a promising compound to inhibit the progression of OSF.

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Introduction

Oral submucous fibrosis (OSF) is a chronic inflammatory condition characterized by the accumulation of fibrous tissue in the oral mucosa, possibly leading to restricted mouth opening and difficulties with mastication. It has been recognized as an oral potentially malignant disorder with a has a high rate of malignant transformation (5.7%–9.13%) into oral squamous cell carcinoma (OSCC).^{1–3} A substantial amount of evidence has been accrued to show that the development of OSF is attributable to the habit of areca nut chewing as the constituents in areca nut has been demonstrated to increase TGF- β production, a central driver in fibroblast-to-myofibroblast conversion, in epithelial cells and gingival fibroblasts.^{4,5} Moreover, several studies have revealed that arecoline, a nicotinic acid-based alkaloid in areca nut, induced latent TGF- β 1 activation and production of reactive oxygen species, resulting in myofibroblast activation of buccal mucosal fibroblasts (BMFs).^{6,7} As such, approaches to diminish the TGF- β 1-associated myofibroblast transdifferentiation may represent attractive anti-fibrotic strategies in OSF.

Osthole, 7-methoxy-8-(3-methyl-2-butenyl), is a member of coumarins that can be obtained through extraction from *Cnidium monnieri* (L.) or via chemical synthesis.⁸ Several studies have shown that osthole has numerous cellular activities, such as anti-oxidative or anti-inflammatory features.^{9,10} In addition, it holds the potential to suppress various types of cancers by prohibiting angiogenesis in hepatocellular carcinoma,¹¹ triggering cell autophagy and pyroptosis in ovarian carcinoma,¹² and inducing cell cycle arrest and apoptosis in head and neck squamous cell carcinoma.¹³ Osthole also has been demonstrated to inhibit fibrosis diseases, such as myocardial fibrosis and renal fibrosis,^{14–17} by attenuating collagen expression via the TGF- β /Smad signaling pathway. Moreover, it was found that osthole mitigated myofibroblast activation of cardiac fibroblasts,¹⁵ kidney interstitial fibroblasts,¹⁷ and hepatic stellate cells.¹⁸ Nevertheless, the suppressive effect of osthole on precancerous OSF has not been investigated.

Non-coding RNA has exhibited its significant role in regulating the development of OSF and OSCC.¹⁹ Both lncRNAs (>200 nucleotides) and short non-coding RNAs (such as microRNAs; ~22 nucleotides) are reported to be the targets of osthole treatments.^{20,21} The lncRNA, non-catalytic region of tyrosine kinase adaptor protein 1 antisense 1 (NCK1-AS1), was upregulated in OSCC patients and correlated with increased invasion and migration in OSCC

cells.²² Additionally, the positive correlation between NCK1-AS1 and TGF- β 1 in both ESCC and prostate cancer cells suggests a potential regulatory role for NCK1-AS in the development of OSF, which might be a target for osthole.^{23,24} However, the role of NCK1-AS1 in OSF has not been elucidated.

Given that several studies have shown that osthole treatment downregulated the activation of TGF- β /Smad signaling and myofibroblast transdifferentiation, we sought to examine whether osthole can exhibit anti-fibrotic property in OSF and elucidate the mechanism by which osthole decreases myofibroblast activation. Herein, we tested numerous myofibroblast activities of osthole-treated fibrotic buccal mucosal fibroblasts (fBMFs) derived from OSF tissues and the activation of the TGF- β /Smad pathway. Subsequently, we used RNA sequencing for screening of differentially expressed genes between cells with or without osthole in order to select a putative target that accounts for the inhibitory property of osthole in OSF.

Materials and methods

Tissue collection and cell culture

Isolation of fBMFs and normal BMFs from buccal mucosa tissues was carried out among the OSF patients and healthy individuals, respectively. The primary cell isolation and culture methods were previously detailed in reference.⁷ Briefly, buccal mucosa tissues attained from surgery were placed in Hanks' Balanced Salt Solution (Thermo Fisher Scientific, Waltham, MA, USA) at 4 °C and immediately transferred to the laboratory for further processing. Following trypsinization, the tissues were cultured in DMEM medium (Thermo Fisher Scientific) containing 10% fetal bovine serum (Thermo Fisher Scientific), and 1% penicillin-streptomycin cocktail and plated into 25-T flasks for 14 days. The cells that were spindle-shaped and migrated out of the tissues were identified as buccal fibroblasts. These cells were continuously passaged and used for subsequent experiments between the 3rd and 8th passages, confirmed to be free of mycoplasma contamination using short tandem repeat (STR) DNA profiling.

