Mol Cell Biochem (2014) 385:145–157
DOI 10.1007/s11010-013-1823-3

Characterization of a unique motif in LIM mineralization protein-1 that interacts with jun activation-domain-binding protein 1

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Received: 25 June 2013 / Accepted: 13 September 2013 / Published online: 29 September 2013

Abstract Development and repair of the skeletal system and other organs are highly dependent on precise regulation of the bone morphogenetic protein (BMP) pathway. The use of BMPs clinically to induce bone formation has been limited in part by the requirement of much higher doses of recombinant proteins in primates than were needed in cell culture or rodents. Therefore, increasing cellular responsiveness to BMPs has become our focus. We determined that an osteogenic LIM mineralization protein, LMP-1 interacts with Smurf1 (Smad ubiquitin regulatory factor 1) and prevents ubiquitination of Smads resulting in potentiation of BMP activity. In the region of LMP-1 responsible for bone formation, there is a motif that directly interacts with the Smurf1 WW2 domain and thus effectively competes for binding with Smad1 and Smad5, key signaling proteins in the BMP pathway. Here we show that the same region also contains a motif that interacts with Jun activation-domain-binding protein 1 (Jab1) which targets a common Smad, Smad4, shared by both the BMP and transforming growth factor-β (TGF-β) pathways, for proteasomal degradation. Jab1 was first identified as a coactivator of the transcription factor c-Jun. Jab1 binds to Smad4, Smad5, and Smad7, key intracellular signaling molecules of the TGF-β superfamily, and causes ubiquitination and/or degradation of these Smads. We confirmed a direct interaction of Jab1 with LMP-1 using recombinantly expressed wild-type and mutant proteins in slot-blot-binding assays. We hypothesized that LMP-1 binding to Jab1 prevents the binding and subsequent degradation of these Smads causing increased accumulation of osteogenic Smads in cells. We identified a sequence motif in LMP-1 that was predicted to interact with Jab1 based on the MAME/MAST sequence analysis of several cellular signaling molecules that are known to interact with Jab-1. We further mutated the potential key interacting residues in LMP-1 and showed loss of binding to Jab1 in binding assays in vitro. The activities of various wild-type and mutant LMP-1 proteins were evaluated using a BMP-responsive luciferase reporter and alkaline phosphatase assay in mouse myoblastic cells that were differentiated toward the osteoblastic phenotype. Finally, to strengthen physiological relevance of LMP-1 and Jab1 interaction, we showed that overexpression of LMP-1 caused nuclear accumulation of Smad4 upon BMP treatment which is reflective of increased Smad signaling in cells.

Keywords BMP-2 · Smad · Smurf1 · Jab1

Abbreviations

BMP	Bone morphogenetic protein
Jab1	Jun activation domain-binding protein 1
RT-PCR	Reverse transcriptase polymerase chain reaction
ALP	Alkaline phosphatase
RUL	Relative units of luciferase
FBS	Fetal bovine serum
hMSCs	Human mesenchymal stem cells
ECL	Enhanced chemiluminescence

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