Skin anti-aging Effects of Exosomes Derived from Mesenchymal Stem Cells

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Introduction:

Skin aging is characterized by wrinkles, roughness, laxity and loss of elasticity, which is associated with intrinsic and extrinsic factors. Intrinsic aging is genetically determined, which occurs inevitably as time passes. While extrinsic aging is caused by external factors such as ultraviolet and air pollution [1].

Mesenchymal stem cells (MSCs) have been demonstrated to be potential seed cells for skin wound healing and skin anti-aging. MSCs are a kind of pluripotent stem cells that are able to self-renew and have potential to differentiate into multiple types of cells [2]. However, the direct use of stem cells are limited by many safety concerns such as tumorigenicity, the transmission of adventitious agents, and unwanted immune responses [3]. In addition, related studies have shown that the wound healing promotion effects of stem cells are mainly attributed to paracrine secretion of some active substances, of which exosomes play important roles. As a cell-free agent, exosomes could overcome possible safety concerns on cell therapy [4]. Thus the effects of MSCs derived exosomes on aging skin remain to be further studied for its application. This study was aimed to evaluate the anti-aging effect of MSCs derived exosomes on human skin cells.

Materials & Methods:

Senescence-associated β-galactosidase (SA-β-gal) staining: SA-β-gal activity was characterized by SA-β-gal assay. The percentage of SA-β-gal positive cells were quantified with the Cell Counter plugin of ImageJ 1.52f software.

The expression of collagen I assay: The cultured fibroblasts were 2% paraformaldehyde fixed and incubated with monoclonal mouse anti-human collagen I antigen. The secondary antibody used was Dako EnVision TMG. DAPI was applied to counterstain the cell nuclei. The immunocytochemistry staining images were obtained with Zeiss Scope A1 microscope, and the differential expression of collagen I between treated and non-treated cells was compared.

The expression of laminin 332 ELISA assay: Keratinocytes were seeded into a 96-well-plate with growth medium supplemented without / with the exosomes. The expression of laminin 332 in keratinocytes monolayer based on cell-based ELISA assay. Read the plates with Multimode Plate Reader. Choose the top-read mode. Excitation filter 485nm/Emission filter 535nm.

The intracellular level of reactive oxygen species (ROS) Assay: Keratinocytes were seeded into a 96-well-plate and induced cell oxidative injury by UVB. The intracellular level of ROS was measured by DCFDA assay. Measure plate immediately on a fluorescence plate reader at Ex/Em = 485/535 nm in end point mode in the presence of compounds.

The three-dimensional (3D) full-thickness skin equivalent model based on collagen matrix assay: The porcine collagen and normal human fibroblasts were mixed and seeded into the culture dish and cultured for 3-4 days to obtain a dermal structure. Then, the normal keratinocytes were seeded onto the dermis surface and cultured without/with the exosomes for 14 days to obtain a full-thickness skin model. The morphology and tissue structure of the skin model were evaluated by Hematoxylin-eosin (HE) staining. Immunostaining was performed on paraffin-embedded formalin-fixed samples to detect the expression of Ki-67.

Conclusions:

MSCs derived exosomes could reduce β-galactosidase activity, promote expression of collagen I and Laminin332, and accelerate the skin remodeling on 3D equivalents. These data indicated the attractive anti-aging effect of MSCs derived exosomes, and its potential on skin care applications.

Results & Discussion:

Inhibition effect of MSCs derived exosomes against senescence

After treatment with 10~80mg/mL MSCs derived exosomes, SA-β-gal activity was significantly reduced (Figure 1), demonstrating that MSCs derived exosomes can inhibit cellular senescence.

