ABSTRACT

Transforming growth factor-beta1 (TGF-β1) is a most potent and abundant growth factor, which is highly present in skeletal tissues and its plays a key role in the development of bone. Matrix metalloproteinase-13 (MMP13) or collagenase-3 involves the degradation of extracellular matrix (ECM) and plays a crucial role in bone remodeling. A defect in this gene leads to the development of skeletal dysplasia. Runx2 is a bone transcription factor involved in osteogenesis process. We previously reported that TGF-β1 stimulates MMP13 expression in osteoblastic cells, and this effect was mediated by Runx2. We also showed that there is no change in the expression of Runx2 RNA or protein, but Runx2 protein undergoes phosphorylation, in osteoblastic cells in response to TGF-β1-treatment. Thus, Runx2 is subjected to covalent modifications that might affect its activity. Several studies reported that Runx2 undergoes various modifications such as phosphorylation, acetylation, and sumoylation. In addition, MAP kinases have been shown to phosphorylate Runx2, and ERK-mediated phosphorylation was shown to maintain the stability of Runx2 in osteoblastic cells.

In this study, we dissected and identified the signaling pathways responsible for TGF-β1-induced phosphorylation of Runx2 in osteoblastic cells. TGF-β1 stimulated the phosphorylation of Runx2 at serine amino acids, and ERK inhibition blocked this effect in rat (UMR106-01) and human (MG-63) osteoblastic cells. Pretreatment with okadaic acid, a serine-threonine phosphatase inhibitor, increased Runx2 serine phosphorylation in osteoblastic cells. When cells were pretreated with an ERK inhibitor, TGF-β1-mediated stimulation of MMP13 mRNA expression decreased. Nano-ESI/LC/MS analysis identified that TGF-β1 stimulates Runx2 phosphorylation at three serine amino acids. Transient transfection of mouse mesenchymal stem cells (C3H10T1/2) with Runx2 serine mutant constructs decreased TGF-β1-mediated Runx2 serine phosphorylation. A luciferase reporter
assay identified that TGF-β1 stimulated MMP13 promoter activity as well as Runx2 activity in these cells only in the presence of the wild Runx2 construct, and not with mutant Runx2. Thus, TGF-β1 stimulates the phosphorylation of Runx2 at three serine amino acids, and this event is required for MMP13 expression in osteoblastic cells. Hence, this study contributes to the knowledge of events governing bone remodeling and bone-related diseases.