

Additional therapeutic agents that target VEGF or immune checkpoints are needed.

SUMMARY ~~OF THE INVENTION~~

In an aspect, there is provided a multifunctional protein molecule comprising at least one decorin molecule having at least 95% sequence identity to SEQ ID NO: 7 linked to an antibody molecule selected from the group consisting of bevacizumab, ranibizumab, ipilimumab, atezolizumab, avelumab, durvalumab, nivolumab and pembrolizumab.

In another aspect, there is provided a nucleic acid or set of nucleic acids encoding a multifunctional protein molecule as defined above.

In another aspect, there is provided a vector or vectors comprising the nucleic acid or set of nucleic acids as defined above.

In another aspect, there is provided a host cell comprising the vector or vectors as defined above.

In another aspect, there is provided a multifunctional protein molecule as defined above for use in treating a disorder characterized by angiogenesis or tumor growth.

In another aspect, there is provided the use of the multifunctional protein molecule as defined above in the manufacture of a medicament for the treatment of a disorder characterized by angiogenesis or tumor growth.

The present ~~invention~~disclosure relates to multifunction protein molecules comprising decorin and uses thereof. In particular, the present ~~invention~~disclosure relates to multifunctional protein molecules comprising decorin and a targeting polypeptide such as an antibody and methods of their production and uses thereof.

Accordingly, in some ~~embodiments~~examples, the present ~~invention~~disclosure provides multifunctional protein molecules comprising at least one decorin molecule or functional portion thereof linked to an antigen binding protein.

In some preferred ~~examples~~embodiments, the antigen binding protein is selected from the group consisting of a VEGF-A antigen binding protein and a checkpoint inhibitor antigen binding protein. In some preferred ~~embodiments~~examples, the checkpoint inhibitor antigen binding protein binds a checkpoint inhibitor protein selected from the group consisting of PD-1, PD-L1, CTLA-4, PD-L2, CD27, CD28, CD40, CD47, CD115, CD122, CD137, OX40, GITR, ICOS, A2AR, B7-H3, B7-H4, BTLA, IDO, KIR, LAG3, NOX2, TIM-3, VISTA, SIGLEC-7,

TIGIT and 4-1BB. In some particularly preferred [embodimentsexamples](#), the checkpoint inhibitor antigen binding protein binds a checkpoint inhibitor protein selected from the group consisting of PD-1, PD-L1, CTLA-4, PD-L2.

In some preferred [embodimentsexamples](#), the antigen binding protein is an antibody. In some preferred [embodimentsexamples](#), the antibody is a monoclonal antibody. In some preferred [embodimentsexamples](#), the monoclonal antibody is selected from the group consisting of bevacizumab, ranibizumab, ipilimumab, atezolizumab, avelumab, durvalumab, nivolumab and pembrolizumab.

In some preferred [embodimentsexamples](#), the decorin polypeptide is a decorin core protein. In some preferred [embodimentsexamples](#), the decorin core protein comprises a mutation at position 4 of the mature decorin core protein. In some preferred [examplesembodiments](#), the mutation is a serine to alanine mutation. In some preferred [embodimentsexamples](#), the decorin core protein lacks substantial modification by glycosaminoglycans molecules at position 4 of the mature decorin core protein. In some preferred [embodimentsexamples](#), the fusion protein comprises two or more copies of the decorin polypeptide.

In some preferred [embodimentexamples](#), the at least a functional portion of a decorin molecule comprises a decorin domain or domains that binds a signaling molecule selected from the group consisting of Transforming Growth Factor- β (TGF- β), Connective Tissue Growth Factor (CTGF), Platelet-Derived Growth Factor (PDGF), Vascular Endothelial Growth Factor Receptor 2 (VEGFR2), Hepatocyte Growth Factor Receptor (HGFR), Insulin-like Growth Factor 1 Receptor (IGF-1R), Epidermal Growth Factor Receptors (EGFRs), myostatin and C1q. In some preferred [embodimentexamples](#), the TGF- β binding domain comprises amino acids Asp45-Lys359 of full length endogenous human decorin or amino acids Leu155-Val260 of full length endogenous human decorin. In some preferred [embodimentexamples](#), the multifunctional protein molecule comprises two or more copies of the at least a functional portion of a decorin molecule.

