BMP activity is required for TGF beta-stimulated periosteal osteogenesis

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INTRODUCTION: Periosteum covers the outer surfaces of bones. The inner cambium layer of periosteum is populated by committed osteoprogenitors. Periosteal osteoprogenitors are the major cellular contributors to appositional bone growth and bone repair by callus formation [1]. Murine deletion models have emphasised the importance of BMP-2 in postnatal periosteal appositional bone growth and fracture repair by callus formation [2]. Accepting this, our previous work has shown that, in vivo, osteogenically active periosteum is distinguished from quiescent periosteum by increased TGF-β ligand and receptor expression, not BMP signalling factors, and that periosteal-derived cells have little or no osteogenic activity under standard in vitro osteogenic culture conditions. This study was conducted to test the hypothesis that TGF beta-stimulated periosteal osteogenesis requires BMP signalling activity.

METHODS: This study was completed with IACUC approval. Periosteum was collected from the craniomedial tibial and dorsal metacarpal bone surfaces of eight healthy adult horses. Periosteal cells were isolated by collagenase digestion, expanded through two monolayer passages in DMEM/10% FBS (control medium). P3 cells were maintained in control medium or were exposed to 10 ng/ml TGF-β3 for 72 hours. After 72 hours, cells were transferred to osteogenic medium (ascorbic acid, dexamethasone and b-glycerophosphate supplementation). Changes in osteogenic gene expression (Runx2, OSX and ALP) and putative osteogenic growth factor expression (BMPs 2, 4, 6 and 7, TGF betas 1, 2 and 3) were assessed by qPCR. Osteogenic status was assessed by Alizarin Red staining for mineralized matrix, and by ALP enzymatic activity. To determine whether TGF-β-stimulated periosteal osteogenesis requires intrinsic BMP activity, TGF-β-pre-treated osteogenic periosteal cultures were co-treated with BMP receptor kinase inhibitors, DMH1, LDH 212854 or K 02288, at low or high concentrations that spanned the reported IC_{50} values.

RESULTS: Pre-exposure to TGF-β 3 for 72 hours induced prominent alizarin red staining of mineralized matrix (Fig 1; left panels) and significantly up-regulated Osterix and ALP mRNA expression and ALP activity (Fig 1; right panels) under subsequent osteogenic conditions, consistent with previous RNA seq data. Periosteal cells exposed to TGF-β3 significantly up-regulated expression of BMP-2 (five-fold), BMP-7 (12-fold) and TGF-β3 (5-fold), in the context of osteogenic activation. Co-administration of BMP receptor kinase inhibitors after TGF-β pre-exposure suppressed Runx-2, OSX and ALP mRNA expression [Fig 2; upper panels] and also inhibited cell aggregation, matrix mineralization [Fig 2; lower panels] and ALP enzymatic induction.

DISCUSSION: These findings corroborate the outcomes of our previous RNAseq-derived data and indicate that TGF-beta signaling is a potent osteogenic stimulus for periosteal cells. The impact of BMP receptor kinase inhibition indicates that the up-regulation of BMPs-2 and -7 are critical for the downstream effects of TGF beta stimulation, or that a critical level of BMP signaling activity is necessary for periosteal osteogenesis independent of any other stimulus.

SIGNIFICANCE/CLINICAL RELEVANCE: The outcomes of this study reconcile the documented importance of BMP-2 activity in periosteal-derived bone formation with our own preliminary data that emphasizes the importance of TGF beta signaling in periosteal osteogenesis. These outcomes support the clinical development of TGF beta signaling factor technologies for the stimulation of bone repair.

REFERENCES: 1. Colnot C (2009) Skeletal cell fate decisions within periosteum and bone marrow during bone regeneration. J Bone Miner Res 24:274–82. 2. Tsuji K et al (2006). BMP2 activity, although dispensable for bone formation, is required for the initiation of fracture healing. Nat Genet 38:1424–9.

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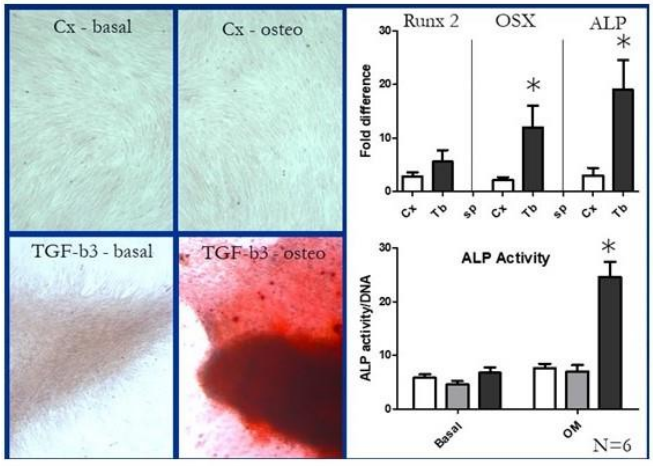

Figure 1. After 72 hours' pre-exposure to basal medium (Cx) or medium with TGF beta 3, P3 periosteal cells were transferred to osteogenic medium for 7 days. Pre-exposure to TGF beta 3 increased matrix mineralization (left panels), OSX and ALP expression and ALP activity, compared to control cultures or cells pre-treated with BMP-2.

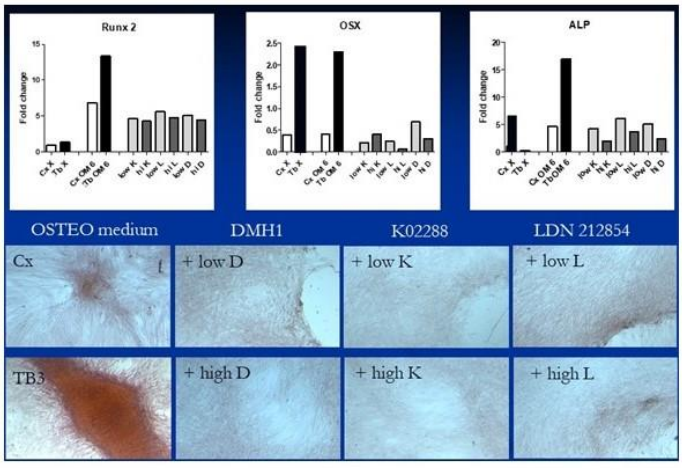

Figure 2. After 72 hours' pre-exposure to basal medium (Cx) or medium with TGF beta 3 (Tb), P3 periosteal cells were transferred to osteogenic medium for 7 days -/+ BMP inhibitors DMH 1 (D), K02288 (K) or LDH212854 (L) in osteogenic at low or high concentrations. BMP inhibitors suppressed OSX and ALP expression, cell aggregation and matrix mineralization.