NOTCH-BINDING AGENTS AND ANTAGONISTS AND METHODS OF USE THEREOF

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] The present invention relates to compositions comprising an agent that binds a human Notch receptor and methods of using those compositions for the treatment of cancer and other diseases. More specifically, the present invention provides, for example, antibodies that specifically bind to a non-ligand binding region of the extracellular domain of a human Notch receptor and inhibit tumor growth. The present invention further provides methods of treating cancer, the method comprising administering a therapeutically effective amount of an antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human Notch receptor protein and inhibits tumor growth.

Background

[0002] The Notch signaling pathway is one of several critical regulators of embryonic pattern formation, post-embryonic tissue maintenance, and stem cell biology. More specifically, Notch signaling is involved in the process of lateral inhibition between adjacent cell fates and plays an important role in cell fate determination during asymmetric cell divisions. Unregulated Notch signaling is associated with numerous human cancers where it can alter the developmental fate of tumor cells to maintain them in an undifferentiated and proliferative state (Brennan and Brown, 2003, *Breast Cancer Res.* 5:69). Thus carcinogenesis can proceed by usurping homeostatic mechanisms controlling normal development and tissue repair by stem cell populations (Beachy et al., 2004, *Nature* 432:324).

[0003] The Notch receptor was first identified in Drosophila mutants with haploinsufficiency resulting in notches at the wing margin, whereas loss-of-function produces an embryonic lethal "neurogenic" phenotype where cells of the epidermis switch fate to neural tissue (Moohr, 1919, *Genet.* 4:252; Poulson, 1937, *PNAS* 23:133; Poulson, 1940, *J. Exp. Zool.* 83:271). The Notch receptor is a single-pass transmembrane receptor containing numerous tandem epidermal growth factor (EGF)-like repeats and three cysteine-rich Notch/LIN-12 repeats within a large extracellular domain (Wharton et al., 1985, *Cell* 43:567; Kidd et al., 1986, *Mol. Cell Biol.* 6:3094; reviewed in Artavanis et al., 1999, *Science* 284:770). Four mammalian Notch proteins have been identified (Notch1, Notch2, Notch3, and Notch4), and mutations in these receptors invariably result in developmental abnormalities and human pathologies including several cancers as described in detail below (Gridley, 1997, *Mol. Cell Neurosci.* 9:103; Joutel & Tournier-Lasserve, 1998, *Semin. Cell Dev. Biol.* 9:619-25).

[0004] Notch receptors are activated by single-pass transmembrane ligands of the Delta, Serrated, Lag-2 (DSL) family. There are five known Notch ligands in mammals: Delta-like 1 (DLL1), Delta-like 3 (DLL3), Delta-like 4 (DLL4), Jagged 1 (JAG1) and Jagged 2 (JAG2) characterized by a DSL domain and

tandem EGF-like repeats within the extracellular domain. The extracellular domain of the Notch receptor interacts with that of its ligands, typically on adjacent cells, resulting in two proteolytic cleavages of Notch, one extracellular cleavage mediated by an ADAM (A Disintegrin And Metallopeptidase) protease and one cleavage within the transmembrane domain mediated by gamma secretase. This latter cleavage generates the Notch intracellular domain (ICD), which then enters the nucleus where it activates the CBF1, Suppressor of Hairless [Su(H)], Lag-2 (CSL) family of transcription factors as the major downstream effectors to increase transcription of nuclear basic helix-loop-helix transcription factors of the Hairy and Enhancer of Split [E(spl)] family (Artavanis et al., 1999, *Science* 284:770; Brennan and Brown, 2003, *Breast Cancer Res.* 5:69; Iso et al., 2003, Arterioscler. *Thromb. Vasc. Biol.* 23:543). Alternative intracellular pathways involving the cytoplasmic protein Deltex identified in Drosophila may also exist in mammals (Martinez et al., 2002, *Curr. Opin. Genet. Dev.* 12:524-33), and this Deltex-dependent pathway may act to suppress expression of Wnt target genes (Brennan et al., 1999, *Curr. Biol.* 9:707-710; Lawrence et al., 2001, *Curr. Biol.* 11:375-85).

[0005] Mammalian Notch receptors undergo cleavage to form the mature receptor and also following ligand binding to activate downstream signaling. A furin-like protease cleaves the Notch receptors during maturation to generate juxtamembrane heterodimers that comprise a non-covalently associated extracelluar subunit and a transmembrane subunit held together in an auto-inhibitory state. Ligand binding relieves this inhibition and induces cleavage of the Notch receptor by an ADAM-type metalloprotease and a gamma-secretase, the latter of which releases the intracellular domain (ICD) into the cytoplasm, allowing it to translocate into the nucleus to activate gene transcription. Cleavage by ADAM occurs within the non-ligand binding cleavage domain within the membrane proximal negative regulatory region.

[0006] Hematopoietic stem cells (HSCs) are the best understood stem cells in the body, and Notch signaling is implicated in their normal maintenance as well as in leukemic transformation (Kopper & Hajdu, 2004, *Pathol. Oncol. Res.* 10:69-73). HSCs are a rare population of cells that reside in a stromal niche within the adult bone marrow. These cells are characterized both by a unique gene expression profile as well as an ability to continuously give rise to more differentiated progenitor cells to reconstitute the entire hematopoietic system. Constitutive activation of Notch1 signaling in HSCs and progenitor cells establishes immortalized cell lines that generate both lymphoid and myeloid cells *in vitro* and in long-term reconstitution assays (Varnum-Finney et al., 2000, *Nat. Med.* 6:1278-81), and the presence of Jagged1 increases engraftment of human bone marrow cell populations enriched for HSCs (Karanu et al., 2000, *J. Exp. Med.* 192:1365-72). More recently, Notch signaling has been demonstrated in HSCs *in vivo* and shown to be involved in inhibiting HSC differentiation. Furthermore, Notch signaling appears to be required for Wnt-mediated HSC self-renewal (Duncan et al., 2005, *Nat. Immunol.* 6:314).

[0007] The Notch signaling pathway also plays a central role in the maintenance of neural stem cells and is implicated in their normal maintenance as well as in brain cancers (Kopper & Hajdu, 2004, *Pathol. Oncol. Res.* 10:69-73; Purow et al., 2005, *Cancer Res.* 65:2353-63; Hallahan et al., 2004, *Cancer Res.* 64:7794-800). Neural stem cells give rise to all neuronal and glial cells in the mammalian nervous system during development, and more recently have been identified in the adult brain (Gage, 2000, *Science* 287:1433-8). Mice deficient for Notch1; the Notch target genes Hes1, 3, and 5; and a regulator of Notch signaling presenilin1 (PS1) show decreased numbers of embryonic neural stem cells. Furthermore, adult neural stem cells are reduced in the brains of PS1 heterozygote mice (Nakamura et al., 2000, *J. Neurosci.* 20:283-93; Hitoshi et al., 2002, *Genes Dev.* 16:846-58). The reduction in neural stem cells appears to result from their premature differentiation into neurons (Hatakeyama et al., 2004, *Dev.* 131:5539-50) suggesting that Notch signaling regulates neural stem cell differentiation and self-renewal.

[0008] Aberrant Notch signaling is implicated in a number of human cancers. The Notch1 gene in humans was first identified in a subset of T-cell acute lymphoblastic leukemias as a translocated locus resulting in activation of the Notch pathway (Ellisen et al., 1991, *Cell* 66:649-61). Constitutive activation of Notch1 signaling in T-cells in mouse models similarly generates T-cell lymphomas suggesting a causative role (Robey et al., 1996, *Cell* 87:483-92; Pear et al., 1996, *J. Exp. Med.* 183:2283-91; Yan et al., 2001, *Blood* 98:3793-9; Bellavia et al., 2000, *EMBO J.* 19:3337-48). Notch1 point mutations, insertions, and deletions producing aberrant Notch1 signaling have also been found to be frequently present in both childhood and adult T-cell acute lymphoblastic leukemia/lymphoma (Pear & Aster, 2004, *Curr. Opin. Hematol.* 11:416-33).

[0009] The frequent insertion of the mouse mammary tumor virus into both the Notch1 and Notch4 locus in mammary tumors and the resulting activated Notch protein fragments first implicated Notch signaling in breast cancer (Gallahan & Callahan, 1987, *J. Virol.* 61:66-74; Brennan & Brown, 2003, *Breast Cancer Res.* 5:69; Politi et al., 2004, *Semin. Cancer Biol.* 14:341-7). Further studies in transgenic mice have confirmed a role for Notch in ductal branching during normal mammary gland development, and a constitutively active form of Notch4 in mammary epithelial cells inhibits epithelial differentiation and results in tumorigenesis (Jhappan et al., 1992, *Genes & Dev.* 6:345-5; Gallahan et al., 1996, *Cancer Res.* 56:1775-85; Smith et al., 1995, *Cell Growth Differ.* 6:563-77; Soriano et al., 2000, *Int. J. Cancer* 86:652-9; Uyttendaele et al., 1998, *Dev. Biol.* 196:204-17; Politi et al., 2004, *Semin. Cancer Biol.* 14:341-7). Evidence for a role for Notch in human breast cancer is provided by data showing the expression of Notch receptors in breast carcinomas and their correlation with clinical outcome (Weijzen et al., 2002, *Nat. Med.* 8:979-86; Parr et al., 2004, *Int. J. Mol. Med.* 14:779-86). Furthermore, overexpression of the Notch pathway has been observed in cervical cancers (Zagouras et al., 1995, *PNAS* 92:6414-8), renal cell carcinomas (Rae et al., 2000, *Int. J. Cancer* 88:726-32), head and neck squamous cell carcinomas (Leethanakul et al., 2000, *Oncogene* 19:3220-4), endometrial cancers (Suzuki et al., 2000, *Int. J. Oncol.*

17:1131-9), and neuroblastomas (van Limpt et al., 2000, *Med. Pediatr. Oncol.* 35:554-8), suggestive of a potential role for Notch in the development of a number of neoplasms. Interestingly, Notch signaling may play a role in the maintenance of the undifferentiated state of Apc-mutant neoplastic cells of the colon (van Es & Clevers, 2005, *Trends in Mol. Med.* 11:496-502).

[0010] The Notch pathway is also involved in multiple aspects of vascular development including proliferation, migration, smooth muscle differentiation, angiogenesis and arterial-venous differentiation (Iso et al., 2003, *Arterioscler. Thromb. Vasc. Biol.* 23:543). For example, homozygous null mutations in Notch1/4 and Jagged1 as well as heterozygous loss of DLL4 result in severe though variable defects in arterial development and yolk sac vascularization. Furthermore, DLL1-deficient and Notch2-hypomorphic mice embryos show hemorrhaging that likely results from poor development of vascular structures (Gale et al., 2004, *PNAS*, 101:15949-54; Krebs et al., 2000, *Genes Dev.* 14:1343-52; Xue et al., 1999, *Hum. Mel. Genet.* 8:723-30; Hrabe de Angelis et al., 1997, *Nature* 386:717-21; McCright et al., 2001, *Dev.* 128:491-502). In humans, mutations in Jagged1 are associated with Alagille syndrome, a developmental disorder that includes vascular defects, and mutations in Notch3 are responsible for an inherited vascular dementia (Cadasil) in which vessel homeostasis is defective (Joutel et al., 1996, *Nature* 383:707-10).

[0011] Anti-Notch antibodies and their possible use as anti-cancer therapeutics have been previously reported. See, e.g., U.S. Patent Application Publication No. 2008/0131434, which is incorporated by reference herein in its entirety. See also International Publication Nos. WO 2008/057144 and WO 2008/076960, as well as U.S. Patent Application Publication Nos. 2008/0226621, 2008/0118520, and 2008/0131908.

BRIEF SUMMARY OF THE INVENTION

[0012] The present invention provides novel Notch-binding agents and novel antagonists of one or more human Notch receptors, as well as methods of using those agents and antagonists. The present invention further provides novel polypeptides, such as antibodies that bind one or more human Notch receptors, fragments of such antibodies, and other polypeptides related to such antibodies. In certain embodiments, the invention provides antagonists of human Notch2 and/or human Notch3, including, but not limited to, antibodies that specifically bind human Notch2 and/or human Notch3. As used herein, the phrase "Notch2 and/or Notch3" means "Notch2," "Notch3," or "both Notch2 and Notch3." In certain embodiments, the antibodies or other antagonists bind to a region of the Notch receptor that is outside of the ligand-binding domain (e.g., EGF10 of Notch2 or EGF9 of Notch3). In certain embodiments, the antibodies specifically bind human Notch2. In certain embodiments, the antibodies specifically bind both human Notch2 and human Notch3. In some embodiments, the antibodies specifically bind human Notch3. Polynucleotides comprising nucleic acid sequences encoding the polypeptides are also provided,

as are vectors comprising the polynucleotides. Cells comprising the polypeptides and/or polynucleotides of the invention are further provided. Compositions (e.g., pharmaceutical compositions) comprising the novel Notch antagonists are also provided. Methods of using the agents and antagonists are also provided, such as methods of using the Notch antagonists to inhibit tumor growth, reduce the tumorigenicity of tumors, inhibit angiogenesis, and/or treat cancer or other diseases associated with angiogenesis.

[0013] In one aspect, the invention provides an agent (e.g., an antibody) that specifically binds to an EGF10 domain (or an equivalent of an EGF10 domain) of one or more human Notch receptors. In certain embodiments, the agent is an antibody. In certain embodiments, the agent is an antagonist. In certain embodiments, the agent specifically binds to EGF10 of human Notch2 and/or EGF9 of human Notch3. EGF9 is the EGF within human Notch3 that is equivalent to EGF10 in the other human Notch receptors Notch1, Notch2, and Notch4. In some embodiments, the agent specifically binds to EGF10 of Notch 2. In some embodiments, the agent specifically binds to EGF10 of Notch 2 and to EGF9 of Notch 3. In some embodiments, the agent specifically binds to EGF9 of Notch 3. In other embodiments, the agent binds to at least part of the sequence HKGAL (SEQ ID NO:28) within Notch2 EGF10. In some embodiments, the agent binds to at least part of the sequence HEDAI (SEQ ID NO:29) within Notch3 EGF9.

[0014] In certain embodiments of each of the aforementioned aspects or embodiments, as well as other aspects and/or embodiments described elsewhere herein, the agent inhibits binding of a ligand to human Notch2 and/or Notch3. In some embodiments, the agent inhibits binding of a ligand to human Notch2. In some embodiments, the agent inhibits binding of a ligand to Notch2 and Notch3. In other embodiments, the agent inhibits binding of a ligand to Notch3. In certain embodiments, the ligand is DLL4, JAG1 or JAG2. In other embodiments, the agent inhibits signaling of human Notch2 and/or Notch3. In some embodiments, the agent inhibits signaling of human Notch2. In some embodiments, the agent inhibits signaling of Notch2 and Notch3. In other embodiments, the agent inhibits signaling of Notch3. In some embodiments Notch2 and/or Notch3 signaling is induced by DLL4, JAG1 or JAG2. Pharmaceutical compositions comprising the agent and methods of using the agent for such uses as inhibiting angiogenesis, inhibiting tumor growth, reducing the tumorigenicity of a tumor, and/or treating cancer are also provided.

[0015] In a further aspect, the invention provides an antibody that specifically binds human Notch2 and/or Notch3, wherein the antibody comprises (a) a heavy chain CDR1 comprising SSSGMS (SEQ ID NO:5), a heavy chain CDR2 comprising VIASSGSNTYYADSVKG (SEQ ID NO:6), and/or a heavy chain CDR3 comprising SIFYTT (SEQ ID NO:51); and/or (b) a light chain CDR1 comprising RASQSVRSNYLA (SEQ ID NO:8), a light chain CDR2 comprising GASSRAT (SEQ ID NO:9), and/or a light chain CDR3 comprising QQYSNFPI (SEQ ID NO:10). In some embodiments, the antibody comprises (a) a heavy chain CDR1 comprising SSSGMS (SEQ ID NO:5), or a variant thereof comprising

1, 2, 3, or 4 conservative amino acid substitutions; a heavy chain CDR2 comprising VIASSGSNTYYADSVKG (SEQ ID NO:6), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; and/or a heavy chain CDR3 comprising SIFYTT (SEQ ID NO:51), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; and/or (b) a light chain CDR1 comprising RASQSVRSNYLA (SEQ ID NO:8), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; a light chain CDR2 comprising GASSRAT (SEQ ID NO:9), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; and/or a light chain CDR3 comprising QQYSNFPI (SEQ ID NO:10), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions. Pharmaceutical compositions comprising the antibody and methods of using the antibody for such uses as inhibiting angiogenesis, inhibiting tumor growth, reducing the tumorigenicity of a tumor, and/or treating cancer are also provided.

[0016] In a further aspect, the invention provides an antibody that specifically binds human Notch2 and/or Notch3, wherein the antibody comprises (a) a heavy chain CDR1 comprising SSSGMS (SEQ ID NO:5), a heavy chain CDR2 comprising VIASSGSNTYYADSVKG (SEQ ID NO:6), and/or a heavy chain CDR3 comprising GIFFAI (SEQ ID NO:7); and/or (b) a light chain CDR1 comprising RASQSVRSNYLA (SEQ ID NO:8), a light chain CDR2 comprising GASSRAT (SEQ ID NO:9), and/or a light chain CDR3 comprising QQYSNFPI (SEQ ID NO:10). In certain embodiments, the antibody specifically binds Notch2. In some embodiments, the antibody comprises (a) a heavy chain CDR1 comprising SSSGMS (SEQ ID NO:5), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; a heavy chain CDR2 comprising VIASSGSNTYYADSVKG (SEQ ID NO:6), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; and/or a heavy chain CDR3 comprising GIFFAI (SEQ ID NO:7), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; and/or (b) a light chain CDR1 comprising RASQSVRSNYLA (SEQ ID NO:8), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; a light chain CDR2 comprising GASSRAT (SEQ ID NO:9), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; and/or a light chain CDR3 comprising QQYSNFPI (SEQ ID NO:10), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions. Pharmaceutical compositions comprising the antibody and methods of using the antibody for such uses as inhibiting angiogenesis, inhibiting tumor growth, reducing the tumorigenicity of a tumor, and/or treating cancer are also provided.

[0017] In another aspect, the invention provides an antibody that specifically binds human Notch2 and/or Notch3, wherein the antibody comprises (a) a heavy chain CDR1 comprising SSSGMS (SEQ ID NO:5), a heavy chain CDR2 comprising VIASSGSNTYYADSVKG (SEQ ID NO:6), and/or a heavy chain CDR3 comprising (G/S)(I/S)F(F/Y)(A/P)(I/T/S/N) (SEQ ID NO:30); and/or (b) a light chain CDR1 comprising RASQSVRSNYLA (SEQ ID NO:8), a light chain CDR2 comprising GASSRAT (SEQ ID NO:9), and/or a light chain CDR3 comprising QQYSNFPI (SEQ ID NO:10). In some embodiments, the antibody

comprises a heavy chain CDR3 comprising SIFYPT (SEQ ID NO:22). In some embodiments, the antibody comprises a heavy chain CDR3 comprising SSSFFAS (SEQ ID NO:23). In other embodiments, the antibody comprises a heavy chain CDR3 comprising SSFYAS (SEQ ID NO:24). In certain embodiments, the antibody comprises a heavy chain CDR3 comprising SSFFAT (SEQ ID NO:25). In some embodiments, the antibody comprises a heavy chain CDR3 comprising SIFYPS (SEQ ID NO:26). In yet other embodiments, the antibody comprises a heavy chain CDR3 comprising SSFFAN (SEQ ID NO:27). Pharmaceutical compositions comprising the antibody and methods of using the antibody for such uses as inhibiting angiogenesis, inhibiting tumor growth, reducing the tumorigenicity of a tumor, and/or treating cancer are also provided.

[0018] In another aspect, the invention provides a polypeptide that comprises: (a) a polypeptide (e.g., a heavy chain variable region) having at least about 80% sequence identity to SEQ ID NO:50, SEQ ID NO:14, SEQ ID NO:40, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, or SEQ ID NO:20 (with or without signal sequence); and/or (b) a polypeptide (e.g., a light chain variable region) having at least about 80% sequence identity to SEQ ID NO:13, SEQ ID NO:19 or SEQ ID NO:39 (with or without signal sequence). In certain embodiments, the polypeptide is an antibody. In certain embodiments, the polypeptide specifically binds human Notch2 and/or Notch3. In some embodiments, the polypeptide specifically binds to human Notch2. In some embodiments, the polypeptide binds to Notch2 and Notch3. In other embodiments, the polypeptide binds to Notch3. In certain embodiments, the polypeptide comprises a polypeptide having at least about 85%, at least about 90%, at least about 95%, at least about 98%, or about 100% sequence identity to SEQ ID NO:14, SEQ ID NO:13, or SEQ ID NO:50. Pharmaceutical compositions comprising the polypeptide and methods of using the polypeptide for such uses as inhibiting angiogenesis, inhibiting tumor growth, reducing the tumorigenicity of a tumor, and/or treating cancer are also provided.

[0019] In still another aspect, the invention provides a polypeptide (e.g., an antibody or a heavy chain or light chain of an antibody) comprising: (a) a polypeptide having at least about 80% sequence identity to SEQ ID NO:49, SEQ ID NO:16, or SEQ ID NO:2 (with or without signal sequence); and/or (b) a polypeptide having at least about 80% sequence identity to SEQ ID NO:18, or SEQ ID NO:4 (with or without signal sequence. In certain embodiments, the polypeptide comprises a polypeptide having at least about 85%, at least about 90%, at least about 95%, at least about 98%, or about 100% sequence identity to SEQ ID NO:39 or SEQ ID NO:40. Pharmaceutical compositions comprising the antibodies and methods of treating cancer comprising administering therapeutically effective amounts of the antibodies are also provided.

[0020] In another aspect, the invention provides a polypeptide (e.g., an antibody or a heavy chain or light chain of an antibody) comprises: (a) a polypeptide having at least about 80% sequence identity to SEQ ID NO:50; and/or (b) a polypeptide having at least about 80% sequence identity to SEQ ID NO:13. In

certain embodiments, the polypeptide comprises a polypeptide having at least about 85%, at least about 90%, at least about 95%, at least about 98%, or about 100% sequence identity to SEQ ID NO:50 or SEQ ID NO:13. In certain embodiments, the polypeptide is an antibody that binds human Notch2 and/or human Notch3. Pharmaceutical compositions comprising the antibodies and methods of treating cancer comprising administering therapeutically effective amounts of the antibodies are also provided.

[0021] In another aspect, the invention provides an antibody that comprises, consists, or consists essentially of a 59R1 IgG2 antibody comprising the heavy chain and light chain of SEQ ID NOs:16 and 18 (with or without signal sequence), respectively, or as encoded by the DNA deposited with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA, USA, under the conditions of the Budapest Treaty on October 15, 2008, and assigned designation number PTA-9547. Pharmaceutical compositions comprising the antibody and methods of using the antibody for such uses as inhibiting angiogenesis, inhibiting tumor growth, reducing the tumorigenicity of a tumor, and/or treating cancer are also provided.

[0022] In an additional aspect, the invention provides an antibody that comprises, consists or consists essentially of a 59R5 IgG2 antibody comprising the heavy chain and light chain of SEQ ID NO:49 and SEQ ID NO:18 (with or without signal sequence), respectively, or as encoded by the DNA deposited with the ATCC on July 6, 2009, and assigned designation number [...]. Pharmaceutical compositions comprising the antibody and methods of using the antibody for such uses as inhibiting angiogenesis, inhibiting tumor growth, reducing the tumorigenicity of a tumor, and/or treating cancer are also provided.

[0023] In another aspect, the invention provides an antibody that competes for specific binding to human Notch2 and/or Notch3 with an antibody comprising a heavy chain variable region comprising SEQ ID NO:14 and a light chain variable region comprising SEQ ID NO:13. In certain embodiments, the antibody competes for specific binding with a 59R1 IgG2 antibody comprising the heavy chain and light chain of SEQ ID NOs:16 and 18 (with or without signal sequence), respectively, or as encoded by the DNA deposited with the ATCC on October 15, 2008, and assigned designation number PTA-9547. In some embodiments, the antibody competes for binding to human Notch2. In some embodiments, the antibody competes for binding to human Notch2 and Notch3. In other embodiments, the antibody competes for binding to human Notch3. Pharmaceutical compositions comprising the antibody and methods of using the antibody for such uses as inhibiting angiogenesis, inhibiting tumor growth, reducing the tumorigenicity of a tumor, and/or treating cancer are also provided.

[0024] In a further aspect, the antibody competes for specific binding to human Notch2 and/or Notch3 with an antibody comprising a heavy chain variable region comprising SEQ ID NO:50 and a light chain variable region comprising SEQ ID NO:13. In some embodiments, the antibody competes for specific binding with a 59R5 antibody comprising the heavy chain and light chain of SEQ ID NOs: 49 and 18, respectively, or as encoded by the DNA deposited with the ATCC on July 6, 2009, and assigned

designation number [...]. In some embodiments, the antibody competes for binding to human Notch2. In some embodiments, the antibody competes for binding to human Notch2 and Notch3. In other embodiments, the antibody competes for binding to human Notch3. Pharmaceutical compositions comprising the antibody and methods of using the antibody for such uses as inhibiting angiogenesis, inhibiting tumor growth, reducing the tumorigenicity of a tumor, and/or treating cancer are also provided.

[0025] In certain other aspects, the invention provides a polypeptide (with or without a signal sequence) comprising a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, and SEQ ID NO:57, as well as a polynucleotide encoding such a polypeptide. In certain embodiments, the polypeptide is an antibody. In certain embodiments, the antibody specifically binds to human Notch2 and/or human Notch3. In certain embodiments, the antibody specifically binds to human Notch2. In certain embodiments, the antibody specifically binds to human Notch2 and human Notch3. In certain embodiments, the antibody specifically binds to human Notch3. In another aspect, the invention provides a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:58, SEQ ID NO: 59 and SEQ ID NO:60.

[0026] In another aspect, the invention provides a method of modulating the function of pericytes and/or vascular smooth muscle cells in a subject (e.g., at the site of a tumor or other aberrant angiogenesis in the subject). In certain embodiments, the method comprises administering an effective amount of an agent that specifically binds human Notch2 and/or human Notch3 to the subject. In certain embodiments, the agent is an antibody. In some embodiments, the agent is an antibody described in any one of the aforementioned aspects and/or embodiments, as well as other aspects and/or embodiments described herein. In certain embodiments, the agent is an antagonist. In certain embodiments, the agent specifically binds to and is an antagonist of human Notch3. In certain embodiments, the modulation of the function of the pericytes and/or vascular smooth muscle cells results in inhibition of angiogenesis and/or tumor growth.

[0027] In still another aspect, the invention provides a method of inhibiting angiogenesis (e.g., tumor angiogenesis) in a subject. In certain embodiments, the method comprises administering to the subject an effective amount of an agent that specifically binds human Notch2 and/or human Notch3. In certain embodiments, the agent is an antagonist. In some embodiments, the agent specifically binds to and is an antagonist of human Notch2. In certain embodiments, the agent specifically binds to and is an antagonist of human Notch3. In some embodiments, the agent is an antagonist of both Notch2 and Notch3. In some embodiments, the antagonist is an antibody. In certain embodiments, the agent is an antibody. In some embodiments, the agent is an antibody described in any one of the aforementioned aspects and/or

embodiments, as well as other aspects and/or embodiments described herein. In some embodiments, the antagonist is not an antibody. In some embodiments, the method of inhibiting angiogenesis further comprises administering to the subject an antagonist of vascular endothelial cell growth factor (VEGF) or of a VEGF receptor. In certain embodiments, the method is a method of inhibiting angiogenesis by modulating the function of pericytes and/or vascular smooth muscle cells.

[0028] In a further aspect, the invention provides a method of inhibiting growth of a tumor in a subject. In certain embodiments, the method comprises administering to the subject a therapeutically effective amount of an antagonist of human Notch2 and/or human Notch3. In certain embodiments, the antagonist is an antibody that specifically binds human Notch2. In some embodiments, the antagonist is an antibody that specifically binds both human Notch2 and human Notch3. In certain embodiments, the antagonist is an antibody that specifically binds human Notch3. In some embodiments, the antagonist is an antibody described in any one of the aforementioned aspects and/or embodiments, as well as other aspects and/or embodiments described herein. In certain embodiments, the tumor comprises a deletion or other mutation in the phosphatase and tensin homolog (PTEN) gene. In certain embodiments, the tumor is a breast tumor.

[0029] In a still further aspect, the invention provides a method of selecting a subject for treatment with a human Notch2 and/or human Notch3 antagonist. In certain embodiments, the method comprises (a) determining if the tumor comprises a deletion or mutation in the phosphatase and tensin homolog (PTEN) gene; and (b) selecting the subject for treatment with a Notch2 and/or Notch3 antagonist if the tumor comprises the deletion or mutation. In some embodiments, the subject is treated with a Notch2 antagonist. In some embodiments, the subject is treated with an antagonist of Notch2 and Notch3. In some embodiments, the subject is treated with an antagonist of Notch3. In some embodiments the antagonist is an antibody. In certain embodiments, the tumor is a breast tumor.

[0030] In another aspect, the invention provides an antibody that specifically binds to a non-ligand binding region of an extracellular domain of at least one human Notch receptor (e.g., 1, 2, 3, or 4 Notch receptors). In certain embodiments, the non-ligand binding region comprises or consists of EGF repeat 10 of a human Notch receptor (or an equivalent of EGF10, such as EGF9 of human Notch3). In some embodiments, the antibody inhibits tumor growth. In some embodiments, the antibody inhibits binding of a ligand to a Notch receptor. In certain embodiments, the antibody inhibits signaling by the Notch receptor. In some embodiments, the Notch receptor is a human Notch1, Notch2, Notch3, or Notch4 receptor. In certain embodiments, the antibody specifically binds to Notch2 (for example, EGF10 of Notch2). In certain embodiments, the antibody specifically binds to Notch2 and at least one additional Notch receptor. In certain embodiments, the additional Notch receptor is Notch3. Pharmaceutical compositions comprising the antibodies and methods of treating cancer comprising administering therapeutically effective amounts of the antibodies are also provided.

[0031] In an additional aspect, the invention provides an antibody that specifically binds to two or more (i.e., at least two or two, three, or four) human Notch receptors. In certain embodiments, the antibody specifically binds to a non-ligand binding region of an extracellular domain of the two or more human Notch receptors. In certain embodiments, if the two or more human Notch receptors comprise Notch1, Notch2, or Notch4, the antibody binds to EGF10 of Notch1, Notch2, or Notch4, and if the two or more human Notch receptors comprise Notch3, the antibody binds to EGF9 of Notch3. In certain embodiments, the non-ligand binding region is not EGF4. In certain embodiments, the two or more human Notch receptors comprise Notch2. In certain embodiments, the two or more human Notch receptors comprise Notch3. In still further embodiments, the two or more human Notch receptors comprise Notch2 and Notch3. In certain embodiments, the antibody is an antagonist of the two or more human Notch receptors. In certain embodiments, the antibody inhibits tumor growth. Pharmaceutical compositions comprising the antibodies and methods of treating cancer comprising administering therapeutically effective amounts of the antibodies are also provided.

[0032] In yet another aspect, the invention provides an isolated antibody that specifically binds to a non-ligand binding region of an extracellular domain of a human Notch2 receptor and inhibits tumor growth, wherein the non-ligand binding region comprises or consists of EGF repeat 10 of the human Notch2 receptor (e.g., SEQ ID NO:36). In some embodiments, the antibody does not bind to any region of human Notch2 outside of EGF repeat 10. In certain embodiments, the antibody also specifically binds to EGF repeat 10 (or equivalent) of at least one additional human Notch receptor (e.g., EGF9 of Notch3). In some embodiments, the antibody binds to human Notch2 EGF10 and Notch3 EGF9. Pharmaceutical compositions comprising the antibodies and methods of treating cancer comprising administering therapeutically effective amounts of the antibodies are also provided.

[0033] In yet another aspect, the invention provides an isolated antibody that specifically binds to a non-ligand binding region of an extracellular domain of a human Notch3 receptor and inhibits tumor growth, wherein the non-ligand binding region comprises or consists of EGF repeat 9 of the human Notch3 receptor (equivalent to EGF10 in the other Notch receptors). In some embodiments, the antibody does not bind to any region of human Notch3 outside of EGF repeat 9. In certain embodiments, the antibody also specifically binds to EGF repeat 10 of at least one additional human Notch receptor. In some embodiments, the antibody binds to human Notch3 EGF9 and Notch2 EGF10. Pharmaceutical compositions comprising the antibodies and methods of treating cancer comprising administering therapeutically effective amounts of the antibodies are also provided.

[0034] In a still further aspect, the invention provides an antibody that binds a non-ligand binding region of an extracellular domain of a human Notch receptor and comprises: (a) a heavy chain CDR1 comprising SSSGMS (SEQ ID NO:5), a heavy chain CDR2 comprising VIASSGSNTYYADSVKG (SEQ ID NO:6), and/or a heavy chain CDR3 comprising GIFFAI (SEQ ID NO:7); and/or (b) a light chain

CDR1 comprising RASQSVRSNYLA (SEQ ID NO:8), a light chain CDR2 comprising GASSRAT (SEQ ID NO:9), and/or a light chain CDR3 comprising QQYSNFPI (SEQ ID NO:10). In certain embodiments, the human Notch receptor is Notch2. In certain embodiments, the antibody binds to EGF10 of a human Notch2 receptor and/or EGF9 of a human Notch3 receptor. In an additional aspect, the invention provides an antibody that competes with such an antibody for specific binding to a non-ligand binding region of an extracellular domain of Notch2 in a competitive binding assay. Pharmaceutical compositions comprising the antibodies and methods of treating cancer comprising administering therapeutically effective amounts of the antibodies are also provided. Methods of inhibiting angiogenesis comprising administering the compositions are also provided.

[0035] In another aspect, the invention provides an antibody that binds a non-ligand binding region of an extracellular domain of a human Notch receptor and comprises: (a) a heavy chain CDR1 comprising SSSGMS (SEQ ID NO:5), a heavy chain CDR2 comprising VIASSGSNTYYADSVKG (SEQ ID NO:6), and/or a heavy chain CDR3 comprising SIFYTT (SEQ ID NO:51); and/or (b) a light chain CDR1 comprising RASQSVRSNYLA (SEQ ID NO:8), a light chain CDR2 comprising GASSRAT (SEQ ID NO:9), and/or a light chain CDR3 comprising QQYSNFPI (SEQ ID NO:10). In certain embodiments, the human Notch receptor is Notch2. In some embodiments, the antibody binds to the human Notch2 and Notch3 receptors. In certain embodiments, the antibody binds to EGF10 of a human Notch2 receptor and/or EGF9 of a human Notch3 receptor. In another embodiment, the invention provides an antibody that competes with such an antibody for specific binding to a non-ligand binding region of an extracellular domain of Notch2 in a competitive binding assay. Pharmaceutical compositions comprising the antibodies and methods of treating cancer comprising administering therapeutically effective amounts of the antibodies are also provided. Methods of inhibiting angiogenesis comprising administering the compositions are also provided.

[0036] In certain embodiments of each of the aforementioned aspects or embodiments, as well as other aspects and/or embodiments described elsewhere herein, the antibody specifically binds to both human Notch2 and human Notch3.

[0037] In certain embodiments of each of the aforementioned aspects or embodiments, as well as other aspects and/or embodiments described elsewhere herein, the antibody is a recombinant antibody. In certain embodiments, the antibody is a monoclonal antibody. In certain embodiments, the antibody is a chimeric antibody. In certain embodiments, the antibody is a humanized antibody. In certain embodiments, the antibody is a human antibody. In some embodiments, the antibody is monovalent, bivalent or multivalent. In certain embodiments, the antibody is a monospecific antibody. In certain embodiments, an individual antigen-binding site of the antibody binds (or is capable of binding) a non-ligand binding region of the extracellular domain of more than one human Notch receptor (e.g., Notch2 and Notch3). In certain alternative embodiments, the antibody is a bispecific antibody. In certain

embodiments, the antibody is an IgG1 antibody. In certain embodiments, the antibody is an IgG2 antibody. In certain embodiments, the antibody is conjugated to a cytotoxic moiety. In certain embodiments, the antibody is isolated. In still further embodiments, the antibody is substantially pure.

[0038] In certain embodiments of each of the aforementioned aspects or embodiments, as well as other aspects and/or embodiments described elsewhere herein, the cancer or tumor treated with the antibody is a breast, colorectal, lung, pancreatic, prostate, or head and neck cancer or tumor. In certain embodiments, the cancer or tumor is melanoma. In certain embodiments, the cancer or tumor is a breast cancer or tumor. In certain embodiments, the cancer or tumor is a colorectal cancer or tumor. In certain embodiments, the cancer or tumor is a pancreatic cancer or tumor. In certain embodiments, the cancer or tumor is a prostate cancer or tumor.

[0039] In certain embodiments of each of the aforementioned aspects or embodiments, as well as other aspects and/or embodiments described elsewhere herein, the methods of treating cancer comprise inhibiting tumor growth. In certain embodiments, the methods of treating cancer comprise reducing the tumorigenicity of tumors (e.g., by reducing the frequency of cancer stem cells in the tumor).

[0040] In certain embodiments of each of the aforementioned aspects or embodiments, as well as other aspects and/or embodiments described elsewhere herein, the antagonist or antibody is administered to a subject in combination with an additional treatment for cancer. In certain embodiments, the additional treatment for cancer comprises radiation therapy, chemotherapy, and/or an additional antibody therapeutic. In some embodiments, the additional treatment for cancer comprises a chemotherapeutic agent. In certain embodiments, the chemotherapy comprises paclitaxel (e.g., TAXOL), irinotecan, gemcitabine, and/or oxaliplatin. In certain embodiments, the additional antibody therapeutic is an antibody that specifically binds a human Notch receptor (e.g., Notch1, 2, 3, or 4) or a human Notch receptor ligand (e.g., DLL4 or JAG1). In some embodiments, the additional antibody therapeutic is an anti-DLL4 antibody. In certain alternative embodiments, the additional antibody therapeutic is an antibody that specifically binds vascular endothelial cell growth factor (VEGF). In certain embodiments, the additional therapeutic binds a VEGF receptor.

[0041] In certain embodiments of each of the aforementioned aspects or embodiments, as well as other aspects and/or embodiments described elsewhere herein, the antibody is administered to a subject in combination with a second therapeutic agent that is an anti-angiogenic agent.

[0042] Cell lines (e.g., hybridoma cell lines) comprising or producing the antibodies or other polypeptides described herein are further provided by the invention. Polynucleotides (e.g., vectors) comprising the polynucleotides described herein, including polynucleotides encoding the polypeptides or the light chain variable regions or heavy chain variable regions of the antibodies described herein are also provided, as are cell lines comprising such polynucleotides.

[0043] In certain embodiments, the present invention provides a method of treating cancer, wherein the cancer comprises cancer stem cells, comprising administering to the subject a therapeutically effective amount of an antibody which binds a Notch receptor. In a more particular aspect, the present invention provides a method of treating cancer, wherein the cancer comprises stem cells expressing one or more Notch receptor family members, comprising administering to the subject a therapeutically effective amount of an antibody that binds those Notch receptor family members. The present invention provides antibodies that bind to the non-ligand binding domain of the extracellular domain of a human Notch receptor and are therapeutically effective against cancer. Thus, in certain embodiments, the present invention provides an antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human Notch receptor and that inhibits tumor growth. In certain embodiments, the present invention further provides a method of treating cancer, the method comprising administering a therapeutically effective amount of an antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human Notch receptor protein and inhibits tumor growth.

[0044] Various advantages in using an antibody that binds Notch receptor family members or the ligands to those Notch receptors to treat such cancers are contemplated herein. In some embodiments, certain Notch receptors are highly expressed in certain solid tumors, for example, breast and colon, and this provides a sink for active drug where the drug binds to the Notch receptor. Antibodies that bind overexpressed Notch receptors are anticipated to have a better safety profile than currently available chemotherapeutic drugs.

[0045] The invention further provides a method of treating cancer in a human, wherein the cancer comprising cancer stem cells is not characterized by overexpression by the cancer stem cell of one or more Notch receptors, comprising administering to the human a therapeutically effective amount of an antibody which binds to a Notch receptor and blocks ligand activation of a Notch receptor.

[0046] The invention further provides a method of treating cancer in a human comprising administering to the human therapeutically effective amounts of (a) a first antibody which binds a Notch receptor and inhibits growth or proliferation of cancer stem cells which overexpress Notch receptors; and (b) a second antibody which binds a Notch receptor and blocks ligand activation of a Notch receptor.

[0047] The invention also provides a method of treating cancer, wherein the cancer is selected from the group consisting of breast, colon, rectal and colorectal cancer, comprising administering a therapeutically effective amount of an antibody which binds Notch. The invention also provides another method of treating cancer, wherein the cancer is selected from the group consisting of breast, colon, pancreatic, prostate, lung, rectal and colorectal cancer, comprising administering a therapeutically effective amount of an antibody that blocks ligand activation of a Notch receptor. The invention also provides still another method of treating cancer, wherein the cancer is selected from the group consisting of breast, colon, pancreatic, prostate, lung, rectal and colorectal cancer, comprising administering a therapeutically

effective amount of an antibody that binds Notch and an antibody that blocks ligand activation of a Notch receptor.

[0048] In further embodiments, the invention provides articles of manufacture for use (among other things) in the above methods. For example, the invention provides an article of manufacture comprising a container and a composition contained therein, wherein the composition comprises an antibody that binds Notch, and further comprises a package insert indicating that the composition can be used to treat a cancer comprising cancer stem cells. In some embodiments, the invention provides an article of manufacture comprising a container and a composition contained therein, wherein the composition comprises an antibody that binds Notch, and further comprises a package insert indicating that the composition can be used to treat cancer comprising cancer stem cells that express one or more Notch receptors.

[0049] In certain embodiments, the invention additionally pertains to an article of manufacture comprising a container and a composition contained therein, wherein the composition comprises an antibody which binds a Notch receptor and blocks ligand activation of a Notch receptor, and further comprises a package insert indicating that the composition can be used to treat cancer, wherein the cancer comprises cancer stem cells that are not characterized by overexpression of the Notch receptor.

[0050] In certain embodiments, an article of manufacture is provided which comprises (a) a first container with a composition contained therein, wherein the composition comprises a first antibody that binds a Notch receptor and inhibits growth of cancer cells comprising cancer stem cells overexpressing Notch; and (b) a second container with a composition contained therein, wherein the composition comprises a second antibody which binds Notch and blocks ligand activation of a Notch receptor.

[0051] In some embodiments, a further article of manufacture is provided which comprises a container and a composition contained therein, wherein the composition comprises an antibody which binds Notch and blocks ligand activation of a Notch receptor, and further comprises a package insert indicating that the composition can be used to treat a cancer selected from the group consisting of colon, pancreatic, prostate, lung, rectal and colorectal cancer.

[0052] The invention additionally provides: an antibody (e.g., a human antibody or a humanized antibody) which binds Notch and blocks ligand activation of a Notch receptor; a composition comprising the antibody and a pharmaceutically acceptable carrier; and an immunoconjugate comprising the antibody conjugated with a cytotoxic agent.

[0053] In one aspect, the invention provides an isolated polynucleotide encoding any of the antibodies or polypeptides of the aforementioned aspects or embodiments, as well as other aspects and/or embodiments described elsewhere herein. In some embodiments, the invention provides a vector comprising the polynucleotide. In some embodiments, a host cell comprises the polynucleotide or the vector. In other embodiments, a process of producing the antibody comprises culturing a host cell comprising the

polynucleotide so that the polynucleotide is expressed and, optionally, further comprises recovering the antibody from the host cell culture (e.g., from the host cell culture medium).

