BRIEF SUMMARY OF THE INVENTION

[0011] The present ~~invention~~disclosure provides a variety of agents that bind to one or more human Wnt proteins, including, but not limited to, soluble FZD receptors and other agents comprising a Fri domain, and novel methods of using those agents. The ~~invention~~disclosure further provides methods of using the agents in the treatment of cancer by administering the agents to a subject in need thereof. In some ~~embodiments~~examples, the methods comprise inhibiting the growth of cancer cells. In certain ~~embodiments~~examples, the Wnt-binding agent is a Wnt antagonist. Novel methods of screening for such Wnt-binding agents are also provided. Polynucleotides encoding the agents, methods of making the agents, and a variety of compositions comprising the agents are likewise provided.

[0011a] In one aspect, there is provided a Wnt-binding agent comprising:

(a) a first polypeptide consisting of SEQ ID NO:39; and

(b) a second polypeptide comprising SEQ ID NO:43;

wherein the first polypeptide is directly linked to the second polypeptide.

[0011b] In another aspect, there is provided a Wnt-binding agent comprising:

(a) a first polypeptide comprising amino acids X to Y of SEQ ID NO:30; and

(b) a second polypeptide comprising SEQ ID NO:43;

wherein the first polypeptide is directly linked to the second polypeptide; and

wherein X = amino acid 25, 26, 27, or 28; and

Y = amino acid 158, 159, 160, 161, 162, 163, or 164.

[0011c] In yet another aspect, there is provided a Wnt-binding agent comprising the amino acid sequence of SEQ ID NO:53.

[0011d] In yet another aspect, there is provided a Wnt-binding agent consisting of SEQ ID NO:53.

[0011e] In yet another aspect, there is provided a polypeptide comprising the Wnt-binding agent as defined herein, and further comprising the amino acid sequence of SEQ ID NO:72.

[0011f] In yet another aspect, there is provided a polypeptide consisting of SEQ ID NO:72 and SEQ ID NO:53.

[0011g] In yet another aspect, there is provided a cell producing the Wnt-binding agent as defined herein.

[0011h] In yet another aspect, there is provided a cell comprising the polypeptide as defined herein.

[0011i] In yet another aspect, there is provided a composition comprising the Wnt-binding agent as defined herein, wherein at least about 80% of the Wnt-binding agent has an N-terminal amino acid sequence of ASA.

[0011j] In yet another aspect, there is provided a pharmaceutical composition comprising the Wnt-binding agent as defined herein, and a pharmaceutically acceptable carrier.

[0011k] In yet another aspect, there is provided a polynucleotide comprising a polynucleotide that encodes the Wnt-binding agent as defined herein.

[0011l] In yet another aspect, there is provided a polynucleotide comprising a polynucleotide that encodes the polypeptide as defined herein.

[0011m] In yet another aspect, there is provided a cell comprising the polynucleotide as defined herein.

[0011n] In yet another aspect, there is provided the use of the Wnt-binding agent as defined herein in the manufacture of a medicament for the treatment of cancer.

[0011o] In yet another aspect, there is provided the use of the Wnt-binding agent as defined herein in the manufacture of a medicament for treatment of a disease associated with Wnt signaling activation.

[0011p] In yet another aspect, there is provided a method of producing the Wnt-binding agent as defined herein, wherein at least about 80% of the Wnt-binding agent has an N-terminal amino acid sequence of ASA, the method comprising using a signal sequence of SEQ ID NO:72for production of the Wnt-binding agent.

[0011q] In yet another aspect, there is provided a polypeptide comprising:
(a) a signal sequence of SEQ ID NO:72;
(b) a Fri domain of human FZD8; and
(c) a human Fc region.

[0011r] In yet another aspect, there is provided a polynucleotide comprising a polynucleotide that encodes the polypeptide as defined herein.

[0011s] In yet another aspect, there is provided a cell comprising the polynucleotide as defined herein.

[0011t] In yet another aspect, there is provided a cell comprising the polypeptide as defined herein.

[0011u] In yet another aspect, there is provided a method of producing a soluble Wnt-binding agent which comprises a Fri domain of human FZD8 in a cell, wherein at least about 80% of the Wnt-binding agent has an N-terminal amino acid sequence of ASA, the method comprising using a signal sequence of SEQ ID NO:72 for production of the Wnt-binding agent.

