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Selenate Exerts the Formation of Skin Fibril through Regulation of TGF-β Signaling Pathway

Myung-Soo Shon [1], Ji-Hye Song [1] and Gyo-Nam Kim [1+]

[1] Department of Food Science and Biotechnology, Kyungnam University, Changwon, Gyeongnam, Korea

Abstract. Selenium has been reported to possess potent anti-oxidant, anti-hyperglycemic and anti-carcinogenic properties. However, the precise biological role of selenium in formation of skin fibril remains unknown. Selenium exists in various forms such as selenate, selenite, and methylseleninic acid. Among them, we found that selenate treatment dose-dependently enhanced mRNA expression levels of skin fibrillar genes such as pro-collagen, collagen I, collagen III, and fibronectin. The type III collagen is the predominant collagen in the granulation tissue of skin health. In addition, we elucidated that the transcriptional growth factor beta (TGF-β) is required in selenate-induced expression of collagen III. TGF-β has long been believed to be the most critical in the process of tissue remodeling. Upon TGF-β binding to its receptor at the cell surface, cytoplasmic transmitters (Smad2 or Smad3) are phosphorylated and then form a heterodimer with a common Smad (Smad4). A few studies have shown activation of TGF-β1 by nutrient in the formation of skin fibril. These results implicate that the selenate could exert the formation of skin fibril through activating TGF-β signaling pathway. Our results also reveal a novel function of selenate in formation of skin fibril and these evidences also provide useful information for the development of skin-related nutraceuticals and nutri-cosmetics design.

Keywords: Selenium, Selenate, Collagen III, Skin fibril, TGF-β

1. Introduction

Human skin is composed of three major layers such as epidermis, dermis, and hypodermis. The approximately 75% of dermis is composed of collagen fibrils and it maintains physiological homeostasis by balance between the processes of biosynthesis and degradation. The pro-collagen is first synthesized as a precursor molecule for composition of collagen fibrils. The collagen III form accounts for 80% of total collagen and the collagen I form exists approximately 10% of total collagen in human dermis. The collagen I and collagen III forms are mainly contributed to responsible for the tensile strength of skin [1], [2].

The transforming growth factor beta (TGF-β) has been well known in association with induction of migration and proliferation, prevention of apoptosis, induction of transformation into myofibroblasts, stimulation of collagen contraction, and promotion of collagen maturation into a highly cross-linked dense matrix in fibroblasts [3]. Recent studies reported that Smads involved in TGF-β signaling, Smad2 and Smad3 are highly homologous direct substrates for activated TGF-β receptor, Smad4 is the common signaling partner. Smad2 and Smad3 are released from receptor complex and associate with Smad4 through phosphorylation by TGF-β receptor kinase at the cell membrane [3].

Selenium is an essential mineral which is exist in small amounts as trace element in organism [4]. Selenium is mainly included in nuts, cereals, meat, mushrooms, fish, and eggs [5]. Selenium is composed of three forms such as selenate, selenite, and methylseleninic acid [4]. Recently, various effects of selenium such as anti-oxidant [4], anti-hyperglycemic [6], and anti-carcinogenic [7] have been widely reported.

[+] Corresponding author. Tel.: +82-10-2498-9933; fax: +82-505-999-2171.
E-mail address: gnkim@kyungnam.ac.kr.

However, the precise biological role and function of selenate in the formation and development of skin fibril remains unclear.

2. Materials and Methods

2.1. Reagents

Selenate, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and chloroform were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Dimethyl sulfoxide (DMSO) and isopropanol were purchased from Junsei (Tokyo, Japan). TRIzol® and Maxime PCR PreMix (i-Taq) were obtained from Life technologies (Carlsbad, CA, USA) and CycleScript RT PreMix was purchased Bioneer (Daejeon, Korea). Dulbecco`s modified Eagle`s medium (DMEM), penicillin-streptomycin, phosphate-buffered saline (PBS), and Rnase free water were purchased from WELGENE Inc. (Daegu, Korea). Fetal bovine serum (FBS) was obtained from Gibco BRL. (Carlsbad, CA, USA). Unless noted, all chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

2.2. Cell Culture

Human skin fibroblasts were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were maintained in DMEM supplemented with 10% FBS, 1% growth factor cocktail and 100 unit/ml penicillin-streptomycin at 37℃ in a humidified 5% CO_2 atmosphere, and the medium was replaced every 2 day.

