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ORIGINAL ARTICLE

Epigallocatechin-3-gallate inhibits transforming-growth-factor-β1-induced collagen synthesis by suppressing early growth response-1 in human buccal mucosal fibroblasts

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Background/purpose: Transforming growth factor (TGF)-β is a key regulator in the pathogenesis of oral submucous fibrosis (OSF). Early growth response (Egr)-1 is essential for fibrotic responses to TGF-β. Because TGF-β signaling is cell-type- and context-dependent, we investigated the signaling involved in TGF-β-induced Egr-1 in primary human buccal mucosal fibroblasts (BMFs).
Methods: TGF-β-induced Egr-1 and its signaling were assessed by western blotting in BMFs. Egr-1 small interfering RNA was used to define the role of Egr-1 on TGF-β-induced mRNAs of the α1- and α2-chains of type I collagen (COL1A1 and COL1A2) and acid-soluble collagen production (via Sircol collagen assay). The effects of epigallocatechin-3-gallate (EGCG) on TGF-β-induced Egr-1 protein and acid-soluble collagen were also evaluated.
Results: TGF-β1 stimulated Egr-1 production in BMFs. Pretreatment with PD98059, SP600125, SB431542, and SIS3, but not SB203580, significantly reduced TGF-β1-induced Egr-1 protein expression. Genetic targeting of Egr-1 completely inhibited TGF-β1-induced type I collagen mRNAs and collagen protein expression. EGCG fully inhibited TGF-β1-induced Egr-1 and TGF-β1-stimulated production of acid-soluble collagens.
Conclusion: We conclude that activin receptor-like kinase (ALK)5, Smad3, extracellular signal-regulated kinase, and c-Jun N-terminal kinase are involved in the TGF-β1-induced Egr-1 protein production in BMFs. Egr-1 mediates TGF-β1-induced COL1A1 and COL1A2 mRNA expression and acid-soluble collagen production in BMFs. EGCG can block TGF-β1-induced collagen

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

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production by attenuating Egr-1 expression in BMFs. Egr-1 is a key mediator in TGF-β1-induced pathogenesis of OSF. EGCG may be useful in the prevention or treatment of OSF.

Introduction

Oral submucous fibrosis (OSF) is a precancerous condition of the oral cavity. In the advanced stage of OSF, type I collagen is the major extracellular matrix constituent in the lamina propria and submucosal layer of the oral cavity.[1] Areca nut (AN; *Areca catechu*) chewing is the most important etiological factor for OSF. The major AN alkaloid, arecoline, causes imbalance between collagen degradation and synthesis.[2] Exposure to AN and stimulation of the transforming growth factor (TGF)-β pathway are responsible for overproduction of collagen and decreased degradation of collagen in OSF.[3] Immunohistochemistry has shown intense TGF-β staining of epithelium, fibroblast, macrophages, and inflammatory cells in early OSF.[4] Arecoline upregulates expression of αvβ6 integrin in oral keratinocytes; the αvβ6-dependent TGF-β1 activation further induces myofibroblast transdifferentiation and contributes to the pathogenesis of OSF.[5] At present, no known treatment completely reverses the process of OSF. Although anti-TGF-β therapy has shown significant antifibrotic effects in animal models, systemically administered and repeated doses of anti-TGF-β1 drug therapy in systemic sclerosis resulted in significant morbidity and mortality in a multicenter, randomized, placebo-controlled clinical trial.[6] TGF-β regulates important physiological processes, including tumor suppression[7] and immunosuppression.[8] Inhibiting TGF-β activity causes spontaneous autoimmunity and epithelial hyperplasia, or interferes with wound healing.[9] Thus, therapeutic targets other than TGF-β need to be evaluated.

Early growth response (Egr)-1 is an immediate early gene located on human chromosome 5q31, encoding an 80-kDa zinc-finger transcription factor that binds to guanine-cytosine (GC)-rich regulatory DNA elements in the promoter region of many target genes.[10] It plays important roles in cellular growth, differentiation, and activation of cell death pathways. It is normally low or undetectable, however, it is induced rapidly and transiently by a wide range of environmental stimuli, including TGF-β.[10,11] Sustained expression of Egr-1 contributes to pathological responses. Increased Egr-1 is detected in lesional human fibrotic tissues from atherosclerotic plaques, idiopathic pulmonary fibrosis, and lung and skin of scleroderma.[11] Our recent study has demonstrated elevated Egr-1 staining in OSF specimens.[12] We have also shown that arecoline stimulates Egr-1 in normal human buccal mucosal fibroblasts (BMFs), implying a role of Egr-1 in the pathogenesis of OSF.[12]

Egr-1 is an important mediator of TGF-β-induced responses.[10] TGF-β induces rapid and transient accumulation of Egr-1 mRNA and protein in normal fibroblasts.[13] Egr-1 subsequently stimulates collagen synthesis, myofibroblast differentiation, and other fibrotic responses, including the secretion of fibrogenic growth factors and cytokines, leading to a positive feedback loop to contribute to the development and persistence of fibrosis.[11] Type I collagen, the principal matrix protein deposited in OSF, is a heterotrimeric molecule composed of two α1-chains and one α2-chain. The α1-chain and α2-chain of type I collagen (COL1A1 and COL1A2) promoters contain binding sites for Egr-1.[13,14] Forced expression of Egr-1 is sufficient by itself to upregulate COL1A2 promoter activity and further enhance the synthesis of type I collagen.[13] Egr-1-null murine embryonic fibroblasts show attenuated synthesis of TGF-β-induced type I procollagen.[13] In explanted Egr-1-null murine skin fibroblasts, TGF-β stimulation of collagen synthesis, cell migration, and myofibroblast transdifferentiation are all significantly impaired.[15] Therefore, Bhattacharyya et al[11] suggested Egr-1 as the new conductor in orchestrating fibrotic responses.

