

CLAIMS

1. A VHH domain targeting VEGF, wherein the VHH domain comprises CDRs 1-3, wherein CDR1 comprises an amino acid sequence set forth in SEQ ID NO: 1, CDR2 comprises an amino acid sequence set forth in SEQ ID NO: 2 with or without one or two amino acid mutations, and CDR3 comprises an amino acid sequence set forth in SEQ ID NO: 3.

2. The VHH domain according to claim 1, wherein the amino acid mutation in CDR2 is at position 58 and/or position 65;

preferably the amino acid mutation in CDR2 is N58Y and/or D65G mutation;

preferably, the VHH domain is a humanized VHH domain;

preferably, the VHH domain further comprises:

1) a first framework region domain FR1 comprising an amino acid sequence set forth in SEQ ID NO: 4 with or without one or two amino acid mutations; and/or

2) a second framework region domain FR2 comprising an amino acid sequence set forth in SEQ ID NO: 5 with or without one amino acid mutation; and/or

3) a third framework region domain FR3 comprising an amino acid sequence set forth in SEQ ID NO: 6 with or without 1-5 amino acid mutations; and/or

4) a fourth framework region domain FR4 comprising an amino acid sequence set forth in SEQ ID NO: 7 with or without one amino acid mutation;

preferably, the one or two amino acid mutations in FR1 are at position 1 and/or position 5; preferably Q1E and/or Q5L mutations; further, the one amino acid mutation in FR2 is at position 49; preferably A49S mutation;

preferably, the 1-5 amino acid mutations in FR3 are at positions selected from the group consisting of positions 74, 82B, 83, 84, 89, and a combination thereof; preferably, the 1-5 amino acid mutations are selected from the group consisting of D74S, V(82B)S, K83R, P84A, M89V and a combination thereof.

preferably, the one amino acid mutation in FR4 is at position 108; preferably Q108L;

preferably, the VHH domain further comprises:

1) a first framework region domain FR1 comprising an amino acid sequence set forth in SEQ ID NO: 4 or SEQ ID NO: 9; and/or

2) a second framework region domain FR2 comprising an amino acid sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 10; and/or

3) a third framework region domain FR3 comprising an amino acid sequence set forth in SEQ ID NO: 6 or SEQ ID NO: 11; and/or

4) a fourth framework region domain FR4 comprising an amino acid sequence set forth in SEQ ID NO: 7 or SEQ ID NO: 12;

preferably, the VHH domain comprises:

1) a framework region combination of FR1 comprising an amino acid sequence set forth in SEQ ID NO: 4, FR2 comprising an amino acid sequence set forth in SEQ ID NO: 5, FR3 comprising an amino acid sequence set forth in SEQ ID NO: 6, and FR4 comprising an amino acid sequence set forth in SEQ ID NO: 7;

2) a framework region combination of FR1 comprising an amino acid sequence set forth in SEQ ID NO: 9, FR2 comprising an amino acid sequence set forth in SEQ ID NO: 10, FR3 comprising an amino acid sequence set forth in SEQ ID NO: 11, and FR4 comprising an amino acid sequence set forth in SEQ ID NO: 12; or

3) a framework region combination of FR1 comprising an amino acid sequence set forth in SEQ ID NO: 9, FR2 comprising an amino acid sequence set forth in SEQ ID NO: 5, FR3 comprising an amino acid sequence set forth in SEQ ID NO: 11, and FR4 comprising an amino acid sequence set forth in SEQ ID NO: 12.

3. The VHH domain according to claim 1, wherein the VHH domain comprises an amino acid sequence set forth in SEQ ID NO: 8 with or without 1-11 amino acid mutations;

preferably, the amino acid mutation is at a position selected from the group consisting of

positions 1, 5, 49, 58, 65, 74, 82B, 83, 84, 89, 108, and a combination thereof.

preferably, the amino acid mutation is selected from the group consisting of Q1E, Q5L, A49S, N58Y, D65G, D74S, V(82B)S, K83R, P84A, M89V, Q108L and a combination thereof.

preferably, the mutation is a mutation combination of

1) Q1E+Q5L+A49S+N58Y+D65G+D74S+V(82B)S+K83R+P84A+M89V+Q108L; or

2) Q1E+Q5L+N58Y+D65G+D74S+V(82B)S+K83R+P84A+M89V+Q108L;

preferably, the VHH domain comprises an amino acid sequence set forth in SEQ ID NO: 8, SEQ ID NO: 13 or SEQ ID NO: 14.

4. Use of the VHH domain according to any one of claims 1 to 3 in the construction of a recombinant protein.

5. A recombinant protein comprising the VHH domain according to any one of claims 1-3:.

preferably, wherein the recombinant protein is selected from the group consisting of a bispecific antibody, a multispecific antibody, and an antibody-drug conjugate.

6. A polynucleotide encoding the VHH domain according to any one of claims 1 to 3, or the recombinant protein according to claim 5.

7. An expression vector comprising the polynucleotide according to claim 6.

8. A host cell comprising the expression vector according to claim 7 or integrated with the polynucleotide according to claim 6 into the genome.

9. A pharmaceutical composition comprising the VHH domain according to any one of

claims 1 to 3, or the recombinant protein according to claim 5, and a pharmaceutically acceptable carrier.

10. Use of the VHH domain according to any one of claims 1 to 3, or the recombinant protein according to claim 5 in the manufacture of a medicament for treating cancer;

preferably the cancer is colorectal cancer.