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Advanced adipose-derived stem cell protein extracts with antioxidant activity modulates matrix metalloproteinases in human dermal fibroblasts

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ABSTRACT

Advanced adipose-derived stem cell protein extracts (AAPE) were used instead of live stem cells to investigate their effect on oxidative stress and matrix metalloproteinases (MMPs) related to tissue repair in human dermal fibroblasts (HDFs). In this study, it was observed that AAPE at 2 μg/ml specifically exhibited scavenging activity of hydrogen peroxide and reducing power. The inhibitory effect of AAPE at 2 μg/ml on MMP-2 activity was increased in the presence of phorbol myristate acetate (PMA). In the absence of PMA, AAPE significantly enhanced activities of MMP-1 and MMP-2 in HDFs, respectively. However, the level of MMP-1 expression was decreased in a dose dependent manner by AAPE. In addition, while the level of extracellular signal-regulated kinases 1 (ERK1) activation was reduced in the presence of AAPE compared to blank, the level that of ERK2 activation was not changed. The expression level of c-Fos, a part of activator protein-1 (AP-1), was increased in nucleus of HDFs. These results reveal that activation of MMPs in the presence of AAPE was increased via AP-1 in HDFs, suggesting that AAPE can be a potential candidate for tissue repair.

1. Introduction

Stem cells have the ability of self-regeneration and differentiation into various cells available for cell therapeutics, regeneration medicine and tissue engineering. In recent years, their availability has further been expanded to face lifting and beauty industry (Huang et al., 2001). In particular, embryonic stem cells are derived from the inner cell mass of the blastocyst and are capable of generating all differentiated cell types in the body. However, tumorigenicity and ethical opposition have impeded the advancement of the embryonic stem cells for clinical application. In contrast, adult stem cells have aroused a great deal of interest. Adult stem cells are multipotent and their differentiation ability is restricted to the cell types of a particular tissue, being responsible for long-life organ regeneration. Currently, they have been studied in a variety of embryonic stem cells application. In

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addition, they are handled as a potential candidate because they do not have a possibility of tumor development, immune rejection response and ethical problem. Various tissues have been studied in order to isolate adult stem cells. Among them adipose tissue is a plentiful and easily accessible source of mesenchymal stem cells that have shown to have multipotent abilities regarding differentiation and possibly immune suppressive capacity (McIntosh et al., 2006; Prichard et al., 2008; Zuk et al., 2002).

Adipose-derived stem cell (ADSC) extracts are therefore a promising alternative to bone marrow derived mesenchymal stem cells (BMSCs) and have been a popular research subject in the field of tissue engineering. In particular, efforts have been directed to investigate the chondrogenic potential of ADSCs and their possible application in repairing dysfunctional cartilage, which is one of the direct causes of disability and chronic pain (Guilaka et al., 2004). In horses and dogs several studies have proven the effectiveness of ADSCs for pain relief and tissue regeneration in the treatment of cartilage defects (Black et al., 2008).

In recent years, dermatology arising interest focuses on anatomical—functional skin damage and possible means to counteract skin defects. Few reports exist on the antioxidant action of stem cells (Baregamian et al., 2006), although evidence supports a protective effect of cytokines on the epithelial cells during oxidative damage. In particular, it has been demonstrated that adipocyte-derived agents could stimulate or suppress tumor cell progression depending on the microenvironment contexts (Iyengar et al., 2003, 2005) and be effective in tissue regeneration (Landskroner-Eiger et al., 2010).

MMPs are a family of zinc-endopeptidases involved in a variety of biological processes and pathological processes such as proliferation, migration and invasion of cells, organ development, tumor metastasis and angiogenesis (Brown and Giavazzi, 1995; Lenz et al., 2000; Liotta et al., 1991). The capacity of cancer cells to metastasize is attributed to their ability to secrete MMPs that degrade the extracellular matrix (ECM) (Zhang et al., 2010). On the other hand, matrix metalloproteinases (MMPs) play an important role in tissue regeneration (Madeddu, 2005).