Collagen gel contraction assay

After dispersing the cells in a collagen gel solution from Sigma-Aldrich (St. Louis, MO, USA), the mixture was placed

in a 24-well plate and incubated at 37 °C for 2 h. Once polymerization was complete, the gels were cultured in 0.5 ml of media for 48 h. The change in collagen gel size, known as the contraction index, was measured using ImageJ software (NIH, Bethesda, MD, USA).²⁵

Transwell migration assays

This assay was carried out according to our previous study.²⁶ A total of 1×10^5 cells in low serum medium were introduced to the top chamber of a transwell (Corning, Acton, MA, USA), while media supplemented with increased serum was used as a chemoattractant in the bottom chamber. After a 24-h incubation, the cells on the bottom surface of the inserted membrane were stained with crystal violet. The number of migratory cells in five randomly selected areas was then counted.

Western blot analysis

As described in our previous study, 20 µg of protein in whole-cell lysate underwent separation via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and was then transferred to a polyvinylidene fluoride membrane (Millipore, Merck, Darmstadt, Germany).²⁷ After the blockade of the non-specific binding sites with 5% bovine serum albumin (Millipore), the membrane was incubated with primary antibodies at 4 °C for 16 h. Subsequently, it was exposed to the corresponding HRP-conjugated secondary antibody at room temperature for 1 h. The signals produced by antibody binding were visualized using ECL substrate (Millipore) and captured using the LAS-1000 plus analyzer (GE Healthcare, Piscataway, NJ, USA). GAPDH was used as an internal reference for normalization purposes. All antibodies used in this study were purchased from Cell Signaling Inc. (Danvers, MA, USA).

Quantitative real-time PCR (qRT-PCR) analysis

RNA extraction, quality assessment, and quantification procedures were previously detailed. The Superscript III first-strand synthesis system (Invitrogen Life Technologies, Carlsbad, CA, USA) was employed to reverse-transcribe RNA into cDNA. PCR experiments using the resulting cDNAs were conducted on ABI StepOne™ Real-Time PCR Systems (Applied Biosystems, Waltham, MA, USA). The specific primer sequences are listed as follows (5'-3'): *NCK-AS1*, TTCCCATTTCTCCAGGTCC (forward), TGGTTACTTTGAGCCTGGC (reverse). The levels of *NCK-AS1* were normalized using *GAPDH*, CTCATGACCACAGTCCATGC (forward), TTCAGCTCTGGGATGACCTT (reverse) and the $2^{-\Delta Ct}$ method was applied.²⁷

Overexpression of NCK-AS1

The *NCK-AS1* cDNA was inserted into the pCDHI-MCS1-EF1-CopGFP plasmid (System Biosciences, Mountain View, CA, USA). Co-transfection of the pCDH plasmid along with two helper plasmids (packaging and envelope plasmids) was performed in 293T cells using Lipofectamine 2000 reagent (Thermo Fisher Scientific) to generate lentiviral

pseudoparticles. GFP-positive cells were sorted via flow cytometry (BD, Becton Drive Franklin Lakes, NJ, USA) following lentivirus infection. The overexpression of *NCK-AS1* was validated using qRT-PCR and Western blot analysis. An empty pCDHI-MCSI-EF1-COpGFP vector served as the control group.²⁷

Statistical analysis

Statistical analyses were conducted using the Statistical Package of Social Sciences software (IBM Corporation, Armonk, NY, USA). Data from at least triplicate experiments are presented as mean $\pm$ SD. Student's t-test, one-way analysis of variance (ANOVA) with post hoc Tukey's test, and other appropriate tests were employed to assess statistical differences. A P -value < 0.05 was considered statistically significant.²⁷

Results

The chemical structure of osthole was shown in Fig. 1A. To evaluate the cytotoxic effect of osthole on BMFs and patient-derived fBMFs, cell proliferation was examined after a 48 h treatment of various concentrations of osthole using a 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Fig. 1B showed that osthole has a concentration-dependent inhibitory effect on cell viability for both BMFs and fBMFs. The half-maximal inhibitory concentrations (IC_{50}) for osthole in BMFs-1, BMFs-2, fBMFs-1 and fBMFs-2 were 83.9 ± 10.8 , 73.1 ± 9.7 , 32.5 ± 4.5 , and 34.7 ± 3.9 µM, respectively (Fig. 1B). These results demonstrated that a lower concentration of osthole was sufficient to reduce the cell viability of fBMFs without harming normal cells. Accordingly, the lower concentrations of osthole (0–20 µM) were used in the following experiments to determine the anti-fibrosis effect of osthole.

