VARIANTS OF HUMAN BMP7 PROTEIN

The present invention is in the field of medicine, particularly in the field of therapeutic proteins. Specifically, the present invention relates to variants of human bone morphogenic protein-7 (BMP7) useful for the treatment of cancer, cartilage damage and degeneration, pain associated with osteoarthritis or bone healing.

Bone morphogenic proteins are a well-known family of growth factors that regulate cell proliferation, migration, differentiation and apoptosis in a number of tissues and organs. Human BMP7 is a secreted signaling molecule of the TGF-beta superfamily and was originally identified for its ability to induce bone formation. It is now recognized as a multifunctional cytokine which mediates growth and differentiation of many different cell types.

BMP7 has been used to promote bone formation, bone fracture healing and spinal fusion. Nevertheless, its use as a therapeutic has been limited primarily to topical administration to the surface of the tissue because of its poor solubility/bioavailability and the propensity to cause ectopic bone formation (EBF) or a rapid formation of new bone in soft tissue as a result of precipitation at the injection site.

BMP7 protein variants with improved properties such as increased expression yield, increased solubility, increased specific activity and decreased immunogenicity have been reported in WO2005/097825. However, there is still a need for human BMP7 protein variants with improved solubility/bioavailability, increased specific activity, decreased binding to endogenous circulating inhibitors, and reduced EBF activity that could be useful as a therapeutic.

Cancer stem-like cells (CSC) in solid tumors are purported to contribute to cancer development and poor treatment outcome. The abilities to self-renew, differentiate, and resist anticancer therapies are hallmarks of these rare cells, and steering them into lineage commitment may be one strategy to curb cancer development or progression. However, despite the acknowledged potential of this approach, there still remains a need for effective cancer therapies which induce tumor cell transdifferentiation and revert CSCs into lineages that are not resistant or less resistant to killing by conventional chemo- and radiotherapy (see, for example, Li, R., et al., Oncotarget. 2016 Oct 18; 7(42): 68360–68370).

Summary

In one aspect, the present disclosure refers to a protein comprising a polypeptide

comprising the amino acid sequence of:
STGSKQRSQNRSKTPKNQEALRMANVAENSSSXaa$_{33}$QRQXaa$_{37}$CKKHELYVSFRDLGWQD
WIIAPXaa$_{60}$GYAAXaa$_{65}$YCEGECAFPLNSYMNATNHAXaa$_{86}$Xaa$_{87}$QXaa$_{89}$LXaa$_{91}$HXaa$_{93}$Xaa$_{94}$
NPETVPKPCCAPTQLXaa$_{110}$AISXaa$_{114}$LYFDDXaa$_{120}$SNVILKKXaa$_{128}$RNMXaa$_{132}$VXaa$_{134}$ACG
CH (SEQ ID NO: 3),

wherein:

Xaa$_{33}$ is D; Xaa$_{37}$ is A; Xaa$_{60}$ is E; Xaa$_{65}$ is Y or G; Xaa$_{86}$ is I, or L; Xaa$_{87}$ is V; Xaa$_{89}$ is T or A; Xaa$_{91}$ is V; Xaa$_{93}$ is F or V; Xaa$_{94}$ is I; Xaa$_{110}$ is G; Xaa$_{114}$ is V or M; Xaa$_{120}$ is S; Xaa$_{128}$ is Y, F or W; Xaa$_{132}$ is V; and, Xaa$_{134}$ is R.

In another aspect, the present disclosure refers to a pharmaceutical composition comprising a protein disclosed herein and one or more pharmaceutically acceptable excipients, diluents, or carriers.

In a further aspect, the present disclosure refers to a use of a pharmaceutical composition disclosed herein in the manufacture of a medicament for the treatment of a cancer selected from colorectal cancer and glioblastoma, cartilage damage and degeneration, pain associated with osteoarthritis, or bone healing.

In another aspect, the present disclosure refers to a protein disclosed herein for use in therapy.

In a further aspect, the present disclosure refers to a protein disclosed herein for use in the treatment of a cancer selected from colorectal cancer and glioblastoma, cartilage damage and degeneration, pain associated with osteoarthritis, or bone healing.

In another aspect, the present disclosure refers to a use of a protein disclosed herein in the manufacture of a medicament for treating a cancer selected from colorectal cancer and glioblastoma, wherein said medicament is to be administered to a patient in need thereof in simultaneous, separate, or sequential combination with an effective amount of one or more chemotherapeutic agents or ionizing radiation.

In a further aspect, the present disclosure refers to a kit for the treatment of a cancer

selected from colorectal cancer and glioblastoma, the kit comprising a first pharmaceutical composition comprising a protein disclosed herein and an acceptable carrier, diluent, or excipient and a second pharmaceutical composition comprising one or more chemotherapeutic agents and an acceptable carrier, diluent, or excipient.

In a further aspect, the present disclosure refers to a use of a protein disclosed herein for the manufacture of a medicament for the treatment of a cancer selected from colorectal cancer and glioblastoma, cartilage damage and degeneration, pain associated with osteoarthritis, or bone healing.

In another aspect, the present disclosure refers to a pharmaceutical composition comprising a protein disclosed herein for use in simultaneous, separate, or sequential combination with a second pharmaceutical composition comprising a chemotherapeutic agent in the treatment of a cancer selected from colorectal cancer and glioblastoma.

In a further aspect, the present disclosure refers to a pharmaceutical composition comprising a protein disclosed herein for use in simultaneous, separate, or sequential combination with ionizing radiation in the treatment of a cancer selected from colorectal cancer and glioblastomaa.

