LRP5 Mutations Linked to High Bone Mass Diseases Cause Reduced LRP5 Binding and Inhibition by SOST*

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The low density lipoprotein (LDL) receptor-related protein 5 (LRP5) is a co-receptor for Wnt proteins and a major regulator in bone homeostasis. Human genetic studies have shown that recessive loss-of-function mutations in LRP5 are linked to osteoporosis, while on the contrary, dominant missense LRP5 mutations are associated with high bone mass (HBM) diseases. All LRP5 HBM mutations are clustered in a single region in the LRP5 extracellular domain and presumably result in elevated Wnt signaling in bone forming cells. Here we show that LRP5 HBM mutant proteins exhibit reduced binding to a secreted bone-specific LRP5 antagonist, SOST, and consequently are more refractory to inhibition by SOST. As loss-of-function mutations in the SOST gene are associated with Sclerosteosis, another disorder of excessive bone growth, our study suggests that the SOST-LRP5 antagonistic interaction plays a central role in bone mass regulation and may represent a nodal point for therapeutic intervention for osteoporosis and other bone diseases.

Bone tissues require constant remodeling throughout life. During post-natal development and into adulthood bones grow in size and strength while retaining their ability to adapt in response to changes associated with physical activities. In aging people, bones lose their strength and often, especially in women, become osteoporotic and with time may lose the ability to provide adequate support for the body. Therefore osteoporosis is a major global health problem, particularly in an increasingly aging population. Osteoporosis results from imbalance of bone tissue homeostasis. A number of signaling pathways are involved in regulation of bone remodeling, among which Wnt signaling via low density lipoprotein (LDL) receptor-related protein 5 (LRP5) and the related LRP6 appears to play a central role (1, 2). LRP5 and LRP6 are cell surface co-receptors for the Wnt family of secreted signaling proteins (3). Upon Wnt stimulation, LRP5 or LRP6 may form complexes with the Frizzled family of seven-pass transmembrane proteins (3–9), leading to the activation of the canonical Wnt/β-catenin pathway.

Human genetic studies have assigned an important role to LRP5 in bone homeostasis, as two distinct classes of LRP5 mutations affecting bone mass have been identified. One class represents loss-of-function mutations associated with the autosomal recessive osteoporosis-pseudoglioma syndrome exhibiting low bone mass (10). These mutations mostly result in premature stop codons or frameshift mutations that preclude the synthesis of the full-length LRP5 protein. In contrast, the other class of LRP5 mutations is linked to autosomal dominant high bone mass (HBM) diseases (11–13). Strikingly, these HBM LRP5 mutations are missense mutations (single amino acid substitutions) clustered in the first YWTD β-propeller domain of the extracellular portion of LRP5 (Fig. 1A). LRP5 mutations in mice recapitulate human bone disorders: knock-out Lrp5−/− mice show low bone mass and osteoporosis (14), whereas transgenic mice expressing a HBM mutant LRP5(G171V) exhibit high bone mass (15, 16). LRP5 controls bone mass in humans and mice most likely through regulation of Wnt signaling activity that is critical for osteoblast production and proliferation (10, 14). Indeed, Wnt signaling activation in bone-forming cells via the overexpression of Wnt10B (17) or β-catenin (18) also leads to high bone mass in transgenic mice.

The molecular mechanism by which LRP5 HBM mutations cause elevated Wnt signaling activation remains to be fully understood (19). The extracellular domain of LRP5, like that of LRP6, is composed of four YWTD β-propeller domains that are each followed by an epidermal growth factor-like domain and of three LDLR type A domains (Fig. 1A) (3). Besides Wnt proteins, secreted antagonists for Wnt signaling, such as Dickkopf1 (Dkk1), bind to LRP5 (5, 20) and by doing so antagonize Wnt signaling through LRP5. In addition, LRP5 binds to an endoplasmic reticulum-resident protein MESD, which may function as a chaperone for LRP5 folding and trafficking through the secretory pathway (21, 22). One study suggests that LRP5(G171V) HBM mutation does not affect LRP5 interaction with Wnts or DKK1 but rather disrupts LRP5 interaction with MESD (22). In this model LRP5 retained inside the cell can activate Wnt signaling via an autocrine mechanism but is resistant to inhibition by DKK1 and other Wnt antagonists present in the extracellular space (22, 23). However, another study shows

