

antibody fragments may have a higher binding affinity to EpCAM in a tumor in comparison with EpCAM present in normal tissues. These anti-EpCAM antibodies or antibody fragments typically have at least comparable efficacy to known anti-EpCAM antibodies. In addition, the present anti-EpCAM antibodies or antibody fragments may exhibit reduced side effects in comparison with monoclonal anti-EpCAM antibodies known in the art for having a relatively low binding affinity to EpCAM in normal tissues. These advantages may provide a more selective targeting of the EpCAM for a tumor and may permit use of higher dosages of these anti-EpCAM antibodies or antibody fragments as a result of the selectivity of the antibodies for EpCAM present in a tumor, whereby more effective therapeutic treatments can be realized without a corresponding increase in undesirable side effects.

SUMMARY

[0010] In one example, the present disclosure provides an isolated polypeptide that specifically binds to EpCAM. The polypeptide comprises a heavy chain variable region including three complementarity determining regions (CDRs) having sequences H1, H2, and H3, wherein:

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

the H2 sequence is X₁IRPSTGYTEYNQKFKD (SEQ ID NO: 2); and

the H3 sequence is GDNWVGAFN (SEQ ID NO: 3);

wherein X₁ is Y or D.

[0011] In the previous example, the H2 sequence may be YIRPSTGYTEYNQKFKD (SEQ ID NO: 22) or the H2 sequence may be DIRPSTGYTEYNQKFKD (SEQ ID NO: 23).

[0012] In another example, the present disclosure includes a product formed by a combination of any of the above-described isolated polypeptides with an isolated polypeptide comprising a light chain variable region including three CDRs having sequences L1, L2, and L3, wherein:

the L1 sequence is SASSSISYMH (SEQ ID NO: 4);

the L2 sequence is STSNLX₂S (SEQ ID NO: 5); and

the L3 sequence is X₃QWSTYX₄T (SEQ ID NO: 6),

wherein X₂ is A or H; X₃ is H or E; and X₄ is H or E.

[0013] In another example, the present disclosure provides isolated polypeptides comprising a heavy chain variable region and a light chain variable region that specifically bind to EpCAM, especially human EpCAM protein, wherein the heavy chain variable region includes three complementarity determining regions having sequences H1, H2, and H3, wherein:

the H1 sequence is GYTFTSYWMH (SEQ ID NO: 1);
the H2 sequence is X₁IRPSTGYTEYNQKFKD (SEQ ID NO: 2); and
the H3 sequence is GDNWVGFAN (SEQ ID NO: 3); wherein X₁ is Y or D; and
the light chain variable region includes three complementarity determining regions having sequences L1, L2, and L3, wherein:
the L1 sequence is SASSSISYMH (SEQ ID NO: 4);
the L2 sequence is STSNLX₂S (SEQ ID NO: 5); and
the L3 sequence is X₃QWSTYX₄T (SEQ ID NO: 6); wherein X₂ is A or H; X₃ is H or E; and X₄ is H or E; and with the proviso that X₁, X₂, X₃ and X₄ cannot be Y, A, H, and H at the same time.

[0013a] In one aspect, the present disclosure refers to isolated polypeptides comprising a heavy chain variable region and a light chain variable region that specifically bind to human EpCAM, wherein the heavy chain variable region includes three complementarity determining regions having sequences H1, H2, and H3, wherein:

the H1 sequence is GYTFTSYWMH (SEQ ID NO: 1);
the H2 sequence is X₁IRPSTGYTEYNQKFKD (SEQ ID NO: 2); and
the H3 sequence is GDNWVGFAN (SEQ ID NO: 3); wherein X₁ is Y or D; and
the light chain variable region includes three complementarity determining regions having sequences L1, L2, and L3, wherein:
the L1 sequence is SASSSISYMH (SEQ ID NO: 4);
the L2 sequence is STSNLX₂S (SEQ ID NO: 5); and
the L3 sequence is X₃QWSTYX₄T (SEQ ID NO: 6); wherein X₂ is A or H; X₃ is H or E; and X₄ is H or E; and with the proviso that X₁, X₂, X₃ and X₄, respectively, cannot be Y, A, H, and H at the same time.

[0014] In the previous example, the H2 sequence may be YIRPSTGYTEYNQKFKD (SEQ ID NO: 22) or the H2 sequence may be DIRPSTGYTEYNQKFKD (SEQ ID NO: 23).