[0054] Moreover, the invention provides an isolated polynucleotide encoding a humanized or human antibody as described in the aforementioned embodiments or aspects, as well as described elsewhere herein; a vector comprising the polynucleotide; a host cell comprising the polynucleotide or the vector; as well as a process of producing the antibody comprising culturing a host cell comprising the polynucleotide so that the polynucleotide is expressed and, optionally, further comprising recovering the antibody from the host cell culture (e.g., from the host cell culture medium).

[0055] The invention further pertains to an immunoconjugate comprising an antibody that binds Notch conjugated to one or more calicheamicin molecules, and the use of such conjugates for treating a Notch expressing cancer, e.g., a cancer in which cancer stem cells overexpress Notch.

[0056] Where aspects or embodiments of the invention are described in terms of a Markush group or other grouping of alternatives, including, but not limited to, groups of alternatives separated by "and/or" or "or," the present invention 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 also envisages the explicit exclusion of one or more of any of the group members in the claimed invention. For example, language such as "X and/or Y" encompasses "X" individually, "Y" individually, as well as "X" and "Y" together.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0057] Figure 1: 59R1 antibodies and variants bind human Notch2 and block ligand binding. (A) FACS analysis of binding by 59R1 Fab to human Notch2. "Clone 1" is 59R1 Fab which was shown to bind human Notch2 on stably transfected HEK293 cells. "Clone 5" is the Fab of a different clone isolated from the phage library which did not bind Notch2. (B) FACS analysis of blocking of ligand (JAG1) binding by 59R1 Fab. "Clone 1" is 59R1 Fab which was shown to block binding of a hJagged1 ECD-Fc fusion to human Notch2 on stably transfected HEK293 cells. "Clone 5" is the Fab of a different clone isolated from the phage library which did not block ligand binding in the assay. (C) FACS analysis of binding of 59R1 IgG2 antibody to human Notch2 on stably transfected HEK293 cells. 59R1 IgG2 antibody was shown to bind human Notch2 on stably transfected HEK293 cells. (D) FACS analysis of blocking of ligand (DLL4) binding by 59R1 IgG2 antibody. 59R1 IgG2 antibody was shown to block binding of a hDLL4 ECD-Fc fusion to human Notch2 on stably transfected HEK293 cells. (E) Affinity maturation strategy for heavy chain CDR3 of 59R1. The parental sequence of the heavy chain CDR3 of 59R1 is shown boxed. Allowed residue changes are as indicated below the parental sequence in the figure. (F) Screening of affinity maturated 59R1 sequences for JAG1 blocking ability. Improved variants are indicated with arrows.

[0058] Figure 2: FACS analysis of cross-reactivity of the 59R1 IgG2 antibody to the four human Notch homologues. 59R1 was found to bind hNotch2 and hNotch3 on transiently transfected HEK-293 cells but was found to not exhibit significant binding to hNotch1 and hNotch4 on the same cells.

[0059] Figure 3: Epitope mapping of 59R1 antibody. (A) Anti-Notch2/3 antibody 59R1 binds to EGF repeat 10 of human Notch2. Supernatant from HEK 293 cells expressing recombinant Notch2-Fc fusion proteins with the indicated EGF repeats of Notch2 between 1 and 12 (x-axis) were used in ELISA with anti-Notch2/3 antibody 59R1. The OD (y-axis) indicated antibody binding (hatched bars) only to Notch2 fusion proteins comprising EGF repeat 10. (The figure shows data obtained from two separate experiments which are shown separately in the top and bottom graphs.) (B) EGF Repeats 11 and 12 are not involved in anti-Notch2/3 antibody 59R1 binding to full length hNotch2. FACS analysis of HEK 293 cells transfected with green fluorescent protein (GFP) (x-axis) alone (top left) or co-transfected with GFP and either full length Notch2 intact or with full length Notch 2 with EGF repeat 11 deleted (ΔEGF11) or EGF repeat 12 deleted (ΔEGF12). Binding of 59R1 is indicated along the y-axis (PE) to all three Notch2 proteins in GFP-expressing cells. (C) EGF repeat 10 is involved in anti-Notch2/3 antibody 59R1 binding to full-length hNotch2, but not in ligand binding. Binding by an anti-Notch2 antibody 59M70 that binds to EGF1-4 of hNotch2 is indicated as "anti-Notch2 binding." Binding by DLL4 is indicated as "ligand binding."

[0060] Figure 4: Anti-Notch2/3 antibody 59R1 inhibits Notch2 signaling in luciferase reporter assays. (A) 59R1 blocks hDLL4-induced Notch2 reporter activity. (B) 59R1 blocks hJAG1-induced Notch2 reporter activity (C) 59R1 blocks hJAG2-induced Notch2 reporter activity

[0061] Figure 5: Notch2/3 Receptor Antibody 59R1 Inhibits Tumor Formation and Growth *In vivo*. (A) Anti-Notch2/3 (59R1) Inhibits the Formation of PE13 Breast Tumors. NOD/SCID mice injected with PE13 breast tumor cells were treated with control antibody (squares) or anti-Notch2/3 antibody 59R1 (open triangles) two days after cell injection and tumor volume (y-axis, mm^3) was measured across time (x-axis, days post cell injection). Treatment with 59R1 antibodies significantly inhibited tumor formation compared to control. ($p < 0.001$) (B) Anti-Notch2/3 (59R1) Inhibits Formation of T3 Breast Tumors. NOD/SCID mice injected with T3 breast tumor cells were treated with control antibody (squares) or anti-Notch2/3 antibody 59R1 (open triangles) two days after cell injection, and tumor volume (y-axis, mm^3) was measured across time (x-axis, days post cell injection). Treatment with 59R1 antibodies significantly inhibited tumor formation compared to control. ($p < 0.001$) (C) Anti-Notch2/3 (59R1) Inhibits the Growth of Colo-205 Colon Tumors. 6-8 week-old immunodeficient *bg/nu XID* female mice on a Swiss CD-1 background injected with Colo-205 colon tumor cells were treated with control antibody (squares) or anti-Notch2/3 antibody 59R1 (diamonds) after tumor volume reached a size between 65 to 200 mm^3. Mean tumor volume (y-axis, mm^3) was measured across time (x-axis, days post cell injection). Treatment with 59R1 antibodies inhibited tumor growth compared to control (*** $p < 0.001$ after day 40). (D) Anti-

Notch2/3 (59R1) Inhibits the Growth of PN4 Pancreatic Tumors. NOD/SCID mice injected with PN4 pancreatic tumor cells were treated with control antibody (squares) or anti-Notch2/3 antibody 59R1 (diamonds) after tumor volume reached an a size between 65 to 200 mm^3. Mean tumor volume (y-axis, mm^3) was measured across time (x-axis, days post cell injection). Treatment with 59R1 antibodies inhibited tumor growth compared to control (*** $p < 0.001$ after day 70). (E) Anti-Notch2/3 (59R1) Inhibits the Growth of PE13 Breast Tumors. NOD/SCID mice injected with PE13 breast tumor cells were treated with control antibody (squares) or anti-Notch2/3 antibody 59R1 (diamonds) after tumor volume reached a size between 65 to 200 mm^3. Mean tumor volume (y-axis, mm^3) was measured across time (x-axis, days post cell injection). Treatment with 59R1 antibodies inhibited tumor growth compared to control (* $p < 0.05$ after day 57). (F) Anti-Notch2/3 (59R1) Inhibits the Growth of T3 Breast Tumors. NOD/SCID mice injected with T3 breast tumor cells were treated with control antibody (solid bars) or anti-Notch2/3 antibody 59R1 (open bars) after tumor volume reached a size between 65 to 200 mm^3. Mean tumor volume was measured on days 18, 25, 39, and 42 post cell injection. Treatment with 59R1 antibodies inhibited tumor growth compared to control (*** $p < 0.001$ on day 42).

[0062] Figure 6: Anti-Notch2/3 antibody 59R1 delays B51 breast tumor recurrence after paclitaxel treatment.

[0063] Figure 7: Anti-Notch2/3 antibody 59R1 decreases cancer stem cell frequency in B51 breast tumor.

[0064] Figure 8: In combination with gemcitabine, anti-Notch2/3 antibody 59R1 inhibits the growth of PN4 pancreatic tumors.

[0065] Figure 9: Anti-Notch2/3 antibody 59R1 inhibits tumor growth in an M4 melanoma xenograft model.

[0066] Figure 10: Anti-Notch2/3 antibody 59R1 inhibits the growth of C28 colon tumors alone and in combination with irinotecan.

[0067] Figure 11: 59R1 IgG2 antibody significantly inhibits tumor growth of established human tumor xenografts *in vivo*. Established Colo-205 (A), C8 (B), PN8 (C), B34 (D), B39 (E), B44 (F), PE-13 (G) and T1 (H) tumors (s.c, n=10 per group) were treated at 15mg/kg once a week with the indicated antibodies (1B711, LZ-1 control antibody, black squares; 59R1, black triangles; AVASTIN, black circles; AVASTIN + 59R1, black diamonds). Tumor volume (x-axis) is plotted over time (y-axis). In the Colo-205 xenograft model, combination therapy of 59R1 with AVASTIN was significantly more effective than either antibody treatment alone. In Figures 11B-11H, asterisks indicate significant tumor-growth inhibition at day shown: *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$, Student's t-test; Symbols, mean; bars, SEM.

[0068] Figure 12: Relative expression levels of selected genes are significantly regulated by 59R1 treatment in various xenograft tumor models. Expression levels of HEYL (A), Notch3 (B), RGS5 (C),

ANGPT1 (D) and ANGPT2 (E) were individually tested by TaqMan® analysis from previously tested xenograft models. Notably, lack of estrogen (ne) abrogates effect of 59R1 in reducing ANGPT1 and ANGPT2 expression in host stroma of T1 harboring mice. Open circles correspond to individual tumors analyzed. Horizontal line, mean.

[0069] Figure 13: The tumor suppressor PTEN gene is deleted in many of the breast tumors in which 59R1 showed anti-tumor efficacy. The PTEN exon, Affymetrix probe distribution, and the deletions in the PTEN gene in chromosome 10 are shown. The thick and thin gray-shaded bars indicate the homozygous and heterozygous deletions of the chromosome fragments, respectively.

[0070] Figure 14. Epitope mapping of 59R1 antibody. (A) Protein alignment of human Notch homologues. The alignment was performed by Clone Manager Software. The EGF 10 repeat of human Notch1, Notch2, and Notch4 and the equivalent EGF in human Notch3, EGF9, is as indicated. The boxed area indicates a region containing one or more amino acid(s) that make up at least part of the 59R1 epitope as defined by FACS binding of 59R1 IgG2 antibody to an hNotch2 H385N AL 388-89 SN mutant (Figure 14B) and to an hNotch1 construct in which aa 382-386 have been mutated to correspond to the hNotch2 sequence (Figure 14C). (B) 59R1 IgG2 antibody binds to hNotch2, but not a mutant hNotch2 in which certain EGF 10 residues have been mutated to hNotch1 residues (H385N AL 388-89 SN). (C) 59R1 IgG2 antibody does not bind to hNotch1, but does bind to a mutant hNotch1 in which certain EGF 10 residues (aa 382-387) have been mutated to match the hNotch2 residues 385-389.

[0071] Figure 15. *In vitro* characterization of 59R5. (A) Figure 15A shows that antibody 59R5 is able to block ligand–induced signaling of Notch2 and Notch3. PC3 tumor cells were transiently transfected with human or mouse Notch receptor (hN2, human Notch2; mN2, murine Notch2; hN3, human Notch3; mN3, murine Notch3) and GFP inducible reporter construct. Transfected cells were incubated with different concentrations of antibody 59R1 and 59R5 in the presence of passively immobilized DLL4 Fc. (B) Figure 15B shows that 59R5 binds to a similar epitope as 59R1. HEK 293 cells were transiently transfected with expression vectors encoding human Notch2, human Notch1, or human Notch1 with residues 382-386 mutated to the corresponding human Notch2 residues. Cells were also co-transfected with a plasmid encoding green fluorescent protein (GFP) to mark those cells that received transfected plasmid. Cells were incubated with 59R1 or 59R5 and fluorescent secondary antibody and then examined by FACS. The regions highlighted by the boxes suggest that cells transfected with the indicated Notch expression vector were able to bind to 59R1 or 59R5.

[0072] Figure 16. Notch receptor antibody 59R5 inhibits tumor formation and growth *in vivo*. Figure 16A shows *in vivo* treatment of PE13 breast tumor cells with antibody 59R5. Figure 16B shows *in vivo* treatment of C28 colon cells with antibody 59R5. Figure 16C shows *in vivo* treatment of Colo205 colon cells with antibody 59R5.

[0073] Figure 17. *In vivo* treatment of tumors using Notch2/3 antibody 59R5 in combination treatment. (A) Mice were injected with PN8 pancreatic tumor cells. Tumors were allowed to grow for 33 days until they had reached an average volume of 120 mm3. The animals were treated with gemcitabine at 20 mg/kg once per week for four week in combination with either control Ab (squares), 59R1 (triangles), or 59R5 (circles). (B) Mice were injected with PE13 breast tumor cells. Tumors were allowed to grow for 40 days before treatments were initiated. The animals were treated with TAXOL at 15 mg/kg twice per week for 5 weeks, plus either control antibody (squares) or 59R5 (circles). After 5 weeks, the TAXOL treatments were stopped and the antibody treatments continued.

[0074] Figure 18. Regulation of gene expression in tumors after treatment with antibody 59R5. Figure 18 shows expression levels of selected genes in stromal cells and selected human genes in PE13 tumor cells after treatment with 59R1, 59R5, or control antibody.

[0075] Figure 19. Reduction of PE13 breast cancer stem cell frequency by 59R1. (A) Established tumors were treated with control antibody, taxol plus control antibody, 59R1, or taxol plus 59R1. Tumors were harvested after three weeks of treatment, processed and serial titrations of human cells from each the four treatment groups were transplanted into a new set of mice (n =10 per cell dose). Tumor growth rate was determined after 75 days. Tumor growth rate after 75 days of growth was used to calculate the CSC frequency using the L-calc program (Stem Cell Technologies, Inc.). (B) Cancer stem cell frequency in PE13 breast tumors after treatment with 59R1 and/or taxol. (C) Cancer stem cell frequency in PN4 pancreatic tumors after treatment with 59R1 and/or gemcitabine. (D) Cancer stem cell frequency in PE13 breast tumors after treatment with 59R5 and/or taxol. A single asterisk indicates a statistically significant difference ($p < 0.05$) vs. the control antibody treated group and a double asterisk indicates a significant difference vs. the taxol and control antibody treated group.

DETAILED DESCRIPTION OF THE INVENTION

[0076] The present invention provides novel agents, including, but not limited to polypeptides such as antibodies, that bind to one or more human Notch receptors, such as Notch2 and/or Notch3. The Notch-binding agents include antagonists of the human Notch receptor(s). Related polypeptides and polynucleotides, compositions comprising the Notch-binding agents, and methods of making the Notch-binding agents are also provided. Methods of using the novel Notch-binding agents, such as methods of inhibiting tumor growth, inhibiting angiogenesis, and/or treating cancer or other angiogenesis-related disease, are further provided.

[0077] The present invention further identifies molecules (e.g., antibodies) that specifically bind to a non-ligand binding region of the extracellular domain of a human Notch receptor and inhibit tumor growth *in vivo*. The ligand binding region of Notch, which is necessary and sufficient for ligand binding, has been

identified as EGF repeats 11 and 12, suggesting this region of the Notch receptor is important in Notch signaling and tumorigenesis (Rebay et al., 1991, *Cell* 67:687; Lei et al., 2003, *Dev.* 130:6411; Hambleton et al., 2004, *Structure* 12:2173). Unexpectedly, antibodies that bind outside the ligand binding domain of the extracellular domain of human Notch receptor have been found to inhibit tumor cell growth *in vivo* (see U.S. Patent Publication No. 2008/0131434, incorporated by reference herein in its entirety). Thus, antibodies that bind outside the ligand binding domain of the extracellular domain of one or more of the human Notch receptors—Notch1, Notch2, Notch3, and Notch4—have value as potential cancer therapeutics.

[0078] An antibody that specifically binds to an epitope containing residues within EGF repeat 10 of human Notch2 has now been identified (Examples 1 and 3 and Figures 3A-3C). The antibody, 59R1, inhibits binding of ligand to Notch2 (Example 1 and Figures 1A-1D) and inhibits ligand-induced Notch2 signaling (Example 4 and Figure 4A-4C), despite binding to Notch2 in a region outside of the ligand-binding region. 59R1 also specifically binds human Notch3 (Example 2 and Figure 2). The antibody has been found to prevent or inhibit tumor cell growth *in vivo* in a variety of different xenograft models, either alone or in combination with a second anti-cancer agent (Examples 5, 6, 7, and 9 and Figures 5A-F, 6, 8-10, and 11A-H). The antibody has also been shown to reduce the tumorigenicity of a tumor *in vivo* in multiple xenograft models by reducing the frequency of cancer stem cells (Examples 8 and 23 and Figures 7 and 19A-C). In addition, treatment with 59R1 was found to downregulate expression of RGS5 (a marker for pericytes and/or vascular smooth muscle cells), Notch3, and HeyL in the stroma of various tumors (Example 10 and Figures 12A-E) and to upregulate hypoxia in breast and colon tumors (Example 11). Without being bound by theory, these data indicate that the 59R1 antibody has an inhibitory effect on tumor angiogenesis that is due, at least in part, to modulation of the function of pericytes and/or vascular smooth muscle cells. Treatment with 59R1 was also found to regulate additional genes in breast tumors. Cell cycle gene pathways, myc-activating genes and several stem cell gene sets were found to be down-regulated by 59R1 (Example 22).

[0079] An additional human antibody, 59R5, has also been developed. 59R5 has properties that are similar to 59R1, such as similar binding affinity to Notch2 and Notch3 and similarities or overlap in their epitopes (Example 13 and Figure 15B). Antibody 59R5 has been shown to have similar activity as 59R1 in blocking Notch2 and Notch 3 signaling (Example 13 and Figure 15A). The 59R5 antibody has also been shown to inhibit tumor growth *in vivo* in several xenograft models, either alone or in combination with a second anti-cancer agent (Examples 14 and 15 and Figures 16A-C and 17A-B). In addition, treatment with 59R5, like 59R1, was found to downregulate expression of RGS5, Notch3, and HeyL in the stroma of various tumors, and 59R5 was also found to regulate the expression of human genes ID4, EDNRA, and EGLN3 in tumor cells to a similar extent as 59R1 (Example 16). 59R5 was further shown

to reduce the tumorigenicity in vivo in a xenograft model by reducing the frequency of cancer stem cells (Example 23 and 19D).

Definitions

[0080] An "antagonist" of a Notch receptor is a term that includes any molecule that partially or fully blocks, inhibits, or neutralizes a biological activity of the Notch pathway. Suitable antagonist molecules specifically include antagonist antibodies or antibody fragments.

[0081] The term "antibody" is used to mean an immunoglobulin molecule that recognizes and specifically binds to a target, such as a protein, polypeptide, peptide, carbohydrate, polynucleotide, lipid, or combinations of the foregoing etc., through at least one antigen recognition site within the variable region of the immunoglobulin molecule. As used herein, the term encompasses intact polyclonal antibodies, intact monoclonal antibodies, antibody fragments (such as Fab, Fab', F(ab')2, and Fv fragments), single chain Fv (scFv) mutants, multispecific antibodies such as bispecific antibodies generated from at least two intact antibodies, fusion proteins comprising an antibody portion, and any other modified immunoglobulin molecule comprising an antigen recognition site so long as the antibodies exhibit the desired biological activity. An antibody can be of any the five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, or subclasses (isotypes) thereof (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2), based on the identity of their heavy-chain constant domains referred to as alpha, delta, epsilon, gamma, and mu, respectively. The different classes of immunoglobulins have different and well known subunit structures and three-dimensional configurations. Antibodies can be naked or conjugated to other molecules such as toxins, radioisotopes, etc.

[0082] As used herein, the term "antibody fragment" refers to a portion of an intact antibody and refers to the antigenic determining variable regions of an intact antibody. Examples of antibody fragments include, but are not limited to Fab, Fab', F(ab')2, and Fv fragments, linear antibodies, single chain antibodies, and multispecific antibodies formed from antibody fragments.

[0083] An "Fv antibody" refers to the minimal antibody fragment that contains a complete antigen-recognition and -binding site either as two-chains, in which one heavy and one light chain variable domain form a non-covalent dimer, or as a single-chain (scFv), in which one heavy and one light chain variable domain are covalently linked by a flexible peptide linker so that the two chains associate in a similar dimeric structure. In this configuration the complementary determining regions (CDRs) of each variable domain interact to define the antigen-binding specificity of the Fv dimer. Alternatively a single variable domain (or half of an Fv) can be used to recognize and bind antigen, although generally with lower affinity.

[0084] A "monoclonal antibody" as used herein refers to homogenous antibody population involved in the highly specific recognition and binding of a single antigenic determinant, or epitope. This is in

contrast to polyclonal antibodies that typically include different antibodies directed against different antigenic determinants. The term "monoclonal antibody" encompasses both intact and full-length monoclonal antibodies as well as antibody fragments (such as Fab, Fab', F(ab')2, Fv), single chain (scFv) mutants, fusion proteins comprising an antibody portion, and any other modified immunoglobulin molecule comprising an antigen recognition site. Furthermore, "monoclonal antibody" refers to such antibodies made in any number of manners including, but not limited to, by hybridoma, phage selection, recombinant expression, and transgenic animals.

[0085] As used herein, the term "humanized antibody" refers to forms of non-human (e.g., murine) antibodies that are specific immunoglobulin chains, chimeric immunoglobulins, or fragments thereof that contain minimal non-human sequences. Typically, humanized antibodies are human immunoglobulins in which residues from the complementary determining region (CDR) are replaced by residues from the CDR of a non-human species (e.g. mouse, rat, rabbit, hamster, etc.) that have the desired specificity, affinity, and capability. In some instances, the Fv framework region (FR) residues of a human immunoglobulin are replaced with the corresponding residues in an antibody from a non-human species that has the desired specificity, affinity, and capability. The humanized antibody can be further modified by the substitution of additional residue either in the Fv framework region and/or within the replaced non-human residues to refine and optimize antibody specificity, affinity, and/or capability. In general, the humanized antibody will comprise substantially all of at least one, and typically two or three, variable domains containing all or substantially all of the CDR regions that correspond to the non-human immunoglobulin whereas all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody can also comprise at least a portion of an immunoglobulin constant region or domain (Fc), typically that of a human immunoglobulin. Examples of methods used to generate humanized antibodies are described in U.S. Pat. No. 5,225,539, herein incorporated by reference.

[0086] A "variable region" of an antibody refers to the variable region of the antibody light chain or the variable region of the antibody heavy chain, either alone or in combination. The variable regions of the heavy and light chain each consist of four framework regions (FR) connected by three complementarity determining regions (CDRs) also known as hypervariable regions. The CDRs in each chain are held together in close proximity by the FRs and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies. There are at least two techniques for determining CDRs: (1) an approach based on cross-species sequence variability (i.e., Kabat et al. Sequences of Proteins of Immunological Interest, (5th ed., 1991, National Institutes of Health, Bethesda Md.)); and (2) an approach based on crystallographic studies of antigen-antibody complexes (Al-lazikani et al 1997, *J. Molec. Biol.* 273:927-948)). In addition, combinations of these two approaches are sometimes used in the art to determine CDRs.

[0087] The term "human antibody" as used herein means an antibody produced by a human or an antibody having an amino acid sequence corresponding to an antibody produced by a human made using any of the techniques known in the art. This definition of a human antibody includes intact or full-length antibodies, fragments thereof, and/or antibodies comprising at least one human heavy and/or light chain polypeptide such as, for example, an antibody comprising murine light chain and human heavy chain polypeptides.

[0088] "Hybrid antibodies" are immunoglobulin molecules in which pairs of heavy and light chains from antibodies with different antigenic determinant regions are assembled together so that two different epitopes or two different antigens can be recognized and bound by the resulting tetramer.

[0089] The term "chimeric antibodies" refers to antibodies wherein the amino acid sequence of the immunoglobulin molecule is derived from two or more species. Typically, the variable region of both light and heavy chains corresponds to the variable region of antibodies derived from one species of mammals (e.g. mouse, rat, rabbit, etc.) with the desired specificity, affinity, and capability while the constant regions are homologous to the sequences in antibodies derived from another (usually human) to avoid eliciting an immune response in that species.

[0090] The term "epitope" or "antigenic determinant" are used interchangeably herein and refer to that portion of an antigen capable of being recognized and specifically bound by a particular antibody. When the antigen is a polypeptide, epitopes can be formed both from contiguous amino acids and noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained upon protein denaturing, whereas epitopes formed by tertiary folding are typically lost upon protein denaturing. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation.

[0091] Competition between antibodies is determined by an assay in which the immunoglobulin under study inhibits specific binding of a reference antibody to a common antigen. Numerous types of competitive binding assays are known, for example: solid phase direct or indirect radioimmunoassay (RIA), solid phase direct or indirect enzyme immunoassay (EIA), sandwich competition assay (see Stahli et al., 1983, *Methods in Enzymology* 9:242-253); solid phase direct biotin-avidin EIA (see Kirkland et al., *J. Immunol.* 1986, 137:3614-3619); solid phase direct labeled assay, solid phase direct labeled sandwich assay (see Harlow and Lane, 1988, *Antibodies, A Laboratory Manual*, Cold Spring Harbor Press); solid phase direct label RIA using I-125 label (see Morel et al., 1988, *Molec. Immunol.* 25(1):7-15); solid phase direct biotin-avidin EIA (Cheung et al., 1990, *Virology* 176:546-552); and direct labeled RIA (Moldenhauer et al., 1990, *Scand. J. Immunol.* 32:77-82). Typically, such an assay involves the use of purified antigen bound to a solid surface or cells bearing either of these, an unlabeled test immunoglobulin and a labeled reference immunoglobulin. Competitive inhibition is measured by determining the amount of label bound to the solid surface or cells in the presence of the test immunoglobulin. Usually the test

immunoglobulin is present in excess. Antibodies identified by competition assay (competing antibodies) include antibodies binding to the same epitope as the reference antibody and antibodies binding to an adjacent epitope sufficiently proximal to the epitope bound by the reference antibody for steric hindrance to occur. Usually, when a competing antibody is present in excess, it will inhibit specific binding of a reference antibody to a common antigen by at least 50 or 75%.

[0092] That an antibody "selectively binds" or "specifically binds" to an epitope or receptor means that the antibody reacts or associates more frequently, more rapidly, with greater duration, with greater affinity, or with some combination of the above to the epitope or receptor than with alternative substances, including unrelated proteins. "Selectively binds" or "specifically binds" means, for instance, that an antibody binds to a protein with a K_D of about 0.1 mM or less, more usually about 1 μM or less. "Selectively binds" or "specifically binds" means at times that an antibody binds to a protein with a K_D of about 0.1 mM or less, at times about 1 μM or less, at times about 0.1 μM or less, at times about 0.01 μM or less, and at times about 1 nM or less. Because of the sequence identity between homologous proteins in different species, specific binding can include an antibody that recognizes a Notch receptor in more than one species. Likewise, because of homology between different Notch receptors (e.g., Notch2 and Notch3) in certain regions of the polypeptide sequences of the receptors, specific binding can include an antibody that recognizes more than one Notch receptor. It is understood that, in certain embodiments, an antibody or binding moiety that specifically binds to a first target may or may not specifically bind to a second target. As such, "specific binding" does not necessarily require (although it can include) exclusive binding, i.e. binding to a single target. Thus, an antibody may, in certain embodiments, specifically bind to more than one target (e.g., human Notch2 and Notch3). In certain embodiments, the multiple targets may be bound by the same antigen-binding site on the antibody. For example, an antibody may, in certain instances, comprise two identical antigen-binding sites, each of which specifically binds two or more human Notch receptors (e.g., human Notch2 and Notch3). In certain alternative embodiments, an antibody may be bispecific and comprise at least two antigen-binding sites with differing specificities. By way of non-limiting example, a bispecific antibody may comprise one antigen-binding site that recognizes an epitope on one Notch receptor, such as human Notch2, and further comprises a second, different antigen-binding site that recognizes a different epitope on a second Notch receptor, such as human Notch3. Generally, but not necessarily, reference to "binding" herein means "specific binding."

[0093] As used herein, the terms "non-specific binding" and "background binding" when used in reference to the interaction of an antibody and a protein or peptide refer to an interaction that is not dependent on the presence of a particular structure (i.e., the antibody is binding to proteins in general rather that a particular structure such as an epitope).

[0094] The terms "isolated" or "purified" refer to material that is substantially or essentially free from components that normally accompany it in its native state. Purity and homogeneity are typically

determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography. A protein (e.g. an antibody) or nucleic acid that is the predominant species present in a preparation is substantially purified. In particular, an isolated nucleic acid is separated from open reading frames that naturally flank the gene and encode proteins other than protein encoded by the gene. An isolated antibody is separated from other non-immunoglobulin proteins and from other immunoglobulin proteins with different antigen binding specificity. It can also mean that the nucleic acid or protein is at least 85% pure, at least 95% pure, and in some embodiments, at least 99% pure.

[0095] As used herein, the terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals in which a population of cells are characterized by unregulated cell growth. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma and various types of head and neck cancer.

[0096] The terms "proliferative disorder" and "proliferative disease" refer to disorders associated with abnormal cell proliferation such as cancer.

[0097] "Tumor" and "neoplasm" as used herein refer to any mass of tissue that result from excessive cell growth or proliferation, either benign (noncancerous) or malignant (cancerous) including pre-cancerous lesions.

[0098] "Metastasis" as used herein refers to the process by which a cancer spreads or transfers from the site of origin to other regions of the body with the development of a similar cancerous lesion at the new location. A "metastatic" or "metastasizing" cell is one that loses adhesive contacts with neighboring cells and migrates via the bloodstream or lymph from the primary site of disease to invade neighboring body structures.

[0099] As used herein, the term "subject" refers to any animal (e.g., a mammal), including, but not limited to humans, non-human primates, rodents, and the like, which is to be the recipient of a particular treatment. Typically, the terms "subject" and "patient" are used interchangeably herein in reference to a human subject.

[00100] The terms "cancer stem cell" or "tumor stem cell" or "solid tumor stem cell" are used interchangeably herein and refer to a population of cells from a solid tumor that: (1) have extensive proliferative capacity; 2) are capable of asymmetric cell division to generate one or more kinds of differentiated progeny with reduced proliferative or developmental potential; and (3) are capable of symmetric cell divisions for self-renewal or self-maintenance. These properties of "cancer stem cells" or

"tumor stem cells" or "solid tumor stem cells" confer on those cancer stem cells the ability to form palpable tumors upon serial transplantation into an immunocompromised mouse compared to the majority of tumor cells that fail to form tumors. Cancer stem cells undergo self-renewal versus differentiation in a chaotic manner to form tumors with abnormal cell types that can change over time as mutations occur.

[00101] The terms "cancer cell" or "tumor cell" and grammatical equivalents refer to the total population of cells derived from a tumor including both non-tumorigenic cells, which comprise the bulk of the tumor cell population, and tumorigenic stem cells (cancer stem cells).

[00102] As used herein "tumorigenic" refers to the functional features of a solid tumor stem cell including the properties of self-renewal (giving rise to additional tumorigenic cancer stem cells) and proliferation to generate all other tumor cells (giving rise to differentiated and thus non-tumorigenic tumor cells) that allow solid tumor stem cells to form a tumor.

[00103] As used herein, the "tumorigenicity" of a tumor refers to the ability of a random sample of cells from the tumor to form palpable tumors upon serial transplantation into immunocompromised mice.

[00104] As used herein, the terms "stem cell cancer marker" or "cancer stem cell marker" or "tumor stem cell marker" or "solid tumor stem cell marker" refer to a gene or genes or a protein, polypeptide, or peptide expressed by the gene or genes whose expression level, alone or in combination with other genes, is correlated with the presence of tumorigenic cancer cells compared to non-tumorigenic cells. The correlation can relate to either an increased or decreased expression of the gene (e.g., increased or decreased levels of mRNA or the peptide encoded by the gene).

[00105] The terms "cancer stem cell gene signature" or "tumor stem cell gene signature" or "cancer stem cell signature" are used interchangeably herein to refer to gene signatures comprising genes differentially expressed in cancer stem cells compared to other cells or population of cells, for example normal breast epithelial tissue. In some embodiments the cancer stem cell gene signatures comprise genes differentially expressed in cancer stem cells versus normal breast epithelium by a fold change, for example by 2 fold reduced and/or elevated expression, and further limited by using a statistical analysis such as, for example, by the P value of a t-test across multiple samples. In another embodiment, the genes differentially expressed in cancer stem cells are divided into cancer stem cell gene signatures based on the correlation of their expression with a chosen gene in combination with their fold or percentage expression change. Cancer stem cell signatures are predictive both retrospectively and prospectively of an aspect of clinical variability, including but not limited to metastasis and death.

[00106] The term "genetic test" as used herein refers to procedures whereby the genetic make-up of a patient or a patient tumor sample is analyzed. The analysis can include detection of DNA, RNA, chromosomes, proteins or metabolites to detect heritable or somatic disease-related genotypes or karyotypes for clinical purposes.

[00107] As used herein, the terms "biopsy" or "biopsy tissue" refer to a sample of tissue or fluid that is removed from a subject for the purpose of determining if the sample contains cancerous tissue. In some embodiments, biopsy tissue or fluid is obtained because a subject is suspected of having cancer. The biopsy tissue or fluid is then examined for the presence or absence of cancer.

[00108] As used herein an "acceptable pharmaceutical carrier" refers to any material that, when combined with an active ingredient of a pharmaceutical composition such as an antibody, allows the antibody, for example, to retain its biological activity. In addition, an "acceptable pharmaceutical carrier" does not trigger an immune response in a recipient subject. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, and various oil/water emulsions. Some diluents for aerosol or parenteral administration are phosphate buffered saline or normal (0.9%) saline.

[00109] The term "therapeutically effective amount" refers to an amount of an antibody, polypeptide, polynucleotide, small organic molecule, or other drug effective to "treat" a disease or disorder in a subject or mammal. In the case of cancer, the therapeutically effective amount of the drug can reduce the number of cancer cells; reduce the tumor size; inhibit or stop cancer cell infiltration into peripheral organs; inhibit and stop tumor metastasis; inhibit and stop tumor growth; relieve to some extent one or more of the symptoms associated with the cancer, or a combination of such effects on cancer cells. To the extent the drug prevents growth and/or kills existing cancer cells, it can be referred to as cytostatic and/or cytotoxic.

[00110] Terms such as "treating" or "treatment" or "to treat" or "alleviating" or "to alleviate" refer to both 1) therapeutic measures that cure, slow down, lessen symptoms of, and/or halt progression of a diagnosed pathologic condition or disorder and 2) prophylactic or preventative measures that prevent or slow the development of a targeted pathologic condition or disorder. Thus those in need of treatment include those already with the disorder; those prone to have the disorder; and those in whom the disorder is to be prevented. In some embodiments, a subject is successfully "treated" for cancer according to the methods of the present invention if the patient shows one or more of the following: a reduction in the number of or complete absence of cancer cells; a reduction in the tumor size; inhibition of or an absence of cancer cell infiltration into peripheral organs including the spread of cancer into soft tissue and bone; inhibition of or an absence of tumor metastasis; inhibition or an absence of tumor growth; relief of one or more symptoms associated with the specific cancer; reduced morbidity and mortality; and improvement in quality of life. Thus, in certain embodiments, treatment of cancer comprises inhibition of tumor growth in a subject.

[00111] As used herein, the terms "polynucleotide" or "nucleic acid" refer to a polymer composed of a multiplicity of nucleotide units (ribonucleotide or deoxyribonucleotide or related structural variants) linked via phosphodiester bonds, including but not limited to, DNA or RNA. The term encompasses sequences that include any of the known base analogs of DNA and RNA including, but not limited to, 4-acetylcytosine, 8-hydroxy-N6-methyladenosine, aziridinylcytosine, pseudoisocytosine,

5-(carboxyhydroxylmethyl) uracil, 5-fluorouracil, 5-bromouracil, 5-carboxymethylaminomethyl 2-thiouracil, 5- carboxymethylaminomethyluracil, dihydrouracil, inosine, N6-isopentenyladenine, 1- methyladenine, 1-methylpseudouracil, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-methyladenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl 2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarbonylmethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, oxybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2 thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, N-uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, and 2,6-diaminopurine.

[00112] The term "gene" refers to a nucleic acid (e.g., DNA) sequence that comprises coding sequences necessary for the production of a polypeptide, precursor, or RNA (e.g., rRNA, tRNA). The polypeptide can be encoded by a full length coding sequence or by any portion of the coding sequence so long as the desired activity or functional properties (e.g., enzymatic activity, ligand binding, signal transduction, immunogenicity, etc.) of the full-length polypeptide or fragment are retained. The term also encompasses the coding region of a structural gene and the sequences located adjacent to the coding region on both the 5' and 3' ends for a distance of about 1 kb or more on either end such that the gene corresponds to the length of the full-length mRNA. Sequences located 5' of the coding region and present on the mRNA are referred to as 5' non-translated sequences. Sequences located 3' or downstream of the coding region and present on the mRNA are referred to as 3' non-translated sequences. The term "gene" encompasses both cDNA and genomic forms of a gene. A genomic form or clone of a gene contains the coding region interrupted with non-coding sequences termed "introns" or "intervening regions" or "intervening sequences". Introns are segments of a gene that are transcribed into nuclear RNA (hnRNA); introns can contain regulatory elements such as enhancers. Introns are removed or "spliced out" from the nuclear or primary transcript; introns therefore are absent in the messenger RNA (mRNA) transcript. The mRNA functions during translation to specify the sequence or order of amino acids in a nascent polypeptide. In addition to containing introns, genomic forms of a gene can also include sequences located on both the 5' and 3' end of the sequences that are present on the RNA transcript. These sequences are referred to as "flanking" sequences or regions (these flanking sequences are located 5' or 3' to the non-translated sequences present on the mRNA transcript). The 5' flanking region can contain regulatory sequences such as promoters and enhancers that control or influence the transcription of the gene. The 3' flanking region can contain sequences that direct the termination of transcription, post transcriptional cleavage, and polyadenylation.

[00113] The term "recombinant" when used with reference to a cell, nucleic acid, protein or vector indicates that the cell, nucleic acid, protein or vector has been modified by the introduction of a heterologous nucleic acid or protein, the alteration of a native nucleic acid or protein, or that the cell is

derived from a cell so modified. Thus, e.g., recombinant cells express genes that are not found within the native (non-recombinant) form of the cell or express native genes that are overexpressed or otherwise abnormally expressed such as, for example, expressed as non-naturally occurring fragments or splice variants. By the term "recombinant nucleic acid" herein is meant nucleic acid, originally formed *in vitro*, in general, by the manipulation of nucleic acid, e.g., using polymerases and endonucleases, in a form not normally found in nature. In this manner, operably linkage of different sequences is achieved. Thus an isolated nucleic acid, in a linear form, or an expression vector formed *in vitro* by ligating DNA molecules that are not normally joined, are both considered recombinant for the purposes of this invention. It is understood that once a recombinant nucleic acid is made and introduced into a host cell or organism, it will replicate non-recombinantly, i.e., using the *in vivo* cellular machinery of the host cell rather than *in vitro* manipulations; however, such nucleic acids, once produced recombinantly, although subsequently replicated non-recombinantly, are still considered recombinant for the purposes of the invention. Similarly, a "recombinant protein" is a protein made using recombinant techniques, i.e., through the expression of a recombinant nucleic acid as depicted above.

[00114] As used herein, the term "vector" is used in reference to nucleic acid molecules that transfer DNA segment(s) from one cell to another. The term "vehicle" is sometimes used interchangeably with "vector." Vectors are often derived from plasmids, bacteriophages, or plant or animal viruses.

[00115] As used herein, the term "gene expression" refers to the process of converting genetic information encoded in a gene into RNA (e.g., mRNA, rRNA, tRNA, or snRNA) through "transcription" of the gene (e.g., via the enzymatic action of an RNA polymerase), and for protein encoding genes, into protein through "translation" of mRNA. Gene expression can be regulated at many stages in the process. "Up-regulation" or "activation" refers to regulation that increases the production of gene expression products (e.g., RNA or protein), while "down-regulation" or "repression" refers to regulation that decrease production. Molecules (e.g., transcription factors) that are involved in up-regulation or down-regulation are often called "activators" and "repressors", respectively.

[00116] The terms "polypeptide" or "peptide" or "protein" or "protein fragment" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymers.

[00117] The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function similarly to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, gamma-carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino

acid, e.g., an alpha carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs can have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions similarly to a naturally occurring amino acid.

[00118] "Conservatively modified variants" applies to both amino acid and nucleic acid sequences. "Amino acid variants" refers to amino acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical or associated (e.g., naturally contiguous) sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode most proteins. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to another of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes silent variations of the nucleic acid. It is recognized that in certain contexts each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, silent variations of a nucleic acid which encodes a polypeptide is implicit in a described sequence with respect to the expression product, but not with respect to actual probe sequences. As to amino acid sequences, it will be recognized that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" including where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention. Tables providing functionally similar amino acids useful for conservative amino acid substitutions are well known in the art. Typical conservative substitutions include: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (see, e.g., Creighton, Proteins (1984)). (See, also, Table 1 herein).

[00119] As used in the present disclosure and claims, the singular forms "a", "an" and "the" include plural forms unless the context clearly dictates otherwise.

[00120] It is understood that wherever embodiments are described herein with the language "comprising," otherwise analogous embodiments described in terms of "consisting of" and/or "consisting essentially of" are also provided.

Certain Embodiments of the Present Invention

[00121] The present invention provides compositions and methods for studying, diagnosing, characterizing, and treating cancer. In particular, in certain embodiments, the present invention provides agents, including antagonists, that bind Notch receptors and methods of using the agents or antagonists to inhibit tumor growth and treat cancer or other disease in human patients. In certain embodiments, the antagonists are antibodies that specifically recognize one or more human Notch receptors.

[00122] In one aspect, the present invention provides an antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human Notch receptor. In some embodiments, the antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human Notch receptor inhibits growth of tumors. In certain embodiments, the antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human Notch receptor and inhibits tumor growth, specifically binds to a non-ligand binding region of the extracellular domain of at least two Notch receptor family members. In certain embodiments, the antibody binds to a non-ligand binding region of the extracellular domain of Notch2 and/or Notch3 receptor. In some embodiments, the antibody binds to a non-ligand binding region of the human Notch2. In some embodiments, the antibody binds to a non-ligand binding region of the extracellular domain of Notch2 and Notch3. In some embodiments, the antibody binds to a non-ligand binding region of the human Notch3. In some embodiments, the antibody binds to Notch1 and/or Notch4.

[00123] In certain embodiments, the antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human Notch receptor and inhibits tumor growth is a monoclonal antibody. In certain embodiments, the antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human Notch receptor and inhibits growth of tumors is a chimeric antibody. In certain embodiments, the antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human Notch receptor and inhibits growth of tumors is a humanized antibody. In certain embodiments, the antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human Notch receptor and inhibits tumor growth is a human antibody. In certain embodiments, the antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human Notch receptor and inhibits tumor growth is a monospecific antibody. In certain embodiments, the antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human Notch receptor and inhibits tumor growth is a bispecific antibody. In certain embodiments, the present invention provides a hybridoma producing an antibody that specifically

binds to a non-ligand binding region of the extracellular domain of a human Notch receptor and inhibits tumor growth.

