nucleotide sequences encoding the T-cell modulatory multimeric polypeptides, and host cells comprising the nucleic acids. The present disclosure provides methods of modulating the activity of a T cell; the methods comprise contacting the T cell with a T-cell modulatory multimeric polypeptide of the present disclosure.

[0004a] In a first aspect, there is provided a multimeric polypeptide comprising a heterodimeric polypeptide, wherein the heterodimeric polypeptide comprises:

a) a first polypeptide comprising:

i) a peptide epitope comprising a human papilloma virus E7 epitope, wherein the peptide epitope comprises the amino acid sequence YMLDLQPETT (SEQ ID NO:13) and has a length of 10 amino acids; and

ii) a first major histocompatibility complex (MHC) polypeptide, wherein the first MHC polypeptide is a β2-microglobulin (β2M) polypeptide; and

b) a second polypeptide comprising:

i) a second MHC polypeptide, wherein the second MHC polypeptide is an MHC class I heavy chain polypeptide; and

ii) an immunoglobulin (Ig) Fc polypeptide,

wherein the multimeric polypeptide comprises one or more immunomodulatory polypeptides, wherein the one or more immunomodulatory polypeptides is:

A) at the C-terminus of the first polypeptide;

B) at the N-terminus of the second polypeptide;

C) at the C-terminus of the second polypeptide; or

D) at the C-terminus of the first polypeptide and at the N-terminus of the second polypeptide, and

wherein at least one of the one or more immunomodulatory polypeptides is a variant IL2 polypeptide that comprises an H16A substitution and an F42A substitution relative to set forth in SEQ ID NO:1 and that exhibits reduced binding affinity to an IL-2 receptor (IL2R) comprising alpha, beta, and gamma polypeptides having amino acid sequences depicted in FIG. 3A-3C, compared to the binding affinity of the IL-2 amino acid sequence set forth in SEQ ID NO: 1 for the IL2R, and

wherein the first polypeptide and the second polypeptide are covalently linked to one another via a disulfide bond.

[0004b] In a second aspect, there is provided a protein comprising two of the multimeric polypeptides of the first aspect.

[0004c] In a third aspect, there is provided a nucleic acid comprising a nucleotide sequence encoding a first and a second polypeptide of the first aspect.

[0004d] In a fourth aspect, there is provided an expression vector comprising the nucleic acid of the third aspect.

[0004e] In a fifth aspect, there is provided a host cell genetically modified with the expression vector of the fourth aspect.

[0004f] In a sixth aspect, there is provided a method of producing the multimeric polypeptide of the first aspect or a protein of the second aspect, the method comprising culturing a host cell of the fifth aspect *in vitro* in a culture medium under conditions such that the host cell synthesizes the multimeric polypeptide.

[0004g] In a seventh aspect, there is provided an *in vitro* method of selectively activating an epitope-specific T cell, the method comprising contacting the T cell with the multimeric polypeptide of the first aspect or a protein of the second aspect wherein said contacting selectively activates the epitope-specific T cell.

[0004h] In an eighth aspect, there is provided a composition comprising:

a) the multimeric polypeptide of the first aspect or a protein of the second aspect; and

b) a pharmaceutically acceptable excipient.

[0004i] In a ninth aspect, there is provided use of the multimeric polypeptide of the first aspect or a protein of the second aspect, or a composition of the eighth aspect in the manufacture of a medicament for treating cancer in an individual.

BRIEF DESCRIPTION OF THE DRAWINGS

[0005] **FIG. 1A-1D** schematically depict various embodiments of a T-cell modulatory multimeric polypeptide of the present disclosure. In these embodiments, disulfide bonds are formed between MHC (e.g., HLA) polypeptides present in separate polypeptides.

[0006] **FIG. 2A-2Q** provide an amino acid sequence of wild-type human IL-2 (FIG. 2A); and amino acid sequences of variant IL-2 polypeptides (FIG. 2B-2Q).

[0007] **FIG. 3A-3C** provide amino acid sequences of IL-2 receptor alpha chain (FIG. 3A), beta chain (FIG. 3B), and gamma chain (FIG. 3C).

[0008] **FIG. 4A-4C** provide amino acid sequences of immunoglobulin Fc polypeptides.

