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Properties of Adipose Tissue Derived Mesenchymal Stem Cells with Long-Term Cryopreservation

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Abstract : Adult mesenchymal stem cells (MSCs) have been investigated using preclinical approaches for tissue regeneration. Porcine MSCs (pMSCs) are capable of growing and attaching to plastic with a fibroblast-like morphology and then differentiating into bone, adipose, and cartilage tissues in vitro. This study was conducted to investigate the proliferating abilities, differentiation potentials, and multipotency of miniature pig adipose tissue-derived MSCs (mpAD-MSCs) with or without long-term cryopreservation, considering that cryostorage has the potential for use in clinical applications. After confirming the characteristics of the mpAD-MSCs, we examined the effect of long-term cryopreservation (> 2 years) on expression of cell surface markers (CD34, CD90 and CD105), proliferating abilities (cumulative population doubling level, doubling time, colony-forming unit, and MTT assay) and differentiation potentials into mesodermal cell lineages. As a result, the expression of cell surface markers is similar between thawed and fresh mpAD-MSCs. However, long-term cryopreservation significantly lowered the differentiation potentials (adipogenic, chondrogenic, and osteogenic) of mpAD-MSCs. When compared with fresh mpAD-MSCs, thawed mpAD-MSCs exhibited lower expression of mesodermal cell lineage-related genes such as peroxisome proliferator-activated receptor-g2, lipoprotein lipase, collagen Type II alpha 1, osteonectin, and osteocalcin. Interestingly, long-term cryostoraged mpAD-MSCs exhibited significantly higher cell viability than the fresh mpAD-MSCs. Long-term cryopreservation induced a 30% increase in the cell viability of mpAD-MSCs when compared with the fresh mpAD-MSCs at 5 days after thawing. However, long-term cryopreservation significantly lowered expression of stemness markers such as Oct3/4, Sox2, and Nanog. Furthermore, long-term cryopreservation negatively affected expression of senescence-associated genes such as telomerase reverse transcriptase and heat shock protein 90 of mpAD-MSCs when compared with the fresh mpAD-MSCs. The results from this study might be important for the successful application of MSCs in clinical trials after long-term cryopreservation.

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