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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

Yu-Ping Hsieh [a], Hsin-Ming Chen [a,b], Hung-Ying Lin [b], Hsiang Yang [b], Jenny Zwei-Chieng Chang [a,b,*]

[a] *Graduate Institute of Clinical Dentistry, School of Dentistry, National Taiwan University, Taipei, Taiwan*
[b] *Department of Dentistry, National Taiwan University Hospital, Taipei, Taiwan*

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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.

* Corresponding author. School of Dentistry, National Taiwan University Hospital, 1 Chang-Te Street, Taipei, 10048, Taiwan.
E-mail address: jennyzc@ms3.hinet.net (J.Z.-C. Chang).

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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