In some preferred [embodimentexamples](#), the decorin molecule is operably linked to a heavy chain of the antibody. In some preferred [embodimentexamples](#), the antigen binding protein is bi-specific. In some preferred [embodimentexamples](#), the antigen binding protein is multi-specific.

In some preferred [embodimentexamples](#), the multifunctional protein molecule is a fusion protein. In some preferred [embodimentexamples](#), the decorin molecule is chemically linked to the antigen binding protein.

In some preferred [embodimentexamples](#), the present [invention-disclosure](#) provides a nucleic acid or set of nucleic acids encoding a multifunctional protein molecule as described above. In some preferred [embodimentexamples](#), the present [invention-disclosure](#) provides a vector or vectors comprising the nucleic acid or set of nucleic acids. In some preferred [embodimentexamples](#), the present [invention-disclosure](#) provides a host cell comprising the vector or vectors.

In some preferred [embodimentexamples](#), the present [invention-disclosure](#) provides methods of inhibiting a target protein and a signaling molecule in a cell, comprising: contacting the cell with a multifunctional protein molecule, nucleic acid molecule, or vector as described above under conditions such that at least one activity of the target protein and at least one activity of a signaling protein are inhibited in the cell, wherein the target protein is selected from the group consisting of VEGF-1, PD-1, PD-L1, CTLA-4, PD-L2, CD27, CD28, CD40, CD47, CD115, CD122, CD137, OX40, GITR, ICOS, A2AR, B7-H3, B7-H4, BTLA, IDO, KIR, LAG3,

NOX2, TIM-3, VISTA, SIGLEC-7, TIGIT and 4-1BB. In some preferred embodimentexamples, the cell is in vitro or in vivo. In some preferred embodimentexamples, the cell is in a subject. In some preferred embodimentexamples, the contacting results in inhibition of an activity selected from the group

consisting of angiogenesis, PD-1 activity, PD-L1 activity, CTLA-4 activity, PD-L2 activity, CD27 activity, CD28 activity, CD40 activity, CD47 activity, CD115 activity, CD122 activity, CD137 activity, OX40 activity, GITR activity, ICOS activity, A2AR activity, B7-H3 activity, B7-H4 activity, BTLA activity, IDO activity, KIR activity, LAG3 activity, NOX2 activity, TIM-3 activity, VISTA activity, SIGLEC-7 activity, TIGIT activity and 4-1BB activity. In some preferred embodimentexamples, the cancer is selected from the group consisting of lung cancer, colorectal cancer, liver cancer, breast cancer, kidney cancer, cervical cancer, ovarian cancer, and glioblastoma. In some preferred embodimentexamples, the signaling protein is selected from the group consisting of Transforming Growth Factor- β (TGF- β), Connective Tissue Growth Factor (CTGF), Platelet-Derived Growth Factor (PDGF), Vascular Endothelial Growth Factor Receptor 2 (VEGFR2), Hepatocyte Growth Factor Receptor (HGFR), Insulin-like Growth Factor 1 Receptor (IGF-1R), various epidermal growth factor receptors (EGFRs), myostatin and C1q. In some preferred embodimentexamples, the signaling protein is Transforming Growth Factor- β (TGF- β).

In some preferred embodimentexamples, the present invention-disclosure provides methods of treating a disorder characterized by angiogenesis or tumor growth, comprising: administering a multifunctional protein molecule, nucleic acid molecule, or vector as described above to a subject under conditions such that angiogenesis or tumor growth is inhibited in the subject. In some preferred embodimentexamples, the tumor is selected from the group consisting of lung cancer, colorectal cancer, liver cancer, breast cancer, kidney cancer, cervical cancer, ovarian cancer, and glioblastoma.

DESCRIPTION OF THE FIGURES

FIG. 1 is a schematic diagram of a fusion protein of the present invention.

FIG. 2 is a map of an expression construct of the present invention.

FIG. 3 is a map of an expression construct of the present invention.