2.3. Cell Viability Assay

MTT assay was performed as previously described with modifications [8]. Proliferating fibroblasts at 80% confluence were treated with selenate for 48 h. After 48 h, the cell culture medium with selenate was aspirated and replaced with fresh 10% FBS-DMEM containing 0.5 mg/ml MTT solution prepared in PBS. The cell culture plate containing MTT was then incubated for 1 h at 37°C. The medium was then aspirated and the remaining purple formazan crystals, present in proportion to the number of viable cells, were solubilized using DMSO and read spectrophotometrically at 595 nm using a microplate reader.

2.4. Isolation of Total RNA and Reverse Transcription-polymerase Chain Reaction (RT-PCR) Analysis

Total RNA from human skin fibroblasts were extracted using TRIzol® according to the manufacturer's instruction. Isolated RNA was subjected to reverse transcription using a CycleScript RT PreMix kit for cDNA synthesis. The primer sequences were: Pro-collagen: forward primer, 5'-TGGTCCTCAGGGGAATTCGG -3' and reverse primer, 5'-AACCTACAGGACCCCGTTCT-3'; Collagen: forward primer, 5'-TGACGAGACCAAGAACTGCC-3' and reverse primer, 5'-GCACCATCATTTCCACGAGC-3'; Collagen Ⅲ: forward primer, 5'-ATGTTGTGCAGTTTGCCCAC-3' and reverse primer, 5'-TCGTCCGGGTCTACCTGATT-3'; TGF-β1: forward primer, 5'-TGGTGGAAACCCACAACGAA-3' and reverse primer, 5'-AGAAGTTGGCATGGTAGCCC-3'; TGF-β receptor: forward primer, 5'-CCGGGATGAAGCCGATCCTA-3' and reverse primer, 5'-GCTTGGCTGTGTTGTCCTTG-3'; Smad: forward primer, 5'-GGGTCAGGTGCCTTAGTGAC-3' and reverse primer, 5'-TCTGAGCCATGCCTGACAAG-3'; Fibronectin: forward primer, 5'-AAGAAGGGCTCGTGTGACAG-3' and reverse primer, 5'-GGGTGTGGAAGGGTAACCAG-3'; Elastin: forward primer, 5'-TCCCTAGTGTCGGAGGTGTT-3' and reverse primer, 5'-ATGGGAGACAATCCGAAGCC-3'; β-Actin: forward primer, 5'-GCAGGAGTATGACGAGTCCG-3' and reverse primer, 5'-AGGGACTTCCTGTAACAATGC-3', respectively. PCR products were analyzed by electrophoresis with 2%(w/v) agarose gel and visualized by ethidium bromide staining.

2.5. Statistical Analysis

All data are presented as mean±SD. Statistical analysis was performed using the Statistical Package for Social Science statistical package (SPSS, Chicago, IL, USA), and the significance of each group was verified

with the analysis of one-way ANOVA followed by Student's *t*-test. A p value<0.05 was considered significant.

3. Results and Discussion

3.1. The Selenate Treatment Does Not Affect the Viability of Human Skin Fibroblasts

The treatment human skin fibroblasts with selenate concentration up to 400 μM for 48 h showed no toxic effect on the viability of human skin fibroblasts (Fig. 1). The treatment of up to 200 μM had only a marginal effect on fibroblasts viability however there was no significant differences between control and selenate treatment groups. Thus, 1-100 μM of selenate was chosen for subsequent studies.

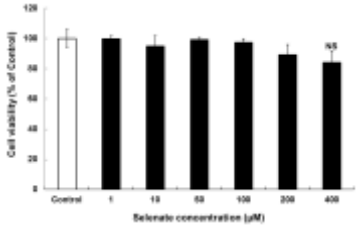

Fig. 1: Cytotoxicity of selenate in human skin fibroblasts.