It is well-known that wound contraction and remodeling of injured tissue involve the differentiation of fibroblasts into α -smooth muscle actin (α -SMA)-expressing myofibroblasts. Since myofibroblasts are evidently more contractile, hydrated collagen lattices have often been used to resemble the stages of wound remodeling and test the intracellular tension on collagen substrates of the pre-incubating cells.²⁸ As shown in Fig. 2A, a higher concentration of osthole attenuated cell contractility, suggesting there was a dose-dependent effect of osthole on collagen gel contraction of fBMFs. Aside from cell contractility, the increased migration capacity is another feature of activated myofibroblasts as they proliferate and migrate to the wound area to restore tissue integrity. We observed that the transwell migration ability was downregulated following osthole treatment (Fig. 2B).

In addition, we measured the secretion of TGF- β 1 in two patient-derived fBMFs and found that the production of TGF- β 1 was markedly repressed in fBMFs incubated with osthole (Fig. 3A). Results from Western blot showed that the expression levels of type I collagen, α -SMA, and phosphorylated-Smad2 were all attenuated in the osthole-treated fBMFs in a dose-dependent manner (Fig. 3B). Collectively, these findings indicated that treatment of osthole may impede the progression of OSF by diminishing

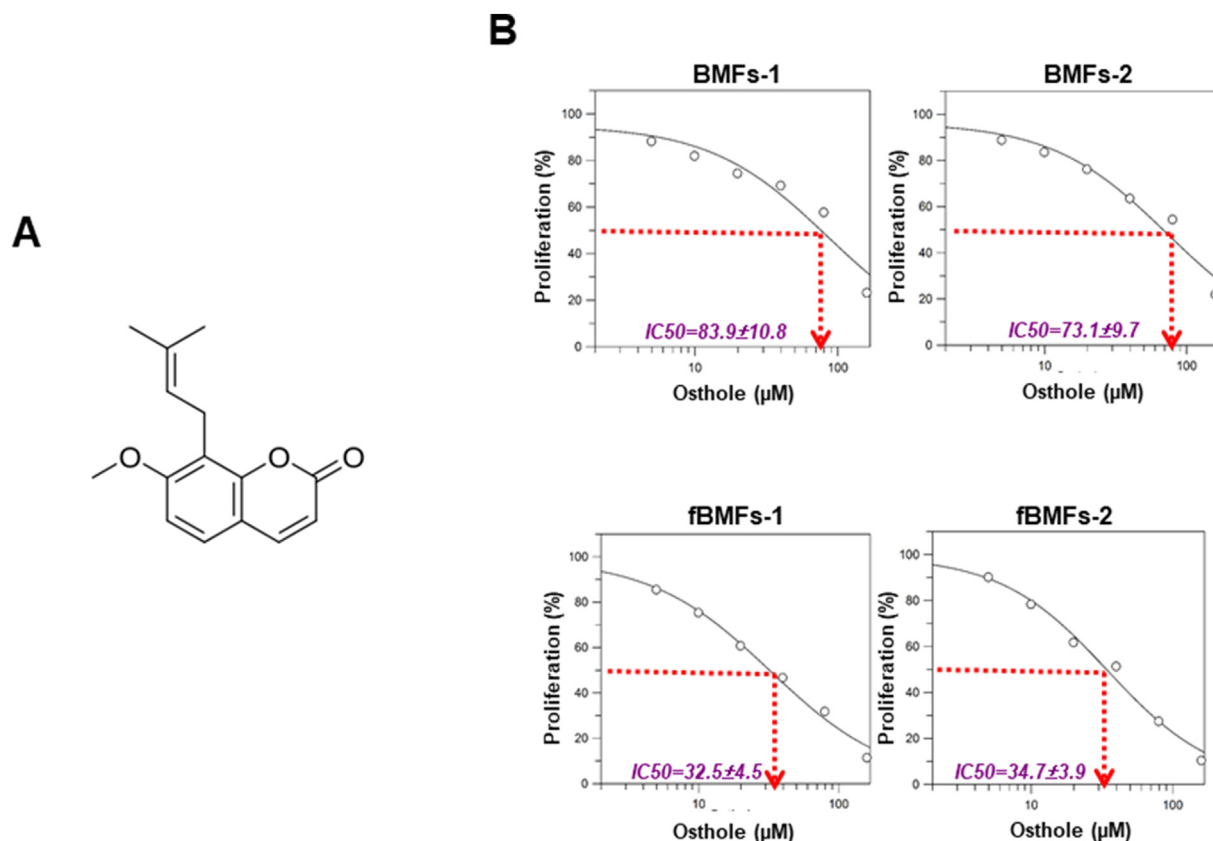


Figure 1 The cytotoxic effect of osthole on the cell viability of buccal mucosal fibroblasts (BMFs) and fibrotic BMFs (fBMFs). (A) The chemical structure of osthole. (B) MTT assay was utilized to examine cell survival/proliferation in response to various concentrations of osthole. The IC₅₀ values were calculated by GraFit software.

