

EGFR BINDING COMPLEX AND METHOD OF MAKING AND USING THEREOF

CLAIMS

WHAT IS CLAIMED IS:

1. A human epithelium growth factor receptor (EGFR) binding peptide having a binding specificity to human EGFR, comprising an amino acid sequence having at least 98%, 95%, or 92% of sequence identity to SEQ ID NO. 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 57, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83.

2. The EGFR binding peptide of Claim 1, comprising a variable heavy (VH) chain and a variable light (VL) chain,

wherein the VH chains comprises an amino acid sequence having at least 98%, 95%, or 92% sequence identity to SEQ ID NO. 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, or 41; and

wherein the VL chain comprises an amino acid sequence having at least 98%, 95%, or 92% sequence identity to SEQ ID NO. 3, 7, 11, 15, 19, 23, 27, 31, 35, 39, or 43.

3. The EGFR binding peptide of Claim 2, comprising a scFv domain, wherein the scFv domain comprises the VH chain and the VL chain, and wherein the scFv domain comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO. 57, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, or 83.

4. The EGFR binding peptide of Claim 3, comprising a histidine residue linked to at least one end of the scFv domain, and wherein the EGFR binding peptide comprises an amino acid sequence having at least 98% sequence identity to 57.

5. The EGFR binding peptide of Claim 2, comprising a Fab domain, wherein the Fab domain comprises the VH chain and the VL chain.

6. The EGFR binding peptide of Claim 5, further comprising a Fc domain linked to the Fab domain to provide a Fab-monoFc fusion protein, wherein the Fc domain comprises a sequence having at least 98% sequence identity to an amino acid sequence selected from SEQ ID NO. 45 and 47.

7. An antibody-like protein having a binding specificity to human EGFR, comprising an EGFR binding domain having a variable heavy (VH) chain and a variable light (VL) chain,

wherein the VH chains comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO. 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, or 41; and

wherein the VL chain comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO. 3, 7, 11, 15, 19, 23, 27, 31, 35, 39, or 43.

8. The antibody-like protein of Claim 7, comprising a scFv domain having an amino acid sequence having at least 98% sequence identity to SEQ ID NO. 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, or a combination thereof.

9. The antibody-like protein of Claim 7, wherein the antibody-like protein is a monospecific antibody comprising an amino acid sequence having at least 98% sequence identity to SEQ ID NO. 137, 139; 141, 143; 141, 149, 151, 139; 145, 147, , or a combination thereof.

10. The antibody-like protein of Claim 7, wherein the antibody-like protein is a bispecific antibody comprising an amino acid sequence having at least 98% sequence identity to SEQ ID NO. 137, 145, 139, 147, 141, 145, 143, 147, 141, 145, 149, 147, 151, 145, 139, 147 or a combination thereof.

11. The antibody-like protein of Claim 7, wherein the antibody-like protein is a penta-specific antibody comprising an amino acid sequence having at least 98% sequence identity to SEQ ID NO. 85, 87; 89, 91; 93, 95, 97, 99, 101, 103, 105, 107, or a combination thereof.

12. The antibody-like protein of Claim 7, wherein the antibody-like protein is a hexa-specific antibody comprising an amino acid sequence having at least 98% sequence identity to SEQ ID NO. 109, 111, 113, 115, 117, 119, or a combination thereof.

13. The antibody-like protein of Claim 7, comprising a heavy chain (HC) and a light chain (LC),
wherein the HC comprises an amino acid sequence having at least 98%, 95%, or 92% of sequence identity to SEQ ID NO. 85, 89, 93, 97, 101, 105, 109, 113, 117, 137, 141, 145, or 151;
and

wherein the LC comprises an amino acid sequence having at least 98%, 95%, or 92% of sequence identity to SEQ ID NO. 87, 91, 95, 99, 103, 107, 111, 115, 119, 139, 143, 147, or 149.

14. The antibody-like protein of Claim 7 comprising a heavy chain monomer and a light chain monomer, wherein the heavy chain monomer having a N-terminus and a C-terminus, comprising in tandem from the N-terminal to the C-terminal,

an optional first binding domain (D1) at the N-terminal,

a Fab domain as a second binding domain(D2) comprising a light chain,

a Fc domain,

an optional third binding domain (D3), and

an optional fourth binding domain (D4) at the C-terminal,

wherein the light chain comprises an optional fifth binding domain (D5) covalently attached to the C-terminus, an optional sixth binding domain (D6) covalently attached to the N-terminus, or a combination thereof, and wherein at least one of D1, D2, D3, D4, D5 and D6 comprises the EGFR binding domain.

15. The antibody-like protein of Claim 14, wherein at least one of the D1 or D2 comprises the EGFR binding domain.

16. The antibody-like protein of Claim 14, wherein each of the D3, D4, D5 and D6 comprises the EGFR binding domain.

17. The antibody-like protein of Claim 14, wherein antibody-like protein is a bispecific antibody comprising an amino acid sequence having at least 98% sequence identity to SEQ ID NO. 137, 145, 139, 147; 141, 145, 143, 147; 141, 145, 149, 147; 151, 145, 139, or 147.

