Chondrogenic Differentiation Of Bone Marrow-derived Mesenchymal Stem Cells Regulated By Wnt/beta-catenin Signaling Pathway

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Introduction: Mesenchymal stem cells (MSCs) from the bone marrow represent a potential source of pluripotent cells for autologous bone tissue engineering. Sequential proliferation, hypertrophy and maturation of bone marrow-derived mesenchymal stem cells are required for proper microenvironment and tightly regulated by cell signaling. A number of signaling pathways have been implicated in the regulation of chondrogenic differentiation and hypertrophy, which include Sox9, parathyroid hormone related peptide, Indian hedgehog, bone morphogenetic protein, transforming growth factor β. One of the signal transduction pathways that has been associated with bone and cartilage formation, but for which relatively little is known with relation to MSCs, is Wnt signaling.

Methods: Passaged-3 MSCs were exposed in lithium chloride (LiCl) and their proliferative activities in the first 7 days were recorded. Additionally, the proliferating cell nuclear antigen (PCNA) at day 7 was measured by western blot. Passaged-3 MSCs were condensed into small pellets and cultivated in the different groups based on the chondrogenic differentiation medium (CDM). The experiment groups had additional lithium chloride (LiCl) or dickkopf-related protein 1 (Dkk-1). The cultures were maintained for 7, 14, 21 days and then analyzed for expression of Sox9, aggrecan, collagen type II (Col 2a), β-catenin, and PCNA genes and proteins. Deposition of glycosaminoglycan (GAG) in the cartilage matrix was also measured for certain cultures.

Results: The proliferative activity curves demonstrate the ability of LiCl-exposed MSCs was much greater than the control. The expression of PCNA in the LiCl-exposed group was significantly higher than the control. (Figure 1).

Time course analysis during chondrogenic differentiation revealed that the chondrogenic markers, such as Sox9, Col 2a, Aggrecan, and cartilaginous extracellular matrix (ECM), such as GAG, were gradually increased. However, the expression of β-catenin protein was not time-dependent, and present at a low ebb in the day 7 and 14. (Figure 2).

At the day 7, the early stage of the chondrogenic differentiation, the protein of Col 2a and Aggrecan were no significance between the induced groups. However, the expression of Sox9 in the LiCl-exposed group was significantly different. In the other hand, the β-catenin protein expression in the chondrogenic induced group was higher than the control; while in the CDM, the β-catenin protein expression in the LiCl-exposed group was much greater than it in the Dkk-1-exposed group. The similar results happened to the Cyclin D and PCNA. (Figure 3).

At the day 21, the later stage of the chondrogenic differentiation. The alcian blue staining and GAG assay demonstrated that LiCl could enhance the cartilaginous extracellular matrix synthesis while Dkk-1 had
an opposite effect. The PCR and western blot results proved the similar conclusion. The protein of Sox9 and Col 2a had a parallel trend. (Figure 3).

**Discussion:** In the current paper, we demonstrate that the canonical Wnt signaling pathway acts through β-catenin not only to enhance mesenchymal stem cells proliferation but also to promote mesenchymal stem cells chondrogenic differentiation in the later stage, while in the early stage, the effect of promote differentiation was not significant.

The Wnt signaling pathway plays important role in patterning and cell fate determination, especially in stem cells. The central protein, β-catenin, is dispensable for stem cells maintenance. On the other hand, the effect of β-catenin in osteogenesis was mostly reported, while it is rare to reported in chondrogenesis. Our results confirm the effect of the Wnt signaling pathway in the chondrogenic differentiation of MSCs, and indicate the effect was upon the regulation of Sox9.

**Significance:** This finding represents an important step towards understanding the precise regulation of MSCs proliferation and differentiation, which may have important applications in cartilage tissue engineering.
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