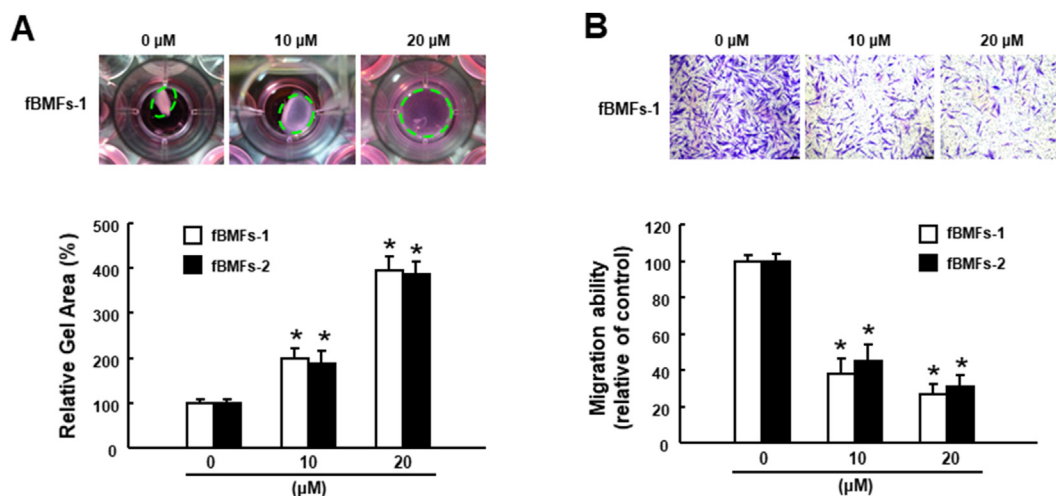


Figure 2 Effects of osthole on collagen gel contractility and migration capacity in fBMFs. The fBMFs were subjected to collagen gel contraction (A) and transwell migration (B) assays. Collagen gel contraction and migration were measured 48 h post-osthole exposure. The experiments were repeated three times and representative results were shown. Results are means ± SD. * $P < 0.05$ compared to no treatment control group.

myofibroblast phenotypes and downregulating the expression of various fibrosis markers.

In an effort to explicate the molecular mechanism underlying the anti-fibrotic effect of osthole, RNA-sequencing

was applied to identify a potential target that mediated the inhibition of myofibroblasts and TGF-β1 activation following osthole treatment. As shown in Fig. 4A, NCK1-AS1 is one of the aberrantly expressed lncRNAs in the osthole-treated

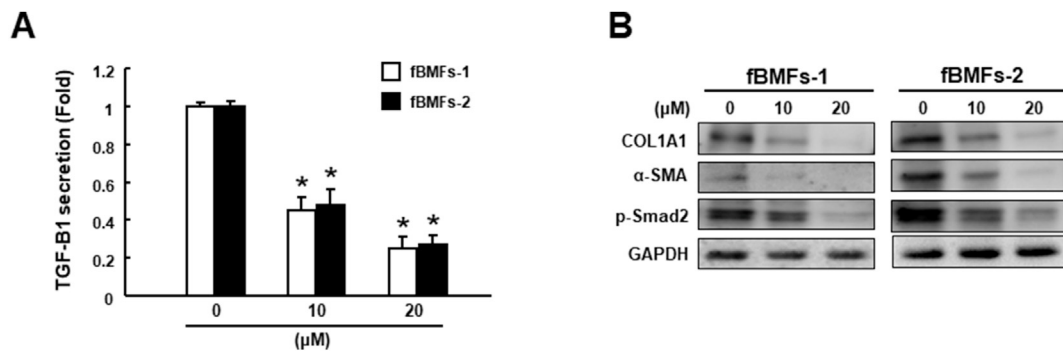


Figure 3 Suppressive property of osthole on the TGF- β /Smad2 signaling and fibrosis markers. (A) ELISA assay was conducted to examine the TGF- β secretion with various concentrations of osthole. (B) The expression of type I collagen alpha I (COL1A1), α -SMA and phosphorylated Smad2 were assessed in the fBMFs treated with the indicated concentration of osthole. The experiments were repeated three times and representative results were shown. Results are means $\pm$ SD. * $P < 0.05$ compared to no treatment group.