Considering the broad range of the biological roles of Egr-1, it is interesting that Egr-1-deficient mice are viable yet without apparent phenotype except for female infertility, reduced body size, impaired liver regeneration, or some altered tissue remodeling.[11] Therefore, in the context of treating fibrosis, Egr-1 should be better than TGF-β as a therapeutic target. Because Egr-1 is crucial for TGF-β-dependent fibrotic responses and because TGF-β signaling is cell-type- and context-dependent,[16] we investigated the signaling pathways of TGF-β-induced Egr-1 expression in normal human BMFs and the effects of blocking Egr-1 on the expression of TGF-β-induced COL1A1 and COL1A2 mRNAs and the production of TGF-β-induced collagen synthesis in BMFs. We further explored whether green tea polyphenol, epigallocatechin-3-gallate (EGCG), affected TGF-β-induced Egr-1 and collagen synthesis in BMFs.

Methods

Cell culture

Under the approval of the Research Ethic Committee of National Taiwan University Hospital (approval number: 201305062RINC), three primary BMFs cultures were established with informed consent obtained from patients as described previously.[12] Cells were plated on 60-mm Petri dishes at a density of 2×10^5 cells, followed by 24 hours serum deprivation before treatment with TGF-β1 (R&D Systems, Minneapolis, MN, USA). To study the potential signaling transduction pathways, BMFs were pretreated with 10μM extracellular signal-regulated kinase (ERK) inhibitor PD98059, 10μM kinase (JNK) inhibitor SP600125, 10μM p38 mitogen-activated protein kinase (MAPK) inhibitor SB203580, 10μM

activin receptor-like kinase (ALK)5 inhibitor SB431542, or 10μM Smad3 phosphorylation inhibitor SIS3 (CalBiochem, San Diego, CA, USA), for 1 hour before exposure to 2 ng/mL TGF-β1 for 2 hours. All tissue culture biological agents were from Invitrogen (Carlsbad, CA, USA).

Western blot analysis

The methods used were similar to those reported in our previous study.[12] Cells were harvested, subjected to lysis, separated, and immunoblotted. The antibodies and dilutions used included: Egr-1 (1:500; Cat. No. sc-189; Santa Cruz Biotechnology, Santa Cruz, CA, USA), β-actin (1:2000; Cat. No. sc-47778; Santa Cruz Biotechnology), goat anti-rabbit secondary antibody (1:10000; Cat. No. ab6721; Abcam, Cambridge, MA, USA), and rabbit antimouse secondary antibody (1:10000; Cat. No. ab6728; Abcam). The immunoreactive signals were visualized using Western Lighting Chemiluminescence Reagent (PerkinElmer, Wellesley, MA, USA) and the Fuji LAS-4000 lumino image analyzer (Fuji Photo Film Co., Tokyo, Japan).

Quantitative real-time polymerase chain reaction

The methods used were similar to those reported in our previous study.[12] TaqMan probes for COL1A1 (ID: Hs00164004_m1), COL1A2 (ID: Hs00164099_m1), and glyceraldehyde 3-phosphate dehydrogenase (GADPH; ID: Hs99999905_m1) were used. GADPH was used as a reference gene.

Sircol collagen assay

BMFs (10^5 cells) were cultured in six-well culture plates and grown to near confluence. After overnight serum starvation, cells were treated with 60nM Egr-1 small interfering RNA (siRNA) or (2.5μM, 5μM, or 10μM) EGCG for 48 hours followed by 2 ng/mL TGF-β1 treatment. Total acid-soluble collagen in BMFs and cell culture supernatants were collected and determined using a Sircol Collagen Assay kit as recommended by the manufacturer (Biocolor Ltd., Carrickfergus, UK).

RNA interference

Endogenous Egr-1 and Smad2/3 expression in BMFs was inhibited with Egr-1 and Smad2/3 siRNA (Santa Cruz Biotechnology) using siRNA Transfection Reagent (Santa Cruz Biotechnology). BMFs were cultured for an additional 24 hours after transfection with fresh medium followed by the indicated treatment and then prepared for immunoblotting, quantitative real-time polymerase chain reaction, or Sircol collagen assay.

Statistical analysis

Group data are expressed as mean ± standard deviation. Differences between treatment groups were analyzed using one-way analysis of variance and Duncan *post hoc* test. A p value < 0.05 was considered significant.

