Journal of Plastic, Reconstructive & Aesthetic Surgery (2015) 68, 372–381

JPRAS
An International Journal of Surgical Reconstruction
www.JPRASurg.com

Improvement of photoaged skin wrinkles with cultured human fibroblasts and adipose-derived stem cells: A comparative study☆

Jae Hoon Jeong [a], Yingfang Fan [a], Ga Young You [a], Tae Hyun Choi [a,b], Sukwha Kim [a,b,*]

[a] *Department of Plastic and Reconstructive Surgery, Institute of Human-Environment Interface Biology, Seoul National University College of Medicine, Seoul, South Korea*
[b] *Department of Pediatric Plastic and Reconstructive Surgery, Seoul National University Children's Hospital, Seoul, South Korea*

Received 16 July 2014; accepted 31 October 2014

KEYWORDS
Stem cells;
Mesenchymal stem cells;
Fibroblasts;
Photoaging of skin

Summary We investigated the antiwrinkle effects of cultured human fibroblasts and adipose-derived stem cells (ADSCs) and the mechanisms underlying the reduction of wrinkles in photoaged skin.

The fibroblasts and ADSCs were isolated from human tissue and cultured. A total of 28 6-week-old female BALB/c nude mice were classified into four groups, including the normal control group and three groups that were irradiated six times a week for 6-weeks using ultraviolet B radiation to induce photoaged wrinkles. ADSCs were injected into the wrinkles in the skin of the second group and fibroblasts were injected into the wrinkles in the skin of the third group. The fourth group was the irradiated negative control group (no therapy). After 4 weeks of injections, the wrinkles were compared by replica analysis, biopsies were performed, and the dermal thickness and collagen densities were measured. We determined the amounts of type 1 collagen and matrix metalloproteinases (MMPs) 1, 2, 3, 9, and 13 using real-time polymerase chain reaction and Western blot analysis, and we assessed tropoelastin and fibrillin-1 expression in the dermis by immunohistochemistry.

Replica analysis showed significant wrinkle reduction in the fibroblast group and the ADSC group. ADSCs stimulated collagen expression and decreased MMP expression. Although fibroblasts stimulated more collagen expression than ADSCs, they also increased MMP expression.

☆ This study was presented at the 11th IQUAM (the International Committee for Quality Assurance and Medical Technologies and Devices in Plastic Surgery) Congress and Consensus Conference, in Budapest, Hungary, April 10–13, 2014.

* Corresponding author. Department of Plastic and Reconstructive Surgery, Institute of Human-Environment Interface Biology, Seoul National University College of Medicine, Department of Pediatric Plastic and Reconstructive Surgery, Seoul National University Children's Hospital, 101 Daehak-ro, Jongno-gu, Seoul 110–744, South Korea. Tel.: +82 2 2072 3480; fax: +82 2 741 3985.
E-mail address: kimsw@snu.ac.kr (S. Kim).

http://dx.doi.org/10.1016/j.bjps.2014.10.045
1748-6815/© 2014 British Association of Plastic, Reconstructive and Aesthetic Surgeons. Published by Elsevier Ltd. All rights reserved.

Overall, the ADSC group showed higher collagen density and had better outcomes in the tropoelastin and fibrillin-1 assessments.

Both cultured fibroblasts and ADSCs could play an important role in wrinkle reduction despite differences in their mechanisms of action.
© 2014 British Association of Plastic, Reconstructive and Aesthetic Surgeons. Published by Elsevier Ltd. All rights reserved.

Introduction

Increasing life expectancy, the phenomenon of population aging, increased income levels, and increased interest in beauty are some of the factors that lead to the high demand for plastic surgery or wrinkle reduction and the increasing interest in antiaging therapies. This is a global trend that is expected to continue. Aging research is mainly divided into studies of chronological aging and photoaging.[1] Chronological aging refers to the structural, functional, and metabolic changes in the skin that occur, along with degenerative changes in other organs, as a result of growing old.[1] While genetic factors are involved in the formation of wrinkles, the amount of exposure to sunlight is also an influencing factor. Photoaging is a separate process from chronological aging and mainly results in the destruction of the collagen and elastin fibers of the skin.[1,2] Several studies have reported that photoaging decreased collagen synthesis and increased transcription of matrix metalloproteinases (MMP), which degraded collagen.[2–9]