[0015] In each of the previous examples, the L2 sequence may be STSNLAS (SEQ ID NO: 24) or the L2 sequence may be STSNLHS (SEQ ID NO: 25).

[0016] In each of the previous examples, the L3 sequence may be HQWSTYHT (SEQ ID NO: 26), the L3 sequence may be HQWSTYET (SEQ ID NO: 27), or the L3 sequence may be EQWSTYHT (SEQ ID NO: 28).

[0017] In each of the previous examples, the heavy chain variable region may have a sequence selected from SEQ ID NOS: 7-13.

[0018] In each of the foregoing examples, the light chain variable region may have a sequence selected from SEQ ID NOS: 14-21.

[0019] In another example, isolated polypeptides of the present disclosure comprise a heavy chain variable region and a light chain variable region having any one pair of sequences selected from: SEQ ID NOS: 8 and 14, SEQ ID NOS: 9 and 14, SEQ ID NOS: 7 and 15, SEQ ID NOS: 7 and 16, SEQ ID NOS: 7 and 17, SEQ ID NOS: 11 and 20, SEQ ID NOS: 12 and 21, SEQ ID NOS: 11 and 18, SEQ ID NOS: 13 and 18, SEQ ID NOS: 10 and 19.

[0020] In another example, the isolated polypeptides of the present disclosure comprise a heavy chain variable region and a light chain variable region each region independently having at least 80%, 85%, 90%, 95%, 98% or 99% identity to a pair of amino acid sequences selected from: SEQ ID NOS: 8 and 14, SEQ ID NOS: 9 and 14, SEQ ID NOS: 7 and 15, SEQ ID NOS: 7 and 16, SEQ ID NOS: 7 and 17, SEQ ID NOS: 11 and 20, SEQ ID NOS: 12 and 21, SEQ ID NOS: 11 and 18, SEQ ID NOS: 13 and 18, and SEQ ID NOS: 10 and 19, respectively; and said isolated polypeptides specifically bind to human EpCAM protein.

[0021] In another example, the present disclosure provides an antibody or antibody fragment comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region includes three complementarity determining regions having sequences H1, H2, and H3, wherein:

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

the H2 sequence is X₁IRPSTGYTEYNQKFKD (SEQ ID NO: 2); and

the H3 sequence is GDNWVGFAN (SEQ ID NO: 3);

wherein X₁ is Y or D; and the light chain variable region includes three complementarity determining regions, having sequences L1, L2, and L3, wherein:

the L1 sequence is SASSSISYMH (SEQ ID NO: 4);

the L2 sequence is STSNLX₂S (SEQ ID NO: 5); and

the L3 sequence is X₃QWSTYX₄T (SEQ ID NO: 6),

wherein X₂ is A or H; X₃ is H or E; and X₄ is H or E; with the proviso that X₁, X₂, X₃ and X₄ cannot be Y, A, H, and H, respectively, at the same time.

[0022] In the previous example, the H2 sequence may be YIRPSTGYTEYNQKFKD (SEQ ID NO: 22) or the H2 sequence may be DIRPSTGYTEYNQKFKD (SEQ ID NO: 23).

[0023] In each of the previous examples, the L2 sequence may be STSNLAS (SEQ ID NO: 24) or the L2 sequence may be STSNLHS (SEQ ID NO: 25).

[0024] In each of the previous examples, the L3 sequence may be HQWSTYHT (SEQ ID NO: 26), the L3 sequence may be HQWSTYET (SEQ ID NO: 27), or the L3 sequence may be EQWSTYHT (SEQ ID NO: 28).

[0025] In each of the previous examples, the heavy chain variable region may have an amino acid sequence selected from SEQ ID NOS: 7-13.

[0026] In each of the previous examples, the light chain variable region may have an amino acid sequence selected from SEQ ID NOS: 14-21.

[0027] In each of the previous examples, the heavy chain variable region and the light chain variable region may have any one pair of sequences selected from: SEQ ID NOS: 8 and 14, SEQ ID NOS: 9 and 14, SEQ ID NOS: 7 and 15, SEQ ID NOS: 7 and 16, SEQ ID NOS: 7 and 17, SEQ ID NOS: 11 and 20, SEQ ID NOS: 12 and 21, SEQ ID NOS: 11 and 18, SEQ ID NOS: 13 and 18, SEQ ID NOS: 10 and 19.

