2009/063965, WO 2009/062690, WO 2011/014457, US 2014/0227283, and U.S. Patent No. 8,853,369. US 2014/0227283 discloses monoclonal anti-Axl antibodies and uses thereof in diagnostic and therapeutic methods. WO 2009/062690 discloses antibodies that bind to the extracellular domain of the Axl protein and can at least partially inhibit Axl activity.

[0008] These monoclonal anti-Axl antibodies will bind to Axl at any location of a patient's body with similar affinity, including at locations of the tumors they are intended to treat. The binding of such antibodies to Axl in non-tumor environments is expected to have an adverse affect on the normal functioning of Axl in these environments and thus may cause significant side-effects. The present invention provides conditionally active anti-Axl antibodies and antibody fragments that have a higher binding affinity to Axl in a tumor microenvironment in comparison with their binding affinities to Axl in a non-tumor environment. The anti-Axl antibodies and antibody fragments of the present invention are expected to have comparable or greater anti-cancer efficacy with reduced side-effects, in comparison with the monoclonal anti-Axl antibodies known in the art. This may also permit administration of higher dosages of the anti-Axl antibodies and antibody fragments or more frequent treatment, thus providing a more effective therapeutic option.

SUMMARY

[0009] Disclosed is an isolated heavy chain variable region polypeptide that specifically binds to the Axl protein. The polypeptide includes three complementarity determining regions H1, H2, and H3 sequences, wherein:

the H1 sequence is $X_1GX_2X_3MX_4$ (SEQ ID NO: 1);

the H2 sequence is $LIKX_5SNGGTX_6YNQKFKG$ (SEQ ID NO: 2); and

the H3 sequence is $GX_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}DYX_{15}X_{16}$ (SEQ ID NO: 3),

wherein

X_1 is T or A or W,

X_2 is H or A,

X_3 is T or I,

X_4 is N or I,

X_5 is P or N,

X_6 is S or I or T,

X_7 is H or D or E or P or R or W,

X_8 is Y or N,

X_9 is E or A or D or F or G or H or I or L or M or N or R or V or Y,

X_{10} is S or D or M or N or Q,

X_{11} is Y or C or E or P,

X_{12} is F or E or N or S or T or V,

X_{13} is A or D or G or L or Y,

X_{14} is M or E or F,

X_{15} is W or A or D or H or L or N or P or R or T, and

X_{16} is G or H.

[0009a] In one aspect, there is provided an isolated polypeptide that specifically binds to Axl protein, said polypeptide comprising a heavy chain variable region including three complementarity determining regions, said regions having H1, H2, and H3 sequences, wherein:

(a) the H1 sequence is $X_1GX_2X_3MX_4$ (SEQ ID NO: 1);

(b) the H2 sequence is $LIKX_5SNGGTX_6YNQKFKG$ (SEQ ID NO: 2); and

(c) the H3 sequence is $GX_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}DYX_{15}X_{16}$ (SEQ ID NO: 3), wherein

X_1 is T or A or W,

X_2 is H or A,

X_3 is T or I,

X_4 is N or I,

X_5 is P or N,

X_6 is S or I or T,

X_7 is H or D or E or P or R or W,

X_8 is Y or N,

X_9 is E or A or D or F or G or H or I or L or M or N or R or V or Y,

X_{10} is S or D or M or N or Q,

X_{11} is Y or C or E or P,

X_{12} is F or E or N or S or T or V,

X_{13} is A or D or G or L or Y,

X_{14} is M or E or F,

X_{15} is W or A or D or H or L or N or P or R or T, and

X_{16} is G or H.

[0010] Disclosed is that, the isolated heavy chain variable region polypeptide is combined with an isolated light chain variable region that includes three complementarity determining regions L1, L2, and L3 sequences, wherein:

the L1 sequence is KASQDX$_{17}$X$_{18}$SX$_{19}$VX$_{20}$ (SEQ ID NO: 4);

the L2 sequence is X$_{21}$X$_{22}$X$_{23}$TRX$_{24}$T (SEQ ID NO: 5); and

the L3 sequence is QEX$_{25}$X$_{26}$SX$_{27}$X$_{28}$X$_{29}$X$_{30}$ (SEQ ID NO: 6),

wherein

X$_{17}$ is V or D or G or N or W,

X$_{18}$ is S or V,

X$_{19}$ is A or L or M,

X$_{20}$ is A or D or N or Q,

X$_{21}$ is W or F,

X$_{22}$ is A or I or N or P or Q,

X$_{23}$ is S or D,

X$_{24}$ is H or D,

X$_{25}$ is H or C or F or I or L or Q or S or T or V or Y,

X$_{26}$ is F or C or D or E or G or N or S,

X$_{27}$ is T or C or P,

X$_{28}$ is P or A or C or D or E or H or K or S or T or V or W,

X$_{29}$ is L or G or R, and

X$_{30}$ is T or I or R.

