TGF-BETAL CALCIUM SIGNALING INCREASES ALPHA 5 INTEGRIN EXPRESSION AND CELL PROLIFERATION IN HUMAN OSTEOBLASTS.

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Introduction: TGF-β1 is a potent osteoactive factor that dramatically affects osteoblast proliferation, differentiation, and maturation. These events are crucial for bone formation, repair, and maintenance and serve as the underlying processes that ultimately influence fracture repair, remodeling, implant fixation, and metabolic bone diseases. The direct effect of TGF-β1 on osteoblast activity has been well documented; however, little is known about the mechanisms through which TGF-β1 exerts these effects. Recently, we have described a novel TGF-β1 Ca2+ signaling pathway in primary human osteoblasts (HOB) and have demonstrated its importance in osteoblast adhesion. Here, we further analyze the role of the TGF-β1 Ca2+ signal in osteoblast protein synthesis and cell proliferation, events that are crucial for proper bone function.

Methods: Cell Preparation: Cultured HOB were isolated using the protocol of Robey and Termine.1,2 HOB were grown in medium containing 1% FBS 48 h prior to treatment. [Ca2+]: Measurement: TGF-β1 induced [Ca2+]i response was measured in Fura-2 loaded HOB (10 μM, 20min, 37°C) by fibronectin-5 integrin expression and distribution in HOB. We also examined TGF-β1 effect on osteoblast proliferation and its mechanism of action. TGF-β1 treatment resulted in a significant increase in osteoblast proliferation at 24 h, an effect that is inhibited in the presence of nifedipine, or a MAPK inhibitor, PD98059 (Fig. 4A). TGF-β1 stimulation of osteoblasts resulted in rapid and transient phosphorylation of ERK (7.5 min) that returned to baseline levels within 15 min after stimulation. Nifedipine blockade of the TGF-β1 Ca2+ signal prevented ERK stimulation (Fig. 4B).

Results and Discussion: The mechanism of TGF-β1 signaling is thought to be mediated by receptor associated Smad proteins. We have recently characterized a TGF-β1 induced Ca2+ signal in osteoblasts and have demonstrated its importance in cell adhesion and independence from Smad signaling (1). Osteoblast interaction with the extracellular matrix protein fibronectin by α5β1 integrin on the cell surface is principally responsible for osteoblast substrate adhesion(6). Our results show that the TGF-β1 induced intracellular Ca2+ signal is responsible, in part, for the stimulation of α5 integrin expression, but not β1 integrin or fibronectin expression in HOB (Figs. 1 & 2). Increased α5 integrin protein and mRNA expression was seen as early as 12 h after TGF-β1 treatment, an effect abrogated by cotreatment of cells with nifedipine, a L-type Ca2+ channel blocker.

These results indicate that TGF-β1 stimulation of osteoblast cell proliferation requires Ca2+ signaling and activation of the MAPK pathway. Taken together, our studies suggest that the TGF-β1 intracellular Ca2+ signal is necessary for events leading to proliferation and differentiation of HOB.


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Fig. 1: WB (A) and temporal expression (B) of α5 integrin.

Fig. 2: WB of β1 integrin (A) and fibronectin (B) over 48 h.

Fig. 3: CLSM of HOB labeled for α5 integrin (A) and actin (B). Combined images (C) demonstrate colocalization (arrows).

Fig. 4: The TGF-β1 Ca2+ signal is important for HOB proliferation (A) and MAPK activation (B).