Claims

1. A recombinant binding protein comprising at least one ankyrin repeat domain, wherein said repeat domain binds VEGF-Axxx with a Kd below 10^{-7}M and inhibits VEGF-Axxx binding to VEGFR-2.

2. The binding protein of claim 1, wherein said ankyrin repeat domain inhibits sprouting of HUVEC spheroids with an IC_{50} value below 10 nM.

3. The binding protein of claim 1, wherein the K_d for the interaction of said ankyrin repeat domain with VEGF-Axxxb is at least 10-fold higher compared to the K_d for the interaction of said repeat domain with the corresponding VEGF-Axxx.

4. The binding protein of claim 1, wherein said ankyrin repeat domain comprises a repeat module represented by the ankyrin repeat sequence motif
1D23GWTPLHL4ADLG5LEIVEVLLK6GADVN7 (SEQ ID NO:4)
wherein
1 represents an amino acid residue selected from the group consisting of K, T and Y;
2 represents the amino acid residue N or M;
3 represents the amino acid residue T or F;
4 represents the amino acid residue S or A;
5 represents the amino acid residue H or R;
6 represents an amino acid residue selected from the group consisting of A, Y, H, and N; and
7 represents the amino acid residue A or T.

5. The binding protein of claim 1, wherein said ankyrin repeat domain comprises a repeat module represented by the ankyrin repeat sequence motif
1D23G4TPLHLAA56GH7EIVEVLLK8GADVNA (SEQ ID NO:5)
wherein
1 represents an amino acid residue selected from the group consisting of A, N, R, V, Y, E, H, I, K, L, Q, S and T;
2 represents an amino acid residue selected from the group consisting of S, A, N, R, D, F, L, P, T and Y;

3 represents an amino acid residue selected from the group consisting of T, V, S, A, L and F;
4 represents an amino acid residue selected from the group consisting of W, F and H;
5 represents an amino acid residue selected from the group consisting of P, I, A, L, S, T, V and Y;
6 represents an amino acid residue selected from the group consisting of W, F, I, L, T and V;
7 represents the amino acid residue L or P; and
8 represents an amino acid residue selected from the group consisting of A, H, N and Y.

6. The binding protein of claim 1, wherein said ankyrin repeat domain comprises a repeat module represented by the ankyrin repeat sequence motif
1D23GWTPLHLAA45GHLEIVEVLLK6GADVNA (SEQ ID NO:7)
wherein
1 represents an amino acid residue selected from the group consisting of K, M, N, R and V;
2 represents an amino acid residue selected from the group consisting of Y, H, M and V;
3 represents an amino acid residue selected from the group consisting of F, L, M and V;
4 represents an amino acid residue selected from the group consisting of R, H, V, A, K and N;
5 represents an amino acid residue selected from the group consisting of F, D, H, T, Y, M and K; and
6 represents an amino acid residue selected from the group consisting of A, H, N and Y.

7. The binding protein of claim 1, wherein said ankyrin repeat domain comprises a repeat module represented by the ankyrin repeat sequence motif
1DFK2DTPLHLAA34GH5EIVEVLLK6GADVNA (SEQ ID NO:10)
wherein
1 represents an amino acid residue selected from the group consisting of L, S and T;
2 represents an amino acid residue selected from the group consisting of G, S and C;
3 represents the amino acid residue S or A;
4 represents an amino acid residue selected from the group consisting of Q, S, M and N;
5 represents an amino acid residue selected from the group consisting of L, M and Q; and
6 represents an amino acid residue selected from the group consisting of A, H, N, Y and D.

8. The binding protein of claim 1, wherein said ankyrin repeat domain comprises a repeat module represented by the ankyrin repeat sequence motif
1D23G4TPL5LAA67GHLEIVEVLLK8GADVNA (SEQ ID NO:12)
wherein
1 represents an amino acid residue selected from the group consisting of K, S, I, N, T and V;
2 represents an amino acid residue selected from the group consisting of K, N, W, A, H, M, Q and S;
3 represents an amino acid residue selected from the group consisting of F, Q, L, H and V;
4 represents the amino acid residue F or T;
5 represents the amino acid residue Q or H;
6 represents the amino acid residue Y or S;
7 represents an amino acid residue selected from the group consisting of N, H, Y and M; and
8 represents an amino acid residue selected from the group consisting of A, H, N and Y.

9. The binding protein of claim 1, wherein said ankyrin repeat domain comprises a repeat module comprising the ankyrin repeat sequence motif of SEQ ID NO:6, wherein said repeat module is preceded by a repeat module represented by the ankyrin repeat sequence motif of SEQ ID NO:5.

10. The binding protein of claim 1, wherein said ankyrin repeat domain comprises a repeat module comprising the ankyrin repeat sequence motif of SEQ ID NO:3, wherein said repeat module is preceded by a repeat module represented by the ankyrin repeat sequence motif of SEQ ID NO:2 and/or followed by a repeat module represented by the ankyrin repeat sequence motif of SEQ ID NO:4.