[00124] In certain embodiments the present invention provides an antibody that specifically binds to a non-ligand binding region comprising EGF repeats 1-10 of the extracellular domain of a human Notch receptor and inhibits tumor growth. In certain embodiments the present invention provides an antibody that specifically binds to a non-ligand binding region comprising EGF repeat 10 (or equivalent) of the extracellular domain of a human Notch receptor and inhibits tumor growth. In certain embodiments the present invention provides an antibody that specifically binds to a non-ligand binding region comprising EGF repeats 13-36 of the extracellular domain of a human Notch receptor and inhibits tumor growth. Certain embodiments provide an antibody that specifically binds to a non-ligand binding region comprising EGF repeats 4 of the extracellular domain of a human Notch receptor and inhibits tumor growth. Certain embodiments provide an antibody that specifically binds to a non-ligand binding region comprising EGF repeat 13 of the extracellular domain of a human Notch receptor and inhibits tumor growth. In certain embodiments, the antibody specifically binds to a non-ligand binding region comprising the LNR-HD domain and inhibits tumor growth.

[00125] In certain embodiments the present invention provides a method of treating cancer in a subject in need thereof comprising administering to the subject a therapeutically effective amount of an antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human Notch receptor protein and inhibits tumor growth in the subject. In certain embodiments, the method of treating cancer comprises administering a therapeutically effective amount of an antibody that specifically binds to at least two Notch receptor family members and inhibits tumor growth. In certain embodiments, the method of treating cancer in a subject in need thereof comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds to a non-ligand binding region of the extracellular domain of Notch2 and/or Notch3 receptor and inhibits tumor growth.

[00126] In certain embodiments, the method of treating cancer comprises administering a therapeutically effective amount of a monoclonal antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human Notch receptor and inhibits tumor growth. In certain embodiments, the method of treating cancer comprises administering a therapeutically effective amount of a chimeric antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human Notch receptor and inhibits tumor growth. In certain embodiments, the method of treating cancer comprises administering a therapeutically effective amount of a humanized antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human Notch receptor and inhibits tumor growth. In certain embodiments, the method of treating cancer comprises administering a therapeutically effective amount of a human antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human Notch receptor and inhibits tumor growth. In some

embodiments, the antibody is a monospecific antibody. In some embodiments, the antibody is a bispecific antibody.

[00127] In certain embodiments, the method of treating cancer comprises administering a therapeutically effective amount of an antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human Notch receptor comprising EGF repeats 1-10 and inhibits tumor growth. In certain embodiments, the method of treating cancer comprises administering a therapeutically effective amount of an antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human Notch receptor comprising EGF repeat 10 (or the equivalent if Notch3) and inhibits tumor growth. In certain embodiments, the method of treating cancer comprises administering a therapeutically effective amount of an antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human Notch receptor comprising EGF repeats 13-36 and inhibits tumor growth. In certain embodiments, the method of treating cancer comprises administering a therapeutically effective amount of an antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human Notch receptor comprising EGF repeat 4 and inhibits tumor growth. In certain embodiments, the method of treating cancer comprises administering a therapeutically effective amount of an antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human Notch receptor comprising EGF repeat 4 and inhibits tumor growth. In certain other embodiments, the antibody that is administered specifically binds to the LNR-HD domain of a human Notch receptor.

[00128] In certain embodiments, the method of treating cancer comprises administering a therapeutically effective amount of an antibody conjugated to a cytotoxic moiety that specifically binds to a non-ligand binding region of the extracellular domain of a human Notch receptor and inhibits tumor growth. In certain embodiments, the method of treating cancer comprises administering a therapeutically effective amount of an antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human Notch receptor and inhibits tumor growth in combination with radiation therapy. In certain embodiments, the method of treating cancer comprises administering a therapeutically effective amount of an antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human Notch receptor and inhibits tumor growth in combination with chemotherapy. In certain embodiments, the method of treating cancer comprises administering a therapeutically effective amount of an antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human Notch receptor and inhibits tumor growth that are from a breast tumor, colorectal tumor, lung tumor, pancreatic tumor, prostate tumor, or a head and neck tumor.

[00129] In certain embodiments, the method of treating cancer comprises identifying patients for treatment with the antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human Notch receptor using a genetic test; and administering a therapeutically effective amount of an antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human

Notch receptor and inhibits tumor growth. In certain embodiments, the method of treating cancer comprises identifying patients for treatment with the antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human Notch receptor using a genetic test that detects a cancer stem cell signature; and administering a therapeutically effective amount of an antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human Notch receptor and inhibits tumor growth.

[00130] In certain embodiments, the present invention provides a method of identifying a molecule that binds to a non-ligand binding region of an extracellular domain of a human Notch receptor and inhibits tumor growth, the method comprising: i) incubating the molecule with the non-ligand binding domain of the extracellular domain of a human Notch receptor; ii) determining if the molecule binds to the non-ligand binding region of the extracellular domain of the human Notch receptor; and iii) determining if the molecule inhibits tumor growth. In certain embodiments, the invention provides a method of identifying a molecule that binds to a non-ligand binding region of an extracellular domain of a human Notch receptor and inhibits tumor growth, the method comprising: i) incubating the molecule with the non-ligand binding domain of the extracellular domain of a human Notch receptor comprising EGF repeats 1-10; ii) determining if the molecule binds to the non-ligand binding region of the extracellular domain of the human Notch receptor comprising EGF repeats 1-10; and iii) determining if the molecule inhibits tumor growth. In certain embodiments, the invention provides a method of identifying a molecule that binds to a non-ligand binding region of an extracellular domain of a human Notch receptor and inhibits tumor growth, the method comprising: i) incubating the molecule with the non-ligand binding domain of the extracellular domain of a human Notch receptor comprising EGF repeat 10 (or equivalent if Notch3); ii) determining if the molecule binds to the non-ligand binding region of the extracellular domain of the human Notch receptor comprising EGF repeat 10 (or equivalent if Notch3); and iii) determining if the molecule inhibits tumor growth. In certain embodiments, the invention provides a method of identifying a molecule that binds to a non-ligand binding region of an extracellular domain of a human Notch receptor and inhibits tumor growth, the method comprising: i) incubating the molecule with the non-ligand binding domain of the extracellular domain of a human Notch receptor comprising EGF repeats 13-36; ii) determining if the molecule binds to the non-ligand binding region of the extracellular domain of the human Notch receptor comprising EGF repeats 13-36; and iii) determining if the molecule inhibits tumor growth.

[00131] In certain embodiments, the present invention provides a pharmaceutical composition comprising an antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human Notch receptor and inhibits tumor growth.

[00132] In certain embodiments, the present invention provides a method of making an antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human Notch receptor and inhibits tumor growth.

[00133] In certain embodiments, the present invention provides an isolated nucleic acid that encodes an antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human Notch receptor and inhibits tumor growth.

[00134] In some embodiments, the invention provides an agent (e.g., an antibody) that specifically binds to an EGF10 domain (or an equivalent of an EGF10 domain if Notch3) of one or more human Notch receptors. In certain embodiments, the agent is an antibody. In certain embodiments, the agent is an antagonist. In certain embodiments, the agent specifically binds to EGF10 of human Notch2 and/or EGF9 of human Notch3. EGF9 is the EGF within human Notch3 that is equivalent to EGF10 in the other human Notch receptors Notch1, Notch2, and Notch4. In certain embodiments, the agent specifically binds human Notch2. In certain embodiments, the agent specifically binds human Notch2 and Notch3. In certain embodiments, the agent specifically binds human Notch3.

[00135] In one aspect, the invention provides a 59R1 antibody comprising the heavy chain and light chain sequences provided in SEQ ID NOs:16 and 18 (with or without signal sequence), respectively, or as encoded by the DNA deposited with ATCC on October 15, 2008, and assigned designation number PTA-9547. The invention further provides polypeptides or antibodies that comprise the heavy chain variable region (e.g., SEQ ID NO:14) and/or the light chain variable region (e.g., SEQ ID NO:13) of such a 59R1 antibody. The invention further provides polypeptides or antibodies comprising one or more (e.g., 1, 2, or 3) of the heavy chain CDRs, and/or one or more of the light chain CDRs of the 59R1 antibody. In still further embodiments, the invention provides antibodies that bind to the same epitope as the 59R1 antibody or antibodies that compete for specific binding to human Notch2 and/or Notch3 with the 59R1 antibody.

[00136] In another aspect, the invention provides a 59R5 antibody comprising the heavy chain and light chain sequences provided in SEQ ID NOs:49 and 18 (with or without signal sequence), respectively, or as encoded by the DNA deposited with ATCC on July 6, 2009 and assigned designation number [...]. The invention further provides polypeptides or antibodies that comprise the heavy chain variable region and/or the light chain variable region sequences SEQ ID NO:50 and/or SEQ ID NO:13. The invention further provides polypeptides or antibodies comprising one or more (e.g., 1, 2, or 3) of the heavy chain CDRs and/or one or more of the light chain CDRs of the 59R5 antibody. In still further embodiments, the invention provides antibodies that bind to the same epitope as the 59R5 antibody or antibodies that compete for specific binding to human Notch2 and/or Notch3 with the 59R5 antibody.

[00137] In certain additional embodiments, the invention provides an antibody that specifically binds to two or more (i.e., at least two or two, three, or four) human Notch receptors. In certain embodiments, the antibody specifically binds to a non-ligand binding region of an extracellular domain of the two or more

human Notch receptors. In certain embodiments, the antibody is a monospecific antibody that specifically binds to a non-ligand binding region of an extracellular domain of the two or more human Notch receptors. In certain embodiments, the antibody binds to EGF10 of Notch1, Notch2, or Notch4, and/or to EGF9 of Notch3. In certain embodiments, the non-ligand binding region to which the antibody binds is not EGF4 or does not comprise EGF4. In certain embodiments, the two or more human Notch receptors comprise Notch2 and/or Notch3. In certain embodiments, the two or more human Notch receptors comprise Notch2 and Notch3. In certain embodiments, the antibody is an antagonist of the two or more human Notch receptors.

[00138] The invention further provides a method of modulating the function of pericytes and/or vascular smooth muscle cells in a subject, wherein the method comprises administering to the subject an effective amount of an agent that specifically binds human Notch2 and/or human Notch3. In certain embodiments, the agent is an antibody. In certain embodiments, the agent is an antagonist.

[00139] The invention further provides a method of inhibiting angiogenesis in a subject, comprising the step of administering to the subject an effective amount of an agent that specifically binds human Notch2 and/or human Notch3. In certain embodiments, the agent is an antibody. In certain embodiments, the agent is an antagonist. In certain embodiments, the antagonist is an antagonist of Notch2. In certain embodiments, the antagonist is an antagonist of Notch3. In certain embodiments, the antagonist is an antagonist of Notch2 and Notch3. In some embodiments, the method of inhibiting angiogenesis comprises modulating the function of pericytes and/or vascular smooth muscle cells. In some embodiments, the angiogenesis is tumor angiogenesis.

[00140] In certain embodiments, the Notch-binding agent is an antagonist of the human Notch receptor(s) to which it specifically binds. In some alternative embodiments, the Notch-binding agent is an agonist of the human Notch receptor(s) to which it specifically binds.

[00141] In certain embodiments, the agent that specifically binds to one or more Notch receptor(s) and is an antagonist of the one or more Notch receptor(s) inhibits at least about 10%, at least about 20%, at least about 30%, at least about 50%, at least about 75%, at least about 90%, or about 100% of one or more activities of the bound Notch receptor(s).

[00142] In certain embodiments, the antagonist of one or more human Notch receptor(s) (e.g., Notch2 and/or Notch3) has one or more of the following effects: inhibit ligand binding to the one or more human Notch receptors, inhibit ligand-induced signaling by the one or more Notch receptors; inhibit proliferation of tumor cells; reduce the tumorigenicity of a tumor by reducing the frequency of cancer stem cells in the tumor; inhibit tumor growth; increase survival, trigger cell death of tumor cells; inhibit angiogenesis; or prevent metastasis of tumor cells.

[00143] In certain embodiments, the antagonist has one or more of the following effects: interference with the expression of a Notch receptor; interference with activation of a Notch receptor signal transduction

pathway by, for example, sterically inhibiting interactions between the Notch receptor and one or more of its ligands, or binding to a human Notch receptor and triggering cell death or inhibiting cell proliferation.

[00144] In certain embodiments, antagonists against a Notch receptor, such as Notch2 or Notch3, act extracellularly to act upon or inhibit the function of the Notch receptor. In certain embodiments, an antagonist is a small molecule that binds to the extracellular domain of a Notch receptor. In certain embodiments, an antagonist of a Notch receptor is proteinaceous. In some embodiments, proteinaceous antagonists of a Notch receptor are antibodies that specifically bind to an extracellular epitope of a Notch receptor. Extracellular binding of an antagonist against a Notch receptor can inhibit the signaling of a Notch receptor protein by inhibiting intrinsic activation (e.g., kinase activity) of a Notch receptor and/or by sterically inhibiting the interaction, for example, of a Notch receptor with one of its ligands. Furthermore, extracellular binding of an antagonist against a Notch receptor can down-regulate cell-surface expression of a Notch receptor such as, for example, by internalization of a Notch receptor and/or decreasing cell surface trafficking of a Notch receptor.

[00145] In certain embodiments, the Notch-binding agent or antagonist (e.g., antibody) specifically binds to a non-ligand binding region of an extracellular domain of at least one human Notch receptor, wherein the non-ligand binding region comprises EGF repeat 10 (or the equivalent if Notch3). In certain embodiments, the agent or antagonist specifically binds to Notch2. In certain embodiments, the agent or antagonist specifically binds to Notch3. In certain embodiments, the agent or antagonist specifically binds to both human Notch2 and human Notch3.

[00146] In certain embodiments, the Notch-binding agent or antagonist (e.g., antibody) specifically binds to an EGF10 domain of human Notch2. In certain embodiments, the Notch-binding agent or antagonist does not bind to any region of the human Notch2 outside of the EGF10 domain. In certain alternative embodiments, the Notch-binding agent or antagonist that specifically binds to an EGF10 domain of human Notch2, also further binds to another region of human Notch2. In other words, in some embodiments, the entire epitope of the agent or antagonist falls within EGF10. In certain other embodiments, the epitope of the agent or antagonist that binds to human Notch2 partially overlaps with EGF10. In certain embodiments, the agent or antagonist binds to at least part of the sequence HKGAL (SEQ ID NO:28) within human Notch2 EGF10. In certain embodiments, the agent or antagonist also binds to other amino acids within human Notch2 EGF10 (e.g., the entire epitope of an anti-Notch2 antibody is not necessarily contained entirely within the sequence HKGAL). In certain embodiments, the Notch-binding agent or antagonist further specifically binds to at least one additional human Notch receptor (e.g., Notch1, Notch3, or Notch4). In certain embodiments, the Notch-binding agent or antagonist that binds to EGF10 of human Notch2 further binds to an EGF10 domain of human Notch1, an EGF9 domain of human Notch3, and/or an EGF10 domain of human Notch4. In certain embodiments, the additional human Notch receptor is human Notch3.

[00147] In certain embodiments, the Notch-binding agent or antagonist (e.g., antibody) binds to an EGF9 domain of human Notch3. As is apparent from the homology between the sequences of the extracellular domains of human Notch2 and human Notch3, EGF9 is the EGF that is the functional/structural equivalent of Notch2 EGF10 in human Notch3. In certain embodiments, the Notch-binding agent or antagonist does not bind to any region of the human Notch3 outside of EGF9. In certain embodiments, the agent or antagonist binds to at least part of the sequence HEDAI (SEQ ID NO:29) within the human Notch3 EGF9 domain. HEDAI (SEQ ID NO:29) is the sequence within the Notch3 EGF9 domain that corresponds to sequence HKGAL (SEQ ID NO:28) within human Notch2 EGF10. In certain embodiments, the agent or antagonist also binds to other amino acids within human Notch3 EGF9. In certain embodiments, the Notch-binding agent or antagonist binds to an EGF10 domain of at least one additional human Notch receptor (e.g., Notch1, Notch2, and/or Notch4). In certain embodiments, the additional human Notch receptor is human Notch2, such as an agent or antagonist that binds to an EGF10 domain of human Notch2. In certain embodiments, the Notch-binding agent or antagonist does not bind to any region of the human Notch2 outside of EGF10. In certain embodiments, the agent or antagonist binds to at least part of the sequence HKGAL (SEQ ID NO:28) within human Notch2 EGF10. In some embodiments, the agent or antagonist is a monospecific antibody that binds to at least part of the sequence HKGAL (SEQ ID NO:28) in Notch2 and also binds to at least part of the sequence HEDAI (SEQ ID NO:29) in Notch3.

[00148] In certain alternative embodiments, the Notch-binding agent or antagonist binds to a portion of the non-ligand binding region of an extracellular domain of a Notch1, Notch2, or Notch4 receptor in a region other than EGF10 or a Notch3 receptor in a region other than EGF9. For example, in certain embodiments, the agent or antagonist binds to the LNR-HD domain of one or more Notch receptors. In certain embodiments, the agent or antagonist binds to EGF1, EGF2, EGF3, EGF4, EGF5, EGF6, EGF7, EGF9, EGF10, EGF13, EGF14, EGF15, EGF16, EGF17, EGF18, EGF19, EGF20, EGF21, EGF22, EGF23, EGF24, EGF25, EGF26, EGF27, EGF28, EGF29, EGF30, EGF31, EGF32, EGF33, EGF34, EGF35, and/or EGF36 of an extracellular domain of one or more Notch receptors.

[00149] In certain embodiments, the Notch-binding agent or antagonist binds to the ligand binding region of an extracellular domain of one or more human Notch receptors. Thus, in certain embodiments, the Notch-binding agent or antagonist may bind to EGF11 and/or EGF12 of Notch 1, 2, or 4 (Rebay et al., 1991, *Cell* 67:687; Lei et al., 2003, *Dev.* 130:6411; Hambleton et al., 2004, *Structure* 12:2173) or EGF10 and/or EGF11 of Notch3 (Peters et al., 2004, *Experimental Cell Research*, 299:454-464).

[00150] In certain embodiments, the Notch-binding agent (e.g., antibody) specifically binds to two or more human Notch receptors (e.g., Notch1, Notch2, Notch3, and/or Notch4). In other words, in certain embodiments, the agent or antibody binds at least two human Notch receptors (i.e., two, three, or four human Notch receptors). Encompassed are agents and antibodies that specifically bind to two human

Notch family receptors (e.g., Notch2 and Notch3, Notch1 and Notch2, Notch1 and Notch3, Notch1 and Notch4, Notch2 and Notch4, or Notch3 and Notch4). Agents and antibodies that specifically bind to three human Notch receptor family members are also envisioned (e.g., agents and antibodies that specifically bind to Notch1, Notch2, and Notch3, Notch1, Notch2, and Notch4, or Notch2, Notch3, and Notch4), as are agents and antibodies that specifically bind to four human Notch receptor family members (e.g., agents and antibodies that specifically bind to Notch1, Notch2, Notch3 and Notch4). In certain embodiments, the agent or antibody specifically binds to both human Notch2 and Notch3. In certain alternative embodiments, the agent or antibody specifically binds to both human Notch1 and Notch2. In some embodiments, the agent or antibody specifically binds to both human Notch1 and Notch3. In still further embodiments, the agent or antibody specifically binds to both human Notch1 and Notch4. In certain embodiments, the agent or antibody is an antagonist of the two or more human Notch receptors.

[00151] In certain embodiments, the Notch-binding agent or antagonist binds to a Notch receptor (e.g., Notch2 and/or Notch3) with a dissociation constant of about 1 μM or less, about 100 nM or less, about 40 nM or less, about 20 nM or less, or about 10 nM or less. In certain embodiments, the agent or antagonist binds one or more human Notch receptors, such as human Notch2 and/or human Notch3, with a K_D of 1 nM or less. In some embodiments, the Notch binding agent is an antibody that binds to Notch2 with a K_D of about 1 nM or less. In some embodiments, the Notch binding agent is an antibody that binds to Notch3 with a K_D of about 1 nM or less. In certain embodiments, the dissociation constant for the agent or antagonist with respect to a particular Notch receptor is the dissociation constant determined using a Notch-Fc fusion protein comprising the Notch extracellular domain and/or a portion of the extracellular domain comprising EGF10 immobilized on a Biacore chip.

[00152] In certain embodiments, the antagonist specifically binds to human Notch3 and inhibits binding of a ligand (e.g., DLL4, JAG1, and/or JAG2) to human Notch3 and/or inhibits signaling of human Notch3. In certain embodiments, the antagonist specifically binds to human Notch2 and inhibits binding of a ligand (e.g., DLL4, JAG1, and/or JAG2) to human Notch2 and/or inhibits signaling of human Notch2. In certain embodiments, the antagonist inhibits DLL4-induced Notch2 signaling. In certain embodiments, the antagonist inhibits DLL4-induced Notch3 signaling. In certain embodiments, the antagonist inhibits JAG2-induced Notch2 signaling. In certain embodiments, the antagonist inhibits JAG2-induced Notch3 signaling. In certain embodiments, the signaling by Notch2 and/or Notch3 is reduced by at least about 10%, by at least about 25%, by at least about 50%, by at least about 75%, by at least about 90%, or by at least about 95%. In certain embodiments, the binding of one or more ligands to Notch2 and/or Notch3 is reduced by at least about 10%, by at least about 25%, by at least about 50%, by at least about 75%, by at least about 90%, or by at least about 95%.

[00153] In some embodiments, antagonists against a Notch receptor bind to a Notch receptor and have one or more of the following effects: inhibit proliferation of tumor cells, trigger cell death directly in tumor

cells, or prevent metastasis of tumor cells. In certain embodiments, antagonists of a Notch receptor trigger cell death via a conjugated toxin, chemotherapeutic agent, radioisotope, or other such agent. For example, an antibody against a Notch receptor is conjugated to a toxin that is activated in tumor cells expressing the Notch receptor by protein internalization. In other embodiments, antagonists of a Notch receptor mediate cell death of a cell expressing the Notch receptor via antibody-dependent cellular cytotoxicity (ADCC). ADCC involves cell lysis by effector cells that recognize the Fc portion of an antibody. Many lymphocytes, monocytes, tissue macrophages, granulocytes and eosinophils, for example, have Fc receptors and can mediate cytolysis (Dillman, 1994, *J. Clin. Oncol.* 12:1497). In some embodiments, an antagonist of a Notch receptor is an antibody that triggers cell death of cell expressing a Notch receptor by activating complement-dependent cytotoxicity (CDC). CDC involves binding of serum complement to the Fc portion an antibody and subsequent activation of the complement protein cascade, resulting in cell membrane damage and eventual cell death. Biological activity of antibodies is known to be determined, to a large extent, by the constant domains or Fc region of the antibody molecule (Uananue and Benacerraf, Textbook of Immunology, 2nd Edition, Williams & Wilkins, p. 218 (1984)). Antibodies of different classes and subclasses differ in this respect, as do antibodies of the same subclass but from different species. Of human antibodies, IgM is the most efficient class of antibodies to bind complement, followed by IgG1, IgG3, and IgG2 whereas IgG4 appears quite deficient in activating the complement cascade (Dillman, 1994, *J. Clin. Oncol.* 12:1497; Jefferis et al., 1998, *Immunol. Rev.* 163:59-76). According to the present invention, antibodies of those classes having the desired biological activity are prepared.

[00154] The ability of any particular antibody against a Notch receptor to mediate lysis of the target cell by complement activation and/or ADCC can be assayed. The cells of interest are grown and labeled *in vitro*; the antibody is added to the cell culture in combination with either serum complement or immune cells which can be activated by the antigen antibody complexes. Cytolysis of the target cells is detected, for example, by the release of label from the lysed cells. In fact, antibodies can be screened using the patient's own serum as a source of complement and/or immune cells. The antibody that is capable of activating complement or mediating ADCC in the *in vitro* test can then be used therapeutically in that particular patient.

[00155] In certain embodiments, the Notch-binding agent or antagonist is an antibody that does not have one or more effector functions. For instance, in some embodiments, the antibody has no antibody-dependent cellular cytoxicity (ADCC) activity and/or no complement-dependent cytoxicity (CDC) activity. In certain embodiments, the antibody does not bind to an Fc receptor and/or complement factors. In certain embodiments, the antibody has no effector function.

[00156] In other embodiments, antagonists of a Notch receptor can trigger cell death indirectly by inhibiting angiogenesis. Angiogenesis is the process by which new blood vessels form from pre-existing

vessels and is a fundamental process required for normal growth, for example, during embryonic development, wound healing, and in response to ovulation. Solid tumor growth larger than 1-2 mm^2 also requires angiogenesis to supply nutrients and oxygen without which tumor cells die. Thus, in certain embodiments, an antagonist of a Notch receptor targets vascular cells that express the Notch receptor including, for example, endothelial cells, smooth muscle cells or components of the extracellular matrix required for vascular assembly. In certain embodiments, an antagonist of a Notch receptor (e.g., Notch2 and/or Notch3) targets pericytes and/or vascular smooth muscle cells. In other embodiments, an antagonist of a Notch receptor inhibits growth factor signaling required by vascular cell recruitment, assembly, maintenance or survival. In certain embodiments, the antagonist modulates the function of pericytes and/or vascular smooth muscle cells.

[00157] In certain embodiments the Notch-binding agents or antagonists (e.g., antibodies), either alone or in combination with a second therapeutic agent, are capable of inhibiting tumor growth. In certain embodiments, the Notch-binding agents or antagonists are capable of inhibiting tumor growth *in vivo* (e.g., in a xenograft mouse model and/or in a human having cancer). In certain embodiments, the Notch-binding agents or antagonists are capable of inhibiting tumor growth by at least about 10%, at least about 25%, at least about 50%, at least about 75%, at least about 90% at a given time point in a xenograft model. In certain embodiments, the Notch-binding agents or antagonists prevent tumor growth. In certain embodiments, the Notch-binding agents or antagonists inhibit tumor recurrence.

[00158] In certain embodiments, the Notch-binding agents are capable of reducing the tumorigenicity of a tumor. In certain embodiments, the agent or antibody is capable of reducing the tumorigenicity of a tumor comprising cancer stem cells in an animal model, such as a mouse xenograft model. In certain embodiments, the number or frequency of cancer stem cells in a tumor is reduced by at least about two-fold, about three-fold, about five-fold, about ten-fold, about 50-fold, about 100-fold, or about 1000-fold (e.g., in a xenograft model). In certain embodiments, the reduction in the frequency of cancer stem cells is determined by limiting dilution assay using an animal model. An example of a limiting dilution assay used to test the efficacy of an anti-Notch antibody is provided in Example 8, below. Additional examples and guidance regarding the use of limiting dilution assays to determine a reduction in the number or frequency of cancer stem cells in a tumor can be found, e.g., in International Publication No. WO 2008/042236, U.S. Patent Application Publication Nos. 2008/0064049, and 2008/0178305, each of which is incorporated by reference herein in its entirety.

[00159] The present invention provides a variety of polypeptides, including but not limited to, antibodies and fragments of antibodies. In certain embodiments, the polypeptide is isolated. In certain alternative embodiments, the polypeptide is substantially pure.

[00160] In certain embodiments, the polypeptides of the present invention can be recombinant polypeptides, natural polypeptides, or synthetic polypeptides comprising the sequence of SEQ ID NOs:2,

4, 13, 14, 16, 18, 19, 20, 39, 40, 49, 50, 52, 53, 54, 55, 56, or 57 (with or without the indicated signal sequences), as well as the polypeptides comprising the polypeptides encoded by the polynucleotides of SEQ ID NOs: 1, 3, 15, 17, 47, 48, 58, 59, or 60 (with or without the indicated signal sequences).

[00161] The invention provides a polypeptide comprising the heavy chain and/or the light chain of 59R1 provided in SEQ ID NO:16 and/or SEQ ID NO:18, respectively. In certain embodiments, the polypeptide is an antibody. In certain embodiments, the polypeptide specifically binds Notch2 and/or Notch3. In some embodiments, the polypeptide specifically binds Notch2 and Notch3.

[00162] The invention provides a polypeptide comprising the heavy chain and/or the light chain of 59R5 provided in SEQ ID NO:49 and/or SEQ ID NO:18, respectively. In certain embodiments, the polypeptide is an antibody. In certain embodiments, the polypeptide specifically binds Notch2 and/or Notch3. In some embodiments, the polypeptide specifically binds Notch2 and Notch3.

[00163] The invention further provides a polypeptide comprising SEQ ID NO:13 and/or SEQ ID NO:14. In certain embodiments, the polypeptide comprises a variable light chain sequence comprising SEQ ID NO:13 and/or a variable heavy chain sequence comprising SEQ ID NO:14. In some embodiments, the polypeptide comprises a variable light chain sequence comprising SEQ ID NO:13 and a variable heavy chain sequence comprising SEQ ID NO:14. In certain embodiments, the polypeptide comprises a variable light chain sequence comprising SEQ ID NO:13 and/or a variable heavy chain sequence comprising SEQ ID NO:50. In some embodiments, the polypeptide comprises a variable light chain sequence comprising SEQ ID NO:13 and a variable heavy chain sequence comprising SEQ ID NO:50. In certain embodiments, the polypeptide comprises a variable light chain sequence comprising SEQ ID NO:13 and/or a variable heavy chain sequence comprising SEQ ID NO:52. In certain embodiments, the polypeptide comprises a variable light chain sequence comprising SEQ ID NO:13 and/or a variable heavy chain sequence comprising SEQ ID NO:53. In certain embodiments, the polypeptide comprises a variable light chain sequence comprising SEQ ID NO:13 and/or a variable heavy chain sequence comprising SEQ ID NO:54. In certain embodiments, the polypeptide comprises a variable light chain sequence comprising SEQ ID NO:13 and/or a variable heavy chain sequence comprising SEQ ID NO:55. In certain embodiments, the polypeptide comprises a variable light chain sequence comprising SEQ ID NO:13 and/or a variable heavy chain sequence comprising SEQ ID NO:56. In certain embodiments, the polypeptide comprises a variable light chain sequence comprising SEQ ID NO:13 and/or a variable heavy chain sequence comprising SEQ ID NO:57. In certain embodiments, the polypeptide is an antibody. In certain embodiments, the polypeptide specifically binds Notch2 and/or Notch3. In some embodiments, the polypeptide specifically binds Notch2 and Notch3. In some embodiments, the polypeptide specifically binds human Notch2. In some embodiments, the polypeptide specifically binds human Notch3.

[00164] It will be recognized in the art that some amino acid sequences of the invention can be varied without significant effect of the structure or function of the protein. If such differences in sequence are

contemplated, it should be remembered that there will be critical areas on the protein which determine activity. Thus, the invention further includes variations of the polypeptides which show substantial activity. Such mutants include deletions, insertions, inversions, repeats, and type substitutions. Guidance concerning which amino acid changes are likely to be phenotypically silent can be found in Bowie et al., Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions, 1990, *Science* 247:1306-1310.

[00165] Thus, the fragments, derivatives, or analogs of the polypeptides of the invention can be: (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (often a conserved amino acid residue) and such substituted amino acid residue can or cannot be one encoded by the genetic code; or (ii) one in which one or more of the amino acid residues includes a substituent group; or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol); or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, derivatives, and analogs are deemed to be within the scope of the teachings herein.

[00166] Of particular interest are substitutions of a charged amino acid with another charged amino acid and with neutral or negatively charged amino acid. The latter results in proteins with reduced positive charge. Reduced positive charge on a protein can lead to reduction in protein aggregation and the prevention of aggregation is highly desirable. Aggregation of proteins can not only result in a loss of activity but can also be problematic when preparing pharmaceutical formulations, because aggregates can be immunogenic. (Pinckard et al., 1967, *Clin. Exp. Immunol.* 2:331-340; Robbins et al., 1987, *Diabetes* 36:838-845; Cleland et al., 1993, *Crit. Rev. Therapeutic Drug Carrier Systems* 10:307-377).

[00167] As indicated, amino acid changes are typically of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the protein (see Table 1.)

Table 1. Conservative Amino Acid Substitutions

Original Amino Acid	Exemplary Conservative Substitutions
Alanine	Valine, Isoleucine, Leucine, Glycine, Serine
Arginine	Lysine, Histidine, Glutamine, Asparagine
Asparagine	Glutamine, Histidine, Lysine, Arginine
Aspartic Acid	Glutamic Acid, Asparagine
Cysteine	Serine, Alanine, Methionine
Glutamine	Asparagine
Glutamic Acid	Aspartic Acid, Glutamine
Glycine	Proline, Alanine
Histidine	Asparagine, Glutamine, Lysine, Arginine
Isoleucine	Leucine, Valine, Methionine, Alanine, Phenylalanine, Norleucine
Leucine	Norleucine, Isoleucine, Valine, Methionine, Alanine, Phenylalanine
Lysine	Arginine, Glutamine, Asparagine, Histidine
Methionine	Leucine, Phenylalanine, Isoleucine, Valine, Cysteine
Phenylalanine	Leucine, Valine, Isoleucine, Alanine, Tyrosine
Proline	Alanine, Glycine
Serine	Threonine
Threonine	Serine
Trytophan	Tyrosine, Phenylalanine
Tyrosine	Tryptophan, Phenylalanine, Threonine, Serine
Valine	Isoleucine, Methionine, Leucine, Phenylalanine, Alanine, Norleucine

[00168] Of course, the number of amino acid substitutions made depends on many factors, including those described above. In certain embodiments, the number of substitutions for any given polypeptide will not be more than 50, 40, 30, 25, 20, 15, 10, or 3.

[00169] In certain embodiment, the polypeptides and polynucleotides of the present invention are provided in an isolated form, and at times are purified to homogeneity.

[00170] The polypeptides of the present invention include the polypeptides of SEQ ID NOs: 2, 4, 13, 14, 16, 18, 19, 20, 39, 40, 49, 50, 52, 53 54, 55, 56, or 57 as well as polypeptides which have at least 90% similarity (at certain times at least 90% sequence identity) to the polypeptides of SEQ ID NOs: 2, 4, 13, 14, 16, 18, 19, 20, 39, 40, 49, 50, 52, 53 54, 55, 56, or 57 and at least 95% similarity (at certain times at least 95% sequence identity) to the polypeptides of SEQ ID NOs: 2, 4, 13, 14, 16, 18, 19, 20, 49, 50, 52, 53 54, 55, 56, or 57 and in still other embodiments, polypeptide which have at least 96%, 97%, 98%, or 99% similarity (at certain times 96%, 97%, 98%, or 99% sequence identity) to the polypeptides of SEQ ID NOs: 2, 4, 13, 14, 16, 18, 19, 20, 39, 40, 49, 50, 52, 53 54, 55, 56, or 57. As known in the art, "similarity" between two polypeptides is determined by comparing the amino acid sequence and its conserved amino acid substitutes of one polypeptide to the sequence of a second polypeptide.

[00171] Fragments or portions of the polypeptides of the present invention can be employed for producing the corresponding full-length polypeptide by peptide synthesis; therefore, the fragments can be employed as intermediates for producing the full-length polypeptides. Fragments or portions of the polynucleotides of the present invention can be used to synthesize full-length polynucleotides of the present invention.

[00172] In certain embodiments, a fragment of the proteins of this invention is a portion or all of a protein which is capable of binding to a Notch receptor protein. This fragment has a high affinity for a Notch receptor or a ligand of a Notch receptor. Certain fragments of fusion proteins are protein fragments comprising at least part of the Notch binding domain of the polypeptide agent or antagonist fused to at least part of a constant region of an immunoglobulin. The affinity is typically in the range of about 10^{-11} to 10^{-12} M, although the affinity can vary considerably with fragments of different sizes, ranging from 10^{-7} to 10^{-13} M. In some embodiments, the fragment is about 10-110 amino acids in length and comprises the Notch binding domain of the polypeptide agent or antagonist linked to at least part of a constant region of an immunoglobulin.

[00173] The polypeptides and analogs can be further modified to contain additional chemical moieties not normally part of the protein. Those derivatized moieties can improve the solubility, the biological half life or absorption of the protein. The moieties can also reduce or eliminate any undesirable side effects of the proteins and the like. An overview for those moieties can be found in Remington's Pharmaceutical Sciences, 20th ed., Mack Publishing Co., Easton, PA (2000).

[00174] The isolated polypeptides described herein can be produced by any suitable method known in the art. Such methods range from direct protein synthesis methods, to constructing a DNA sequence encoding isolated polypeptide sequences and expressing those sequences in a suitable transformed host.

[00175] In some embodiments of a recombinant method, a DNA sequence is constructed by isolating or synthesizing a DNA sequence encoding a wild-type protein of interest. Optionally, the sequence can be mutagenized by site-specific mutagenesis to provide functional analogs thereof. See, e.g. Zoeller et al., 1984, *Proc. Nat Acad. Sci. USA* 81:5662-5066 and U.S. Pat. No. 4,588,585. Another method of

constructing a DNA sequence encoding a polypeptide of interest would be by chemical synthesis using an oligonucleotide synthesizer. Such oligonucleotides can be designed based on the amino acid sequence of the desired polypeptide and selecting those codons that are favored in the host cell in which the recombinant polypeptide of interest will be produced.

[00176] Standard methods can be applied to synthesize an isolated polynucleotide sequence encoding an isolated polypeptide of interest. For example, a complete amino acid sequence can be used to construct a back-translated gene. Further, a DNA oligomer containing a nucleotide sequence coding for the particular isolated polypeptide can be synthesized. For example, several small oligonucleotides coding for portions of the desired polypeptide can be synthesized and then ligated. The individual oligonucleotides typically contain 5' or 3' overhangs for complementary assembly.

[00177] Once assembled (by synthesis, site-directed mutagenesis, or another method), the mutant DNA sequences encoding a particular isolated polypeptide of interest will be inserted into an expression vector and operatively linked to an expression control sequence appropriate for expression of the protein in a desired host. Proper assembly can be confirmed by nucleotide sequencing, restriction mapping, and expression of a biologically active polypeptide in a suitable host. As is well known in the art, in order to obtain high expression levels of a transfected gene in a host, the gene is operatively linked to transcriptional and translational expression control sequences that are functional in the chosen expression host.

[00178] Recombinant expression vectors may be used to amplify and express DNA encoding polypeptides. Recombinant expression vectors are replicable DNA constructs which have synthetic or cDNA-derived DNA fragments encoding a Notch receptor fusion or a bioequivalent analog operatively linked to suitable transcriptional or translational regulatory elements derived from mammalian, microbial, viral or insect genes. A transcriptional unit generally comprises an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, transcriptional promoters or enhancers, (2) a structural or coding sequence which is transcribed into mRNA and translated into protein, and (3) appropriate transcription and translation initiation and termination sequences, as described in detail below. Such regulatory elements can include an operator sequence to control transcription. An origin of replication which usually confers the ability to replicate in a host and a selection gene to facilitate recognition of transformants can additionally be incorporated. DNA regions are operatively linked when they are functionally related to each other. For example, DNA for a signal peptide (secretory leader) is operatively linked to DNA for a polypeptide if it is expressed as a precursor which participates in the secretion of the polypeptide; a promoter is operatively linked to a coding sequence if it controls the transcription of the sequence; or a ribosome binding site is operatively linked to a coding sequence if it is positioned so as to permit translation. Generally, "operatively linked" means contiguous and, in the case of secretory leaders, means contiguous and in reading frame. Structural elements intended for use in yeast

expression systems include a leader sequence enabling extracellular secretion of translated protein by a host cell. Alternatively, where recombinant protein is expressed without a leader or transport sequence, it can include an N-terminal methionine residue. This residue can optionally be subsequently cleaved from the expressed recombinant protein to provide a final product.

[00179] The choice of expression control sequence and expression vector will depend upon the choice of host. A wide variety of expression host/vector combinations can be employed. Useful expression vectors for eukaryotic hosts, include, for example, vectors comprising expression control sequences from SV40, bovine papilloma virus, adenovirus and cytomegalovirus. Useful expression vectors for bacterial hosts include known bacterial plasmids, such as plasmids from *Esherichia coli*, including pCR1, pBR322, pMB9 and their derivatives, and wider host range plasmids, such as M13 and filamentous single-stranded DNA phages.

[00180] Suitable host cells for expression of a polypeptide include prokaryotes, yeast, insect or higher eukaryotic cells under the control of appropriate promoters. Prokaryotes include gram negative or gram positive organisms, for example *E. coli* or *Bacilli*. Higher eukaryotic cells include established cell lines of mammalian origin as described herein. Cell-free translation systems could also be employed. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described by Pouwels et al. (Cloning Vectors: A Laboratory Manual, Elsevier, N.Y., 1985), the relevant disclosure of which is hereby incorporated by reference.

[00181] Various mammalian or insect cell culture systems are also advantageously employed to express recombinant protein. Expression of recombinant proteins in mammalian cells can be performed because such proteins are generally correctly folded, appropriately modified and completely functional. Examples of suitable mammalian host cell lines include the COS-7 lines of monkey kidney cells, described by Gluzman 1981, *Cell* 23:175, and other cell lines capable of expressing an appropriate vector including, for example, L cells, C127, 3T3, Chinese hamster ovary (CHO), HeLa and BHK cell lines. Mammalian expression vectors can comprise nontranscribed elements such as an origin of replication, a suitable promoter and enhancer linked to the gene to be expressed, and other 5' or 3' flanking nontranscribed sequences, and 5' or 3' nontranslated sequences, such as necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, and transcriptional termination sequences. Baculovirus systems for production of heterologous proteins in insect cells are reviewed by Luckow and Summers, 1988, *Bio/Technology* 6:47.

[00182] The proteins produced by a transformed host can be purified according to any suitable method. Such standard methods include chromatography (e.g., ion exchange, affinity and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for protein purification. Affinity tags such as hexahistidine, maltose binding domain, influenza coat sequence and glutathione-S-transferase can be attached to the protein to allow easy purification by passage over an

appropriate affinity column. Isolated proteins can also be physically characterized using such techniques as proteolysis, nuclear magnetic resonance and x-ray crystallography.

[00183] For example, supernatants from systems which secrete recombinant protein into culture media can be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a suitable purification matrix. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Finally, one or more reversed-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify a cancer stem cell protein-Fc composition. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a homogeneous recombinant protein.

[00184] Recombinant protein produced in bacterial culture is usually isolated by initial extraction from cell pellets, followed by one or more concentration, salting-out, aqueous ion exchange or size exclusion chromatography steps. High performance liquid chromatography (HPLC) can be employed for final purification steps. Microbial cells employed in expression of a recombinant protein can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

[00185] In certain embodiments, the Notch-binding agent or antagonist comprises an antibody. In certain embodiments, the antibody is isolated. In certain embodiments, the antibody is substantially pure.

[00186] The present invention provides antibodies that compete for specific binding to human Notch2 and/or Notch3 with an antibody comprising a heavy chain variable region comprising SEQ ID NO:14 and a light chain variable region comprising SEQ ID NO:13. The present invention also provides antibodies that compete for specific binding to human Notch2 and/or Notch3 with an antibody that comprises, consists, or consists essentially of a 59R1 IgG2 antibody comprising the heavy chain and light chain of SEQ ID NOs:16 and 18 (with or without signal sequence), respectively, or as encoded by the DNA deposited with the ATCC on October, 15, 2008, and assigned designation number PTA-9547.

[00187] The invention further provides antibodies that specifically bind to one or more Notch receptors, that comprise one, two, three, four, five, and/or six CDRs of SEQ ID NOs:5-10, 22-27, 30 or 51 with up to four (i.e., 0, 1, 2, 3, or 4) conservative amino acid substitutions (see, e.g., Table 1) per CDR. The invention also provides antibodies that specifically bind to one or more Notch receptors, that comprise one, two, three, four, five, and/or six CDRs of 59R1 (i.e., SEQ ID NOs:5-10), with up to four conservative amino acid substitutions per CDR. Thus, the invention provides antibodies that specifically bind to one or

more human Notch receptors that comprise one, two, three, four, five and/or six of the CDRs of 59R1. In certain embodiments, the antibodies comprise the heavy chain CDR3 of 59R1, with up to four conservative amino acid substitutions, and/or the light chain CDR3 of 59R1, with up to four conservative amino acid substitutions. In some embodiments, the antibody comprises (a) a heavy chain CDR1 comprising SSSGMS (SEQ ID NO:5), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; a heavy chain CDR2 comprising VIASSGSNTYYADSVKG (SEQ ID NO:6), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; and/or a heavy chain CDR3 comprising GIFFAI (SEQ ID NO:7), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; and/or (b) a light chain CDR1 comprising RASQSVRSNYLA (SEQ ID NO:8), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; a light chain CDR2 comprising GASSRAT (SEQ ID NO:9), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; and/or a light chain CDR3 comprising QQYSNFPI (SEQ ID NO:10), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions.