Description

The present invention provides alternative human BMP7 protein variants. Particularly, the present invention provides variants of human BMP7 protein with increased specific activity, improved solubility/bioavailability characteristics, decreased binding to endogenous circulating

We claim:

1. A protein comprising a polypeptide comprising the amino acid sequence of: STGSKQRSQNRSKTPKNQEALRMANVAENSSSXaa$_{33}$QRQXaa$_{37}$CKKHELYVSFR DLGWQDWIIAPXaa$_{60}$GYAAXaa$_{65}$YCEGECAFPLNSYMNATNHAXaa$_{86}$Xaa$_{87}$QXaa $_{89}$LXaa$_{91}$HXaa$_{93}$Xaa$_{94}$NPETVPKPCCAPTQLXaa$_{110}$AISXaa$_{114}$LYFDDXaa$_{120}$SNVILK KXaa$_{128}$RNMXaa$_{132}$VXaa$_{134}$ACGCH (SEQ ID NO: 3),
wherein:
Xaa$_{33}$ is D; Xaa$_{37}$ is A; Xaa$_{60}$ is E; Xaa$_{65}$ is Y or G; Xaa$_{86}$ is I, or L; Xaa$_{87}$ is V; Xaa$_{89}$ is T or A; Xaa$_{91}$ is V; Xaa$_{93}$ is F or V; Xaa$_{94}$ is I; Xaa$_{110}$ is G; Xaa$_{114}$ is V or M; Xaa$_{120}$ is S; Xaa$_{128}$ is Y, F or W; Xaa$_{132}$ is V; and, Xaa$_{134}$ is R.

2. The protein of claim 1 wherein Xaa$_{65}$ is G; Xaa$_{86}$ L; Xaa$_{89}$ is T; Xaa$_{93}$ is V; and Xaa$_{128}$ is F or W.

3. The protein of claim 2 wherein Xaa$_{114}$ is V.

4. The protein of claim 3 wherein the amino acid sequence of the polypeptide is SEQ ID NO: 8.

5. The protein of claim 1 wherein the amino acid sequence of the polypeptide is selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 9, and SEQ ID NO: 10.

6. The protein of any one claims 1-5 wherein the N-terminus of the polypeptide is covalently fused to the C-terminus of a human BMP7 pro-domain polypeptide comprising an amino acid sequence of SEQ ID NO: 18.

7. The protein of any one of claims 1-5 wherein the amino acid sequence of the polypeptide is selected from the group consisting of SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, and SEQ ID NO: 16.

8. A pharmaceutical composition comprising a protein of any one of claims 1-5 and one or more pharmaceutically acceptable excipients, diluents, or carriers.

9. The pharmaceutical composition of claim 8 wherein the protein is a disulfide linked homodimer.

10. The pharmaceutical composition of claim 9 wherein the composition further comprises a polypeptide having the amino acid sequence of SEQ ID NO: 18.

11. Use of a pharmaceutical composition of either of claims 9 or 10 in the manufacture of a medicament for the treatment of a cancer selected from colorectal cancer and glioblastoma, cartilage damage and degeneration, pain associated with osteoarthritis, or bone healing.

12. The protein of any one of claims 1-5 for use in the treatment of a cancer selected from colorectal cancer and glioblastoma, cartilage damage and degeneration, pain associated with osteoarthritis, or bone healing.

13. The protein for use of claim 12, wherein the protein is to be administered in simultaneous, separate, or sequential combination with ionizing radiation.

14. The protein for use claim 12, wherein the protein is to be administered in simultaneous, separate, or sequential combination with one or more chemotherapeutic agents.

15. Use of a protein of any one of claims 1-5 in the manufacture of a medicament for treating a cancer selected from colorectal cancer and glioblastoma, wherein said medicament is to be administered to a patient in need thereof in simultaneous, separate, or sequential combination with an effective amount of one or more chemotherapeutic agents or ionizing radiation.

16. A kit for the treatment of a cancer selected from colorectal cancer and glioblastoma, the kit comprising a first pharmaceutical composition comprising a protein of any one of claims 1-5 and an acceptable carrier, diluent, or excipient and a second pharmaceutical composition comprising one or more chemotherapeutic agents and an acceptable carrier, diluent, or excipient.

17. The kit of claim 16, wherein the first pharmaceutical composition further comprises a polypeptide having the amino acid sequence of SEQ ID NO: 18.

18. Use of a protein of any one of claims 1-5 for the manufacture of a medicament for the treatment of a cancer selected from colorectal cancer and glioblastoma, cartilage damage and degeneration, pain associated with osteoarthritis, or bone healing.

19. The use of claim 18, wherein the protein is to be administered in simultaneous, separate, or sequential combination with ionizing radiation.

20. The use of either of claims 18 or 19, wherein the protein is to be administered in simultaneous, separate, or sequential combination with one or more chemotherapeutic agents.

21. A pharmaceutical composition comprising a protein of any one of claims 1-5 for use in simultaneous, separate, or sequential combination with a second pharmaceutical composition comprising a chemotherapeutic agent in the treatment of a cancer selected from colorectal cancer and glioblastoma.

22. The pharmaceutical composition for use of claim 21, wherein either pharmaceutical composition is to be administered in simultaneous, separate, or sequential combination with ionizing radiation.

23. A pharmaceutical composition comprising a protein of any one of claims 1-5 for use in simultaneous, separate, or sequential combination with ionizing radiation in the treatment of a cancer selected from colorectal cancer and glioblastoma.