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**MICRORNA-10A CAN RESTORE HUMAN  
MESENCHYMAL STEM CELL DIFFERENTIATION  
THROUGH KLF4**

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**Objectives** Human mesenchymal stem cells (hMSC) are thought to be multipotent cells, which have the properties of self-regeneration

and differentiation plasticity, that can replicate and differentiate into lineages of mesenchymal tissues, including bone, fat, cartilage, tendon, muscle and marrow stroma. Human aging is a highly complex process that is characterised by an increase in age-associated diseases. Some studies have found that aging affects MSC function.

MicroRNAs (miRNAs) are posttranscriptional modulators of gene expression that are small, non-coding (typically 20 nt) and incorporate into the miRNA-induced silencing complex (RISC) to play an important role in many developmental processes. In a variety of cellular processes, such as cell survival, replicative senescence, proliferation and differentiation, miRNAs are key regulators. MiRNAs expression patterns change with age, and miRNA may directly affect the aging process. Recent studies have suggested that miRNAs change during MSC differentiation and that miRNAs play a critical role in MSC differentiation.

At present, few data exist to confirm the role of aging-related miRNAs in hMSC differentiation. This study profiled the miRNA expression of hMSC derived from young and old individuals and directly assessed the effects of these miRNAs during hMSC differentiation.

**Methods** Human bone marrow aspirates were obtained from the sternums of patients undergoing cardiac surgery. Young bone marrow was collected from patients aged 17–30, and old bone marrow was obtained mainly from patients aged 65–80 with valve disease. HMSC was immunostained with fluorescein conjugated antibodies to identify hMSC. Cell growth was evaluated using the cell proliferation assay for 7 consecutive days after plating. Growth curves were generated for young and old hMSC and compared. HMSC was induced to three lineage differentiation (include of adipogenic, osteogenic and chondrogenic differentiation) by culturing in the differentiation medium. Immunohistochemistry stain and real time PCR were used to identify the differentiated cell and cell specific gene expression of adipocyte, osteoblast and chondroblast. The hMSC senescence was analysed by the  $\beta$ -galactosidase staining. The miRNAs expression in young and old hMSC was analysed by Affymetrix GeneChip 2.0 miRNA arrays and identified by real time PCR. Dual-luciferase gene report system was used to identify the target of miR-10a. miR-10a lentiviral constructs for over-expressing miR-10a, inhibiting the expression of miR-10a and KLF4 were used to detect the effect of miR-10a and KLF4 in hMSC proliferation, differentiation and cell senescence.

**Results** Compared with the young hMSC, the proliferate and differentiate potential of old hMSC were decreased. In old hMSC, both the percentage of SA- $\beta$ -gal positive cells and the staining intensity increased. Although aged hMSC became senescent, the composition and expression level of MSC specific surface markers were not varied. Hsa-miR-196a, hsa-miR-378, hsa-miR-378-star, hsa-miR-486-5p and hsa-miR-664-star were up-regulated and that hsa-miR-10a, hsa-miR-708 and hsa-miR-3197 were down-regulated in old subjects compared with young subjects. KLF4, identified by dual-luciferase gene report system, was the target of miR-10a. Over-expression of miR-10a can increase the differentiation of all three cell lineages in old and young hMSC and reduce cell senescence; conversely, proliferation was inhibited. Contrastingly, inhibiting miR-10a expression produced the opposite result. Directly suppressing KLF4 expression resulted in differentiation, reduced cell senescence and inhibit proliferation in both young and old hMSC.

#### Conclusions

1. In old hMSC, accompany with the age increased, the composition and expression level of MSC specific surface markers were not varied; the proliferate and three lineage differentiate potential were decreased; the cell senescence increased; miRNA expression was varied.
2. MicroRNA-10a can restore human mesenchymal stem cell differentiation through KLF4.