fBMFs. In view of the oncogenic role of NCK1-AS1 in OSCC and positive relationship with TGF- β 1, we then assessed whether NCK1-AS1 was implicated in the osthole-inhibited myofibroblast activation.

qRT-PCR was conducted to validate the RNA-sequencing data, and we found that the expression of NCK1-AS1 was dose-dependently downregulated in fBMFs by osthole (Fig. 4B). Moreover, we demonstrated that ectopic expression of NCK1-AS1 counteracted the inhibitory property of osthole on collagen gel contractility (Fig. 5A). Likewise, the suppressive effect of osthole on migration capacity was declined in fBMFs overexpressed NCK1-AS1 (Fig. 5B). Taken together, our results demonstrated that the anti-fibrosis role of osthole in OSF may be associated with the downregulation of NCK1-AS1.

Discussion

Over the past few decades, non-coding RNAs have emerged as critical determinants of gene expression during the

development of various diseases, including OSF.^{26,27} Several studies have examined the therapeutic effect of naturally derived ingredients on inhibition of myofibroblast activation in OSF via modulation of non-coding RNAs.^{25,29} In this study, we found that the expression of lncRNA NCK1-AS1 was downregulated in osthole-receiving fBMFs. Furthermore, we demonstrated that inhibition of NCK1-AS1 was essential to the anti-fibrotic effect of osthole in fBMFs, as evidenced by the reappearance of higher migration capacity and collagen gel contractility in NCK1-AS1-overexpressing fBMFs in the presence of osthole. Currently, the majority of the existing studies regarding NCK1-AS1 focus on its oncogenic role and there is a lack of research that explores the role of NCK1-AS1 in the development of fibrosis.^{30–32} Our results demonstrated that NCK1-AS1 may contribute to oral fibrogenesis by promoting myofibroblast activities.

The lncRNA NCK1-AS1, alternatively referred to as NCK1 divergent transcript (NCK1-DT), is located on chromosome 3q22.3 in humans.³³ Aside from its tumor-promoting role in OSCC,²² NCK1-AS1 in plasma has been used to differentiate

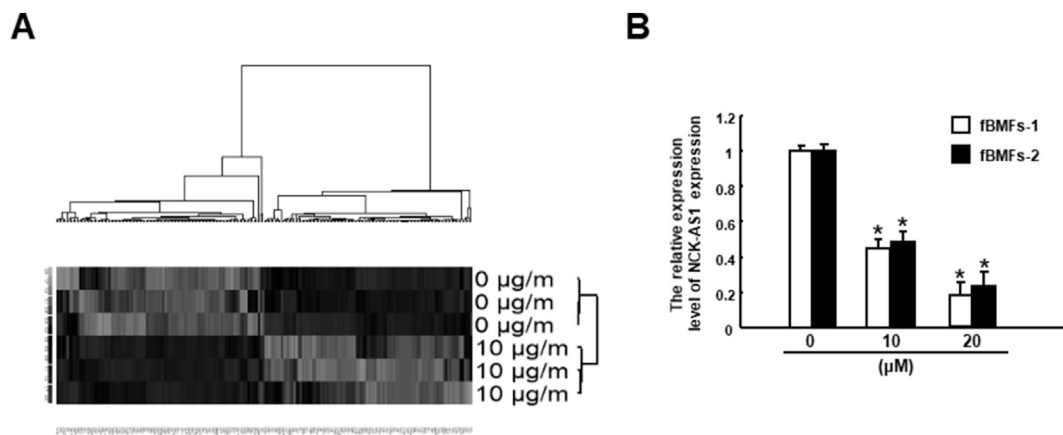


Figure 4 Osthole treatment decreased the expression of NCK1-AS1. (A) A heatmap showing NCK1-AS1 was one of the differentially expressed genes in fBMFs treated with or without osthole. (B) The expression level of NCK1-AS1 in two fBMFs was dose-dependently reduced in response to osthole. The experiments were repeated three times and representative results were shown. Results are means $\pm$ SD. * $P < 0.05$ compared to no treatment group.

