a YWTD-type β-propeller and EGF-like domain (Jeon et al., 2001). Each repeat at a similar position in LRP6 and LRP5 is highly conserved, whereas the different repeats within the same protein are considerably more divergent. Interestingly, Bourhis et al. (2010) demonstrated that Wnt9b binds exclusively within the E1-E2 region *in vitro*, whereas Wnt3a binds only to a fragment containing E3-E4, suggesting that each repeat, or a combination of two adjacent repeats, binds to a different subset of Wnt isoforms. This arrangement may accommodate the diversity of Wnt proteins, and possibly also allow for their differential regulation by LRP5/6 antagonist ligands. In Notch and VEGF receptors, whose extracellular regions contain repeats of EGF-like and Ig domains, respectively, binding of multiple ligand isoforms is localized to the same region of one or two repeats, although the presence of other repeats can enhance binding. (Rebay et al., 1991; Davis-Smyth et al., 1996; Cunningham et al., 1997).

For receptor tyrosine kinases, ligand-induced dimerization initiates stimulation of the kinase activity and signaling. While ligand-induced receptor-coreceptor heterodimerization is necessary for canonical Wnt signaling, there is no clearly defined role for LRP5/6 or FZD homodimerization. Forced dimerization of different recombinant LRP6 proteins can either activate or inhibit Wnt signaling.

β-catenin-dependent Wnt signaling is initiated by a Wnt isoform binding to both the receptor FZD and coreceptor LRP5/6, which then assembles a multimeric complex at the cytoplasmic membrane face to recruit and inactivate the kinase GSK3. Whether and how mechanistically different interactions between Wnt isoforms and receptors might modulate this process remains to be determined.

SUMMARY

Disclosed is~~One aspect of the invention provides for~~ an isolated antibody that binds to LRP6, wherein the antibody inhibits signaling induced by a first Wnt isoform and potentiates signaling induced by a second Wnt isoform. In one embodiment, the first Wnt isoform is selected from the group consisting of Wnt3 and Wnt3a. In one embodiment, the second Wnt isoform is selected from the group consisting of Wnt 1, 2, 2b, 4, 6, 7a, 7b, 8a, 9a, 9b, 10a, and 10b. In another embodiment, the first Wnt isoform is selected from the group consisting of Wnts 1, 2, 2b, 6, 8a, 9a, 9b, and 10b and the second Wnt isoform is selected from the group consisting of Wnt3 and Wnt3a.

One ~~aspect~~embodiment of the ~~invention~~disclosure provides for an antibody binds to the E3-E4 region of LRP6. Another ~~aspect~~embodiment of the ~~invention~~disclosure provides for an antibody binds to the E1-E2 region of LRP6. Yet another ~~aspect~~embodiment provides for an antibody that binds to two different regions of LRP6, such as the E1-E2 region of LRP6 and the E3-E4 region. In one ~~aspect~~embodiment these antibodies inhibit Wnt signaling induced by the combination of Wnt1 and Wnt3a. In one ~~aspect~~embodiment these antibodies inhibit autocrine Wnt signaling.

One ~~aspect~~embodiment of the ~~invention~~disclosure provides a method of treating an individual having cancer comprising administering to the individual an effective amount of an isolated antibody that binds to LRP6 and inhibits signaling induced by a Wnt isoform selected from the group consisting of Wnt3 and Wnt3a, and an isolated antibody that binds to LRP6 and inhibits signaling induced by a Wnt isoform selected from the group consisting of Wnt 1, 2, 2b, 6, 8a, 9a, 9b, and 10b.

Another ~~aspect~~embodiment of the ~~invention~~disclosure provides for a method of treating an individual having cancer comprising administering to the individual an effective amount of an isolated antibody that binds to LRP6 and inhibits signaling induced by Wnt3 and Wnt3a, and an isolated antibody that binds to LRP6 and inhibits signaling induced by Wnt 1, 2, 2b, 6, 8a, 9a, 9b, and 10b.

Another ~~aspect~~embodiment of the ~~invention~~disclosure provides for a method of treating an individual having cancer comprising administering to the individual an effective amount of an isolated antibody that binds to LRP6 and inhibits signaling induced byWnt3 and Wnt3a, and an isolated antibody that binds to LRP6 and inhibits signaling induced by Wnt 1, 2, 2b, 4, 6, 7a, 7b, 8a, 9a, 9b, 10a, and 10b.

One ~~aspect~~embodiment of the ~~invention~~disclosure provides a method of treating an individual having a skeletal disorder, such as osteoporosis, osteoarthritis, bone fractures, and bone lesions, comprising administering to the individual an effective amount of an anti-LRP6 antibody described herein.

Another ~~aspect~~embodiment of the ~~invention~~disclosure provides for a method of potentiating Wnt signaling induced by a Wnt isoform in an individual comprising administering to the individual an effective amount of an anti-LRP6 antibody described herein and the Wnt isoform to potentiate Wnt signaling induced by the Wnt isoform.

