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[0006] Amino acid substitutions that reduce binding of the Fc region to FcγR may result in a reduced thermal stability of the Fc region (see, for example, International Publication No. WO 2020/086776 and J Biol Chem; 292(5):1865-75 (2017)).

[0007] International Publication No. WO 2013/118858 describes amino acid substitutions in the Fc region that improve thermal stability as well as increase or decrease an effector function. WO 2013/118858 shows that a combination of amino acid substitutions, each of which independently improves thermal stability, does not necessarily improve thermal stability in an additive manner.

SUMMARY

[0008] Described herein are Fc variants with high thermal stability and attenuated effector function. One example of the disclosure relates to a polypeptide comprising one or more variant Fc regions, each of the variant Fc regions comprising two CH2 domains, wherein at least one CH2 domain in at least one of the variant Fc regions is a variant CH2 domain comprising amino acid substitutions at positions 234, 235 and 265 (all positions expressed by EU numbering), wherein the amino acid residues at positions 234, 235, and 265 of the variant CH2 domain are Ala, Ala, and Gly, respectively, or Ala, Ala, and Asn, respectively, and wherein each of the one or more variant Fc regions is a variant IgG1 Fc region, a variant IgG4 Fc region or a variant IgG1/IgG4 Fc region.

[0008a] In one aspect, provided herein is a polypeptide comprising one or more variant Fc regions, each of the variant Fc regions comprising two CH2 domains, wherein the CH2 domains in at least one of the variant Fc regions are variant CH2 domains comprising amino acid substitutions at positions 234, 235 and 265, wherein the amino acid residues at positions 234, 235, and 265 of the variant CH2 domains are Ala, Ala,

and Gly, respectively, or Ala, Ala, and Asn, respectively, wherein each of the one or more variant Fc regions is a variant IgG1 Fc region, a variant IgG4 Fc region or a variant IgG1/IgG4 Fc region, and wherein the polypeptide has reduced binding affinity to an FcγRI, an FcγRII and an FcγRIII as compared to a parent polypeptide comprising parent CH2 domains without amino acid substitutions at positions 234, 235 and 265, and wherein all positions are expressed by EU numbering.

[0008b] In another aspect, provided herein is a fusion protein comprising the polypeptide described herein and a heterologous polypeptide

[0008c] In another aspect, provided herein is a polypeptide-drug conjugate comprising the polypeptide described herein conjugated to one or more drugs or modifying agents.

[0008d] In another aspect, provided herein is a nucleic acid comprising a nucleotide sequence encoding the polypeptide described herein.

[0008e] In another aspect, provided herein is a host cell comprising the nucleic acid described herein.

[0008f] In another aspect, provided herein is a method for producing the polypeptide described herein, the method comprising culturing the host cell described herein in a medium under conditions suitable for expression of the polypeptide, and recovering the polypeptide from the medium or the host cell.

[0008g] In another aspect, provided herein is a pharmaceutical composition comprising the polypeptide described herein, the fusion protein described herein, or the polypeptide-drug conjugate described herein, and a pharmaceutically acceptable carrier.

[0008h] In another aspect, provided herein is a polypeptide described herein, a fusion protein described herein, or a polypeptide-drug conjugate described herein, for use in therapy.

[0008i] In another aspect, provided herein is the use of the polypeptide described herein, the fusion protein described herein or polypeptide-drug conjugate described herein in the manufacture of a medicament for treatment of a cancer, an immune disease, an inflammatory disease or an infectious disease.

[0009] Another example of the disclosure relates to a molecule comprising a polypeptide as described herein.

[0010] Another example of the disclosure relates to a polypeptide-drug conjugate comprising a polypeptide as described herein conjugated to one or more drugs or modifying agents.

[0011] Another example of the disclosure relates to a nucleic acid comprising a nucleotide sequence encoding a polypeptide as described herein or a portion thereof.

[0012] Another example of the disclosure relates to a host cell comprising a nucleic acid that comprises a nucleotide sequence encoding a polypeptide as described herein or a portion thereof.

[0013] Another example relates to a method for producing a polypeptide as described herein comprising culturing a host cell comprising a nucleic acid that comprises a nucleotide sequence encoding the polypeptide or a portion thereof in a medium under conditions suitable for expression of the polypeptide, and recovering the polypeptide from the medium or the host cell.

[0014] Another example of the disclosure relates to a pharmaceutical composition comprising a polypeptide as described herein, a molecule comprising the polypeptide, or a polypeptide-drug conjugate comprising the polypeptide conjugated to a drug or a modifying agent, and a pharmaceutically acceptable carrier.

[0015] Another example relates to a use of a polypeptide as described herein, a molecule comprising the polypeptide, or a polypeptide-drug conjugate comprising the polypeptide conjugated to a drug or a modifying agent, in therapy.

BRIEF DESCRIPTION OF DRAWINGS

[0016] **Figure 1** is a schematic illustration showing a structure of a general IgG antibody. The IgG antibody is composed of heavy chains, each consisting of VH, CH1, hinge, CH2, and CH3, and light chains, each consisting of VL and CL.