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3 The abbreviations used are: LDL, low density lipoprotein; LDLR, LDL receptor; LRP, LDL receptor-related protein; HBM, high bone mass; CM, conditioned medium; WT, wild type; GFP, green fluorescent protein; PBS, phosphate-buffered saline.
that LRP5 HBM mutants exhibit reduced binding to DKK1 and are resistant to inhibition by DKK1 (24).

SOST is a newly characterized secreted Wnt signaling antagonist that binds to and inhibits LRP5 (23, 25). SOST is encoded by the Sclerosteosis gene, whose loss-of-function or down-regulation mutations are linked to Sclerosteosis and Van Buchem disease, respectively (26–28). These two autosomal recessive disorders exhibit excessive bone growth and share many similarities with LRP5 high bone mass diseases. We recently showed that SOST, like DKK1 (5), is able to bind LRP5 and to disrupt Wnt-induced Fz–LRP5 complexes in vitro (25). Here we show that all six LRP5 HBM mutations analyzed in our study exhibit reduced binding to SOST and are resistant to SOST mediated Wnt inhibition.

**MATERIALS AND METHODS**

Wnt10B/pcDNA3.1 (24), Wnt6/LNCX (29), MESD-flag/pcDNA3.1 (21), LRP5/pcDNA3 (30), LRP5N-myc/pcDNA3, DKK1-IgG/pcDNA3.1+ (5), DKK1-flag/pcS2+ (31), SOST/pcDNA3.1+, SOST-IgG/pcDNA3.1 (25) and Kremen2-V5/pCS (32) plasmids were described earlier. HBM mutations G171V (11, 12), N198S (12), D111Y, A214T, and T235I (13), and R154M (33) and DKK1 binding mutations E721A and W781A (22) were introduced into LRP5 via primer-directed mutagenesis of the LRP5 cDNA fragment cloned into the BlueScript plasmid. The entire insertion was sequenced to confirm mutations before cloning back into LRP5- or LRP5N-myc-expressing vectors. Fz8/pcDNA3 plasmid was made by cloning Fz8 cDNA into pcDNA3 vector. Full-length LRP5-myc constructs were made by cloning the myc tag consisting of six tandem myc epitopes before the stop codon. DKK1-AP (alkaline phosphatase) and SOST-AP fusion constructs were generated by inserting DKK1 and SOST cDNAs into the ATag5 vector (34), respectively.

HEK293T cells were obtained from ATCC and grown in DMEM supplemented with 10% newborn calf serum and antibiotics. Precipitation, immunoblotting, and conditioned media (CM) production were performed as described earlier (25). HEK293T cells were seeded into 24-well plates and transfected with 200 ng of the SuperTOPflash reporter plasmid (35), 20 ng of pRL-TK (Promega), and other plasmids as indicated in the legends. All experiments were repeated at least three times. We examined whether HBM mutations affect LRP5 cell surface presentation. For this purpose we employed LRP5-myc, which harbors the myc tag at the C terminus of the full-length LRP5, and identically tagged LRP5 HBM mutants. Upon transfection into HEK293T cells, we performed cell surface protein biotinylation to label LRP5 and mutants that are expressed on the plasma membrane. As observed for secreted LRP5N (Fig. 1B), we did not notice any appreciable changes in the mobility of LRP5 mutants compared with that of the wild type (WT) LRP5, suggesting that LRP5 post-translational modifications are not significantly affected by these mutations (Fig. 1B).