[00188] The invention also provides antibodies that specifically bind to one or more Notch receptors, that comprise one, two, three, four, five, and/or six CDRs of 59R5 (i.e., SEQ ID NOs: 5, 6, 8-10, 51), with up to four conservative amino acid substitutions per CDR. In certain embodiments, the antibodies comprise the heavy chain CDR3 of 59R5, with up to four conservative amino acid substitutions, and/or the light chain CDR3 of 59R5, with up to four conservative amino acid substitutions. In some embodiments, the antibody comprises (a) a heavy chain CDR1 comprising SSSGMS (SEQ ID NO:5), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; a heavy chain CDR2 comprising VIASSGSNTYYADSVKG (SEQ ID NO:6), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; and/or a heavy chain CDR3 comprising SIFYTT (SEQ ID NO:51), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; and/or (b) a light chain CDR1 comprising RASQSVRSNYLA (SEQ ID NO:8), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; a light chain CDR2 comprising GASSRAT (SEQ ID NO:9), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; and/or a light chain CDR3 comprising QQYSNFPI (SEQ ID NO:10), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions. In certain embodiments, the antibody comprises a heavy chain CDR1 comprising SSSGMS (SEQ ID NO:5), a heavy chain CDR2 comprising VIASSGSNTYYADSVKG (SEQ ID NO:6), and/or a heavy chain CDR3 comprising SIFYTT (SEQ ID NO:51).

[00189] Also provided is an antibody that specifically binds human Notch2 and/or Notch3, wherein the antibody comprises a heavy chain CDR1 comprising SSSGMS (SEQ ID NO:5), a heavy chain CDR2 comprising VIASSGSNTYYADSVKG (SEQ ID NO:6), and/or a heavy chain CDR3 comprising (G/I)(I/S)F(F/Y)(A/P)(I/T/S/N) (SEQ ID NO:30). In certain embodiments, the heavy chain CDR3 is selected from the group consisting of SIFYPT (SEQ ID NO:22), SSFFAS (SEQ ID NO:23), SSFYAS

(SEQ ID NO:24), SSFFAT (SEQ ID NO:25), SIFYPS (SEQ ID NO:26), and SSFFAN (SEQ ID NO:27). In certain embodiments, the antibody comprises a heavy chain CDR1 comprising SSSGMS (SEQ ID NO:5), a heavy chain CDR2 comprising VIASSGSNTYYADSVKG (SEQ ID NO:6), and/or a heavy chain CDR3 comprising GIFFAI (SEQ ID NO:7). In certain embodiments, the heavy chain CDR(s) are contained within a variable region of an antibody heavy chain. In certain embodiments, the antibody further comprises a light chain CDR1 comprising RASQSVRSNYLA (SEQ ID NO:8), a light chain CDR2 comprising GASSRAT (SEQ ID NO:9), and/or a light chain CDR3 comprising QQYSNFPI (SEQ ID NO:10). In certain embodiments, the light chain CDR(s) are contained within a variable region of an antibody light chain. In certain embodiments, the heavy chain CDR(s) and/or the light chain CDR(s) have been modified with 1, 2, 3, or 4 conservative amino acid substitutions. In certain embodiments, each of the CDR(s) have been modified by no more than 1-2 conservative amino acid substitutions.

[00190] For example, in certain embodiments, the invention provides an antibody that specifically binds human Notch2 and/or Notch3, wherein the antibody comprises: (a) a heavy chain CDR1 comprising SSSGMS (SEQ ID NO:5), a heavy chain CDR2 comprising VIASSGSNTYYADSVKG (SEQ ID NO:6), and a heavy chain CDR3 comprising GIFFAI (SEQ ID NO:7); and/or (b) a light chain CDR1 comprising RASQSVRSNYLA (SEQ ID NO:8), a light chain CDR2 comprising GASSRAT (SEQ ID NO:9), and a light chain CDR3 comprising QQYSNFPI (SEQ ID NO:10). In some embodiments, the antibody comprises both the indicated light and heavy chain CDRs.

[00191] In some embodiments, the invention provides an antibody that specifically binds human Notch2 and/or Notch3, wherein the antibody comprises: (a) a heavy chain CDR1 comprising SSSGMS (SEQ ID NO:5), a heavy chain CDR2 comprising VIASSGSNTYYADSVKG (SEQ ID NO:6), and a heavy chain CDR3 comprising SIFYTT (SEQ ID NO:51); and/or (b) a light chain CDR1 comprising RASQSVRSNYLA (SEQ ID NO:8), a light chain CDR2 comprising GASSRAT (SEQ ID NO:9), and a light chain CDR3 comprising QQYSNFPI (SEQ ID NO:10). In certain embodiments, the antibody comprises both the indicated light and heavy chain CDRs.

[00192] The invention further provides an antibody that specifically binds human Notch2 and/or Notch3, wherein the antibody comprises a light chain CDR1 comprising RASQSVRSNYLA (SEQ ID NO:8), a light chain CDR2 comprising GASSRAT (SEQ ID NO:9), and/or a light chain CDR3 comprising QQYSNFPI (SEQ ID NO:10).

[00193] The invention also provides an antibody that specifically binds human Notch2 and/or Notch3, wherein the antibody comprises: (a) a polypeptide having at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 98% sequence identity to SEQ ID NO:14 or SEQ ID NO:20; and/or (b) a polypeptide having at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 98% sequence identity to SEQ ID NO:13 or SEQ ID NO:19. Accordingly, in certain embodiments, the antibody comprises (a) a heavy chain variable region having at least about 95%

sequence identity to SEQ ID NO:14; and/or (b) a light chain variable region having at least about 95% sequence identity to SEQ ID NO:13. In certain embodiments, the antibody comprises: (a) a polypeptide (e.g., a heavy chain variable region) comprising SEQ ID NO:14 or SEQ ID NO:20; and/or (b) a polypeptide (e.g., a light chain variable region) comprising SEQ ID NO:13 or SEQ ID NO:19.

[00194] The invention also provides an antibody that specifically binds human Notch2 and/or Notch3, wherein the antibody comprises: (a) a polypeptide having at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 98% sequence identity to SEQ ID NO:50; and/or (b) a polypeptide having at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 98% sequence identity to SEQ ID NO:13. Accordingly, in certain embodiments, the antibody comprises (a) a heavy chain variable region having at least about 95% sequence identity to SEQ ID NO:50; and/or (b) a light chain variable region having at least about 95% sequence identity to SEQ ID NO:13. In certain embodiments, the antibody comprises: (a) a polypeptide (e.g., a heavy chain variable region) comprising SEQ ID NO:50; and/or (b) a polypeptide (e.g., a light chain variable region) comprising SEQ ID NO:13.

[00195] In certain embodiments, the antagonists are antibodies that can mediate complement-dependent cytotoxicity or antibody-dependent cellular cytotoxicity to kill tumors expressing a target antigen. In certain alternative embodiments, the antibodies are directly conjugated to toxins or radioisotopes to mediate tumor cell killing. Furthermore, tumor survival depends on neo-vascularization, and in certain embodiments, the antibodies have an anti-angiogenic effect.

[00196] The present invention provides isolated antibodies against a Notch receptor such as human Notch2 and/or Notch3. The antibody, or antibody fragment, can be any monoclonal or polyclonal antibody that specifically recognizes the described Notch receptor. In some embodiments, the present invention provides monoclonal antibodies, or fragments thereof, that specifically bind to a Notch receptor described herein. In some embodiments, the monoclonal antibodies, or fragments thereof, are chimeric or humanized antibodies that specifically bind to the extracellular domain of a Notch receptor described herein. In other embodiments, the monoclonal antibodies, or fragments thereof, are human antibodies that specifically bind to the extracellular domain of a Notch receptor described herein. In certain embodiments, the antibodies are IgG1 or IgG2 antibodies.

[00197] The antibodies against a Notch receptor find use in the experimental, diagnostic and therapeutic methods described herein. In certain embodiments, the antibodies of the present invention are used to detect the expression of a Notch receptor in biological samples such as, for example, a patient tissue biopsy, pleural effusion, or blood sample. Tissue biopsies can be sectioned and protein detected using, for example, immunofluorescence or immunohistochemistry. Alternatively, individual cells from a sample are isolated, and protein expression detected on fixed or live cells by FACS analysis. Furthermore, the antibodies can be used on protein arrays to detect expression of a Notch receptor, for example, on tumor

cells, in cell lysates, or in other protein samples. In other embodiments, the antibodies of the present invention are used to inhibit the growth of tumor cells by contacting the tumor cells with the antibodies either in *in vitro* cell based assays or *in vivo* animal models. In still other embodiments, the antibodies are used to treat cancer in a human patient by administering a therapeutically effective amount of an antibody against a Notch receptor.

[00198] Polyclonal antibodies can be prepared by any known method. Polyclonal antibodies are raised by immunizing an animal (e.g. a rabbit, rat, mouse, donkey, goat, etc.) by multiple subcutaneous or intraperitoneal injections of the relevant antigen (a purified peptide fragment, full-length recombinant protein, fusion protein, etc.) optionally conjugated to keyhole limpet hemocyanin (KLH), serum albumin, etc. diluted in sterile saline and combined with an adjuvant (e.g. Complete or Incomplete Freund's Adjuvant) to form a stable emulsion. The polyclonal antibody is then recovered from blood, ascites and the like, of an animal so immunized. Collected blood is clotted, and the serum decanted, clarified by centrifugation, and assayed for antibody titer. The polyclonal antibodies can be purified from serum or ascites according to standard methods in the art including affinity chromatography, ion-exchange chromatography, gel electrophoresis, dialysis, etc.

[00199] Monoclonal antibodies can be prepared using hybridoma methods, such as those described by Kohler and Milstein, 1975, *Nature* 256:495. Using the hybridoma method, a mouse, hamster, or other appropriate host animal, is immunized as described above to elicit the production by lymphocytes of antibodies that will specifically bind to an immunizing antigen. Alternatively, lymphocytes can be immunized *in vitro*. Following immunization, the lymphocytes are isolated and fused with a suitable myeloma cell line using, for example, polyethylene glycol, to form hybridoma cells that can then be selected away from unfused lymphocytes and myeloma cells. Hybridomas that produce monoclonal antibodies directed specifically against a chosen antigen as determined by immunoprecipitation, immunoblotting, or by an *in vitro* binding assay such as radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA) can then be propagated either *in vitro* culture using standard methods (Goding, *Monoclonal Antibodies: Principles and Practice*, Academic Press, 1986) or *in vivo* as ascites tumors in an animal. The monoclonal antibodies can then be purified from the culture medium or ascites fluid as described for polyclonal antibodies above.

[00200] Alternatively monoclonal antibodies can also be made using recombinant DNA methods as described in U.S. Pat. No. 4,816,567. The polynucleotides encoding a monoclonal antibody are isolated from mature B-cells or hybridoma cell, such as by RT-PCR using oligonucleotide primers that specifically amplify the genes encoding the heavy and light chains of the antibody, and their sequence is determined using conventional procedures. The isolated polynucleotides encoding the heavy and light chains are then cloned into suitable expression vectors, which when transfected into host cells such as E. coli cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce

immunoglobulin protein, express monoclonal antibodies in the host cells. Also, recombinant monoclonal antibodies or fragments thereof of the desired species can be isolated from phage display libraries, e.g., as described herein.

[00201] The polynucleotide(s) encoding a monoclonal antibody can further be modified in a number of different manners using recombinant DNA technology to generate alternative antibodies. In some embodiments, the constant domains of the light and heavy chains of, for example, a mouse monoclonal antibody can be substituted 1) for those regions of, for example, a human antibody to generate a chimeric antibody or 2) for a non-immunoglobulin polypeptide to generate a fusion antibody. In other embodiments, the constant regions are truncated or removed to generate the desired antibody fragment of a monoclonal antibody. Furthermore, site-directed or high-density mutagenesis of the variable region can be used to optimize specificity, affinity, etc. of a monoclonal antibody.

[00202] More generally, modified antibodies useful in the present invention may be obtained or derived from any antibody. Further, the parent or precursor antibody, or fragment thereof, used to generate the disclosed modified antibodies may be murine, human, chimeric, humanized, non-human primate or primatized. In other embodiments the modified antibodies of the present invention can comprise single chain antibody constructs (such as that disclosed in U.S. Pat. No. 5,892,019, which is incorporated herein by reference) having altered constant domains as described herein. Consequently, any of these types of antibodies modified in accordance with the teachings herein are compatible with this invention.

[00203] According to the present invention, techniques can be adapted for the production of single-chain antibodies specific to a polypeptide of the invention (see U.S. Pat. No. 4,946,778). In addition, methods can be adapted for the construction of Fab expression libraries (Huse et al., 1989, *Science* 246:1275-1281) to allow rapid and effective identification of monoclonal Fab fragments with the desired specificity for Notch, or derivatives, fragments, analogs or homologs thereof. Antibody fragments that contain the idiotypes to a polypeptide of the invention may be produced by techniques in the art including, but not limited to: (a) an $F(ab')_2$ fragment produced by pepsin digestion of an antibody molecule; (b) an Fab fragment generated by reducing the disulfide bridges of an $F(ab')_2$ fragment, (c) an Fab fragment generated by the treatment of the antibody molecule with papain and a reducing agent, and (d) Fv fragments.

[00204] Bispecific antibodies are also within the scope of the invention. Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens (or, in certain embodiments, two different epitopes on the same antigen). In the present case, one of the binding specificities is for an antigenic polypeptide of the invention (Notch, or a fragment thereof), while the second binding target is any other antigen, and advantageously is a cell surface protein, or receptor or receptor subunit. Bispecific antibodies that comprise one antigen-binding site that

specifically binds one human Notch receptor (e.g., Notch2) and further comprise a second, different antigen-binding site that specifically binds a second human Notch receptor (e.g., Notch3) are provided.

[00205] Methods for making bispecific antibodies are known in the art. Traditionally the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy chain/light chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, 1983, *Nature* 305:537-539). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography.

[00206] Alternatively, in certain embodiments, the antibodies described herein may be monospecific. For example, in certain embodiments, each of the one or more antigen-binding sites that an antibody contains is capable of binding (or binds) the same one or more human Notch receptors (e.g., Notch2, Notch3, or homologous epitopes on both Notch2 and Notch3). In certain embodiments, an antigen-binding site of a monospecific antibody described herein is capable of binding (or binds) both the EGF repeat 9 of human Notch3 and EGF repeat 10 of Notch2.

[00207] Antibody variable domains with the desired binding specificities can be fused to immunoglobulin constant domain sequences. The fusion is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH2 and CH3 regions. The first heavy chain constant region (CH1) containing the site necessary for light chain binding can be present in at least one of the fusions. DNA encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. Further details of generating bispecific antibodies can be found in Suresh et al., 1986, *Methods in Enzymology* 121:210.

[00208] Bispecific antibodies can be prepared as full-length antibodies or antibody fragments. Techniques for generating bispecific antibodies from antibody fragments have been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. In addition, Brennan et al., 1985, *Science* 229:81 describe a procedure wherein intact antibodies are proteolytically cleaved to generate $F(ab')_2$ fragments.

[00209] Additionally, Fab' fragments can be directly recovered from E. coli and chemically coupled to form bispecific antibodies (Shalaby et al., 1992, *J. Exp. Med.* 175:217-225). These methods can be used in the production of a fully humanized bispecific antibody $F(ab')_2$ molecule.

[00210] Antibodies with more than two valencies are also contemplated. For example, trispecific antibodies can be prepared (Tutt et al., 1991, *J. Immunol.* 147:60).

[00211] Exemplary bispecific antibodies can bind to two different epitopes, at least one of which originates in a polypeptide of the invention. Alternatively, an anti-antigenic arm of an immunoglobulin

molecule can be combined with an arm which binds to a triggering molecule on a leukocyte such as a T cell receptor molecule (e.g. CD2, CD3, CD28, or B7), or Fc receptors for IgG so as to focus cellular defense mechanisms to the cell expressing the particular antigen. Bispecific antibodies can also be used to direct cytotoxic agents to cells which express a particular antigen. These antibodies possess an antigen-binding arm and an arm which binds a cytotoxic agent or a radionuclide chelator, such as EOTUBE, DPTA, DOTA, or TETA.

[00212] Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune cells to unwanted cells (U.S. Pat. No. 4,676,980). It is contemplated that the antibodies can be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins can be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate.

[00213] For the purposes of the present invention, it should be appreciated that modified antibodies can comprise any type of variable region that provides for the association of the antibody with the polypeptides of Notch. In this regard, the variable region may comprise or be derived from any type of mammal that can be induced to mount a humoral response and generate immunoglobulins against the desired tumor associated antigen. As such, the variable region of the modified antibodies can be, for example, of human, murine, non-human primate (e.g. cynomolgus monkeys, macaques, etc.) or lupine origin. In some embodiments both the variable and constant regions of the modified immunoglobulins are human. In other embodiments the variable regions of compatible antibodies (usually derived from a non-human source) can be engineered or specifically tailored to improve the binding properties or reduce the immunogenicity of the molecule. In this respect, variable regions useful in the present invention can be humanized or otherwise altered through the inclusion of imported amino acid sequences.

[00214] In some embodiments, of the present invention the monoclonal antibody against a Notch receptor is a humanized antibody. Humanized antibodies are antibodies that contain minimal sequences from non-human (e.g., murine) antibodies within the variable regions. Such antibodies are used therapeutically to reduce antigenicity and HAMA (human anti-mouse antibody) responses when administered to a human subject. In practice, humanized antibodies are typically human antibodies with minimum to no non-human sequences. A human antibody is an antibody produced by a human or an antibody having an amino acid sequence corresponding to an antibody produced by a human.

[00215] Humanized antibodies can be produced using various techniques known in the art. An antibody can be humanized by substituting the CDR of a human antibody with that of a non-human antibody (e.g., mouse, rat, rabbit, hamster, etc.) having the desired specificity, affinity, and/or capability (Jones et al., 1986, *Nature* 321:522-525; Riechmann et al., 1988, *Nature* 332:323-327; Verhoeyen et al., 1988, *Science*

239:1534-1536). The humanized antibody can be further modified by the substitution of additional residues either in the Fv framework region and/or within the replaced non-human residues to refine and optimize antibody specificity, affinity, and/or capability.

[00216] As an alternative to humanization, human antibodies can be generated. Human antibodies can be prepared using various techniques known in the art, including from transgenic animals, phage libraries, and *in vitro* activated human B cells.

[00217] For example, it is now possible to produce transgenic animals (e.g., mice) containing human immunoglobulin loci that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array into such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, e.g., Jakobovits et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:2551; Jakobovits et al., 1993, *Nature* 362:255-258; Bruggemann et al., 1993, *Year in Immuno.* 7:33; U.S. Pat. Nos. 5,545,806; 5,569,825; 5,591,669; 5,545,807; 5,545,807; 5,625,126; 5,633,425; and 5,661,016; and WO 97/17852.

[00218] Alternatively, phage display technology can be used to produce human antibodies and antibody fragments *in vitro*, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B-cell. Phage display can be performed in a variety of formats. Several sources of V-gene segments can be used for phage display. A diverse array of anti-oxazolone antibodies have been isolated from a small random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated. Methods of selecting human antibodies from a phage library, where that phage library expresses human antibodies are well known in the art (Vaughan et al., 1996, *Nature Biotechnology* 14:309-314; Sheets et al., 1998, *PNAS* 95:6157-6162; Hoogenboom and Winter, 1991, *J. Mol. Biol.* 227:381; McCafferty et al., 1990, *Nature* 348:552-554; Clackson et al., 1991, *Nature* 352:624-628; and Marks et al., 1991, *J. Mol. Biol.*, 222:581-597). Techniques for the generation and use of antibody phage libraries are also described in U.S. Patent Nos. 5,969,108; 6,172,197; 5,885,793; 6,521,404; 6,544,731; 6,555,313; 6,582,915; 6,593,081; 6,300,064; 6,653,068; 6,706,484; and 7,264,963; and Rothe et al., 2008, *J. Mol. Bio.* 376:1182-

1200 (each of which is incorporated by reference in its entirety). Affinity maturation strategies, such as chain shuffling (Marks et al., 1992, *Bio/Technology* 10:779-783, incorporated by reference in its entirety), are known in the art and may be employed to generate high affinity human antibodies.

[00219] Human antibodies can also be directly prepared using various techniques known in the art. Immortalized human B lymphocytes immunized *in vitro* or isolated from an immunized individual that produce an antibody directed against a target antigen can be generated. (See, for example, Cole et al., 1985, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77; Boemer et al., 1991, *J. Immunol.*, 147 (1):86-95; U.S. Pat. Nos. 5,750,373; 5,567,610; and 5,229,275).

[00220] It will be appreciated that grafting the entire non-human variable domains onto human constant regions will produce "classic" chimeric antibodies. In the context of the present application the term "chimeric antibodies" will be held to mean any antibody wherein the immunoreactive region or site is obtained or derived from a first species and the constant region (which may be intact, partial or modified in accordance with this invention) is obtained from a second species. In some embodiments, the antigen binding region or site will be from a non-human source (e.g., mouse) and the constant region is human. While the immunogenic specificity of the variable region is not generally affected by its source, a human constant region is less likely to elicit an immune response from a human subject than would the constant region from a non-human source.

[00221] The variable domains in both the heavy and light chains are altered by at least partial replacement of one or more CDRs and, if necessary, by partial framework region replacement and sequence modification. Although the CDRs may be derived from an antibody of the same class or even subclass as the antibody from which the framework regions are derived, it is envisaged that the CDRs will be derived from an antibody of different class and preferably from an antibody from a different species. It must be emphasized that it may not be necessary to replace all of the CDRs with the complete CDRs from the donor variable region to transfer the antigen binding capacity of one variable domain to another. Rather, it may only be necessary to transfer those residues that are necessary to maintain the activity of the antigen binding site. Given the explanations set forth in U.S. Pat. Nos. 5,585,089; 5,693,761; and 5,693,762, it will be well within the art, either by carrying out routine experimentation or by trial and error testing to obtain a functional antibody with reduced immunogenicity.

[00222] Alterations to the variable region notwithstanding, it will be appreciated that the modified antibodies of this invention will comprise antibodies, or immunoreactive fragments thereof, in which at least a fraction of one or more of the constant region domains has been deleted or otherwise altered so as to provide desired biochemical and/or biological characteristics such as increased tumor localization or reduced serum half-life when compared with an antibody of approximately the same immunogenicity comprising a native or unaltered constant region. In some embodiments, the constant region of the modified antibodies will comprise a human constant region. Modifications to the constant region

compatible with this invention comprise additions, deletions or substitutions of one or more amino acids in one or more domains. That is, the modified antibodies disclosed herein may comprise alterations or modifications to one or more of the three heavy chain constant domains (CH1, CH2 or CH3) and/or to the light chain constant domain (CL). In some embodiments of the invention, modified constant regions wherein one or more domains are partially or entirely deleted are contemplated. In other embodiments, the modified antibodies will comprise domain deleted constructs or variants wherein the entire CH2 domain has been removed (ΔCH2 constructs). In still other embodiments, the omitted constant region domain will be replaced by a short amino acid spacer (e.g., 10 residues) that provides some of the molecular flexibility typically imparted by the absent constant region.

[00223] Besides their configuration, it is known in the art that the constant region mediates several effector functions. For example, binding of the C1 component of complement to antibodies activates the complement system. Activation of complement is important in the opsonization and lysis of cell pathogens. The activation of complement also stimulates the inflammatory response and can also be involved in autoimmune hypersensitivity. Further, antibodies bind to cells via the Fc region, with a Fc receptor site on the antibody Fc region binding to a Fc receptor (FcR) on a cell. There are a number of Fc receptors which are specific for different classes of antibody, including IgG (gamma receptors), IgE (epsilon receptors), IgA (alpha receptors) and IgM (mu receptors). Binding of antibody to Fc receptors on cell surfaces triggers a number of important and diverse biological responses including engulfment and destruction of antibody-coated particles, clearance of immune complexes, lysis of antibody-coated target cells by killer cells (called antibody-dependent cell-mediated cytotoxicity, or ADCC), release of inflammatory mediators, placental transfer and control of immunoglobulin production. Although various Fc receptors and receptor sites have been studied to a certain extent, there is still much which is unknown about their location, structure and functioning.

[00224] While not limiting the scope of the present invention, it is believed that antibodies comprising constant regions modified as described herein provide for altered effector functions that, in turn, affect the biological profile of the administered antibody. For example, the deletion or inactivation (through point mutations or other means) of a constant region domain may reduce Fc receptor binding of the circulating modified antibody thereby increasing tumor localization. In other cases it may be that constant region modifications, consistent with this invention, moderate complement binding and thus reduce the serum half life and nonspecific association of a conjugated cytotoxin. Yet other modifications of the constant region may be used to eliminate disulfide linkages or oligosaccharide moieties that allow for enhanced localization due to increased antigen specificity or antibody flexibility. Similarly, modifications to the constant region in accordance with this invention may easily be made using well known biochemical or molecular engineering techniques.

[00225] It will be noted that the modified antibodies may be engineered to fuse the CH3 domain directly to the hinge region of the respective modified antibodies. In other constructs it may be desirable to provide a peptide spacer between the hinge region and the modified CH2 and/or CH3 domains. For example, compatible constructs could be expressed wherein the CH2 domain has been deleted and the remaining CH3 domain (modified or unmodified) is joined to the hinge region with a 5-20 amino acid spacer. Such a spacer may be added, for instance, to ensure that the regulatory elements of the constant domain remain free and accessible or that the hinge region remains flexible. However, it should be noted that amino acid spacers may, in some cases, prove to be immunogenic and elicit an unwanted immune response against the construct. Accordingly, any spacer added to the construct should be relatively non-immunogenic or, even omitted altogether if the desired biochemical and/or biological qualities of the modified antibodies are to be maintained.

[00226] Besides the deletion of whole constant region domains, it will be appreciated that the antibodies of the present invention may be provided by the partial deletion or substitution of a few or even a single amino acid. For example, the mutation of a single amino acid in selected areas of the CH2 domain may be enough to substantially reduce Fc binding and thereby increase tumor localization. Similarly, it may be desirable to simply delete that part of one or more constant region domains that control the effector function (e.g., complement Clq binding) to be modulated. Such partial deletions of the constant regions may improve selected characteristics of the antibody (serum half-life) while leaving other desirable functions associated with the subject constant region domain intact. Moreover, as alluded to above, the constant regions of the disclosed antibodies may be modified through the mutation or substitution of one or more amino acids that enhances the profile of the resulting construct. In this respect it may be possible to disrupt the activity provided by a conserved binding site (e.g., Fc binding) while substantially maintaining the configuration and immunogenic profile of the modified antibody. Yet other embodiments may comprise the addition of one or more amino acids to the constant region to enhance desirable characteristics such as effector function or provide for more cytotoxin or carbohydrate attachment. In such embodiments it can be desirable to insert or replicate specific sequences derived from selected constant region domains.

[00227] This invention also encompasses bispecific antibodies that specifically recognize a Notch receptor. Bispecific antibodies are antibodies that are capable of specifically recognizing and binding at least two different epitopes. The different epitopes can either be within the same molecule (e.g., the same Notch receptor polypeptide) or on different molecules. For example, the antibodies can specifically recognize and bind a Notch receptor as well as, for example, 1) an effector molecule on a leukocyte such as a T-cell receptor (e.g., CD3) or Fc receptor (e.g., CD64, CD32, or CD16) or 2) a cytotoxic agent as described in detail herein. Bispecific antibodies can be intact antibodies or antibody fragments. Techniques for making bispecific antibodies are common in the art (Millstein et al., 1983, *Nature*

305:537-539; Brennan et al., 1985, *Science* 229:81; Suresh et al, 1986, *Methods in Enzymol.* 121:120; Traunecker et al., 1991, *EMBO J.* 10:3655-3659; Shalaby et al., 1992, *J. Exp. Med.* 175:217-225; Kostelny et al., 1992, *J. Immunol.* 148:1547-1553; Gruber et al., 1994, *J. Immunol.* 152:5368; and U.S. Pat. No. 5,731,168).

[00228] In certain embodiments of the invention, it can be desirable to use an antibody fragment, rather than an intact antibody, to increase tumor penetration, for example. Various techniques are known for the production of antibody fragments. Traditionally, these fragments are derived via proteolytic digestion of intact antibodies (for example Morimoto et al., 1993, *Journal of Biochemical and Biophysical Methods* 24:107-117 and Brennan et al., 1985, *Science*, 229:81). However, these fragments are now typically produced directly by recombinant host cells as described herein. Thus Fab, Fv, and scFv antibody fragments can all be expressed in and secreted from *E. coli* or other host cells, thus allowing the production of large amounts of these fragments. Alternatively, such antibody fragments can be isolated from the antibody phage libraries discussed herein. The antibody fragments can also be linear antibodies as described in U.S. Pat. No. 5,641,870, for example, and can be monospecific or bispecific. Other techniques for the production of antibody fragments will be apparent.

[00229] It can further be desirable, especially in the case of antibody fragments, to modify an antibody in order to increase its serum half-life. This can be achieved, for example, by incorporation of a salvage receptor binding epitope into the antibody fragment by mutation of the appropriate region in the antibody fragment or by incorporating the epitope into a peptide tag that is then fused to the antibody fragment at either end or in the middle (e.g., by DNA or peptide synthesis).

[00230] The present invention further embraces variants and equivalents which are substantially homologous to the chimeric, humanized and human antibodies, or antibody fragments thereof, set forth herein. These can contain, for example, conservative substitution mutations, i.e. the substitution of one or more amino acids by similar amino acids. For example, conservative substitution refers to the substitution of an amino acid with another within the same general class such as, for example, one acidic amino acid with another acidic amino acid, one basic amino acid with another basic amino acid or one neutral amino acid by another neutral amino acid. What is intended by a conservative amino acid substitution is well known in the art.

[00231] The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent. Cytotoxic agents include chemotherapeutic agents, growth inhibitory agents, toxins (e.g., an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), radioactive isotopes (i.e., a radioconjugate), etc. Chemotherapeutic agents useful in the generation of such immunoconjugates include, for example, methotrexate, adriamicin, doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents. Enzymatically active toxins and fragments thereof that can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin,

exotoxin A chain, ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), Momordica charantia inhibitor, curcin, crotin, Sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes. Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyidithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutareldehyde), bis-azido compounds (such as bis(p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). Conjugates of an antibody and one or more small molecule toxins, such as a calicheamicin, maytansinoids, a trichothene, and CC1065, and the derivatives of these toxins that have toxin activity, can also be used.

[00232] Conjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune cells to unwanted cells (U.S. Pat. No. 4,676,980). It is contemplated that the antibodies can be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins can be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate.

[00233] In some embodiments, the antibody of the invention contains human Fc regions that are modified to enhance effector function, for example, antigen-dependent cell-mediated cytotoxicity (ADCC) and/or complement dependent cytotoxicity (CDC). This can be achieved by introducing one or more amino acid substitutions in an Fc region of the antibody. For example, cysteine residue(s) can be introduced in the Fc region to allow interchain disulfide bond formation in this region to improve complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC) (Caron et al., 1992, *J. Exp Med.* 176:1191-1195; Shopes, 1992, *Immunol.* 148:2918-2922). Homodimeric antibodies with enhanced anti-tumor activity can also be prepared using heterobifunctional cross-linkers as described in Wolff et al., 1993, *Cancer Research* 53:2560-2565. Alternatively, an antibody can be engineered which has dual Fc regions (Stevenson et al., 1989, *Anti-Cancer Drug Design* 3:219-230).

[00234] Regardless of how useful quantities are obtained, the antibodies of the present invention can be used in any one of a number of conjugated (i.e. an immunoconjugate) or unconjugated forms. The antibodies of this invention can be used in a nonconjugated or "naked" form to harness the subject's natural defense mechanisms including complement-dependent cytotoxicity (CDC) and antibody dependent cellular toxicity (ADCC) to eliminate the malignant cells. In some embodiments, the antibodies can be conjugated to radioisotopes, including, but not limited to, ^{90}Y, ^{125}I, ^{131}I, ^{123}I, ^{111}In, ^{131}In, ^{212}Bi, ^{105}Rh, ^{153}Sm, ^{67}Cu, ^{67}Ga, ^{166}Ho, ^{177}Lu, ^{186}Re and ^{188}Re using any one of a number of well known

chelators or direct labeling. In other embodiments, the disclosed compositions can comprise antibodies coupled to drugs, prodrugs or biological response modifiers such as methotrexate, adriamycin, and lymphokines such as interferon. Still other embodiments of the present invention comprise the use of antibodies conjugated to specific biotoxins such as ricin or diptheria toxin. In yet other embodiments the modified antibodies can be complexed with other immunologically active ligands (e.g., antibodies or fragments thereof) wherein the resulting molecule binds to both the neoplastic cell and an effector cell such as a T cell. The selection of which conjugated or unconjugated modified antibody to use will depend of the type and stage of cancer, use of adjunct treatment (e.g., chemotherapy or external radiation) and patient condition. It will be appreciated that one could readily make such a selection in view of the teachings herein.

[00235] The preparation and characterization of anti-Notch antibodies is also taught, e.g., in U.S. Patent Application Publication No. 2008/0131434, which is incorporated by reference herein in its entirety.

[00236] In certain embodiments, the Notch-binding agent or antagonist is a polypeptide that is not an antibody. A variety of methods for identifying and producing non-antibody polypeptides that bind with high affinity to a protein target are known in the art. See, e.g., Skerra, 2007, *Curr. Opin. Biotechnol.*, 18:295-304; Hosse et al., 2006, *Protein Science*, 15:14-27; Gill et al., 2006, *Curr. Opin. Biotechnol.*, 17:653-658; Nygren, 2008, *FEBS J.*, 275:2668-76; and Skerra, 2008, *FEBS J.*, 275:2677-83, each of which is incorporated by reference herein in its entirety. In certain embodiments, phage display technology has been used to identify/produce the Notch-binding polypeptide. In certain embodiments, the polypeptide comprises a protein scaffold of a type selected from the group consisting of protein A, a lipocalin, a fibronectin domain, an ankyrin consensus repeat domain, and thioredoxin.

[00237] In some embodiments, the agent is a non-protein molecule. In certain embodiments, the agent is a small molecule. Combinatorial chemistry libraries and techniques useful in the identification of non-protein Notch-binding agents are known to those skilled in the art. See, e.g., Kennedy et al., 2008, *J. Comb. Chem*, 10:345-354; Dolle et al, 2007, *J. Comb. Chem.*, 9:855-902; and Bhattacharyya, 2001, *Curr. Med. Chem.*, 8:1383-404, each of which is incorporated by reference herein in its entirety. In certain further embodiments, the agent is a carbohydrate, a glycosaminoglycan, a glycoprotein, or a proteoglycan.

[00238] In certain embodiments, the agent is a nucleic acid aptamer. Aptamers are polynucleotide molecules that have been selected (e.g., from random or mutagenized pools) on the basis of their ability to bind to another molecule. In some embodiments, the aptamer comprises a DNA polynucleotide. In certain alternative embodiments, the aptamer comprises an RNA polynucleotide. In certain embodiments, the aptamer comprises one or more modified nucleic acid residues. Methods of generating and screening nucleic acid aptamers for binding to proteins are well known in the art. See, e.g., U.S. Pat. Nos. 5,270,163; 5,683,867; 5,763,595; 6,344,321; 7,368,236; 5,582,981; 5,756,291; 5,840,867; 7,312,325; and 7,329,742; International Patent Publication Nos. WO 02/077262; WO 03/070984; U.S. Patent Application

Publication Nos. 2005/0239134; 2005/0124565; and 2008/0227735, each of which is incorporated by reference herein in its entirety.

[00239] The antibodies of the present invention can be assayed for immunospecific binding by any method known in the art. The immunoassays which can be used include, but are not limited to, competitive and non-competitive assay systems using techniques such as Biacore analysis, FACS analysis, immunofluorescence, immunocytochemistry, Western blot analysis, radioimmunoassay, ELISA, "sandwich" immunoassay, immunoprecipitation assay, precipitin reaction, gel diffusion precipitin reaction, immunodiffusion assay, agglutination assay, complement-fixation assay, immunoradiometric assay, fluorescent immunoassay, and protein A immunoassay. Such assays are routine and well known in the art (see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety).

[00240] In some embodiments, of the present invention the immunospecificity of an antibody against a Notch receptor is determined using ELISA. An ELISA assay comprises preparing antigen, coating wells of a 96 well microtiter plate with antigen, adding the antibody against a Notch receptor conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the well, incubating for a period of time and detecting the presence of the antigen. Alternatively the antibody against a Notch receptor is not conjugated to a detectable compound, but instead a second conjugated antibody that recognizes the antibody against a Notch receptor is added to the well. Further, instead of coating the well with the antigen, the antibody against a Notch receptor can be coated to the well and a second antibody conjugated to a detectable compound can be added following the addition of the antigen to the coated well. The parameters that can be modified to increase the signal detected, as well as other variations of ELISAs are well known in the art (see e.g. Ausubel et al., eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1).

[00241] The binding affinity of an antibody to a Notch receptor and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g., ^{3}H or ^{125}I), or fragment or variant thereof, with the antibody of interest in the presence of increasing amounts of unlabeled antigen followed by the detection of the antibody bound to the labeled antigen. The affinity of the antibody against a Notch receptor and the binding off-rates can be determined from the data by Scatchard plot analysis. In some embodiments, Biacore kinetic analysis is used to determine the binding on and off rates of antibodies against a Notch receptor. Biacore kinetic analysis comprises analyzing the binding and dissociation of antibodies from chips with immobilized Notch antigens on their surface.

[00242] The invention provides isolated polynucleotides encoding the polypeptides of SEQ ID NOs:2, 4, 13, 14, 16, 18, 19, 20, 39, 40, 49, 50, 52, 53, 54, 55, 56, or 57 as well as the polynucleotides of SEQ ID NOs: 1, 3, 15, 17, 47 or 48. The polynucleotides of the invention can be in the form of RNA or in the

form of DNA, wherein DNA includes cDNA, genomic DNA, and synthetic DNA. The DNA can be double-stranded or single-stranded, and if single-stranded it can be the coding strand or non-coding (anti-sense) strand. Thus, the term "polynucleotide encoding a polypeptide" encompasses a polynucleotide which includes only coding sequences for the polypeptide as well as a polynucleotide which includes additional coding and/or non-coding sequences. In some embodiments, the invention provides a polynucleotide that hybridizes to a polynucleotide encoding the polypeptides of SEQ ID NOs:2, 4, 13, 14, 16, 18, 19, 20, 39, 40, 49, 50, 52, 53, 54, 55, 56, or 57. In some embodiments, the polynucleotides hybridize to the polynucleotides of SEQ ID NOs: 1, 3, 15, 17, 47, 48, 58, 59 or 60. In some embodiments, the polynucleotides hybridize under stringent hybridization conditions.

[00243] As used herein, the phrases "hybridizes" or "selectively hybridizes" or "specifically hybridizes" refer to the binding or duplexing of a molecule only to a particular nucleotide sequence under stringent hybridization conditions when that sequence is present in a complex mixture (e.g., a library of DNAs or RNAs). See, e.g., Andersen (1998) Nucleic Acid Hybridization Springer-Verlag; Ross (ed. 1997) Nucleic Acid Hybridization Wiley.

[00244] As used herein, the phrase "stringent hybridization conditions" refers to conditions under which a probe or other polynucleotide will hybridize to its target subsequence or other complementary sequence, typically in a complex mixture of nucleic acid, but generally to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Probes, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993). Generally, stringent conditions are selected to be about 5-10 °C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength. The Tm is the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at Tm, 50% of the probes are occupied at equilibrium). Stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30 °C for short probes (e.g., 10 to 50 nucleotides) and at least about 60 °C for long probes (e.g., greater than 50 nucleotides). Stringent conditions can also be achieved with the addition of destabilizing agents such as formamide. For high stringency hybridization, a positive signal is at least two times background, or 10 times background hybridization. Exemplary high stringency or stringent hybridization conditions include: 50% formamide, 5x SSC, and 1% SDS incubated at 42 °C or 5x SSC and 1% SDS incubated at 65 °C, with a wash in 0.2x SSC and 0.1% SDS at 65 °C. For PCR, a temperature of about 36 °C is typical for low stringency amplification, although annealing temperatures can vary from about 32 °C to about 48 °C depending on primer length. For high stringency PCR

amplification, a temperature of about 62 °C is typical, although high stringency annealing temperatures can range from about 50 °C to about 65 °C, depending on the primer length and specificity. Typical cycle conditions for both high and low stringency amplifications include a denaturation phase of 90 °C to 95 °C for 30-120 sec, an annealing phase lasting 30-120 sec, and an extension phase of about 72 °C for 1-2 min.

[00245] The present invention further relates to variants of the hereinabove described polynucleotides which encode for fragments, analogs, and derivatives. The variant of the polynucleotide can be a naturally occurring allelic variant of the polynucleotide or a non-naturally occurring variant of the polynucleotide.

[00246] As hereinabove indicated, the polynucleotide can have a coding sequence which is a naturally occurring allelic variant of the coding sequence of the disclosed polypeptides. As known in the art, an allelic variant is an alternate form of a polynucleotide sequence which has a substitution, deletion or addition of one or more nucleotides that does not substantially alter the function of the encoded polypeptide.

[00247] The present invention also includes polynucleotides, wherein the coding sequence for the mature polypeptide can be fused in the same reading frame to a polynucleotide which aids in expression and secretion of a polypeptide from a host cell, for example, a leader sequence which functions as a secretory sequence for controlling transport of a polypeptide from the cell. The polypeptide having a leader sequence is a preprotein and can have the leader sequence cleaved by the host cell to form the mature form of the polypeptide. The polynucleotides can also encode for a proprotein which is the mature protein plus additional 5' amino acid residues. A mature protein having a prosequence is a proprotein and is an inactive form of the protein. Once the prosequence is cleaved an active mature protein remains.

[00248] Thus, for example, the polynucleotide of the present invention can encode for a mature protein, or for a protein having a prosequence or for a protein having both a prosequence and presequence (leader sequence).

[00249] The polynucleotides of the present invention can also have the coding sequence fused in frame to a marker sequence which allows for purification of the polypeptide of the present invention. For example, the marker sequence can be a hexa-histidine tag supplied by a pQE-9 vector to provide for purification of the mature polypeptide fused to the marker in the case of a bacterial host. Or for example, the marker sequence can be a hemagglutinin (HA) tag when a mammalian host, e.g. COS-7 cells, is used. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., 1984, *Cell* 37:767).

[00250] Further embodiments of the invention include isolated nucleic acid molecules comprising a polynucleotide having a nucleotide sequence at least 90% identical, 95% identical, and in some embodiments, at least 96%, 97%, 98% or 99% identical to SEQ ID NOs:1, 3, 15, 17, 47, 48, 58, 59 or 60. In some embodiments, the polynucleotides comprising a nucleotide sequence at least 90% identical, 95%

identical, and in some embodiments, at least 96%, 97%, 98% or 99% identical hybridize to the polynucleotides of SEQ ID NOs:1, 3, 15, 17, 47, 48, 58, 59 or 60. In some embodiments, the polynucleotides comprising a nucleotide sequences at least 90% identical, 95% identical, and in some embodiments, at least 96%, 97%, 98% or 99% identical hybridize to the polynucleotides of SEQ ID NOs:58, 59 or 60. In some embodiments, the polynucleotides hybridize under stringent hybridization conditions. In some embodiments, the polynucleotides hybridize to the polynucleotides of SEQ ID NO:58, 59 or 60 under stringent hybridization conditions. By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence can include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence can be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence can be inserted into the reference sequence. These mutations of the reference sequence can occur at the amino- or carboxy-terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

[00251] As a practical matter, whether any particular nucleic acid molecule has a certainty percent sequence identity to a reference sequence (for example, has at least about 80%, at least about 90%, at least about 95%, or at least about 97% sequence identity to a reference sequence or is 95%, 96%, 97%, 98% or 99% identical to the reference sequence) can be determined conventionally using known computer programs such as the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). Bestfit uses the local homology algorithm of Smith and Waterman, 1981, *Advances in Applied Mathematics* 2: 482-489, to find the best segment of homology between two sequences. When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed.

