Interaction of IGFBP6 with LMP-1 may define a novel intracellular mechanism for regulation of osteoblast differentiation

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Introduction: Insulin-like growth factor binding protein (BP6) is a member of a family of six IGFBPs that are widely expressed and affect cell growth, survival and differentiation (1). Effects of BP6 in a number of in vitro systems have been inhibitory for differentiation including myoblasts and osteoblasts (2). Likewise, we have previously found that increased BP6 expression reduces ALP activity in osteoblast-like cells (3). Using a yeast-2-hybrid screen and coimmunoprecipitation, we identified LMP-1 as a binding partner of BP6 and determined that BP6 bound to the C-terminal of LMP-1. LIM mineralization protein-1 (LMP-1) is an intracellular protein that is a critical positive regulator of osteoblast differentiation (4), and we previously demonstrated that gene therapy with LMP-1 enhances fracture repair (5). The exact mechanism by which BP6 affects differentiation has not yet been elucidated. We hypothesized that the interaction of LMP-1 and BP6 may define a novel intracellular mechanism for regulation of osteoblast differentiation.

To study this hypothesis we studied 1) the effect of BP6 and LMP-1 on MC3T3-E1 osteoblast differentiation, 2) the intracellular localization of BP6 and LMP-1, and 3) the effect of BP6 and LMP-1 on human collagen transcription.

Materials and Methods: Primary human osteoblasts and ROS 17/2.8 cells were maintained in DMEM 10% CS. MC3T3-E1 cells were maintained in αMEM 10% FBS 1% P/S. To induce differentiation of MC3T3-E1 osteoblasts, 10mM βGP and 50μg/ml AA-P were added to the media.

A murine leukemia virus (MLV)-based retroviral vector was used to target the expression of the human LMP-1, hBP6 or GFP control transgenes in cultured osteoblasts. Additionally, LMP-1 was 5' tagged with influenza hemaglutinin (HA) to facilitate identification. Effects of increased LMP-1, hBP6 and control transgene expression on osteoblast mineralization in vitro were evaluated after 14 or 21 days of culture in osteogenic media by alizarin red staining and expression of osteocalcin and ostein. Western blots confirmed expression of LMP-1, BP6, or control transgene.

Plasmid expression vectors were prepared that produced BP6-RFP and GFP-LMP-1 fusion protein constructs, including GFP-based constructs containing each of the LIM domains of LMP-1. Indirect immunofluorescence was also used to determine subcellular localization of LMP-1 and BP6. Fluorescent microscopy and data capture were used to identify subcellular localization of constructs 48h after transient transfection.

For promoter analysis, effectene (Qiagen) was used to transfect cells with the collagen promoter (hCol1n2-pGL3basic) and/or VR1012 based LMP-1 and BP6 expression constructs. Cells were extracted for luciferase and protein assays 48h after transfection. Significance was evaluated by the Student’s two tailed t-test.

Discussion: First, we found that BP6 and LMP-1 have opposite effects on osteoblast differentiation with BP6 being inhibitor and LMP-1 being stimulatory. Secondly, we found that the binding of LMP-1 and BP6 altered the subcellular location of LMP-1 and that the relocation of LMP-1 to the nucleus required the second and third LIM domains. The movement of LMP-1 from the cytoplasm into the nucleus may play a role in the effects of BP6 on osteoblast differentiation. Finally, we found that LMP-1 and BP6 have opposing effects on the collagen promoter activity and that LMP-1 can abrogate the suppression of hCol1n2 promoter activity by BP6 when they were co-expressed. To our knowledge this is the first time that the alteration of subcellular location of LMP-1 by BP6 as well as the opposing effects of BP6 and LMP-1 on collagen transcription has been described. Taken together, these data imply that the physical interaction of LMP-1 and BP6 may be physiologically relevant and define an important intracellular mechanism for regulation of osteoblast differentiation.

References: