論 文 要 旨

Hif-1α Expression is essential for BMP9-mediated Osteoblast Differentiation through the induction of a Glycolytic Enzyme, PDK1

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OBJECTIVE. Tissue engineering of bone has received much interest, as bone is one of the tissues with the highest regenerative potential in human body. Bone healing or regeneration involves an intricate network of molecules including bone morphogenetic proteins (BMPs). Recent reports showed that BMP-9 has the strongest osteoinductive potential among BMP members, and it may be considered as a useful cytokine for bone regeneration therapy. In recent years, the influence of oxygen tension on bone functions has become a major research focus. Changes in oxygen concentrations act as a signal that regulates expression of various hypoxia-responsive genes through Hypoxia inducible factor (Hif) pathway. Hif-1α is a well-established hypoxia-responsive transcription factor inducing the gene expression of many angiogenic and glycolytic proteins. Notably, Hif-1α often coordinates various developmental processes of the human body, including skeletal development. However, it is not clear if Hif-1α exerts any effect on BMP9-induced osteogenic differentiation. In this study, we aimed to investigate the functional roles of Hif-1α in the signaling pathway of BMP9-induced osteoblast differentiation.

METHODS. MC3T3-E1 cells (a mouse osteoblast cell line) were stimulated with recombinant BMP9 or BMP2 for the time course analysis of Hif-1α protein expression. We then stimulated MC3T3-E1 cells with BMP9 under normoxic or hypoxic (2% O_2) condition with or without Chrysin (PHD activator) or NSC697923 (Ubc13 inhibitor). Hif-1α and PDK1 gene knockdown was performed by transfecting MC3T3-E1 cells with Hif-1α -or PDK1-specific siRNA. Protein and mRNA expression levels of Hif-1α, glycolytic, angiogenic and osteogenic genes were analyzed by Western blotting and real-time PCR, respectively.

RESULTS. Hif-1α protein expression was significantly induced by both BMP9 and BMP2 in MC3T3-E1 cells within 2 hours. Combination of BMP9 and hypoxia resulted in synergistic increase of Hif-1α protein level. Although Chrysin inhibited both BMP9- and hypoxia-induced Hif-1α protein expressions, NSC697923 failed to inhibit BMP9-induced HIF1α protein expression. When Hif-1α expression was knocked down by siRNA, the mRNA expression level of a glycolytic enzymes, PDK1and LDHa, but not that of ALP, Runx2, Osterix, or VEGFα, was significantly

inhibited in BMP9-stimulated MC3T3-E1 cells. We then assessed the roles of Hif-1a and PDK1 in BMP9-mediated osteoblast differentiation by transducing Hif-1a-specific, PDK-1 specific, or control siRNA into MC3T3-E1 cells, followed by BMP9 stimulation for 3 days to induce osteoblastic differentiation. Significant decrease of ALP mRNA level, but not that of Runx2 or Osterix, was detected with both Hif-1α and PDK1 siRNA treatments, indicating that Hif-1α and PDK-1 play important roles in BMP9-induced osteoblast differentiation in a manner independent of Runx2 or Osterix.

DISCUSSION. Here, we have revealed for the first time that the protein expression of Hif-1α is rapidly induced by osteogenic BMPs under normoxic condition. The Hif-1α induction mechanism by BMP9 seems to differ from that by hypoxia. Unexpectedly, Hif-1α expression is essential for the induction of glycolytic enzymes, PDK1 and LDHa, but not that of an angiogenic cytokine VEGFα, in BMP9-stimulated osteoblasts. Being consistent with previous reports that increased glycolysis is an essential feature of differentiated osteoblasts, our findings indicate that Hif-1α expression is important for BMP9-mediated osteoblast differentiation through the induction of PDK1.