SUMMARY

[0007] Described herein are methods and compositions for targeting tumor cells with an immunoglobulin fusion protein specific for both a tumor cell antigen and CD47. Specifically, the immunoglobulin fusion protein includes an immunoglobulin moiety that is specific for a tumor cell antigen and has a second moiety that is specific for CD47.

[0007a] In one aspect, there is provided a SIRPα immunoglobulin fusion protein comprising: (i) an IgV extracellular domain of SIRPα or a SIRPα variant comprising an amino acid sequence at least 80% identical to (a) residues 3-115 of SEQ ID NO:6, or (b) residues 3-114 of SEQ ID NO:8, or (c) residues 1-115 of SEQ ID NO:190, wherein the SIRPα variant comprises a modification to an amino acid at one or more positions corresponding to positions 6, 27, 31, 37, 54, 56, or 72 of SEQ ID NO:6, SEQ ID NO: 8 or SEQ ID NO:190, wherein the modification is a substitution selected from the group consisting of: V6I; V27I; A27I; I31R; I31T; Q37W; Q37H; E54P; H56P; S66Q; L66Q; and M72R; and (ii) an immunoglobulin molecule or portion thereof that binds to a surface antigen on a disease promoting cell, wherein the disease promoting cell is a tumor cell and the surface antigen is a tumor antigen.

[0008] Also dislosed are SIRPα immunoglobulin fusion proteins. The fusion protein includes an IgV extracellular domain of SIRPα or a SIRPα variant having an amino acid sequence at least 80% identical to residues 3-115 of SEQ ID NO:6 or to 3-114 of SEQ ID NO:8. The fusion protein also includes an immunoglobulin molecule or portion thereof that binds to a surface antigen on a disease promoting cell. In one example, the disease promoting cell is a tumor cell and the surface antigen is a tumor antigen.

[0009] In certain examples, the immunoglobulin fusion protein includes a SIRPα variant with an amino acid sequence at least 85%, at least 90%, or at least 95% identical to residues 3-115 of SEQ ID NO:6 or to 3-114 of SEQ ID NO:8.

[0010] In certain examples, the immunoglobulin fusion protein includes a SIRPα variant with an amino acid sequence at least 80%, at least 85%, at least 90%, or at least 95% identical to residues 1-115 of SEQ ID NO:6 or to 1-114 of SEQ ID NO:8.

[0011] In some examples, the IgV extracellular domain is residues 1-115 of SEQ ID NO: 6, while in other examples, the IgV extracellular domain is residues 1-114 of SEQ ID NO:8. In some examples, the IgV extracellular domain is residues 3-115 of SEQ ID NO: 6 while in other examples, the IgV extracellular domain is residues 3-114 of SEQ ID NO:8. In yet other examples, the IgV extracellular domain is residues 1-114 of SEQ ID NO:193 or residues 1-115 of SEQ ID NO:194 or residues 1-115 of SEQ ID NO:195 or residues 1-115 of SEQ ID NO:196 or

[0024a] In another example, the present invention provides a SIRPα immunoglobulin fusion protein comprising: an IgV extracellular domain of SIRPα or of a SIRPα variant comprising an amino acid sequence at least 80% identical to residues 1-115 of SEQ ID NO:190; and an immunoglobulin molecule or portion thereof that binds to a surface antigen on a disease promoting cell.

[0025] In another example, the disclosure is directed to a SIRPα immunoglobulin fusion protein that includes an IgV extracellular domain of SIRPα or of a SIRPα variant having an amino acid sequence at least 80% identical to residues 1-115 of SEQ ID NO:190; and an immunoglobulin molecule or portion thereof that binds to a surface antigen on a disease promoting cell. The disease promoting cell may be a tumor cell and the surface antigen may be a tumor antigen.

[0026] In one example, the SIRPα variant has an amino acid sequence at least 85%, at least 90%, or at least 95% identical to residues 1-115 of SEQ ID NO:190.