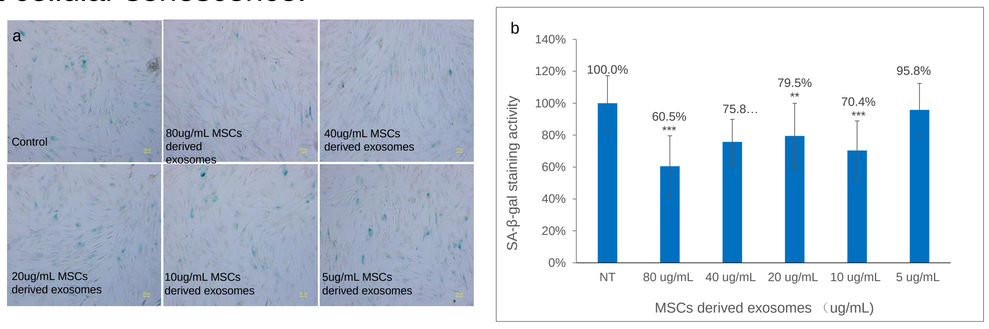
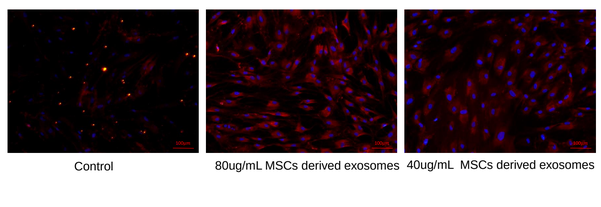


Figure 1. Effects of MSCs derived exosomes on SA-β-gal activity

Effect of MSCs derived exosomes on collagen I

Results shown that a much higher expression of collagen I was observed in human dermal fibroblasts cultured with MSCs derived exosomes, which indicates that MSCs derived exosomes could induce the synthesis of collagen I in human dermal fibroblast.



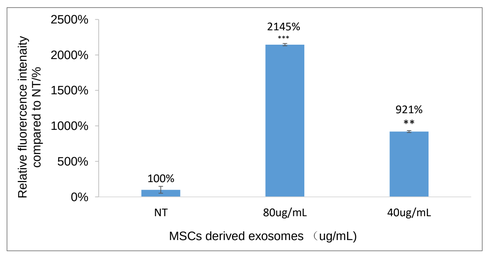


Figure 2. Effects of MSCs derived exosomes on collagen I

Effects of MSCs derived exosomes on the expression of laminin 332

MSCs derived exosomes could promote the expression of laminin 332 with concentration of 0.3125~5 mg/mL. Among which, the expression of laminin 332 increased to 151% when treated with 5mg/mL MSCs derived exosomes.

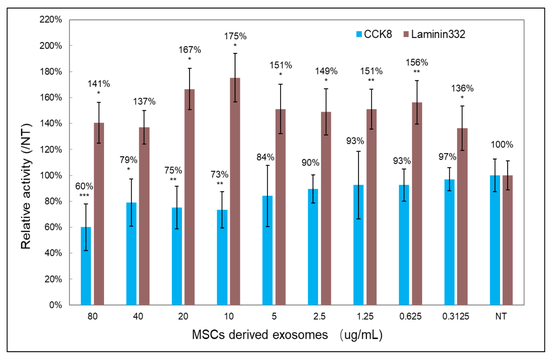


Figure 3. Effects of MSCs derived exosomes on the expression of laminin 332

Influence of MSCs derived exosomes on 3D skin equivalent model

Compared with control group, Ki67 expression was obviously increased after treated with 5ug/mL MSCs derived exosomes. Meanwhile, at epidermal level, skin equivalent models treated with MSCs derived exosomes displayed a more regular arrangement and cuboidal basal keratinocytes in histologic sections. And there were more active cells with mitotic activity in the basal layer, indicating an increased stability of epidermal architecture compared to the untreated control.

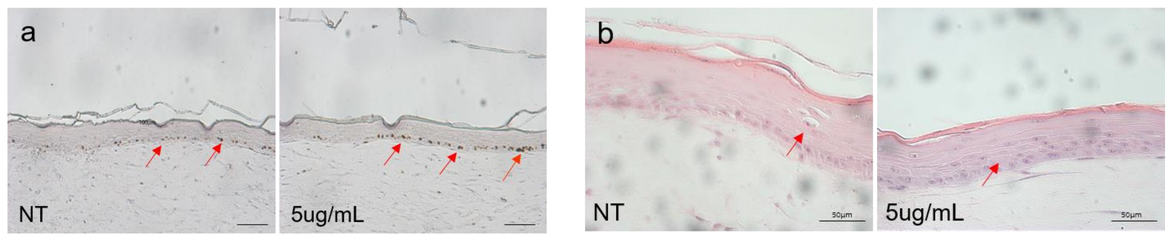


Figure 5. Effects of MSCs derived exosomes on 3D full-thickness skin equivalent model. a. Optical microscopy observation of epidermal proliferation Ki-67. b. influence of MSCs derived exosomes on 3D skin equivalent model.

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