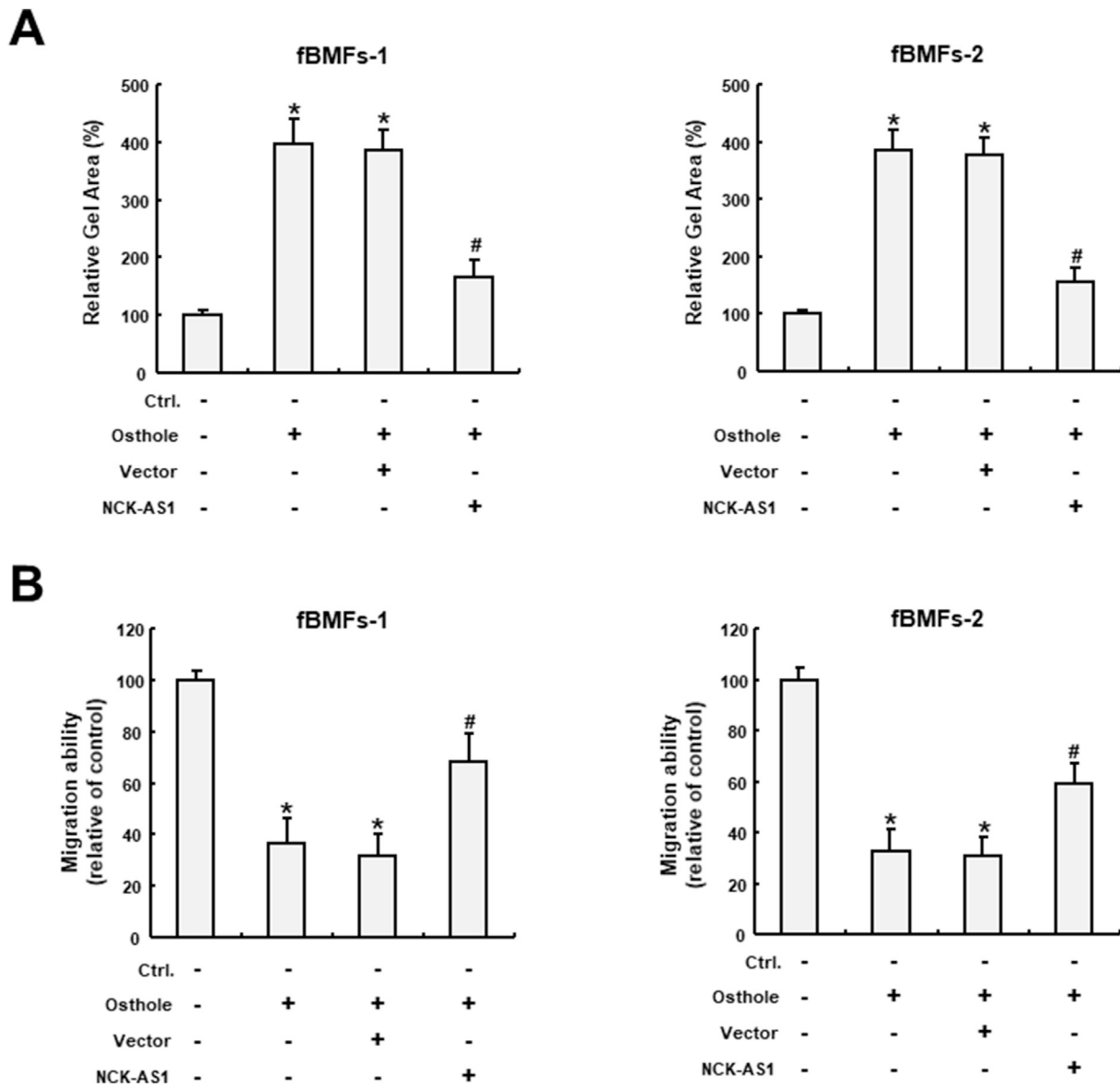


Figure 5 The inhibitory effects of osthole on myofibroblast activities in fBMFs were hindered by overexpression of NCK1-AS1. (A) Collagen gel contraction and (B) Tranwell migration assays were carried out to examine whether suppression of NCK1-AS1 was required for the osthole-induced downregulation of myofibroblast activities. The experiments were repeated three times and representative results were shown. Results are means $\pm$ SD. * $P < 0.05$ compared to no treatment group. # $P < 0.05$ compared to osthole treatment group.

oral ulcers from the early stages of OSCC and holds early diagnostic value.²² Here, we showed that NCK1-AS1 may play a part in the development of precancerous OSF. Although we did not elucidate the detailed molecular mechanism underlying the effect of NCK1-AS1 on myofibroblast activation, there were several studies showing the relationship between NCK1-AS1 and TGF- β 1. As aforementioned, activation of TGF- β 1 signaling is pivotal in the development of fibrosis diseases by inducing fibroblast differentiation into myofibroblasts, which overproduce extracellular matrix. It has been revealed that NCK1-AS1 showed a positive correlation with TGF- β 1 in ESCC and upregulation of NCK1-AS1 led to an increase in TGF- β 1 production of ESCC and prostate cancer cells, while TGF- β 1

overexpression failed to affect NCK1-AS1 expression.^{23,24} In line with these findings, we showed that both the expression of lncRNA NCK1-AS1 and TGF- β 1 signaling were inhibited in fBMFs following osthole treatment. Accordingly, it is plausible that suppression of NCK1-AS1 in response to osthole restrained myofibroblast activation by downregulation of TGF- β 1 pathway.