[0027] In another example, the SIRPα variant has a modification to an amino acid at one or more positions corresponding to positions 6, 27, 31, 37, 54, 56, 66, or 72 of SEQ ID NO:190. In a further example, the modification is selected from the group consisting of: V6I; V27I; A27I; I31R; I31T; Q37W; Q37H; E54P; H56P; S66Q; L66Q; and M72R.

[0028] In another example, the disclosure is directed to a SIRPα immunoglobulin fusion protein that includes an anti-EGFR antibody or an antigen binding portion thereof and an IgV extracellular domain of SIRPα or of a SIRPα variant having an amino acid sequence at least 80% identical to residues 3-115 of SEQ ID NO:6 or to 3-114 of SEQ ID NO:8.

[0029] In a further example, the SIRPα or of a SIRPα variant has an amino acid sequence at least 85% identical, at least 90% identical, or at least 95% identical to residues 3-115 of SEQ ID NO:6 or to 3-114 of SEQ ID NO:8.

[0030] In yet another example, the SIRPα or of a SIRPα variant has an amino acid sequence at least 80% identical, at least 85% identical, at least 90% identical, or at least 95% identical to residues 1-115 of SEQ ID NO:6 or to 1-114 of SEQ ID NO:8.

variable region from an antibody selected from cetuximab, panitumumab, nimotuzumumab, matuzumab, futuximab, imgatuzumab, or necitumumab. In yet a further example, the anti-EGFR antibody is selected from cetuximab, panitumumab, nimotuzumumab, matuzumab, futuximab, imgatuzumab, or necitumumab. In another example, the anti-EGFR antibody is cetuximab.

[0032] In another example, the SIRPα or SIRPα variant is connected to the N-terminus of the heavy or light chain of the anti-EGFR antibody or antigen binding portion thereof, optionally via a linker. In another example, the SIRPα or SIRPα variant is connected to the C-terminus of the heavy or light chain of the anti-EGFR antibody or antigen binding portion thereof, optionally via a linker.

[0033] In a further example, the SIRPα-anti-EGFR immunoglobulin fusion protein includes an IgV extracellular domain of SIRPα or of a SIRPα variant having a modification at one or more of positions corresponding to positions 6, 27, 31,37, 54,56, 66 or 72 of SEQ ID NO:6 or SEQ ID NO:8. In one example, the modification is a substitution corresponding to Q37W. In another example, the modification is one or more substitutions corresponding to substitutions selected from V6I, C27I, A27I, I31R, Q37W, Q37H, E54P, H56P, S66Q, L66Q, and M72R.

[0033a] In yet another example, the present invention provides an immunoglobulin fusion protein comprising: an immunoglobulin molecule or portion thereof that binds a tumor cell antigen; and a CD47 binding moiety comprising an IgV extracellular domain of SIRPα or a SIRPα variant comprising an amino acid sequence at least 80% identical to residues 3-115 of SEQ ID NO:6 or 3-114 of SEQ ID NO:8; wherein the fusion protein has a % red blood cell (RBC) binding mean fluorescence intensity (MFI) of 35% or less when % RBC binding MFI to an anti-CD47 antibody is calibrated at 100%, wherein the anti-CD47 antibody is B6H12/huIgG1, wherein the fusion protein also binds to a CD47 antigen on a non-red blood cell.

[0034] In a further example, the disclosure is directed to an immunoglobulin fusion protein having an immunoglobulin molecule or portion thereof that binds a tumor cell antigen and a CD47 binding moiety comprising an IgV extracellular domain of SIRPα or a SIRPα variant having an amino acid sequence at least 80% identical to residues 3-115 of SEQ ID NO:6 or 3-114 of SEQ ID NO:8. The fusion protein has a % red blood cell (RBC) binding

85%, at least 90%, at least 95% identical to residues 1-115 of SEQ ID NO:6 or 1-114 of SEQ ID NO:8.

[0037] In some examples, the tumor cell antigen is EGFR. In some examples, the immunoglobulin molecule is an intact antibody. For example, the intact antibody is an anti-EGFR antibody in some examples, while in certain examples the anti-EGFR-antibody is cetuximab.