[0009] **FIG. 5A-5C** provide amino acid sequences of human leukocyte antigen (HLA) Class I heavy chain polypeptides. Signal sequences are underlined.

CLAIMS

What is claimed is:

1. A multimeric polypeptide comprising a heterodimeric polypeptide, wherein the heterodimeric polypeptide comprises:

a) a first polypeptide comprising:

i) a peptide epitope comprising a human papilloma virus E7 epitope, wherein the peptide epitope comprises the amino acid sequence YMLDLQPETT (SEQ ID NO:13) and has a length of 10 amino acids; and

ii) a first major histocompatibility complex (MHC) polypeptide, wherein the first MHC polypeptide is a β2-microglobulin (β2M) polypeptide; and

b) a second polypeptide comprising:

i) a second MHC polypeptide, wherein the second MHC polypeptide is an MHC class I heavy chain polypeptide; and

ii) an immunoglobulin (Ig) Fc polypeptide,

wherein the multimeric polypeptide comprises one or more immunomodulatory polypeptides, wherein the one or more immunomodulatory polypeptides is:

A) at the C-terminus of the first polypeptide;

B) at the N-terminus of the second polypeptide;

C) at the C-terminus of the second polypeptide; or

D) at the C-terminus of the first polypeptide and at the N-terminus of the second polypeptide, and

wherein at least one of the one or more immunomodulatory polypeptides is a variant IL2 polypeptide that comprises an H16A substitution and an F42A substitution relative to set forth in SEQ ID NO:1 and that exhibits reduced binding affinity to an IL-2 receptor (IL2R) comprising alpha, beta, and gamma polypeptides having amino acid sequences depicted in FIG. 3A-3C, compared to the binding affinity of the IL-2 amino acid sequence set forth in SEQ ID NO: 1 for the IL2R, and

wherein the first polypeptide and the second polypeptide are covalently linked to one another via a disulfide bond.

2. A multimeric polypeptide of claim 1, wherein the Ig Fc polypeptide comprises L234A and L235A substitutions.

3. A multimeric polypeptide of claim 1 or 2, wherein the β2M polypeptide comprises amino acids 21-119 of any one of the amino acid sequences set forth in FIG.6, optionally wherein the amino acid sequence comprises an R12C substitution based on the amino acid numbering of the amino acid sequence set forth in SEQ ID NO:16.

4. A multimeric polypeptide of any one of claims 1-3, wherein the MHC class I heavy chain polypeptide is an HLA-A heavy chain.

5. A multimeric polypeptide of any one of claims 1-4, wherein the MHC class I heavy chain polypeptide comprises an amino acid sequence having at least 95% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:14, optionally wherein the MHC class I heavy chain polypeptide comprises the amino acid sequence set forth in SEQ ID NO:19.

6. A multimeric polypeptide of any one of claims 1-5, wherein the first polypeptide and the second polypeptide are covalently linked to one another.

7. A multimeric polypeptide of claim 6, wherein the covalent linkage is via a disulfide bond.

8. A multimeric polypeptide of claim 7, wherein the disulfide bond joins a Cys residue in the β2M polypeptide and a Cys residue in the MHC heavy chain polypeptide.

9. A multimeric polypeptide of claim 8, wherein a Cys at amino acid residue 12, based on the amino acid numbering of SEQ ID NO:17, of the β2M polypeptide is disulfide bonded to a Cys at amino acid residue 236, based on the amino acid numbering of SEQ ID NO:19, of the MHC heavy chain polypeptide.

10. A multimeric polypeptide of claim 7, wherein the first polypeptide chain comprises a linker between the peptide epitope and the β2M polypeptide, and wherein the disulfide bond links a Cys present in the linker with a Cys of the MHC heavy chain polypeptide.