Results

TGF-β1 stimulates Egr-1 protein expression in normal BMFs

Human BMFs were treated with various concentrations of TGF-β1. TGF-β1 significantly increased Egr-1 synthesis (Figure 1). The levels of Egr-1 protein increased approximately 3.5-fold after stimulation with 2 ng/mL TGF-β1 for 2 hours and declined thereafter.

ERK, JNK, and Smad3 involvement in TGF-β1-induced Egr-1 signaling

PD98059, SP600125, SB431542, and SIS3 significantly abolished the TGF-β1-induced levels of Egr-1, but not SB203580 (Figure 2A). We further knocked down Smad3 using Smad2/3 siRNA and found that TGF-β1-induced Egr-1 was completely abrogated (Figure 2B).

Egr-1 mediating TGF-β1-induced fibrotic gene expression and collagen production

We explored the effect of knocking down Egr-1 on the transcript expression levels of COL1A1 and COL1A2 genes using quantitative real-time polymerase chain reaction in BMFs. As shown in Figures 3A and 3B, COL1A1 and COL1A2

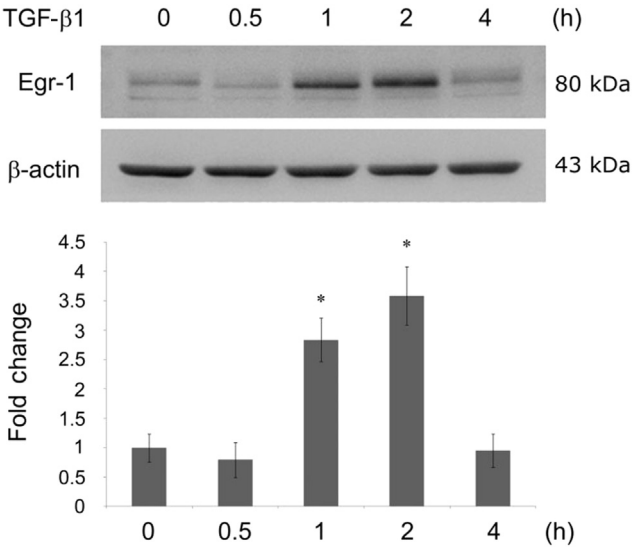

Figure 1 TGF-β1 stimulated Egr-1 protein expression in normal human BMFs. BMFs were treated with 2 ng/mL TGF-β1. Egr-1 protein levels were measured by western blot analysis. Experiments were repeated three times with BMF Strain 1 for statistical analyses and were verified once with each of the other two strains. All of the experimental results were similar. A representative result is shown. Results were further quantified by densitometric analysis, normalized by the level of β-actin, and expressed as the fold-change relative to the untreated controls. * $p < 0.05$ compared with the control. BMF = buccal mucosal fibroblast; Egr = early growth response; TGF = transforming growth factor.