Research on photoaging treatments is also important for the field of cell therapy.[5,7–13] The cells that are receiving the most attention for use in cell therapies related to photoaging are adipose-derived stem cells (ADSCs).[5,7–9] Many studies about ADSCs have been conducted recently.[5,7–9] Some researchers have indicated that injected ADSCs were effective for treating photoaged wrinkles, as they significantly increased collagen synthesis, collagen density, and dermal thickness.[5–9] Similar studies have found that ADSCs lead to an increase in messenger RNA (mRNA) transcription for procollagen type I protein while decreasing MMP.[3,5,8,9] The most likely mechanisms underlying wrinkle reduction after injection of ADSCs are fibroblast activation, the creation of collagen, and decreased MMP expression.[5,8,9]

There have been basic research studies and clinical trials related to the injection of fibroblasts; however, the researchers only assessed patient satisfaction, dermis thickness in a biopsy, and the conformation of collagen.[10–17] The exact mechanisms underlying the antiwrinkle activity of fibroblasts on photoaged skin remain unknown.[16,17] We investigated the effects of cultured human fibroblast injections on photoaged skin wrinkles in a nude mouse model, and we compared the antiwrinkle effects between fibroblasts and ADSCs. We also examined the changes in elastic fibers, which have not previously been studied.

Materials and methods

Isolation and culture of fibroblasts and ADSCs

Human tissue samples were obtained from a woman undergoing the transverse rectus abdominis myocutaneous (TRAM) flap surgery with informed consent. Protocols were approved by the institutional review boards of the Seoul National University Hospital (No. H-1108-098-374) and cultured.[5,7,9] ADSCs were directly isolated from fat tissue, and fibroblasts were isolated from dermis.[5,7,9] Cell isolation and culture were performed as follows: The suctioned fat was washed twice using phosphate-buffered saline (PBS). The samples were digested with 0.1% collagenase type I (Worthington Biochemicals, Lakewood, NJ, USA) under agitation at 37 °C (ADSC 1 h and fibroblast 4 h) and centrifuged for 10 min at 800 × *g*. The pellet was washed and resuspended in PBS.[5,9] After another round of centrifugation, the supernatant was removed and the cell band buoyant over histopaque was collected.[6] Cells were cultured overnight at 37 °C and 5% CO_2 in a control medium (10% fetal bovine serum, 100 IU penicillin, 100 mg/ml streptomycin, 5 μg/ml heparin, and 2 ng/ml acidic fibroblast factor).[5] The adherent cell population containing ADSCs and fibroblasts was maintained over 3 days and then expanded and cultured.[5]

Animal experiments

Twenty-eight 6-week-old female BALB/c nude mice were provided by ORENT Inc. (Seongnam, Korea). The mice were fed a standard diet and were rested for 1 week in the animal facility before the experiment. A total of 28 mice were assigned to four groups (each group consisting of seven mice): the normal control group and three irradiated groups.[18] We determined the minimal erythema dose (MED) of BALB/c nude mice as the minimal intensity of ultraviolet B (UVB) radiation that produced erythema on a given test area.[19] The recorded MED was 150 mJ/cm,[2] which is higher than the MED for the hairless mice that are frequently used as the animal model of photoaging. Wrinkles were induced in the mice in the three groups by irradiation with UVB radiation (290–320 nm) six times a week for 6-weeks using four UVB lamps (TL20W/12RS; Philips, NY, USA) fitted with a Kodacel filter (TA401/407; Kodak, Rochester, NY, USA) to remove the ultraviolet C radiation. The irradiation amounts were slightly increased weekly for a total UVB dosage of 7200 mJ/cm^2.

The three groups of irradiated mice were then subjected to different treatments. ADSCs (1×10^5 cells, 0.5 ml) were injected into the back skin wrinkles of mice in one of the irradiated groups, whereas fibroblasts (1×10^5 cells, 0.5 ml) were injected into the back skin wrinkles of another group. The final group was considered the irradiated negative control group (no therapy).[1,5] A 30-gauge needle was used for the injection of cells in 0.5 ml Hank's Balanced Salt Solution (HBSS). We injected the cells parallel to deep wrinkles in a retrograde fashion. The cells were injected into intradermal, subdermal, and panniculus adiposus layers. Three or five injections were administered per

Download English Version:

https://daneshyari.com/en/article/4117281

Download Persian Version:

https://daneshyari.com/article/4117281

Daneshyari.com