Osthole has been shown to ameliorate fibrosis diseases and suppress myofibroblast activation through various mechanisms. For instance, osthole demonstrated anti-fibrosis effects by reducing cellular oxidation, inflammation-related genes and chemokines production in hepatic fibrosis.¹⁸ Another study showed that osthole exerted an inhibitory effect in renal fibrosis by repression

of epithelial-mesenchymal transition and hindrance of myofibroblast proliferation via downregulating DNA synthesis and expressions of proliferation- and cell-cycle-associated proteins.¹⁷ Besides, most of the studies suggested that osthole exhibited a protective effect against fibrosis diseases through the downregulation of TGF- β 1 signaling and collagen I and III expression.^{9,14–16,34} Our results were consistent with these findings and showed that osthole blocked the TGF- β 1/Smad pathway and suppressed collagen expression. In addition, we showed that the myofibroblast activities and the expression of myofibroblast marker, α -SMA, were all decreased after exposure to osthole.

In conclusion, the present study demonstrated the promising results of using osthole to alleviate the progression of OSF by repressing myofibroblast activation and TGF- β 1/Smad signaling, possibly through downregulation of NCK1-AS1.

Declaration of competing interest

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

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References

- Chuang SL, Wang CP, Chen MK, et al. Malignant transformation to oral cancer by subtype of oral potentially malignant disorder: a prospective cohort study of Taiwanese nationwide oral cancer screening program. *Oral Oncol* 2018;87:58–63.
- 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.
- Chiang WF, Lin SY, Lin JF, et al. Malignant development in patients with oral potentially malignant disorders detected through nationwide screening: outcomes of 5-year follow-up at a single hospital. *Head Neck* 2020;42:67–76.
- 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.
- Pant I, Kumar N, Khan I, Rao SG, Kondaiah P. Role of areca nut induced TGF- β and epithelial-mesenchymal interaction in the pathogenesis of oral submucous fibrosis. *PLoS One* 2015;10:e0129252.
- Hsieh YP, Wu KJ, Chen HM, Deng YT. Arecoline activates latent transforming growth factor β 1 via mitochondrial reactive oxygen species in buccal fibroblasts: suppression by epigallocatechin-3-gallate. *J Formos Med Assoc* 2018;117:527–34.
- Liao YW, Yu CC, Hsieh CW, Chao SC, Hsieh PL. Aberrantly downregulated FENDRR by arecoline elevates ROS and myofibroblast activation via mitigating the miR-214/MFN2 axis. *Int J Biol Macromol* 2024;264:130504.
- Sun M, Sun M, Zhang J. Osthole: an overview of its sources, biological activities, and modification development. *Med Chem Res* 2021;30:1767–94.
- Li Q, Wang Y, Yan J, et al. Osthole ameliorates early diabetic kidney damage by suppressing oxidative stress, inflammation and inhibiting TGF- β 1/Smads signaling pathway. *Int Immunopharm* 2024;133:112131.
- Singh L, Bhatti R. Signaling pathways involved in the neuro-protective effect of osthole: evidence and mechanisms. *Mol Neurobiol* 2024;61:1100–18.
- Yao F, Zhang L, Jiang G, Liu M, Liang G, Yuan Q. Osthole attenuates angiogenesis in an orthotopic mouse model of hepatocellular carcinoma via the downregulation of nuclear factor- κ B and vascular endothelial growth factor. *Oncol Lett* 2018;16:4471–9.
- Liang J, Zhou J, Xu Y, et al. Osthole inhibits ovarian carcinoma cells through LC3-mediated autophagy and GSDME-dependent pyroptosis except for apoptosis. *Eur J Pharmacol* 2020;874:172990.
- Yang J, Zhu XJ, Jin MZ, Cao ZW, Ren YY, Gu ZW. Osthole induces cell cycle arrest and apoptosis in head and neck squamous cell carcinoma by suppressing the PI3K/AKT signaling pathway. *Chem Biol Interact* 2020;316:108934.
- Liu JC, Zhou L, Wang F, Cheng ZQ, Rong C. Osthole decreases collagen I/III contents and their ratio in TGF- β 1-overexpressed mouse cardiac fibroblasts through regulating the TGF- β /Smad signaling pathway. *Chin J Nat Med* 2018;16:321–9.
- Liu JC, Wang F, Xie ML, et al. Osthole inhibits the expressions of collagen I and III through Smad signaling pathway after treatment with TGF- β 1 in mouse cardiac fibroblasts. *Int J Cardiol* 2017;228:388–93.
- Chen R, Xue J, Xie ML. Reduction of isoprenaline-induced myocardial TGF- β 1 expression and fibrosis in osthole-treated mice. *Toxicol Appl Pharmacol* 2011;256:168–73.
- Zhang S, Huang Q, Cai X, et al. Osthole ameliorates renal fibrosis in mice by suppressing fibroblast activation and epithelial-mesenchymal transition. *Front Physiol* 2018;9:1650.
- Liu YW, Chiu YT, Fu SL, Huang YT. Osthole ameliorates hepatic fibrosis and inhibits hepatic stellate cell activation. *J Biomed Sci* 2015;22:63.
- Zhou S, Zhu Y, He Z, et al. Long non-coding RNA expression profile associated with malignant progression of oral submucous fibrosis. *JAMA Oncol* 2019;2019:6835176.
- Lv X, Yang H, Zhong H, He L, Wang L. Osthole exhibits an antitumor effect in retinoblastoma through inhibiting the PI3K/AKT/mTOR pathway via regulating the hsa_circ_0007534/miR-214-3p axis. *Pharm Biol* 2022;60:417–26.
- Niu Z, Fu M, Li Y, Ren H, Zhang X, Yao L. Osthole alleviates pulmonary vascular remodeling by modulating microRNA-22-3p mediated lipid metabolic reprogramming. *Phytomedicine* 2022;96:153840.
- Le F, Ou Y, Luo P, Zhong X. LncRNA NCK1-AS1 in plasma distinguishes oral ulcer from early-stage oral squamous cell carcinoma. *J Biol Res (Thessalon)* 2020;27:16.
- Fu X, Chen X, Si Y, Yao Y, Jiang Z, Chen K. Long non-coding RNA NCK1-AS1 is overexpressed in esophageal squamous cell carcinoma and predicts survival. *Bioengineered* 2022;13:8302–10.
- Guan Z, Song Y, Ma J, et al. Altered expression of lncRNA NCK1-AS1 distinguished patients with prostate cancer from those with benign prostatic hyperplasia. *Oncol Lett* 2019;18:6379–84.
- Lee YH, Hsieh PL, Chao SC, Liao YW, Liu CM, Yu CC. α -Mangostin inhibits the activation of myofibroblasts via downregulation of linc-ROR-mediated TGF β 1/Smad signaling. *Nutrients* 2023;15:1321.
- Chou MY, Hsieh PL, Chao SC, Liao YW, Yu CC, Tsai CY. MiR-424/TGIF2-mediated pro-fibrogenic responses in oral submucous fibrosis. *Int J Mol Sci* 2023;24:5811.