For biotinylation of cell surface proteins HEK293T cells were seeded on 10-cm dishes and transfected with 2 μg of LRP5-myc-expressing plasmids plus 2 μg of GFP- or MESD-expressing plasmid. Cell labeling, protein extraction, and precipitation were performed in the cold room at +4°C. 36 h after transfection cells were rinsed once with ice-cold PBS and incubated with 1 ml of 0.5 mg/ml of sulfom-NHS-LC-biotin (Pierce) for 30 min. After incubation non-reacting NHS-biotin was neutralized by 50 mM Tris, 150 mM NaCl, pH 8.8, for 15 min. Cells were washed two times with PBS and membrane proteins were extracted by 2% Triton X-100 on PBS for 2 h on the rocking platform. Cell extracts were cleared by centrifugation and diluted three times with PBS. Biotinylated proteins were precipitated with the neutravidin-Sepharose (Pierce) for 2 h. Precipitates were washed with TBST four times and used for immunoblotting.

For measuring DKK1-AP and SOST-AP binding to cells expressing different forms of LRP5, HEK293T cells were seeded on 24-well plates and transfected with 0.2 μg of LRP5-expressing plasmids plus 0.2 μg of GFP- or MESD-expressing plasmid. 36 h after transfection cell media were replaced with 0.5 ml of conditioned media containing DKK1-AP or SOST-AP fusion protein supplemented with 0.5 mg/ml heparin (Sigma). Binding was performed for 30 min at room temperature. Cells were washed three times with PBS. Alkaline phosphatase activity was determined using a reporter system from Pierce (catalog number 37621).

**RESULTS**

We introduced six HBM mutations, D111Y, R154M, G171V, N198S, A214T, and T235I, identified in four independent studies (11–13, 33) into the first YWTD β-propeller domain of LRP5 (Fig. 1A). We also introduced in the third YWTD β-propeller two missense mutations, E721A and W781A, which have been shown to affect DKK1 binding to LRP5 (22) (Fig. 1A). We first found that all these LRP5 mutations exhibit similar levels of protein expression when transfected in HEK293T cells (Fig. 1B). We did not notice any appreciable changes in the mobility of LRP5 mutants compared with that of the wild type (WT) LRP5, suggesting that LRP5 post-translational modifications are not significantly affected by these mutations (Fig. 1B).

We also introduced the same mutations into LRP5N, which encodes a secreted LRP5 ectodomain with the myc tag at the C terminus (5). LRP5N secretion into conditioned media reflects the folding status of the LRP5 extracellular domain, as it is generally postulated that only properly folded proteins can reach the cell surface or be secreted. All mutant LRP5N proteins, with the exception of G171V, were secreted similarly to the WT LRP5N (Fig. 1C). Co-expression of MESD significantly increased the maturation and secretion of all LRP5N proteins, and the extent of the increase was similar among the mutants and the WT LRP5N (Fig. 1C). LRP5N(G171V) secretion was also stimulated by MESD as seen on films with a longer exposure, although the amount of matured LRP5N(G171V) still remained significantly lower than that of the WT LRP5N (Fig. 1C). We estimated that less than 5% of all expressed LRP5Ns were secreted into conditioned media even in the presence of MESD, and correspondingly we did not observe detectable decrease of LRP5N amounts retained by cells associated with MESD co-expression (Fig. 1C, panel c).

We examined whether HBM mutations affect LRP5 cell surface presentation. For this purpose we employed LRP5-myc, which harbors the myc tag at the C terminus of the full-length LRP5, and identically tagged LRP5 HBM mutants. Upon transfection into HEK293T cells, we performed cell surface protein biotinylation to label LRP5 and mutants that are expressed on the plasma membrane. As observed for secreted LRP5N (Fig. 1B), we did not notice any appreciable changes in the mobility of LRP5 mutants compared with that of the wild type (WT) LRP5, suggesting that LRP5 post-translational modifications are not significantly affected by these mutations (Fig. 1B).

