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Vegetable peptones increase production of type I collagen in human fibroblasts by inducing the RSK-CCAAT/enhancer binding protein-ß phosphorylation pathway☆

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ABSTRACT

Skin aging appears to be principally attributed to a decrease in type I collagen level and the regeneration ability of dermal fibroblasts. We hypothesized that vegetable peptones promote cell proliferation and production of type I collagen in human dermal fibroblasts. Therefore, we investigated the effects of vegetable peptones on cell proliferation and type I collagen production and their possible mechanisms in human dermal fibroblasts. Vegetable peptones significantly promoted cell proliferation in a concentration-dependent manner. In addition, the human luciferase type I collagen α2 promoter and type I procollagen synthesis assays showed that the vegetable peptones induced type I procollagen production by activating the type I collagen α2 promoter. Moreover, the vegetable peptones activated p90 ribosomal s6 kinase, which was mediated by activating the Raf-p44/42 mitogen-activated protein kinase signaling pathway. Furthermore, the vegetable peptone–induced increase in cell proliferation and type I collagen production decreased upon treatment with the ERK inhibitor PD98059. Taken together, these findings suggest that increased proliferation of human dermal fibroblasts and enhanced production of type I collagen by vegetable peptones occur primarily by inducing the p90 ribosomal s6 kinase–CCAAT/enhancer binding protein β phosphorylation pathway, which is mediated by activating Raf-ERK signaling.

Abbreviations: COL1A2, type I collagen α2; C/EBP-β, CCAAT/enhancer binding protein β; ECM, extracellular matrix; ERK, p44/42 MAPK; MAPK, mitogen-activated protein kinase; MMPs, matrix metalloproteinases; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; RSK, p90 ribosomal s6 kinase.

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1. Introduction

Skin aging appears to be principally attributed to a decrease in type I collagen level and the regenerative ability of dermal fibroblasts. Type I collagen is the main structural component of the extracellular matrix (ECM), which has a pivotal function in maintaining structure of the skin dermis [1-3]. Aged skin exhibits numerous alterations in the dermis. Destruction and loss of ECM constituents at the dermal epidermal junction and in the dermis by matrix metalloproteinases (MMPs) are characteristic biochemical features of aging skin. Changes include deposition of dystrophic elastic fibers in the papillary dermis, termed *solar elastosis* [4]; decreases in the major fibrillar collagen types I and III [5]; reduced numbers of anchoring fibrils; and a reduced fibrillin-rich microfibrillar network [6] proximal to the dermal epidermal junction. In addition, the proliferating potential of dermal fibroblasts decreases as skin ages [7].

The Ras–mitogen-activated protein kinase (MAPK)–p90 ribosomal protein S6 kinase (RSK)–CCAAT-enhancer binding proteins β (C/EBP-β) pathway controls important aspects of cell growth, proliferation, survival, and collagen production. The RSKs are a group of serine/threonine kinases and constituents of the cyclic AMP and cyclic GMP-dependent protein kinases and protein kinase C (AGC) subfamily in the human kinome. There are 4 RSK isoforms (RSK1-4), and each is a product of a separate gene. The RSK isoforms are characterized by 75% to 80% sequence identity [8-11]. Although the RSK isoforms are broadly distributed in human tissues, they exhibit variable tissue expression, indicating that they may be involved in different functions. The RSK isoforms are activated by extracellular signaling molecules that stimulate the Ras-ERK pathway. These molecules include a variety of different growth factors, cytokines, peptide hormones, and neurotransmitters [12,13]. The RSK isoforms play an important role in the MAPK signaling cascade [14], which is responsible for regulating cellular growth, differentiation, and expression of the collagen gene.

