**Sclerostin Induction in Human Osteoblasts by the Proinflammatory Cytokines TWEAK and TNF-alpha**

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**INTRODUCTION**

We recently showed that a member of the TNF superfamily, TNF-like weak inducer of apoptosis (TWEAK), is a novel mediator in a mouse model of inflammatory bone destruction.\(^1\) We sought to investigate a role for TWEAK in human osteoblast biology, and how TWEAK might interact with TNF in this context. The canonical Wnt-signalling pathway regulates osteoblast differentiation and bone formation. Wnt ligands act via frizzled (Fzd) and LRP5/6 co-receptors on target cells, resulting in the downstream transcription of osteogenesis-related genes. Several inhibitors of the Wnt pathway have been identified, including Dkkopf-1 (DKK-1), secreted Frizzled-related protein (sFRP) and sclerostin. Sclerostin is the product of the SOST gene, mutations in which cause diseases with high bone mass, indicating that this molecule has a key role in the regulation of bone formation. Negative regulators of Sclerostin expression include anabolic stimuli such as bone loading and PTH treatment. Chronic exposure to inflammatory stimuli can reduce bone formation. The aim of these experiments was to investigate the effect of TWEAK and TNF on Sclerostin expression in human osteoblasts.

**METHODS**

Adult Human Osteoblasts (HO) were isolated from femoral neck trabecular bone and maintained in αMEM medium containing 10% FCS (αMEM-10). Experiments were performed using cells at or before passage 3. Cells were treated with TWEAK or TNF-alpha and analysed for proliferation using Carboxyfluorescein diacetate succinimidyl ester (CFSE) cell labeling and Fluorescence Activated Cell Sorting (FACS) analysis, for gene expression using RT-PCR, and for mineralization by measurement of cell layer-associated calcium. Student’s t-Test was used to analyse differences in mineralisation and cell proliferation experiments. One way analysis of variance (ANOVA) followed by Tukey’s post-hoc analysis was used to examine differences in gene expression studies. A p value < 0.05 was considered to be significant.

**RESULTS**

Human Osteoblasts were found to express TWEAK at both the mRNA and protein level, the latter as revealed by cell immunofluorescence. In addition, the TWEAK receptor, Fn14, was abundant in HO, as assessed by FACS. Both TWEAK (Figure 1) and TNF were mitogenic for HO, and evidence was obtained that this was of the osteoblast differentiation inhibitor, sclerostin. In later cultures, TWEAK might interact with TNF in this context. The canonical Wnt signalling pathway regulates osteoblast differentiation and bone formation. Wnt ligands act via frizzled (Fzd) and LRP5/6 co-receptors on target cells, resulting in the downstream transcription of osteogenesis-related genes. Several inhibitors of the Wnt pathway have been identified, including Dkkopf-1 (DKK-1), secreted Frizzled-related protein (sFRP) and sclerostin. Sclerostin is the product of the SOST gene, mutations in which cause diseases with high bone mass, indicating that this molecule has a key role in the regulation of bone formation. Negative regulators of Sclerostin expression include anabolic stimuli such as bone loading and PTH treatment. Chronic exposure to inflammatory stimuli can reduce bone formation. The aim of these experiments was to investigate the effect of TWEAK and TNF on Sclerostin expression in human osteoblasts.

**TWEAK and TWEAK/TNF effects on RUNX2 and osteocalcin expression.**

**DISCUSSION**

These results suggest that TWEAK, alone and in conjunction with TNF, acts in part by promoting sclerostin expression, which in turn regulates the expression of key osteoblast transcription factors. The results further suggest that the persistent presence of TWEAK may be both catabolic and anti-anabolic, and that TWEAK and TNF need to be considered together in the aetiology of inflammatory bone remodelling.

**Fig 1.** Cell Proliferation: HO were labeled with CFSE and then remained untreated or were treated for 7 days with 100ng/ml TWEAK before analysis of proliferation, in terms of the number of cell doublings.

**Fig 2.** Gene expression of Sclerostin in response to TWEAK and TNF-alpha: HO were treated for up to 20 days with TWEAK (50 ng/ml) or TNF-alpha (1 ng/ml). RNA was extracted and analysed for steady state levels of sclerostin mRNA at the times indicated, as a function of the housekeeping gene, GAPDH.

**REFERENCES**