NUTRITION RESEARCH 36 (2016) 925–936

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Apple ethanol extract promotes proliferation of human adult stem cells, which involves the regenerative potential of stem cells

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ARTICLE INFO

Article history:
Received 12 February 2016
Revised 17 June 2016
Accepted 20 June 2016

Keywords:
Adult stem cells
Apple extract
ERK
mTOR pathway
Cytokines

ABSTRACT

Tissue regeneration using adult stem cells (ASCs) has significant potential as a novel treatment for many degenerative diseases. Previous studies have established that age negatively affects the proliferation status and differentiation potential of ASCs, suggesting a possible limitation in their potential therapeutic use. Therefore, we hypothesized that apple extract might exert beneficial effects on ASCs. The specific objectives were to investigate the proliferative effect of apple ethanol extract on human adipose tissue-derived mesenchymal stem cells (ADSCs) and human cord blood-derived mesenchymal stem cells (CB-MSCs), and identify the possible molecular mechanisms. Apple extract promoted proliferation of ADSCs and CB-MSCs as determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and Click-iT 5-ethynyl-2′-deoxyuridine flow cytometry assays. In addition, phosphorylation of p44/42 MAPK (ERK), mammalian target of rapamycin (mTOR), p70 S6 kinase (p70S6K), S6 ribosomal protein (S6RP), eukaryotic initiation factor (eIF) 4B and eIF4E was induced stepwise in ADSCs. Furthermore, apple extract significantly induced the production of vascular endothelial growth factor and interleukin-6 in both ADSCs and CB-MSCs. Similarly, apple extract-induced phosphorylation of the mTOR/p70S6K/S6RP/eIF4B/eIF4E pathway was blocked by pretreatment with PD98059, a specific ERK inhibitor. These results indicate that apple extract-induced proliferation of ADSCs under serum-free conditions is mediated by ERK-dependent cytokine production. Moreover, the beneficial effect of apple extract on proliferation of ASCs may overcome the limitation in therapeutic use of stem cells in tissue regeneration and maintenance of stem cell homeostasis.

Abbreviations: DMEM, Dulbecco's modified Eagle's medium; ERK, p44/42 MAPK; ADSCs, human adipose tissue-derived mesenchymal stem cells; FBS, fetal bovine serum; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; p70S6K, p70 S6 kinase; CB-MSCs, human cord blood-derived mesenchymal stem cells; S6RP, S6 ribosomal protein; eIF, eukaryotic initiation factor; VEGF, vascular endothelial growth factor; IL-6, interleukin-6; mTOR, mammalian target of rapamycin; ASCs, adult stem cells.

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http://dx.doi.org/10.1016/j.nutres.2016.06.010
0271-5317/

1. Introduction

Stem cells are essential for the development and continued maintenance of tissues and organs. Stem cells hold great promise as a novel therapeutic option for use in tissue regeneration. Because of their multilineage differentiation capacity, stem cells may be useful for treatment of diverse degenerative diseases. [1,2]. Stem cells also exert an immunomodulatory effect that is dependent on cell–cell contact or mediated by secreted immunosuppressive molecules [3,4]. Stem cells have been isolated from many sources, including adipose tissue, the umbilical cord, bone marrow, and so on. Among these, the most common and effective way of obtaining stem cells is from adipose tissue.

The capacity for self-renewal and differentiation is a major characteristic of stem cell biology. The fate of stem cells, including their renewal and differentiation, is regulated by regulation of cell cycling. Cell proliferation is an extensively coordinated process that is regulated both temporally and spatially. Proliferation is regulated by multiple extracellular growth and differentiation factors, including soluble or membrane-bound factors and extracellular matrix components. As a result, when growth conditions are favorable, target of rapamycin (TOR) is active and yeast cells maintain a robust rate of ribosome biogenesis, translation initiation, and nutrient import. Accordingly, when growth conditions permit, rapamycin-sensitive TOR signaling promotes anabolic processes and antagonizes catabolic processes. Many of these rapamycin-sensitive readouts of TOR are conserved in mammals [5]. Mammalian target of rapamycin (mTOR) kinase phosphorylates p70 S6 kinase (p70S6K), which regulates the phosphorylation of S6 ribosomal protein (S6RP), the functional site of the 40S ribosomal subunit. Thus, mTOR/p70S6K is required for cell growth and cell cycle progression [6,7].