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LRP5 HBM Mutants Show Reduced Binding and Inhibition by SOST

To test whether any of the LRP5 HBM mutations may cause an increase in ligand-independent signaling activity of LRP5, we transfected the WT and mutant LRP5 expression plasmids into HEK293T cells and monitored the activation of Wnt signaling using TOP-flash reporter assays (35). All LRP5 mutants, similar to the WT LRP5, were able to only weakly activate the TOP-flash reporter (Fig. 3A). In contrast, LRP5ΔN, which lacks the extracellular domain and behaves as a constitutively activated receptor (36), strongly activated the TOP-flash reporter (Fig. 3A). These results, which are consistent with previous studies (12, 24), suggest that LRP5 HBM mutants do not exhibit a higher basal signaling activity.

We next examined whether the LRP5 HBM mutants may affect the folding and cell surface presentation of LRP5. The G171V mutation causes a significant decrease of LRP5 expression on the cell surface, although MESD co-expression can still enhance LRP5(G171V) surface expression.

FIGURE 2. The cell surface expression of LRP5 HBM mutants. HEK293T cells were transfected with LRP5-myc plus GFP (panels a, c, e, and g) or MESD (panels b, d, f, and h)-expressing plasmids. Cell surface proteins were biotinylated, and whole cell extracts were tested for the presence of LRP5-myc (panels c and d) or the endogenous transferrin receptor, which was used as a control cell surface protein (panel g and h). Biotinylated proteins were precipitated from cell extracts with avidin-Sepharose and analyzed for LRP5-myc (panels a and b) or the transferrin receptor (panels e and f). Note that MESD enhanced the cell surface expression of all LRP5-myc proteins (panel a versus panel b) but not of the transferrin receptor (panels e and f). Lanes 11 and 12 on panel b were re-runs of samples from lane 1 of panels a and c, respectively, and show that the MESD co-expression causes an increase in the amount of LRP5 on the cell surface and that LRP5-myc is expressed on the cell surface mostly as a slower electrophoretic mobility form, which is barely detectable in the whole cell extract.
which Wnts are involved remains to be fully elucidated (1, 2). Mice lacking Wnt10B gene have decreased bone mass whereas overexpression of Wnt10B in transgenic mice increases bone mass (17), suggesting that Wnt10B plays a role in the process. Other Wnt genes may also be involved. For example, Wnt6 is expressed during long bone development (37) and in a calvarial cell line stimulated for osteogenesis (38). Therefore, we chose these two candidate Wnts to investigate the effect of LRP5 HBM mutations on activation of Wnt signaling. Both Wnt10B and Wnt6 require exogenous Frizzled8 to activate the TOP-flash reporter, likely reflecting the absence of an appropriate endogenous Frizzled receptor for these Wnts in HEK293T cells (Fig. 3B). Thus Frizzled8 expression plasmid is included in all subsequent experiments. We found that none of the LRP5 HBM mutants exhibited stronger synergistic effect with either Wnt10B or Wnt6 than the WT LRP5 (Fig. 3, C and D). In fact, some of LRP5 HBM mutants showed a slightly or moderately reduced capability to mediate Wnt10B or Wnt6 signaling (Fig. 3, C and D). Thus LRP5 HBM mutants do not appear to exhibit stronger signaling activation than the WT LRP5.

The G171V mutation leads to decreased LRP5 presentation on the cell surface (Fig. 2) and at the same time does not significantly affect its ability to synergize with Wnt10B and Wnt6 (Fig. 3, C and D). To examine this issue further we compared activation of Wnt signaling by different amounts of the WT and LRP5(G171V). Surprisingly, the WT and LRP5(G171V) showed very similar abilities in Wnt signaling at all doses tested (Fig. 4). Both started to exhibit stimulatory effect on Wnt signaling at 0.1 ng/well and reached the maximal activation between 1 and 3 ng/well (Fig. 4). However at higher doses both LRP5 and LRP5(G171V) became inhibitory toward Wnt signaling (Fig. 4), possibly due to the formation of non-productive