11. A multimeric polypeptide of claim 1, comprising:

a) a first polypeptide comprising, in order from N-terminus to C-terminus:

i) the peptide epitope; and

ii) the β2M polypeptide;

b) a second polypeptide comprising in order from N-terminus to C-terminus:

i) a first immunomodulatory polypeptide, wherein the first immunomodulatory polypeptide is a variant IL-2 polypeptide comprising an H16A substitution and an F42A substitution relative to set forth in SEQ ID NO:1;

ii) a second immunomodulatory polypeptide, wherein the second immunomodulatory polypeptide is a variant IL-2 polypeptide comprising an H16A substitution and an F42A substitution relative to set forth in SEQ ID NO:1;

iii) the MHC class I heavy chain polypeptide; and

iv) the Ig Fc polypeptide,

and wherein:

the β2M polypeptide comprises amino acids 21-119 of any one of the amino acid sequences set forth in FIG. 6, optionally wherein the amino acid sequence comprises an R12C substitution based on the amino acid numbering of SEQ ID NO:16,

the MHC class I heavy chain polypeptide comprises an amino acid sequence having at least 95% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:14, optionally wherein the MHC class I heavy chain polypeptide comprises the amino acid sequence set forth in SEQ ID NO:19, and

wherein the disulfide bond joins Cys at amino acid residue 12 of the β2M polypeptide to a Cys at amino acid residue 236 of the MHC heavy chain polypeptide.

12. A multimeric polypeptide of claim 11, wherein

i.) the β2M polypeptide comprises the amino acid sequence depicted in FIG. 34A;

ii.) the first and second variant IL-2 polypeptide comprise the amino acid sequence depicted in FIG. 34B;

iii) the MHC Class I heavy chain polypeptide comprises the amino acid sequence depicted in FIG. 34C; and

iv) the Ig Fc polypeptide comprises an amino acid sequence having at least 95% amino acid sequence identity to the amino acid sequence depicted in FIG. 33A, 33B, 33C, or 33D.

13. A multimeric polypeptide of claim 11 or 12, wherein the first polypeptide comprises a linker between the epitope and the β2M polypeptide, and the second polypeptide comprises a peptide linker between one or more of:

a) a first copy of the variant IL-2 polypeptide and a second copy of the variant IL-2 polypeptide;

b) the variant IL-2 polypeptide and the MHC class I heavy chain polypeptide; and

c) the MHC heavy chain polypeptide and the Ig Fc polypeptide.

14. A multimeric polypeptide of claim 13, wherein the peptide linker is selected from $(GGGGS)_3$, $(GGGGS)_4$, and AAAGG.

15. A multimeric polypeptide of any of claims 11-14, comprising:

a) a first polypeptide comprising, in order from N-terminus to C-terminus:

i) an HPV16 E7 epitope comprising amino acid sequence YMLDLQPETT (SEQ ID NO:13);

ii) a linker comprising the amino acid sequence GGGGSGGGGSGGGGS (SEQ ID NO:89); and

iii) a β2M polypeptide comprising the amino acid sequence set forth in SEQ ID NO:17; and

b) a second polypeptide comprising an amino acid sequence selected from the amino acid sequence depicted in FIG. 22, the amino acid sequence depicted in FIG. 25, and the amino acid sequence depicted in FIG. 28.

16. A protein comprising two of the multimeric polypeptides of any one of claims 1 to 15.

17. A protein of claim 16, wherein the two heterodimers are disulfide linked to one another via the Fc polypeptides present in the heterodimers.

18. A nucleic acid comprising a nucleotide sequence encoding a first and a second polypeptide according to any one of claims 1-15.

19. An expression vector comprising the nucleic acid of claim 18.

20. A host cell genetically modified with the expression vector of claim 19.

21. A method of producing the multimeric polypeptide of any one of claims 1-15 or a protein of claim 16 or 17, the method comprising culturing a host cell of claim 20 *in vitro* in a culture medium under conditions such that the host cell synthesizes the multimeric polypeptide.

22. An *in vitro* method of selectively activating an epitope-specific T cell, the method comprising contacting the T cell with the multimeric polypeptide of any one of claims 1-15 or a protein of claim 16 or 17

wherein said contacting selectively activates the epitope-specific T cell.

23. A composition comprising:

a) the multimeric polypeptide of any one of claims 1-15 or a protein of claim 16 or 17; and

b) a pharmaceutically acceptable excipient.

24. Use of the multimeric polypeptide of any one of claims 1-15 or a protein of claim 16 or 17, or a composition of claim 23 in the manufacture of a medicament for treating cancer in an individual.

25. The use of claim 24, wherein the medicament is to be administered subcutaneously, intravenously, or peritumorally.

26. The use of claim 24 or 25, wherein the medicament is to be administered systemically, distally to a treatment site, locally, or at or near a treatment site.

27. The use of claim 25, wherein the medicament is to be administered intravenously.