[0028] In another example, the antibody or antibody fragment of the present disclosure comprises a heavy chain variable region and a light chain variable region each region independently having at least 80%, 85%, 90%, 95%, 98% or 99% identity to a pair of amino acid sequences selected from: SEQ ID NOS: 8 and 14, SEQ ID NOS: 9 and 14, SEQ ID

NOS: 7 and 15, SEQ ID NOS: 7 and 16, SEQ ID NOS: 7 and 17, SEQ ID NOS: 11 and 20, SEQ ID NOS: 12 and 21, SEQ ID NOS: 11 and 18, SEQ ID NOS: 13 and 18, and SEQ ID NOS: 10 and 19, respectively; and said antibody or antibody fragment specifically binds to human EpCAM protein.

[0028a] In one aspect, the present disclosure refers to an antibody or antigen-binding antibody fragment which comprises a heavy chain variable region and a light chain variable region each region independently having at least 80%, 85%, 90%, 95%, 98% or 99% identity to a pair of amino acid sequences selected from: SEQ ID NOS: 8 and 14, SEQ ID NOS: 9 and 14, SEQ ID NOS: 7 and 15, SEQ ID NOS: 7 and 16, SEQ ID NOS: 7 and 17, SEQ ID NOS: 11 and 20, SEQ ID NOS: 12 and 21, SEQ ID NOS: 11 and 18, SEQ ID NOS: 13 and 18, and SEQ ID NOS: 10 and 19, respectively; said antibody or antigen-binding antibody fragment specifically binds to human EpCAM protein.

[0028b] In one aspect, the present disclosure refers to an anti-EpCAM antibody or antigen-binding antibody fragment that specifically binds to human EpCAM protein comprising the isolated polypeptide(s) as disclosed herein.

[0029] In each of the previous examples, the antibody or antibody fragment may have a higher binding affinity to EpCAM 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. In one example, the condition is pH.

[0030] In each of the previous examples, the antibody or antibody fragment may have a higher antigen binding activity to EpCAM 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. In one example, the condition is pH.

[0031] In each of the previous examples, the conditionally active antibody or antibody fragment may have at least 70% of the antigen binding activity at pH 6.0 as compared to the same antigen binding activity of the parent antibody or antibody fragment at pH 6.0 and the conditionally active antibody or antibody fragment may have less than 50%, or less than 40%, or less than 30%, or less than 20% or less than 10% of the antigen binding activity at pH 7.4 as compared to the same antigen binding activity of the parent antibody or antibody fragment at pH 7.4. The antigen binding activity may be binding to EpCAM protein or binding to CD3.

[0032] In each of the previous examples, the antigen binding activity may be measured by an ELISA assay.

[0033] In yet another example, the present disclosure provides an immunoconjugate that includes any of the antibody or antibody fragments of the disclosure described above. In the immunoconjugate, the antibody or antibody fragment may be conjugated to an agent selected from a chemotherapeutic agent, a radioactive atom, a cytostatic agent and a cytotoxic agent.

[0033a] In one aspect, the present disclosure refers to an immunoconjugate comprising the antibody or antibody fragment as disclosed herein.

[0034] In yet another example, the present disclosure provides a pharmaceutical composition that includes any of the polypeptides, the antibody or antibody fragments, or the immunoconjugates of the disclosure described above, together with a pharmaceutically acceptable carrier.

[0034a] In one aspect, the present disclosure refers to a pharmaceutical composition comprising:
the polypeptide as disclosed herein, the antibody or antigen-binding antibody fragment as disclosed herein, or the immunoconjugate as disclosed herein; and
a pharmaceutically acceptable carrier, and optionally, further comprising a tonicity agent.

[0035] A single dose of the pharmaceutical composition of may include an amount of the polypeptide, the antibody or antibody fragment, or the immunoconjugate of about 135 mg, 235 mg, 335 mg, 435 mg, 535 mg, 635 mg, 735 mg, 835 mg, 935 mg, 1035 mg, 1135 mg, 1235 mg, or 1387 mg.

[0036] A single dose of the pharmaceutical composition of may include an amount of the polypeptide, the antibody or antibody fragment, or the immunoconjugate in a range of 135-235 mg, 235-335 mg, 335-435 mg, 435-535 mg, 535-635 mg, 635-735 mg, 735-835 mg, 835-935 mg, 935-1035 mg, 1035-1135 mg, 1135-1235 mg, or 1235-1387 mg.