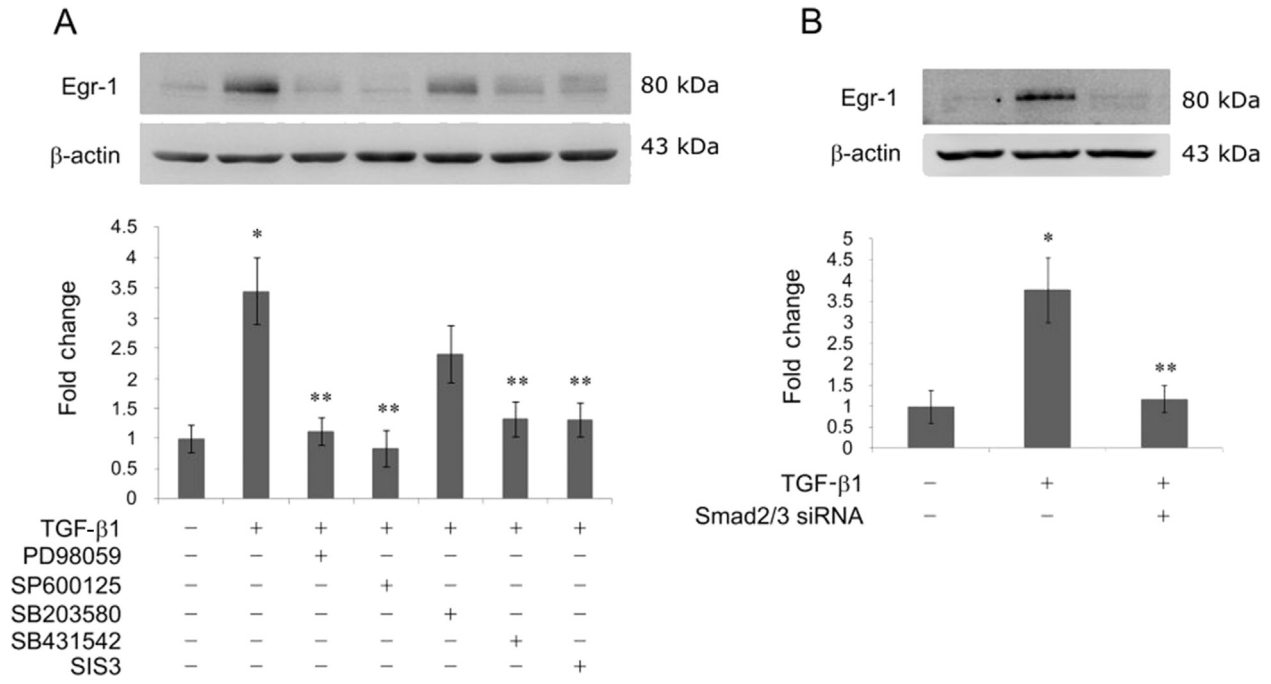

Figure 2 ERK, JNK, and Smad3 were involved in the TGF-β1-induced Egr-1 in BMFs. (A) BMFs were pretreated with 10μM ERK inhibitor PD98059, 10μM JNK inhibitor SP600125, 10 μM p38 mitogen-activated protein kinase inhibitor SB203580, 10μM activin receptor-like kinase 5 inhibitor SB431542, or 10μM Smad3 inhibitor SIS3 for 1 hour before exposure to 2 ng/mL TGF-β1 for 2 hours. (B) BMFs were transfected with 60nM Smad2/3 small interfering RNA for 24 hours and then incubated with 2 ng/mL TGF-β1 for 2 hours. Egr-1 protein levels in (A) and (B) were measured by western blot analysis. Experiments were repeated three times with BMF Strain 1 for statistical analyses and were verified once with each of the other 2 strains. All the experimental results were similar. A representative result is shown. Results were further quantified by densitometric analysis, normalized by the level of β-actin, and expressed as the fold change relative to the untreated controls as shown in the lower panels of (A) and (B). * $p < 0.05$ compared with the control. ** $p < 0.05$ compared with 2 ng/mL TGF-β1. BMF = buccal mucosal fibroblast; Egr = early growth response; ERK = extracellular signal-regulated kinase; JNK = c-Jun N-terminal kinase; TGF = transforming growth factor.

mRNAs significantly increased when treated with TGF-β1 alone. Knocking down Egr-1 with Egr-1 siRNA completely inhibited the levels of TGF-β1-induced COL1A1 and COL1A2 mRNAs. The total amount of TGF-β1-induced collagen accumulation also significantly decreased in BMFs transfected with Egr-1 siRNA as assessed by the Sircol assay (Figure 3C).

EGCG inhibiting TGF-β1-stimulated Egr-1 and collagen production

We investigated the effects of lovastatin, curcumin, or EGCG on TGF-β1-induced Egr-1 protein expression in BMFs. Results showed that 20μM lovastatin, 10μM curcumin, and 10μM EGCG inhibited the TGF-β1-induced Egr-1 protein expression by 69.4%, 64%, and 100%, respectively (Figure 4A). EGCG inhibited TGF-β1-stimulated Egr-1 expression and collagen production in a dose-dependent manner (Figures 4B and 4C). At a concentration of 10μM, EGCG almost completely abrogated the TGF-β1-stimulated production of collagens (Figure 4C).

Discussion

Many animal models of fibrosis have shown increased Egr-1 expression in affected organs and active Egr-1-dependent signaling.[11] Moreover, several models show improvement after attenuating the effects of Egr-1. However, controversies concerning the profibrotic function of Egr-1 do exist. TGF-α-induced lung fibrosis,[17] α-naphthylisothiocyanate-induced liver fibrosis,[18] and carbon-tetrachloride-induced hepatic fibrosis[19] are exacerbated in Egr-1 null mice. These contrasting results suggest that the initiating nature of injury, the extracellular stimuli involved, and the tissue or cell type of its expression could influence the role of Egr-1 in fibrogenesis. Our previous study has shown Egr-1 staining in OSF specimen and elevated Egr-1 induced by arecoline in BMFs.[12] In this present study, we showed that TGF-β1 induced Egr-1 expression and that Egr-1 mediated TGF-β1-induced COL1A1 and COL1A2 mRNA expression and collagen production in BMFs. Therefore, our results implicate Egr-1 as a key mediator of TGF-β-regulated fibrosis in the pathogenesis of OSF.

To initiate its cellular action in fibroblasts, TGF-β first binds to TGF-β receptor II and then recruits and phosphorylates TGF-β receptor I (ALK5) to subsequently activate Smad2/3.[20,21] This Smad3 pathway mediates most of the profibrotic activities of TGF-β.[22] In addition to the canonical Smad pathway, non-Smad signaling is also activated by the TGF-β receptor complex.[23] Furthermore, the ability of TGF-β to activate MAPKs (ERK1/2, JNK, or p38) has gained increasing interest. TGF-β-induced MAPK pathways may activate collagen synthesis independently or in