[00252] The polynucleotide variants can contain alterations in the coding regions, non-coding regions, or both. In some embodiments the polynucleotide variants contain alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. In some embodiments, nucleotide variants are produced by silent substitutions due to the degeneracy of the genetic code. Polynucleotide variants can be produced for a variety of reasons, e.g., to

optimize codon expression for a particular host such as changing codons in the human mRNA to those preferred by a bacterial host such as *E. coli*.

[00253] The present invention further provides pharmaceutical compositions comprising antagonists (e.g., antibodies) that target a Notch receptor. These pharmaceutical compositions find use in inhibiting tumor cell growth and treating cancer in human patients.

[00254] Formulations are prepared for storage and use by combining a purified Notch-binding agent or antagonist (e.g., antibody) of the present invention with a pharmaceutically acceptable carrier, excipient, and/or stabilizer as a sterile lyophilized powder, aqueous solution, etc. (Remington, The Science and Practice of Pharmacy 20th Edition Mack Publishing, 2000). Suitable carriers, excipients, or stabilizers comprise: nontoxic buffers such as phosphate, citrate, and other organic acids; salts such as sodium chloride; antioxidants such as ascorbic acid and methionine; preservatives such as octadecyldimethylbenzyl ammonium chloride, hexamethonium chloride, benzalkonium chloride, benzethonium chloride, phenol, butyl or benzyl alcohol, alkyl parabens, such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, 3-pentanol, and m-cresol; low molecular weight polypeptides (less than about 10 amino acid residues); proteins such as serum albumin, gelatin, and immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, and lysine; carbohydrates such as monosacchandes, disaccharides, glucose, mannose, and dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose and sorbitol; salt-forming counter-ions such as sodium; metal complexes such as Zn-protein complexes; and/or non-ionic surfactants such as TWEEN and polyethylene glycol (PEG).

[00255] The pharmaceutical composition of the present invention can be administered in any number of ways for either local or systemic treatment. Administration can be topical (such as to mucous membranes including vaginal and rectal delivery) using transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders; pulmonary (e.g., by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal); oral; or parenteral including intravenous, intraarterial, subcutaneous, intraperitoneal, intratumoral, or intramuscular injection or infusion; or intracranial (e.g., intrathecal or intraventricular) administration.

[00256] The therapeutic formulation can be in unit dosage form. Such formulations include tablets, pills, capsules, powders, granules, solutions or suspensions in water or non-aqueous media, or suppositories for oral, parenteral, or rectal administration or for administration by inhalation. In solid compositions such as tablets the principal active ingredient is mixed with a pharmaceutical carrier. Conventional tableting ingredients include corn starch, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, dicalcium phosphate or gums, and other diluents (e.g., water) to form a solid preformulation composition containing a homogeneous mixture of a compound of the present invention, or a non-toxic pharmaceutically acceptable salt thereof. The solid preformulation composition is then subdivided into unit dosage forms

of the type described above. The tablets, pills, etc. of the novel composition can be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner composition covered by an outer component. Furthermore, the two components can be separated by an enteric layer that serves to resist disintegration and permits the inner component to pass intact through the stomach or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol and cellulose acetate.

[00257] Pharmaceutical formulations include antagonists (e.g., antibodies) of the present invention complexed with liposomes (Epstein, et al., 1985, *Proc. Natl. Acad. Sci. USA* 82:3688; Hwang, et al., 1980, *Proc. Natl. Acad. Sci. USA* 77:4030; and U.S. Pat. Nos. 4,485,045 and 4,544,545). Liposomes with enhanced circulation time are disclosed in U.S. Pat. No. 5,013,556. Some liposomes can be generated by the reverse phase evaporation with a lipid composition comprising phosphatidylcholine, cholesterol, and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter.

[00258] The antagonist (e.g., antibody) can also be entrapped in microcapsules. Such microcapsules are prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nanoparticles and nanocapsules) or in macroemulsions as described in Remington's, The Science and Practice of Pharmacy, 20th Ed., Mack Publishing (2000).

[00259] In addition sustained-release preparations can be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles (e.g., films or microcapsules). Examples of sustained-release matrices include polyesters, hydrogels such as poly(2-hydroxyethyl-methacrylate) or poly(v nylalcohol), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and 7 ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the Lupron Depot (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), sucrose acetate isobutyrate, and poly-D(-)-3-hydroxybutyric acid.

[00260] In certain embodiments, the pharmaceutical compositions comprise both the Notch-binding agent or antagonist and a second therapeutic agent. In certain embodiments, the second therapeutic agent is an anti-cancer agent and/or an anti-angiogenic agent.

[00261] The present invention provides methods for inhibiting the growth or proliferation of tumorigenic cells expressing a Notch receptor using the Notch receptor antagonists described herein. In some embodiments, the methods comprise inhibiting the growth of tumorigenic cells expressing a Notch2 and/or Notch3 receptor using any one the antibodies or polypeptides described herein. In some

embodiments, the method of inhibiting the growth of tumorigenic cells expressing a Notch receptor comprises contacting the cell with an antagonist against a Notch receptor *in vitro*. For example, an immortalized cell line or a cancer cell line that expresses a Notch receptor is cultured in medium to which is added an antibody which specifically binds to Notch2 and/or Notch3 and inhibits cell growth. Or tumor cells and/or tumor stem cells are isolated from a patient sample such as, for example, a tissue biopsy, pleural effusion, or blood sample and cultured in medium to which is added an antibody which specifically binds to Notch2 and/or Notch3 and inhibits cell growth. In some embodiments, the antagonist is an antibody that specifically recognizes an epitope of a Notch2 and/or Notch3 receptor.

[00262] In some embodiments, the method of inhibiting the growth or proliferation of tumorigenic cells expressing a Notch receptor comprises contacting the cell with an antagonist against a Notch receptor (e.g., an antagonist of Notch2 and/or Notch3) *in vivo*. In certain embodiments, contacting a tumorigenic cell with an antagonist to a Notch receptor is undertaken in an animal model. For example, xenografts expressing a Notch receptor are grown in immunocompromised mice (e.g., NOD/SCID mice). The mice are administered an antagonist to the Notch receptor to inhibit tumor growth. Alternatively, cancer stem cells that express a Notch receptor are isolated from a patient sample such as, for example, a tissue biopsy, pleural effusion, or blood sample and injected into immunocompromised mice. The mice are then administered an antagonist against the Notch receptor to inhibit tumor cell growth. In some embodiments, the antagonist of a Notch receptor is administered at the same time or shortly after introduction of tumorigenic cells into the animal to prevent tumor growth. In other embodiments, the antagonist of a Notch receptor is administered as a therapeutic after the tumorigenic cells have grown to a specified size. In some embodiments, the antagonist is a Notch receptor protein fusion that specifically binds to a Notch receptor. In certain embodiments, the antagonist is an antibody that specifically recognizes an epitope of a Notch receptor. In some embodiments, the antibody is any one of the antibodies or polypeptides described herein.

[00263] In certain embodiments, contacting a tumorigenic cell with an antagonist to a Notch receptor is undertaken in a human patient diagnosed with cancer. In some embodiments, the antagonist is an antibody that specifically binds to a Notch receptor. In other embodiments, the antagonist is an antibody that specifically recognizes an epitope of a Notch receptor. For example, the invention provides method of inhibiting growth of a tumor in a subject, comprising administering to the subject a therapeutically effective amount of an antagonist of human Notch2 and/or Notch3. In some embodiments, the antagonist is an antibody that binds to Notch2. In some embodiments, the antagonist is an antibody that binds to Notch3. In some embodiments, the antagonist is an antibody that binds to Notch2 and Notch3. In some embodiments, the antagonist is an antibody or polypeptide as described in any one of the aforementioned aspects or embodiments, as well as any other aspects or embodiments described herein. In certain

embodiments, the tumor comprises an inactivating deletion or mutation in the phosphatase and tensin homolog (PTEN) gene.

[00264] The invention further provides methods of inhibiting Notch signaling (e.g., Notch2 and/or Notch3) in a cell, comprising contacting the cell with an effective amount of the Notch antagonist. These methods may be *in vivo* or *in vitro*. In some embodiments, the Notch antagonist is an antibody. In some embodiments, the methods comprise inhibiting Notch2 signaling in a cell comprising contacting the cell with an effective amount of any one of the antibodies or polypeptides of the aforementioned aspects or embodiments, as well as any other aspects or embodiments described herein. In some embodiments, the Notch antagonist is an antibody. In some embodiments, the methods comprise inhibiting Notch3 signaling in a cell comprising contacting the cell with an effective amount of any of the antibodies or polypeptides of the aforementioned aspects or embodiments, as well as any other aspects or embodiments described herein.

[00265] The invention further provides a method of modulating the function of pericytes and/or vascular smooth muscle cells, comprising administering an effective amount of an antagonist of human Notch3 to the subject. In some embodiments, the method inhibits angiogenesis by modulating the function of pericytes and/or vascular smooth muscle cells. In some embodiments, the antagonist is an antibody or polypeptide as described in any of the aforementioned aspects or embodiments, as well as any other aspects or embodiments described herein. In certain embodiments, the vascular development that is inhibited is aberrant vascular development. In certain embodiments, the vascular development that is inhibited is in a tumor. In certain embodiments, the method further comprises administering to the subject an antagonist of VEGF or of a VEGF receptor.

[00266] In addition, the invention provides methods of inhibiting angiogenesis or vascular development in a subject, comprising administering an effective amount of a Notch antagonist to the subject. In certain embodiments, the Notch antagonist is a Notch3 antagonist. In certain embodiments, the Notch antagonist is a Notch2 antagonist. In certain embodiments, the antagonist is an antagonist of Notch2 and/or 3. In some embodiments, the antagonist is an anti-Notch2/3 antibody. In some embodiments, the methods of inhibiting angiogenesis comprises administering an antibody or polypeptide of any of the aforementioned aspects or embodiments, as well as any other embodiments or aspects described herein. In certain embodiments, the angiogenesis is tumor angiogenesis. In certain embodiments, the vascular development is at the site of a tumor. In certain alternative embodiments, the angiogenesis is not tumor angiogenesis. In certain embodiments, the inhibition of angiogenesis or vascular development is due, at least in part, to modulation of the function of pericytes and/or vascular smooth muscle cells. In certain embodiments, the method further comprises administering to the subject an antagonist of vascular endothelial cell growth factor (VEGF) or of a VEGF receptor.

[00267] Methods of reducing the tumorigenicity of a tumor (e.g., a tumor that comprises cancer stem cells) are also provided. In certain embodiments, the methods comprise administering to a subject in need thereof (e.g., subject has a tumor) a therapeutically effective amount of the Notch antagonist. In certain embodiments, the Notch antagonist is an antibody that binds Notch2. In certain embodiments, the Notch antagonist is an antibody that binds Notch3. In certain embodiments, the Notch antagonist is an antibody that binds Notch2 and Notch3. In certain embodiments, the Notch antagonist is an antibody or polypeptide of any of the aforementioned aspects or embodiments, as well as any other embodiments or aspects described elsewhere herein. In certain embodiments, the frequency of cancer stem cells in the tumor is reduced by administration of the antibody. In some embodiments, the tumor is a colorectal tumor, breast tumor, pancreatic tumor or melanoma.

[00268] It is further envisioned that the agents and antagonists of the present invention can be used to treat various conditions characterized by expression of and/or increased responsiveness of cells to a Notch receptor. The invention provides methods of treating proliferative disease, such as cancer, diseases associated with angiogenesis (e.g., angiogenesis-dependent diseases), and diseases in which the upregulation or deregulation of Notch signaling plays a role.

[00269] In certain embodiments the disease to be treated with the Notch-binding agents or antagonists is a Notch-related disease. In certain embodiments, the disease is characterized by upregulation or deregulation of Notch signaling (e.g., Notch2 and/or Notch3 signaling). In certain embodiments, the disease or tumor is Notch2 and/or Notch3-dependent.

[00270] Particularly, it is envisioned that the antagonists (e.g., antibodies) against a Notch receptor will be used to treat proliferative disorders including, but not limited to, benign and malignant tumors of the kidney, liver, bladder, breast, stomach, ovary, colon, rectum, prostate, lung, vulva, thyroid, head and neck, brain (glioblastoma, astrocytoma, medulloblastoma, etc.), blood and lymph (leukemias and lymphomas). In certain embodiments, the proliferative disorder that Notch-binding agent or antagonist is used to treat is colorectal cancer, breast cancer, pancreatic cancer, or melanoma. In certain embodiments, the cancer comprises cancer stem cells.

[00271] In certain embodiments, the tumors treated are solid tumors. Examples of solid tumors that can be treated using a therapeutic composition of the instant invention, for example, an antibody that binds Notch include, but are not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma,

choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma. The invention is applicable to sarcomas and epithelial cancers, such as ovarian cancers and breast cancers. In certain embodiments, the tumor is a colorectal tumor, breast tumor, pancreatic tumor, or melanoma. In certain embodiments, the tumor is an ovarian tumor. In certain embodiments, the tumor is a medulloblastoma. In certain embodiments, the tumor comprises cancer stem cells.

[00272] In certain embodiments, the disease to be treated with the Notch-binding agent or antagonist is a disease associated with angiogenesis. In certain embodiments, the disease is cancer. In certain other embodiments, the disease is not a cancerous condition. For example, the disease may be wet macular degeneration, age related macular degeneration, diabetic retinopathy, a hemangioma, rheumatoid arthritis, psoriasis, neovascular glaucoma, polycystic ovary disease, endometriosis and inflammatory bowel disorders.

[00273] In certain embodiments, the tumor expresses the Notch receptor or receptors to which the Notch-binding agent or antagonist is targeted. In certain embodiments, the tumor expresses Notch2 and/or Notch3. In certain embodiments, the tumor overexpresses Notch2 and/or Notch3. In certain embodiments, the tumor is dependent upon one or more Notch receptors to which the antibody administered specifically binds. For example, in certain embodiments, an antibody that specifically binds Notch2 (or Notch2 and Notch3) may be used to inhibit the growth or otherwise target the Notch2-dependent tumor. In certain embodiments, an antibody that specifically binds Notch3 (or Notch2 and Notch3) may be used to inhibit the growth or otherwise target the Notch3-dependent tumor. In certain embodiments, the tumor comprises cancer stem cells.

[00274] In certain embodiments, the tumor is homozygotic or heterozygotic for an inactivating deletion or mutation in the gene encoding the tumor suppressor phosphatase and tensin homolog (PTEN). In certain embodiments, the tumor comprising the deletion or mutation is a breast tumor.

[00275] The antagonists are administered as an appropriate pharmaceutical composition to a human patient according with known methods. Suitable methods of administration include intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intravenous, intratumoral, intraarterial, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes.

[00276] In certain embodiments, in addition to administering a Notch antagonist, the method or treatment further comprises administering a second therapeutic agent (prior to, concurrently with, and/or subsequently to administration of the Notch antagonist). In certain embodiments, the second therapeutic

agent is an anti-cancer and/or anti-angiogenic agent. Pharmaceutical compositions comprising the Notch antagonist and the second therapeutic agent are also provided.

[00277] It will be appreciated that the combination of a Notch antagonist (e.g., antibody) and a second therapeutic agent may be administered in any order or concurrently. In selected embodiments, the Notch antagonists will be administered to patients that have previously undergone treatment with the second anti-cancer agent. In certain other embodiments, the Notch antagonist and the second therapeutic agent will be administered substantially simultaneously or concurrently. For example, a subject may be given the Notch antagonist while undergoing a course of treatment with the second therapeutic agent (e.g., chemotherapy). In certain embodiments, the Notch antagonist will be administered within 1 year of the treatment with the second therapeutic agent. In certain alternative embodiments, the Notch antagonist will be administered within 10, 8, 6, 4, or 2 months of any treatment with the second therapeutic agent. In certain other embodiments, the Notch antagonist will be administered within 4, 3, 2, or 1 week of any treatment with the second therapeutic agent. In some embodiments, the Notch antagonist will be administered within 5, 4, 3, 2, or 1 days of any treatment with the second therapeutic agent. It will further be appreciated that the two agents or treatment may be administered to the subject within a matter of hours or minutes (i.e., substantially simultaneously).

[00278] Useful classes of anti-cancer agents include, for example, antitubulin agents, auristatins, DNA minor groove binders, DNA replication inhibitors, alkylating agents (e.g., platinum complexes such as cisplatin, mono(platinum), bis(platinum) and tri-nuclear platinum complexes and carboplatin), anthracyclines, antibiotics, antifolates, antimetabolites, chemotherapy sensitizers, duocarmycins, etoposides, fluorinated pyrimidines, ionophores, lexitropsins, nitrosoureas, platinols, performing compounds, purine antimetabolites, puromycins, radiation sensitizers, steroids, taxanes, topoisomerase inhibitors, vinca alkaloids, or the like. In certain embodiments, the second anti-cancer agent is an antimetabolite, a topoisomerase inhibitor, or an angiogenesis inhibitor.

[00279] Anticancer agents that may be administered in combination with the Notch antagonists include chemotherapeutic agents. Thus, in some embodiments, the treatment involves the combined administration of an antagonist of the present invention and a chemotherapeutic agent or cocktail of multiple different chemotherapeutic agents. Treatment with an antagonist can occur prior to, concurrently with, or subsequent to administration of chemotherapies. Chemotherapies contemplated by the invention include chemical substances or drugs which are known in the art and are commercially available, such as doxorubicin, 5-fluorouracil, cytosine arabinoside (Ara-C), cyclophosphamide, thiotepa, busulfan, cytoxin, taxol, methotrexate, cisplatin, melphalan, vinblastine and carboplatin. Combined administration can include co-administration, either in a single pharmaceutical formulation or using separate formulations, or consecutive administration in either order but generally within a time period such that all active agents can exert their biological activities simultaneously. Preparation and dosing schedules for such

chemotherapeutic agents can be used according to manufacturers' instructions or as determined empirically. Preparation and dosing schedules for such chemotherapy are also described in Chemotherapy Service Ed., M. C. Perry, Williams & Wilkins, Baltimore, Md. (1992).

[00280] Chemotherapeutic agents useful in the instant invention also include, but are not limited to, alkylating agents such as thiotepa and cyclophosphamide (CYTOXAN); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphaoramide and trimethylolomelamime; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabicin, caminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK.; razoxane; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside (Ara-C); cyclophosphamide; thiotepa; taxoids such as paclitaxel (TAXOL) and doxetaxel (TAXOTERE, Rhone); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoic acid; esperamicins; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Chemotherapeutic agents also include anti-hormonal agents that act to regulate or

inhibit hormone action on tumors such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (Fareston); and antiandrogens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

[00281] In certain embodiments, the chemotherapeutic agent is a topoisomerase inhibitor. Topoisomerase inhibitors are chemotherapy agents that interfere with the action of a topoisomerase enzyme (e.g., topoisomerase I or II). Topoisomerase inhibitors include, but are not limited to, doxorubicin HCL, daunorubicin citrate, mitoxantrone HCL, actinomycin D, etoposide, topotecan HCL, teniposide (VM-26), and irinotecan. In certain embodiments, the second anticancer agent is irinotecan. In certain embodiments, the tumor to be treated is a colorectal tumor and the second anticancer agent is a topoisomerase inhibitor, such as irinotecan.

[00282] In certain embodiments, the chemotherapeutic agent is an anti-metabolite. An anti-metabolite is a chemical with a structure that is similar to a metabolite required for normal biochemical reactions, yet different enough to interfere with one or more normal functions of cells, such as cell division. Anti-metabolites include, but are not limited to, gemcitabine, fluorouracil, capecitabine, methotrexate sodium, ralitrexed, pemetrexed, tegafur, cytosine arabinoside, thioguanine, 5-azacytidine, 6-mercaptopurine, azathioprine, 6-thioguanine, pentostatin, fludarabine phosphate, and cladribine, as well as pharmaceutically acceptable salts, acids, or derivatives of any of these. In certain embodiments, the second anticancer agent is gemcitabine. In certain embodiments, the tumor to be treated is a pancreatic tumor and the second anticancer agent is an anti-metabolite (e.g., gemcitabine).

[00283] In other embodiments, the treatment involves the combined administration of an antagonist of the present invention and radiation therapy. Treatment with an antagonist can occur prior to, concurrently with, or subsequent to administration of radiation therapy. Any dosing schedules for such radiation therapy can be used.

[00284] In other embodiments, the treatment can involve the combined administration of antibodies of the present invention with other antibodies against additional tumor associated antigens including, but not limited to, antibodies that bind to the EGF receptor (EGFR) (e.g., Erbitux®), the erbB2 receptor (HER2) (e.g., Herceptin®), and vascular endothelial growth factor (VEGF) (e.g., Avastin®). In certain alternative embodiments, the second anti-cancer agent comprises an antibody that specifically binds to human DLL4 or other ligand of a Notch receptor or an antibody that specifically binds to an additional human Notch receptor. Exemplary, anti-DLL4 antibodies, are described, for example, in U.S. Patent Application Publication No. US 2008/0187532, incorporated by reference herein in its entirety. Additional anti-DLL4 antibodies are described in, e.g., International Patent Publication Nos. WO 2008/091222 and WO 2008/0793326, and U.S. Patent Application Publication Nos. US 2008/0014196, US 2008/0175847; US 2008/0181899; and US 2008/0107648, each of which is incorporated by reference herein in its entirety.

Exemplary anti-Notch antibodies, are described, for example, in U.S. Patent Application Publication No. US 2008/0131434, incorporated by reference herein in its entirety. In certain embodiments, the second anti-cancer agent is an inhibitor of Notch signaling. In certain embodiments, the second anti-cancer agent is an antibody that is an angiogenesis inhibitor (e.g., an anti-VEGF antibody). In certain embodiments, the second therapeutic agent is an antibody that specifically binds a VEGF receptor. In certain embodiments, the second therapeutic agent is AVASTIN (bevacizumab), HERCEPTIN (trastuzumab), VECTIBIX (panitumumab), or ERBITUX (cetuximab). Combined administration can include co-administration, either in a single pharmaceutical formulation or using separate formulations, or consecutive administration in either order but generally within a time period such that all active agents can exert their biological activities simultaneously.

[00285] Furthermore, treatment can include administration of one or more cytokines (e.g., lymphokines, interleukins, tumor necrosis factors, and/or growth factors) or can be accompanied by surgical removal of cancer cells or any other therapy deemed necessary by a treating physician.

[00286] For the treatment of the disease, the appropriate dosage of an antagonist of the present invention depends on the type of disease to be treated, the severity and course of the disease, the responsiveness of the disease, whether the antagonist is administered for therapeutic or preventative purposes, previous therapy, patient's clinical history, and so on, all at the discretion of the treating physician. The antagonist can be administered one time or over a series of treatments lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved (e.g., reduction in tumor size). Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient and will vary depending on the relative potency of an individual antagonist. The administering physician can easily determine optimum dosages, dosing methodologies and repetition rates. In general, dosage is from 0.01 μg to 100 mg per kg of body weight, and can be given once or more daily, weekly, monthly or yearly. The treating physician can estimate repetition rates for dosing based on measured residence times and concentrations of the drug in bodily fluids or tissues.

[00287] In certain embodiments, the patients under consideration for treatment with the Notch antagonist are screened prior to treatment with the Notch antagonist. In certain embodiments, a tumor in a patient or a tumor that has been removed from a patient is tested for the presence of cancer stem cells. In certain embodiments, the tumor is tested for expression of the one or more Notch receptors (e.g., Notch2 and/or Notch3) to which the antagonist binds. In certain embodiments, the tumor is tested for the presence of an inactivating deletion or mutation in the gene encoding the tumor suppressor phosphatase and tensin homolog (PTEN). In certain embodiments, the tumor so tested is a breast tumor.

[00288] For example, the invention provides a method of selecting a subject for treatment with a Notch2 and/or Notch 3 antagonist, wherein the subject has a tumor or has had a tumor removed. In certain embodiments, the method comprises (a) determining if the tumor comprises a deletion or mutation in the

PTEN gene, and (b) selecting the subject for treatment with the Notch 3 antagonist if the tumor comprises the deletion or mutation.

[00289] In certain alternative embodiments of the present invention, patients screened for the presence of colon adenomas or polyps are tested for allelic loss and somatic mutations via a genetic test. In some embodiments the genetic test screens for loss or mutations in the Wnt pathway including, for example, in APC, Axin2 or beta-catenin.

[00290] In another aspect, the present invention provides kits that can be used to perform the methods described herein. In some embodiments, a kit comprises an antibody or antibodies specific for a Notch receptor, a purified antibody or antibodies, in one or more containers. In some embodiments, a kit further comprises a substantially isolated Notch receptor comprising an epitope that is specifically immunoreactive with the antibody or antibodies included in the kit, a control antibody that does not react with the Notch receptor, and/or a means for detecting the binding of an antibody to a Notch receptor (such as, for example, a fluorescent chromophore, an enzymatic substrate, a radioactive compound or a luminescent compound conjugated to the antibody against a Notch receptor or to a second antibody that recognizes the antibody against a Notch receptor). In other embodiments, a kit comprises reagents specific for the detection of mRNA or cDNA (e.g., oligonucleotide probes or primers) of one or more Notch receptor. In some embodiments, the kits contain all of the components necessary and/or sufficient to perform a detection assay, including all controls, directions for performing assays, and any necessary software for analysis and presentation of results.

[00291] A compartment kit includes any kit in which reagents are contained in separate containers. Such containers include small glass containers, plastic containers or strips of plastic or paper. Such containers allows one to efficiently transfer reagents from one compartment to another compartment such that the samples and reagents are not cross-contaminated, and the agents or solutions of each container can be added in a quantitative fashion from one compartment to another. Such containers will include a container which will accept the test sample, a container which contains the antibodies or probes used in the methods, containers which contain wash reagents (such as phosphate buffered saline, Tris-buffers, etc.), and containers which contain the reagents used to detect the bound antibody or probe. One will readily recognize that the disclosed polynucleotides, polypeptides and antibodies of the present invention can be readily incorporated into one of the established kit formats which are well known in the art.

[00292] In some embodiments, the invention further provides a kit comprising a Notch-binding agent or antagonist and a second therapeutic agent. In certain embodiments, the Notch-binding agent or antagonist is an antibody that specifically binds to Notch 2 and/or Notch3. In certain embodiments, the Notch-binding agent or antagonist is an antibody that specifically binds to Notch 2 and Notch3. In certain embodiments, the second therapeutic agent is an anti-cancer agent and/or an anti-angiogenic agent.

[00293] In one aspect, the present invention provides a method of identifying a molecule that binds to a non-ligand binding region of an extracellular domain of a human Notch receptor and inhibits tumor growth, the method comprising: i) incubating the molecule with the non-ligand binding domain of the extracellular domain of the human Notch receptor; ii) determining if the molecule binds to the non-ligand binding region of the extracellular domain of the human Notch receptor; and iii) determining if the molecule inhibits tumor growth. Molecules that specifically bind a non-ligand binding region of an extracellular domain of a human Notch receptor include, but are not limited to, small organic molecules, polypeptides, and antibodies.

[00294] Screening can be performed using any suitable method known in the art. In certain embodiments, screening is performed *in vitro*. In some embodiments, cells expressing a non-ligand binding region of the extracellular domain of a human Notch receptor are incubated with a labeled molecule and specific binding of the labeled molecule to a non-ligand binding region of the extracellular domain of a human Notch receptor is determined by FACS analysis. In some embodiments, a non-ligand binding region of the extracellular domain of a human Notch receptor is expressed by phage display, and molecules that specifically bind to a non-binding region of the extracellular domain of a human Notch receptor are identified. Other suitable methods for identifying molecules that specifically bind to a non-ligand binding region of a human Notch receptor include, but are not limited to, ELISA, Western (or immuno) blotting, and yeast-two-hybrid.

[00295] Molecules that specifically bind to a non-ligand binding region of an extracellular domain of a human Notch receptor are then tested for inhibition of tumor cell growth. Testing can be performed using any suitable method known in the art. In certain embodiments, molecules that specifically bind to non-ligand binding region of the extracellular domain of a human Notch receptor are tested for the ability to inhibit tumor growth *in vitro*. In some embodiments, molecules that specifically bind a non-ligand binding region of the extracellular domain of a human Notch receptor are incubated with tumor cells in culture and proliferation of tumor cells in the presence of a molecule that specifically binds a non-ligand binding region of the extracellular domain of a human Notch receptor is determined and compared to tumor cells incubated with a non-binding control molecule. In certain embodiments, molecules that specifically bind to non-ligand binding region of the extracellular domain of a human Notch receptor are tested for the ability to inhibit tumor growth *in vivo*. In certain embodiments, molecules that specifically bind a non-ligand binding region of the extracellular domain of a human Notch receptor are injected into an animal xenograft model and the growth of tumors in animals treated with molecules that specifically bind to non-ligand binding region of the extracellular domain of a human Notch receptor is determined and compared to animals treated with a non-binding control molecule.

EXAMPLES

[00296] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application.

Example 1

Production of human antibodies to Notch2

[00297] Human antibodies that specifically recognize the non-ligand binding portion of the extracellular domain of a Notch2 receptor were isolated using phage display technology. A synthetic antibody library containing human antibody variable domains was screened for specific and high affinity recognition of a Notch2 receptor.

[00298] Briefly, $2x10^{13}$ Fab displaying phage particles were incubated with a passively immobilized, recombinant Notch2 Fc fusion protein (SEQ ID NO:21) comprising the extracellular ligand binding site of Notch2 and surrounding EGF repeats (EGF1-12) in round one. The non-specific phage were washed off, and then the specific phage were eluted with DTT. The eluted output was used to infect TG1 F+ bacteria, rescued with helper phage, and then Fab display induced with IPTG (0.25 mM). This process was repeated for two additional rounds and then round three was screened in ELISA against passively immobilized recombinant Notch2 (EGF1-12) Fc fusion (5 μg/ml).

[00299] A particular Fab (59R1) was identified that bound the human Notch2 receptor and blocked binding of Jagged1 to human Notch2. Binding of the 59R1 Fab to human Notch2 was verified by FACS assay using a stable human cell line HEK-293 which overexpressed human Notch2 (hN2) (Figure 1A). Fab binding was detected by phycoerythrin (PE)-conjugated goat anti-human Fab (Jackson Immunochemicals). The 59R1 Fab (referred to in Figure 1A as clone 1) demonstrated good binding to hN2. The 59R1 Fab also demonstrated good blocking activity against the Notch ligand human Jagged1 in a binding assay using the same stable cell line (Figure 1B). Ligand binding and blocking was determined by incubating hJagged1 extracellular domain (ECD) fused to human Fc constant region with the cells and Fabs selected from the phage library and using PE-conjugated goat anti-human Fc gamma specific antibodies (Jackson Immunochemicals) for detection.

[00300] The sequences of the VH and VL of the 59R1 Fab are provided in SEQ ID NO: 11 and SEQ ID NO: 12 (including N-terminus bacterial signal sequences that are cleaved upon secretion), respectively. The CDRs of the 59R1 Fab are as indicated in Table 2 below.

Table 2. CDRs of 59R1 human Fab and IgG antibodies

Lead	Heavy Chain			Light Chain		
	CDR1	CDR2	CDR3	CDR1	CDR2	CDR3
59R1	SSSGMS (SEQ ID NO:5)	VIASSGSNTYYADSVKG (SEQ ID NO:6)	GIFFAI (SEQ ID NO:7	RASQSVRSNYLA (SEQ ID NO:8)	GASSRAT (SEQ ID NO:9	QQYSNFPI (SEQ ID NO:10)

[00301] Variable regions based on those of the 59R1 Fab were cloned into Ig expression vectors containing human IgG2 heavy-chain and kappa light-chain along with their respective mammalian signal sequences for expression in Chinese Hamster Ovary (CHO) cells. The VH and VL of the 59R1 IgG antibody are provided as SEQ ID NO: 13 and SEQ ID NO: 14, respectively. The amino acid sequence of the heavy chain and light chain of the 59R1 IgG antibody (including signal sequences) are provided as SEQ ID NO: 16 and SEQ ID NO: 18, respectively. The signal sequence at the N-terminus of the amino acid sequence of each of the chains is cleaved upon secretion. The nucleic acid sequences encoding the heavy and light chains of the 59R1 IgG antibody are provided as SEQ ID NO: 1 and SEQ ID NO: 3, respectively. Protein A purification was used to purify the antibodies. Bacterial plasmid DNA containing a synthetic DNA insert encoding the heavy and light chain of the 59R1 IgG2 antibody DNA was deposited as "59R1" with the ATCC, 10801 University Boulevard, Manassas, VA, USA, under the conditions of the Budapest Treaty on October 15, 2008, and assigned designation number PTA-9547.

[00302] In addition, the 59R1 IgG2 antibody was assayed for its ability to block binding of DLL4 to the human Notch 2 receptor by FACS analysis. HEK-293 cells stably overexpressing human Notch2 were incubated with the antibody at various concentration and then detected for hNotch2 binding (Figure 1C) by PE-conjugated goat anti-human Fc gamma specific antibody, or ligand blocking activity (Figure 1D). Ligand blocking was determined by incubating the cells with human DLL4 ECD tagged with the rabbit Fc constant region and the 59R1 antibody at a range of concentrations, and then detecting the hDLL4 by PE-conjugated donkey anti-rabbit antibody. Binding of hNotch2 and ligand blocking activity were thus confirmed for the 59R1 IgG2 antibody.

[00303] A germlined variant of 59R1 (referred to herein as "59RGV") was also expressed and purified. The VH and VL of the 59RGV antibody are provided as SEQ ID NO: 19 and SEQ ID NO: 20, respectively. The amino acid sequence of the heavy chain and light chain of the 59RGV antibody (including signal sequences) are provided as SEQ ID NO: 2 and SEQ ID NO: 4, respectively. The signal sequence at the N-terminus of the amino acid sequence of each of the chains is cleaved upon secretion. The nucleic acid sequences encoding the heavy and light chains of the 59RGV antibody are provided as SEQ ID NO: 15 and SEQ ID NO: 17, respectively.

[00304] Highly hydrophobic CDRs have the potential, in certain instances, to allow for unfavorable, non-specific binding by an antibody. Since the amino acid sequence of the heavy

chain CDR3 of 59R1 had an unusual degree of hydrophobic character, variants of 59R1 that contained heavy chain CDR3 sequences with decreased hydrophobic character were produced. Heavy chain CDR3 affinity maturation was conducted by allowing restricted changes from the parental sequence (GIFFAI; SEQ ID NO:7) as shown in Figure 1E. Allowed amino acids at each position were allowed to change from parental residues to the residues indicated in Figure 1E. Improved variants were isolated by screening them for improved JAG1 blocking ability as shown in Figure 1F (indicated with arrows). Briefly, Fabs (1 and 10 μg/ml) were mixed with hJAG1-rb Fc (preclustered 5 μg/ml to 2μl/ml PE-conjugated donkey anti-rabbit) and then added to hNotch2 stably transfected 293 cells. hJAG1 binding was then assessed using flow cytometry. Six improved variants (versus 59R1 Fab) were isolated and their HC CDR3 sequences were as follows: SIFYPT (SEQ ID NO:22), SSFFAS (SEQ ID NO:23), SSFYAS (SEQ ID NO:24), SSFFAT (SEQ ID NO:25), SIFYPS (SEQ ID NO:26), and SSFFAN (SEQ ID NO:27). The sequences of the heavy chain variable regions for these variants are sequences SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, and SEQ ID NO:57.

Example 2

Cross-reactivity and binding affinity of anti-Notch2/3 59R1 antibody

[00305] The ability of the 59R1 IgG2 antibody to cross-react with other Notch receptors was determined by FACS assay using HEK-293 cells transiently transfected with the human Notch1, Notch2, Notch3 or Notch4 expression plasmid and green fluorescent protein (GFP) as a transfection control. GFP positive cells indicated expression of the transgene. The 59R1 IgG2 was added to the cells at 2 μg/ml and detected by PE-conjugated goat anti-human Fc gamma specific (Jackson Immunochemicals). All Notch constructs were full length. The results are shown in Figure 2. As shown in Figure 2, the 59R1 IgG2 antibody specifically binds to both human Notch3 and human Notch2, but does not bind significantly to full-length human Notch1 or human Notch4.

[00306] The affinities for human and mouse Notch1, Notch2, Notch3, and Notch4 were determined using a Biacore 2000 instrument. Briefly, recombinant human and mouse Notch proteins (EGF10-15 for Notch1, 2, & 4; EGF9-14 for Notch3) were immobilized on a CM5 chip using standard amine based chemistry (NHS/EDC). For hNotch2, EGF1-12 was also tested for binding. Different antibody concentrations (1 – 100 nM) were injected over the protein surfaces and kinetic data were collected over time. The data was fit using the simultaneous global fit equation to yield dissociation constants (K_D, nM) for each Notch (Table 3).

Table 3

59R1 IgG Dissociation Constants (K_D)								
Ab	mNotch1 (nM)	hNotch1 (nM)	mNotch2 (nM)	hNotch2 (nM)*	hNotch2 (nM)**	mNotch3 (nM)	hNotch3 (nM)	hNotch4 (nM)
59R1	>10	86.4	0.35	<0.1	<0.1	0.13	0.12	NB

*N2(EGF1-12)

**N2(EGF 10-155)

Example 3

Epitope mapping of anti-Notch2/3 antibody 59R1

[00307] To identify antibodies that recognize specific non-ligand binding regions of the Notch receptor extracellular domains, epitope mapping was performed.

[00308] The binding of anti-Notch2/3 antibodies to supernatant from HEK 293 cells transfected with sequences encoding recombinant human Notch2 Fc fusion proteins comprising the full length human Notch2 protein or various human Notch2 deletion constructs containing various deletions of EGF repeats one to twelve were tested by ELISA. See Table 4 below. HEK-293 cells were transiently transfected with pcDNA 3.1 (Invitrogen) with hNotch2 cDNA's encoding for the indicated amino acids fused to the constant region of human IgG (hFc). Supernatants were harvested 48 hours later. To capture hN2–hfc proteins, 96 well plates were first coated with goat anti-human Fc gamma specific IgG (Jackson Immunochemicals, #109-006-098) at 100 ng per well in sodium bicarbonate buffer overnight at 4°. Plates were washed and blocked in 5% bovine serum/PBS-Tween-20. Supernatants were added to plates and incubated at room temperature for 1 hour. Plates were washed in PBS-T. 59R1 Fab was added at 10 μg/ml in 5% serum/PBS-T and incubated at room temperature for 1 hour. Plates were washed in PBS-T. Fab binding was detected by goat anti-human Fab specific antibody conjugated to horseradish peroxidase (Thermo, # 31414) diluted 1:5000 in 5% serum/PBS-T for 1 hour at room temperature. Plates were washed and developed with 1 Step Ultra TMB (Thermo, # 34028). Plates were read on a Perkin Elmer Victor 1420 plate reader. Anti-Notch2/3 59R1 antibody bound only to supernatant from cells expressing recombinant Notch2 proteins comprising EGF10, which consists of amino acids 375-417 of human Notch 2. (Figure 3A).

Table 4. Human Notch2 deletion constructs

Construct	amino acids
hN2 1-3	1-144
hN2 1-4	1-181
hN2 1-5	1-221
hN2 1-6	1-263
hN2 1-7	1-301
hN2 1-8	1-341
hN2 1-9	1-378
hN2 1-10	1-417
hN2 1-11	1-456
hN2 1-12	1-493
hN2 8-12	296-493
hN2 9-12	326-493
hN2 10-12	375-493
hN2 11-12	413-493
hN2 12-12	454-493

[00309] Moreover, FACS analysis shows that 59R1 Fab antibody binding was retained when EGF11 or EGF12 were deleted from full length Notch2 recombinant protein expressed by HEK 293 cells (Figure 3B). Point mutations were made within EGF10 of Notch2 fusion proteins and binding of 59R1 to each EGF10 mutant was determined by FACS analysis. HEK-293 cells were transiently transfected with the indicated Notch expression plasmid and GFP as a transfection control. GFP positive cells indicated expression of the transgene. The 59R1 Fab antibody was added to the cells at 10 μg/ml and detected by PE-conjugated goat anti-human (Jackson Immunochemicals).

[00310] To verify that loss of EGF repeat 10 does not interfere with ligand binding, a mutant hNotch2 missing amino acids 375-412 was generated and tested for binding to 59R1, a hNotch2 monoclonal 59M70 directed against EGF 1-4, and binding to the ligand human DLL4 (Figure 3C). FACS analysis of HEK-293 cells transiently transfected with the indicated Notch expression plasmid and GFP as a transfection control. GFP positive cells indicate expression of the transgene. Anti-Notch2 (59M70) was added at 20 μg/ml and detected by PE-conjugated goat anti-mouse (Caltag, #3004-4). 59R1 (IgG2) was added to the cells at 2 μg/ml and detected by PE-conjugated goat anti-human Fc gamma specific (Jackson Immunochemicals). Ligand binding was determined by incubation of the cells with human DLL4 extracellular domain (ECD) fused to rabbit IgG constant region at 5 μg/ml and detected by PE-conjugated donkey anti-rabbit. As shown in Figure 3C, ligand and 59M70 both bind to hNotch2 in the absence of EGF 10, but 59R1 does not.

[00311] A comparison analysis of the EGF 10 regions of human Notch1, Notch2, and Notch4 and the EGF 9 region of human Notch3 (the equivalent of EGF 10 in the other Notch receptors) was performed to determine likely binding sites for 59R1 (Figure 14A). As a result of the analysis, several point mutants were created within full-length Notch2, converting residues within EGF10 to the corresponding amino acids in human Notch 1. Also, conversely, point mutations were made in hNotch1 EGF 10 converting

residues to the corresponding hN2 residues. Mutants in full-length Notch sequences were generated by QuikChange® mutagenesis (Stratagene) and verified by sequencing. Binding to the mutants was determined by FACS analysis (Figure 14B and 14C). 59R1 was detected by PE-conjugated goat anti-human Fc gamma specific antibody (Jackson Immunochemicals, #109-116-170). The amino acids necessary for 59R1 binding to hNotch 2 were thus determined to be histidine 385, alanine 388, and leucine 389 (residues within the boxed hNotch2 sequence shown in Figure 14A). The corresponding residues in hNotch3 are histidine 361, alanine 364, and isoleucine 365.

Example 4

Anti-Notch2/3 antibody 59R1 inhibits Notch2 signaling

[00312] Luciferase reporter assays were used to assay the 59R1 antibody for its ability to block hDLL4-, hJAG1-, and hJAG2-induced Notch2 signaling.

[00313] Hela cells that stably overexpress human Notch2 were transiently transfected with firefly luciferase with a synthetic 8X CBS promoter (Ong et al., 2006, *J. of Biological Chemistry*, 281:5106-5119), pSPORT6 MAML-1, and Renilla luciferase-CMV as a transfection control. Cells were incubated with 100 ng of immobilized hDLL4 (R&D systems) with the indicated antibodies for 16 hours and then assayed using Dual-Glo (Promega) according to the manufacturer's instructions. Control antibody was at a concentration of 40 μg/ml. 59R1 IgG2 antibody was titrated, starting at 40 μg/ml, and then diluted by one-fourth. The gamma secretase inhibitor (GSI) dibenzazepine (DBZ) was used as a control at 1 μM. As shown in Figure 4A, the 59R1 antibody was found to inhibit hDLL4-induced Notch2 reporter activity.