[0017] **Figure 2A** shows binding activity to human FcγRI for IgG1 WT obtained by replacing the IgG2 Fc region of the anti-EGFR antibody, panitumumab, with a human wildtype IgG1 Fc region; a variant obtained by introducing L234A/L235A (LALA) mutations into the Fc region of IgG1 WT; a variant obtained by introducing L234A/L235A/D265A (LALA-DA) mutations into the Fc region of IgG1 WT; and a variant obtained by introducing L234A/L235A/P329G (LALA-PG) mutations into the Fc region of IgG1 WT, as evaluated by an SPR method.

WHAT IS CLAIMED IS:

1. A polypeptide comprising one or more variant Fc regions, each of the variant Fc regions comprising two CH2 domains, wherein the CH2 domains in at least one of the variant Fc regions are variant CH2 domains comprising amino acid substitutions at positions 234, 235 and 265, wherein the amino acid residues at positions 234, 235, and 265 of the variant CH2 domains are Ala, Ala, and Gly, respectively, or Ala, Ala, and Asn, respectively, wherein each of the one or more variant Fc regions is a variant IgG1 Fc region, a variant IgG4 Fc region or a variant IgG1/IgG4 Fc region, and wherein the polypeptide has reduced binding affinity to an FcγRI, an FcγRII and an FcγRIII as compared to a parent polypeptide comprising parent CH2 domains without amino acid substitutions at positions 234, 235 and 265, and wherein all positions are expressed by EU numbering.
2. The polypeptide according to claim 1, further comprising an amino acid substitution at position 329 of the variant CH2 domain, wherein the amino acid residue at position 329 of the variant CH2 domain is Ala.
3. The polypeptide according to claim 1 or 2, wherein the FcγRI, FcγRII and FcγRIII are human FcγRI, FcγRII and FcγRIII.
4. The polypeptide according to claim 1 or 2, wherein the FcγRI, FcγRII and FcγRIII are cynomolgus FcγRI, FcγRII and FcγRIII.
5. The polypeptide according to any one of claims 1 to 4, wherein the polypeptide comprises one or more variant IgG1 Fc regions.

6. The polypeptide according to any one of claims 1 to 4, wherein the polypeptide comprises one variant Fc region.
7. The polypeptide according to any one of claims 1 to 4, wherein the polypeptide comprises one variant IgG1 Fc region.
8. The polypeptide according to any one of claims 1 to 7, wherein the amino acid residues at positions 234, 235, and 265 of the variant CH2 domain are Ala, Ala, and Gly, respectively.
9. The polypeptide according to any one of claims 1 to 7, wherein the amino acid residues at positions 234, 235, and 265 of the variant CH2 domain are Ala, Ala, and Asn, respectively.
10. The polypeptide according to claim 9, wherein a thermal denaturation midpoint temperature (T_m) of the variant CH2 domain is within 1°C of the T_m of a parent CH2 domain without amino acid substitutions at positions 234, 235 and 265.
11. The polypeptide according to claim 8, wherein a thermal denaturation midpoint temperature (T_m) of the variant CH2 domain is higher by 1°C or more than the T_m of a parent CH2 domain without amino acid substitutions at positions 234, 235 and 265.
12. The polypeptide according to claim 11, wherein the T_m of the variant CH2 domain is higher by 3°C or more than the T_m of the parent CH2 domain.
13. The polypeptide according to any one of claims 1 to 12, wherein the polypeptide is an antibody or a functional fragment thereof.

14. The polypeptide according to claim 13, wherein the antibody is a bispecific or multispecific antibody.
15. The polypeptide according to claim 13 or claim 14, wherein the antibody or functional fragment thereof binds a tumor antigen.
16. A fusion protein comprising the polypeptide according to any one of claims 1 to 14 and a heterologous polypeptide.
17. A fusion protein comprising the polypeptide according to claim 15 and a heterologous polypeptide.
18. A polypeptide-drug conjugate comprising the polypeptide according to any one of claims 1 to 15 conjugated to one or more drugs or modifying agents.
19. The polypeptide-drug conjugate according to claim 18, wherein the drug is a chemotherapeutic agent or a cytotoxic agent.
20. The polypeptide-drug conjugate according to claim 18, wherein the modifying agent is a non-proteinaceous polymer.
21. A nucleic acid comprising a nucleotide sequence encoding the polypeptide according to any one of claims 1 to 15.
22. A host cell comprising the nucleic acid according to claim 21.

23. A method for producing the polypeptide according to any one of claims 1 to 15, the method comprising culturing the host cell according to claim 22 in a medium under conditions suitable for expression of the polypeptide, and recovering the polypeptide from the medium or the host cell.
24. A pharmaceutical composition comprising the polypeptide according to any one of claims 1 to 15, the fusion protein according to claim 16 or claim 17, or the polypeptide-drug conjugate according to any one of claims 18 to 20, and a pharmaceutically acceptable carrier.
25. A polypeptide according to claim 15, a fusion protein according to claim 17, or a polypeptide-drug conjugate according to claim 19, for use in therapy.
26. Use of the polypeptide according to claim 15, the fusion protein according to claim 17 or polypeptide-drug conjugate according to claim 19 in the manufacture of a medicament for treatment of a cancer, an immune disease, an inflammatory disease or an infectious disease.