Mis-expressions of SOX9 during Skeletogenesis Lead to Accelerated Adipogenesis in Transgenic Mice

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Introduction: During development mesenchymal cells can differentiate into adipocytes, chondrocytes, myoblasts, and osteoblasts as determined by different cell-type specific transcription factors. Among them SOX9 is a positive regulator for chondrogenesis while RUNX2 acts as a transcriptional activator essential for osteoblasts differentiation and chondrocytes maturation. RUNX2 also represses adipocytes differentiation and RUNX2 deficiency in chondrocytes led to enhanced adipogenesis (Enomoto H et al., J. Cell Sci. 117: 417-25, 2004). Our previous studies demonstrated that SOX9 and RUNX2 directly interacted with each other via their respective DNA binding domain. SOX9 decreased RUNX2 binding to its target sequence and drastically inhibited RUNX2 transactivation of osteoblast-specific and hypertrophic chondrocyte-specific enhancers. We have also generated transgenic mice mis-expressing FLAG-tagged SOX9 in osteoblasts by utilizing a 2.3kb osteoblast-specific *Col1a1* promoter. The *Col1a1-Flag-Sox9* transgenic mice displayed severe dwarfism and osteopenia. Expression of osteoblast differentiation markers such as *Col1a1* and *Osteocalcin* were all downregulated in transgenic mice. *Ex vivo* culture experiments demonstrated that osteoblasts from transgenic mice were less active compared with wild-type mice. Bone histomorphometry analysis revealed that osteoblast numbers and bone formation rates were greatly reduced in transgenic mice. (Zhou G. et al., Proc. Natl. Acad. Sci. USA 103:19004-9, 2006).

Materials and Methods: The bone marrow is constituted of two separate and distinct stem cells. The hematopoietic stem cells (HSC) are responsible for the production and maintenance of all the mature blood cells while mesenchymal stem cells (MSC) are capable of osteogenic, chondrogenic, adipogenic, myogenic and neuronal differentiation. HSCs reside in the so-called "stem-cell niche" in close contact with supporting cells like osteoblasts and vascular endothelium and its fate is determined by signals from their niche through cell-surface or secreted molecules. Thus osteoblasts play important roles in the regulation of hematopoiesis. However the effect of osteoblasts on MSC differentiation remains poorly understood. In this study to investigate the effects of SOX9 on mesenchymal stem cells differentiation, we isolated pluripotent bone marrow stromal cells (BMSC) from 8-10 week-old *Col1a1-SOX9* transgenic mice and induced adipocytes and osteoblasts differentiation *in vitro*. To directly investigate inhibitory effects of SOX9 on RUNX2 during chondrocytes hypertrophy, we have also generated a novel *Col10a1-SOX9* transgenic mouse model in which SOX9 is specifically expressed in hypertrophic chondrocytes driven by a cell-type-specific 10kb *Col10a1* promoter.

Results: While there were no significant differences in the number of osteoblast precursor cells by alkaline phosphatase assay between wild-type and transgenic mice, *Col1a1-SOX9* BMSCs exhibited decreased osteogenetic differentiation by Alzarin Red staining. Interestingly *Col1a1-SOX9* BMSCs also displayed enhanced adipocytes differentiation by Oil Red-O staining (Figure A). Furthermore, In monolayer culture of rib hypertrophic chondrocytes (HC) isolated from 6-day-old *Col10a1-SOX9* transgenic mice with SOX9 mis-expression in hypertrophic chondrocytes, expression of *Col10a1*, a downstream target of RUNX2, was decreased by 55% by quantitative RT-PCR. Additionally mineralized hypertrophic cartilage areas were significantly reduced by 10% ($p<0.05$) in 10-week-old proximal tibia of *Col10a1-SOX9* transgenic mice by von Kossa staining (Figure B). Furthermore in comparison with wild-type mice, *Col10a1-SOX9* HC culture also displayed increased adipogenesis with more lipid droplets accumulation by Oil Red-O staining and higher expression levels of adipogenic genes including *C/EBPα*, *PPARγ2*, *SCD1* and *Glut4* (Figure C).

Discussion: In conclusion, our work suggests that during mesenchymal cells differentiation repression of RUNX2 function by SOX9 not only results in impaired osteogenesis and hypertrophic cartilage mineralization, it also leads to enhanced adipogenesis via relieving RUNX2 inhibition of adipocytes differentiation. Further studies of the effect of SOX9 on pluripotent bone marrow stem cell differentiation and the interaction between SOX9 and RUNX2 in this process will deepen our understanding of the molecular and genetic controls of proper cell fate determination and differentiation during endochondral ossification. This is also crucial to the development of novel diagnostic and therapeutic approaches for various birth defects including skeletal disorders.

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