[00314] Hela cells that stably overexpress human Notch2 were transiently transfected with firefly luciferase with a synthetic 8X CBS promoter, pSPORT6 MAML-1, and Renilla luciferase-CMV as a transfection control. Cells were incubated with either 200 ng of immobilized hJAG1 (R&D systems) or hJAG2 (R&D systems) for 16 hours and then assayed using Dual-Glo (Promega) according to the manufacturer's instructions. 59R1 IgG2 antibody was at a concentration of 40 μg/ml. The gamma secretase inhibitor (GSI) dibenzazepine (DBZ) was used as a control at 1 μM. As shown in Figures 4B and 4C, the 59R1 antibody was found to inhibit both hJAG1- and hJAG2-induced Notch2 reporter activity, respectively.

Example 5

Anti-Notch2/3 antibody 59R1 prevents *in vivo* tumor growth

[00315] This example describes the use of an anti-Notch2/3 receptor antibody (59R1) that binds a non-ligand binding region of the Notch receptors (EGF10 of Notch2 and EGF10 of Notch3) to prevent tumor growth in a xenograft model.

[00316] In certain embodiments, NOD/SCID mice injected with 50,000 PE13 or T3 breast tumor cells were treated with anti-Notch2/3 antibody 59R1 or control antibody 1B7.11 two days following cell injections. Antibodies were dosed at 10 mg/kg twice week. Anti-Notch2/3 antibody 59R1 significantly reduced both PE13 (Figure 5A) and T3 (Figure 5B) tumor growth compared to control.

Example 6

In vivo treatment of tumors using anti-Notch 2/3 antibody 59R1

[00317] This example describes the use of anti-Notch 2/3 antibodies to treat cancer in a xenograft model.

[00318] In one experiment, the $1x10^7$ viable Colo-205 colon tumor cells were injected into 6-8 week-old immunodeficient *bg/nu XID* female mice on a Swiss CD-1 background. Tumors were allowed to grow to a size of between 65 to 200 mm^3 after which mice were randomized (n=10 per experimental group), and antibodies administration begun. Animals were treated with 15 mg/kg of either control 1B7.11 antibodies or anti-Notch2/3 59R1 antibodies once weekly. Tumor size was measured twice weekly, and tumor volume was calculated as described (*see* Michieli et al., 2004, *Cancer Cell*, 6:61-73). Anti-Notch2/3 antibody 59R1 significantly reduced Colo-205 tumor growth compared to control (Figure 5C).

[00319] In another experiment, anti-Notch2/3 antibodies were tested for an effect on pancreatic tumor growth. NOD/SCID mice were injected with 30,000 PN4 pancreatic tumor cells sub-cu in the right flank, and tumors were allowed to grow until they had reached an average volume of 100 mm^3. Animals were randomized and dosing of anti-Notch2/3 antibody 59R1 or control antibody 1B711 was initiated. Antibodies were dosed at 15 mg/kg given once per week. Anti-Notch2/3 antibody 59R1 significantly reduced PN4 tumor growth compared to control (Figure 5D).

[00320] In a further experiment, anti-Notch2/3 antibodies were tested for an effect on breast tumor growth. NOD/SCID mice were injected with 50,000 PE13 or T3 breast tumor cells, and tumors were allowed to grow to a size of between 65 to 200 mm^3 after which mice were randomized (n=10 per experimental group), and antibodies administration begun. Animals were treated with 15 mg/kg of either control 1B7.11 antibodies or anti-Notch2/3 59R1 antibodies twice weekly. Tumor size was measured twice weekly, and tumor volume was calculated as described (*see* Michieli et al., 2004). Anti-Notch2/3 antibody 59R1 significantly reduced growth of both PE13 (Figure 5E) and TE (Figure 5F) tumors compared to control.

[00321] At the end point of antibody treatment, tumors may be harvested for further analysis. In some embodiments, a portion of the tumor is analyzed by immunofluorescence to assess antibody penetration into the tumor and tumor response. A portion of each harvested tumor from anti-Notch2/3 antibody treated and control antibody treated mice is fresh-frozen in liquid nitrogen, embedded in O.C.T., and cut on a cryostat as 10 μm sections onto glass slides. Alternatively a portion of each tumor is formalin-fixed,

paraffin-embedded, and cut on a microtome as 10 μm section onto glass slides. Sections are post-fixed and incubated with chromophore labeled antibodies that specifically recognize injected antibodies to detect anti-Notch2/3 antibody or control antibodies present in the tumor biopsy. Furthermore, antibodies that detect different tumor and tumor recruited cell types such as, for example, anti-VE cadherin (CD144) or anti-PECAM-1 (CD31) antibodies to detect vascular endothelial cells, anti-smooth muscle alpha-actin antibodies detect vascular smooth muscle cells, anti-Ki67 antibodies to detect proliferating cells, TUNEL assays to detect dying cells, and anti-intracellular domain (ICD) Notch fragment antibodies to detect Notch signaling can be used to assess affects of antibody treatment on angiogenesis, tumor growth, and tumor morphology.

[00322] The effect of anti-Notch2/3 antibody treatment on tumor cell gene expression may also be assessed. Total RNA is extracted from a portion of each harvested tumor from Notch2/3 antibody treated and control antibody treated mice and used for quantitative RT-PCR. Expression levels of Notch2/3, components of the Notch2 and/or Notch3 signaling pathway, as well as cancer stem cell markers including, for example, CD44, are analyzed relative to the house-keeping gene GAPDH as an internal control. Changes in tumor cell gene expression upon Notch2/3 antibody treatment are thus determined.

[00323] In addition, the effect of anti-Notch2/3 antibody treatment on the presence of cancer stem cells in a tumor may be assessed. Tumor samples from Notch 2/3 antibody versus control antibody treated mice are cut up into small pieces, minced completely using sterile blades, and single cell suspensions obtained by enzymatic digestion and mechanical disruption. Dissociated tumor cells are then analyzed by FACS analysis for the presence of tumorigenic cancer stem cells based on ESA+, CD44+, CD24-/low, Lin- surface cell marker expression as described in detail above.

[00324] The tumorigenicity of cells isolated based on ESA+, CD44+, CD24-/low, Lin- expression following anti-Notch2/3 antibody treatment can then assessed. In one example, 5,000, 1,000, 500, and 100 isolated ESA+, CD44+, CD24-/low, Lin- cancer stem cells from Notch 2/3 antibody treated versus control antibody treated mice are re-injected subcutaneously into the mammary fat pads of NOD/SCID mice. The tumorigenicity of cancer stem cells based on the number of injected cells required for consistent tumor formation is thus determined.

Example 7

Anti-Notch 2/3 antibody 59R1 delays tumor recurrence *in vivo* following paclitaxel treatment

[00325] B51 breast tumor cells (50,000 cells per mouse) were injected sub-cutaneously into the mammary fat pad of NOD-SCID mice. Tumors were allowed to grow for 50 days until they had reached an average volume of ~100 mm^3. Animals were randomized (n = 10/group) and treatments were initiated. One group received a control antibody (1B711) at 10 mg/kg twice per week and paclitaxel (Taxol) at 15 mg/kg

twice per week and the other group received 59R1 at 10 mg/kg twice per week and paclitaxel at 15 mg/kg twice per week. Tumor volumes were measured on the indicated days. Treatments were carried out for 38 days until the tumor volumes had regressed to ~ 50 mm^3, after which the paclitaxel treatments were halted and the antibody treatments continued for the duration of the experiment.

[00326] The results are shown in Figure 6. Tumors were observed to recur more rapidly in the control group compared with the group treated with 59R1.

Example 8

Anti-Notch 2/3 antibody 59R1 decreases the frequency of cancer stem cells in a tumor *in vivo*

[00327] Limiting dilution assays (LDAs) can be used to assess the effect of a Notch-binding agent on solid tumor cancer stem cells and on the tumorigenicity of a tumor comprising the cancer stem cells. The assays can be sued to determine the frequency of cancer stem cells in tumors from animals treated with the Notch-binding agent or other agent and to compare that frequency to the frequency of cancer stem cells in tumors from control animals.

[00328] An LDA was used to assess the effect on the tumorigenicity of the B51 breast tumors that were treated with the combination of control antibody (1B711) plus paclitaxel (Taxol) or treated with the combination of 59R1 and paclitaxel, as described above in Example 7. In addition, the effect of treatment of B51 breast tumors with the control antibody alone or 59R1 alone was also determined by LDA. The doses of antibodies and paclitaxel and the schedule of dosing for the control antibody group and the 59R1 group were the same as described in Example 7, above for the other two treatment groups. After three doses of antibodies and/or paclitaxel, tumors were harvested, processed and dissociated into single cells. The human tumor cells were isolated from the xenograft tumor cells by incubation with biotinylated mouse antibodies (α-mouse CD45-biotin 1:200 dilution and rat α-mouse H2Kd-biotin 1:100 dilution, BioLegend, San Diego, CA) on ice for 30 min, followed by addition of streptavidin-labeled magnetic beads and removal of the mouse cells with the aid of a magnet. The human cells in the suspension were harvested and counted.

[00329] A serial titration of cells (30, 90, 270, and 810 cells) from each of the four treatment groups was injected in a 1:1 (v/v) mixture of FACS buffer and Matrigel into a new set of NOD-SCID mice (n=10/group). Tumors were allowed to grow for 72 days. The percentage of mice with detectable tumors was determined in all groups. The cancer stem cell frequency was then calculated using L-Calc™ software (StemCell Technologies Inc.; downloadable from www.stemcell.com/search/default.asp).

[00330] The results are shown in Figure 7. The frequency of cancer stem cells in the tumor in the control-treated mice ("Control") was determined to be 1:66. The frequency of cancer stem cells in the tumor in the paclitaxel-treated mice ("Taxol") was shown to be 1:25, indicating that treatment with paclitaxel had

actually increased the frequency of cancer stem cells in the tumor by more than two-fold relative to the control. Treatment with the 59R1 antibody, either alone ("59R1") or in combination with paclitaxel ("Taxol+59R1"), on the other hand, reduced the frequency of cancer stem cells in the tumors. The 59R1 antibody alone reduced the cancer stem cell frequency in the breast tumors by more than two-fold relative to the control. Treatment with the combination of 59R1 antibody and paclitaxel reduced the frequency of cancer stem cells in the tumor by more than about two-fold relative to treatment with 59R1 alone ($p<0.0001$), by about 4.5-fold relative to treatment with the control antibody, and by about twelve-fold relative to treatment with paclitaxel alone. These results indicate that treatment with the 59R1 antibody is effective at reducing the tumorigenicity of a breast tumor, whether given alone or in combination with paclitaxel, even though treatment with paclitaxel alone has the opposite effect.

Example 9

Additional *in vivo* treatment of tumors using anti-Notch 2/3 antibody 59R1

[00331] PN4 pancreatic tumor cells (50,000 cells per mouse) were injected subcutaneously into the flank region of Nod-Scid mice. Tumors were allowed to grow for 27 days until they had reached an average volume of ~120 mm^3. Animals were randomized into four treatment groups (n = 10/group) and treatments were initiated. One group received a control antibody (1B711) at 10 mg/kg twice per week; one group received gemcitabine at 40 mg/kg once per week plus the control antibody at 10 mg/kg twice per week; one group received 59R1 at 10 mg/kg twice per week, and the fourth group received the combination of 59R1 at 10 mg/kg twice per week and gemcitabine 40 mg/kg once per week. Tumor volumes were measured on the indicated days. The results are shown in Figure 8. Tumor growth was found to be inhibited by the combination of 59R1 and gemcitabine ($p < 0.001$).

[00332] M4 melanoma tumor cells (10,000 cells per mouse) were injected subcutaneously into the flank region of NOD-SCID mice. Tumors were allowed to grow for 25 days until they had reached an average volume of ~80 mm^3. Animals were randomized into treatment groups (n = 10/group) and treatments were initiated. One group received a control antibody (1B711) at 10 mg/kg twice per week and one group received 59R1 at 10 mg/kg twice per week. Tumor volumes were measured on the indicated days. The results are shown in Figure 9. Tumor growth was found to be inhibited by 59R1.

[00333] C28 colon tumor cells (10,000 cells per mouse) were injected subcutaneously into the flank region of NOD-SCID mice. Tumors were allowed to grow for 24 days until they had reached an average volume of ~130 mm^3. Animals were randomized into four treatment groups (n = 10/group) and treatments were initiated. One group received a control antibody (1B711) at 10 mg/kg twice per week; one group received irintoecan at 7.5 mg/kg once per week plus the control antibody at 10 mg/kg twice per week; one group received 59R1 at 10 mg/kg twice per week, and the fourth group received the

combination of 59R1 at 10 mg/kg twice per week and irinotecan at 7.5 mg/kg once per week. Tumor volumes were measured on the indicated days. The results are shown in Figure 10. Tumor growth was found to be inhibited by 59R1 alone relative to the control antibody group and by the combination of 59R1 and irinotecan relative to the irinotecan group.

[00334] The 59R1 IgG2 antibody was also tested *in vivo* in the breast tumor xenograft lines OMP-B34, OMP-B39, OMP-B44, PE13, and UM-T1, the pancreas tumor xenograft line OMP-PN8, and the colon tumor xenograft line OMP-C8. These tumor xenograft lines were established by adhering to procedures described in Al-Hajj et al., 2003, *Proc. Natl. Acad. Sci. USA*, 100:3983-3988. Female NOD/SCID immuno-compromised mice 7–10 weeks old were used for the establishment of the breast tumor xenografts and male NOD/SCID mice were used for the OMP-Pn8 and OMP-C8 tumor-models (Harlan, Indianapolis, Indiana). The 59R1 IgG2 antibody was also tested *in vivo* in a Colo-205 colon tumor xenograft model. Female immunodeficient *bg/nu XID* mice on a Swiss CD-1 background were used for the Colo-205 xenograft tumor model. In case of the breast cancer models, slow-releasing estrogen pellets (0.36 mg) had to be implanted. Mice were subcutaneously injected on the right flank with 50,000 (OMP-B34, OMP-B39, OMP-B44, PE13, and UM-T1) or $1x10^7$ (Colo-205) viable cells, respectively, in a mixture of PBS (without magnesium or calcium) and Matrigel at a 1:1 ratio. The injected total volume per mouse was 200 µl with 50% being Matrigel. Once the tumor had reached a size between 65-200 mm^3, the mice were randomized. Antibodies were administered weekly and tumors measured twice weekly. LZ1 (a human antibody that recognizes lyzozyme) or 1B711 (a murine IgG1 antibody that recognizes the hapten trinitrophenol) was used as a control antibody for treatment of each tumor type. Tumor volume was calculated as described Al-Hajj et al. (2003). Data are expressed as the mean and the mean ±S.E.M. Group means were compared using Student's two-tailed, unpaired t-test. Probability (P) values of <0.05 were interpreted as significantly different. All statistical analysis was performed using Microsoft EXCEL and Graph Pad PRISM.

[00335] The results of the additional *in vivo* experiments in Colo205, C8, PNA, B34, B39, B44, PE13, and T1 xenograft models are shown in Figures 11A-11H, respectively. As shown in Figure 11A, monotherapy with the 59R1 antibody significantly inhibited growth of the Colo205 tumor relative to the control antibody (LZ1) ($p<0.01$). Combination therapy with 59R1 plus the anti-VEGF antibody bevacizumab (AVASTIN) provided an even greater inhibition of tumor growth ($p<0.001$) than either 59R1 or bevacizumab alone. In another colorectal xenograft model, C8, 59R1 was likewise shown to inhibit tumor growth relative to LZ1 control antibody (Figure 11B). Similarly, 59R1 was found to inhibit pancreatic tumor growth (relative to control antibody) in the PN8 xenograft model (Figure 11C). 59R1 was also shown to inhibit breast cancer growth relative to a control antibody in each of the five breast cancer xenograft models B34 (Figure 11D), B39 (Figure 11E), B44 (Figure 11F), and PE13 (Figure 11G). The 59R1 antibody was likewise found to be effective at inhibiting tumor growth in the T1 breast cancer

model (Figure 11H), although it was only effective in the presence of estrogen, despite T1 being an estrogen receptor negative tumor.

Example 10

Effect of treatment with anti-Notch2/3 antibody 59R1 on gene regulation in xenograft tumor models

[00336] Gene expression levels in various xenograft tumor models treated with the 59R1 IgG2 antibody were analyzed by microarray analysis. Global gene expression profiling analysis was performed on Affymetrix HG-U133 plus 2.0 microarray (Affymetrix, Santa Clara, CA). Three independent RNA samples of xenograft whole tumors from the control and treatment groups were isolated and hybridized to the microarrays according to the manufacturer's instructions. Scanned array background adjustment and signal intensity normalization were performed with GCRMA algorithm in the open-source bioconductor software (www.bioconductor.org). The expression level of each gene was normalized by z-score transformation across the samples in the control (CTRL) and treatment (59R1) groups. Genes differentially expressed ($p < 0.05$ and fold change > 2.0) between the two groups were identified with Bayesian t-test (Baldi et al., 2001, *Bioinformatics*, 17:509-519. The expression patterns of selected associated differentially regulated genes in selected tumor xenograft models (Colo205, B44, PE13, and T1) are shown in Table 5 below. The P-value (PVal) of each gene is the probability of significant regulation of the gene by 59R1 by chance using Bayesian t-test. A number of genes including the genes encoding regulator of G-protein signaling 5 (RGS5), Notch3, and hairy/enhancer-of-split related with YRPW motif-like (HEYL) protein were shown to be significantly down-regulated in the stroma of the 59R1-treated mice relative to the control mice. (By contrast, these particular genes encoding RGS5, Notch3, and HEYL were not found to be significantly down-regulated in the human cells of the tumors.)

Table 5. Differentially expressed genes in stroma of 59R1-treated tumors

	Colo205		B44		PE13		T1	
Gene	Fold	pVal	Fold	pVal	Fold	pVal	Fold	pVal
1420942_s_at (Rgs5)	-5.52	7.65E-07	-2.43	5.59E-04	-4.23	2.86E-05	-1.18	9.82E-04
1417466_at (Rgs5)	-3.39	6.62E-07	-2.22	3.11E-04	-4.03	1.31E-10	-1.99	4.11E-04
1420941_at (Rgs5)	-5.10	1.66E-03	-2.09	1.18E-03	-2.99	1.35E-05	-1.97	2.07E-03
1421964_at (Notch3)	-3.26	3.70E-06	-2.03	2.30E-03	-1.91	1.67E-03	-1.01	8.86E-01
1416286_at (Rgs4)	-3.08	2.69E-03	-1.57	3.84E-02	-1.83	6.71E-05	-1.13	4.47E-01
1434141_at (Gucy1a3)	-2.49	2.87E-03	-1.74	1.07E-02	-4.18	1.49E-07	1.20	5.95E-01
1459713_s_at (Tmem16a)	-1.90	1.90E-03	-1.70	1.01E-02	-7.28	9.89E-10	-2.14	1.79E-04
1420872_at (Gucy1b3)	-1.94	1.90E-02	-1.65	7.68E-03	-3.06	8.52E-10	-1.01	7.13E-01
1422789_at (Aldh1a2)	-1.73	1.20E-02	-4.92	2.42E-08	-2.17	1.58E-04	-2.16	9.27E-04
1419302_at (Heyl)	-3.28	5.61E-03	-1.12	2.36E-01	-1.77	5.72E-04	-1.07	2.39E-02
1451501_a_at (Ghr)	-1.83	1.69E-02	-2.24	2.71E-04	-1.66	8.90E-04	-1.12	3.38E-01

1417714_x_at (Hba-a1/Hba-a2)	-8.37	2.49E-02	-2.56	4.63E-04	-1.92	1.06E-02	1.42	9.24E-01
1428361_x_at (Hba-a1/Hba-a2)	-8.91	1.93E-02	-2.42	1.08E-03	-1.73	4.27E-02	1.73	4.67E-01
1452590_a_at (Plac9)	-1.61	1.07E-02	-1.64	1.22E-02	-1.62	6.17E-03	1.20	7.36E-01
1449632_s_at (Fkbp10)	-1.72	1.69E-02	-1.57	1.12E-02	-1.63	1.80E-04	1.07	5.97E-01
1449280_at (Esm1)	2.07	1.06E-02	1.55	3.48E-02	1.56	4.35E-02	1.18	2.44E-01
1418829_a_at (Eno2)	1.79	2.92E-02	1.71	1.02E-02	1.54	5.43E-03	1.29	9.92E-02

[00337] The expression levels in the stroma from the xenograft models Colo205, B29, B34, B44, PE13, T1 (without estrogen treatment), T1 (with estrogen treatment), C8, and PN8 of selected genes that had been identified in the microarray analysis as being regulated by treatment with 59R1 (*heyl*, *notch3*, *rgs5*, *angpt1*, and *angpt2*) were further analyzed by TaqMan® analysis. The results are shown in Figures 12A (*heyl*), 12B (*notch3*), 12C (*rgs5*), 12D (*angpt1*), and 12E (*angpt2*).

[00338] The results of the TaqMan® analysis confirm that *notch3* and *rgs5* are down-regulated in the stroma of each of the various tumor types in response to treatment with 59R1 (relative to control) (Figures 12B and C). RGS5 is a well-known marker of pericytes and vascular smooth muscle cells (Berger et al., 2005, *Blood*, 105:1094-1101; Lovschall et al., 2007, *Int. J. Dev. Biol.*, 51: 715-721; Cho et al., 2003, *FASEB J.*, 17:440-2). Notch3 has been identified as being coexpressed with RGS5 in pericytes during angiogenesis and plyaing an important role in the regulation of the fate of pericytes and vascular smooth muscle cells (Lovschall et al., 2007, *Int. J. Dev. Biol.*, 51: 715-721; Domenga et al., 2004, *Genes & Dev.*, 18:2730-2735; Sweeney et al., 2004, *FASEB J.*, 18:1421-3; Morrow et al., 2005, *Am. J. Physiol. Cell Physiol.*, 289:C1188-C1196).

[00339] In addition, *heyl* was also confirmed to be downregulated in the stroma of each of the xenograft models except B34 (Figure 12A). HeyL belongs to the Hey family of downstream transcription factors of Notch signaling (Hey1, Hey2, and HeyL). The downregulation of *heyl* by 59R1 suggests that the 59R1 antibody directly affects Notch signaling by downregulating *heyl*.

[00340] *Angiopoietin-1 (angpt1)* and *angiopoietin-2 (angpt2)* were also determined to be down-regulated in the stroma of a number of the breast cancer models (Figures 12D and E). ANGPT1 and 2 (angiopoietin-1 and -2) are ligands for the TIE 1 and 2 receptors. TIE receptors, like VEGF, are crucial signaling molecules in neoangiogenesis processes (Jones et al., 2001, *Nature Reviews*, 2:257-267).

[00341] Notably, however, *angpt1* and *angpt2* were down-regulated in the stroma of the T1 model when estrogen treatment was used ("T1 e"), conditions under which treatment with 59R1 was effective against tumor growth, but not in the stroma of the same model in the absence of estrogen treatment ("T1 ne"), conditions under which treatment with 59R1 was ineffective against tumor growth (see Example 9, above). Thus, the effect of 59R1 on the down-regulation of angiopoietin-1 and angiopoietin-2 in the stroma of the T1 tumor is abrogated in the absence of estrogen treatment. One possible explanation of this effect is that in the absence of estrogen treatment, the levels of the growth factors angiopoietin-1 and

angiopoietin-2 in the T1 stroma are not sufficiently elevated to provide for measurable decreases in expression levels upon treatment with the 59R1 antibody. Estrogen has been shown to have significant effects on the tumor microenvironment (Banka et al., 2006, *Cancer Res.* 66:3667-3672). One possible explanation of this data is that estrogen leads to a dependence of the tumor on ANGPT2 signaling, which then leads to sensitivity to 59R1 treatment.

Example 11

Anti-Notch2/3 antibody 59R1 significantly induces hypoxia in colon and breast tumors

[00342] Staining for hypoxic regions was performed in Colo-205 colon tumors and PE-13 breast tumors that had been treated either with 59R1 IgG2 antibody or with 1B711 control antibody. The staining was performed as described in Ridgway et al., 2006, *Nature* 444:1083-1087. Briefly, to measure hypoxia, pimonidazole-hydrochloride (HypoxyProbe, NPI, Burlington, MA), which forms long-lived protein adducts at partial pressure of oxygen less than approximately 10 mm Hg, was injected intraperitoneally at 60 mg/kg 1 hr prior to sacrifice. Tumors were then processed for histological analysis, and tumor sections were stained using anti-pimonidazole antibody following manufacturer's protocol (NPI). Photographs were taken using a BX51 microscope (Olympus, Center Valley, PA).

[00343] Viable tumor cells were found to be equally present in 1B711 and 59R1-treated tumors, as indicated by a relatively uniform and dense DAPI stain (data not shown). The number of CD31-positive cells also remained unchanged, suggesting that endothelial cell number was not affected by 59R1 treatment. In 59R1-treated Colo-205 and PE13 tumors, however, hypoxic regions (as detected by anti-pimonidazole antibody) were significantly more pronounced than in 1B711 treated tumors (data not shown). AF594-conjugated goat anti-rat $F(ab')_2$ was used to detect anti-CD31 antibody and FITC-conjugated goat anti-rabbit antibody was used to detect anti-pimonidazole antibody.

Example 12

Breast tumors comprising deletions in the PTEN tumor suppressor gene are responsive to treatment with 59R1

[00344] DNA samples were prepared from tumor cells of xenograft breast cancers. Before the DNA isolation, mouse stroma cells in the xenograft tumors were depleted using magnetic beads conjugated with mouse cell specific antibodies. The purified DNA samples were hybridized to Affymetrix Genome-Wide Human SNP Array 6.0 genechip (Affymetrix, Santa Clara, CA), which has more than 946,000 probes for the detection of copy number variations (CNVs), according to the manufacturer's instructions. The copy number state changes were estimated by Hidden Markov Model (HMM) and their variations (CNVs) of each sample were obtained by rank segmentation analysis using Hapmap270 as baseline. Due to the

inherent noise in the array, -0.5 and -1.0 log2 ratios were used as the cutoffs for the heterozygous deletion and homozygous deletion under the significance threshold $<1.0\times10^{-6}$ and minimum number of probes per segment =5.

[00345] Figure 13 shows the exon, Affymetrix probe distribution, and the deletions in the gene of the tumor suppressor phosphatase tensin homolog (PTEN) in chromosome 10. The B29, B34, B37, B40, B51, T2, T3, and T6 breast tumors were found to have intact PTEN genes in their genomes. The PTEN gene was determined to harbor homozygous deletions in B39 tumor, while B44, PE13, and T1 tumors were determined to have heterozygous deletions of this gene. As discussed above, 59R1 was determined to have anti-tumor efficacy in each of these four breast tumors comprising homozygous or heterozygous deletions of PTEN. These results suggest that tumors, especially breast tumors, harboring homozygous or heterozygous PTEN deletions may be particularly suitable for treatment with an anti-Notch2/3 antibody such as 59R1.

Example 13

Characterization of 59R5 Antibody

[00346] An additional human antibody 59R5 that specifically binds human Notch 2 and human Notch 3 was identified. The sequences of the heavy chain and light chain are provided in SEQ ID NO: 49 and SEQ ID NO:18, respectively. The heavy chain variable region is provided in SEQ ID NO:50 and the light chain variable region is provide SEQ ID NO:13. The heavy chain CDR3 sequence of 59R5 comprises SIFYTT, SEQ ID NO:51. The other CDR sequences of 59R5 are identical to 59R1. Biacore analysis of 59R1 and 59R5 binding affinities indicated that 59R5 had similar binding properties for both Notch2 and Notch3 as 59R1. Both antibodies bind human and murine Notch2 and Notch3 receptors with sub-nanomolar affinity (see Table 6).

Table 6

IgG Dissociation Constants (K_D, nM)							
	m-Notch1	h-Notch1	m-Notch2	h-Notch2	m-Notch3	h-Notch3	h-Notch4
59R1	>10	>10	0.65	0.05	0.32	0.19	NB
59R5	>10	>10	0.26	0.05	0.29	0.22	NB

[00347] 59R5 was determined to have similar activity in blocking Notch2 and Notch3 signaling as 59R1. Receptor activation was determined in luciferase-based assays. PC3 tumor cells were transiently transfected with a human or mouse Notch receptor (human Notch2, murine Notch2, human Notch3, or murine Notch3) and GFP inducible reporter construct. Transfected cells were incubated with different concentrations of 59R1 or 59R5 antibody in the presence of passively immobilized DLL4-Fc protein.

Notch receptor activation was determined by measuring luciferase activity. As shown in Figure 15A, 59R5 blocked ligand-induced activation of human Notch2, murine Notch2, human Notch3 and murine Notch3 receptor signaling at similar levels as 59R1.

[00348] The binding epitope of 59R5 was determined. As was described in Example 3 for analysis of antibody 59R1, several point mutants were created within full-length Notch1, converting residues within EGF10 to the corresponding amino acids in human Notch 2. Mutants in full-length Notch sequences were generated by QuikChange® mutagenesis (Stratagene) and verified by sequencing. HEK 293 cells were transiently transfected with expression vectors encoding human Notch2, human Notch1, or human Notch1 with residues 382-386 mutated to the corresponding human Notch2 residues. Cells were also co-transfected with a plasmid encoding green fluorescent protein (GFP) to mark those cells that received transfected plasmid. Cells were incubated with 59R1 or 59R5 and fluorescent secondary antibody and then examined by FACS. 59R1 and 59R5 were detected by PE-conjugated goat anti-human Fc gamma specific antibody (Jackson Immunochemicals, #109-116-170). As shown in Figure 15B, 59R5 bound to Notch2 and did not bind to Notch1. However, when amino acids corresponding to Notch2 amino acids 385-389 were substituted into Notch1, 59R5 was able to bind to the mutated Notch1. This suggested that at least one or more amino acids necessary for 59R5 binding to human Notch 2 were positioned within amino acids 385-389 (residues in the boxed hNotch2 sequence shown in Figure 14A) and suggested that 59R5 binds the same epitope as 59R1, or an epitope similar to, or overlapping with, the epitope of 59R1.

Example 14

In vivo treatment of tumors using Notch2/3 antibody 59R5

[00349] In one embodiment, NOD/SCID mice were injected with PE13 breast tumor cells. The mice were treated with anti-Notch2/3 antibody 59R1, anti-Notch2/3 antibody 59R5, or control antibody. Antibodies were dosed at 15 mg/kg once per week in a "preventative" mode where dosing was initiated two days after cell injection. Figure 16A shows that 59R5 treatment inhibited tumor growth by greater than 80%, similar to the effects seen with 59R1.

[00350] In another embodiment, NOD/SCID mice were injected with C28 colon tumor cells. The mice were treated with anti-Notch2/3 antibody 59R1, anti-Notch2/3 antibody 59R5 or control antibody. Antibodies were dosed at 15 mg/kg once per week in a "preventative" mode where dosing was initiated two days after cell injection. Figure 16B shows that both 59R1 and 59R5 inhibited the growth of C28 colon tumors.

[00351] In another embodiment, NOD/SCID mice were injected with Colo205 colon tumor cells. The mice were treated with anti-Notch2/3 antibody 59R1, anti-Notch2/3 antibody 59R5 or control antibody. Antibodies were dosed at 15 mg/kg once per week after tumors had been established. Figure

16C shows that both 59R1 and 59R5 inhibited the growth of Colo208 colon tumors at similar levels.

Example 15

In vivo treatment of tumors using Notch2/3 antibody 59R5 in combination treatment

[00352] In one embodiment, NOD/SCID mice were injected with PN8 pancreatic tumor cells. The tumors were allowed to grow for approximately 33 days until they had reached an average tumor volume of 150mm^3. The mice were treated with gemcitabine at 20 mg/kg once per week for four weeks in combination with control antibody, anti-Notch2/3 antibody 59R1, or anti-Notch2/3 antibody 59R5. As shown in Figure 17A, antibody 59R5 inhibited tumor growth at a similar level as antibody 59R1 and that combination treatment prolonged tumor recurrence longer than gemcitabine alone.

[00353] In one embodiment, to evaluate the effect of 59R5 on cancer stem cells, a tumor recurrence study was carried out in the PE13 breast tumor model. NOD/SCID mice were injected with PE13 breast tumor cells. The tumors were allowed to grow for 40 days before treatments were initiated. The mice were treated with taxol at 15 mg/kg twice per week for 5 weeks, in combination with either control antibody or anti-Notch 2/3 antibody 59R5. After 5 weeks, the taxol treatments were stopped and the antibody treatments were continued. 59R5 was observed to significantly delay tumor recurrence after high-dose taxol treatment (Fig. 17B). These results suggest that 59R5 treatment reduces cancer stem cell frequency.

[00354] A summary of the *in vivo* activity of 59R1 and 59R5 as described in the preceding embodiments is shown in Table 7. Tumor volumes and p values for each experiment are shown relative to the control group. The PE13, C28 and Colo205 studies were carried out as described in Example 14. PN8 studies were carried out as described above. For the PN8 experiment, the control is gemcitabine alone and values for 59R1 and 59R5 are the combinations with gemcitabine. Antibodies were dosed once per week at 15 mg/kg for all experiments.

Table 7

	PE13		**C28**		**Colo205**		**PN8**	
	Tumor vol	p value	Tumor vol	p value	Tumor vol	p value	Tumor vol	p value
59R1	0.25	<0.0001	0.29	<0.0001	0.68	0.003	0.27	0.026
59R5	0.18	<0.0001	0.38	<0.0001	0.61	0.001	0.11	0.036

Example 16

Regulation of Gene Expression in Tumors after 59R5 Treatment

[00355] To determine if 59R5 and 59R1 were functioning by the same mechanisms *in vivo*, the expression of key target genes in tumor cells and tumor stroma were examined. Gene expression was assayed by

quantitative PCR in PE13 tumor cells and stromal cells. Gene expression levels relative to the control antibody treated group are shown in Figure 18. 59R1 and 59R5 regulated the expression of murine HeyL, Notch3, and RGS5 in stromal cells to a similar extent (left panel). The same pattern of regulation was observed in C28 tumors (data not shown). Thus, the mechanism of action previously identified for 59R1 in regulating genes in the tumor stromal critical for function of the tumor vasculature and pericytes was retained by 59R5. Similarly, 59R5 and 59R1 regulated the expression of the human genes ID4, EDNRA, and EGLN3 in tumor cells to the same degree (right panel).

[00356] Unlike other members of this gene family, ID4 is generally underexpressed in tumors, and ID4 has been shown to be a tumor suppressor in breast cancer that is frequently silenced by methylation. Loss of expression of ID4 is correlated with a worse prognosis in breast cancer patients (Noetzel et al., 2008, *BMC Cancer* 8:154). Thus, up-regulation of ID4 in PE13 breast tumor cells may be part of the anti-tumor mechanism of anti-Notch2/3. EDNRA is the gene encoding endothelin receptor which promotes growth of both endothelial and tumor cells and stimulates metastatic activity of tumor cells (Bagnato and Rosano 2008, *Int. J. Biochem. Cell. Biol.* 40:1443-51). EGLN3 (also known as HIF-3α) is a hypoxia inducible gene. Induction of EGLN3 by anti-Notch2/3 is consistent with disruption of functional vasculature in the treated tumors. These data indicated that the biological activities and mechanism of action of 59R1 and 59R5 were very similar.

[00357] Table 8 shows results from a microassay analysis of 59R1 and 59R5 treated PE13 tumors. The numbers are mean differential expression values for treated vs. control animals, with 3 animals per group.

Table 8

59R1		59R5			
Fold	**pVal**	**Fold**	**pVal**	**Symbol**	**Gene Title**
-5.10	3.21E-05	-3.00	1.10E-03	Foxc2	forkhead box C2
-4.40	1.26E-05	-2.46	7.45E-04	Hey2	hairy/enhancer-of-split related with YRPW motif 2
-4.32	8.03E-06	-2.14	1.00E-04	Rgs5	regulator of G-protein signaling 5
-3.33	5.59E-04	-2.79	3.18E-03	Heyl	hairy/enhancer-of-split related with YRPW motif-like
-2.71	4.80E-04	-2.90	1.02E-04	Rgs4	regulator of G-protein signaling 4
-2.10	2.17E-04	-1.86	4.33E-05	Notch3	Notch gene homolog 3 (Drosophila)
-1.92	3.16E-02	-2.35	3.43E-03	Mmp9	matrix metallopeptidase 9
2.36	3.06E-02	4.97	3.35E-02	Pdcd1lg2	programmed cell death 1 ligand 2
7.42	6.25E-07	2.80	2.07E-03	Gzma	granzyme A

[00358] Microarray analysis reveals that 59R5 significantly inhibited the Notch pathway ($p<0.01$) as measured by gene expression (e.g., *Foxc2, Hey2, Heyl, Notch3*). These results were comparable to 59R1. *Foxc2* is a down-stream target of the Hedgehog pathway and is involved in cell differentiation. Additional genes involved in apoptosis (e.g., granzyme A) and tumor-associated tissue remodeling

(MMP-9) were also similarly expressed between 59R1 and 59R5. These data suggested that the biological activities and mechanism of action of 59R1 and 59R5 are very similar.

Example 17

Production of additional Notch2 and/or Notch3 antibodies

Antigen Production

[00359] In certain embodiments, recombinant polypeptide fragments of the human Notch2 or human Notch3 extracellular domain are generated as antigens for antibody production. For example, standard recombinant DNA technology can be used to isolate a polynucleotide encoding amino acids 1-493 of Notch2 (SEQ ID NO: 33), encompassing EGF 1-12. This polynucleotide can be ligated in-frame N-terminal to either a human Fc-tag or histidine-tag and cloned into a transfer plasmid vector for baculovirus mediated expression in insect cells. Standard transfection, infection, and cell culture protocols can be used to produce recombinant insect cells expressing the corresponding Notch2 polypeptide (SEQ ID NO: 34) (O'Reilly et al., 1994, Baculovirus expression vectors: A Laboratory Manual, Oxford: Oxford University Press).

[00360] Cleavage of the endogenous signal sequence of human Notch2 was approximated using cleavage prediction software SignalP 3.0, however the actual *in vivo* cleavage point can differ by a couple of amino acids either direction. The predicted cleavage of Notch2 is between amino acids 1 and 26, thus Notch2 antigen protein comprises approximately amino acid 27 through amino acid 493. Antigen protein can be purified from insect cell conditioned medium using Protein A and Ni^{++}-chelate affinity chromatography. Purified antigen protein is then dialyzed against PBS (pH=7), concentrated to approximately 1 mg/ml, and sterile filtered in preparation for immunization.

Immunization

[00361] Mice can be immunized with purified Notch2 or Notch3 antigen protein using standard techniques. Blood from individual mice can be screened approximately 70 days after initial immunization for antigen recognition using ELISA and FACS analysis (described in detail below). The animals with the highest antibody titers are then selected for final antigen boost after which spleen cells are isolated for hybridoma production. Hybridoma cells are plated at 1 cell per well in 96 well plates, and the supernatant from each well screened by ELISA and FACS analysis against antigen protein. Several hybridomas with high antibody titer are selected and scaled up in static flask culture. Antibodies are purified from the hybridoma supernatant using protein A or protein G agarose chromatography and antibodies are tested by FACS as described below.

FACS analysis

[00362] To select monoclonal antibodies produced by hybridomas, clones that recognize native cell-surface Notch2 (and/or Notch3) protein, FACs analysis is used. HEK293 cells are co-transfected with expression vectors encoding a full-length cDNA clone of Notch2 and the transfection marker GFP. Twenty-four to forty-eight hours post-transfection, cells are collected in suspension and incubated on ice with anti-Notch2 (or anti-Notch3 or anti-Notch2/3) antibodies or control IgG to detect background antibody binding. The cells are washed and primary antibodies detected with anti-mouse secondary antibodies conjugated to a fluorescent chromophore. Labeled cells are then sorted by FACS to identify anti-Notch2, anti-Notch3, or anti-Notch2/3 antibodies that specifically recognize cell surface expression of native cell-surface Notch2 and/or Notch3 protein.

Chimeric antibodies

[00363] After monoclonal antibodies that specifically recognize a non-ligand binding domain of a Notch receptor are identified, these antibodies are modified to overcome the human anti-mouse antibody (HAMA) immune response when rodent antibodies are used as therapeutics agents. The variable regions of the heavy-chain and light-chain of the selected monoclonal antibody are isolated by RT-PCR from hybridoma cells and ligated in-frame to human IgG1 heavy-chain and kappa light chain constant regions, respectively, in mammalian expression vectors. Alternatively a human Ig expression vector such as TCAE 5.3 is used that contains the human IgG1 heavy-chain and kappa light-chain constant region genes on the same plasmid (Preston et al., 1998, *Infection & Immunity* 66:4137-42). Expression vectors encoding chimeric heavy- and light-chains are then co-transfected into Chinese hamster ovary (CHO) cells for chimeric antibody production. Immunoreactivity and affinity of chimeric antibodies are compared to parental murine antibodies by ELISA and FACS.

Humanized antibodies

[00364] As chimeric antibody therapeutics are still frequently antigenic, producing a human anti-chimeric antibody (HACA) immune response, chimeric antibodies against a Notch2 or Notch3 receptor can require further humanization. To generate humanized antibodies the three short hypervariable sequences, or complementary determining regions (CDRs), of the chimeric antibody heavy- and light-chain variable domains described above are engineered using recombinant DNA technology into the variable domain framework of a human heavy- and light-chain sequences, respectively, and then cloned into a mammalian expression vector for expression in CHO cells. The immunoreactivity and affinity of the humanized antibodies are compared to parental chimeric antibodies by ELISA and FACS. Additionally, site-directed or high-density mutagenesis of the variable region can be used to optimize specificity, affinity, etc. of the humanized antibody.

Example 18

Additional *in vitro* assays to evaluate antibodies against a Notch receptor

[00365] This example describes methods for *in vitro* assays to test the activity of antibodies generated against a Notch2 and/or Notch3 receptor on cell proliferation and cytotoxicity.

Proliferation Assay

[00366] Antibodies against Notch2 and/or Notch3 are tested for their effect on tumor cell growth *in vitro* using a BrdU based assay. Freshly dissociated, Lin-depleted breast tumor cells are cultured in low oxygen for between 2-5 days. Cells are then cultured at 20,000 cells/well with 2.5 μg/mL or 5.0 μg/mL anti-Notch antibody, control non-specific murine IgG, or no antibody for three days followed by 18 hours BrdU labeling. All experiments are performed with multiple replicates. The ability of anti-Notch antibodies to inhibit cell proliferation compared to control antibodies is then determined.

Complement-dependent cytotoxicity assay

[00367] Cancer cell lines expressing a Notch2 receptor and/or a Notch3 receptor or, alternatively, cancer stem cells isolated from a patients sample passaged as a xenograft in immunocompromised mice are used to measure complement dependent cytotoxicity (CDC) mediated by an antibody against a Notch 2 and/or Notch3 receptor. Cells are suspended in 200 μl RPMI 1640 culture medium supplemented with antibiotics and 5% FBS at 106 cells/ml. Suspended cells are then mixed with 200 μl serum or heat-inactivated serum with antibodies against a Notch2 and/or Notch3 receptor or control antibodies in triplicate. Cell mixtures are incubated for 1 to 4 hours at 37°C in 5% CO_2. Treated cells are then collected, resuspended in 100 μl FITC-labeled annexin V diluted in culture medium and incubated at room temperature for 10 min. One hundred μl of a propidium iodide solution (25 μg/ml) diluted in HBSS is added and incubated for 5 min at room temperature. Cells are collected, resuspended in culture medium and analyzed by flow cytometry. Flow cytometry of FITC stained cells provides total cell counts, and propidium iodide uptake by dead cells as a percentage of total cell numbers is used to measure cell death in the presence of serum and antibodies against a Notch2 and/or Notch3 receptor compared to heat-inactivated serum and control antibodies. The ability of anti-Notch2/3 antibodies to mediated complement-dependent cytotoxicity is thus determined.

