Editorial: Sclerostin and Wnt Signaling—The Pathway to Bone Strength

The realization that Wnt signaling was critical to bone strength came to those in the bone field suddenly and from several directions. Whereas a search of ASBMR (American Society for Bone and Mineral Research) abstracts about Wnt, LRP5 (low-density-lipoprotein-receptor-related protein 5), or Dickkopf (Dkk) returned a void in 2000, there were 111 reports at the most recent meetings. The various discoveries read like a story, with surprises, coincidences, and promise for a better treatment of osteoporosis.

In 1997 clinical investigators from Creighton University reported a kindred with high bone mass. The proband was an 18-yr-old girl who had back pain after an automobile accident. Radiographs showed dense but otherwise normal bones, and the bone mineral density was 5.6 SD values above average for age. Her mother had similarly dense bones with a bone density z-score of 4.98 but no skeletal symptoms. This led to an extended survey of this family whose members carried the “high bone mass” gene (1). Using linkage analysis, they demonstrated that the trait was located to a region on chromosome 11q12–13. Recombinant events in two of the individuals significantly refined the interval, and further analysis, including a search for mutations, revealed that the family members with high bone mass had a single point mutation in the LRP5 gene (2). This was not on any list of candidate genes involved in bone metabolism. Shortly afterward, an unrelated kindred was reported that carried the identical mutation, G171V, which was also associated with high bone density (3). In both studies, the inheritance was autosomal dominant. Meanwhile, another group of investigators discovered that a different mutation in the LRP5 gene caused the osteoporosis-pseudoglioma syndrome, the protein is functional, so the Wnt-signaling pathway is inhibited. The heterozygotic carriers had low bone density and increased risk of fractures (4).

LRP5, despite its name, does not play a role in lipoprotein metabolism, but is a member of the Wnt-signaling pathway. This complex pathway is well illustrated and animated on web pages by Dr. Randall Moon (http://faculty.washington.edu/rmoon/) and Dr. Roel Nusse (http://www.stanford.edu/~rnusse/wntwindow.html) and includes many proteins named after unfortunate fruit flies. The Wnt genes are homologous to segment-polarity genes that are critical in embryonic development (the 1995 Nobel prize for medicine was given to Nusslein-Volhard and Wieschaus for their work with these genes).

Already several of the Wnt-related proteins have been shown to be important in the regulation of bone metabolism (as will be discussed below). The signal is Wnt, a protein secreted by many cell types (the name is a combination of the *Drosophila* gene *wingless* and the mouse gene *int*). There are over a dozen forms of Wnt. The seven-transmembrane domain receptor is Frizzled. Its side-kick or coreceptor, a single transmembrane protein, is LRP5 (product of the “high bone mass gene,” which is the homolog of the *Drosophila* protein Arrow). Intracellular proteins include Dishevelled, Axin, glycogen synthase kinase (GSK)3, and β-catenin (Armadillo). When there is no signal, the intracellular GSK phosphorylates β-catenin so it is ubiquitinated and broken down by the proteasome. When Wnt binds the Frizzled receptor, it phosphorylates and activates Dishevelled, which represses GSK3, which releases Axin from the β-catenin. The Axin is then restrained by the intracellular part of LRP5, and so the β-catenin accumulates and enters the nucleus and binds TCF transcription factors. This pathway, which involves Wnt and β-catenin, is called the canonical Wnt-signaling pathway. Several extracellular proteins can inhibit this process. Dkk ties up LRP5 by binding to it and another membrane protein called Kremen. Sclerostin, homolog of Wise, also binds to the LRP5 protein. These each prevent the LRP5 from restraining Axin. Secreted Frizzled-related protein (sFRP) acts as a decoy receptor and binds to Wnt.

In patients with the G171V mutation and high bone mass, the mutated LRP5 resists binding to Dkk. Therefore, the Wnt-signaling pathway is more active. In the patients with osteoporosis pseudoglioma syndrome, the protein is non-functional, so the Wnt-signaling pathway is inhibited.

While investigators were discovering the LRP5 mutations using linkage analysis in kindred studies, a completely different approach was used in a study of multiple myeloma. Tian et al. (5) performed gene array analysis on bone marrow cells from patients with myeloma and compared those who had bone lesions on magnetic resonance imaging scans to those without lesions. Of 10,000 genes, 57 could distinguish the two groups of patients. Four of these were significantly overexpressed in the plasma cells of patients with magnetic resonance imaging lesions, and the one that coded for a secreted protein was Dkk1. Recall that Dkk is one of the extracellular proteins that inhibits Wnt signaling, and that patients with high bone mass are resistant to Dkk. The investigators further showed that the myeloma cells secreted Dkk. Thus, inhibition of Wnt signaling facilitates the development of lytic bone lesions in patients with multiple myeloma.

Once the Wnt-signaling pathway had been shown to be important to bone disease, many investigators began working to identify the effects of this pathway on bone cells. A recent study (6), again employing gene array technology, examined the genes that were expressed in stromal cells.
They found 879 of 39,000 genes that showed statistically significant expression differences after treatment with Wnt3a. These genes included some that control cell differentiation (promotion of osteoblastic as opposed to adipocytic phenotypes) and inhibition of apoptosis (longer lifespan), and other proteins in the Wnt-signaling pathway (feedback). Of particular interest is the finding that Wnt3a up-regulates osteoprotegerin, a secreted protein that inhibits bone resorption. Thus, the Wnt-signaling pathway not only increases osteoblastic cell differentiation and bone formation, but also inhibits bone resorption by blocking the receptor activator of nuclear factor-κB-ligand (RANK-L)/RANK interaction (6). Furthermore, preosteoblasts are more likely to secrete RANK-L, so if Wnt signaling is inhibited there are more preosteoblasts, more RANK-L but less osteoprotegerin, and fewer mature osteoblasts capable of refilling resorption cavities. This combination contributes to the bone lesions in multiple myeloma. Exciting new abstracts from the 2005 ASBMR meetings show that small compounds that inhibit Dkk can improve the bone lesions in experimental myeloma (7, 8). These compounds also increase bone density in mice without myeloma (9). Transgenic mice with heterozygous Dkk knockout also have increased bone formation and strong bones, probably because they have lower levels of the inhibitory protein Dkk. Homozygous knockout mice, however, are not viable and have abnormal calvariae (Dickkopf means “thick head” because it promotes head formation in vertebrates) (10).