[0036a] In one aspect, the present disclosure refers to a single dose of the pharmaceutical composition as disclosed herein, comprising an amount of the polypeptide, the antibody or antigen-binding antibody fragment, or the immunoconjugate in a range of 135-235 mg, 235-335 mg, 335-435 mg, 435-535 mg, 535-635 mg, 635-735 mg, 735-835 mg, 835-935 mg, 935-1035 mg, 1035-1135 mg, 1135-1235 mg, or 1235-1387 mg, optionally, further comprising an immune checkpoint inhibitor molecule.

[0037] Each of the foregoing pharmaceutical compositions may further include an immune checkpoint inhibitor molecule. The immune checkpoint inhibitor molecule may be an antibody or antibody fragment against an immune checkpoint. The immune checkpoint may be selected from LAG3, TIM3, TIGIT, VISTA, BTLA, OX40, CD40, 4-1BB, CTLA4, PD-1, PD-L1, GITR, B7-H3, B7-H4, KIR, A2aR, CD27, CD70, DR3, and ICOS or the immune checkpoint may be CTLA4, PD-1 or PD-L1.

[0038] Each of the foregoing pharmaceutical compositions may further include an antibody or antibody fragment against an antigen selected from PD1, PD-L1, CTLA4, AXL, ROR2, CD3, HER2, B7-H3, ROR1, SFRP4 and a WNT protein. The WNT protein may be selected from WNT1, WNT2, WNT2B, WNT3, WNT4, WNT5A, WNT5B, WNT6, WNT7A, WNT7B, WNT8A, WNT8B, WNT9A, WNT9B, WNT10A, WNT10B, WNT11 and WNT16.

[0039] In yet another example, the present disclosure provides a kit for diagnosis or treatment including any of the polypeptides, the antibody or antibody fragments, or the immunoconjugates of the present disclosure described above.

BRIEF DESCRIPTION OF THE DRAWINGS

[0040] FIG. 1 shows a schematic representation of the functions of EpCAM in cancer metastasis and progression.

[0041] FIG. 2 shows a sequence alignment of exemplary heavy chain variable regions of anti-EpCAM antibodies of the present invention.

[0042] FIG. 3 shows a sequence alignment of exemplary light chain variable regions of anti-EpCAM antibodies of the present invention.

[0043] FIG. 4 shows the binding activity of exemplary anti-EpCAM antibodies of the present invention to human EpCAM at different values of pH, as measured by enzyme linked immunosorbent assay (ELISA).

[0044] FIGS. 5A-5D show binding activities of anti-EpCAM antibodies of the present invention to Colo205 cells that express human EpCAM at different values of pH for two different amounts of the antibodies as measured by fluorescence activated cell sorting (FACS).

WHAT IS CLAIMED IS:

1. Isolated polypeptides comprising a heavy chain variable region and a light chain variable region that specifically bind to human EpCAM, wherein the heavy chain variable region includes three complementarity determining regions having sequences H1, H2, and H3, wherein:

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

the H2 sequence is X₁IRPSTGYTEYNQKFKD (SEQ ID NO: 2); and

the H3 sequence is GDNWVGfAN (SEQ ID NO: 3); wherein X₁ is Y or D; and the light chain variable region includes three complementarity determining regions having sequences L1, L2, and L3, wherein:

the L1 sequence is SASSSISYMH (SEQ ID NO: 4);

the L2 sequence is STSNLX₂S (SEQ ID NO: 5); and

the L3 sequence is X₃QWSTYX₄T (SEQ ID NO: 6); wherein X₂ is A or H; X₃ is H or E; and X₄ is H or E; and with the proviso that X₁, X₂, X₃ and X₄, respectively, cannot be Y, A, H, and H at the same time.

2. The isolated polypeptides of claim 1, wherein the H2 sequence is YIRPSTGYTEYNQKFKD (SEQ ID NO: 22), or wherein the H2 sequence is DIRPSTGYTEYNQKFKD (SEQ ID NO: 23).

3. The isolated polypeptides of claim 1, wherein the heavy chain variable region has an amino acid sequence selected from SEQ ID NOS: 7-13.

4. The isolated polypeptides of claim 1, wherein the L2 sequence is STSNLAS (SEQ ID NO: 24), or wherein the L2 sequence is STSNLHS (SEQ ID NO: 25).

