Smads, p38 and ERK1/2 are involved in BMP9-induced osteogenic differentiation of C3H10T1/2 mesenchymal stem cells

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INTRODUCTION

Mesenchymal stem cells (MSCs) are non-hematopoietic stem cells capable of differentiating into osteoblastic, chondrogenic, myogenic, or adipogenic lineages (1). Bone morphogenetic proteins (BMPs), members of transforming growth factors β (TGFβ) superfamily, are believed to perform pivotal functions in the areas of stem cell self-renew and differentiation during development (2, 3). Mesenchymal stem cells (MSCs) are non-hematopoietic stem cells capable of differentiating into osteoblastic, chondrogenic, myogenic, or adipogenic lineages (1). Bone morphogenetic proteins (BMPs), members of transforming growth factors β (TGFβ) superfamily, are believed to perform pivotal functions in the areas of stem cell self-renew and differentiation during development (2, 3).

Although previous studies have demonstrated that BMP9 is highly capable of inducing osteogenic differentiation of mesenchymal stem cells, the molecular mechanism involved remains to be fully elucidated. In this study, we showed that BMP9 simultaneously promotes the activation of Smad1/5/8, p38 and ERK1/2 in C3H10T1/2 cells. Knockdown of Smad4 with RNA interference reduced nuclear translocation of Smad1/5/8, and disrupted BMP9-induced osteogenic differentiation. BMP9-induced osteogenic differentiation was blocked by p38 inhibitor SB203580, whereas enhanced by ERK1/2 inhibitor PD98059. SB203580 decreased BMP9-activated Smads singling, and yet PD98059 stimulated Smads singling in C3H10T1/2 cells. The effects of inhibitor were reproduced with adenovirus expressing siRNA targeted p38 and ERK1/2, respectively. Taken together, our findings revealed that Smads, p38 and ERK1/2 are involved in BMP9-induced osteogenic differentiation. Also, it is noteworthy that p38 and ERK1/2 may play opposing regulatory roles in mediating BMP9-induced osteogenic differentiation of C3H10T1/2 cells. [BMB reports 2012; 45(4): 247-252]

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act in opposition to regulate BMP9-osteogenic differentiation partly through influence on Smads signaling cascade.

RESULTS

BMP9 promoted activation of transcription factors Smad1/5/8, p38 and ERK1/2 MAPKs in C3H10T1/2 mesenchymal stem cells

In this present study, exogenous BMP9 was introduced into C3H10T1/2 cells using recombinant adenovirus assay. ALP activity and calcium deposition were selected as early osteogenic and late osteogenic marker, respectively. Consisted with our previous reports (7-9), BMP9 was found to intensively increase ALP activity and calcium deposition of C3H10T1/2 cells (Fig. 1A, B). These results re-confirmed that BMP9 was capable of promoting osteogenic differentiation of MSCs.

Next, after BMP9-treatment, the levels of phosphorylated/activated Smads, p38 and ERK1/2 were detected by western blotting. As illustrated in Fig. 1C, BMP9 simultaneously stimulated the phosphorylation/activation of transcription factors Smad1/5/8, p38 and ERK1/2 MAPKs, without affecting the total amounts of these proteins. Our recently reports have demonstrated that dominant negative (dn) mutant of TGFβ receptors ALK1, ALK2, BMPRII and ActRII, which lack the kinase domain, remarkably inhibited osteoinductive activity and signal transduction of BMP9 (8, 9). Here, when exogenous dn-ALK1, dn-ALK2, dn-BMPRII and dn-ActRII were introduced into C3H10T1/2 cells in conjunction with BMP9 (Fig. 1D), a significant reduction in the levels of phosphorylated Smad1/5/8, p38 and ERK1/2 was observed (Fig. 1E). These findings indicated that BMP9 was capable of effectively activating Smad1/5/8, p38 and ERK1/2 in C3H10T1/2 cells.

Smads signaling was required for BMP9-induced osteogenic differentiation of C3H10T1/2 mesenchymal stem cells

It has been well demonstrated that phosphorylated/activated Smad1/5/8 form a complex with Smad4, which translocates to the nucleus to regulate target gene transcription. To ascertain whether this signaling pathway is required for BMP9-induced osteogenic differentiation of C3H10T1/2 cells, Smad4 expression was dis...