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.

CLAIMS

1. An isolated 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) or 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).

2. The antibody of claim 1, which 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 SIFYTT (SEQ ID NO:51).

3. The antibody of claim 1, which 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 GIFFAI (SEQ ID NO:7).

4. 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

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

5. The antibody of claim 4, which comprises:

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

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

6. The antibody of claim 4, which comprises:

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

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

7. The antibody of claim 4, which comprises:

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

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

8. The antibody of claim 4, which comprises:

(a) a heavy chain variable region comprising SEQ ID NO:14; and

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

9. An isolated antibody that specifically binds human NOTCH2 and/or human NOTCH3, wherein the antibody:

(a) binds at least part of the sequence HKGAL (SEQ ID NO:28) within the EGF repeat 10 of human NOTCH2; and/or

(b) binds at least part of the sequence HEDAI (SEQ ID NO:29) within the EGF repeat 9 of human NOTCH3.

10. An antibody that competes for specific binding to human NOTCH2 and/or human NOTCH3 with a second antibody in a competitive binding assay, wherein the second antibody comprises:

(a) a heavy chain variable region comprising SEQ ID NO:50 and a light chain variable region comprising SEQ ID NO:13; or

(b) a heavy chain variable region comprising SEQ ID NO:14 and a light chain variable region comprising SEQ ID NO:13.

11. An antibody that competes for specific binding to human NOTCH2 and/or human NOTCH3 with antibody 59R5 or antibody 59R1.

12. The antibody of any one of claims 1 to 11, which is a monoclonal antibody, a chimeric antibody, a humanized antibody, a human antibody, a bispecific antibody, or an antibody fragment.

13. The antibody of claim 1 encoded by the polynucleotide deposited with the ATCC as PTA-10170.

14. The antibody of claim 1 encoded by the polynucleotide deposited with the ATCC as PTA-9547.

15. The antibody of any one of claims 1 to 11, 13, or 14, which is conjugated to a cytotoxic agent.

16. An isolated cell producing the antibody of any one of claims 1 to 15.

17. A pharmaceutical composition comprising the antibody of any one of claims 1 to 15 and a pharmaceutically acceptable carrier.

18. An isolated polynucleotide encoding the antibody of any one of claims 1 to 15.

19. A vector comprising the polynucleotide of claim 18.

20. An isolated cell comprising the polynucleotide of claim 18.

21. An isolated cell comprising the vector of claim19.

22. Use of the antibody of any one of claims 1 to 15, in the manufacture of a medicament for the treatment of cancer.

23. Use of the antibody of any one of claims 1 to 15, in the manufacture of a medicament for inhibiting tumor growth.

24. The use of claim 22 or claim 23, wherein the treatment of cancer or inhibiting tumor growth comprises use of the antibody in combination with a second therapeutic agent.

25. The use of claim 24, wherein the second therapeutic agent is a chemotherapeutic agent, a second antibody, or an anti-angiogenesis agent.

26. Use of the antibody of any one of claims 1 to 15, in the manufacture of a medicament for inhibiting angiogenesis.

27. The use of claim 26, wherein the angiogenesis is tumor angiogenesis.

28. 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.

29. The antibody of any one of claims 1-15, for use in the treatment of cancer.

30. The antibody of any one of claims 1-15, for use in inhibiting tumor growth.

31. The antibody of any one of claims 1-15, for use in inhibiting angiogenesis.