Other patients with high or low bone mass have been studied to determine whether they might have abnormalities in the Wnt-signaling pathway. Heterozygous missense or frameshift mutations in the LRP5 gene have been found in children with juvenile osteoporosis (11). On the other end of the molecule, six different missense mutations were found in families with increased bone density (12). These mutations were all related to the same extracellular region of the protein, on the outermost propellar structure, which has been shown in one case to prevent Dkk binding. It has not yet been shown whether the other mutations for that region also inhibit Dkk binding, or whether they enhance Wnt signaling by an alternate mechanism. Gain-of-function mutations that cause dense bones are not always beneficial, and in some patients the thick jaws and lingual exostoses have caused dental symptoms (12, 13).

Within the group of bone diseases with high bone mass are Van Buchem’s disease and sclerosteosis. Patients with Van Buchem’s disease have abnormally thick skulls, square jaws, and may have abnormalities in fingers. The bones can be painful. Some of the patients were found to have mutations in the LRP5 gene, but others did not. Sclerosteosis is a serious, autosomal recessive disease. Patients have very thick bones, especially in the skull, with entrapment of cranial nerves leading to deafness and facial nerve palsy, increased intracranial pressure, and greater risk of stroke. Bones in the rest of the skeleton are also thick and dense, and frequently syndactyly is present. Most of the patients are Afrikkan from South Africa. In a description of the natural history of these patients, Hamersma et al. (14) noted that none of their 63 patients had ever fractured a bone. Also, the obligatory heterozygous gene carriers were resistant to fractures and did not have degenerative osteoarthropathy.

Recently patients with sclerosteosis (15) and some patients with hyperostosis resembling Van Buchem’s disease (16, 17) were found to have homozygous mutations in the SOST gene. Sclerostin, the SOST gene product, was initially thought to function as a bone morphogenetic protein antagonist. This antagonistic function, however, is weak and does not really explain the disease. Now we know that sclerostin is a circulating inhibitor of the Wnt-signaling pathway, which acts to inhibit LR5P5 function (18, 19). This protein is expressed almost exclusively in the osteocytes. In fact, a recent histochemical study of human iliac crest biopsies has shown that the sclerostin is not present in osteocytes near the bone surface but only in the more mature osteocytes that are deeper in the bone (20). Osteocytes are terminally differentiated osteoblasts that become embedded within newly mineralized matrix during bone formation. Each osteocyte has long cell processes that connect with older osteocytes deeper within the bone, contemporary osteocytes at the same depth, and new osteocytes and surface lining cells. Thus, the osteocytes form a network in the bone reminiscent of the neural network in the body. These osteocytes are able to detect mechanical strain by sensing fluid movement within the canaliculi. It is not clear exactly how they direct the formation of new bone; the cells can respond to mechanical strain by secreting nitric oxide, prostaglandins, and growth factors. Now we know they also secrete sclerostin. One theory is that the osteocytes tonically suppress the lining cells via sclerostin and then stop secreting it when the need arises to form new bone. When larger stresses are applied to bone, small cracks appear, followed by bone resorption that removes the damaged bone. It is possible that the osteocytes undergo apoptosis when there has been damage to the bone. Because the dead cells can’t make sclerostin, the surface cells are released from sclerostin inhibition and preosteoblasts can start to differentiate into osteoblasts in time to fill in the resorption cavity. Although the exact mechanism is not known, it now seems likely that sclerostin plays an important role in skeletal adaptation to mechanical forces.

The current report by Gardner et al. (21) in this issue extends these fascinating observations. Relatives of patients with sclerosteosis were tested to determine whether they were heterozygous for the mutation in the SOST gene. The bone densities of these heterozygous persons were high, and they did not have fractures. The carriers were healthy and had no symptoms of skeletal dysfunction. Sclerosteosis is therefore a human model of a gene knockout showing a beneficial effect in heterozygotic carriers but serious disease in homozygote knockouts.

It is not surprising that companies are racing to develop pharmacological agents directed toward the Wnt-signaling pathway to treat osteoporosis. Current therapies are almost all antiresorptive and at best have been able to prevent only half of the fractures. After bone resorption is blocked, bone formation decreases to very low levels that seriously limit this method of treatment. The only anabolic agent currently approved is intermittent PTH [which actually acts, at least in part, as a stimulator of the Wnt-signaling pathway (22)]. Inhibition of sclerostin is a particularly promising anabolic
approach because the human model of partial inhibition is already demonstrated. Indiscriminate stimulation of Wnt signaling could lead to complications in other tissues that rely on this pathway but, because sclerostin is expressed only in the bone, the effects of agents directed toward sclerostin would be targeted to the bone. Antibodies to sclerostin have already been shown to increase the bone formation rate and bone density in mice (23). This is encouraging news to those with a goal of preventing osteoporotic fractures.

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