In one aspect, there is provided an isolated antibody that binds to LRP6, wherein the antibody is selected from the group consisting of :

(i) an antibody comprising
a VH comprising
(a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 17;
(b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 18; and
(c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 19,
and a VL comprising
(g) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 25;
(h) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 26; and
(i) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 27;

(ii) an antibody comprising
a VH comprising
(a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 17;
(b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 18; and
(c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 21,
and a VL comprising
(g) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 25;
(h) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 26; and
(i) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 28; and

(iii) an antibody comprising
a VH comprising
(a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 20;
(b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 18; and
(c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 19,
and a VL comprising
(g) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 25;
(h) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 26; and
(i) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 27.

In one aspect, there is provided an isolated antibody that binds to LRP6, wherein the antibody comprises a VH comprising
(a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 22;
(b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 23; and
(c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 24,
and a VL comprising
(g) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 25;
(h) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 26; and
(i) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 27.

Also provided are specific anti-LRP6 antibodies, including bispecific ant-LRP6 antibodies. In one embodiment, the isolated antibody that binds to LRP6 comprises a VH

comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, and SEQ ID NO: 15. In one embodiment, the antibody further comprises a VL comprising an amino acid sequence selected from the group consisting SEQ ID NO: 10 and SEQ ID NO: 12. In one embodiment, the isolated antibody that binds to LRP6 comprises a VH comprising an amino acid sequence having at least 90% homology to an amino acid sequence of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, and SEQ ID NO: 15. In one embodiment, the isolated antibody that binds to LRP6 further comprises a VL comprising an amino acid sequence having at least 90% homology to an amino acid sequence selected from the group consisting of SEQ ID NO: 10 and SEQ ID NO: 12.

In one aspect, there is provided an isolated bispecific antibody that binds to two different regions of LRP6, wherein the antibody comprises a first VH domain comprising
(a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 20;
(b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 18; and
(c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 19,
a first VL domain comprising
(g) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 25;
(h) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 26; and
(i) HVR-L3 comprising the amino acid sequence SEQ ID NO: 27,
a second VH domain comprising
(d) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 22;
(e) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 23; and
(f) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 24,
and a second VL domain comprising
(g) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 25;
(h) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 26; and
(i) HVR-L3 comprising the amino acid sequence SEQ ID NO: 27.

In one embodiment, the antibody is an isolated bispecific antibody that binds to two different regions of LRP6 wherein the antibody comprises a VH comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, and SEQ ID NO: 15. In one embodiment, the bispecific antibody comprises a first VH comprising the amino acid sequence of SEQ ID NO: 15 and a second VH comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, and SEQ ID NO: 13. In one embodiment, the bispecific antibody further comprises a VL comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 10 and SEQ ID NO: 12.

In one embodiment, the bispecific antibody that binds to two different regions of LRP6 comprises a VH comprising an amino acid sequence having at least 90% homology to an amino acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, or SEQ ID NO: 15. In one embodiment, the bispecific antibody that binds to two different regions of LRP6 comprises a first VH comprising an amino acid sequence having at least 90% homology to an amino acid sequence of SEQ ID NO: 15 and a second VH comprising an amino acid sequence having at least 90% homology to an amino acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, and SEQ ID NO: 13. In one embodiment, the bispecific antibody further comprises a VL comprising an amino acid sequence having at least 90% homology to an amino acid sequence selected from the group consisting of SEQ ID NO: 10, and SEQ ID NO: 12.

In one embodiment, the isolated bispecific antibody that binds to two different regions of LRP6 comprises a first VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID

In one embodiment the bispecific antibody further inhibits signaling induced by a Wnt isoform selected from the group consisting of Wnt 4, 7a, 7b, and 10a. In one embodiment the bispecific antibody inhibits autocrine Wnt signaling.

One ~~aspect~~embodiment of the ~~invention~~disclosure provides a bispecific antibody that inhibits signaling induced by a Wnt isoform selected from the group consisting of Wnt3 and Wnt3a and inhibits signaling induced by a Wnt isoform selected from the group consisting of Wnt 1, 2, 2b, 6, 8a, 9a, 9b, and 10b. In one embodiment, the bispecific antibody further inhibits signaling induced by a Wnt isoform selected from the group consisting of Wnt 4, 7a, 7b, and 10a.

In one aspect, there is provided an antibody that competes for binding to LRP6 with the bispecific antibody as defined above.

One ~~aspect~~embodiment of the ~~invention~~disclosure provides for an antibody that competes for binding to LRP6 with any of the anti-LRP6 antibodies, including the bispecific antibodies, described herein.

In one aspect, there is provided an antibody that binds to the same two epitopes as the bispecific antibody as defined above.