18. The antibody-like protein of Claim 17, wherein the bispecific antibody is asymmetric with the D2 comprising the EGFR binding domain and the D3 has a binding specificity to CD3.

19. The antibody-like protein of Claim 7 having a N-terminus and a C-terminus, comprising,

a first monomer, comprising, from the N-terminus to the C-terminus, a first binding domain (mD1), a variable heavy (VH) chain, a CH1 domain, a first hinge, a first CH2 domain, a first CH3 domain, and a fourth binding domain (mD4),

a second monomer, comprising, from the N-terminus to the C-terminus, a second binding domain (mD2), a variable light (VL) chain, a CL domain, a second hinge, a second CH2 domain, and a second CH3 domain, and a fifth binding domain (mD5),

wherein the CH chain and CL chain forms a third binding domain (mD3),

wherein the first monomer and the second monomer are covalently paired through at least one disulfide bond between the CH1 domain and the CL domain and at least one disulfide bond between the first hinge and the second hinge, and

wherein the multi-specific antibody-like protein is at least bi-specific.

20. The antibody-like protein of Claim 19, wherein at least one of the mD1, mD2, mD3, mD4, and mD5 comprises the EGFR binding domain.

21. The antibody-like protein of Claim 19, wherein at least one of the mD3 or mD2 domain comprises the EGFR binding domain.
22. The antibody-like protein of Claim 19, wherein the mD2, mD4, mD5 each comprises the EGFR binding domain.
23. The antibody-like protein of Claim 19, comprising an amino acid sequence having at least 98% sequence identity to SEQ ID NO 121, 123, 125, 127, 129, 131, 133, 135, or a combination thereof.
24. A heavy chain, comprising an amino acid sequence having at least 98% sequence identity to SEQ ID NO 121, 125, 129, or 133.
25. A light chain, comprising an amino acid sequence having at least 98% sequence identity to 123, 127, 131, or 135.
26. An isolated nucleic acid sequence encoding the antibody-like protein of Claim 7.
27. An expression vector comprising the isolated nucleic acid sequences of Claim 26.
28. A host cell comprising the isolated nucleic acid sequence of Claim 26.
29. A pharmaceutical composition, comprising the antibody-like protein of Claim 7 and a pharmaceutically acceptable carrier.
30. An immunoconjugate comprising the antibody-like protein of Claim 7 and a cytotoxic agent.
31. A pharmaceutical composition, comprising the immunoconjugate of Claim 30 and a pharmaceutically acceptable carrier.
32. A method for treating or preventing a cancer, an autoimmune disease, or an infectious disease in a subject, said method comprising administering to the subject a pharmaceutical composition comprising a purified antibody-like protein of Claim 7.
33. A method for producing the antibody-like protein of Claim 7, comprising culturing a host cell such that the DNA sequence encoding the antibody-like protein of Claim 9 is expressed, and purifying said multi-specific antibody-like protein.
34. A solution comprising an effective concentration of the antibody-like protein of Claim 7, wherein the solution is blood plasma in a subject.

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1. A human epithelium growth factor receptor (EGFR) binding peptide having a binding specificity to human EGFR, comprising an amino acid sequence having at least 98% of sequence identity to SEQ ID NO. 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 57, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, or 83.

2. The EGFR binding peptide of Claim 1, comprising a variable heavy (VH) chain and a variable light (VL) chain,

wherein the VH chain comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO. 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, or 41; and

wherein the VL chain comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO. 3, 7, 11, 15, 19, 23, 27, 31, 35, 39, or 43.

3. The EGFR binding peptide of Claim 2, comprising a scFv domain, wherein the scFv domain comprises the VH chain and the VL chain, and wherein the scFv domain comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO. 57, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, or 83.

4. The EGFR binding peptide of Claim 3, comprising a histidine residue linked to at least one end of the scFv domain, and wherein the human EGFR binding peptide comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO. 57.

5. The EGFR binding peptide of Claim 2, comprising a Fab domain, wherein the Fab domain comprises the VH chain and the VL chain.

6. The EGFR binding peptide of Claim 5, further comprising a Fc domain linked to the Fab domain to provide a Fab-monoFc fusion protein, wherein the Fc domain comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO. 45 or 47.

7. An antibody-like protein having a binding specificity to human EGFR, comprising an EGFR binding domain having a variable heavy (VH) chain and a variable light (VL) chain,

wherein the VH chain comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO. 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, or 41; and

wherein the VL chain comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO. 3, 7, 11, 15, 19, 23, 27, 31, 35, 39, or 43.

8. The antibody-like protein of Claim 7, comprising a scFv domain having an amino acid sequence having at least 98% sequence identity to SEQ ID NO. 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, or 83.

9. The antibody-like protein of Claim 7, wherein the antibody-like protein is a monospecific antibody comprising an amino acid sequence having at least 98% sequence identity to SEQ ID NO. 137, 139; 141, 143; 141, 149, 151, 139; 145, or 147.