FIG. 4 is a SDS-PAGE gel of media from the pooled CHO cell line expressing the bevacizumab fusion protein. Lane 10. Molecular weight standards. Lane 11. Non-reduced media sample. Lane 12. Reduced media sample.

CLAIMS

What is claimed is:

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1. A multifunctional protein molecule comprising at least one decorin molecule ~~or~~ functional portion thereof having at least 95% sequence identity to SEQ ID NO: 7 linked to an antigen-binding protein/antibody molecule selected from the group consisting of bevacizumab, ranibizumab, ipilimumab, atezolizumab, avelumab, durvalumab, nivolumab and pembrolizumab.

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2. ~~— The multifunctional protein molecule of claim 1, wherein the antigen-binding protein is selected from the group consisting of a VEGF-A antigen-binding protein and a checkpoint inhibitor antigen-binding protein.~~

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3. ~~— The multifunctional protein molecule of claim 2, wherein the checkpoint inhibitor antigen-binding protein binds a checkpoint inhibitor protein selected from the group consisting of PD-1, PD-L1, CTLA-4, PD-L2, CD27, CD28, CD40, CD47, CD115, CD122, CD137, OX40, GITR, ICOS, A2AR, B7-H3, B7-H4, BTLA, IDO, KIR, LAG3, NOX2, TIM-3, VISTA, SIGLEC-7, TIGIT and 4-1BB.~~

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4. ~~— The multifunctional protein molecule of claim 2, wherein the checkpoint inhibitor antigen-binding protein binds a checkpoint inhibitor protein selected from the group consisting of PD-1, PD-L1, CTLA-4, PD-L2.~~

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5. ~~— The multifunctional protein molecule of any one of claims 1 to 4, wherein the antigen-binding protein is an antibody.~~

6. ~~— The multifunctional protein molecule of claim 5, wherein the antibody is a monoclonal antibody.~~

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~~7. The multifunctional protein molecule of claim 6, wherein the monoclonal antibody is selected from the group consisting of bevacizumab, ranibizumab, ipilimumab, atezolizumab, avelumab, durvalumab, nivolumab and pembrolizumab.~~

5 ~~82.~~ The multifunctional protein molecule of ~~any one of claims 1 to 7~~, wherein the decorin molecule ~~is~~comprises a mutation at position 4 as compared to wild-type decorin-core protein.

~~9. The multifunctional protein molecule of claim 8, wherein the decorin-core protein comprises a mutation at position 4 of the mature decorin-core protein.~~

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~~103.~~ The multifunctional protein molecule of claim ~~29~~, wherein the mutation is a serine to alanine mutation.

15 ~~114.~~ The multifunctional protein molecule of claim ~~82~~, wherein the decorin ~~core protein~~molecule lacks ~~substantial~~ modification by glycosaminoglycans molecules at position 4 of the ~~mature decorin core protein~~molecule.

20 ~~125.~~ The multifunctional protein molecule of ~~any one of claims 1 to 11~~, wherein the ~~fusion multifunctional~~ protein molecule comprises two or more copies of the decorin molecule~~polypeptide~~.

25 ~~136.~~ The multifunctional protein molecule of ~~any one of claims 1 to 12~~, wherein the ~~functional portion of a~~ decorin molecule comprises a decorin domain or domains that binds a signaling molecule selected from the group consisting of Transforming Growth Factor- β (TGF- β), Connective Tissue Growth Factor (CTGF), Platelet-Derived Growth Factor (PDGF), Vascular Endothelial Growth Factor Receptor 2 (VEGFR2), Hepatocyte Growth Factor Receptor (HGFR), Insulin-like Growth Factor 1 Receptor (IGF-1R), Epidermal Growth Factor Receptors (EGFRs), myostatin and C1q.

~~147.~~ The multifunctional protein molecule of claim ~~136~~, wherein the TGF- β binding domain comprises amino acids Asp45-Lys359 of full length endogenous human decorin or amino acids Leu155-Val260 of full length endogenous human decorin.

5 ~~158.~~ The multifunctional protein molecule of ~~any one of claims 13 or 146~~, wherein the multifunctional protein molecule comprises two or more copies of the ~~functional portion of a~~ decorin molecule.