3.2. The Selenate Treatment Enhance Expression Levels of Skin Fibril-Related Genes

To examine whether selenate could exert the expression levels of skin fibril-relate genes, human skin fibroblasts were exposed to 1, 10, 50, and 100 μM of selenate for 24 h. As shown in Fig. 2, selenate treatment showed dramatically enhanced mRNA expression levels of pro-collagen, collagen I, collagen III, and fibronectin. Fibrillar collagenes, and fibronectin comprise the structural component of the extracellular matrix (ECM), which is important in maintaining architecture and preservation of skin health [9]. Collagens are synthesized as pro-collagen forms, which are secreted in to the interstitial space where they undergo cleavage of their end-terminal propeptide sequences to enable collagen fiber formation. Fibronectin is a multifunctional adhesive glycoprotein produced by fibroblasts, macrophages, and endothelial cells and it regulate cellular shape and movement. Our result indicates the selenate has the positive potential in maintaining or increasing skin health through activation of skin fibril-related genes.

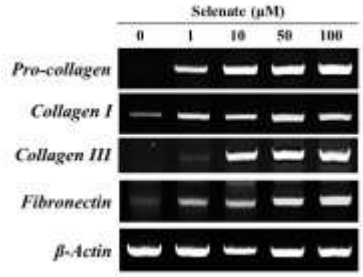

Fig. 2: The selenate treatment enhance the mRNA expression levels of skin fibril-related genes in human skin fibroblasts.

3.3. The Selenate Treatment Activate TGF-β Signaling Pathway

TGF-β is a multipotent cytokine that signals through its receptors for autocrine and paracrine effects on cellular growth, differentiation, inflammation, apoptosis, and matrix synthesis [10]. In general, once TGF-β bind to the receptors, the activated serine/threonine kinase activity of the receptors subsequently phosphorylates several cytosolic transcription factors Smads, which the form a complex with the common Smad, to interact with nuclear transcription factors in the nucleus for transcription of TGF-β downstream genes [11]. Previous studies showed that the TGF-β could induce the expression of α-smooth muscle actin (SMA), modulates the expression of adhesive receptors, and the synthesis of ECM molecules including collagen I and fibronectin [12], [13]. These results lead us to hypothesize that TGF signaling mediates the expressions of skin fibril-related genes in selenate-treated fibroblasts. Human skin fibroblasts were exposed

to 1, 10, 50, and 100 of selenate for 24 h and we observed markedly increased mRNA expression levels of TGF-1, Smad2, and Collagen III (Fig. 3).

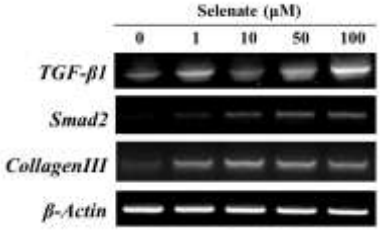

Fig. 3: The selenate treatment activate TGF-β signaling pathway.

3.4. Activation of TGF-β signaling pathway is required in enhancement of collagen III expression by selenate treatment in human fibroblasts

To test whether TGF-β is required in selenate-induced enhancement of expression levels of skin fibrillar genes in fibroblasts, we employed selective TGF-β inhibitor, SB431542, to identify the potential molecular mechanism. Human skin fibroblasts were treated with 10 and 100 μM of selenate for 24 h in the presence or absence of 5 μM SB431542 and these cells were subjected to the PCR analysis. While selenate treatment markedly exerted mRNA expression levels of TGF-1 and collagen III, cotreatment with SB431542 reversed selenate-induced TGF-β and collagen III expression levels (Fig. 4). This result implies that the activation of TGF-β1 is required in enhancement of expression levels of skin fibrillar genes such as collagen III by selenate treatment in human fibroblasts.

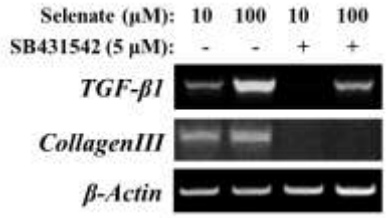

Fig. 4: The selenate treatment activate the skin-fibril gene such as collagen III through regulation of TGF-β signaling pathway.

