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MEETING REPORTS

Hypoxia Signaling: Meeting Report from the 3rd Joint Meeting of the European Calcified Tissue Society and the International Bone and Mineral Society

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At the 3rd Joint Meeting of the European Calcified Tissue Society and the International Bone and Mineral Society, many exciting current findings were presented. In particular, tissue-specific manipulation of gene expression was used in several important studies. This brief *Meeting Report* summarizes a couple of reports, using this technique, that focused on oxygen signaling in bone and cartilage, particularly as presented during a symposium at the meeting on hypoxia signaling.

Oxygen is required for various cellular metabolism and bioenergetics processes. However, oxygen tension is not homogenous in the body and some cells/tissues, such as chondrocytes and the renal medulla, are hypoxic compared to others. Thus, there is an adaptive system to hypoxia (1;2). In normoxic conditions, HIF-1α binds to an E3 ubiquitin ligase called von Hippel-Lindau (VHL) protein and is rapidly degraded. In contrast, HIF-1α is stabilized, translocates to the nucleus and modifies gene expression in hypoxic conditions. This means that some molecules sense oxygen tension and modify HIF-1α protein. Prolyl-hydroxylase domain proteins (PHDs) are one of these oxygen sensors. PHDs hydroxylate two prolyl residues in the HIF-1α protein and mediate the binding of HIF-1α and VHL. Dr. Geert Carmeliet (Leuven, Belgium) addressed whether PHDs have physiological roles in growth plate chondrocytes. While mice with global knockout of *PHD1* and *PHD3* did not show any bone phenotypes, *PHD2* knockout mice were embryonic lethal. Therefore, the investigators created chondrocyte-specific knockout mice missing *PHD2* using Col2-Cre. This resulted in the stabilization of HIF-1α in growth plate chondrocytes indicating that PHD2 is important for oxygen sensing in these cells. In addition, these mice also exhibited growth retardation, shortening of hypertrophic and proliferative zones in the growth plate, and increased chondrocyte remnants and trabecular bone mass. Metabolically, these mice showed various changes including increased anaerobic glycolysis, reduced glucose oxidation, and decreased energy generation and protein synthesis in chondrocytes. The activation of the unfolded protein response caused inhibition of mRNA translation and increased protein folding and removal. These results indicate that PHD2 is critical for normal cellular metabolism of growth plate chondrocytes.

Dr. Ernestina Schipani (Boston, USA) addressed the importance of HIFs also using tissue-specific knockout mice. Tissue-specific knockout of *Hif1a* encoding HIF-1α in chondrocytes using Col2-Cre caused cell death of chondrocytes in the central part of the growth plate (3). In addition, conditional inactivation of *Hif1a* in limb bud mesenchyme using Prx1-Cre resulted in delayed differentiation into chondrocytes and an impairment of joint development (4). In contrast, conditional knockout of *Epas1* encoding HIF-2α caused only a modest impairment of differentiation of hypertrophic cells into late hypertrophic chondrocytes (5), indicating the importance of HIF-1α in cartilage development. Dr. Schipani then examined the result of enhanced actions of HIFs by deleting *Vhlh* encoding VHL protein. Deletion of *Vhlh* in chondrocytes caused

reduced cell proliferation and increased matrix deposition (6). On the other hand, deletion of *Vhlh* in limb bud mesenchyme using Prx1-Cre caused many abnormal phenotypes including shortening of limbs, deranged columnar structure of growth plate chondrocytes, fibrosis in the joint cavity, fusion of small bones, expansion of trabecular bone and dilated blood vessels in bone. Furthermore, deletion of *Vhlh* in osteoblastic cells using Osterix-Cre caused increased trabecular bone, dilatation of blood vessels in bone marrow and increased the hematopoietic cell pool. While the mechanisms of some of these phenotypes such as dilated vessels remain to be clarified, these results confirm the importance of oxygen signaling in physiological control of cartilage and bone metabolism.

Similar to *Hif1a* deletion, deletion of *Vegfa* encoding vascular endothelial growth factor (VEGF) also causes cell death in the central part of the growth plate. Tissue-specific overexpression of *Vegfa* using Col2-Cre in cartilage-specific *Vegfa* knockout mice prevented this cell death and hypoxia in the growth plate. However, there are several isoforms of VEGF and insoluble VEGF(188) was shown to be unable to rescue this apoptosis (7). Introduction of soluble VEGF(164) fully prevented chondrocyte death in *Vegfa* null mice. In contrast, this soluble VEGF(164) only partially rescued cell death and hypoxia in chondrocytes of conditional *Hif1a*-deleted mice. These results indicate that HIF-1α prevents cell death and hypoxia in both a VEGF-dependent and VEGF-independent manner.

All of these studies show the importance of PHD2, HIFs, VHL protein and VEGF as well as the power of molecular genetic studies. However, several phenotypes of these genetically modified mice are not explained and it is not completely understood how HIFs control bone and cartilage metabolism. Future studies are necessary to clarify these issues.

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