J Musculoskelet Neuronal Interact 2005; 5(4):338-339

Original Article

Hylonome

Wdr5, a novel WD repeat protein, regulates osteoblast and chondrocyte differentiation *in vivo*

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Keywords: Wdr5, Osteoblasts, Endochondral Bone Formation, Wnt Signaling

Skeletal development *in vivo* occurs via two major processes, intramembranous and endochondral ossification. Intramembranous bone formation in mammals is restricted to the flat bones of the cranial vault and the medial part of the clavicles. Endochondral bone formation occurs in bones that are involved in weight bearing and in forming joints and provides a mechanism for longitudinal growth of the skeleton during development. Both intramembranous and endochondral ossification begin with condensation of mesenchymal cells that form a template for the skeleton and end with formation of calcified skeleton. However, while intramembranous ossification occurs by direct differentiation of mesenchymal cells into osteoblasts, endochondral bone formation occurs through a highly coordinated sequence of events beginning with chondrocyte proliferation and deposition of cartilage matrix, continuing with hypertrophy and mineralization of the cartilage matrix and ending with cartilage matrix degradation, apoptosis of the hypertrophic chondrocytes, vascular invasion and formation of an ossification center containing type I collagen-expressing osteoblasts. The growth plate consists of zones of cells that follow a precise program of differentiation during endochondral bone formation. At the upper end of the growth plate is the reserve zone, consisting of a pool of chondrocytes that replenish the cells in the zone of the proliferating, type II collagen-expressing, chondrocytes. Proliferating chondrocytes, in turn, differentiate into prehypertrophic chondrocytes and then hypertrophic chondrocytes, which synthesize a specialized extracellular matrix containing type X collagen. Terminally differentiated hypertrophic chondrocytes undergo apoptosis and, following vascular invasion, are replaced by trabecular bone (primary spongiosa).

Several signaling pathways, including the parathyroid hormone related-peptide (PTHrP), Indian hedgehog (Ihh), and the Wnt signaling pathways are known to cooperate in regulating chondrogenesis and osteogenesis. Ihh, a member of the conserved hedgehog family of signaling factors, induces chondrocyte proliferation and osteoblast differentiation and prevents hypertrophic differentiation of chondrocytes[1-3]. In mice lacking Ihh, chondrocytes differentiate prematurely, resulting in a short-limbed dwarfism[4]. PTHrP is expressed in the proliferating chondrocytes and prevents hypertrophic differentiation of chondrocytes in the growth plate, therefore maintaining the pool of proliferative chondrocytes[5-8]. Targeted disruption of PTHrP in mice results in dwarfism characterized by a reduced zone of proliferating chondrocytes, accompanied by acceleration of hypertrophic differentiation[5,6]. Members of the Wnt family, secreted, lipid-modified glycoproteins that activate cell surface receptor-mediated signal transduction pathways, have been recently found to promote postnatal bone accrual as well as several aspects of skeletal development[9-12]. Wnt proteins activate gene transcription via three distinct intracellular signaling pathways: the Wnt/β-catenin pathway (canonical pathway) that activates Lef/Tcf target genes, the Wnt/Ca^{2+} pathway, that activates protein kinase C and the Wnt/planar polarity pathway that regulates gene expression via Rho/Rac GTPase and Jun N-terminal kinase.

Using differential display PCR we identified a novel BMP-2-induced gene named BIG-3 (BMP-2 Induced Gene 3kb) and recently renamed Wdr5[13]. Wdr5 is a new member of a family of structurally conserved proteins, the WD-40 repeat proteins. This family of proteins has been implicated in numerous cellular functions including signal transduction, mRNA processing, gene regulation, vesicular trafficking and regulation of the cell cycle[14-16]. WD repeats are conserved Trp-Asp motifs and each of the WD repeats is thought to fold into four antiparallel β strands radiating outward from a central axis, leading to the description of "β-propeller"[14-16]. Wdr5 mRNA is expressed in marrow stromal cells, osteoblasts,

The authors have no conflict of interest.

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Accepted 31 July 2005

osteocytes and chondrocytes[13]. Wdr5 dramatically accelerates the program of osteoblastic and chondrocyte differentiation *in vitro* and is developmentally expressed in osteoblasts, proliferating and hypertrophic chondrocytes[13,17]. Since the WD-40 repeat proteins are involved in the recruitment of other proteins, it is likely that the β-propeller structure of Wdr5 (that contains 7 WD repeats) is involved in the assembly of a multimeric protein complex that interacts with or modulates the expression of novel or known osteogenic factors and thereby accelerating osteoblast differentiation.

To investigate whether Wdr5 has a functional role during endochondral bone formation *in vivo*, transgenic mice overexpressing Wdr5 under the control of the 2.3-kb fragment of the mouse α (1) I collagen promoter were generated. The expression of the transgene resulted in an overall bigger skeleton. Histological analyses demonstrated that the humeri of transgene positive embryos were longer than those isolated from wild type littermates, and displayed an expansion of the hypertrophic chondrocyte layer. While no difference was observed in the expression domain of type X collagen, a marker for hypertrophic chondrocytes, transgenic mice displayed an expansion of the expression domain of osteopontin, a marker for terminally differentiated hypertrophic chondrocytes and osteoblasts as well as an acceleration in osteoblast differentiation, shown by an increased and more extensive expression of type I collagen, a specific marker for osteoblasts. The acceleration in chondrocyte and osteoblast differentiation was also reflected by more extensive mineral deposition in both the terminally differentiated hypertrophic chondrocytes and the bone collar of the transgene positive mice. Acceleration of osteoblast differentiation was also observed in primary calvarial osteoblast cultures isolated from transgene positive mice. These data suggest that targeted expression of Wdr5 to osteoblasts results in accelerated osteoblast differentiation and expansion of the hypertrophic chondrocyte layer. The canonical Wnt pathway was activated earlier in the bone collar of Wdr5 transgenic mice suggesting that targeted overexpression of Wdr5 to osteoblasts has autocrine actions on osteoblast differentiation, likely mediated by the canonical Wnt pathway.

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