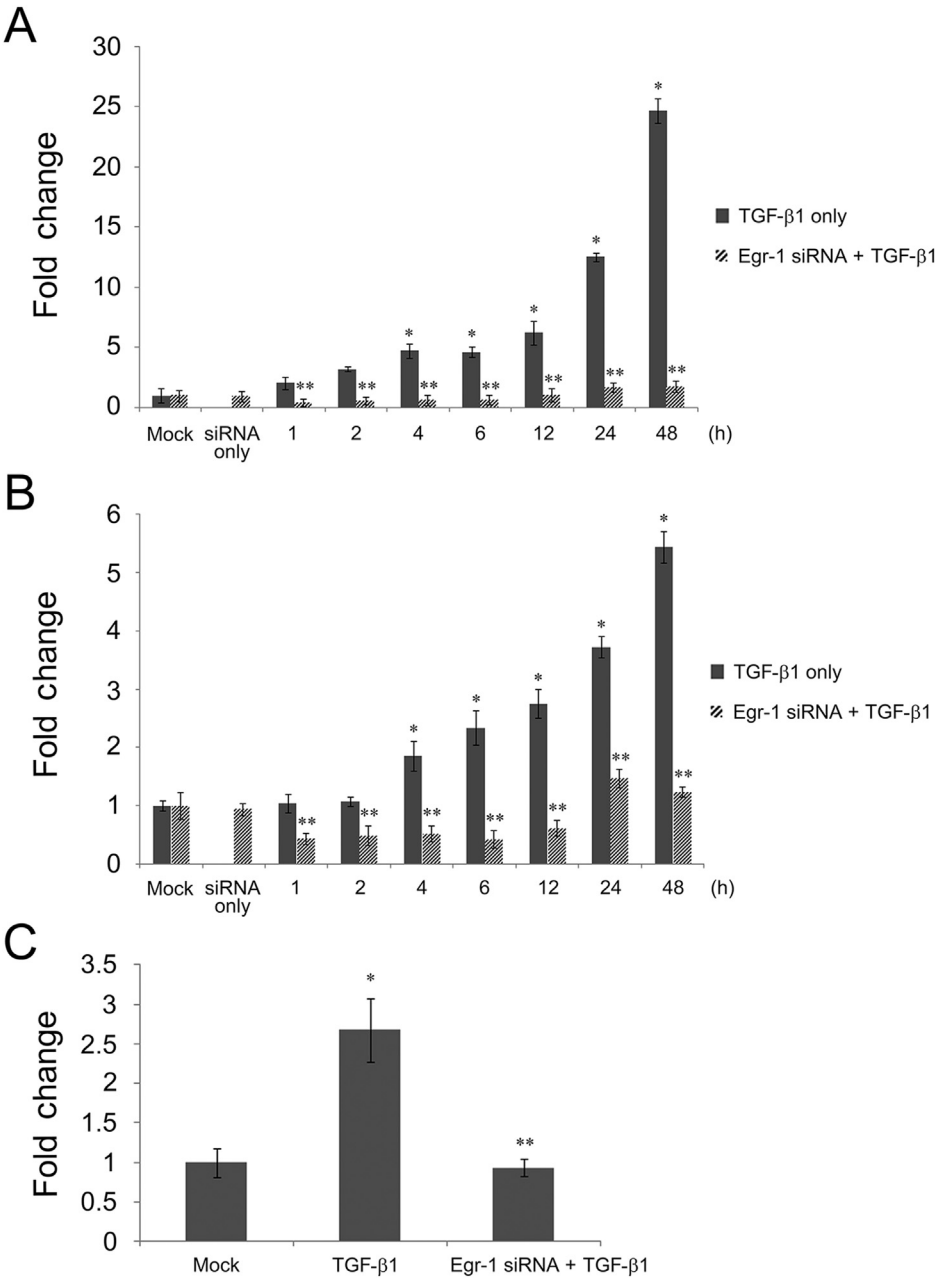

Figure 3 Egr-1 mediated TGF-β1-induced fibrotic gene expression and collagen production. BMFs were transfected with 60nM Egr-1 siRNA followed by 2 ng/mL TGF-β1 treatment for 0—48 hours to study the TGF-β1-induced (A) COL1A1 and (B) COL1A2 mRNA expression using TaqMan Gene Expression Assays. Experiments were repeated three times with BMF Strain 1 for statistical analyses and were verified once with each of the other two strains. All experimental results were similar. Data are presented as means ± SD (* $p < 0.05$ compared to control, ** $p < 0.05$ compared to 2 ng/mL TGF-β1). (C) BMFs were transfected with 60nM Egr-1 siRNA followed by 2 ng/mL TGF-β1 treatment for 48 hours and then subjected to Sircol collagen assay. Bars represent means ± SD of collagen level, normalized to the level in untreated BMFs, and expressed as fold change relative to untreated controls (* $p < 0.05$ compared to control, ** $p < 0.05$ compared to 2 ng/mL TGF-β1). BMF = buccal mucosal fibroblast; COL1A1 = type 1 collagen α1 chain; COL1A2 = type 1 collagen α2 chain; Egr = early growth response; SD = standard deviation; siRNA = small interfering RNA; TGF = transforming growth factor.

coordination with TGF-β-activated Smads.[24] Using Affymetrix microarrays, Chen et al[13] identified Egr-1 as Smad3-inducible gene in hTERT-BJ1 cells (human foreskin fibroblasts). Later, the results from Bhattacharyya et al[25] using ALK5 inhibition in normal primary human dermal fibroblasts and Smad-deficient mouse primary dermal fibroblasts indicated that TGF-β-stimulated Egr-1 occurred independently of ALK5/Smad3 but required ERK1/2 signaling. Our results showed that SB431542, SIS3, PD98059, and SP600125 attenuated the TGF-β1-induced Egr-1, indicating the involvement of ALK5/Smad3, ERK, and JNK pathways in TGF-β1-induced Egr-1 signaling in BMFs. We further showed

