Smad Signaling Determines Chondrogenic Differentiation of Bone-Marrow Derived Mesenchymal Stem Cells
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Introduction
The aim of this study was to investigate the roles of Smad2/3 and Smad1/5/8 phosphorylation in chondrogenic differentiation of bone-marrow derived mesenchymal stem cells (BMSCs) by addition of TGF-β in culture, and to assess whether specific targeting of the Smad signaling pathways may offer possibilities to prevent terminal differentiation and mineralization of chondrogenically differentiated BMSCs.

Materials and Methods
BMSC were chondrogenically differentiated in pellet culture in chondrogenic medium including TGF-β2. SB-505124 was added to the medium to study the effect of inhibition of Smad2/3 phosphorylation on chondrogenic differentiation of BMSCs, while dorsomorphin was added to study inhibition of Smad1/5/8 phosphorylation. Immunohistochemistry and gene-expression analysis was performed for markers of chondrogenic and terminal differentiation, as well as for Smad2/3P and Smad1/5/8P. The ability to mineralize of the tissue-engineered constructs was studied in-vitro by adding 10 mM β-glycerophosphate (BGP) to the medium.

Results
Terminally differentiated chondrocytes in cartilage produced in vitro by chondrogenic differentiation of BMSCs or studied ex vivo during murine embryonic limb formation, stained positive for both Smad2/3P and Smad1/5/8P. On the other hand, hyaline-like cartilage that lacks expression for MMP13 and collagen X only expressed Smad2/3P. When either Smad2/3 or Smad1/5/8 phosphorylation was blocked by addition of SB-505124 or dorsomorphin throughout culture, no collagen II expression was observed, indicating that both pathways are involved in early chondrogenesis. Distinct functions for these pathways were demonstrated when Smad signaling was blocked after the onset of chondrogenesis. Blocking Smad2/3P from day 14-35 resulted in a halt in collagen II production. On the other hand, blocking Smad1/5/8P during this time resulted in decreased expression of MMP13, collagen X and alkaline phosphatase without inhibiting further collagen II production. Moreover, blocking Smad1/5/8P completely prevented mineralization.

Discussion and Conclusions
Our data strongly suggest that Smad2/3 and Smad1/5/8 phosphorylation in chondrogenesis is stage dependent. Both pathways are crucial for initial chondrogenesis. While Smad2/3 is important for further chondrogenic differentiation and matrix production, Smad1/5/8 is crucial for terminal differentiation and mineralization. Moreover, our data imply that in-vitro cartilage tissue engineering could greatly benefit from blocking the Smad1/5/8 route after chondrogenic differentiation of BMSCs is induced, to prevent terminal differentiation and mineralization while sustaining further cartilage-matrix production.

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Disclosures
No conflict of interest.