Plant-derived peptides (vegetable peptones), manufactured by enzymatic hydrolysis of selected vegetal raw materials rich in proteins, are used in microbial broth and solid culture media [15]. Vegetable peptones have been used in cell culture medium formulations to fortify amino acid content in small peptide form as a substitute for serum [16,17]. Our research group found that vegetable peptones significantly promote adult stem cell proliferation, suggesting that vegetable peptones may be used as a serum-free culture medium for adult stem cells [18]. Physiologic uptake of peptones is linked to specialized transport systems, which are different from amino acid transporters. Intracellular peptones are first clipped by proteases, and the resulting free amino acids are used as nutrients in the tricarboxylic acid (TCA) cycle or as precursors for other amino acids, nucleic acids, or incorporated into proteins [19]. Franek et al [16] reported that the small peptides and free amino acids are used as a nutritive supply, but they are also considered a possible beneficial effect of larger and more specific peptides, that is, bioactive peptides [20].

In this study, we assessed the effects of vegetable peptones on cell proliferation and type I collagen production, which are involved in skin aging. We investigated the biological potential of vegetable peptones to address our hypothesis that vegetable peptones promote cell proliferation and production of type I collagen in human dermal fibroblasts. The samples were tested for cell proliferation and type I collagen production activities as well as the possible mechanism underlying the vegetable peptone–mediated effects using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, human type I collagen α2 (COL1A2) promoter luciferase and type I procollagen synthesis assays, and Western blot analysis.

2. Methods and materials

2.1. Materials

Anti–phospho–c-Raf (Ser259) antibody, anti–phospho–S6 ribosomal protein (Ser240/244) antibody, anti–phospho–p90 RSK (Ser380) antibody, anti–phospho–ERK 1/2 (p42/44 MAPK) antibody (Thr202/Tyr204) (E10), anti–ERK 1/2 (p42/44 MAPK) antibody, and the PathoScan Total cyclin D1 Sandwich enzyme-linked immunosorbent assay (ELISA) kit were obtained from Cell Signaling Technology (Beverly, MA, USA). Anti–β-actin antibody and the phospho–ERK 1/2 ELISA kit were obtained from Sigma-Aldrich (St Louis, MO, USA) and Thermo Fisher (Rockford, IL, USA), respectively. The C/EBP-β (pThr235 and pThr188) cell-based ELISA kit was purchased from Antibodies-online GmbH (Aachen, Germany). The COL1A2 luciferase reporter (COL1A2-Luc) plasmid was constructed by fusing the human COL1A2 promoter region into the pLuc vector (Stratagene, La Jolla, CA, USA). The COL1A2-Luc contains 376 base pairs of the α2(I) collagen (COL1A2) promoter and 58 base pairs of the transcribed sequence. BI-D1870 (RSK-specific inhibitor) was purchased from Stemgent (Cambridge, MA, USA). LY294002 was obtained from Calbiochem-Novabiochem (Darmstadt, Germany). Wheat peptone and pea peptone were obtained from Fluka (St Quentin Fallavier, France).

2.2. Cell lines and cell culture

Human dermal fibroblasts (derived from neonatal foreskin) were acquired from the Amore-Pacific Corp R&D Center (Yongin, South Korea). The cells were cultured in Dulbecco Modified Eagle Medium containing 10% fetal bovine serum (Gibco-BRL, Sparks, MD, USA) and penicillin streptomycin at 37°C in a humidified atmosphere containing 95% air/5% carbon dioxide [21].

2.3. Transfection and luciferase reporter gene assay

Human dermal fibroblasts were transiently transfected with 2 μg of the firefly luciferase reporter gene under control of COL1A2-responsible elements and 0.2 μg of the Renilla luciferase expression vector driven by the thymidine kinase promoter (Promega, Madison, WI, USA) and the Superfect reagent (Invitrogen, Carlsbad, CA, USA) [22]. The transfected cells were transferred to 6-well plates and incubated for 24 hours at a density of 8×10^5 cells/mL. After 24 hours, the cells were further cultured in the presence or absence of vegetable peptones for 5 hours. Luciferase activity was determined using the Dual Luciferase Assay system (Promega) and an LB953 luminometer (Berthold, Wildbad, Germany) and expressed as the ratio of COL1A2-dependent firefly luciferase activity divided by control thymidine kinase Renilla luciferase