

## Reporter-Vector Systems to Monitor Osteogenic Differentiation

G. Feichtinger, B. Kloesch, I. Kehrer, D. Dopler, A. Banerjee, M. van Griensven, H. Redl.

*Ludwig Boltzmann Institute for Experimental and Clinical Traumatology,*

AUVA

*Research Centre, – Austrian Cluster of Tissue Regeneration - Vienna, Austria*

**INTRODUCTION:** BMPs trigger osteoblastic differentiation of capable mammalian cells through TGF- $\beta$ -family signaling pathways. The transcription factor Cbfa-1/Osf2 that is initially activated by this signal transduction cascade drives the expression of osteoblastic marker genes like osteocalcin, osteopontin, bone sialoprotein and collagen I through tissue specific *cis*-acting elements.

The bioactivity of recombinantly expressed BMPs and other osteogenic substances for bone tissue engineering applications needs to be assayed *in vivo* and *in vitro*. The employed standard procedures are the ectopic bone formation assay *in vivo* and the alkaline phosphatase assay *in vitro* and *ex vivo*. The systems presented herein could provide useful alternatives to these procedures.

Our primary aim was to design and evaluate new reporter-vector systems based on Cbfa-1/Osf2 activated promoters *in vitro* concerning their possible application for BMP bioactivity assays. We recently investigated a transiently transfected reporter-vector based on the murine osteocalcin-2 (mOG-2) promoter linked to the open reading frame of the Enhanced Yellow Fluorescent Protein (EYFP) that showed high specificity but weak reporter gene expression upon stimulation with rhBMP-2 in C2C12 murine myoblasts.

Therefore as second aim we evaluated the signal amplification potential of viral enhancers.

The new construct employs a cytomegalovirus enhancer cloned directly upstream of the murine OG2-promoter that drives expression of the reporter gene EYFP.

Additionally we designed a murine collagen I  $\alpha$ 1 reporter-vector. This construct drives osteoblast specific expression of the reporter gene dsRed via collagen I  $\alpha$ 1 - enhancer and promoter elements.

**METHODS:** Both vectors were transiently transfected in C2C12 murine myoblasts and rhBMP-2 induced reporter gene expression was detected by fluorescence microscopy.

Marker gene and reporter gene expression monitoring (e.g. osteocalcin, BSP, Cbfa-1/Osf2, osterix, EYFP, dsRed) by RT-PCR and alkaline phosphatase assays were carried out for a period

of 14 days to prove the osteoblast restricted activity of our reporter-vectors. Furthermore, tissue specific activity of the CMV-enhanced construct was confirmed by transfection of the HEPG2 cell line (hepatocytic origin).

**RESULTS:** C2C12 myoblasts transfected with the CMV-enhanced construct showed strong reporter gene expression upon stimulation with rhBMP-2 (500 ng/ml) and clustering of EYFP positive cells was observed by day 7 and more prominent by day 14.

HEPG2 cells transfected with the same construct exhibited weak background expression of the reporter gene confirming its tissue-specificity. The collagen I  $\alpha$ 1 reporter vector is currently under investigation.

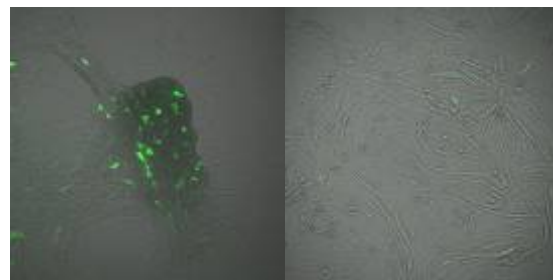


Fig. 1: CMV-enhanced mOG2P reporter signal in C2C12 cells: rhBMP2 treated (left) vs. untreated control (right).

**DISCUSSION & CONCLUSIONS:** We conclude from our *in vitro* results concerning the CMV-enhanced mOG2-vector that viral enhancers amplify reporter gene expression without altering the behavior of the employed tissue specific promoters.

The newly developed CMV-enhanced system allows the real time monitoring of osteoblastic differentiation *in vitro* by fluorescence microscopy therefore providing a sensitive bioactivity assay for osteogenic substances in C2C12 cells.

**ACKNOWLEDGEMENTS:** This work was supported by the European projects Hippocrates (NMP3-CT-2003-505758) and Expertissues (NMP-CT-2004-500283).