Different Effects of Transforming Growth Factor – beta Subtypes on Mineralisation of Cartilage Formed by Bone Marrow Stromal Cells

FLJ Cals, CA Hellingman, JLM Koevoet, RJ Baatenburg de Jong, GJVM van Osch

Corresponding Author: f.cals@erasmusmc.nl

Department of Otorhinolaryngology and Orthopaedics, Erasmus MC Rotterdam, the Netherlands

Introduction
Bone Marrow Stromal Cells (BMSCs) chondrogenically differentiate in response to defined medium containing Transforming Growth Factor – beta (TGF-β). Three mammalian subtypes of TGF-β (TGF-β1, -β2, and -β3) are known. Contradictory results in literature¹²³, suggest that TGF-β subtypes might provoke certain subtype-specific activities. Therefore, the aim of our study was to investigate whether there are TGF-β subtype-dependent effects on inducing in vitro chondrogenesis, hypertrophy and mineralisation.

Materials and Methods
Human BMSC pellets were cultured for 5 weeks in chondrogenic medium containing either 2.5 ng/ml, 10 ng/ml or 25 ng/ml of TGF-β1, -β2 or -β3. Collagen type II staining and quantification of total collagen and GAG/DNA were performed. The expression of hypertrophy markers, such as collagen type X, VEGF and MMP activity were studied. Mineralisation was demonstrated at day 35 by alkaline phosphatase (ALP) activity and Alizarin Red (AR) staining after addition of β-glycerophosphate (βGP) from day 21 on. Four donors with variable chondrogenic capacity were compared. Statistical analyses were conducted with SPSS 15.0. A t-test was performed to detect significant differences. A p-value less than 0.05 was considered significant.

Results
Cells from a donor with low chondrogenic capacity uniformly induced less cartilage, while cells with good chondrogenic capacity performed good with all TGF-β subtypes. All subtypes showed a comparable dose-response, with significantly less cartilage when 2.5 ng/ml was used, compared to 10 ng/ml and 25 ng/ml. No consistent differences were observed in expression of hypertrophy markers between the TGF-β subtypes. However, focusing on mineralisation, it was obvious that TGF-β1 treated pellets contained less than TGF-β2 or -β3. Representative samples of one donor are shown in figure 1 and figure 2.

Discussion and Conclusions
Although the observations confirm a large variability in chondrogenic differentiation between cells from different human BMSCs donors, the amount of produced cartilage and presence of hypertrophy do not consistently differ between various TGF-β subtypes. We believe that the contribution of our study to the field of cartilage tissue engineering is the conclusion that TGF-β subtypes however have differential effects on mineralization, with TGF-β1 being least potent.

References

Acknowledgement
This study is financially supported by Dutch Program for Tissue Engineering.

Disclosures
The authors indicate no potential conflicts of interest.