[0010a] In another aspect, there is provided an isolated polypeptide that specifically binds to Axl protein, said polypeptide comprising an light chain variable region including three complementarity determining regions having L1, L2, and L3 sequences, wherein:

(a) the L1 sequence is KASQDX$_{17}$X$_{18}$SX$_{19}$VX$_{20}$ (SEQ ID NO: 4);

(b) the L2 sequence is X$_{21}$X$_{22}$X$_{23}$TRX$_{24}$T (SEQ ID NO: 5); and

(c) the L3 sequence is QEX$_{25}$X$_{26}$SX$_{27}$X$_{28}$X$_{29}$X$_{30}$ (SEQ ID NO: 6),

wherein

X$_{17}$ is V or D or G or N or W,

X$_{18}$ is S or V,

X$_{19}$ is A or L or M,

X$_{20}$ is A or D or N or Q,

X$_{21}$ is W or F,

X$_{22}$ is A or I or N or P or Q,

X$_{23}$ is S or D,

X$_{24}$ is H or D,

X$_{25}$ is H or C or F or I or L or Q or S or T or V or Y,

X_{26} is F or C or D or E or G or N or S,

X_{27} is T or C or P,

X_{28} is P or A or C or D or E or H or K or S or T or V or W,

X_{29} is L or G or R, and

X_{30} is T or I or R.

[0011] Disclosed is an anti-Axl antibody or antibody fragment that includes the isolated heavy chain variable region polypeptide of the disclosure.

[0011a] In another aspect, there is provided an anti-Axl antibody or antibody fragment comprising the isolated heavy chain variable region polypeptide as described herein.

[0012] Disclosed is an immunoconjugate that includes the antibody or antibody fragment of the disclosure, optionally conjugated to an

agent selected from a chemotherapeutic agent, a radioactive atom, a cytostatic agent and a cytotoxic agent.

[0013] Disclosed is a pharmaceutical composition that includes the polypeptide, the antibody or antibody fragment, or the immunoconjugate of the disclosure, together with a pharmaceutically acceptable carrier.

[0014] Disclosed is a kit for diagnosis or treatment including the polypeptide, the antibody or antibody fragment, or the immunoconjugate of the present disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] FIGS. 1A-1B show sequence alignments of the heavy chain variable regions and the light chain variable regions, respectively, of anti-Axl antibodies of the present invention.

[0016] FIG. 2 shows binding (OD450) of various conditionally active antibodies of the invention to Axl's extracellular domain at pH 6.0 and pH 7.4. These conditionally active antibodies were more active at pH 6.0 than at pH 7.4.

[0017] FIG. 3 shows the selectivity of various conditionally active antibodies of the invention to Axl's extracellular domain. The selectivity was measured as the ratio of the binding affinity to a binding partner at pH6.0 to the binding affinity to the same binding partner at pH 7.4.

[0018] FIG. 4 shows by size exclusion chromatograph indicating that conditionally active antibodies of the invention do not aggregate, as described in Example 1.

[0019] FIG. 5 shows thermostability of conditionally active antibodies of the invention before and after heat shock as measured by an ELISA assay, as described in Example 1.

[0020] FIGS. 6A-6B show selectivity of conditionally active antibodies of the invention as measured by SPR assay in Example 1.

[0021] FIG. 7 shows pH dependent binding profiles for binding of anti-Axl antibodies of the present invention to Axl in KREBS buffer.

[0022] FIGS. 8A-8E show results of another cell killing study using A549 cells wherein anti-Axl antibodies of the present invention were employed for cell killing at pH 6.0 and pH 7.4 and at different antibody concentrations.

[0023] FIGS. 9A-9D show binding affinity to human Axl and cynomolgus Axl for anti-Axl antibodies of the present invention in different buffers and at different pH levels.

[0024] FIGS. 10A-10H show cell killing of different cell lines at different pH levels by anti-Axl antibodies of the present invention that were conjugated to duomycin.

WHAT IS CLAIMED IS:

1. An isolated polypeptide that specifically binds to Axl protein, said polypeptide comprising a heavy chain variable region including three complementarity determining regions, said regions having H1, H2, and H3 sequences, wherein:

(a) the H1 sequence is $X_1GX_2X_3MX_4$ (SEQ ID NO: 1);

(b) the H2 sequence is $LIKX_5SNGGTX_6YNQKFKG$ (SEQ ID NO: 2); and

(c) the H3 sequence is $GX_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}DYX_{15}X_{16}$ (SEQ ID NO: 3), wherein

X_1 is T or A or W,

X_2 is H or A,

X_3 is T or I,

X_4 is N or I,

X_5 is P or N,

X_6 is S or I or T,

X_7 is H or D or E or P or R or W,

X_8 is Y or N,

X_9 is E or A or D or F or G or H or I or L or M or N or R or V or Y,

X_{10} is S or D or M or N or Q,

X_{11} is Y or C or E or P,

X_{12} is F or E or N or S or T or V,

X_{13} is A or D or G or L or Y,

X_{14} is M or E or F,

X_{15} is W or A or D or H or L or N or P or R or T, and

X_{16} is G or H.

2. The polypeptide of claim 1, wherein the heavy chain variable region is encoded by a DNA sequence selected from sequences of SEQ ID NOS: 11-13.