[0038] In one example, the fusion protein has a % RBC binding MFI of less than 30%, less than 25%, less than 20%, less than 15%, less than 10%, less than 5%, less than 4%, less than 3%, less than 2%, or less than 1%. In one example, the %RBC binding MFI is less than 10%.

[0039] In yet another example, the fusion protein has a % RBC binding MFI of between 0-1%, 0-2%, 0-3%, 0-4%, 1-2%, 1-3%, 1-4%, 2-3%, 2-4%, 3-4%, 3-7%, 3-10% or 5-10%.

[0040] In yet another example, the fusion protein has a % RBC binding MFI of 5% or less, 4% or less, 3% or less, 2% or less, or 1% or less.

[0041] In a further example, the antibody moiety is an anti-EGFR antibody. For example, the anti-EGFR antibody is cetuximab, whereas in another example, the anti-EGFR antibody is panitumumab, nimotuzumumab, matuzumab, futuximab, imgatuzumab, or necitumumab.

[0041a] In yet another aspect, the present invention provides a nucleic acid or nucleic acids encoding the SIRPα immunoglobulin fusion protein disclosed herein.

[0041b] In yet another aspect, the present invention provides a cell comprising the nucleic acid or nucleic acids disclosed herein.

[0041c] In yet another aspect, the present invention provides a method of producing an SIRPα immunoglobulin fusion protein by maintaining the cell disclosed herein under conditions that permit expression of the nucleic acid or nucleic acids disclosed herein.

[0042] Also disclosed are nucleic acids encoding the SIRPα immunoglobulin fusion proteins described herein. Because immunoglobulin

[0042a] In yet another aspect, the present invention provides a pharmaceutical composition comprising a pharmaceutically effective amount of the SIRPα immunoglobulin fusion protein disclosed herein and a pharmaceutically acceptable carrier.

[0043] Also disclosed are pharmaceutical compositions that include pharmaceutically effective amounts of an immunoglobulin fusion protein described herein including a pharmaceutically acceptable carrier.

[0043a] In yet another aspect, the present invention provides the use of an effective amount of the SIRPα immunoglobulin fusion protein disclosed herein in the manufacture of a medicament for treating cancer in a mammal in need thereof.

[0044] Also disclosed are methods of treating cancer by administering an effective amount of an immunoglobulin fusion protein described herein. The cancers that can be treated include breast, colorectal, lung, pancreatic, endometrial, ovarian, gastric, prostate, renal, cervical, myeloma, lymphoma, leukemia, thyroid, uterine, bladder, neuroendocrine, head and neck, liver, nasopharyngeal, testicular, small cell lung cancer, non-small cell lung cancer, melanoma, basal cell skin cancer, squamous cell skin cancer, dermatofibrosarcoma protuberans, Merkel cell carcinoma, glioblastoma, glioma, sarcoma, mesothelioma, or myelodisplastic syndromes.

BRIEF DESCRIPTION OF THE DRAWINGS

[0045] FIGS. 1A-O schematically illustrate the different DNA and protein constructs of the fusion proteins of the invention.

[0046] FIG. 1A shows two schematic diagrams of DNA constructs for the expression of an antibody-SIRPα fusion protein. DNA construct 1 (top) encodes the heavy chain variable domain of the antibody (VH) followed by heavy chain constant domains (CH1, hinge (H)-CH2-CH3) genetically fused via an optional linker (L) to SIRPα. DNA construct 2 (bottom) encodes the light chain variable domain of the antibody (VL) followed by light chain constant domain (CL).

[0047] FIG. 1B is a schematic drawing of an antibody-SIRPα fusion protein having a tetrameric structure where the two polypeptide components (i.e., light chain and heavy chain) are each encoded by a DNA construct shown in FIG. 1A.