4. Conclusions

Selenium is an essential micronutrient and dietary selenium is abundant in nuts, cereals, and meat. Various biological role and function of selenium have been reported. However, precise role of selenium, particularly selenate, in formation and development of skin fibril is unclear. Human skin is composed of three major layers such as epidermis, dermis, and hypodermis. The collagen I, collagen III, and fibronectin crucially contribute to the skin health through maintaining tensile strength.

In this study, we showed that selenate treatment dose-dependently enhanced mRNA expression levels of skin fibrillar genes such as pro-collagen, collagen I, collagen III, and fibronectin. Our inhibitor assay elucidated that the transcriptional growth factor beta (TGF-β) is required in selenate-induced expression of collagen III. These results suggest that the selenate could exert the formation of skin fibril through activating TGF-β signaling pathway. Our results reveal a novel function of selenate in formation of skin fibril and these evidences also provide useful information for the development of skin-related nutraceuticals and nutria-cosmetics design.

5. Acknowledgements

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6. References

[1] Y. Oishi, Z. W. Fu, Y. Ohnuki, H. Kato, T. Noguchi. Molecular basis of the alteration in skin collagen metabolism in response to *in vivo* dexamethasone treatment: effects on the synthesis of collagen type Ⅰ and Ⅲ, collagenase, and tissue inhibitors of metalloproteinases. *Br. J. Dermatol.* 2002, 147: 859-868.

[2] H. J. Chung, A. Steplewski, K. Y. Chung, J. Uitto, A. Fertala. Collagen fibril formation: a new target to limit fibrosis. *J. Biol. Chem*. 2008, 283 (38): 25879-25886.

[3] G. Lakos, S. Takagawa, S. J. Chen, A. M. Ferreira, G. W. Han, K. Masuda, X. J. Wang, L. A. DiPietro, J. Varga. Targeted disruption of TGF-β/Smad3 signaling modulates skin fibrosis in a mouse model of scleroderma. *Am. J. Pathol.* 2004, 165 (1): 203-217.

[4] Y. Mehdi, J. L. Hornick, L. Istasse, I. Dufrasne. Selenium in the environment, metabolism and involvement in body functions. *Molecules*. 2013, 18: 3292-3311.

[5] M. N. I. Barclay, A. MacPherson, J. Dixon. Selenium content of a range of UK foods. *J. Food Comp. Anal*. 1995, 8: 307-318.

[6] S. Bo, A. Lezo, G. Menato, M. L. Gallo, C. Bardelli, A. Signorile, C. Berutti, M. Massobrio, G. F. Pagano. Gestational hyperglycemia, zine, selenium, and antioxidant vitamins. *Nutrition*. 2005, 21: 186-191.

[7] N. Y. Alwahaibi, S. B. Budin, J. Mohamed, A. Alhamdani. Nuclear factor-kappa B as promising target for selenium chemoprevention in rat hepatocarcinogenesis. *J. Gastroen. hepatol*. 2010, 25: 786-791.

[8] CY. Kim, GN. Kim, J. L. Wiacek, CY. Chen, KH. Kim. Selenate inhibits adipogenesis through induction of transforming growth factor-β1 (TGF-β1) signaling. *Biochem. Bioph. Res. Co.* 2012, 326: 551-557.

[9] Y. Ma, G. V. Halade, M. L. Lindsey. Extracellular matrix and fibroblast communication following myocardial infarction. *J. Cardiovasc. Transl. Res.* 2012, 5 (6): 848–857.

[10] Y. Shi, J. Massague. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. *Cell.* 2003, 113: 685-700.

[11] K. Wharton, R. Derynck. TGFβ family signaling: novel insights in development and disease. *Development.* 2009, 136: 3691-3697.

[12] R. A. Ignotz, J. Massague. Transforming growth factor-beta stimulates the expression of fibronectin and collagen and their incorporation into the extracellular matrix. *J Biol. Chem.* 1986, 261 (9): 4337–4345.

[13] V. John, R. Joel, A. J. Sergio. Transforming growth factor β (TGFβ) causes a persistent increase in steady-state amounts of type Ⅰ and type Ⅲ collagen and fibronectin mRNAs in normal human dermal fibroblasts. *Biochem. J.* 1987, 247: 597-604.