Another ~~aspect~~embodiment of the ~~invention~~disclosure provides for an antibody that binds to the same two epitopes as a bispecific antibody described herein. In one embodiment, one of the two epitopes comprises amino acid residues R28, E51, D52, V70, S71, E73, L95, S96, D98, E115, R141, and N185 of LRP6. In one embodiment, one of the two epitopes comprises amino acid residues R28, E51, D52, V70, S71, E73, L95, S96, D98, E115, R141, N185, R29, W188, K202, P225, H226, S243, and F266 of LRP6.

In one aspect, there is provided an isolated nucleic acid encoding the antibody or bispecific antibody as defined above.

In one aspect, there is provided a host cell comprising the nucleic acid as defined above.

Another ~~aspect~~embodiment of the ~~invention~~disclosure provides an isolated nucleic acid encoding an anti-LRP6 antibody described herein. Another ~~aspect~~embodiment provides for a host cell comprising such a nucleic acid.

In one aspect, there is provided an immunoconjugate comprising the antibody or bispecific antibody as defined above and a cytotoxic agent.

One ~~aspect~~embodiment of the ~~invention~~disclosure provides for an immunoconjugate comprising an anti-LRP6 antibody described herein and a cytotoxic agent. Another ~~aspect~~embodiment provides for a pharmaceutical formulation comprising an anti-LRP6 antibody described herein and a pharmaceutically acceptable carrier.

In one aspect, there is provided a pharmaceutical formulation comprising the antibody or bispecific antibody as defined above and a pharmaceutically acceptable carrier.

In one aspect, there is provided the antibody or bispecific antibody as defined above for use as a medicament.

In one aspect, there is provided the antibody or bispecific antibody as defined above for use in treating cancer.

In one aspect, there is provided the antibody as defined above for use as a medicament useful in the treatment of a skeletal disorder.

In one aspect, there is provided the use of the antibody or bispecific antibody as defined above in the manufacture of a medicament.

In one aspect, there is provided the use of the antibody or bispecific antibody as defined above in the manufacture of a medicament for treating cancer.

In one aspect, there is provided the use of the antibody as defined above in the manufacture of a medicament for treating a skeletal disorder.

One ~~aspect~~embodiment of the ~~invention~~disclosure provides for an anti-LRP6 antibody described herein for use as a medicament. One ~~aspect~~embodiment provides for an anti-LRP6 antibody described herein for use in treating cancer or a skeletal disorder. One ~~aspect~~embodiment provides for an anti-LRP6 antibody described herein for use in inhibiting signaling induced by a first Wnt isoform and potentiating signaling induced by a second Wnt isoform. One ~~aspect~~embodiment provides for use of an anti-LRP6 antibody described herein for use in the manufacture of a medicament, useful in treating, for example, cancer or a skeletal disorder.

One ~~aspect~~embodiment of the ~~invention~~disclosure provides a method of treating an individual having cancer, such as non-small cell lung cancer, breast cancer, pancreatic cancer, ovarian cancer, kidney cancer, and prostate cancer, comprising administering to the individual an effective amount of an anti-LRP6 antibody described herein.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1A. Graph showing inhibition of Wnt luciferase reporter activity in HEK293 cells induced with 0.1 mg/ml purified Wnt3a by antibodies against LRP6.E3-E4 protein.

Figure 1B. Western blot analysis of HEK293 cells either unstimulated or induced with Wnt3a and treated with the indicated LRP6 antibody or purified protein

Figure 1C. Graph showing that the YW210.09 antibody potentiates Wnt reporter gene activity in a manner proportional to Wnt3a concentration in HEK293 cells.

Figure 2A. Graph showing concentration-dependent inhibition and potentiation of autocrine Wnt signaling in PA-1 teratocarcinoma cells transfected with luciferase reporters and treated with LRP6 antibodies, either individually or in combination, or Fzd8CRD-Fc protein.

Figure 2B. Graph showing result of qPCR expression analysis of Wnt-induced genes SAX1 and GAD1 and Wnt-repressed gene LEFTY2 in PA-1 cells treated with or without 0.3 mg/ml Wnt3a protein, and treated with 10 mg/ml YW211.31 antibody, anti-gD monoclonal antibody (negative control) or Fzd8CRD-Fc protein (positive control). Data is normalized to samples from cells with no addition (NA) of Wnt3a .

Figure 3. Summary table showing the effects of LRP6 antibodies and Fzd8CRD-Fc protein on autocrine signaling in cell lines.

Figure 4A. Graph depicting the result of qPCR expression analysis of AXIN2 mRNA in four cell lines treated with 25 μg/ml YW211.31.57 antibody or Fzd8CRD-Fc protein, with and without (NA) 0.2 μg/ml Wnt3a.

Figure 4B. Graph depicting expression of Wnt-induced genes in NCI-H23cells is potentiated by YW211.31.57 and antagonized by YW210.09 antibody (30 μg/ml). CD4-Fc protein (30 μg/ml) serves as a negative control.

Figure 4C. Graph depicting expression of Wnt-induced genes in M14 cells is potentiated by YW211.31.57 and antagonized by YW210.09 antibody (30 μg/ml).