An *in Vitro* Chondrogenic Differentiation Model Using KS483 Mouse Mesenchymal Progenitor Cell Line

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Introduction

KS483 cells are multipotent mouse mesenchymal progenitor cells that are able to differentiate into chondrocytes, adipocytes and osteoblasts and are a well-characterized model for the study of osteoblast differentiation and bone formation.[1-3] *In vitro* chondrogenic differentiation can be accomplished by the widely used pellet culture system where the cells are maintained in high-density pellets to mimic mesenchymal condensation during development.

Aim of the Study

To study the use of KS483 cell line in an *in vitro* model of chondrogenic differentiation.

Materials and Methods

KS483 cells were cultured in three-dimensional (3D) pellet culture system in serum-free differentiation medium in the presence and absence of BMP-6 for 17-28 days. The pellets were cultured in 96-well suspension culture plates and the medium was changed in 2-3 day intervals. The pellets were embedded in paraffin, sectioned and stained for Safranin O and Alcain blue for assessment of sulfated glycosaminoglycan (sGAG) content, and processed for immunohistochemistry to visualize type II collagen. Pellet area was measured using MetaMorph 6.1 image analysis software, and chondrogenic differentiation was determined by percentage of positive staining.

For biochemical analysis, the pellets were digested with proteinase K and assayed for sGAG (Wieslab® sGAG quantitative kit, Euro-Diagnostica) and DNA content (DNA Quantitation Kit, Sigma-Aldrich) at days 17, 24 and 28. Type II collagen carboxyterminal propeptide (CP-II; IB-CPII-SCATM Cartilage Synthesis Competitive Assay, IBEX Pharmaceuticals Inc) was determined in the culture medium collected at day 17.

Histological analysis

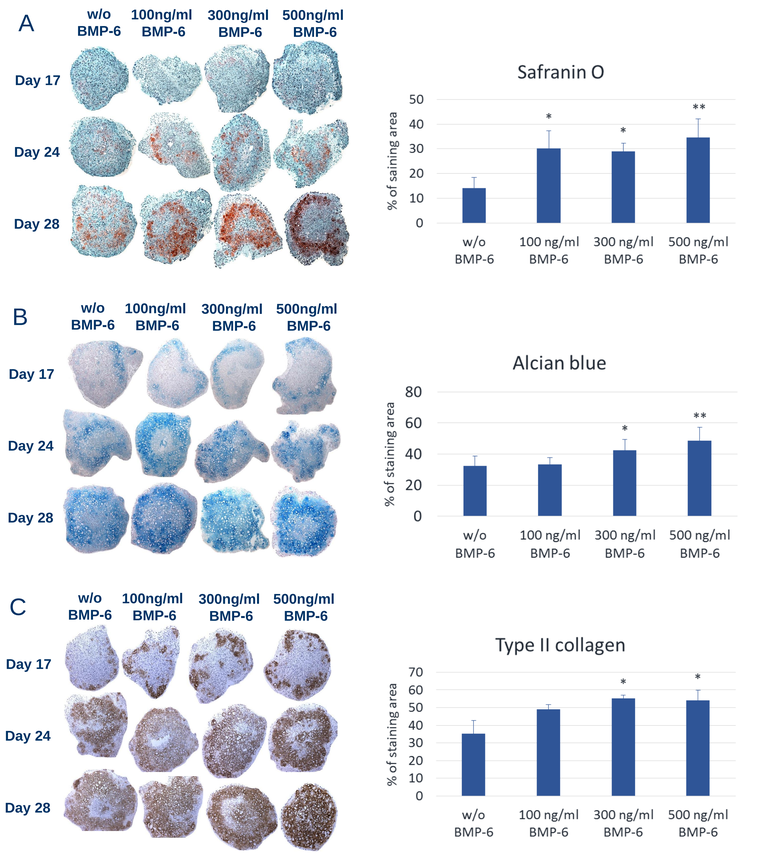

Figure 1: The effects of BMP-6 on chodrogenic differentiation visualized by A) Safranin O, B) Alcian Blue and C) Collagen type II immunostaining at days 17, 24 and 28. Chondrogenic differentiation was determined by percentage of positive staining of three replicate pellets. Statistical analysis was performed between the w/o BMP-6 and treatment groups. * p<0.05 and ** p<0.01 compared to the group w/o BMP-6.

Biochemical analysis

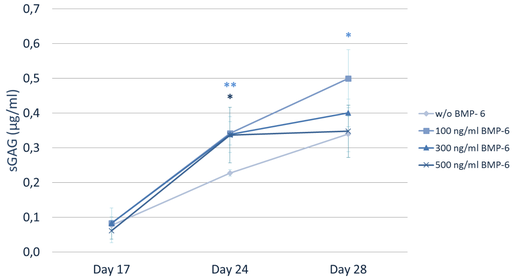

Figure 2: sGAG content of the pellets. The results are shown as mean and SD of three replicates. Statistical analysis was performed between the w/o BMP-6 and treatment groups. * p<0.05 and ** p<0.01 compared to the group w/o BMP-6.

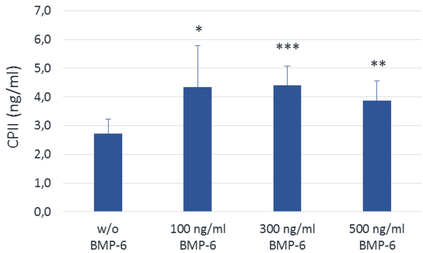

Figure 3: Type II collagen carboxyterminal propeptide (CP-II) released into the culture media at day 17. The results are shown as mean and SD of six replicates. Statistical analysis was performed between the w/o BMP-6 and treatment groups. * p<0.05 and ** p<0.01, ***p<0.001 compared to the group w/o BMP-6.

Summary

- BMP-6 showed concentration-dependent stimulatory effects on chondrogenic differentiation based on Safranin O, Alcian blue and collagen type II histological / immunohistochemical stainings.
- BMP-6 increased sGAG content of the pellets with 100 ng/ml and 300 ng/ml concentrations at day 24, and with 100 ng/ml at day 28.
- BMP-6 increased CP-II release with all concentrations tested at day 17.

Conclusions

These results suggest that the KS483 cell line can be used for setting up an *in vitro* model for studying chondrogenic differentiation and for identifying novel compounds with anabolic effects on chondrogenesis. As these cells can rapidly proliferate, they provide an unlimited source of easily expanded progenitor cells for conducting large number of replicates and studies. The pellets are simple to produce and have experimental conditions that are easy to control.

Acknowledgements

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References

1. Yamashita T, Ishii H, Shimoda K, Sampath TK, Katagiri T, Wada M, Osawa T, Suda T (1996) Subcloning of three osteoblastic cell lines with distinct differentiation phenotypes from the mouse osteoblastic cell line KS-4. Bone 19:429-436.
2. Dang ZC, van Bezooijen RL, Karperien M, Papapoulos SE, Löwik CWGM (2002) Exposure of KS483 cells to estrogen enhances osteogenesis and inhibits adipogenesis. J Bone Miner Res 17:394-404.
3. Fagerlund KM, Rissanen JP, Suutari T, Chan A, Halleen JM (2009) Validation of an in vitro osteoblast culture model using estrogen responsive KS483 mouse osteoblast precursor cell line. J Bone Miner Res 24 (Suppl 1).