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**FIGURE 3.** LRP5 HBM mutants do not exhibit elevated basal/ligand-independent or Wnt-mediated signaling activity. *A*, LRP5 HBM mutants do not activate the TOP-flash reporter in the absence of exogenous Wnt ligands. HEK293T cells were transfected with a TOP-flash reporter and the WT (100 ng), mutant LRP5 (100 ng), or a LRP5ΔN-expressing plasmid (1 ng). Lower doses of WT LRP5 and LRP5 HBM mutants (1–10 ng) resulted in even lower TOP-flash activation (data not shown). B, Wnt10B and Wnt6 activate TOP-flash reporter only in the presence of exogenous Frizzled8. HEK293T were transfected with Wnt10B- or Wnt6-expressing plasmid (1 ng) with or without Fz8-expressing plasmid (1 ng). C and D, LRP5 HBM mutants synergize with Wnt10B (C) and Wnt6 (D) in the activation of TOP-flash reporter similarly or somewhat less efficiently than the WT LRP5. HEK293T were transfected with Wnt10B/Fz8 or Wnt6/Fz8-expressing plasmid (0.2 ng/1 ng) alone or with LRP5-expressing plasmid (0.3 ng) as indicated. Asterisks above the bars represent statistically significant difference (p < 0.05) between the indicated LRP5 mutant and the WT LRP5.
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FIGURE 4. LRP5(G171V) cooperates with Wnt10B and Wnt6 in the activation of the TOP-flash reporter similarly as the WT LRP5. HEK293T cells were transfected with Fz8-expressing plasmid (1 ng), different amounts of the WT LRP5 (light gray bars), or LRP5(G171V) (dark gray bars) as indicated plus 1 ng of Wnt10B (A) or Wnt6 (B)-expressing plasmid. Insets are displaying schematic presentation TOP-flash results using linear scale instead of logarithm scale used in the main panel. Asterisks shown above the bars represent statistically significant difference (p < 0.05) between the indicated LRP5 mutant and the WT LRP5.

FIGURE 5. LRP5 HBM mutants and the WT LRP5 bind DKK1 similarly. A, LRP5 HBM mutations do not affect LRP5 binding to DKK1. DKK1-IgG CM was mixed with the WT LRP5N-myc or mutant LRP5N-myc CM, and DKK1-IgG was precipitated with protein G-agarose beads. Precipitates were immunoblotted with anti-myc antibodies (panels a and e) or anti-human IgG antibodies (panel d). CM mixtures before precipitation (input) were immunoblotted with anti-myc antibodies (panels b and f) or anti-human IgG antibodies (panel c). Note that although LRP5(G171V)N-myc was secreted into CM less efficiently than the WT LRP5N-myc (lanes 1 and 4, panel b), they bound similarly to DKK1 (panel e) when their input was adjusted to the same level (panel f). B, DKK1-AP binding to HEK293T cells expressing the WT or LRP5 mutants. HEK293T cells were transfected with 200 ng of the WT or mutant LRP5-expressing plasmids without (light gray bars) or with (dark gray bars) MESD-expressing plasmid (200 ng). 36 h later DKK1-AP binding to cells were determined, background reading of DKK1-AP binding to mock-transfected cells was subtracted from all measurements, and results were normalized against cells expressing the WT LRP5. Asterisks shown above the bars represent statistically significant difference (p < 0.05) between the indicated LRP5 mutant and the WT LRP5. IP, immunoprecipitation.

signaling complexes. Therefore, although distinct from the rest of LRP5 HBM mutants, LRP5(G171V) nonetheless has similar signaling activity as WT LRP5 and the other LRP5 HBM mutants. We next examined whether LRP5 HBM mutations compromise the inhibitory activity of secreted Wnt signaling antagonists, of which several distinct families have been identified (39). Some Wnt antagonists act via direct binding to Wnts, thus their anti-Wnt activities are not expected to be affected by LRP5 HBM mutations. On the other hand, DKK1 is known to directly bind to LRP5 and inhibit Wnt signaling (5, 20). A previous study showed that the G171V mutation does not affect LRP5 binding to DKK1 (22). In contrast, another study demonstrated that several LRP5 HBM mutants, including LRP5(G171V), exhibit a reduction in DKK1 binding and DKK1-mediated inhibition (24). To evaluate this issue further, we compared the DKK1 interaction with the WT LRP5, LRP5 HBM mutants, and two LRP5 mutants, LRP5(E721A) and LRP5(W781A), which are known to be defective in DKK1-binding (22). We found that all LRP5 HBM mutants bound to DKK1 similar to that of the WT LRP5 (Fig. 5A). LRP5N(G171V) was poorly secreted, but if presented in equal amounts as the WT LRP5N in the conditioned media, LRP5N(G171V) showed similar binding to DKK1 as the WT LRP5N (Fig. 5A, lanes 11 and 12). In contrast, LRP5N(E721A) and LRP5N(W781A) displayed a dramatic reduction in DKK1 binding (Fig. 5A). We also tested DKK1 binding to HEK293T cells expressing the WT and various mutant LRP5 using a DKK1-AP (alkaline phosphatase) fusion protein. DKK1-AP exhibited binding to cells expressing the WT LRP5 (Fig. 5B). This binding was greatly enhanced when MESD was co-expressed with LRP5.
LRP5 HBM Mutants Show Reduced Binding and Inhibition by SOST