[0011v] In yet another aspect, there is provided a soluble Wnt-binding agent produced by the method as defined herein.

[0011w] In yet another aspect, there is provided a composition comprising a soluble Wnt-binding agent which comprises a Fri domain of human FZD8, wherein at least about 80% of the Wnt-binding agent has an N-terminal sequence of ASA.

[0012] Also disclosed is~~Thus, in one aspect, the invention provides~~ a method of inhibiting the growth of a tumor. In certain ~~embodiments~~examples, the method comprises contacting the tumor with an effective amount of an agent that binds to one or more Wnt proteins (e.g., human Wnt proteins). The method may be *in vivo* or *in vitro*. In certain ~~embodiments~~examples, the tumor is in a subject, and the contacting of the tumor with the agent comprises administration of a therapeutically effective amount of the Wnt-binding agent to the subject.

[0013] Also disclosed is~~In another aspect, the invention provides~~ a method of reducing the frequency of cancer stem cells in a tumor comprising cancer stem cells. Accordingly, the ~~invention~~disclosure also provides methods of reducing the tumorigenicity of tumors. In some ~~embodiments~~examples, the methods comprise contacting the tumor with an effective amount of an agent that binds to one or more Wnt proteins (e.g., human Wnt proteins). The method may be *in vivo* or *in vitro*. For example, the contacting may comprise administration of the effective amount of the Wnt-binding agent to a human having the tumor.

[0014] Also disclosed is~~In another aspect, the invention provides~~ a method of inducing cells in a tumor to differentiate or inducing expression of differentiation markers in a tumor. In certain ~~embodiments~~examples, the method comprises contacting the tumor with an effective amount of an agent that binds to one or more Wnt proteins (e.g., human Wnt proteins). The method may be *in vivo* or *in vitro*.

[0015] Also disclosed is~~In a still further aspect, the invention provides~~ a method of treating a cancer in a subject, comprising administering a therapeutically effective amount of the Wnt-binding agent to the subject.

[0016] Also disclosed is~~In an additional aspect, the invention provides~~ a method of treating a disease in a subject wherein the disease is associated with Wnt signaling activation, comprising administering a therapeutically effective amount of the Wnt-binding agent to the subject.

[0017] Also disclosed is~~In yet another aspect, the invention provides~~ a method of treating a disorder in a subject, wherein the disorder is characterized by an increased level of stem cells and/or progenitor cells, comprising administering a therapeutically effective amount of the Wnt-binding agent to the subject.

[0018] In certain ~~embodiments~~examples of each of the aforementioned aspects, as well as other aspects provided herein, the Wnt-binding agent is a polypeptide. In certain ~~embodiments~~examples, the agent is a soluble receptor.

[0019] Also disclosed~~In certain embodiments of each of the aforementioned aspects, as well as other aspects provided~~ herein, the agent comprises a Fri domain of a FZD receptor, or a fragment of a FZD Fri domain that binds one or more Wnt proteins. In certain ~~embodiments~~examples, the FZD receptors are human FZD receptors. For example, the Fri domain may be from human FZD4 or human FZD5. In certain alternative ~~embodiments~~examples, the agent may comprise a Fri domain of a human FZD8 receptor or a fragment of that Fri domain that binds one or more Wnt proteins. In certain ~~embodiments~~examples, the Wnt-binding agent is a soluble FZD receptor. In alternative ~~embodiments~~examples, the Wnt-binding agent does not comprise a Fri domain of a FZD receptor.

[0020] Also disclosed~~In certain embodiments of each of the aforementioned aspects, as well as other aspects provided~~ herein, the agent comprises a Fri domain of a soluble Frizzled-related protein (SFRP), or a fragment of a SFRP Fri domain that binds one or more Wnt proteins. In certain ~~embodiments~~examples the SFRP is a human SFRP.

[0021] Also disclosed~~In certain embodiments of each of the aforementioned aspects, as well as other aspects provided~~ herein, the agent comprises a Fri domain of a Ror protein or a fragment of a Ror Fri domain that binds one or more Wnt proteins. In certain ~~embodiments~~examples the Ror protein is a human Ror protein.

