OSTEOGENIC DIFFERENTIATION OF MESENCHYMAL STEM CELLS INDUCED BY BONE MORPHOGENETIC PROTEIN-4: CROSS-TALK AMONG DIFFERENT SIGNALING PATHWAYS

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Introduction: Bone morphogenetic protein-4 (BMP-4) possesses osteoinductive activities and is known to induce osteogenic differentiation in mesenchymal stem cells (MSCs)\(^1\). It has been reported that BMP signaling is associated with the PTEN/PI3 Kinase/AKT signaling pathway which subsequently alters the Wnt signaling pathway, an important regulator which plays multiple functions in stem cell maintenance, proliferation and cell fate determination\(^2\). Our purpose in this study is to investigate whether BMP-4 alters the PTEN/PI3 Kinase/AKT signaling when MSCs undergo osteogenic differentiation by it. We also investigate whether nuclear β-catenin translocation, which is downstream of PTEN/PI3 Kinase/AKT signaling, is associated with osteogenic differentiation of MSCs induced by BMP-4.

Materials and Methods: Human bone marrow MSCs were isolated according to a method described previously\(^4\). To induce osteogenic differentiation, MSCs were pretreated with starvation medium which consists of IMDM supplemented with 20ng/ml bFGF and 20ng/ml EGF for 3 days and then treated with IMDM and human recombinant BMP-4 (20ng/ml) for 3 weeks with medium changes twice weekly. Osteogenesis was assessed by alkaline phosphatase (ALP) activity assay normalized by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) assay and osteogenic gene expression was assessed by reverse transcription-polymerase chain reaction (RT-PCR) at weekly intervals. The early effects of exogenous BMP-4 in the osteogenic induction were assessed after 24, 60 and 96 hours of treatment by detecting the expression of PTEN and phosphorylated AKT with western blot analysis. Immunofluorescent staining was also performed to assess the levels of β-catenin after BMP-4 treatment. MSCs treated with osteogenic medium, which consists of IMDM supplemented with 0.1 M dexamethasone (Sigma-Aldrich, St Louis, MO), 10 mM β-glycerophosphate (Sigma-Aldrich) and 0.2 mM ascorbic acid (AsA; Sigma-Aldrich) was used for comparison.

Results: Under BMP-4 and osteogenic medium conditions, morphological changes were observed after 7 days treatment. MSCs became flat and had a larger nucleus contrasting with the starvation group (Figure 1). Osteogenic gene expression showed an increased level of type I collagen after BMP-4 treatment (Figure 2), and ALP activity assay of the MSCs showed a significant increase in BMP-4 group (Figure 3). Western blot of PTEN and phosphorylated AKT showed that BMP-4 treatment didn’t make significant changes of PTEN levels in MSCs. However, phosphorylated AKT was decreased after 96 hours BMP-4 treatment (Figure 4). Immunofluorescent staining showed decreased levels of β-catenin after 96 hours BMP-4 treatment (Figure 5).

Discussion: Recent studies have suggested that BMP signaling is related to Wnt signaling through PTEN/PI3 Kinase/AKT signaling. It has been reported that BMP-2 decreased PTEN degradation and increases PTEN levels\(^2\). According to our results, during the osteogenic differentiation induced by BMP-4, PTEN expression in MSCs did not significantly change. However, p-AKT was decreased under BMP-4 treatment at 96 hours. Immunofluorescent staining also showed a decreased level of β-catenin in MSCs after BMP-4 treatment. The decreased levels of p-AKT and β-catenin by BMP-4 suggest that BMP-4 may regulate AKT and Wnt signaling without the involvement of PTEN. Further work is required to elucidate the more detailed signaling pathways between BMP-4 and Wnt signaling.

References