27. 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.
28. Arora PD, Narani N, McCulloch CA. The compliance of collagen gels regulates transforming growth factor-beta induction of alpha-smooth muscle actin in fibroblasts. *Am J Pathol* 1999; 154:871–82.
29. Lin CY, Hsieh PL, Liao YW, Peng CY, Yu CC, Lu MY. Arctigenin reduces myofibroblast activities in oral submucous fibrosis by LINC00974 inhibition. *Int J Mol Sci* 2019;20.
30. Wang B, Wang K, Jin T, et al. NCK1-AS1 enhances glioma cell proliferation, radioresistance and chemoresistance via miR-22-3p/IGF1R ceRNA pathway. *Biomed Pharmacother* 2020;129: 110395.
31. Huang L, Li X, Ye H, et al. Long non-coding RNA NCK1-AS1 promotes the tumorigenesis of glioma through sponging microRNA-138-2-3p and activating the TRIM24/Wnt/ β -catenin axis. *J Exp Clin Cancer Res* 2020;39:63.
32. Lin Q, Jia Y, Zhang D, Jin H. NCK1-AS1 promotes the progression of melanoma by accelerating cell proliferation and migration via targeting miR-526b-5p/ADAM15 axis. *Cancer Cell Int* 2021;21:367.
33. Wang Y, Pan J, Sun Z. LncRNA NCK1-AS1-mediated regulatory functions in human diseases. *Clin Transl Oncol* 2023;25: 323–32.
34. Chen R, Xue J, Xie M. Osthole regulates TGF- β 1 and MMP-2/9 expressions via activation of PPAR α / γ in cultured mouse cardiac fibroblasts stimulated with angiotensin II. *J Pharm Pharmacol* 2013;16:732–41.