[0022] Also disclosed~~In certain embodiments of each of the aforementioned aspects, as well as other aspects provided~~ herein, the agent further comprises a human Fc region. In certain ~~embodiments~~examples, the Wnt-binding agent is a fusion protein. In certain ~~embodiments~~examples, the Wnt-binding agent comprises SEQ ID NO:1. In certain ~~embodiments~~examples, the Wnt-binding agent comprises SEQ ID NO:46. In certain ~~embodiments~~examples, the Wnt-binding agent comprises SEQ ID NO:48. In certain ~~embodiments~~examples, the Wnt-binding agent comprises SEQ ID NO:50. In certain ~~embodiments~~examples, the Wnt-binding agent comprises SEQ ID NO:53. In some ~~embodiments~~examples, the Wnt-binding agent (before signal sequence cleavage) comprises SEQ ID NO:50 and a signal sequence selected from the group consisting of SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, and SEQ ID NO:74. In some ~~embodiments~~examples, the Wnt-binding agent (before signal sequence cleavage) comprises SEQ ID NO:50 and a signal sequence selected from the group consisting of SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, and SEQ ID NO:74. In some ~~embodiments~~examples, the Wnt-binding agent (before signal sequence cleavage) comprises SEQ ID NO:50 and SEQ ID NO:71. In some ~~embodiments~~examples, the Wnt-binding agent (before signal sequence cleavage) comprises SEQ ID NO:53 and a signal sequence selected from the group consisting of SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, and SEQ ID NO:74. In some ~~embodiments~~examples, the Wnt-binding agent (before signal sequence cleavage) comprises SEQ ID NO:53 and a signal sequence selected from the group consisting of SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, and SEQ ID NO:74. In some ~~embodiments~~examples, the Wnt-binding agent (before signal sequence cleavage) comprises SEQ ID NO:53 and SEQ ID NO:71.

[0023] Also disclosed~~In certain embodiments of each of the aforementioned aspects, as well as other aspects provided~~ herein, the Wnt-binding agent binds to one or more, two or more, three or more, or four or more human Wnt proteins selected from the group consisting of Wnt1, Wnt2, Wnt2b, Wnt3, Wnt3a, Wnt7a, Wnt7b, Wnt8a, Wnt8b, Wnt10a, and Wnt10b. In certain ~~embodiments~~examples, the agent binds to Wnt1, Wnt2, Wnt3, Wnt3a, and Wnt7b.

[0024] Also disclosed~~In certain embodiments of each of the aforementioned aspects, as well as other aspects provided~~ herein, the agent is a Wnt antagonist. In certain ~~embodiments~~examples, the agent inhibits Wnt signaling. In certain ~~embodiments~~examples, the agent inhibits Wnt canonical Wnt signaling.

[0025] Also disclosed~~In certain embodiments of each of the aforementioned aspects, as well as other aspects provided~~ herein, the tumor or cancer is a tumor/cancer selected from the group consisting of colorectal tumor/cancer, pancreatic tumor/cancer, lung tumor/cancer, ovarian tumor/cancer, liver tumor/cancer, breast tumor/cancer, kidney tumor/cancer, prostate tumor/cancer, gastrointestinal tumor/cancer, melanoma, cervical tumor/cancer, bladder tumor/cancer, glioblastoma, and head and neck tumor/cancer.

[0026] Also disclosed~~In certain embodiments of each of the aforementioned aspects, as well as other aspects provided~~ herein, the methods further comprise contacting the tumor with a second therapeutic agent or administering a second therapeutic agent to the subject. In certain ~~embodiments~~examples, the second therapeutic agent is a chemotherapeutic agent. In certain ~~embodiments~~examples, the second chemotherapeutic agent is an antimetabolite (e.g., gemcitabine) or an antimitotic agent (e.g., a taxane such as paclitaxel).