Antibody-dependent cellular cytotoxicity assay

[00368] Cancer cell lines expressing a Notch2 receptor and/or a Notch3 receptor or, alternatively, cancer stem cells isolated from a patients sample passaged as a xenograft in immunocompromised mice (described in detail below) are used to measure antibody dependent cellular cytotoxicity (ADCC)

mediated by an antibody against a Notch2 and/or Notch3 receptor. Cells are suspended in 200 µl phenol red-free RPMI 1640 culture medium supplemented with antibiotics and 5% FBS at 106 cells/ml. Peripheral blood mononuclear cells (PBMCs) are isolated from heparinized peripheral blood by Ficoll-Paque density gradient centrifugation for use as effector cells. Target cells (T) are then mixed with PBMC effector cells (E) at E/T ratios of 25:1, 10:1 and 5:1 in 96-well plates in the presence of a Notch2 or Notch3 receptor or control antibodies. Controls include incubation of target cells alone and effector cells alone in the presence of antibody. Cell mixtures are incubated for 1 to 6 hours at 37°C in 5% CO_2. Released lactate dehydrogenase (LDH), a stable cytosolic enzyme released upon cell lysis, is then measured by a colorimetric assay (e.g., CytoTox96 Non-radioactive Cytotoxicity Assay; Promega; Madison, WI). Absorbance data at 490 nm are collected with a standard 96-well plate reader and background corrected. The percentage of specific cytotoxicity is calculated according to the formula: % cytotoxicity = 100 x (experimental LDH release – effector spontaneous LDH release – target spontaneous LDH release) / (target maximal LDH release – target spontaneous LDH release). The ability of antibodies against a Notch2 and/or Notch3 receptor to mediated antibody dependent cellular cytotoxicity is thus determined.

Example 19

Production of antibodies against EGF10 (or equivalent EGF) of Notch receptors

[00369] Identification of an antibody that specifically binds the tenth EGF repeat of Notch2 and the corresponding EGF repeat of Notch3 (the ninth EGF repeat) that reduces tumor growth in animals suggests the importance of the non-ligand binding domain, and the tenth EGF repeat (or its equivalent) in particular, for effective cancer therapies. To target the EGF repeat 10 (or equivalent EGF) in Notch receptor family members, antibodies against EGF10 of Notch1, Notch2, or Notch4 or against EGF9 of Notch3 are produced and analyzed. Specifically, mice are immunized with antigens comprising the tenth EGF repeat of Notch1 (SEQ ID NO:35); Notch2 (SEQ ID NO:36), or Notch4 (SEQ ID NO:38) or the ninth EGF repeat of Notch3 (SEQ ID NO:37). Antibodies that recognize specific Notch receptors as well as antibodies that recognize different combinations of the four Notch receptors are identified using FACS analysis of HEK 293 cells transfected with each Notch receptor as described in detail above. Antibodies that recognize the tenth EGF repeat (or equivalent EGF) of two Notch receptor family members are envisioned (e.g. antibodies that recognize the Notch1 EGF10 and Notch2 EGF10; Notch1 EGF10 and Notch3 EGF9; Notch1 EGF10 and Notch4 EGF10; Notch2 EGF10 and Notch3 EGF9; Notch2 EGF10 and Notch4 EGF10; or Notch3 EGF9 and Notch4 EGF10). Antibodies that recognize the tenth EGF repeat (or equivalent EGF) of three Notch receptor family members are likewise contemplated (e.g., antibodies that recognize the Notch1 EGF10, Notch2 EGF10, and Notch3 EGF9; Notch1 EGF10, Notch2 EGF10, and

Notch4 EGF10; or Notch2 EGF10, Notch3 EGF9, and Notch4 EGF10). And antibodies that recognize the tenth EGF repeat (or equivalent EGF) of four Notch receptor family members are envisioned (e.g. antibodies that recognize the Notch1 EGF10, Notch2 EGF10, Notch3 EGF9 and Notch4 EGF10).

Example 20

Treatment of human cancer using anti-Notch receptor antibodies

[00370] This example describes methods for treating cancer using antibodies against a Notch receptor to target tumors comprising cancer stem cells and/or tumor cells in which Notch receptor expression has been detected.

[00371] The presence of cancer stem cell marker expression can first be determined from a tumor biopsy. Tumor cells from a biopsy from a patient diagnosed with cancer are removed under sterile conditions. In some embodiments, the tissue biopsy is fresh-frozen in liquid nitrogen, embedded in O.C.T., and cut on a cryostat as 10 μm sections onto glass slides. Alternatively the tissue biopsy is formalin-fixed, paraffin-embedded, and cut on a microtome as 10 μm section onto glass slides. Sections are incubated with antibodies against a Notch receptor to detect protein expression. Additionally, the presence of cancer stem cells can be determined. Tissue biopsy samples are cut up into small pieces, minced completely using sterile blades, and cells subject to enzymatic digestion and mechanical disruption to obtain a single cell suspension. Dissociated tumor cells are then incubated with anti-ESA, -CD44, -CD24, and –Lin, antibodies to detect cancer stem cells, and the presence of ESA+, CD44+, CD24-/low, Lin- tumor stem cells is determined by flow cytometry as described in detail above.

[00372] Cancer patients whose tumors are diagnosed as expressing a Notch receptor are treated with anti-Notch receptor antibodies. Humanized or human monoclonal anti-Notch receptor antibodies generated as described above are purified and formulated with a suitable pharmaceutical carrier in PBS for injection. Patients are treated with the Notch antibodies once a week for at least 10 weeks, but in certain cases once a week for at least about 14 weeks. Each administration of the antibody should be a pharmaceutically effective dose about 2 to about 100 mg/ml and in certain cases between about 5 to about 40 mg/ml. The antibody can be administered prior to, concurrently with, or after standard radiotherapy regimens or chemotherapy regimens using one or more chemotherapeutic agent, such as paclitaxel, gemcitabine, irinotecan, oxaliplatin, fluorouracil, leucovorin, or streptozocin. Patients are monitored to determine whether such treatment has resulted in an anti-tumor response, for example, based on tumor regression, reduction in the incidences of new tumors, lower tumor antigen expression, decreased numbers of cancer stem cells, or other means of evaluating disease prognosis.

Example 21

Production of antibodies against Notch1, Notch2, Notch3, and/or Notch4 EGF repeat 4

[00373] To target the EGF repeat 4 in Notch receptor family members, antibodies against Notch1, Notch2, Notch3, and/or NOTCH4 EGF repeat 4 are produced and analyzed. Specifically, mice are immunized with antigens comprising the fourth EGF repeat of Notch1 (SEQ ID NO:41), Notch2 (SEQ ID NO:42), Notch3 (SEQ ID NO: 43), or Notch4 (SEQ ID NO:44). Antibodies that recognize specific Notch receptors as well as antibodies that recognize different combinations of the four Notch receptors are identified using FACS analysis of HEK 293 cells transfected with each Notch receptor as described in detail above. Antibodies that recognize the fourth EGF repeat of two Notch receptor family members are envisioned (e.g. antibodies that recognize the fourth EGF repeat of Notch1 and Notch2; Notch1 and Notch3; Notch1 and Notch4; Notch2 and Notch3; Notch2 and Notch4; or Notch3 and Notch4). Antibodies that recognize the fourth EGF repeat of three Notch receptor family members are envisioned (e.g. antibodies that recognize the fourth EGF repeat of Notch1, Notch2, and Notch3; Notch1, Notch2, and Notch4; or Notch2, Notch3, and Notch4). And antibodies that recognize the fourth EGF repeat of four Notch receptor family members are envisioned (e.g. antibodies that recognize the fourth EGF repeat of Notch1, Notch2, Notch3 and Notch4).

[00374] A description of the exemplary production and characterization of a monoclonal antibody, 13M57, that binds EGF4 of Notch1 can be found in U.S. Patent Application Publication No. 2008/0131434, which is incorporated by reference herein in its entirety.

Example 22

Additional gene expression assays in tumor cells treated with 59R1

[00375] Changes in gene expression in response to 59R1 treatment in tumor cells in xenograft models were identified.

[00376] Several pathways/gene sets that are regulated by antibody 59R1 in tumor cells were identified (Table 9) using Gene Set Enrichment Analysis (Mootha et al., 2003, *Nature Genetics* 34:267-73; Subramanian et al., 2005, *Proc. Natl. Acad. Sci. USA* 102:15545-50) in the breast tumors T1, PE13, and B51. Notably, cell cycle gene pathways, myc-activating genes and several stem cell gene sets are down-regulated by 59R1 in this analysis. cMyc has been shown to be a direct target of the Notch pathway (Weng et al., 2006, *Genes Dev.* 20:2096-109). The stem cell gene sets down-regulated by 59R1 were derived from a molecular signature derived from five distinct populations: human fetal hematopoietic stem cells (HCS), murine fetal and adult HSCs, neural stem cells (NSC), and embryonic stem cells (ESC) (Ivanova et al., 2002, *Science* 298:601-604), and also a recently described core ESC gene set (Ben-Porath et al., 2008, *Nature Genetics* 40:499-507) and an ESC self-renewal gene set whose down-regulation causes differentiation (Hu et al., 2009, *Genes Dev.* 23:837-48).

Table 9

Name	Size	FDR	Description
NGUYEN KERATO UP NOTCH	27	0.0774	Genes concomitantly modulated by activated Notch1 in mouse and human primary keratinocytes-Up
CELLCYCLEPATHWAY	22	0.0798	Cyclins interact with cyclin-dependent kinases to form active kinase complexes that regulate progression thr
YU CMYC UP	28	0.0884	Myc-activated genes
HSC STHSC FETAL	27	0.0885	Up-regulated in mouse short-term functional hematopoietic stem cells from fetal liver (ST-HSC Shared)
HSC STHSC SHARED	27	0.0907	Up-regulated in mouse short-term functional hematopoietic stem cells from both adult bone marrow an
WEINBERG ESC EXP2	30	0.1001	40 genes specifically overexpressed in hES cells according to Meta-analysis of 8 profiling studies (Natu
ESC SELF RENEWAL	30	0.1087	Genes identified by a genome-wide RNAI screen, whose downregulation caused mESC differentiation
BRENTANI REPAIR	33	0.1122	Cancer related genes involved in DNA repair

FDR <15%

Example 23

Reduction of cancer stem cell frequency by Notch2/3 antibodies

[00377] Using a similar experimental study as described in Example 8, an analysis of cancer stem cell frequency by limiting dilution analysis was carried out in PE13 breast cancer cells. Animals bearing PE13 breast tumors were treated with control antibody, taxol plus control antibody, 59R1, or taxol plus 59R1 for three weeks. Tumors were harvested after three weeks, and CSC frequency in the treated tumors was analyzed. Serial titrations of human cells from each the four treatment groups were transplanted into a new set of mice (n =10 per cell dose). Tumor growth rate after 75 days of growth (Figure 19A) was used to calculate the CSC frequency using the L-calc program (Stem Cell Technologies, Inc.). The control antibody treated tumors were determined to have a tumor initiating cell frequency of 1:74. Treatment with taxol alone increased the CSC frequency to 1:30. In contrast, treatment with 59R1 decreased CSC frequency to 1:179 and the combination of 59R1 plus taxol decreased CSC frequency to 1:319 (Figure 19B). A single asterisk indicates a statistically significant difference ($p < 0.05$) vs. the control antibody treated group and a double asterisk indicates a significant difference vs. the taxol and control antibody treated group. This experiment indicated that 59R1 treatment of PE13 breast tumors reduced CSC frequency as a single agent and more dramatically, in combination with taxol treatment. In contrast, treatment with taxol alone, while effective at reducing tumor volume, increased the CSC frequency of treated tumors indicating that tumor initiating cells are preferentially resistant to the effects of this chemotherapeutic agent.

[00378] In addition to investigating the effects of 59R1 in tumors and the effect on CSC frequency, gene changes were studied following 59R1 treatment in combination with taxol. The experiment was performed in PE13 breast tumors where a decrease in CSC frequency after treatment with 59R1 alone or 59R1 plus taxol treatment had previously been observed (and described herein). Microarray analysis was

performed on tumors from the same experiment where limiting dilution analyses of PE13 were carried out for CSC quantification (Figure 19). Animals bearing PE13 breast tumors were treated three weeks with 59R1 plus taxol, control and taxol prior to harvesting for microarray analysis. Mean differential expression values for taxol vs. control and 59R1 plus taxol vs. taxol treated animals (3 animals per group) were calculated. Strikingly, in the gene expression microarray data, it was found that 59R1 in combination with taxol affected apoptosis, hypoxia, differentiation, and stem-cell related genes in the opposite fold direction than the gene changes observed following with taxol alone (Table 10) consistent with the effects of these compounds on the CSC frequency.

Table 10

Taxol vs. control		59R1 Taxol vs. Taxol			
Fold	pval	Fold	pval	Symbol	Name
10.2	6.8E-03	-4.3	2.3E-01	BMPR1B	bone morphogenetic protein receptor, type IB
-2.1	4.2E-05	1.8	6.9E-05	BNIP3	BCL2/adenovirus E1B 19kDa interacting protein 3
-21.1	1.0E-02	11.9	3.9E-04	EGLN3	egl nine homolog 3
13.4	5.9E-05	-1.8	1.2E-01	HSPB6	heat shock protein, alpha-crystallin-related, B6
2.2	1.5E-02	-2.5	1.9E-03	ITGAM	integrin, alpha M
4.6	3.6E-03	-4.4	5.2E-03	LHX8	LIM homeobox 8
-9.0	2.0E-06	6.4	1.8E-05	NDRG1	N-myc downstream regulated gene 1
6.4	6.9E-06	-2.2	7.4E-03	RARRES1	retinoic acid receptor responder 1
2.6	3.5E-04	-1.7	1.1E-03	RARRES3	retinoic acid receptor responder 3
4.8	3.9E-05	-2.2	1.6E-02	RBP2	retinol binding protein 2, cellular
10.6	1.3E-10	-1.5	5.9E-02	XAF1	XIAP associated factor 1

[00379] The apoptosis-related genes regulated in this dataset include BNIP3, NDRG1 HSPB6, and XAF1. BNIP3 (Bcl-2/E1B 19 kDa interacting protein) is a pro-apoptotic member of the Bcl-2 family that is expressed in hypoxic regions of tumors (Kothari et al., 2003, *Oncogene* 22:4734-44). BNIP3 is down-regulated by taxol alone and up-regulated by the combination therapy, suggesting that 59R1 plus taxol may promote apoptosis. Consistent with this idea is the observation that HSPB6 is down-regulated in taxol treated tumors; HSPB6 over-expression may protect against apoptosis in some biological systems (Fan et al., 2005, *Trends Cardiovasc. Med.* 15:138-41). NDRG1 (N-myc downstream regulated gene1), which is up-regulated in the combination treatment, is necessary for p53-dependent apoptosis (Stein et al., 2004, *J. Biol. Chem.* 279:48930-40). Interestingly, NDRG1 is also a putative suppressor of colorectal cancer metastases. Its increased expression is associated with improved survival in prostate and breast cancer (Shah et al., 2005, *Clin. Cancer Res.* 11:3296-302). Additionally, NDRG1 is involved in promoting differentiation. The expression of NDRG1 has been shown to be highly expressed in well-differentiated pancreatic cancer cells, and not expressed in the less differentiated tumor cells (Angst et al., 2006, *Br. J. Cancer* 95:307-13). It was also observed that other stem cell-related genes such BMPR1B

and homeobox containing gene, LHX8, were up-regulated by taxol alone, and then down-regulated with 59R1 treatment in combination with taxol.

[00380] Several genes involved in the metabolism of retinoids (RARRES1, RARRES3, RBP2), which are similar functionally to the putative stem cell marker, ALDH1a1, were up-regulated by taxol, and then down-regulated with taxol plus 59R1 treatment. Retinoic acid signaling has been shown to be linked to cellular differentiation (Appel and Eisen, 2003, *Neuron* 40:461-4). Taken together, these data show that 59R1 has significant effects on gene expression in PE13 breast tumor cells and may begin to elucidate some of the mechanisms that underlie the observed decrease in cancer stem cell frequency in this tumor following treatment with 59R1 and taxol combination therapy.

[00381] In another embodiment, a PN4 pancreatic tumor model was used to test for reduction in cancer stem cell frequency after treatment with 59R1. PN4 pancreatic tumors were treated with control antibody, anti-Notch2/3 59R1, gemcitabine, or a combination of 59R1 and gemcitabine for a period of three weeks. Antibodies were dosed at 10 mg/kg, twice per week and gemcitabine was dosed at 50 mg/kg, twice per week. Tumors from each group were harvested and processed to obtain single cell suspensions. The human tumors cells in the xenograft were isolated and counted. A titration of cells (30, 90 or 210 cells) were re-injected into NOD-SCID mice (n=10 per group). Tumor growth was assayed on day 84 and tumor initiating cell frequency was calculated from the tumor take rate. The control antibody treated tumors were determined to have a tumor initiating cell frequency of 1:137. Treatment with gemcitabine alone increased the CSC frequency to 1:61. In contrast, treatment with 59R1 decreased CSC frequency to 1:281 and the combination of 59R1 plus gemcitabine decreased CSC frequency to 1:675 (Figure 19C). A single asterisk indicates a statistically significant difference ($p < 0.05$) vs. the control antibody treated group and a double asterisk indicates a significant difference vs. the gemcitabine and control antibody treated group.

[00382] In another embodiment, a PE13 breast tumor model was used to test for reduction in cancer stem cell frequency after treatment with 59R5. PE13 breast tumors were treated with control antibody, anti-Notch2/3 59R5, taxol, or a combination of 59R5 and taxol for a period of three weeks. Antibodies were dosed at 20 mg/kg, once per week and taxol was dosed at 15 mg/kg, twice per week. Tumors from each group were harvested and processed to obtain single cell suspensions. The human tumors cells in the xenograft were isolated and counted. A titration of cells (50, 150 or 450 cells) were re-injected into NOD-SCID mice (n=10 per group). Tumor growth was assayed on day 39 and tumor initiating cell frequency was calculated from the tumor take rate. The control antibody treated tumors were determined to have a tumor initiating cell frequency of 1:70. Treatment with taxol alone increased the CSC frequency to 1:30. In contrast, treatment with 59R5 decreased CSC frequency to 1:202 and the combination of 59R5 plus taxol decreased CSC frequency to 1:382 (Figure 19D). A single asterisk indicates a statistically

significant difference ($p < 0.05$) vs. the control antibody treated group and a double asterisk indicates a significant difference vs. the taxol and control antibody treated group.

[00383] As observed in other experiments, these results indicated that 59R1 treatment of PN4 pancreatic tumors and 59R5 treatment of PE13 breast tumors reduced CSC frequency as a single agent and more dramatically, in combination with gemcitabine or taxol treatment, respectively. In contrast, treatment with taxol or gemcitabine alone, while effective at reducing tumor volume, increased the CSC frequency of treated tumors indicating that tumor initiating cells are preferentially resistant to the effects of these chemotherapeutic agents.

[00384] All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those in the relevant fields are intended to be within the scope of the following claims.

SEQUENCES

Human anti-Notch2/3 antibody sequences

SEQ ID NO:1: Nucleotide sequence encoding anti-Notch2/3 IgG2 59R1 heavy chain, plus signal sequence. The sequence encoding the signal sequence is underlined.

ATGAAACACCTGTGGTTCTTCCTCCTGCTGGTGGCAGCTCCCAGATGGGTCCTGTCCCAG
GTGCAATTGGTGGAAAGCGGCGGCGGCCTGGTGCAACCGGGCGGCAGCCTGCGTCTGAGC
TGCGCGGCCTCCGGATTTACCTTTTCTTCTTCTGGTATGTCTTGGGTGCGCCAAGCCCCT
GGGAAGGGTCTCGAGTGGGTGAGCGTTATCGCTTCTTCTGGTAGCAATACCTATTATGCG
GATAGCGTGAAAGGCCGTTTTACCATTTCACGTGATAATTCGAAAAACACCCTGTATCTG
CAAATGAACAGCCTGCGTGCGGAAGATACGGCCGTGTATTATTGCGCGCGTGGTATTTTT
TTTGCTATTTGGGGCCAAGGCACCCTGGTGACGGTTAGCTCAGCCAGCACAAAGGGCCCT
AGCGTCTTCCCTCTGGCTCCCTGCAGCAGGAGCACCAGCGAGAGCACAGCCGCCCTGGGC
TGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCTCTG
ACCAGCGGCGTGCACACCTTCCCAGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGC
AGCGTGGTGACCGTGCCCTCCAGCAACTTCGGCACCCAGACCTACACCTGCAACGTAGAT
CACAAGCCCAGCAACACCAAGGTGGACAAGACAGTTGAGCGCAAATGTTGTGTCGAGTGC
CCACCGTGCCCAGCACCACCTGTGGCAGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCC
AAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGACGTGAGC
CACGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCC
AAGACAAAGCCACGGGAGGAGCAGTTCAACAGCACGTTCCGTGTGGTCAGCGTCCTCACC
GTTGTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGGC
CTCCCAGCCCCCATCGAGAAAACCATCTCCAAAACCAAAGGGCAGCCCCGAGAACCACAG
GTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGC
CTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCG
GAGAACAACTACAAGACCACACCTCCCATGCTGGACTCCGACGGCTCCTTCTTCCTCTAC
AGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTG
ATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA

TGA

SEQ ID NO:16: Predicted protein sequence of anti-Notch2/3 59R1 IgG2 heavy chain, plus signal sequence. The signal sequence is underlined.
MKHLWFFLLLVAAPRWVLSQVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIA
SSGSNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGIFFAIWGQGTLVTVSSASTKGPSV
FPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQ
TYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP
EVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQ
PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:3: Nucleotide sequence encoding anti-Notch2/3 59R1 light chain, plus signal sequence. The sequence encoding the signal sequence is underlined.
ATGGTGTTGCAGACCCAGGTCTTCATTTCTCTGTTGCTCTGGATCTCTGGTGCCTACGGG
GATATCGTGCTGACCCAGAGCCCGGCGACCCTGAGCCTGTCTCCGGGCGAACGTGCGACC
CTGAGCTGCAGAGCGAGCCAGTCTGTTCGTTCTAATTATCTGGCTTGGTACCAGCAGAAA
CCAGGTCAAGCACCGCGTCTATTAATTTATGGTGCTTCTTCTCGTGCAACTGGGGTCCCG
GCGCGTTTTAGCGGCTCTGGATCCGGCACGGATTTTACCCTGACCATTAGCAGCCTGGAA
CCTGAAGACTTTGCGGTTTATTATTGCCAGCAGTATTCTAATTTTCCTATTACCTTTGGC
CAGGGTACGAAAGTTGAAATTAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCG
CCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTC
TATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCC
CAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTG
ACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAG
GGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG

SEQ ID NO:18: Predicted protein sequence of anti-Notch2/3 59R1 light chain, plus signal sequence. The signal sequence is underlined.
MVLQTQVFISLLLWISGAYGDIVLTQSPATLSLSPGERATLSCRASQSVRSNYLAWYQQKPGQAPRLLIYG
ASSRATGVPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQYSNFPITFGQGTKVEIKRTVAAPSVFIFPPS
DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY
ACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:5: 59R1Heavy chain CDR1
SSSGMS

SEQ ID NO:6: 59R1Heavy chain CDR2
VIASSGSNTYYADSVKG

SEQ ID NO:7: 59R1Heavy chain CDR3
GIFFAI

SEQ ID NO:8: 59R1 Light chain CDR1
RASQSVRSNYLA

SEQ ID NO:9: 59R1 Light chain CDR2
GASSRAT

SEQ ID NO:10: 59R1 Light chain CDR3
QQYSNFPI

SEQ ID NO:11: 59R1 Light chain VL of 59R1 Fab plus signal sequence. The signal sequence is underlined.
MKKTAIAIAVALAGFATVAQADIVLTQSPATLSLSPGERATLSCRASQSVRSNYLAWYQQKPGQAPRLLIY
GASSRATGVPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQYSNFPITFGQGTKVEIKR

SEQ ID NO:12: 59R1 Heavy chain VH of 59R1 Fab plus signal sequence. The signal sequence is underlined.
MKQSTIALALLPLLFTPVTKAQVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSV
IASSGSNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGIFFAIWGQGTLVTVSSA

SEQ ID NO:13: 59R1 Light chain VL of 59R1 IgG antibody
DIVLTQSPATLSLSPGERATLSCRASQSVRSNYLAWYQQKPGQAPRLLIYGASSRATGVPARFSGSGSGTD
FTLTISSLEPEDFAVYYCQQYSNFPITFGQGTKVEIKR

SEQ ID NO:14: 59R1 Heavy chain VH of 59R1 IgG antibody
QVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIASSGSNTYYADSVKGRFTIS
RDNSKNTLYLQMNSLRAEDTAVYYCARGIFFAIWGQGTLVTVSSA

SEQ ID NO:39: 59R1 light chain VL plus mammalian signal sequence (underlined)
MVLQTQVFISLLLWISGAYGDIVLTQSPATLSLSPGERATLSCRASQSVRSNYLAWYQQK
PGQAPRLLIYGASSRATGVPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQYSNFPITFG
QGTKVEIKR

SEQ ID NO:40: 59R1 heavy chain VH plus mammalian signal sequence (underlined)
MKHLWFFLLLVAAPRWVLSQVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAP
GKGLEWVSVIASSGSNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGIF
FAIWGQGTLVTVSSA

SEQ ID NO:15: Nucleotide sequence encoding the heavy chain of anti-Notch2/3 59RGV IgG2 antibody (germlined variant of 59R1), plus signal sequence. The sequence encoding the signal sequence is underlined.
ATGAAGCACCTGTGGTTCTTTCTGCTGCTGGTCGCCGCTCCTAGATGGGTGCTGTCCGAG
GTGCAGCTGGTCGAGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCTCCCTGAGACTGTCC
TGCGCTGCCTCCGGCTTCACCTTCTCCTCCTCCGGCATGTCCTGGGTGCGCCAGGCTCCC
GGCAAGGGCCTGGAGTGGGTGTCCGTGATCGCCTCCAGCGGCTCCAACACCTACTACGCC
GACTCCGTGAAGGGCCGGTTCACCATCTCCCGGGACAACTCCAAGAACACCCTGTACCTG
CAGATGAACTCCCTGCGGGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGGCATCTTC
TTCGCCATCTGGGGCCAGGGCACCCTGGTGACCGTGTCCTCCGCCTCCACCAAGGGCCCT
TCCGTGTTCCCTCTGGCCCCTTGCTCCCGGTCCACCTCCGAGTCCACCGCCGCTCTGGGC
TGCCTGGTGAAGGACTACTTCCCTGAGCCTGTGACAGTGTCCTGGAACTCTGGCGCCCTG
ACCTCCGGCGTGCACACCTTCCCTGCCGTGCTGCAGTCCTCCGGCCTGTACTCCCTGTCC
TCCGTGGTGACAGTGCCTTCCTCCAACTTCGGCACCCAGACCTACACCTGCAACGTGGAC
CACAAGCCTTCCAACACCAAGGTGGACAAGACCGTGGAGCGGAAGTGCTGCGTGGAGTGC
CCTCCTTGCCCTGCCCCTCCTGTGGCTGGCCCTAGCGTGTTCCTGTTCCCTCCTAAGCCT
AAGGACACCCTGATGATCTCCCGGACCCCTGAGGTGACCTGCGTGGTGGTGGACGTGTCC
CACGAGGACCCTGAGGTGCAGTTCAATTGGTACGTGGACGGCGTGGAGGTGCACAACGCC
AAGACCAAGCCTCGGGAGGAACAGTTCAACTCCACCTTCCGGGTGGTGTCCGTGCTGACC
GTGGTGCACCAGGACTGGCTGAACGGCAAGGAATACAAGTGCAAAGTCTCCAACAAGGGC
CTGCCTGCCCCTATCGAGAAAACCATCAGCAAGACCAAGGGCCAGCCTCGCGAGCCTCAG
GTGTACACCCTGCCTCCATCCAGGGAGGAAATGACCAAGAACCAGGTGTCCCTGACCTGT
CTGGTGAAGGGCTTCTACCCTTCCGATATCGCCGTGGAGTGGGAGTCCAACGGCCAGCCT
GAGAACAACTACAAGACCACCCCTCCTATGCTGGACTCCGACGGCTCCTTCTTCCTGTAC

TCCAAGCTGACAGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTG
ATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGTCCCTGAGCCCTGGCAAG
TAG

SEQ ID NO:2: Predicted protein sequence of the heavy chain of anti-Notch2/3 59RGV (germlined variant of 59R1), plus signal sequence. The signal sequence is underlined.
MKHLWFFLLLVAAPRWVLSEVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIA
SSGSNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGIFFAIWGQGTLVTVSSASTKGPSV
FPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQ
TYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP
EVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQ
PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:17: Nucleotide sequence of the anti-Notch2/3 59RGV antibody (germlined variant of 59R1), plus signal sequence. The sequence encoding the signal sequence is underlined.
ATGGTGCTGCAGACCCAGGTGTTCATCTCCCTGCTGCTGTGGATCTCCGGCGCCTACGGC
GAGATCGTGCTGACCCAGTCCCCTGCCACACTGAGCCTGAGCCCTGGCGAGAGAGCCACC
CTGAGCTGCAGGCGGGCCTCCCAGTCCGTGCGGTCCAACTACCTGGCTTGGTATCAGCAG
AAACCCGGACAGGCCCCTCGGCTGCTGATCTACGGCGCCTCCTCCCGGGCTACCGGCATC
CCTGCCCGGTTCTCCGGCTCCGGCAGCGGCACCGACTTCACCCTGACCATCTCCTCCCTG
GAGCCTGAGGACTTCGCCGTGTACTACTGCCAGCAGTACTCCAACTTCCCTATCACCTTC
GGCCAGGGCACCAAGGTGGAGATCAAGCGGACCGTGGCCGCTCCTTCCGTGTTCATCTTC
CCCCCTTCCGACGAGCAGCTGAAGTCCGGCACCGCCTCCGTGGTGTGCCTGCTGAACAAC
TTCTACCCTCGGGAGGCCAAGGTGCAGTGGAAGGTGGACAACGCCCTGCAGTCCGGCAAC
TCCCAGGAATCCGTCACCGAGCAGGACTCCAAGGACAGCACCTACTCCCTGTCCTCCACC
CTGACCCTGTCCAAGGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGAGGTGACCCAC
CAGGGCCTGTCCAGCCCTGTGACCAAGTCCTTCAACCGGGGCGAGTGCTAG

SEQ ID NO:4: Predicted protein sequence of the light chain of anti-Notch2/3 59RGV antibody (germlined variant of 59R1), plus signal sequence. The signal sequence is underlined.
MVLQTQVFISLLLWISGAYGEIVLTQSPATLSLSPGERATLSCRRASQSVRSNYLAWYQQKPGQAPRLLIY
GASSRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQYSNFPITFGQGTKVEIKRTVAAPSVFIFPP
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV
YACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:19: 59R1 Light chain VL of 59RGV antibody (germlined variant of 59R1)
EIVLTQSPATLSLSPGERATLSCRRASQSVRSNYLAWYQQKPGQAPRLLIYGASSRATGIPARFSGSGSGT
DFTLTISSLEPEDFAVYYCQQYSNFPITFGQGTKVEIKR

SEQ ID NO:20: 59R1 Heavy chain VH of 59RGV antibody (germlined variant of 59R1)
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIASSGSNTYYADSVKGRFTIS
RDNSKNTLYLQMNSLRAEDTAVYYCARGIFFAIWGQGTLVTVSSA

SEQ ID NO:22 (alternative heavy chain CDR3)
SIFYPT

SEQ ID NO:23 (alternative heavy chain CDR3)
SSFFAS

SEQ ID NO:24 (alternative heavy chain CDR3)
SSFYAS

SEQ ID NO:25 (alternative heavy chain CDR3)
SSFFAT

SEQ ID NO:26 (alternative heavy chain CDR3)
SIFYPS

SEQ ID NO:27(alternative heavy chain CDR3)
SSFFAN

SEQ ID NO:30 (heavy chain CDR3 consensus sequence):
(G/S) (I/S)F(F/Y) (A/P) (I/T/S/N)

SEQ ID NO:47: 59R5 Light Chain nucleotide sequence (without signal sequence)
GACATCGTGCTGACCCAGTCCCCCGCCACACTGTCCCTGTCTCCCGGCGAGAGAGCCACC
CTGAGCTGTCGGGCCTCCCAGTCCGTGCGGTCCAACTACCTGGCCTGGTATCAGCAGAAG
CCCGGCCAGGCCCCTCGGCTGCTGATCTACGGCGCCTCCTCCAGGGCTACCGGCGTGCCT
GCCCGGTTCTCCGGCTCCGGCTCTGGCACCGACTTCACCCTGACCATCTCCAGCCTGGAG
CCTGAGGACTTCGCCGTGTACTACTGCCAGCAGTACTCCAACTTCCCTATCACCTTCGGC
CAGGGCACCAAGGTGGAGATCAAGCGGACCGTGGCCGCTCCTTCCGTGTTCATCTTCCCC
CCTTCCGACGAGCAGCTGAAGTCCGGCACCGCCTCCGTGGTGTGCCTGCTGAACAACTTC
TACCCTCGGGAGGCCAAGGTGCAGTGGAAGGTGGACAACGCCCTGCAGTCCGGCAACTCC
CAGGAGTCCGTCACCGAGCAGGACTCCAAGGACTCTACCTACTCCCTGTCCTCCACCCTG
ACCCTGAGCAAGGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGAGGTGACCCACCAG
GGCCTGTCCTCTCCCGTGACCAAGTCCTTCAACCGGGGCGAGTGC
SEQ ID NO:48: 59R5 Heavy chain nucleotide sequence (without signal sequence)
GAGGTGCAGCTGGTCGAGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCTCCCTGAGACTG
TCCTGCGCCGCTTCCGGCTTCACCTTCTCCTCCAGCGGCATGTCCTGGGTGCGCCAGGCA
CCTGGCAAAGGACTCGAGTGGGTGTCCGTGATCGCCTCCTCCGGCTCCAACACCTACTAC
GCCGACTCCGTGAAGGGCCGGTTCACCATCTCCCGGGACAACTCCAAGAACACCCTGTAC
CTGCAGATGAACTCCCTGCGGGCCGAGGACACCGCCGTGTACTACTGCGCCCGGTCCATC
TTCTACACCACCTGGGGCCAGGGCACCCTGGTGACCGTGTCCTCCGCCTCCACCAAGGGC
CCCTCCGTGTTCCCTCTGGCCCCTTGCTCCCGGTCCACCTCTGAGTCTACCGCCGCTCTG
GGCTGCCTGGTGAAGGACTACTTCCCTGAGCCTGTGACCGTGTCCTGGAACTCTGGCGCC
CTGACCTCTGGCGTGCACACCTTCCCTGCCGTGCTGCAGTCCTCCGGCCTGTACTCCCTG
TCCTCCGTGGTGACCGTGCCTTCCTCCAACTTCGGCACCCAGACCTACACCTGCAACGTG
GACCACAAGCCTTCCAACACCAAGGTGGACAAGACCGTGGAGCGGAAGTGCTGCGTGGAG
TGCCCTCCTTGTCCTGCTCCTCCTGTGGCTGGCCCTTCTGTGTTCCTGTTCCCTCCTAAG
CCTAAGGACACCCTGATGATCTCCCGGACCCCTGAAGTGACCTGCGTGGTGGTGGACGTG
TCCCACGAGGACCCTGAGGTGCAGTTCAATTGGTACGTGGACGGCGTGGAGGTGCACAAC
GCCAAGACCAAGCCTCGGGAGGAACAGTTCAACTCCACCTTCCGGGTGGTGTCTGTGCTG
ACCGTGGTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGTCCAACAAG
GGCCTGCCTGCCCCTATCGAAAAGACCATCTCTAAGACCAAGGGCCAGCCTCGCGAGCCT
CAGGTCTACACCCTGCCTCCTAGCCGGGAGGAAATGACCAAGAACCAGGTGTCCCTGACC
TGTCTGGTGAAGGGCTTCTACCCTTCCGATATCGCCGTGGAGTGGGAGTCTAACGGCCAG
CCTGAGAACAACTACAAGACCACCCCTCCTATGCTGGACTCCGACGGCTCCTTCTTCCTG
TACTCCAAGCTGACAGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCC
GTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGTCCCTGTCTCCTGGC
AAG
SEQ ID NO:49: 59R5 Heavy chain
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIASSGSNTYY
ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARSIFYTTWGQGTLVTVSSASTKG
PSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPK

PKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVL
TVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPGK

SEQ ID NO:50: 59R5 Heavy chain variable region
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIASSGSNTYY
ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARSIFYTTWGQGTLVTVSSAST

SEQ ID NO:51: 59R5 Heavy chain CDR3
SIFYTT

SEQ ID NO:52: Variant 59R1 Heavy chain variable region
QVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIASSGSNTYYADSVKGRFTIS
RDNSKNTLYLQMNSLRAEDTAVYYCARSIFYPTWGQGTLVTVSSA

SEQ ID NO:53: Variant 59R1 Heavy chain variable region
QVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIASSGSNTYYADSVKGRFTIS
RDNSKNTLYLQMNSLRAEDTAVYYCARSSFFASWGQGTLVTVSSA

SEQ ID NO:54: Variant 59R1 Heavy chain variable region
QVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIASSGSNTYYADSVKGRFTIS
RDNSKNTLYLQMNSLRAEDTAVYYCARSSFYASWGQGTLVTVSSA

SEQ ID NO:55: Variant 59R1 Heavy chain variable region
QVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIASSGSNTYYADSVKGRFTIS
RDNSKNTLYLQMNSLRAEDTAVYYCARSSFFATWGQGTLVTVSSA

SEQ ID NO:56: Variant 59R1 Heavy chain variable region
QVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIASSGSNTYYADSVKGRFTIS
RDNSKNTLYLQMNSLRAEDTAVYYCARSIFYPSWGQGTLVTVSSA

SEQ ID NO:57: Variant 59R1 Heavy chain variable region
QVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIASSGSNTYYADSVKGRFTIS
RDNSKNTLYLQMNSLRAEDTAVYYCARSSFFANWGQGTLVTVSSA
SEQ ID NO:58: 59R5 Heavy chain variable region nucleotide sequence (without signal sequence)
GAGGTGCAGCTGGTCGAGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCTCCCTGAGACTG
TCCTGCGCCGCTTCCGGCTTCACCTTCTCCTCCAGCGGCATGTCCTGGGTGCGCCAGGCA
CCTGGCAAAGGACTCGAGTGGGTGTCCGTGATCGCCTCCTCCGGCTCCAACACCTACTAC
GCCGACTCCGTGAAGGGCCGGTTCACCATCTCCCGGGACAACTCCAAGAACACCCTGTAC
CTGCAGATGAACTCCCTGCGGGCCGAGGACACCGCCGTGTACTACTGCGCCCGGTCCATC
TTCTACACCACCTGGGGCCAGGGCACCCTGGTGACCGTGTCCTCCGCCTCCACC

SEQ ID NO:59: 59R1 Light chain variable region nucleotide sequence (without signal sequence)
GATATCGTGCTGACCCAGAGCCCGGCGACCCTGAGCCTGTCTCCGGGCGAACGTGCGACC
CTGAGCTGCAGAGCGAGCCAGTCTGTTCGTTCTAATTATCTGGCTTGGTACCAGCAGAAA
CCAGGTCAAGCACCGCGTCTATTAATTTATGGTGCTTCTTCTCGTGCAACTGGGGTCCCG
GCGCGTTTTAGCGGCTCTGGATCCGGCACGGATTTTACCCTGACCATTAGCAGCCTGGAA
CCTGAAGACTTTGCGGTTTATTATTGCCAGCAGTATTCTAATTTTCCTATTACCTTTGGC
CAGGGTACGAAAGTTGAAATTAAACGT

SEQ ID NO:60: 59R1 Heavy chain variable region nucleotide sequence (without signal sequence)
CAGGTGCAATTGGTGGAAAGCGGCGGCGGCCTGGTGCAACCGGGCGGCAGCCTGCGTCTG

AGCTGCGCGGCCTCCGGATTTACCTTTTCTTCTTCTGGTATGTCTTGGGTGCGCCAAGCC
CCTGGGAAGGGTCTCGAGTGGGTGAGCGTTATCGCTTCTTCTGGTAGCAATACCTATTAT
GCGGATAGCGTGAAAGGCCGTTTTACCATTTCACGTGATAATTCGAAAAACACCCTGTAT
CTGCAAATGAACAGCCTGCGTGCGGAAGATACGGCCGTGTATTATTGCGCGCGTGGTATT
TTTTTTGCTATTTGGGGCCAAGGCACCCTGGTGACGGTTAGCTCAGCC

Human Notch-related sequences:

SEQ ID NO:21: Notch2(EGF1-12) Fc fusion protein amino acid sequence
MPALRPALLWALLALWLCCAAPAHALQCRDGYEPCVNEGMCVTYHNGTGYCKCPEGFLGEYCQHRDPCEKN
RCQNGGTCVAQAMLGKATCRCASGFTGEDCQYSTSHPCFVSRPCLNGGTCHMLSRDTYECTCQVGFTGKEC
QWTDACLSHPCANGSTCTTVANQFSCKCLTGFTGQKCETDVNECDIPGHCQHGGTCLNLPGSYQCQCPQGF
TGQYCDSLYVPCAPSPCVNGGTCRQTGDFTFECNCLPGFEGSTCERNIDDCPNHRCQNGGVCVDGVNTYNC
RCPPQWTGQFCTEDVDECLLQPNACQNGGTCANRNGGYGCVCVNGWSGDDCSENIDDCAFASCTPGSTCID
RVASFSCMCPEGKAGLLCHLDDACISNPCHKGALCDTNPLNGQYICTCPQGYKGADCTEDVDECAMANSNP
CEHAGKCVNTDGAFHCECLKGYAGPRCEMDINECHSDPCQNDATCLDKIGGFTCLCMPGFKGVHCELGRAD
KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR
EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV
SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN
HYTQKSLSLSPGK

SEQ ID NO:28 (potential component of 59R1 binding site within EGF10 of human Notch2): HKGAL

SEQ ID NO:29 (site within human Notch3 EGF9 that corresponds to the potential component of 59R1 binding site within human Notch2 EGF10): HEDAI

