

# UBIQUITIN LIGASE CBL-B DOWNREGULATES BONE FORMATION THROUGH SUPPRESSION OF IGF-1 SIGNALING IN OSTEOBLASTS DURING DENERVATION

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## INTRODUCTION

Unloading, such as a long-term bed rest, cast-immobilization and weightlessness, suppresses bone formation by osteoblasts, leading to osteopenia(1). The resistance of osteoblasts to growth factors contributes to such an unloading-mediated osteopenia(1, 2). Therefore, it is important to elucidate the molecular mechanism of the resistance of osteoblasts to growth factors during unloading. In a separate study, we found that in unloading conditions, a RING-type ubiquitin ligase Cbl-b ubiquitinated and degraded insulin receptor substrate-1 (IRS-1) in skeletal muscle cells, resulting in the resistance to insulin-like growth factor-1 (IGF-1)(3). In this study, we reasoned that Cbl-b also played an important role in the unloading-mediated resistance of osteoblasts to growth factors, especially IGF-1.

## MATERIALS AND METHODS

6-week-old Cbl-b-deficient (Cbl-b<sup>-/-</sup>) or wild-type mice were subjected to sciatic neurectomy. Bone formation of these mice was assessed by calcein double labeling and histomorphometric analyses of hindlimb bones. We examined the amounts of the IGF-1 signaling molecules in femur of these mice by RT-PCR, Western blotting and immunohistochemical analyses. We also examined the response of Cbl-b-overexpressing or -deficient osteoblastic cells to IGF-1.

## RESULTS

Sciatic neurectomy increased the amount of Cbl-b protein about twice in osteoprogenitor cells from hindlimb bones and the number of Cbl-b-positive osteoblastic cells in metaphysis of femur.

In wild-type mice, the denervation decreased femur wet weight and bone formation indicated by calcein double labeling. In contrast, in Cbl-b<sup>-/-</sup> mice, the bone wet weight and bone formation were sustained during denervation.

Expression of bone formation marker genes (alkaline phosphatase, collagen type I and osteocalcin) in femur of wild-type mice was significantly suppressed by denervation to 30, 20 and 60%, respectively, of undenervated control whereas deficiency of Cbl-b gene cancelled the decline.

Furthermore, denervation decreased the number of IRS-1-containing cells in metaphysis and the amounts of IGF-1 signaling molecules, such as phosphatidylinositol 3-phosphate kinase (PI3K) and Akt, in femur of wild-type mice, although their mRNA levels were constant. Interestingly, denervation failed to decrease the amounts of these signaling molecules in femur of Cbl-b<sup>-/-</sup> mice.

On a cellular level, primary osteoblastic cells from Cbl-b<sup>-/-</sup> mice were more sensitive to IGF-1 treatment than those from wild-type mice. The proliferation rate of Cbl-b<sup>-/-</sup> osteoblastic cells after IGF-1 treatment was enhanced about twice, compared with that of wild-type cells. In Cbl-b-overexpressing osteoblastic cells, IGF-1 treatment induced binding of Cbl-b to the IGF-1 signaling molecules (IRS-1, PI3K and Akt) and ubiquitination of IRS-1 and PI3K. Furthermore, IGF-1 treatment led to only loss of IRS-1 protein among these ubiquitinated proteins in Cbl-b-overexpressing osteoblastic cells.

## DISCUSSION

Activation of the IGF receptor I stimulates two distinct pathways in osteoblasts: the Ras/mitogen-activated protein kinase (MAPK) pathway and the IRS-1/PI3K/Akt pathway. Both pathways promote proliferation of osteoblasts, although the latter has a critical role in regulating their apoptosis(4). To conquer bone atrophic diseases, finding a target gene relating these pathways is important. In skeletal muscle of rats subjected to spaceflight or tail-suspension, we found that highly expressed Cbl-b preferentially ubiquitinated and degraded IRS-1, leading to the resistance of skeletal muscle cells to IGF-1(3). The results of this study suggest that Cbl-b also induces resistance of osteoblasts to IGF-1, at least in part, by suppressing IRS-1/PI3K/Akt pathway, resulting in decreased bone formation of osteoblastic cells in these diseases.

Based on these finding, Cbl-b is one of the key proteins for downregulating IGF-1-mediated bone formation as well as muscle protein synthesis during unloading conditions. Targeting Cbl-b may be a more efficient therapeutic option for attenuating bone and muscle atrophy during unloading.

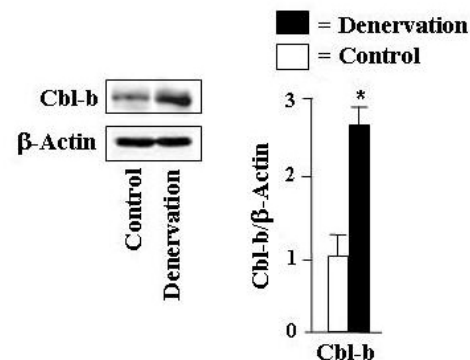


FIGURE 1. Expression of Cbl-b protein in bone marrow osteoprogenitor cells.

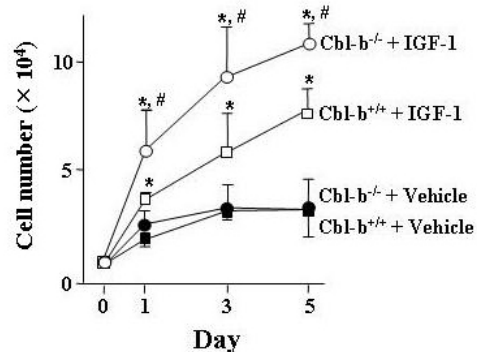


FIGURE 2. Response of Cbl-b-deficient osteoblastic cells to IGF-1 stimulation.

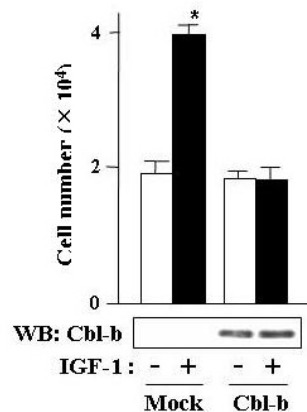


FIGURE 3. Response of Cbl-b-overexpressing osteoblastic cells to IGF-1 stimulation.

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