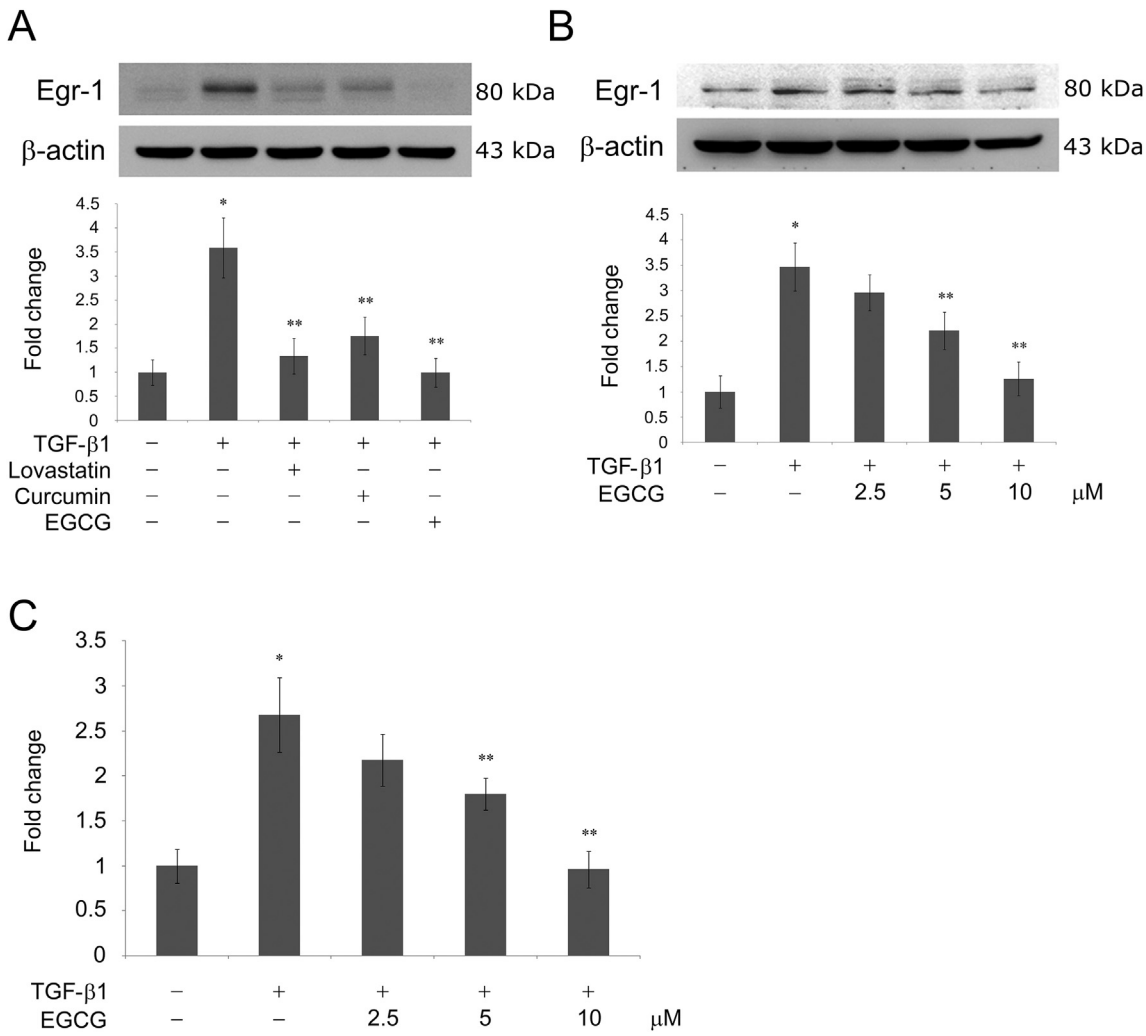

Figure 4 EGCG inhibited TGF-β1-induced Egr-1 expression and collagen production. (A) BMFs were pretreated with 20μM lovastatin, 10μM curcumin, or 10μM EGCG, for 1 hour before exposure to 2 ng/mL TGF-β1 for 2 hours. (B) BMFs were pretreated with increasing concentrations of EGCG (0—10μM) for 1 hour, and then with 2 ng/mL TGF-β1 for 2 hours. Egr-1 protein levels in (A) and (B) were measured by western blot analysis. Experiments were repeated three times with BMF Strain 1 for statistical analyses and were verified once with each of the other two strains. All experimental results were similar. A representative result is shown. Results were further quantified by densitometric analysis, normalized by the level of β-actin, and expressed as the fold-change relative to the untreated controls as shown in the lower panels of (A) and (B) (* $p < 0.05$ compared with the control, ** $p < 0.05$ compared with 2 ng/mL TGF-β1). (C) BMFs were pretreated with increasing concentrations of EGCG (0—10μM) for 1 hour, and then incubated with 2 ng/mL TGF-β1 in the presence or absence of EGCG for 48 hours. Collagen from BMFs was quantified with Sircol collagen assay. Bars represent means ± standard deviation of collagen level, normalized to the level in untreated BMFs, and expressed as fold change relative to untreated controls (* $p < 0.05$ compared to control, ** $p < 0.05$ compared to 2 ng/mL TGF-β1). BMF = buccal mucosal fibroblast; EGCG = epigallocatechin-3-gallate; Egr = early growth response; TGF = transforming growth factor.

that knocking down Smad3 using Smad2/3 siRNA completely abolished TGF-β1-induced Egr-1. These results confirm that in BMFs, upregulation of Egr-1 by TGF-β depends on ALK5/Smad3 signaling. Our results suggest the different signaling between dermal and buccal fibroblasts.