FIGURE 6. DKK1 inhibits LRP5 HBM mutants and the WT LRP5 similarly. HEK293T cells were transfected with expression plasmid for Wnt10B (3 ng) (A) or Wnt6 (1 ng) (B) alone (lane 2) or together with DKK1 (10 ng) or DKK1 (10 ng) plus LRP5 (1 ng) plasmids as indicated. LRP5(E721A) and LRP5(W781A) are resistant to DKK1 inhibition (lanes 11 and 12). Note that expression plasmids for Fz8 (1 ng) and Kremen2 (3 ng) were co-transfected in all lanes. Asterisks shown above the bars represent statistically significant difference (p < 0.05) between the indicated LRP5 mutant and the WT LRP5.

DISCUSSION

Loss-of-function mutations of LRP5 are associated with familial osteoporosis (10), whereas LRP5 HBM mutations are associated with high bone mass diseases (11–13, 33). LRP5 HBM mutations, which have been identified in several independent studies, are all missense mutations clustered in the first YWTD β-propeller domain of the LRP5 ectodomain (Fig. 1A). These HBM diseases share many common features to Sclerosteosis and Van Buchem disease, which are also disorders of bone overgrowth and are linked to loss-of-function mutations and down-regulation of the SOST gene expression, respectively (26–28). In this study we have shown that LRP5 HBM muta-
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Our data supports previous studies showing that LRP5 HBM mutations do not increase the basal or ligand-dependent activity of LRP5 (22, 24). Our results further suggest that reduced SOST-binding and consequently an escape from SOST mediated inhibition may account for the higher Wnt signaling ability of most, if not all, LRP5 HBM mutants identified thus far. Our data also highlight a critical role of the first YWTD β-propeller of LRP5 in SOST-binding. This conclusion is fully consistent with the emerging picture that distinct β-propeller domains are involved in mediating SOST and DKK1 binding and inhibition of LRP5/LRP6. Indeed DKK1 inhibition of LRP5 relies primarily on the third YWTD β-propeller of LRP5, given that deletion of this β-propeller domain does not affect Wnt1/LRP5 signaling but renders LRP5 resistant to DKK1 inhibition (22). Furthermore, E721A missense mutation in the third β-propeller decreases the affinity of LRP5 for DKK1 and blocks DKK1 inhibitory activity (22). Similarly for LRP6, deletion of the third and the fourth β-propellers, but not of the first and the second β-propellers, abolishes DKK1 binding to LRP6 (41). Consistent with these studies, we found that both missense mutations introduced into the third β-propeller, E721A or W781A, disrupts LRP5 binding to DKK1 (Fig. 5, A and C) and renders LRP5 resistant to DKK1 inhibition (Fig. 6, A and B). In contrast, six...
LRP5 HBM mutations in the first β-propeller do not affect DKK1 binding to LRP5 and have no effect on DKK1 mediated inhibition of LRP5 (Fig. 5, A and C, and Fig. 6, A and B).

