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Gene targeting approaches in mice: Assessing the roles of LRP5 and LRP6 in osteoblasts

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The ability to genetically manipulate the mouse has revolutionized biomedical research. This is certainly the case in the field of skeletal tissue research. The ability to create mice carrying germline deletions has led to important insights into gene function during skeletal development. Many recent advances have been facilitated by the development of cre-lox recombination systems. These allow for deletions of genes at specific points in cell lineages and have been invaluable in working out the requirements for genes during different stages of osteoblast, chondrocyte, and osteoclast differentiation. The cre-lox system was identified in bacteria in the early 1980s[1]. The P1 bacteriophage protein called cyclization recombination (Cre) is 38 kDa and catalyzes recombination between two of its sequence recognition (LoxP) sites. A loxP (locus of X-over P1) site is a 34-base-pair consensus sequence containing a core domain of 8 base pairs flanked on each side by a 13-base-pair palindrome sequence[2]. Cre-mediated recombination results in the elimination of sequences flanked by the loxP sites. The utility of this system in eukaryotic cells was first demonstrated in the late 1980s[3-5], and further confirmation of its activity in transgenic mice was shown in 1992[6,7]. This has led to the development of numerous mouse strains in which essential portions on the gene are flanked by loxP sites (so-called "floxed" strains). If the floxed alleles are properly designed, Cre-mediated recombination leads to the creation of a null gene in a specific tissue or cell type. Numerous cre-expressing strains have been developed to facilitate studying components of the mouse skeleton[8].

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My laboratory's work has focused on examining the role of the Wnt signaling pathway in development and disease, with a particular interest in the role of this pathway in bone[9-12]. Wnts activate several signal transduction cascades upon engaging their cognate receptors[13-16]. The Wnt pathway that is frequently deregulated in human cancer, the so-called "canonical" pathway, initiates a signal when a Wnt ligand binds to a receptor complex containing a member of the frizzled family of seven-transmembrane receptors and either low-density lipoprotein receptor-related protein 5 (LRP5) or LRP6[14]. This signal downregulates glycogen synthase kinase-3 (GSK-3) activity[17,18]. Normally, GSK-3 phosphorylates β-catenin, targeting it for ubiquitin-mediated degradation. Inhibition of GSK-3 by Wnt signaling increases the level of cytosolic β-catenin, which translocates to the nucleus and activates target genes. This pathway can also directly activate the mammalian target of rapamycin (mTOR)[19]. Mutations in the Wnt co-receptors LRP5 and LRP6 produce striking alterations in bone mass in both humans and mice.

Osteoporosis pseudoglioma (OPPG) is a rare syndrome associated with premature, generalized osteoporosis leading to bone fracturing and progressive blindness. Inactivation of LRP5 was identified as the causative genetic alteration underlying OPPG[20]. LRP5 is expressed in osteoblasts, but not in osteoclasts[20,21]; therefore, subsequent work has focused on this cell type as the one in which LRP5 was required to regulate normal bone development and/or homeostasis. Additional support for the role of LRP5 in bone growth was provided when two groups independently reported that a point mutation in LRP5 (G171V) was present in affected individuals of families displaying an autosomally dominant high-bone-mass trait[22,23]. These two genetically independent families have bone density approximately five standard deviations above that of unaffected family members and the general population. Importantly, affected individuals have a normal lifespan and only limited morbidity associated with this mutation. The glycine normally at

position 171 in the LRP5 protein lies within the fourth blade of the first six-bladed β-propeller[24]. The G171V mutation does not directly increase signaling activity; instead it inhibits the ability of Dkk1, Sclerostin, and potentially other proteins to bind LRP5/6 and inhibit Wnt signaling[25]. In addition, it may interfere with Mesd binding and transit of LRP5 to the cell surface, facilitating increased autocrine signaling within the endoplasmic reticulum[26]. Numerous other alterations in human LRP5 have subsequently been identified and correlated with changes in bone mass[27]. Mutations in LRP6 have also been linked to changes in bone mass in humans. Members of a family in which a putative partial loss-of-function mutation in LRP6 was identified were predisposed to early cardiovascular-related death (associated with dramatically elevated levels of plasma LDL and triglycerides, hypertension, diabetes, and osteoporosis[28]. In addition, several mutant mouse models with targeted or spontaneous point mutations in LRP6 have been found to have altered bone development[10,29,30].

Consistent with other reports[31], we have found that mice carrying a conditional deletion of β-catenin in mature osteoblasts developed severe osteopenia accompanied by increased osteoclastogenesis[11]. We also know that LRP5-deficient mice have low bone mass but are viable and fertile and do not exhibit alterations in osteoclast function[10,21,29,32,33]. One potential explanation for these differences is that the highly related LRP6 might exert overlapping, or distinct, roles in bone development. Consistent with this, we found that mice carrying global mutations in both LRP5 and LRP6 display synergistic deficiencies in bone mass[10]. However, since these mutations were present in all cells, it did not allow us to unambiguously determine that the defects were due to altered osteoblast regulation. To address this, we created mice carrying a conditional allele of *LRP6* (*LRP6*^{flox}). The generation of mice expressing Cre driven by the human osteocalcin promoter (*OC-cre*$^{TG/+}$)[34] and homozygous for the *LRP6*^{flox} allele has revealed that such mice have significantly low bone mass, demonstrating that *LRP6* is required for the normal bone acquisition. When the *OC-cre*$^{TG/+}$;*LRP6*$^{flox/flox}$ mice are mated with mice globally deficient in *LRP5*, the *OC-cre*$^{TG/+}$;*LRP6*$^{flox/flox}$;*LRP5*$^{ko/ko}$ offspring develop severe osteopenia and essentially phenocopy mice lacking β-catenin in osteoblasts (*OC-cre*$^{TG/+}$;*β-catenin*$^{flox/flox}$ mice); the mice die within four weeks of birth with severe osteopenia associated with reduced bone formation and increased bone resorption. This suggests that both LRP5 and LRP6 are required to fully activate β-catenin in mature osteoblasts.

Previous studies have shown that loss of β-catenin in mesenchymal progenitors leads to excess chondrocyte formation at the expense of osteoblastogenesis, while activation of β-catenin leads to the reciprocal phenotype[35-37]. We have examined the role of LRP5 and LRP6 at these early developmental stages by gene deletion mediated by the *Dermo1-Cre* transgene. *OC-cre*$^{TG/+}$-*LRP6*$^{flox/'flox}$ mice are viable and fertile, however, DEXA analysis reveals a significant reduction of bone mass (approximately 10%) by 3 months of age. Preliminary analysis finds that *Dermo-*$^{TG/+}$*-LRP6*$^{flox/flox}$;*LRP5*$^{ko/ko}$ do not survive embyrogenesis and have a number of abnormalities.

In summary, our work provides clear evidence for a role of LRP6 in regulating osteoblast differentiation and/or function. We have also found that LRP5 and LRP6 play overlapping roles in both early and late stages of osteoblast differentiation.

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