11. The binding protein of claim 1, wherein said repeat domain competes for binding to VEGF-Axxx with an ankyrin repeat domain selected from the group consisting of SEQ ID NOs:16, 22, 23, 29, 30, 33, 34, 36, 39 and 40.

12. The binding protein of claim 1, wherein said ankyrin repeat domain is selected from the group consisting of SEQ ID NOs:14 to 40.

13. The binding protein of claim 1, wherein said ankyrin repeat domain comprises an amino acid sequence that has at least 75% amino acid sequence identity with one ankyrin repeat domain selected from the group consisting of SEQ ID NOs:14 to 40.

14. The binding protein of claim 1, wherein one or more of the amino acid residues of the repeat modules of said repeat domain are exchanged by an amino acid residue found at the corresponding position on alignment of a repeat unit.

15. A binding protein of any one of claims 1 to 14 additionally comprising a non-proteinaceous polymer moiety.

16. A nucleic acid encoding a binding protein according to any one of claims 1 to 14.

17. A pharmaceutical composition comprising the binding protein of any one of claims 1 to 15 or the nucleic acid of claim 16, and optionally a pharmaceutical acceptable carrier and/or diluent.

18. Use of a binding protein of any one of claims 1 to 15 or the nucleic acid of claim 16 in the manufacture of a medicament for the treatment of an eye disorder.

19. Use of a binding protein of any one of claims 1 to 15 or the nucleic acid of claim 16 in the manufacture of a medicament for treating pathological angiogenesis.

Claims

1. A recombinant binding protein comprising at least one ankyrin repeat domain, wherein said ankyrin repeat domain binds VEGF-A165 with a Kd below 10^{-7}M, and wherein said ankyrin repeat domain comprises a repeat module with the ankyrin repeat sequence motif 1D23GWTPLHL4ADLG5LEIVEVLLK6GADVN7 (SEQ ID NO:4)
wherein
1 represents an amino acid residue selected from the group consisting of K, T and Y;
2 represents the amino acid residue N or M;
3 represents the amino acid residue T or F;
4 represents the amino acid residue S or A;
5 represents the amino acid residue H or R;
6 represents an amino acid residue selected from the group consisting of A, Y, H, and N; and
7 represents the amino acid residue A or T.

2. A recombinant binding protein comprising at least one ankyrin repeat domain, wherein said ankyrin repeat domain binds VEGF-A165 with a Kd below 10^{-7}M, and wherein said ankyrin repeat domain comprises a repeat module with the ankyrin repeat sequence motif 1D23GWTPLHLAA45GHLEIVEVLLK6GADVNA (SEQ ID NO:7)
wherein
1 represents an amino acid residue selected from the group consisting of K, M, N, R and V;
2 represents an amino acid residue selected from the group consisting of Y, H, M and V;
3 represents an amino acid residue selected from the group consisting of F, L, M and V;
4 represents an amino acid residue selected from the group consisting of R, H, V, A, K and N;
5 represents an amino acid residue selected from the group consisting of F, D, H, T, Y, M and K; and
6 represents an amino acid residue selected from the group consisting of A, H, N and Y.

3. A recombinant binding protein comprising at least one ankyrin repeat domain, wherein said ankyrin repeat domain binds VEGF-A165 with a Kd below 10^{-7}M, and wherein said ankyrin repeat domain comprises a repeat module with the ankyrin repeat sequence motif 1DFK2DTPLHLAA34GH5EIVEVLLK6GADVNA (SEQ ID NO:10)
wherein

1 represents an amino acid residue selected from the group consisting of L, S and T;
2 represents an amino acid residue selected from the group consisting of G, S and C;
3 represents the amino acid residue S or A;
4 represents an amino acid residue selected from the group consisting of Q, S, M and N;
5 represents an amino acid residue selected from the group consisting of L, M and Q; and
6 represents an amino acid residue selected from the group consisting of A, H, N, Y and D.

4. A recombinant binding protein comprising at least one ankyrin repeat domain, wherein said ankyrin repeat domain binds VEGF-A165 with a Kd below 10^{-7}M, and wherein said ankyrin repeat domain comprises a repeat module with the ankyrin repeat sequence motif 1D23G4TPL5LAA67GHLEIVEVLLK8GADVNA (SEQ ID NO:12)
wherein
1 represents an amino acid residue selected from the group consisting of K, S, I, N, T and V;
2 represents an amino acid residue selected from the group consisting of K, N, W, A, H, M, Q and S;
3 represents an amino acid residue selected from the group consisting of F, Q, L, H and V;
4 represents the amino acid residue F or T;
5 represents the amino acid residue Q or H;
6 represents the amino acid residue Y or S;
7 represents an amino acid residue selected from the group consisting of N, H, Y and M; and
8 represents an amino acid residue selected from the group consisting of A, H, N and Y.