5. The isolated polypeptides of any one of claims 2 - 4, wherein the L3 sequence is HQWSTYHT (SEQ ID NO: 26), or wherein the L3 sequence is HQWSTYET (SEQ ID NO: 27), or wherein the L3 sequence is EQWSTYHT (SEQ ID NO: 28).

6. The isolated polypeptides of claim 1 or 3, wherein the light chain variable region has an amino acid sequence selected from SEQ ID NOS: 14-21.

7. An anti-EpCAM antibody or antigen-binding antibody fragment that specifically binds to human EpCAM protein comprising the isolated polypeptide(s) of any one of claims 1-6.
8. An antibody or antigen-binding antibody fragment which comprises a heavy chain variable region and a light chain variable region each region independently having at least 80%, 85%, 90%, 95%, 98% or 99% identity to a pair of amino acid sequences selected from: SEQ ID NOS: 8 and 14, SEQ ID NOS: 9 and 14, SEQ ID NOS: 7 and 15, SEQ ID NOS: 7 and 16, SEQ ID NOS: 7 and 17, SEQ ID NOS: 11 and 20, SEQ ID NOS: 12 and 21, SEQ ID NOS: 11 and 18, SEQ ID NOS: 13 and 18, and SEQ ID NOS: 10 and 19, respectively; said antibody or antigen-binding antibody fragment specifically binds to human EpCAM protein.
9. The antibody or antigen-binding antibody fragment of claim 8 wherein the heavy chain variable region and the light chain variable region have any one pair of sequences selected from the pairs:, SEQ ID NOS: 8 and 14, SEQ ID NOS: 9 and 14, SEQ ID NOS: 7 and 15, SEQ ID NOS: 7 and 16, SEQ ID NOS: 7 and 17, SEQ ID NOS: 11 and 20, SEQ ID NOS: 12 and 21, SEQ ID NOS: 11 and 18, SEQ ID NOS: 13 and 18, SEQ ID NOS: 10 and 19.
10. The antibody or antigen-binding antibody fragment of any one of claims 7 - 9, wherein the antibody or antigen-binding antibody fragment has a higher binding affinity to EpCAM protein at a value of a condition in a tumor microenvironment in comparison with a different value of a same condition that occurs in a non-tumor microenvironment.
11. The antibody or antigen-binding antibody fragment of claim 10, wherein the condition is pH, and wherein the pH in the tumor microenvironment is in a range of from 5.0 to 6.8 and the pH in the non-tumor microenvironment is in a range of from 7.0 to 7.6.
12. The antibody or antigen-binding antibody fragment of any one of claims 10 - 11, wherein the antibody or antigen-binding antibody fragment has a ratio of binding affinity to the EpCAM protein at a value of a condition in a tumor microenvironment to a binding affinity to the EpCAM 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 antigen-binding antibody fragment of any one of claims 7 - 12, wherein the antibody is a multispecific antibody or antigen-binding antibody fragment, or wherein the antibody is a bispecific antibody or antigen-binding antibody fragment, and the bispecific antibody or antigen-binding antibody fragment comprises an anti-CD3-ScFv.

14. An immunoconjugate comprising the antibody or antibody fragment of any one of claims 7 - 13.

15. The immunoconjugate of claim 14, wherein the immunoconjugate comprises at least one agent selected from a chemotherapeutic agent, a radioactive atom, a cytostatic agent and a cytotoxic agent.

16. The immunoconjugate of claim 14, comprising at least two said agents, and at least one agent is a radioactive agent, and the radioactive agent is selected from an alpha emitter, a beta emitter, and a gamma emitter.

17. The immunoconjugate of any one of claims 15 – 16, wherein the antibody or antigen-binding antibody fragment and the at least one agent are covalently bonded to a linker molecule.

18. A pharmaceutical composition comprising:
the polypeptide of any one of claims 1 - 6, the antibody or antigen-binding antibody fragment of any one of claims 7 - 13, or the immunoconjugate of any one of claims 14 - 17;
and
a pharmaceutically acceptable carrier, and optionally, further comprising a tonicity agent.

19. A single dose of the pharmaceutical composition of claim 18, comprising an amount of the polypeptide, the antibody or antigen-binding antibody fragment, or the immunoconjugate in a range of 135-235 mg, 235-335 mg, 335-435 mg, 435-535 mg, 535-635 mg, 635-735 mg, 735-835 mg, 835-935 mg, 935-1035 mg, 1035-1135 mg, 1135-1235 mg, or 1235-1387 mg, optionally, further comprising an immune checkpoint inhibitor molecule.