

Proceedings of German Society for Stem Cell Research (PGSSCR)

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

Published on 16 May 2007

Introduction:

Hypertrophic differentiation of mesenchymal stem cells (MSCs) presents a major problem in TGF- β -driven *in vitro* chondrogenesis. BMPs have been described as inducers 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- β 3 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 conditions micromasses

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- β 3. None of the other growth factors (BMP-2, -4, -6, -7, aFGF, IGF-I) led to chondrogenesis, alone, whereas combination with TGF- β results in chondrogenesis without suppressing collagen type X expression. Combinations of TGF- β 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- β on MSC *in vitro*. A fine tuning to collagen type II-positive pellet cultures with low content of collagen type X seems feasible.

JSRM
www.pubstemcell.com