The history of the use of natural and dietary agents as medicinal tools for management of many diseases including cancer can be traced back to the Greek physician Hippocrates, who was an early proponent of nutritional healing. Evidence suggests that a diet high in fruits and vegetables reduces the risk of various chronic diseases [8]. Apples contain several classes of polyphenolic antioxidants, including catechins, epicatechins, and procyanidins, which are the major phytochemicals in the human diet. Several lines of evidence suggest that apples and apple products possess a wide range of biological activities that may prevent cardiovascular disease, asthma and pulmonary dysfunction, diabetes, obesity, and cancer [9,10].

Previous studies reported that aging impacts various properties of stem cells [11,12]. This effect is most focused on two specific parameters—differentiation and proliferation [13-16]. In addition, there is evidence to suggest that the amount of stem cells, and their proliferation rate, diminish with age. Moreover, an age-dependent correlation between the proliferative potential of stem cells and cell apoptosis has been reported [17]. These findings suggest that the regenerative potential of stem cells is down-regulated with age and points to a possible limitation in their potential therapeutic use. To date, no study has evaluated the influence of apple extract on ASC proliferation under serum-free conditions. However, our group previously demonstrated that phloridzin is involved in the proliferation of human CD49f(+)/CD29(+) keratinocytes [18]. In addition, we found that phloridzin was one of the main compounds in apple extract. Therefore, we hypothesized that apple extract might exert beneficial effects on ASCs. The specific objectives were to investigate the proliferative effect of apple ethanol extract on human adipose tissue-derived mesenchymal stem cells (ADSCs) and human cord blood-derived mesenchymal stem cells (CB-MSCs), and to identify the possible molecular mechanisms of the proliferation-promoting activity. In this study, among ASCs, we used ADSCs) and CB-MSCs, which have received much attention as a therapeutic candidate for cell therapy because, compared to other mesenchymal stem cells, they are relatively easy to obtain in large quantities. In addition, adipose tissue is a dynamic participant in endocrine physiology, serving as the source of secreted cytokines. Furthermore, extensive research has recently focused on adipose tissue due to the increased prevalence of obesity. Because of the above, ADSCs were used in this study.

2. Methods and materials

2.1. *Cells and reagents*

Human adipose tissue-derived mesenchymal stem cells (ADSCs) were purchased from Invitrogen (Invitrogen, Carlsbad, CA, USA). Cells were cultured in MesenPRO RS medium (Gibco; Invitrogen) at 37°C in a 5% CO_2 incubator. We obtained human cord blood-derived stem cells (CB-MSCs) from Cha University (Seongnam, Gyunggi, Korea). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing low glucose levels supplemented with 15% fetal bovine serum (FBS), 1% penicillin and streptomycin at 37°C in a 5% CO_2 incubator. The medium was changed every 3 days until the cells reached confluence, at which time they were passaged. In the experiments involving inhibitors, apple extract and inhibitors were simultaneously treated. Specifically, cells were incubated in a combination culture medium that contained apple extract (1%) or apple extract (1%) with the indicated concentrations of inhibitors for the indicated times under serum-free conditions. In addition, ADSCs and CB-MSCS were characterized using ASC markers by flow cytometry analysis. We used ADSCs and CB-MSCs before they lost their ability to grow or differentiate into all potential phenotypes.

2.2. *Preparation of apple extract*

Apple (*Malus pumila* Mill.) was harvested in Geochang Gun, Gyeongsangnam Do, Korea, and was thoroughly washed with water, shade-dried and ground into powder. The dried powder (90 g) was extracted with 70% ethanol (9 L) for 24 hours, and the extract was incrassated by a rotary evaporator for 3 hours. To remove ethanol from the extract, it was mixed with water and incrassated again. Subsequently, the extracted liquid was filtered through filter paper and frozen on a freezing tray for 48 hours. Freeze-drying for 60 hours afforded a perfectly dried extract of apple (20 g), which was dissolved in