The Effects of Wnt Inhibitors on the Chondrogenesis from Human Mesenchymal Stem Cell

Yoon-Jin, Roh; Zhejiu Quan; Jong-Min Lee; Soyoung, Kim; +Gun-Il, Im
+ Department of Orthopaedics, University of Dongguk Ilsan Hospital, Goyang, Korea
gunil@duih.org

INTRODUCTION:
As many of the molecular events of embryogenesis are recapitulated during the chondrogenesis of MSCs in three dimensional in-vitro cultures, it is possible that modulations of these pathways could be used to enhance chondrogenesis and inhibit hypertrophic changes in cartilage tissue engineering. Considering that β-catenin is an important messenger in modulating the early differentiation of MSCs in the developing embryo, the modulation of the Wnt pathway may present one of the powerful means for this purpose. The purpose of this study was to test the hypothesis that the inhibition of canonical Wnt pathway promoted chondrogenesis from hMSCs. We first tested various concentrations of two known inhibitors of canonical Wnt signaling, DKK-1 and sFRP-1 for toxicity and early chondrogenesis, then further tested the effectiveness of one chosen concentration of these factors to enhance the chondrogenic differentiation after three week period of in-vitro culture.

MATERIALS AND METHODS:
The bone marrow samples used to isolate MSCs were obtained from three patients (mean age: 50 years) who were undergoing total hip replacement due to osteoarthritis. In order to measure the change in cell viability, Alamar Blue assay was performed. MSCs at passage 3 were plated at a density of 5 x 10⁵ cells/300µl/well in 96-well plates. To induce chondrogenesis, in-vitro pellet cultures were carried out using 2.5 x 10⁵ MSCs at passage 3 in DMEM/F-12 supplemented with 1% ITS, 10⁻⁷ M dexamethasone, 50 µM ascorbate-2-phosphate, 50 µM L-proline and 1 mM sodium pyruvate for the control subset; in other subsets, 100ng/ml, 200ng/ml and 300ng/ml of DKK-1 or sFRP-1 were additionally treated. The pellets were harvested after 3 and 6 days for analysis. For standard chondrogenic culture, 2.5 x 10⁵ MSCs at passage 3 were cultured for 21 days in the same condition as described above with different combination of sFRP-1 and TGF-β. The subsets are 1) no treatment 2) 200ng/ml of sFRP-1 for initial 7 days 3) 10ng/ml of TGF-β for whole culture period 4) 200ng/ml of sFRP-1 for initial 7 days plus 10ng/ml of TGF-β for whole culture period. The pellets were harvested 7 days, 14 days, and 21 days for the analysis after the start of pellet culture. The DNA and GAG content was determined. All the PCR reactions were performed on the Light Cycler 480 system² (Roche): for collagen type I (COL1A1), collagen type II (COL2A1), collagen type 10 (COL10A1), SOX-9, β-catenin and GAPDH. To determine the early protein expression of β-catenin, collagen type II, and SOX-9, MSCs in pellet culture were treated with 200ng/ml of either DKK-1 or sFRP-1 for 6 days, and harvested for analysis.

RESULT:
Both DKK-1 and sFRP-1 negligibly reduced cell viability, but DNA content decreased with greater doses. Both DKK-1 and sFRP-1 did not affect the β-catenin gene expression while they decreased the β-catenin protein expression (Fig. 1). Wnt inhibitors increased GAG synthesis at 3 and 6 days after treatment (Fig. 2). Wnt inhibitors increased the gene and protein expressions of type II collagen and SOX-9, more prominently by sFRP-1 than DKK-1 in the early chondrogenesis (Fig. 3). sFRP-1 did not show synergistic effect to TGF-β for the chondrogenic gene expression. sFRP-1 did not significantly affect SOX gene expression in the presence or absence of TGF-β. COL1A1 and COL10A1 also increased with passage of time, but sFRP-1 treatment did not significantly affect the COL1A1 or COL10A1 gene expression regardless of TGF-β treatment (Fig. 4).

DISCUSSION:
The overall results of this study demonstrated that Wnt inhibitors promoted early chondrogenesis from MSCs while lacking synergistic effect with TGF-β in the culture of longer term. It is suggested that Wnt inhibitors do not provide an ultimately enhancing role in the cartilage tissue engineering from MSCs.