5. A recombinant binding protein comprising at least one ankyrin repeat domain, wherein said ankyrin repeat domain binds VEGF-A165 with a Kd below 10^{-7}M, and wherein said ankyrin repeat domain comprises a repeat module with the ankyrin repeat sequence motif 1D23G4TPLHLAA56GH7EIVEVLLK8GADVNA (SEQ ID NO:5)
wherein
1 represents an amino acid residue selected from the group consisting of A, N, R, V, Y, E, H, I, K, L, Q, S and T;
2 represents an amino acid residue selected from the group consisting of S, A, N, R, D, F, L, P, T and Y;

3 represents an amino acid residue selected from the group consisting of T, V, S, A, L and F;
4 represents an amino acid residue selected from the group consisting of W, F and H;
5 represents an amino acid residue selected from the group consisting of P, I, A, L, S, T, V and Y;
6 represents an amino acid residue selected from the group consisting of W, F, I, L, T and V;
7 represents the amino acid residue L or P; and
8 represents an amino acid residue selected from the group consisting of A, H, N and Y;
and/or a repeat module with the ankyrin repeat sequence motif
1D23G4TPLHLAA56GHLEIVEVLLK7GADVNA (SEQ ID NO:6)
wherein
1 represents an amino acid residue selected from the group consisting of H, Q, A, K, R, D, I, L, M, N, V and Y;
2 represents an amino acid residue selected from the group consisting of Y, F and H;
3 represents an amino acid residue selected from the group consisting of Q, F and T;
4 represents an amino acid residue selected from the group consisting of W, M, G, H, N and T;
5 represents an amino acid residue selected from the group consisting of T, A, M, L and V;
6 represents an amino acid residue selected from the group consisting of I, L, V, D and T; and
7 represents an amino acid residue selected from the group consisting of A, H, N and Y.

6. The binding protein of claim 5, wherein said ankyrin repeat domain comprises the repeat module with the ankyrin repeat sequence motif of SEQ ID NO:6 preceded by the repeat module with the ankyrin repeat sequence motif of SEQ ID NO:5.

7. The binding protein of claim 5, wherein said repeat domain competes for binding to VEGF-A165 with an ankyrin repeat domain selected from the group consisting of SEQ ID NOs:16, 22, 23, 29, 30, 33, 34, 36, 39 and 40.

8. The binding protein of claim 5 additionally comprising a non-proteinaceous polymer moiety.

9. A pharmaceutical composition comprising the binding protein of claim 5, and optionally a pharmaceutical acceptable carrier and/or diluent.

10. The binding protein of claim 5 wherein, in the ankyrin repeat sequence motif 1D23G4TPLHLAA56GH7EIVEVLLK8GADVNA (SEQ ID NO:5),
1 represents an amino acid residue selected from the group consisting of A, N, R, V, Y, E, H, I, K, L, Q, S and T;
2 represents an amino acid residue selected from the group consisting of S, A, N and R;
3 represents an amino acid residue selected from the group consisting of T, V, S, A and L;
4 represents an amino acid residue selected from the group consisting of W, F and H;
5 represents the amino acid residue P or I;
6 represents an amino acid residue selected from the group consisting of W, F, I, L, T and V;
7 represents the amino acid residue L or P; and
8 represents an amino acid residue selected from the group consisting of A, H, N and Y.

11. The binding protein of claim 5 wherein, in the ankyrin repeat sequence motif 1D23G4TPLHLAA56GH7EIVEVLLK8GADVNA (SEQ ID NO:5),
1 represents the amino acid residue selected from the group consisting of A, N, R, V, Y, E, H, I, K, L, Q, S and T;
2 represents an amino acid residue selected from the group consisting of S, A, N and R;
3 represents an amino acid residue selected from the group consisting of T, V and S;
4 represents an amino acid residue selected from the group consisting of W, F and H;
5 represents the amino acid residue P or I;
6 represents an amino acid residue selected from the group consisting of W, F, I, L, T and V;
7 represents the amino acid residue L or P; and
8 represents an amino acid residue selected from the group consisting of A, H, N and Y.

12. The binding protein of claim 5 comprising the repeat domain of SEQ ID NO: 29.

13. The binding protein of claim 5 wherein, in the ankyrin repeat sequence motif 1D23G4TPLHLAA56GHLEIVEVLLK7GADVNA (SEQ ID NO:6),
1 represents an amino acid residue selected from the group consisting of H, Q, A, K, R, D, I, L, M, N, V and Y;
2 represents an amino acid residue selected from the group consisting of Y, F and H;

3 represents an amino acid residue selected from the group consisting of Q, F and T;
4 represents the amino acid residue W or M;
5 represents an amino acid residue selected from the group consisting of T, A and M;
6 represents an amino acid residue selected from the group consisting of I, L and V; and
7 represents an amino acid residue selected from the group consisting of A, H, N and Y.

14. The binding protein of claim 5 wherein, in the ankyrin repeat sequence motif 1D23G4TPLHLAA56GHLEIVEVLLK7GADVNA (SEQ ID NO:6),
1 represents the amino acid residue H, Q, A, K and R;
2 represents an amino acid residue selected from the group consisting of Y, F and H;
3 represents an amino acid residue selected from the group consisting of Q, F and T;
4 represents the amino acid residue W or M;
5 represents an amino acid residue selected from the group consisting of T, A and M;
6 represents an amino acid residue selected from the group consisting of I, L and V; and
7 represents an amino acid residue selected from the group consisting of A, H, N and Y.