10. The antibody-like protein of Claim 7, wherein the antibody-like protein is a bispecific antibody comprising an amino acid sequence having at least 98% sequence identity to SEQ ID NO. 137, 145, 139, 147, 141, 145, 143, 147, 141, 145, 149, 147, 151, 145, 139, or 147.

11. The antibody-like protein of Claim 7, wherein the antibody-like protein is a penta-specific antibody comprising an amino acid sequence having at least 98% sequence identity to SEQ ID NO. 85, 87; 89, 91; 93, 95, 97, 99, 101, 103, 105, or 107.

12. The antibody-like protein of Claim 7, wherein the antibody-like protein is a hexa-specific antibody comprising an amino acid sequence having at least 98% sequence identity to SEQ ID NO. 109, 111, 113, 115, 117, or 119.

13. The antibody-like protein of Claim 7, comprising a heavy chain (HC) and a light chain (LC), wherein the HC comprises an amino acid sequence having at least 98% of sequence identity to SEQ ID NO. 85, 89, 93, 97, 101, 105, 109, 113, 117, 137, 141, 145, or 151; and

wherein the LC comprises an amino acid sequence having at least 98% of sequence identity to SEQ ID NO. 87, 91, 95, 99, 103, 107, 111, 115, 119, 139, 143, 147, or 149.

14. The antibody-like protein of Claim 7 comprising a heavy chain monomer and a light chain monomer, wherein the heavy chain monomer having a N-terminus and a C-terminus, comprising in tandem from the N-terminal to the C-terminal,

an optional first binding domain (D1) at the N-terminal,

a Fab domain as a second binding domain(D2) comprising a light chain,

a Fc domain,

an optional third binding domain (D3), and

an optional fourth binding domain (D4) at the C-terminal.

wherein the light chain comprises an optional fifth binding domain (D5) covalently attached to the C-terminus, an optional sixth binding domain (D6) covalently attached to the N-terminus, or a combination thereof, and wherein at least one of D1, D2, D3, D4, D5 and D6 comprises the EGFR binding domain.

15. The antibody-like protein of Claim 14, wherein at least one of the D1 or D2 comprises the EGFR binding domain.

16. The antibody-like protein of Claim 14, wherein each of the D3, D4, D5 and D6 comprises the EGFR binding domain.

17. The antibody-like protein of Claim 14, wherein antibody-like protein is a bispecific antibody comprising an amino acid sequence having at least 98% sequence identity to SEQ ID NO. 137, 145, 139, 147; 141, 145, 143, 147; 141, 145, 149, 147; 151, 145, 139, or 147.

18. The antibody-like protein of Claim 17, wherein the bispecific antibody is asymmetric with the D2 comprising the EGFR binding domain and the D3 has a binding specificity to CD3.

19. The antibody-like protein of Claim 7 having a N-terminus and a C-terminus, comprising,

a first monomer, comprising, from the N-terminus to the C-terminus, a first binding domain (mD1), a variable heavy (VH) chain, a CH1 domain, a first hinge, a first CH2 domain, a first CH3 domain, and a fourth binding domain (mD4),

a second monomer, comprising, from the N-terminus to the C-terminus, a second binding domain (mD2), a variable light (VL) chain, a CL domain, a second hinge, a second CH2 domain, and a second CH3 domain, and a fifth binding domain (mD5),

wherein the CH chain and CL chain forms a third binding domain (mD3),

wherein the first monomer and the second monomer are covalently paired through at least one disulfide bond between the CH1 domain and the CL domain and at least one disulfide bond between the first hinge and the second hinge,

wherein the multi-specific antibody-like protein is at least bi-specific, and

wherein at least one of the mD1, mD2, mD3, mD4, and mD5 comprises the EGFR binding domain.

20. The antibody-like protein of Claim 19, wherein at least one of the mD3 or mD2 domain comprises the EGFR binding domain.

21. The antibody-like protein of Claim 19, wherein the mD2, mD4, mD5 each comprises the EGFR binding domain.

22. The antibody-like protein of Claim 19, comprising an amino acid sequence having at least 98% sequence identity to SEQ ID NO. 121, 123, 125, 127, 129, 131, 133, 135, or a combination thereof.

23. An isolated nucleic acid sequence encoding the antibody-like protein of any one of Claims 7 to 22.

24. An expression vector comprising the isolated nucleic acid sequences of Claim 23.

25. A host cell comprising the isolated nucleic acid sequence of Claim 23.

26. A pharmaceutical composition, comprising the antibody-like protein of any one of Claims 7 to 22 and a pharmaceutically acceptable carrier.

27. An immunoconjugate comprising the antibody-like protein of any one of Claims 7 to 22 and a cytotoxic agent.

28. A pharmaceutical composition, comprising the immunoconjugate of Claim 27 and a pharmaceutically acceptable carrier.

29. Use of a purified antibody-like protein of any one of Claims 7 to 22 in the preparation of a medicament for treating or preventing a cancer, an autoimmune disease, or an infectious disease in a subject.

30. A method for producing the antibody-like protein of any one of Claims 7 to 22, comprising

culturing a host cell such that the DNA sequence encoding the antibody-like protein of any one of Claims 7 to 22 is expressed, and

purifying said multi-specific antibody-like protein.