To date, no known treatment for OSF is effective in reversing the disease. Considering the role of Egr-1 in the pathogenesis of OSF, blocking Egr-1 may be a novel strategy to control pathological fibrogenesis in OSF. Although there are no specific inhibitors of Egr-1, multiple pharmacological agents such as statins, curcumin, and EGCG have shown potent effects of Egr-1 inhibition.[11,13,26,27] All these three agents modulate cellular signaling to exert anti-inflammatory, antioxidative, and chemopreventive effects.[28,29] In addition, they suppress fibrotic diseases in various animal models. Our previous studies have demonstrated these agents may be capable of blocking fibrogenic signaling in oral tissues.[30—32] Therefore, we examined the effects of these agents on the expression of Egr-1 in BMFs. Our results revealed that lovastatin, curcumin, and EGCG all significantly inhibited the TGF-β1-induced Egr-1 protein expression. EGCG (10μM) completely inhibited TGF-β1-stimulated Egr-1 and abrogated TGF-β1-stimulated collagen production. Our previous study has shown that

EGCG completely blocks the arecoline-induced Egr-1 and collagen gel contraction in BMFs.[12] We have also shown that EGCG fully blocks the TGF-β1-induced connective tissue growth factor (CCN2/CTGF) in BMFs.[31] Taken together, EGCG, with its history of safe consumption in green tea, may be useful as prevention or therapy for OSF.

In conclusion, this study demonstrates that TGF-β1 stimulates Egr-1 production in BMFs. ALK5/Smad3, ERK1/2, and JNK pathways mediate TGF-β1-induced Egr-1 in BMFs. Egr-1 subsequently induces COL1A1 and COL1A2 mRNAs expression and collagen production in BMFs. EGCG blocks the ability of TGF-β1 to produce collagen by attenuating the TGF-β1-induced Egr-1 activation in BMFs. EGCG potentially serves as a novel agent for prevention or treatment of OSF.