~~169.~~ The multifunctional protein molecule of ~~any of claims 2 to 151~~, wherein the decorin molecule is operably linked to a heavy chain of the antibody.

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~~17.—The multifunctional protein molecule of any one of claims 1 to 16, wherein the antigen binding protein is bi-specific.~~

15 ~~18.—The multifunctional protein molecule of any one of claims 1 to 16, wherein the antigen binding protein is multi-specific.~~

~~1910.~~ The multifunctional protein molecule of ~~any one of claims 1 to 18~~, wherein the multifunctional protein molecule is a fusion protein.

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~~2011.~~ The multifunctional protein molecule of ~~any one of claims 1 to 18~~, wherein the decorin molecule ~~or functional portion thereof~~ is chemically linked to the antigen binding protein.

~~2412.~~ A nucleic acid or set of nucleic acids encoding a multifunctional protein molecule of ~~any one of Cclaims 1 to 20~~.

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~~2213.~~ A vector or vectors comprising the nucleic acid or set of nucleic acids of ~~Cclaim 2412~~.

~~2314.~~ A host cell comprising the vector or vectors of ~~Cclaim 2213~~.

30 ~~24.—Mutifunctional protein molecule, nucleic acid molecule or vector according to any one of claims 1 to 22 for use to inhibit a target protein and a signaling molecule in a cell.~~

25. — Use of claim 24, wherein the target protein is selected from the group consisting of VEGF-1, PD-1, PD-L1, CTLA-4, PD-L2, CD27, CD28, CD40, CD47, CD115, CD122, CD137, OX40, GITR, ICOS, A2AR, B7-H3, B7-H4, BTLA, IDO, KIR, LAG3, NOX2, TIM-3, VISTA, SIGLEC-7, TIGIT and 4-1BB.

26. — Use of any one of claims 24 to 25, wherein the cell is in vitro or in vivo.

27. — Use of any one of claims 24 or 25, wherein the cell is in a subject.

28. — Use of any one of claims 24 to 27, wherein the contacting results in inhibition of an activity selected from the group consisting of angiogenesis, PD-1 activity, PD-L1 activity, CTLA-4 activity, PD-L2 activity, CD27 activity, CD28 activity, CD40 activity, CD47 activity, CD115 activity, CD122 activity, CD137 activity, OX40 activity, GITR activity, ICOS activity, A2AR activity, B7-H3 activity, B7-H4 activity, BTLA activity, IDO activity, KIR activity, LAG3 activity, NOX2 activity, TIM-3 activity, VISTA activity, SIGLEC-7 activity, TIGIT activity and 4-1BB activity.

29. — Use of any one of claims 24 to 28, wherein the cells is a cancer cell selected from the group consisting of lung cancer, colorectal cancer, liver cancer, breast cancer, kidney cancer, cervical cancer, ovarian cancer, and glioblastoma cells.

30. — Use of any one of claims 24 to 29, wherein the signaling protein is selected from the group consisting of Transforming Growth Factor- β (TGF- β), Connective Tissue Growth Factor (CTGF), Platelet Derived Growth Factor (PDGF), Vascular Endothelial Growth Factor Receptor 2 (VEGFR2), Hepatocyte Growth Factor Receptor (HGFR), Insulin-like Growth Factor-1 Receptor (IGF-1R), various epidermal growth factor receptors (EGFRs), myostatin and C1q.

31. — Use of any one of claims 24 to 30, wherein the signaling protein is Transforming Growth Factor- β (TGF- β).

~~3215.~~ Multifunctional protein molecule, ~~nucleic acid molecule or vector according to any one~~
of claims ~~1 to 22~~ for use in treating a disorder characterized by angiogenesis or tumor growth.

5 16. Use of the multifunctional protein molecule of claim 1 in the manufacture of a
medicament for the treatment of a disorder characterized by angiogenesis or tumor growth.

~~3317.~~ The Use of claim ~~3216~~, wherein the tumor is selected from the group consisting of lung
10 cancer, colorectal cancer, liver cancer, breast cancer, kidney cancer, cervical cancer, ovarian
cancer, and glioblastoma.