Reduction of hypertrophy by PTHrP and bFGF during in vitro chondrogenesis of mesenchymal stem cells from bone marrow

Weiss S, Bock R, Hennig T, Richter W

Division of Experimental Orthopaedics, Orthopaedic Clinic University of Heidelberg, Schlierbacher Landstr. 200 a, D-69118 Heidelberg, Germany

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Introduction:
Hypertrophic differentiation of mesenchymal stem cells (MSCs) presents a major problem in TGF-b-driven in vitro chondrogenesis. BMPs have been described as inductors of chondrogenesis while PTHrP or bFGF are associated with a reduction of hypertrophy. We aimed to identify chondrogenic culture conditions avoiding cell hypertrophy by analyzing the effect of growth factors alone or in combination with TGF-b3 on MSC pellets cultured in vitro and after transplantation in SCID mice in vivo.

Materials and Methods:
Chondrogenic induction of hBMSC spheroid cultures was modified by addition of factors suspected to stimulate or inhibit chondrogenic hypertrophy. Hypertrophic differentiation was assessed by immunohistochemical analysis (collagen type I, -II, -X, alcian blue), RT-PCR (Col1A1, Col2A1, Col10A1, MMP-13) and quantification of ALP activity up to 6 weeks of differentiation. After 6 weeks of culture under chondrogenic conditionsmicromasses were transplanted subcutaneously in SCID mice for 4 weeks and analyzed histologically (alizarin red) thereafter.

Results:
Chondrogenic differentiation as confirmed by positive staining of type II collagen and alcian blue was achieved after supplementing chondrogenic medium with TGF-b3. None of the other growth factors (BMP-2, -4, -6, -7, aFGF, IGF-I) led to chondrogenesis, alone, whereas combination with TGF-b results in chondrogenesis without suppressing collagen type X expression. Combinations of TGF-b with PTHrP or bFGF suppressed collagen type X staining and ALP induction. However, they also prevented the differentiation to chondrocyte-like cells when added from day 0. Delayed addition of PTHrP or bFGF rescued chondrogenesis and suppressed ALP activity along with expression of other hypertrophic markers. In vivo, delayed PTHrP or bFGF treatment could not inhibit calcification.
Conclusion:

PTHrP and bFGF are attractive anti-hypertrophic factors able to modulate the chondrogenic effect of TGF-b on MSC in vitro. A fine tuning to collagen type II-positive pellet cultures with low content of collagen type X seems feasible.