3. The polypeptide of any one of claims 1-2 in combination with an isolated light chain variable region including three complementarity determining regions L1, L2, and L3 sequences, wherein:

(a) the L1 sequence is $KASQDX_{17}X_{18}SX_{19}VX_{20}$ (SEQ ID NO: 4);

(b) the L2 sequence is $X_{21}X_{22}X_{23}TRX_{24}T$ (SEQ ID NO: 5); and

(c) the L3 sequence is $QEX_{25}X_{26}SX_{27}X_{28}X_{29}X_{30}$ (SEQ ID NO: 6),

wherein

X_{17} is V or D or G or N or W,

X_{18} is S or V,

X_{19} is A or L or M,

X_{20} is A or D or N or Q,

X_{21} is W or F,

X_{22} is A or I or N or P or Q,

X_{23} is S or D,

X_{24} is H or D,

X_{25} is H or C or F or I or L or Q or S or T or V or Y,

X_{26} is F or C or D or E or G or N or S,

X_{27} is T or C or P,

X_{28} is P or A or C or D or E or H or K or S or T or V or W,

X_{29} is L or G or R, and

X_{30} is T or I or R.

4. The polypeptide of claim 3, wherein the light chain variable region is encoded by a DNA sequence selected from SEQ ID NOS: 7-10.

5. An isolated polypeptide that specifically binds to Axl protein, said polypeptide comprising an light chain variable region including three complementarity determining regions having L1, L2, and L3 sequences, wherein:

(a) the L1 sequence is $KASQDX_{17}X_{18}SX_{19}VX_{20}$ (SEQ ID NO: 4);

(b) the L2 sequence is $X_{21}X_{22}X_{23}TRX_{24}T$ (SEQ ID NO: 5); and

(c) the L3 sequence is $QEX_{25}X_{26}SX_{27}X_{28}X_{29}X_{30}$ (SEQ ID NO: 6),

wherein

X_{17} is V or D or G or N or W,

X_{18} is S or V,

X_{19} is A or L or M,

X_{20} is A or D or N or Q,

X_{21} is W or F,

X_{22} is A or I or N or P or Q,

X_{23} is S or D,

X_{24} is H or D,

X_{25} is H or C or F or I or L or Q or S or T or V or Y,

X_{26} is F or C or D or E or G or N or S,

X_{27} is T or C or P,

X_{28} is P or A or C or D or E or H or K or S or T or V or W,

X_{29} is L or G or R, and

X_{30} is T or I or R.

6. The polypeptide of claim 5, wherein the light chain variable region is encoded by a DNA sequence selected from SEQ ID NOS: 7-10.

7. An anti-Axl antibody or antibody fragment comprising the isolated heavy chain variable region polypeptide of claim 1.

8. The antibody or antibody fragment of claim 7, further comprising an isolated light chain variable region comprising three complementarity determining regions L1, L2, and L3 sequences, wherein:

(a) the L1 sequence is $KASQDX_{17}X_{18}SX_{19}VX_{20}$ (SEQ ID NO: 4);

(b) the L2 sequence is $X_{21}X_{22}X_{23}TRX_{24}T$ (SEQ ID NO: 5); and

(c) the L3 sequence is $QEX_{25}X_{26}SX_{27}X_{28}X_{29}X_{30}$ (SEQ ID NO: 6),

wherein

X_{17} is V or D or G or N or W,

X_{18} is S or V,

X_{19} is A or L or M,

X_{20} is A or D or N or Q,

X_{21} is W or F,

X_{22} is A or I or N or P or Q,

X_{23} is S or D,

X_{24} is H or D,

X_{25} is H or C or F or I or L or Q or S or T or V or Y,

X_{26} is F or C or D or E or G or N or S,

X_{27} is T or C or P,

X_{28} is P or A or C or D or E or H or K or S or T or V or W,

X_{29} is L or G or R, and

X_{30} is T or I or R.

9. The antibody or antibody fragment of any one of claims 7-8, wherein the antibody or antibody fragment has a higher binding affinity to Axl protein at a value of a condition in a tumor microenvironment in comparison with a different value of the same condition that occurs in a non-tumor microenvironment.

10. The antibody or antibody fragment of claim 9, wherein the condition is pH.

11. The antibody or antibody fragment of claim 10, wherein the pH in the tumor microenvironment is in a range of from 5.8 to 7.0 and the pH in the non-tumor microenvironment is in a range of 7.0-7.6.

12. The antibody or antibody fragment of any one of claims 7-11, wherein the antibody or antibody fragment has a ratio of binding affinity to the Axl protein at a value of a condition in a tumor microenvironment and a binding affinity to the Axl protein at a different value of the same condition in a non-tumor microenvironment of at least about 1.5:1, at least about 2:1, at least about 3:1, at least about 4:1, at least about 5:1, at least about 6:1, at least about 7:1, at least about 8:1, at least about 9:1, at least about 10:1, at least about 20:1, at least about 30:1, at least about 50:1, at least about 70:1, or at least about 100:1.

13. The antibody or antibody fragment of any of one of claims 7-12, wherein the antibody or antibody fragment is a chimeric antibody, a multispecific antibody, or a humanized antibody.