Porcine bone marrow stromal cells

Short and long term in vitro induction of bone, cartilage and fat tissue

Lijin Zou, MD

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Official opponents: Søren Overgaard, Benny Dahl, and Kristian Steengaard-Pedersen.

Tutors: Cody Bünger, Haisheng Li, and Tina Mygind.

Correspondence: Lijin Zou, Orthopaedic Research Laboratory, Department of Orthopaedics, Aarhus University Hospital, 8000 Århus C, Denmark.

E-mail: zou.lijin@ki.au.dk

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ABSTRACT

Bone marrow stromal cells (BMSC) are an ideal source for cell therapy and tissue engineering since they are relatively easy to harvest and have multilineage differentiation potency. BMSC have been evaluated for clinical applications.

However, several issues still need to be further studied. Successful use of BMSC requires a continuous in vitro expansion due to the low frequency of BMSC in bone marrow. Moreover, their use for gene and cell therapy of skeletal diseases such as osteogenesis imperfecta requires the long-lasting engraftment of BMSC endowed with a residual proliferation potential sufficient to sustain the low, but continuous bone turnover. On the other hand, in the case of tissue engineering, understanding the mutual control of the osteoblast/chondroblast/adipocyte differentiation of BMSC is of importance in the control of skeletal remodeling, because bone growth and repair processes strongly depend on the appropriate differentiation of BMSC in the bone and cartilage lineage pathways. Finally, how to improve osteogenic differentiation of BMSC should be also considered.

In the interest of mechanism on multilineage differentiation, optimization of osteogenic differentiation condition, and changes about biological properties of BMSC during long-term culture, we investigated the reciprocal relationship among transcription factors and marker genes during multilineage differentiation, the role of hyaluronan (HA) in osteogenic differentiation of BMSC, and alterations about proliferation and the multipotency during long-term passage in an in vitro pig model.

We demonstrate that the differentiation of porcine BMSC towards one pathway restricts expression of other lineage-specific genes. This particularly involved that osteoblast, adipocyte and chondroblast pathways are mutually regulated. Exogenous HA stimulates endogenous HA, which together may play a synergetic role in osteogenic differentiation under osteoinducing conditions. Porcine BMSC can undergo spontaneous transformation following long-term culture due to loss of P53 and Fas, and activation of c-Myc instead of telomerase. On the other hand, although a large number of BMSC can be available through a continuous expansion, late stage cells (after P4, two months) are not suitable for bone tissue engineering due to progressively loss of osteogenic potency. Chondrogenic potency maintains till P15 at least, however, late stage cells from recovery of cell growth (after P12) are not either suitable for cartilage tissue engineering due to spontaneous transformation.

These findings may have important implications on the clinical application of BMSC, which means that only early stages BMSC should be considered from in vitro expansion.