# 論文審査の要旨

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

(BMP9 による骨芽細胞分化誘導において，Hif-1α は解糖系酵素 PDK1 の発現誘導を介して必須の役割を果たす)

Bone is one of the tissues with the highest regenerative potential. In cases where healing is disrupted or critical bone defects exist, autologous bone graft is still considered as the gold standard. Autologous bone graft contains 3 properties which are osteogenic, osteoconductive and osteoinductive. The present study focused on the osteoinductive profile using Bone Morphogenetic Protein (BMP). BMP is one of the most promising and intensively studied groups of growth factors that are involved in bone healing. Among BMPs identified, BMP-9 has been demonstrated to have the highest osteoinductive profile. Therefore, the present study chose BMP9 as the research topic. Inside the bone marrow, osteoblasts experience various oxygen tensions. Changes in oxygen concentration act as a signal that regulates expression of various hypoxia-responsive genes through Hypoxia inducible factor (Hif) pathway. Hif-1α protein activates expression of target genes including angiogenesis and glucose metabolism genes. Active glycolysis and the glycolytic shift may in turn be important for driving osteoblast differentiation. Hif-1α protein expression is negatively regulated by proteasomal degradation involving hydroxylation by PHD in normoxic conditions. 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. The experiment objective was to explore the possible role of Hif-1α in BMP9 mediated osteoblast differentiation. The present study used MC3T3-E1 pre-osteoblastic cell line and stimulated it with BMP9 in combination with chemical inhibitors and RNA interference to knockdown Hif-1α and PDK1 genes.

As a result, the following findings were clarified in this research.

1. Protein expression of Hif-1α was induced in BMP9-stimulated cells and hypoxia cells. Synergistic pattern was revealed by the combined stimulation with BMP9 and hypoxia. Furthermore, comparison results showed that stimulation with BMP2 induced Hif-1α protein in a similar time course to that of BMP9.
2. There was no obvious induction of Hif-1α and Hif-2α mRNA in either BMP9 or hypoxia stimulated cells.
3. BMP9-induced Hif-1α protein expression was blocked almost completely by PHD activator (Chrysin) but not by Ubc13 inhibitor.
4. Upon Hif-1α knockdown, PDK1 induction by BMP9 was inhibited. PGK1 and LDHa showed no induction upon BMP9 stimulation, but their expression was slightly decreased upon Hif-1α knockdown. Using the same Hif-1α knockdown system, the induction of VEGFα by treatment with BMP9 was not affected by Hif-1α knockdown.
5. Matrix mineralization process was inhibited in the presence of Hif-1α and PDK1 inhibitors.
6. Significant decrease of ALP mRNA level was detected with both Hif-1α and PDK1 knockdown. However, mRNA expression of either Runx2 or Osterix, two transcription factors known as master regulators of osteoblast differentiation, was unaffected.

The present study concludes that BMP9 stimulation induces the protein expression of Hif-1α through the inactivation of PHD and that Hif-1α protein expression is essential for the induction of PDK1, which is essential for osteoblasts differentiation. This is a novel finding demonstrating an alternate mechanism of BMP9-mediated osteogenesis. The second conclusion is that the expression of VEGFα in BMP9-induced osteoblasts is independent of Hif-1α protein induction. An interesting matter that needs to be elucidated in future study is the mechanism of the osteoblast differentiation promoted by Hif-1α and PDK1 is independent of Runx2 and Osterix induction.

In conclusion, we decided that this research has sufficient value as a thesis dissertation.