SEQ ID NO:45: hNotch1
Amino Acids 1-1732 Extracellular domain. (underlined)
Amino Acids 372-414 EGF repeat 10 (double underlined and italicized)
MPPLLAPLLCLALLPALAARGPRCSQPGETCLNGGKCEAANGTEACVCGGAFVGPRCQDPNPCLSTPCKNA
GTCHVVDRRGVADYACSCALGFSGPLCLTPLDNACLTNPCRNGGTCDLLTLTEYKCRCPPGWSGKSCQQAD
PCASNPCANGGQCLPFEASYICHCPPSFHGPTCRQDVNECGQKPGLCRHGGTCHNEVGSYRCVCRATHTGP
NCERPYVPCSPSPCQNGGTCRPTGDVTHECACLPGFTGQNCEENIDDCPGNNCKNGGACVDGVNTYNCRCP
PEWTGQYCTEDVDECQLMPNACQNGGTCHNTHGGYNCVCVNGWTGEDCSENIDDCASAACFHGATCHDRVA
SFYCECPHGRTGLLCH*LNDACISNPCNEGSNCDTNPVNGKAICTCPSGYTGPACSQDVD*ECSLGANPCEHA
GKCINTLGSFECQCLQGYTGPRCEIDVNECVSNPCQNDATCLDQIGEFQCICMPGYEGVHCEVNTDECASS
PCLHNGRCLDKINEFQCECPTGFTGHLCQYDVDECASTPCKNGAKCLDGPNTYTCVCTEGYTGTHCEVDID
ECDPDPCHYGSCKDGVATFTCLCRPGYTGHHCETNINECSSQPCRHGGTCQDRDNAYLCFCLKGTTGPNCE
INLDDCASSPCDSGTCLDKIDGYECACEPGYTGSMCNINIDECAGNPCHNGGTCEDGINGFTCRCPEGYHD
PTCLSEVNECNSNPCVHGACRDSLNGYKCDCDPGWSGTNCDINNNECESNPCVNGGTCKDMTSGYVCTCRE
GFSGPNCQTNINECASNPCLNQGTCIDDVAGYKCNCLLPYTGATCEVVLAPCAPSPCRNGGECRQSEDYES
FSCVCPTGWQGQTCEVDINECVLSPCRHGASCQNTHGGYRCHCQAGYSGRNCETDIDDCRPNPCHNGGSCT
DGINTAFCDCLPGFRGTFCEEDINECASDPCRNGANCTDCVDSYTCTCPAGFSGIHCENNTPDCTESSCFN
GGTCVDGINSFTCLCPPGFTGSYCQHDVNECDSQPCLHGGTCQDGCGSYRCTCPQGYTGPNCQNLVHWCDS
SPCKNGGKCWQTHTQYRCECPSGWTGLYCDVPSVSCEVAAQRQGVDVARLCQHGGLCVDAGNTHHCRCQAG
YTGSYCEDLVDECSPSPCQNGATCTDYLGGYSCKCVAGYHGVNCSEEIDECLSHPCQNGGTCLDLPNTYKC
SCPRGTQGVHCEINVDDCNPPVDPVSRSPKCFNNGTCVDQVGGYSCTCPPGFVGERCEGDVNECLSNPCDA
RGTQNCVQRVNDFHCECRAGHTGRRCESVINGCKGKPCKNGGTCAVASNTARGFICKCPAGFEGATCENDA
RTCGSLRCLNGGTCISGPRSPTCLCLGPFTGPECQFPASSPCLGGNPCYNQGTCEPTSESPFYRCLCPAKF
NGLLCHILDYSFGGGAGRDIPPPLIEEACELPECQEDAGNKVCSLQCNNHACGWDGGDCSLNFNDPWKNCT
QSLQCWKYFSDGHCDSQCNSAGCLFDGFDCQRAEGQCNPLYDQYCKDHFSDGHCDQGCNSAECEWDGLDCA
EHVPERLAAGTLVVVVLMPPEQLRNSSFHFLRELSRVLHTNVVFKRDAHGQQMIFPYYGREEELRKHPIKR
AAEGWAAPDALLGQVKASLLPGGSEGGRRRRELDPMDVRGSIVYLEIDNRQCVQASSQCFQSATDVAAFLG

ALASLGSLNIPYKIEAVQSETVEPPPPAQLHFMYVAAAAFVLLFFVGCGVLLSRKRRRQHGQLWFPEGFKV
SEASKKKRREPLGEDSVGLKPLKNASDGALMDDNQNEWGDEDLETKKFRFEEPVVLPDLDDQTDHRQWTQQ
HLDAADLRMSAMAPTPPQGEVDADCMDVNVRGPDGFTPLMIASCSGGGLETGNSEEEEDAPAVISDFIYQG
ASLHNQTDRTGETALHLAARYSRSDAAKRLLEASADANIQDNMGRTPLHAAVSADAQGVFQILIRNRATDL
DARMHDGTTPLILAARLAVEGMLEDLINSHADVNAVDDLGKSALHWAAAVNNVDAAVVLLKNGANKDMQNN
REETPLFLAAREGSYETAKVLLDHFANRDITDHMDRLPRDIAQERMHHDIVRLLDEYNLVRSPQLHGAPLG
GTPTLSPPLCSPNGYLGSLKPGVQGKKVRKPSSKGLACGSKEAKDLKARRKKSQDGKGCLLDSSGMLSPVD
SLESPHGYLSDVASPPLLPSPFQQSPSVPLNHLPGMPDTHLGIGHLNVAAKPEMAALGGGGRLAFETGPPR
LSHLPVASGTSTVLGSSSGGALNFTVGGSTSLNGQCEWLSRLQSGMVPNQYNPLRGSVAPGPLSTQAPSLQ
HGMVGPLHSSLAASALSQMMSYQGLPSTRLATQPHLVQTQQVQPQNLQMQQQNLQPANIQQQQSLQPPPPP
PQPHLGVSSAASGHLGRSFLSGEPSQADVQPLGPSSLAVHTILPQESPALPTSLPSSLVPPVTAAQFLTPP
SQHSYSSPVDNTPSHQLQVPEHPFLTPSPESPDQWSSSSPHSNVSDWSEGVSSPPTSMQSQIARIPEAFK

SEQ ID NO:31: human Notch2
Amino Acids 1-1677: Extracellular domain (underlined)
Amino Acids 375-417: EGF repeat 10 (double underlined and italicized)
MPALRPALLWALLALWLCCAAPAHALQCRDGYEPCVNEGMCVTYHNGTGYCKCPEGFLGEYCQHRDPCEKN
RCQNGGTCVAQAMLGKATCRCASGFTGEDCQYSTSHPCFVSRPCLNGGTCHMLSRDTYECTCQVGFTGKEC
QWTDACLSHPCANGSTCTTVANQFSCKCLTGFTGQKCETDVNECDIPGHCQHGGTCLNLPGSYQCQCPQGF
TGQYCDSLYVPCAPSPCVNGGTCRQTGDFTFECNCLPGFEGSTCERNIDDCPNHRCQNGGVCVDGVNTYNC
RCPPQWTGQFCTEDVDECLLQPNACQNGGTCANRNGGYGCVCVNGWSGDDCSENIDDCAFASCTPGSTCID
RVASFSCMCPEGKAGLLCH*LDDACISNPCHKGALCDTNPLNGQYICTCPQGYKGADCTEDVD*ECAMANSNP
CEHAGKCVNTDGAFHCECLKGYAGPRCEMDINECHSDPCQNDATCLDKIGGFTCLCMPGFKGVHCELEINE
CQSNPCVNNGQCVDKVNRFQCLCPPGFTGPVCQIDIDDCSSTPCLNGAKCIDHPNGYECQCATGFTGVLCE
ENIDNCDPDPCHHGQCQDGIDSYTCICNPGYMGAICSDQIDECYSSPCLNDGRCIDLVNGYQCNCQPGTSG
VNCEINFDDCASNPCIHGICMDGINRYSCVCSPGFTGQRCNIDIDECASNPCRKGATCINGVNGFRCICPE
GPHHPSCYSQVNECLSNPCIHGNCTGGLSGYKCLCDAGWVGINCEVDKNECLSNPCQNGGTCDNLVNGYRC
TCKKGFKGYNCQVNIDECASNPCLNQGTCFDDISGYTCHCVLPYTGKNCQTVLAPCSPNPCENAAVCKESP
NFESYTCLCAPGWQGQRCTIDIDECISKPCMNHGLCHNTQGSYMCECPPGFSGMDCEEDIDDCLANPCQNG
GSCMDGVNTFSCLCLPGFTGDKCQTDMNECLSEPCKNGGTCSDYVNSYTCKCQAGFDGVHCENNINECTES
SCFNGGTCVDGINSFSCLCPVGFTGSFCLHEINECSSHPCLNEGTCVDGLGTYRCSCPLGYTGKNCQTLVN
LCSRSPCKNKGTCVQKKAESQCLCPSGWAGAYCDVPNVSCDIAASRRGVLVEHLCQHSGVCINAGNTHYCQ
CPLGYTGSYCEEQLDECASNPCQHGATCSDFIGGYRCECVPGYQGVNCEYEVDECQNQPCQNGGTCIDLVN
HFKCSCPPGTRGLLCEENIDDCARGPHCLNGGQCMDRIGGYSCRCLPGFAGERCEGDINECLSNPCSSEGS
LDCIQLTNDYLCVCRSAFTGRHCETFVDVCPQMPCLNGGTCAVASNMPDGFICRCPPGFSGARCQSSCGQV
KCRKGEQCVHTASGPRCFCPSPRDCESGCASSPCQHGGSCHPQRQPPYYSCQCAPPFSGSRCELYTAPPST
PPATCLSQYCADKARDGVCDEACNSHACQWDGGDCSLTMENPWANCSSPLPCWDYINNQCDELCNTVECLF
DNFECQGNSKTCKYDKYCADHFKDNHCDQGCNSEECGWDGLDCAADQPENLAEGTLVIVVLMPPEQLLQDA
RSFLRALGTLLHTNLRIKRDSQGELMVYPYYGEKSAAMKKQRMTRRSLPGEQEQEVAGSKVFLEIDNRQCV
QDSDHCFKNTDAAAALLASHAIQGTLSYPLVSVVSESLTPERTQLLYLLAVAVVIILFIILLGVIMAKRKR
KHGSLWLPEGFTLRRDASNHKRREPVGQDAVGLKNLSVQVSEANLIGTGTSEHWVDDEGPQPKKVKAEDEA
LLSEEDDPIDRRPWTQQHLEAADIRRTPSLALTPPQAEQEVDVLDVNVRGPDGCTPLMLASLRGGSSDLSD
EDEDAEDSSANIITDLVYQGASLQAQTDRTGEMALHLAARYSRADAAKRLLDAGADANAQDNMGRCPLHAA
VAADAQGVFQILIRNRVTDLDARMNDGTTPLILAARLAVEGMVAELINCQADVNAVDDHGKSALHWAAAVN
NVEATLLLLKNGANRDMQDNKEETPLFLAAREGSYEAAKILLDHFANRDITDHMDRLPRDVARDRMHHDIV
RLLDEYNVTPSPPGTVLTSALSPVICGPNRSFLSLKHTPMGKKSRRPSAKSTMPTSLPNLAKEAKDAKGSR
RKKSLSEKVQLSESSVTLSPVDSLESPHTYVSDTTSSPMITSPGILQASPNPMLATAAPPAPVHAQHALSF
SNLHEMQPLAHGASTVLPSVSQLLSHHHIVSPGSGSAGSLSRLHPVPVPADWMNRMEVNETQYNEMFGMVL
APAEGTHPGIAPQSRPPEGKHITTPREPLPPIVTFQLIPKGSIAQPAGAPQPQSTCPPAVAGPLPTMYQIP
EMARLPSVAFPTAMMPQQDGQVAQTILPAYHPFPASVGKYPTPPSQHSYASSNAAERTPSHSGHLQGEHPY
LTPSPESPDQWSSSSPHSASDWSDVTTSPTPGGAGGGQRGPGTHMSEPPHNNMQVYA

SEQ ID NO:32: human Notch3

Amino Acids 1-1640: Extracellular domain (underlined)
Amino Acids 351-393: EGF repeat 9 (double underlined and italicized)

MGPGARGRRRRRRPMSPPPPPPPVRALPLLLLLAGPGAAAPPCLDGSPCANGGRCTQLPSREAACLCPPGW
VGERCQLEDPCHSGPCAGRGVCQSSVVAGTARFSCRCPRGFRGPDCSLPDPCLSSPCAHGARCSVGPDGRF
LCSCPPGYQGRSCRSDVDECRVGEPCRHGGTCLNTPGSFRCQCPAGYTGPLCENPAVPCAPSPCRNGGTCR
QSGDLTYDCACLPGFEGQNCEVNVDDCPGHRCLNGGTCVDGVNTYNCQCPPEWTGQFCTEDVDECQLQPNA
CHNGGTCFNTLGGHSCVCVNGWTGESCSQNIDDCATAVCFHGATCHDRVASFYCACPMGKTGLLCH*LDDAC*
*VSNPCHEDAICDTNPVNGRAICTCPPGFTGGACDQDVD*ECSIGANPCEHLGRCVNTQGSFLCQCGRGYTGP
RCETDVNECLSGPCRNQATCLDRIGQFTCICMAGFTGTYCEVDIDECQSSPCVNGGVCKDRVNGFSCTCPS
GFSGSTCQLDVDECASTPCRNGAKCVDQPDGYECRCAEGFEGTLCDRNVDDCSPDPCHHGRCVDGIASFSC
ACAPGYTGTRCESQVDECRSQPCRHGGKCLDLVDKYLCRCPSGTTGVNCEVNIDDCASNPCTFGVCRDGIN
RYDCVCQPGFTGPLCNVEINECASSPCGEGGSCVDGENGFRCLCPPGSLPPLCLPPSHPCAHEPCSHGICY
DAPGGFRCVCEPGWSGPRCSQSLARDACESQPCRAGGTCSSDGMGFHCTCPPGVQGRQCELLSPCTPNPCE
HGGRCESAPGQLPVCSCPQGWQGPRCQQDVDECAGPAPCGPHGICTNLAGSFSCTCHGGYTGPSCDQDIND
CDPNPCLNGGSCQDGVGSFSCSCLPGFAGPRCARDVDECLSNPCGPGTCTDHVASFTCTCPPGYGGFHCEQ
DLPDCSPSSCFNGGTCVDGVNSFSCLCRPGYTGAHCQHEADPCLSRPCLHGGVCSAAHPGFRCTCLESFTG
PQCQTLVDWCSRQPCQNGGRCVQTGAYCLCPPGWSGRLCDIRSLPCREAAAQIGVRLEQLCQAGGQCVDED
SSHYCVCPEGRTGSHCEQEVDPCLAQPCQHGGTCRGYMGGYMCECLPGYNGDNCEDDVDECASQPCQHGGS
CIDLVARYLCSCPPGTLGVLCEINEDDCGPGPPLDSGPRCLHNGTCVDLVGGFRCTCPPGYTGLRCEADIN
ECRSGACHAAHTRDCLQDPGGGFRCLCHAGFSGPRCQTVLSPCESQPCQHGGQCRPSPGPGGGLTFTCHCA
QPFWGPRCERVARSCRELQCPVGVPCQQTPRGPRCACPPGLSGPSCRSFPGSPPGASNASCAAAPCLHGGS
CRPAPLAPFFRCACAQGWTGPRCEAPAAAPEVSEEPRCPRAACQAKRGDQRCDRECNSPGCGWDGGDCSLS
VGDPWRQCEALQCWRLFNNSRCDPACSSPACLYDNFDCHAGGRERTCNPVYEKYCADHFADGRCDQGCNTE
ECGWDGLDCASEVPALLARGVLVLTVLLPPEELLRSSADFLQRLSAILRTSLRFRLDAHGQAMVFPYHRPS
PGSEPRARRELAPEVIGSVVMLEIDNRLCLQSPENDHCFPDAQSAADYLGALSAVERLDFPYPLRDVRGEP
LEPPEPSVPLLPLLVAGAVLLLVILVLGVMVARRKREHSTLWFPEGFSLHKDVASGHKGRREPVGQDALGM
KNMAKGESLMGEVATDWMDTECPEAKRLKVEEPGMGAEEAVDCRQWTQHHLVAADIRVAPAMALTPPQGDA
DADGMDVNVRGPDGFTPLMLASFCGGALEPMPTEEDEADDTSASIISDLICQGAQLGARTDRTGETALHLA
ARYARADAAKRLLDAGADTNAQDHSGRTPLHTAVTADAQGVFQILIRNRSTDLDARMADGSTALILAARLA
VEGMVEELIASHADVNAVDELGKSALHWAAAVNNVEATLALLKNGANKDMQDSKEETPLFLAAREGSYEAA
KLLLDHFANREITDHLDRLPRDVAQERLHQDIVRLLDQPSGPRSPPGPHGLGPLLCPPGAFLPGLKAAQSG
SKKSRRPPGKAGLGPQGPRGRGKKLTLACPGPLADSSVTLSPVDSLDSPRPFGGPPASPGGFPLEGPYAAA
TATAVSLAQLGGPGRAGLGRQPPGGCVLSLGLLNPVAVPLDWARLPPPAPPGPSFLLPLAPGPQLLNPGTP
VSPQERPPPYLAVPGHGEEYPVAGAHSSPPKARFLRVPSEHPYLTPSPESPEHWASPSPPSLSDWSESTPS
PATATGAMATTTGALPAQPLPLSVPSSLAQAQTQLGPQPEVTPKRQVLA

SEQ ID NO:46: hNotch4
Amino Acids 1-1444 Extracellular domain (underlined)
Amino Acids 392-434 EGF repeat 10 (double underlined and italicized)

MQPPSLLLLLLLLLLLCVSVVRPRGLLCGSFPEPCANGGTCLSLSLGQGTCQCAPGFLGETCQFPDPCQNA
QLCQNGGSCQALLPAPLGLPSSPSPLTPSFLCTCLPGFTGERCQAKLEDPCPPSFCSKRGRCHIQASGRPQ
CSCMPGWTGEQCQLRDFCSANPCVNGGVCLATYPQIQCHCPPGFEGHACERDVNECFQDPGPCPKGTSCHN
TLGSFQCLCPVGQEGPRCELRAGPCPPRGCSNGGTCQLMPEKDSTFHLCLCPPGFIGPDCEVNPDNCVSHQ
CQNGGTCQDGLDTYTCLCPETWTGWDCSEDVDECETQGPPHCRNGGTCQNSAGSFHCVCVSGWGGTSCEEN
LDDCIAATCAPGSTCIDRVGSFSCLCPPGRTGLLCH*LEDMCLSQPCHGDAQCSTNPLTGSTLCLCQPGYSG*
*PTCHQDLD*ECLMAQQGPSPCEHGGSCLNTPGSFNCLCPPGYTGSRCEADHNECLSQPCHPGSTCLDLLATF
HCLCPPGLEGQLCEVETNECASAPCLNHADCHDLLNGFQCICLPGFSGTRCEEDIDECRSSPCANGGQCQD
QPGAFHCKCLPGFEGPRCQTEVDECLSDPCPVGASCLDLPGAFFCLCPSGFTGQLCEVPLCAPNLCQPKQI
CKDQKDKANCLCPDGSPGCAPPEDNCTCHHGHCQRSSCVCDVGWTGPECEAELGGCISAPCAHGGTCYPQP
SGYNCTCPTGYTGPTCSEEMTACHSGPCLNGGSCNPSPGGYYCTCPPSHTGPQCQTSTDYCVSAPCFNGGT
CVNRPGTFSCLCAMGFQGPRCEGKLRPSCADSPCRNRATCQDSPQGPRCLCPTGYTGGSCQTLMDLCAQKP
CPRNSHCLQTGPSFHCLCLQGWTGPLCNLPLSSCQKAALSQGIDVSSLCHNGGLCVDSGPSYFCHCPPGFQ
GSLCQDHVNPCESRPCQNGATCMAQPSGYLCQCAPGYDGQNCSKELDACQSQPCHNHGTCTPKPGGFHCAC

PPGFVGLRCEGDVDECLDQPCHPTGTAACHSLANAFYCQCLPGHTGQWCEVEIDPCHSQPCFHGGTCEATA
GSPLGFICHCPKGFEGPTCSHRAPSCGFHHCHHGGLCLPSPKPGFPPRCACLSGYGGPDCLTPPAPKGCGP
PSPCLYNGSCSETTGLGGPGFRCSCPHSSPGPRCQKPGAKGCEGRSGDGACDAGCSGPGGNWDGGDCSLGV
PDPWKGCPSHSRCWLLFRDGQCHPQCDSEECLFDGYDCETPPACTPAYDQYCHDHFHNGHCEKGCNTAECG
WDGGDCRPEDGDPEWGPSLALLVVLSPPALDQQLFALARVLSLTLRVGLWVRKDRDGRDMVYPYPGARAEE
KLGGTRDPTYQERAAPQTQPLGKETDSLSAGFVVVMGVDLSRCGPDHPASRCPWDPGLLLRFLAAMAAVGA
LEPLLPGPLLAVHPHAGTAPPANQLPWPVLCSPVAGVILLALGALLVLQLIRRRRREHGALWLPPGFTRRP
RTQSAPHRRRPPLGEDSIGLKALKPKAEVDEDGVVMCSGPEEGEEVGQAEETGPPSTCQLWSLSGGCGALP
QAAMLTPPQESEMEAPDLDTRGPDGVTPLMSAVCCGEVQSGTFQGAWLGCPEPWEPLLDGGACPQAHTVGT
GETPLHLAARFSRPTAARRLLEAGANPNQPDRAGRTPLHAAVAADAREVCQLLLRSRQTAVDARTEDGTTP
LMLAARLAVEDLVEELIAAQADVGARDKWGKTALHWAAAVNNARAARSLLQAGADKDAQDNREQTPLFLAA
REGAVEVAQLLLGLGAARELRDQAGLAPADVAHQRNHWDLLTLLEGAGPPEARHKATPGREAGPFPRARTV
SVSVPPHGGGALPRCRTLSAGAGPRGGGACLQARTWSVDLAARGGGAYSHCRSLSGVGAGGGPTPRGRRFS
AGMRGPRPNPAIMRGRYGVAAGRGGRVSTDDWPCDWVALGACGSASNIPIPPPCLTPSPERGSPQLDCGPP
ALQEMPINQGGEGKK

SEQ ID NO:33: Polynucleotide sequence encoding human Notch2 EGF 1-12
ATGCCCGCCCTGCGCCCCGCTCTGCTGTGGGCGCTGCTGGCGCTCTGGCTGTGCTGCGCG
GCCCCCGCGCATGCATTGCAGTGTCGAGATGGCTATGAACCCTGTGTAAATGAAGGAATG
TGTGTTACCTACCACAATGGCACAGGATACTGCAAATGTCCAGAAGGCTTCTTGGGGGAA
TATTGTCAACATCGAGACCCCTGTGAGAAGAACCGCTGCCAGAATGGTGGGACTTGTGTG
GCCCAGGCCATGCTGGGGAAAGCCACGTGCCGATGTGCCTCAGGGTTTACAGGAGAGGAC
TGCCAGTACTCAACATCTCATCCATGCTTTGTGTCTCGACCCTGCCTGAATGGCGGCACA
TGCCATATGCTCAGCCGGGATACCTATGAGTGCACCTGTCAAGTCGGGTTTACAGGTAAG
GAGTGCCAATGGACGGATGCCTGCCTGTCTCATCCCTGTGCAAATGGAAGTACCTGTACC
ACTGTGGCCAACCAGTTCTCCTGCAAATGCCTCACAGGCTTCACAGGGCAGAAATGTGAG
ACTGATGTCAATGAGTGTGACATTCCAGGACACTGCCAGCATGGTGGCACCTGCCTCAAC
CTGCCTGGTTCCTACCAGTGCCAGTGCCCTCAGGGCTTCACAGGCCAGTACTGTGACAGC
CTGTATGTGCCCTGTGCACCCTCACCTTGTGTCAATGGAGGCACCTGTCGGCAGACTGGT
GACTTCACTTTTGAGTGCAACTGCCTTCCAGGTTTTGAAGGGAGCACCTGTGAGAGGAAT
ATTGATGACTGCCCTAACCACAGGTGTCAGAATGGAGGGGTTTGTGTGGATGGGGTCAAC
ACTTACAACTGCCGCTGTCCCCCACAATGGACAGGACAGTTCTGCACAGAGGATGTGGAT
GAATGCCTGCTGCAGCCCAATGCCTGTCAAAATGGGGGCACCTGTGCCAACCGCAATGGA
GGCTATGGCTGTGTATGTGTCAACGGCTGGAGTGGAGATGACTGCAGTGAGAACATTGAT
GATTGTGCCTTCGCCTCCTGTACTCCAGGCTCCACCTGCATCGACCGTGTGGCCTCCTTC
TCTTGCATGTGCCCAGAGGGGAAGGCAGGTCTCCTGTGTCATCTGGATGATGCATGCATC
AGCAATCCTTGCCACAAGGGGGCACTGTGTGACACCAACCCCCTAAATGGGCAATATATT
TGCACCTGCCCACAAGGCTACAAAGGGGCTGACTGCACAGAAGATGTGGATGAATGTGCC
ATGGCCAATAGCAATCCTTGTGAGCATGCAGGAAAATGTGTGAACACGGATGGCGCCTTC
CACTGTGAGTGTCTGAAGGGTTATGCAGGACCTCGTTGTGAGATGGACATCAATGAGTGC
CATTCAGACCCCTGCCAGAATGATGCTACCTGTCTGGATAAGATTGGAGGCTTCACATGT
CTGTGCATGCCAGGTTTCAAAGGTGTGCATTGTGAATTA

SEQ ID NO:34: Human Notch2 EGF 1-12 polypeptide sequence
MPALRPALLWALLALWLCCAAPAHALQCRDGYEPCVNEGMCVTYHNGTGYCKCPEGFLGE
YCQHRDPCEKNRCQNGGTCVAQAMLGKATCRCASGFTGEDCQYSTSHPCFVSRPCLNGGT
CHMLSRDTYECTCQVGFTGKECQWTDACLSHPCANGSTCTTVANQFSCKCLTGFTGQKCE
TDVNECDIPGHCQHGGTCLNLPGSYQCQCPQGFTGQYCDSLYVPCAPSPCVNGGTCRQTG
DFTFECNCLPGFEGSTCERNIDDCPNHRCQNGGVCVDGVNTYNCRCPPQWTGQFCTEDVD
ECLLQPNACQNGGTCANRNGGYGCVCVNGWSGDDCSENIDDCAFASCTPGSTCIDRVASF
SCMCPEGKAGLLCHLDDACISNPCHKGALCDTNPLNGQYICTCPQGYKGADCTEDVDECA
MANSNPCEHAGKCVNTDGAFHCECLKGYAGPRCEMDINECHSDPCQNDATCLDKIGGFTC
LCMPGFKGVHCEL

SEQ ID NO:35: Human Notch1 EGF10
LNDACISNPCNEGSNCDTNPVNGKAICTCPSGYTGPACSQDVD

SEQ ID NO:36: Human Notch2 EGF10
LDDACISNPCHKGALCDTNPLNGQYICTCPQGYKGADCTEDVD

SEQ ID NO:37: Human Notch3 EGF9 (EGF9 is the EGF of human Notch3 that corresponds to EGF10 of the other Notch receptors including Notch2)
LDDACVSNPCHEDAICDTNPVNGRAICTCPPGFTGGACDQDVD

SEQ ID NO:38: Human Notch4 EGF10
LEDMCLSQPCHGDAQCSTNPLTGSTLCLCQPGYSGPTCHQDLD

SEQ ID NO:41: Notch1 EGF repeat 4
QADPCASNPCANGGQCLPFEASYICHCPPSFHGPTCRQ

SEQ ID NO:42: Notch2 EGF repeat 4
TDACLSHPCANGSTCTTVANQFSCKCLTGFTGQKCET

SEQ ID NO:43: Notch3 EGF repeat 4
SDVDECRVGEPCRHGGTCLNTPGSFRCQCPAGYTGPLCEN

SEQ ID NO:44: Notch4 EGF repeat 4
RDFCSANPCVNGGVCLATYPQIQCHCPPGFEGHACER

Applicant's or agent's file reference	International application No.
2293.049PC03	PCT/US2009/003994

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 8, 9, 36, line ____.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution

American Type Culture Collection

Address of depositary institution *(including postal code and country)*

10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
July 6, 2009	PTA-10170

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

Plasmid isolated from E.coli containing human DNA insert 59R5

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☐ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer TYETTA STATON	Authorized officer

Form PCT/RO/134 (July1998; reprint January 2004)

AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

CANADA

The applicant hereby requests that, until either a Canadian patent has been issued on the basis of the application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the furnishing of a sample of deposited biological material referred to in the application only be effected to an independent expert nominated by the Commissioner of Patents.

CROATIA

The applicant hereby requests that a sample of deposited biological material referred to in the application should be made available between the publication of the application and the granting of the patent to an independent expert only. Samples shall be made available only if the person requesting them undertakes, for the term during which the patent is in force, not to make them or any material derived from them available to third parties, and not to use them or any material derived from them except for experimental or research purposes, unless the applicant for or owner of the patent, as applicable, expressly waives such undertaking.

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. Any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent office or any person approved by the applicant in the individual case.

FINLAND

The applicant hereby requests that, until the publication of the mention of the grant of a patent by the National Board of Patents and Registration of Finland or for 20 years from the date of filing if the application has been finally decided upon without resulting in the grant of a patent by the National Board of Patents and Registration of Finland, the furnishing of a sample shall only be effected to an expert in the art. Any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the National Board of Patents and Registration of Finland or any person approved by the applicant in the individual case.

GERMANY

The applicant herby requests that, until the grant of a patent or for 20 years from the date of filing of the application is refused or withdrawn, a sample shall only be issued to an independent expert nominated by the applicant.

ICELAND

The applicant hereby requests that, until a patent has been granted or a final decision taken by the Icelandic Patent Office concerning an application which has not resulted in a patent, the furnishing of a sample shall only be effected to an expert in the art. Any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Icelandic Patent office or any person approved by the applicant in the individual case.

NORWAY

The applicant hereby requests that, until the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. Any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Norwegian Patent office or any person approved by the applicant in the individual case.

SINGAPORE

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert.

SPAIN

The applicant hereby requests that, until the publication of the mention of the grant of a Spanish patent or for 20 years from the date of filing if the application is refused or withdrawn, the biological material shall be made available as provided in Article 45 SPL only by the issue of a sample to an independent expert.

SWEDEN

The applicant hereby requests that, until the patent has been granted by the Swedish Patent and Registration Office or if the application has been finally decided upon without resulting in the grant of the patent, the furnishing of a sample shall only be effected to an expert in the art. The same is applied to rejected or withdrawn applications within a period of 20 years from the filing date.

SWITZERLAND

The applicant hereby requests that, the furnishing of samples to a third party may be subject to the condition that that party indicates to the depository institution its name and address for the purpose of information of the depositor and undertakes: (a) not to make available the deposited culture or a culture derived from it to a third party; (b) not to use the culture outside the purview of the law; (c) to produce, in case of a dispute, evidence that the obligations under items (a) and (b) have not been violated.

THE FORMER YUGOSLAV REPUBLIC OF MACEDONIA

The applicant hereby requests that, the furnishing of samples to a third party may be subject to the condition that that party: (a) has a right to demand that a sample of the viable biological or microbiological material be made available; (b) has undertaken to ensure that the applicant does not authorize access to the sample of the deposited viable biological or microbiological material to any third party before the expiry of the prescribed period of validity of the patent.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert.

EUROPEAN PATENT OFFICE

The applicant hereby requests that, until the publication of the mention of the grant of a European patent or for 20 years from the date of filing if the application is refused or withdrawn or deemed to be withdrawn, the biological material shall be made available as provided in Rule 28(3) EPC only by the issue of a sample to expert nominated by the requester (Rule 28(4) EPC).

CLAIMS

WHAT IS CLAIMED IS:

1. An isolated antibody that specifically binds to a non-ligand binding region of an extracellular domain of human Notch2, wherein the antibody specifically binds to EGF repeat 10 of human Notch2.

2. The antibody of claim 1, which is an antagonist of human Notch2.

3. The antibody of claim 2, which inhibits binding of a ligand to human Notch2.

4. The antibody of claim 3, wherein the ligand is DLL4, JAG1, and/or JAG2.

5. The antibody of any one of claims 2-4, which inhibits signaling of human Notch2.

6. The antibody of any one of claims 1-5, which binds human Notch2 with a K_D of about 1 nM or less.

7. The antibody of any one of claims 1-6, which does not bind to any region of human Notch2 outside of the EGF repeat 10.

8. The antibody of any one of claims 1-7, wherein the antibody binds to at least part of the sequence HKGAL (SEQ ID NO:28) within the Notch2 EGF repeat 10.

9. The antibody of any one of claims 1-8, which further specifically binds to at least one additional human Notch receptor.

10. The antibody of claim 9, which comprises an antigen binding site that binds the non-ligand binding region of the extracellular domain of both the human Notch2 and the additional human Notch receptor(s).

11. The antibody of claim 10, wherein the additional human Notch receptor is human Notch3.

12. The antibody of any one of claims 1-8, which further specifically binds to a non-ligand binding region of an extracellular domain of human Notch3, wherein the antibody specifically binds to EGF repeat 9 of human Notch3.

13. The antibody of claim 12, which comprises an antigen-binding site that binds both the EGF repeat 9 of human Notch3 and the EGF repeat 10 of Notch2.

14. The antibody of claim 12 or 13, which is an antagonist of human Notch3.

15. The antibody of claim 14, which inhibits binding of a ligand to human Notch3.

16. The antibody of claim 15, wherein the ligand is DLL4, JAG1, and/or JAG2.

17. The antibody of any one of claims 14-16, which inhibits signaling of human Notch3.

18. The antibody of any one of claims 12-17, which binds human Notch3 with a K_D of about 1 nM or less.

19. The antibody of any one of claims 12-18, which does not bind to any region of human Notch3 outside of EGF repeat 9.

20. An isolated antibody that specifically binds to a non-ligand binding region of an extracellular domain of human Notch3, wherein the antibody specifically binds to EGF repeat 9 of human Notch3.

21. The antibody of claim 20, which is an antagonist of human Notch3.

22. The antibody of claim 21, which inhibits binding of a ligand to human Notch3.

23. The antibody of claim 22, wherein the ligand is DLL4, JAG1, and/or JAG2.

24. The antibody of any one of claims 21-23, which inhibits signaling of human Notch3.

25. The antibody of any one of claims 20-24, which binds human Notch3 with a K_D of about 1 nM or less.

26. The antibody of any one of claims 20-25, which does not bind to any region of human Notch3 outside of EGF repeat 9.

27. The antibody of any one of claims 20-26, wherein the antibody binds to at least part of the sequence HEDAI (SEQ ID NO:29) within the Notch3 EGF repeat 9.

28. The antibody of any one of claims 20-27, which further specifically binds to EGF repeat 10 of at least one additional human Notch receptor.

29. The antibody of claim 28, which comprises an antigen binding site that binds the non-ligand binding region of the extracellular domain of both the human Notch3 and the additional human Notch receptor(s).

30. An antibody encoded by the polynucleotide deposited with the ATCC as 59R1 or PTA-9547.

31. An antibody that specifically binds human Notch2 and/or Notch3, wherein the antibody comprises

(a) a heavy chain CDR1 comprising SSSGMS (SEQ ID NO:5), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions;

(b) a heavy chain CDR2 comprising VIASSGSNTYYADSVKG (SEQ ID NO:6), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; and

(c) a heavy chain CDR3 comprising either SIFYTT (SEQ ID NO:51) or GIFFAI (SEQ ID NO:7), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions.

32. The antibody of claim 31, wherein the heavy chain CDR1 comprises SSSGMS (SEQ ID NO:5), the heavy chain CDR2 comprises VIASSGSNTYYADSVKG (SEQ ID NO:6), and the heavy chain CDR3 comprises SIFYTT (SEQ ID NO:51).

33. An antibody that specifically binds human Notch2 and/or Notch3, wherein the antibody comprises a heavy chain CDR1 comprising SSSGMS (SEQ ID NO:5), a heavy chain CDR2 comprising VIASSGSNTYYADSVKG (SEQ ID NO:6), and a heavy chain CDR3 comprising (G/S)(I/S)F(F/Y)(A/P)(I/T/S/N) (SEQ ID NO:30).

34. The antibody of claim 33, wherein the heavy chain CDR3 is selected from the group consisting GIFFAI (SEQ ID NO:7), SIFYPT (SEQ ID NO:22), SSFFAS (SEQ ID NO:23), SSFYAS (SEQ ID NO:24), SSFFAT (SEQ ID NO:25), SIFYPS (SEQ ID NO:26), and SSFFAN (SEQ ID NO:27).

35. The antibody of any one of claims 31-34, which further comprises:

(a) a light chain CDR1 comprising RASQSVRSNYLA (SEQ ID NO: 8), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions;

(b) a light chain CDR2 comprising GASSRAT (SEQ ID NO:9), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; and

(c) a light chain CDR3 comprising QQYSNFPI (SEQ ID NO:10), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions.

36. The antibody of claim 35, wherein the light chain CDR1 comprises RASQSVRSNYLA (SEQ ID NO: 8), the light chain CDR2 comprises GASSRAT (SEQ ID NO:9), and/or the light chain CDR3 comprises QQYSNFPI (SEQ ID NO:10).

37. An antibody that specifically binds a non-ligand binding region of an extracellular domain of human Notch2 and/or Notch3, wherein the antibody comprises:

(a) a heavy chain CDR1 comprising SSSGMS (SEQ ID NO:5), a heavy chain CDR2 comprising VIASSGSNTYYADSVKG (SEQ ID NO:6), and a heavy chain CDR3 comprising SIFYTT (SEQ ID NO:51) or GIFFAI (SEQ ID NO:7); and/or

(b) a light chain CDR1 comprising RASQSVRSNYLA (SEQ ID NO:8), a light chain CDR2 comprising GASSRAT (SEQ ID NO:9), and a light chain CDR3 comprising QQYSNFPI (SEQ ID NO:10).

38. The antibody of claim 37, wherein the antibody comprises:

(a) a heavy chain CDR1 comprising SSSGMS (SEQ ID NO:5), a heavy chain CDR2 comprising VIASSGSNTYYADSVKG (SEQ ID NO:6), and a heavy chain CDR3 comprising SIFYTT (SEQ ID NO:51); and

(b) a light chain CDR1 comprising RASQSVRSNYLA (SEQ ID NO:8), a light chain CDR2 comprising GASSRAT (SEQ ID NO:9), and a light chain CDR3 comprising QQYSNFPI (SEQ ID NO:10).

39. The antibody of claim 37, wherein the antibody comprises:

(a) a heavy chain CDR1 comprising SSSGMS (SEQ ID NO:5), a heavy chain CDR2 comprising VIASSGSNTYYADSVKG (SEQ ID NO:6), and a heavy chain CDR3 comprising GIFFAI (SEQ ID NO:7); and

(b) a light chain CDR1 comprising RASQSVRSNYLA (SEQ ID NO:8), a light chain CDR2 comprising GASSRAT (SEQ ID NO:9), and a light chain CDR3 comprising QQYSNFPI (SEQ ID NO:10).

40. An antibody that specifically binds human Notch2 and/or Notch3, wherein the antibody comprises:

(a) a heavy chain variable region having at least about 90% sequence identity to SEQ ID NO:50, SEQ ID NO:14, or SEQ ID NO:20; and/or

(b) a heavy chain variable region having at least about 90% sequence identity to SEQ ID NO:13 or SEQ ID NO:19.

41. The antibody of claim 40, which comprises:

(a) a heavy chain variable region having at least about 95% sequence identity to SEQ ID NO:50 or SEQ ID NO:14; and/or

(b) a light chain variable region having at least about 95% sequence identity to SEQ ID NO:13.

42. The antibody of claim 41, which comprises:

(a) a heavy chain variable region comprising SEQ ID NO:50; and/or

(b) a light chain variable region comprising SEQ ID NO:13.

43. The antibody of claim 41, which comprises:

(a) a polypeptide comprising SEQ ID NO:14; and/or

(b) a polypeptide comprising SEQ ID NO:13.

44. The antibody of any one of claims 31-43, which specifically binds to human Notch2.

45. The antibody of claim 44, which further specifically binds to human Notch3.

46. The antibody of any one of claims 31-43, which binds to EGF repeat 10 of human Notch2 and/or EGF repeat 9 of human Notch3.

47. An isolated antibody that competes for specific binding to human Notch2 and/or Notch3 with the antibody of any one of claims 30 to 43.

48. The antibody of any one of claims 30-43, which is an antagonist of human Notch2 and/or Notch3.

49. An isolated antibody that specifically binds to a non-ligand binding region of an extracellular domain of two or more human Notch receptors, wherein if the two or more human Notch receptors comprise Notch2, Notch1, or Notch4, the antibody specifically binds to EGF10 of Notch2, Notch1, or Notch4, and if the two or more human Notch receptors comprise Notch3, the antibody binds to EGF 9 of Notch3.

50. The antibody of claim 49, wherein the antibody is an antagonist of the two or more human Notch receptors.

51. The antibody of claim 49 or 50, wherein the two or more human Notch receptors comprise both Notch1 and Notch4.

52. The antibody of any one of claims 1-51, which inhibits tumor growth.

53. The antibody of any one of claims 1-52, which is a recombinant antibody, a monoclonal antibody, a chimeric antibody, a humanized antibody, a human antibody, or an antibody fragment.

54. The antibody of any one of claims 1-53, which is a bispecific antibody.

55. The antibody of any one of claims 1-53, which is a monospecific antibody.

56. The antibody of any one of claims 1-55, which is an IgG1 or IgG2 antibody.

57. A cell comprising or producing the antibody of any one of claims 1-56.

58. A pharmaceutical composition comprising the antibody of any one of claims 1-56.

59. A method of inhibiting Notch3 signaling in a cell comprising contacting the cell with an effective amount of the antibody of any one of claims 11-43, 45-48, or 52-56 with the cell.

60. A method of inhibiting Notch 2 signaling in a cell comprising contacting the cell with an effective amount of the antibody of any one of claims 1-19, 30-48, or 52-56 with the cell.

61. A method of inhibiting angiogenesis in a subject, comprising administering an effective amount of the antibody of any one of claims 1-48 or 52-56 to the subject.

62. The method of claim 61, which is a method of inhibiting angiogenesis by modulating the function of pericytes and/or vascular smooth muscle cells.

63. The method of claim 61 or 62, wherein the angiogenesis is tumor angiogenesis.

64. A method of inhibiting tumor growth in a subject, comprising administering an effective amount of the antibody of any one of claims 1-56 to the subject.

65. A method of reducing the tumorigenicity of a tumor in a subject, comprising administering an effective amount of the antibody of any one of claims 1-56 to the subject.

66. The method of claim 65, wherein the frequency of cancer stem cells in the tumor is reduced.

67. The method of claim 64, 65, or 66, wherein the tumor is a colorectal tumor, breast tumor, pancreatic tumor or melanoma.

68. A method of treating an angiogenesis-associated disease in a subject, comprising administering an effective amount of the antibody of any one of claims 1-48 or 52-56 to the subject.

69. A method of treating cancer in a subject, comprising administering an effective amount of the antibody of any one of claims 1-56 to the subject.

70. The method of claim 69, wherein the cancer is colorectal cancer, breast cancer, pancreatic cancer, or melanoma.

71. The method of any one of claims 61-70, further comprising administering a therapeutically effective amount of a second agent to the subject, wherein the second agent is an anti-cancer agent and/or an anti-angiogenic agent.

72. The method of claim 71, wherein the second agent is a chemotherapeutic agent.

73. The method of claim 72, wherein the chemotherapeutic agent is paclitaxel, gemcitabine, or irinotecan.

74. The method of claim 71, wherein the second agent is an antagonist of vascular endothelial cell growth factor (VEGF) or of a VEGF receptor.

75. The method of claim 71, wherein the second agent is an anti-DLL4 antibody.

76. The method of any one of claims 61-75, wherein the subject is human.

77. A polypeptide comprising a sequence selected from the group consisting of SEQ ID NO:50, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:49.

78. A polynucleotide comprising a polynucleotide sequence encoding the polypeptide of claim 77.

79. A polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO:58, SEQ IDNO:59, SEQ ID NO:60, SEQ ID NO:48, SEQ IDNO:47, SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:15, and SEQ ID NO:17.

80. A polynucleotide that hybridizes to a polynucleotide having the sequence of SEQ ID NO:58, SEQ ID NO:59, or SEQ ID NO:60 under stringent hybridization conditions.

81. A vector comprising the polynucleotide of any one of claims 78-80.

82. A method of inhibiting angiogenesis in a subject, comprising administering an effective amount of an antagonist of human Notch 2 and/or Notch3 to the subject.

83. The method of claim 82, which is a method of inhibiting angiogenesis by modulating the function of pericytes and/or vascular smooth muscle cells.

84. The method of claim 82 or 83, wherein the angiogenesis is tumor angiogenesis.

85. The method of any one of claims 82 to 84, wherein the antagonist is an antibody.

86. The method of any one of claims 82 to 84, wherein the antagonist is not an antibody.

87. The method of any one of claims 82 to 86, wherein the antagonist is an antagonist of human Notch3.

88. The method of claim 87, wherein the antagonist is also an antagonist of human Notch2.

89. The method of any one of claims 82 to 88, which further comprises administering to the subject an antagonist of vascular endothelial cell growth factor (VEGF) or of a VEGF receptor.