Acknowledgments

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References

1. Utsunomiya H, Tilakaratne WM, Oshiro K, Maruyama S, Suzuki M, Ida-Yonemochi H, et al. Extracellular matrix remodeling in oral submucous fibrosis: its stage-specific modes revealed by immunohistochemistry and in situ hybridization. *J Oral Pathol Med* 2005;**34**:498—507.
2. Tilakaratne WM, Klinikowski MF, Saku T, Peters TJ, Warnakulasuriya S. Oral submucous fibrosis: review on aetiology and pathogenesis. *Oral Oncol* 2006;**42**:561—8.
3. Rajalalitha P, Vali S. Molecular pathogenesis of oral submucous fibrosis—a collagen metabolic disorder. *J Oral Pathol Med* 2005;**34**:321—8.
4. Kale AD, Mane DR, Shukla D. Expression of transforming growth factor beta and its correlation with lipodystrophy in oral submucous fibrosis: an immunohistochemical study. *Med Oral Patol Oral Cir Bucal* 2013;**18**:e12—8.
5. Moutasim KA, Jenei V, Sapienza K, Marsh D, Weinreb PH, Violette SM, et al. Betel-derived alkaloid up-regulates keratinocyte alphavbeta6 integrin expression and promotes oral submucous fibrosis. *J Pathol* 2011;**223**:366—77.
6. Denton CP, Merkel PA, Furst DE, Khanna D, Emery P, Hsu VM, et al. Recombinant human anti-transforming growth factor beta1 antibody therapy in systemic sclerosis: a multicenter, randomized, placebo-controlled phase I/II trial of CAT-192. *Arthritis Rheum* 2007;**56**:323—33.
7. Tian M, Neil JR, Schiemann WP. Transforming growth factor-beta and the hallmarks of cancer. *Cell Signal* 2011;**23**:951—62.
8. Wrzesinski SH, Wan YY, Flavell RA. Transforming growth factor-beta and the immune response: implications for anticancer therapy. *Clin Cancer Res* 2007;**13**:5262—70.
9. Varga J, Pasche B. Antitransforming growth factor-beta therapy in fibrosis: recent progress and implications for systemic sclerosis. *Curr Opin Rheumatol* 2008;**20**:720—8.
10. Bhattacharyya S, Wu M, Fang F, Tourtellotte W, Feghali-Bostwick C, Varga J. Early growth response transcription factors: key mediators of fibrosis and novel targets for anti-fibrotic therapy. *Matrix Biol* 2011;**30**:235—42.
11. Bhattacharyya S, Fang F, Tourtellotte W, Varga J. Egr-1: new conductor for the tissue repair orchestra directs harmony (regeneration) or cacophony (fibrosis). *J Pathol* 2013;**229**:286—97.
12. Hsieh YP, Chen HM, Chang JZ, Chiang CP, Deng YT, Kuo MY. Arecoline stimulated early growth response-1 production in human buccal fibroblasts: suppression by epigallocatechin-3-gallate. *Head Neck* 2015;**37**:493—7.
13. Chen SJ, Ning H, Ishida W, Sodin-Semrl S, Takagawa S, Mori Y, et al. The early-immediate gene EGR-1 is induced by transforming growth factor-beta and mediates stimulation of collagen gene expression. *J Biol Chem* 2006;**281**:21183—97.
14. Lejard V, Blais F, Guerquin MJ, Bonnet A, Bonnin MA, Havis E, et al. EGR1 and EGR2 involvement in vertebrate tendon differentiation. *J Biol Chem* 2011;**286**:5855—67.
15. Wu M, Melichian DS, de la Garza M, Gruner K, Bhattacharyya S, Barr L, et al. Essential roles for early growth response transcription factor Egr-1 in tissue fibrosis and wound healing. *Am J Pathol* 2009;**175**:1041—55.
16. Massague J. TGFbeta signalling in context. *Nat Rev Mol Cell Biol* 2012;**13**:616—30.
17. Kramer EL, Mushaben EM, Pastura PA, Acciani TH, Deutsch GH, Khurana Hershey GK, et al. Early growth response-1 suppresses epidermal growth factor receptor-mediated airway hyperresponsiveness and lung remodeling in mice. *Am J Respir Cell Mol Biol* 2009;**41**:415—25.
18. Sullivan BP, Cui W, Copple BL, Luyendyk JP. Early growth response factor-1 limits biliary fibrosis in a model of xenobiotic-induced cholestasis in mice. *Toxicol Sci* 2012;**126**:267—74.
19. Pritchard MT, Nagy LE. Hepatic fibrosis is enhanced and accompanied by robust oval cell activation after chronic carbon tetrachloride administration to Egr-1-deficient mice. *Am J Pathol* 2010;**176**:2743—52.
20. Massague J. TGF-beta signal transduction. *Annu Rev Biochem* 1998;**67**:753—91.
21. Kang JS, Liu C, Derynck R. New regulatory mechanisms of TGF-beta receptor function. *Trends Cell Biol* 2009;**19**:385—94.
22. Flanders KC. Smad3 as a mediator of the fibrotic response. *Int J Exp Pathol* 2004;**85**:47—64.
23. Prime SS, Pring M, Davies M, Paterson IC. TGF-beta signal transduction in oro-facial health and non-malignant disease (part I). *Crit Rev Oral Biol Med* 2004;**15**:324—36.
24. Ghosh AK, Quaggin SE, Vaughan DE. Molecular basis of organ fibrosis: potential therapeutic approaches. *Exp Biol Med (Maywood)* 2013;**238**:461—81.
25. Bhattacharyya S, Chen SJ, Wu M, Warner-Blankenship M, Ning H, Lakos G, et al. Smad-independent transforming growth factor-beta regulation of early growth response-1 and sustained expression in fibrosis: implications for scleroderma. *Am J Pathol* 2008;**173**:1085—99.
26. Moon Y, Lee M, Yang H. Involvement of early growth response gene 1 in the modulation of microsomal prostaglandin E synthase 1 by epigallocatechin gallate in A549 human pulmonary epithelial cells. *Biochem Pharmacol* 2007;**73**:125—35.
27. Park JS, Khoi PN, Joo YE, Lee YH, Lang SA, Stoeltzing O, et al. EGCG inhibits recepteur d'origine nantais expression by suppressing Egr-1 in gastric cancer cells. *Int J Oncol* 2013;**42**:1120—6.
28. Siegel-Axel DI. Cerivastatin: a cellular and molecular drug for the future? *Cell Mol Life Sci* 2003;**60**:144—64.
29. Surh YJ, Kundu JK, Na HK, Lee JS. Redox-sensitive transcription factors as prime targets for chemoprevention with anti-inflammatory and antioxidative phytochemicals. *J Nutr* 2005;**135**:2993S—3001S.
30. Chang JZ, Yang WH, Deng YT, Chen HM, Kuo MY. Thrombin-stimulated connective tissue growth factor (CTGF/CCN2) production in human buccal mucosal fibroblasts: inhibition by epigallocatechin-3-gallate. *Head Neck* 2012;**34**:1089—94.
31. Chang JZ, Yang WH, Deng YT, Chen HM, Kuo MY. EGCG blocks TGFbeta1-induced CCN2 by suppressing JNK and p38 in buccal fibroblasts. *Clin Oral Investig* 2013;**17**:455—61.
32. Yang WH, Kuo MY, Liu CM, Deng YT, Chang HH, Chang JZ. Curcumin inhibits TGFbeta1-induced CCN2 via Src, JNK, and Smad3 in Gingiva. *J Dent Res* 2013;**92**:629—34.