Reactive oxygen species (ROS) plays a key role in photoaging. In particular, ultra violet A is mainly responsible for wrinkle formation via production of ROS and activation of MMPs. Therefore, these combined ideas propose that AAPE may contribute to the accelerated wound healing by antioxidant effects and inhibition of MMPs. In the present study, AAPE were originally prepared in our research group. AAPE instead of ADSC was used to investigate the inhibitory effect on the oxidative stress and MMPs related to tissue repair in HDFs. It was found that AAPE can modulate MMPs related to ROS. Here we show how AAPE influence MMPs expression and activity as well as antioxidant ability related to tissue regeneration.

2. Materials and methods

2.1. Materials

Dulbecco's modified Eagle's medium (DMEM), trypsin–EDTA, penicillin/streptomycin/amphotericin (10,000 U/ml, 10 mg/ml, and 2.5 mg/ml, respectively) and fetal bovine serum (FBS) were obtained from Gibco BRL, Life Technologies (NY, USA). Human dermal fibroblastss were kindly donated by LG HG&CM Research Institute (Daejeon, Korea). MTT (3-(4,5-dimethyl-2-yl)-2,5-diphenyl tetrazolium bromide) reagent, gelatin, agarose and PMA were purchased from Sigma Chemical Co. (St. Louis, MO, USA). AAPE were prepared as follows: Adult stem cells were isolated from human adipose. Briefly, human subcutaneous adipose tissues were obtained from healthy women by medical liposuction. The adipose tissues were exposed to collagenase (final 0.075% type II collagenase, Sigma-Aldrich, St. Louis, MO, USA) for 30 min at 37 °C, followed by centrifugation at 400 g for 10 min, washed and resuspended in small volume of PBS (phosphate buffer solution). The stromal cell fraction was filtered through a 70-μm cell strainer (BD Biosciences, San Jose, CA, USA). Using Histopaque-1077 (Sigma-Aldrich, St. Louis, MO, USA), ADSCs were isolated from the filtrate, then cultured at 37 °C, 5% CO_2 in DMEM containing 10% FBS. Cells were finally frozen in aliquots using CellFreezer™ (Genenmed, Korea). To produce a ADSC-CM (AAPE™), a frozen vial containing 1×10^6 cells were launched onto culture medium containing 10% FBS. After repeating subcultures to reach 5×10^8 cells, the expanded ADSCs were introduced into CellFactory™ CF10 (Nunc, USA) in DMEM/F12 serum-free medium (Welgene, Korea). Cultures were conducted under a hypoxia by providing 2% O_2 using N_2 gas supply in a humidified multichannel incubator (Sanyo, Japan) during 2 weeks. The conditioned media were collected and micro-filtered, followed by quantitated total protein content. Finally, for fresh use, 4 ml vials containing equal protein concentration were freeze-dried (Ilshin PVTFD 10RS, Korea) as a single lot sample preparation of AAPE for this study.

2.2. Cell culture

Cell lines were separately grown as monolayers at 5% CO_2 and 37 °C humidified atmosphere using appropriate media supplemented with 10% fetal bovine serum, 2 mM glutamine and 100 μg/ml penicillin–streptomycin. DMEM was used as the culture medium for human dermal fibroblasts (HDFs) cultured primarily from human fetal skin. Cells were passaged 3 times a week by treating with trypsin–EDTA and used for experiments after 5 passages.

2.3. MTT assay

Cytotoxic levels of AAPE were measured using MTT (3-(4,5-dimethyl-2-yl)-2,5-diphenyl tetrazolium bromide) method as previously described (Hansen et al., 1989). Cells were grown in 96-well plates at a density of 5×10^3 cells/well. After 24 h, cells were washed with fresh medium and were treated with AAPE at 0.25, 0.5, 1, 2 and 3 μg/ml. After 48 h of incubation, cells were rewashed and 20 μl of MTT (5 mg/ml) was added and incubated for 4 h. Finally, DMSO (150 μl) was added to solubilize the formazan salt formed and amount of formazan salt was determined by measuring the OD at 540 nm using an GENios® microplate reader (Tecan Austria GmbH, Austria). Relative cell viability was determined by the amount of MTT converted into formazan salt. Viability of cells was quantified