CLAIMS

1. A SIRPα immunoglobulin fusion protein comprising:

(i) an IgV extracellular domain of SIRPα or a SIRPα variant comprising an amino acid sequence at least 80% identical to (a) residues 3-115 of SEQ ID NO:6, or (b) residues 3-114 of SEQ ID NO:8, or (c) residues 1-115 of SEQ ID NO:190, wherein the SIRPα variant comprises a modification to an amino acid at one or more positions corresponding to positions 6, 27, 31, 37, 54, 56, or 72 of SEQ ID NO:6, SEQ ID NO: 8 or SEQ ID NO:190, wherein the modification is a substitution selected from the group consisting of: V6I; V27I; A27I; I31R; I31T; Q37W; Q37H; E54P; H56P; S66Q; L66Q; and M72R; and

(ii) an immunoglobulin molecule or portion thereof that binds to a surface antigen on a disease promoting cell, wherein the disease promoting cell is a tumor cell and the surface antigen is a tumor antigen.

2. The SIRPα immunoglobulin fusion protein of claim 1, wherein the SIRPα variant comprises an amino acid sequence at least 85%, at least 90%, or at least 95% identical to residues 3-115 of SEQ ID NO:6, or to residues 3-114 of SEQ ID NO:8, or to residues 1-115 of SEQ ID NO:190.

3. The SIRPα immunoglobulin fusion protein of claim 1, wherein the SIRPα variant comprises an amino acid sequence at least 80%, at least 85%, at least 90%, or at least 95% identical to residues 1-115 of SEQ ID NO:6, or to residues 1-114 of SEQ ID NO:8.

4. The SIRPα immunoglobulin fusion protein of claim 1, wherein the SIRPα variant comprises an amino acid sequence at least 80%, at least 85%, at least 90%, or at least 95% identical to a sequence selected from the group consisting of: residues 1-114 of SEQ ID NO:193, residues 1-115 of SEQ ID NO:194, residues 1-115 of SEQ ID NO:195, residues 1-115 of SEQ ID NO:196, residues 1-114 of SEQ ID NO:197, residues 1-114 of SEQ ID NO:198, residues 1-115 of SEQ ID NO:199, residues 1-114 of SEQ ID NO:200, and residues 1-115 of SEQ ID NO: 190.

5. The SIRPα immunoglobulin fusion protein of claim 1, wherein the IgV extracellular domain comprises a sequence selected from the group consisting of residues 1-115 of SEQ ID NO: 6, residues 1-114 of SEQ ID NO:8, 1-114 of SEQ ID NO:193, residues 1-115 of SEQ ID NO:194, residues 1-115 of SEQ ID NO:195, residues 1-115 of SEQ ID NO:196, residues

1-114 of SEQ ID NO:197, residues 1-114 of SEQ ID NO:198, residues 1-115 of SEQ ID NO:199, residues 1-114 of SEQ ID NO:200, and residues 1-115 of SEQ ID NO:190.

6. The SIRPα immunoglobulin fusion protein of claim 1, wherein the SIRPα variant comprises an amino acid sequence at least 80%, at least 85%, at least 90%, or at least 95% identical to residues 1-343 of SEQ ID NO:6.

7. The SIRPα immunoglobulin fusion protein of any one of claims 1 to 6, wherein the SIRPα or SIRPα variant is connected to the antibody light chain.

8. The SIRPα immunoglobulin fusion protein of claim 1,

wherein the fusion protein has a % red blood cell (RBC) binding mean fluorescence intensity (MFI) of 10% or less when % RBC binding MFI to an anti-CD47 antibody is calibrated at 100%, wherein the anti-CD47 antibody is B6H12/huIgG1; and

wherein the fusion protein also binds to a CD47 antigen on a non-red blood cell.

9. A nucleic acid or nucleic acids encoding the SIRPα immunoglobulin fusion protein of any one of claims 1-8.

10. A cell comprising the nucleic acid or nucleic acids of claim 9.

11. A method of producing an SIRPα immunoglobulin fusion protein by maintaining the cell of claim 10 under conditions that permit expression of the nucleic acid or nucleic acids of claim 9.

12. A pharmaceutical composition comprising a pharmaceutically effective amount of the SIRPα immunoglobulin fusion protein of any one of claims 1-8 and a pharmaceutically acceptable carrier.

13. The use of an effective amount of the SIRPα immunoglobulin fusion protein of any one of claims 1-8 in the manufacture of a medicament for treating cancer in a mammal in need thereof.