

## Temporal expression of FGF-Receptors in chondrogenesis of mesenchymal stem cells

[CA Hellingman<sup>1</sup>](#), [DA Frenz<sup>2</sup>](#), [GJVM van Osch<sup>1</sup>](#)

<sup>1</sup> Erasmus MC, University Medical Center Rotterdam, The Netherlands <sup>2</sup>Dept. Otorhinolaryngology and Anatomy & Structural Biology, Albert Einstein College of Medicine, Bronx, USA

**INTRODUCTION:** Adult mesenchymal stem cells (MSCs) are considered promising candidate cells for therapeutic cartilage and bone regeneration. Because tissue regeneration and embryonic development may involve similar pathways, understanding common pathways may lead to advances in regenerative medicine. In embryonic limb development Fibroblast Growth Factor Receptors (FGFR) play a role in chondrogenic differentiation. The aim of this study was to compare FGFR expression in in-vivo embryonic limb development and in-vitro chondrogenesis of MSCs for the purpose of better controlling MSC derived cartilage formation.

**METHODS:** Assessment of embryonic chondrogenesis. Murine embryos were excised on E12, E13, E14, E16, E18. Chondrogenesis of MSCs Bone-marrow-derived MSCs were isolated from femur of patients undergoing total hip replacement. After expansion of adherent cells, MSCs were cultured in pellets in chondrogenic differentiation medium (DMEM, 1:100 ITS+, 25 µg/ml ascorbic acid-2-phosphate, 10 ng/mL TGFβ2, 10<sup>-7</sup>M dexamethasone) for 35 days. Modulation of FGF signalling FGF2 or FGF9 (5nM) was added to the chondrogenic differentiation medium either throughout the culture period (day 0-35), during early differentiation (day 3-14), or during late differentiation (day 21-35). Pellets were used for histology and biochemical analysis of glycosaminoglycan (GAG) content. Immunohistochemistry. All samples were embedded in paraffin and stained for N-cadherin (marker for condensation), collagen type II (marker for cartilage), collagen type X (marker for terminally differentiated or hypertrophic cartilage) and FGFR1, 2, 3.

**RESULTS AND DISCUSSION:** Three stages can be discriminated during chondrogenic differentiation of adult MSCs in vitro, similar to embryonic limb development: condensation, differentiation, and hypertrophy. Expression of

FGFR1, 2, 3 was related to the differentiation stage in a similar way in both models. During the condensation phase (N-cadherin expression) a peak in expression of FGFR2 occurred. Differentiating chondrocytes (collagen II positive, collagen X negative) did not express any FGFRs. During hypertrophy (collagen X positive) all FGFRs were expressed.

Different FGFs are known to have different binding affinities for the different FGFRs. To examine potential application of our findings, pellets were treated with FGF2 or FGF9 in a stage specific manner. When added during the entire culture period, both FGF2 and FGF9 treated pellets contained significantly less GAG at day 35 than control pellets. Pellets treated with FGF2 from day 3-14 had a lower GAG content at day 35 than control pellets whereas a trend towards a higher GAG content was seen when FGF 9 was added. Addition of FGF during hypertrophic differentiation is detrimental for cartilage-matrix production. Although all receptors are expressed during hypertrophy, FGF2 and FGF9 have differential effect when added day 21-35. While FGF2 inhibits further matrix deposition, FGF9 increases matrix resorption. This suggest that the FGFRs have specific effects, even during hypertrophy when they are all expressed.

We have demonstrated that different stages can be discerned in chondrogenic differentiation, and that the effect of a growth factor depends on the stage in which it is added in the medium. Since the effects of growth factors and modulation of intracellular signalling are mostly studied throughout culture, more attention to stage-specific effects may be warranted.

**ACKNOWLEDGEMENTS:** This research was financially supported by the Dutch Program for Tissue Engineering.