ACTIVATION OF β-CATENIN SIGNALLING ENHANCES THE OSTEOGENIC GENE RESPONSE TO MECHANICAL LOADING IN MESENCHYMAL STEM CELLS

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Introduction

Wnt/β-catenin signalling and mechanical loading are able to inhibit adipogenesis and to stimulate osteoblastogenesis of mesenchymal stem cells [Christodoulides, 2009; Case and Rubin, 2010]. The involvement of β-catenin signaling in mechanically induced bone formation has already been shown *in vivo* using a tibia loading model [Robinson, 2006]. The aim of the present study was to investigate the influence of the activation of β-catenin on the osteogenic and adipogenic response of mesenchymal stem cells to mechanical loading in vitro.

Methods

C3H10T1/2 cells were cultivated in adipogenic medium. SB415286 was added for activating β-catenin signalling. Cells were loaded by daily homogenous cyclic stretching for 5 days. Real Time RT-PCR and Western blotting were performed for expression analysis. Three independent experiments in duplicate (n=6) were performed. Data were analyzed for significance (value $p \leq 0.05$) using Student's *t*-test.

Results

Mechanical loading and the β-catenin signalling activator SB415286 significantly upregulated the relative gene expression of the osteogenic markers Runx2, Ptgs2, and Cyr61, as well as the expression of Wnt10b (Tab. 1). Mechanical loading and SB415286 downregulated the adipogenic markers Cebpa and Pparg. Mechanical loading in addition to SB415286 treatment enhanced the mechanically induced expression of Runx2, Ptgs2, Cyr61, Wnt10b, and reduced expression of Pparg and Cebpa. Real-time RT-PCR results were verified by Western blotting (Fig. 1).

Gene of interest	x-fold change of relative gene expression after cell stimulation		
	S	SB	S + SB
Runx2	1.7* ± 0.35	2.6* ± 0.16	2.8* ± 0.47
Ptgs2	5.3* ± 3.78	5.7* ± 1.30	13.3* ± 3.91
Cyr61	5.1* ± 3.11	6.6* ± 1.20	17.8* ± 5.18
Wnt10b	1.3* ± 0.31	2.7* ± 0.12	3.1* ± 0.25
Pparg	0.9* ± 0.14	0.6* ± 0,11	0.6* ± 0.07
Cebpa	0.8* ± 0.31	0.6* ± 0.23	0.3* ± 0.06

Table 1: Expression of osteogenic and adipogenic genes. ($p \leq 0.05$ compared to untreated control cells)*

Figure 1: Expression of osteogenic and adipogenic proteins

Discussion

SB415286 and mechanical loading led to an increase of osteogenic marker expression and to a reduction of adipogenic marker expression. SB415286 provoked a sensitizing effect on the mechanically induced osteogenic gene expression as well as on the mechanically reduced adipogenic gene expression. Interestingly, the expression of Wnt10b, which is known as an inhibitor of adipogenesis and a stimulator of osteoblastogenesis [Christodoulides, 2009], was upregulated by SB415286 and mechanical loading. Sensitizing mechanosensitive pathways, which contribute to the enhancement of osteogenesis and simultaneous impairment of adipogenesis, might represent a therapeutic target for osteoanabolic therapy in patients with osteoporosis.

References

Case and Rubin, J Cell Biochem 110 (2010) 545-553

Christodoulides et al, Trends Endocrinol Metab 20 (2009) 16-24

Robinson et al, J Biol Chem 281 (2006) 31720-31728