[0027] Also disclosed is~~In a still further aspect, the invention provides~~ a polypeptide comprising a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:65 and SEQ ID NO:66, as well as cells producing and compositions comprising the polypeptide. Pharmaceutical compositions comprising the polypeptide and a pharmaceutically acceptable carrier are also provided. In addition, polynucleotides comprising a polynucleotide that encodes the polypeptides of SEQ ID NO:1, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:65 and SEQ ID NO:66 or having the sequence of SEQ ID NO:2 are also provided. Vectors and cells comprising the polynucleotides are likewise provided.

[0028] Also disclosed is~~In an additional aspect, the invention provides~~ a method of screening an agent for anti-tumor activity and/or activity against cancer stem cells. In certain ~~embodiments~~examples, the method comprises comparing the level of one or more differentiation markers and/or one or more stemness markers in a first solid tumor (e.g., a solid tumor comprising cancer stem cells) that has been exposed to an agent to the level of the one or more differentiation markers in a second solid tumor that has not been exposed to the agent. In some ~~embodiments~~examples, the method comprises: (a) exposing a first solid tumor, but not a second solid tumor, to the agent; (b) assessing the level of one or more differentiation markers and/or one or more

stemness markers in the first and second solid tumors; and (c) comparing the level of the one or more differentiation markers in the first tumor to the level of the one or more differentiation markers in the second solid tumor. In certain ~~embodiments~~examples, the (a) increased levels of the one or more differentiation markers in the first solid tumor relative to the second solid tumor indicates anti-tumor or anti-cancer stem cell activity of the agent; and/or (b) decreased levels of the one or more stemness markers indicates anti-tumor or anti-cancer stem cell activity of the agent. In certain ~~embodiments~~examples, the agent binds one or more Wnt proteins. In certain ~~embodiments~~examples, the agent is a soluble FZD receptor. In certain methods, the agent is an antibody, such as an anti-FZD or anti-Wnt antibody. In certain alternative ~~embodiments~~examples, the agent is a small molecule.

[0029] Where ~~aspects or embodiments~~examples of the ~~invention~~disclosure are described in terms of a Markush group or other grouping of alternatives, the present ~~invention~~disclosure encompasses not only the entire group listed as a whole, but each member of the group individually and all possible subgroups of the main group, but also the main group absent one or more of the group members. The present ~~invention~~disclosure also envisages the explicit exclusion of one or more of any of the group members in the claimed ~~invention~~disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0030] Figure 1. Pharmacokinetics of FZD8-Fc (54F03) in the rat. Administration of a single dose (10mg/kg) of FZD8-Fc was followed by assessment of the pharmacokinetic properties of FZD8-Fc. Serum concentrations of FZD8-Fc were determined at 1, 24, 48, 72, 96, 168, 240 and 336 hours post-administration.

[0031] Figure 2. Inhibition of PN4 pancreas tumor growth by FZD8-Fc (54F03) treatment. PN4 pancreas tumor cells were injected subcutaneously into NOD/SCID mice. Mice were treated with FZD8-Fc (-▲-), gemcitabine (-■-), a combination of FZD8-Fc and gemcitabine (-Δ-), or a control antibody (-●-). Data are shown as tumor volume (mm^3), over days post treatment.

[0032] Figure 3. Reduction of $CD44^{hi}$ cell population in PN4 tumors treated with FZD8-Fc (54F03). Cell surface staining for ESA and CD44 on tumor cells treated with control antibody, FZD8-Fc, gemcitabine, or a combination of FZD8-Fc and gemcitabine was performed. For each treatment group, single cell suspensions from five tumors were pooled prior to staining.

[0033] Figure 4. *In vivo* limiting dilution assay of FZD8-Fc (54F03)-treated PN4 pancreatic tumors.

[0034] Figure 5. Increased cell differentiation of PN4 pancreatic tumors treated with FZD8-Fc (54F03). Paraffin embedded sections of PN4 tumors treated with control antibody, FZD8-Fc, gemcitabine, or a combination of FZD8-Fc and gemcitabine were stained with alcian blue to detect mucin-expressing cells.

[0035] Figure 6. Increased cell differentiation of PN8 pancreatic tumors treated with FZD8-Fc (54F03). Paraffin embedded sections of PN8 tumors treated with control antibody, FZD8-Fc, gemcitabine, or a combination of FZD8-Fc and gemcitabine were stained with alcian blue to detect mucin-expressing cells.