A previous study shows that SOST binds to LRP5 via the first and the second β-propellers but not the third and the fourth β-propellers (23). Similar observations have been made for a SOST-related protein 1, SOSTDC1 (WISE, ECTODIN), since SOSTDC1/WISE binding to LRp6 fully depends on the presence of the first and the second β-propellers (42). In agreement with these data we found that LRP5 HBM mutations in the first β-propeller significantly reduce SOST binding to and inhibition of LRP5, whereas missense mutations in the third β-propeller of LRP5 have no effect on SOST binding to and inhibition of LRP5 (Fig. 7, A and C, and Fig. 8, A and B). Therefore our results support the view that SOST binding and inhibition is primarily mediated by the first and possibly the second β-propeller domains of LRP5/LRP6, whereas DKK1 binding and inhibition is mostly via the third and possibly the fourth β-propeller domains of LRP5/6. We note that Ai et al. (24) recently showed that LRP5 HBM mutations might reduce DKK1 binding to LRP5 and make LRP5 more resistant to DKK1 inhibition. At the moment we do not have an explanation for these discrepancies.

G171V was the first identified and studied HBM mutation (11, 12, 22). Paradoxically, LRP5(G171V) shows a significant decrease in cell surface expression compared with the HBM LRP5 mutants (Fig. 2) and to WT LRP5 (22, 24). However, G171V is able to activate Wnt signaling in a similar manner with the WT LRP5 at all different doses tested (Fig. 4). A previous study reported a reduction in G171V binding to MESD (22). On the basis of this observation, a mechanism was proposed that LRP5(G171V), by acquiring ability to signal within a cell, is able to mediate autocrine Wnt signaling while being shielded from inhibition by extracellular Wnt antagonists (22). However, two sets of experimental data indicate that other explanations remain possible. First, cells expressing the WT LRP5 or LRP5(G171V) respond to exogenously added Wnt proteins similarly despite significantly lower surface expression level of LRP5(G171V) (22, 24), suggesting that LRP5(G171V) can reach the cell surface in amounts that are sufficient to mediate Wnt signaling. Second, binding between LRP5 and MESD does not necessarily correlate with the LRP5 folding status (24), as MESD by definition likely interacts with misfolded LRP5 to facilitate LRP5 folding. Indeed we found that MESD is able to promote LRP5(G171V) cell surface expression (Figs. 1C and 2). Despite of the differences discussed above, LRP5(G171V) exhibits reduced SOST binding (Fig. 7A) and is resistant to SOST inhibition similar to the other LRP5 HBM mutants (Fig. 8, A and B). Thus we propose that all LRP5 HBM mutations, including LRP5(G171V), render LRP5 more resistant to SOST inhibition, thereby yielding higher levels of Wnt signaling. However, we cannot rule out the possibility of enhanced autocrine Wnt signaling via LRP5(G171V) as previously proposed for this particular mutation (22).

The increase in bone mineral density found in Sclerosteosis patients is more severe (43) than in patients with HBM diseases linked to LRP5 mutations (11, 12). The Z-scores for the lumbar spine and hip for HBM patients usually measure between 2 and 7, while Z-scores of Sclerosteosis patients ranged from 8 to 14.5 for the lumbar spine and from 8 to 11.5 for the hip. According to our findings, LRP5 HBM mutations render LRP5 resistant to the SOST mediated inhibition of Wnt signaling. However, they cannot affect the SOST-mediated inhibition of LRp6, which is also involved in bone homeostasis (44). On the other hand, a lack of SOST protein in Sclerosteosis patients relieves both LRP5 and LRp6 from all SOST mediated inhibition. This should result in higher Wnt signaling activity in bone tissues than in the case of LRP5 HBM mutations and consequently in higher severity of Sclerosteosis.

In summary, our data show that the primary effect of LRP5 HBM mutations is the disruption of LRP5 interaction with SOST and a consequent reduction of SOST inhibition of Wnt/ LRp5 signaling. Our study suggests that HBM diseases and Sclerosteosis share a common molecular mechanism and further implies that intervention of SOST-LRP5 interaction may represent a therapeutic strategy for the treatment of osteoporosis.

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