Activins A, B and AB are the homodimers and heterdimer respectively of two polypeptide chains, βA and βB (Vale et al., Nature 321, 776-779 (1986), Ling et al., Nature 321, 779-782 (1986)). Activins were originally discovered as gonadal peptides involved in the regulation of follicle stimulating hormone synthesis, and are now believed to be involved in the regulation of a number of biological activities. Activin A is a predominant form of activin.

Activin, myostatin, GDF-11 and other members of the TGF-β superfamily bind and signal through a combination of activin type II and activin type IIB receptors, both of which are transmembrane serine/threonine kinases (Harrison et al., J. Biol. Chem. 279, 28036-28044 (2004)). Cross-linking studies have determined that myostatin is capable of binding the activin type II receptors ActRIIA and ActRIIB *in vitro* (Lee et al., PNAS USA 98:9306-11 (2001)). There is also evidence that GDF-11 binds to both ActRIIA and ActRIIB (Oh et al., Genes Dev 16:2749-54 (2002)).

TGF-β protein expression is known to be associated with a variety of diseases and disorders. Therefore, therapeutic molecules capable of antagonizing several TGF-β proteins simultaneously may be particularly effective for treating these diseases and disorders.

Production of therapeutic proteins on a commercial scale requires proteins that can be efficiently expressed and purified without disruption of the integrity of the protein. Manufacturability can be described as the ability to express and purify a protein in a sufficiently efficient manner to allow for cost-effective production of the protein. In a commercial setting, manufacturability must be determined for each potential therapeutic protein. Although protein expression and purification processes can be optimized for a protein, manufacturability appears to be a function of the intrinsic properties of the protein as well. The present invention provides biologically active therapeutic proteins having improved manufacturability properties, capable of effectively antagonizing TGF-β proteins.

SUMMARY

The present disclosure provides isolated proteins comprising stabilized human activin receptor IIB (designated svActRIIB) polypeptides capable of binding and inhibiting the activities of activin, GDF-11 and myostatin, and characterized by improved manufacturability properties. The stabilized ActRIIB polypeptides are

characterized by having amino acid substitutions at both positions 28 and 44 with respect to SEQ ID NO: 2.

In a first aspect, there is provided an isolated protein comprising a stabilized activin IIB receptor polypeptide, wherein said polypeptide is selected from the group consisting of:

(a) a polypeptide consisting of the sequence set forth in the group consisting of SEQ ID NO: 4, 6, 12 and 14;

(b) a polypeptide having at least 90% sequence identity to (a), wherein the polypeptide has a W or a Y at the position corresponding to position 28 of SEQ ID NO:2 and a T at the position corresponding to position 44 of SEQ ID NO:2, wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11, and

(c) polypeptides having at least 95% sequence identity to (a), wherein the polypeptide has a W or a Y at the position corresponding to position 28 of SEQ ID NO:2 and a T at the position corresponding to position 44 of SEQ ID NO:2, wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11.

In a second aspect, there is provided an isolated protein consisting of the sequence set forth in SEQ ID NO: 10.

In a third aspect, there is provided an isolated protein comprising a stabilized activin IIB receptor polypeptide (svActRIIB) wherein said polypeptide is selected from the group consisting of:

(a) a polypeptide consisting of the sequence set forth in SEQ ID NO: 2, except for a single amino acid substitution at the position corresponding to position 28 of SEQ ID NO:2, and a single amino acid substitution at the position corresponding to position 44 of SEQ ID NO:2, wherein the substitution at position 28 is selected from the group consisting of W and Y, and the substitution at position 44 is T;

(b) a polypeptide consisting of the sequence set forth in amino acids 19 through 134 of SEQ ID NO:2, except for a single amino acid substitution at the position corresponding to position 28 of SEQ ID NO:2, and a single amino acid substitution at the position corresponding to position 44 of SEQ ID NO:2, wherein the substitution at position 28 is selected from the group consisting of W and Y, and the substitution at position 44 is T;

(c) a polypeptide consisting of the sequence set forth in amino acids 23 through 134 of SEQ ID NO:2, except for a single amino acid substitution at the position corresponding to position 28 of SEQ ID NO:2, and a single amino acid substitution at the position corresponding to position 44 of SEQ ID NO:2, wherein the

substitution at position 28 is selected from the group consisting of W and Y, and the substitution at position 44 is T;

(d) a polypeptide consisting of the sequence set forth in amino acids 25 through 134 of SEQ ID NO: 2, except for a single amino acid substitution at the position corresponding to position 28 of SEQ ID NO:2, and a single amino acid substitution at the position corresponding to position 44 of SEQ ID NO:2, wherein the substitution at position 28 is selected from the group consisting of W and Y, and the substitution at position 44 is T;

(e) a polypeptide having at least 80% sequence identity to any one of (a) through (d), except for a single amino acid substitution at the position corresponding to position 28 of SEQ ID NO:2, and a single amino acid substitution at the position corresponding to position 44 of SEQ ID NO:2, wherein the substitution at position 28 is selected from the group consisting of W and Y, and the substitution at position 44 is T, wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11.

In one embodiment, the isolated protein comprises a polypeptide having the sequence set forth in SEQ ID NO: 2, except for a single amino acid substitution at position 28, and a single amino acid substitution at position 44, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T. In another embodiment, the polypeptide has the sequence set forth in amino acids 19 through 134 of SEQ ID NO: 2, except for a single amino acid substitution at position 28, and a single amino acid substitution at position 44, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T. In another embodiment, the polypeptide has the sequence set forth in amino acids 23 through 134 of SEQ ID NO: 2, except for a single amino acid substitution at position 28, and a single amino acid substitution at position 44, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T. In another embodiment, the polypeptide has the sequence set forth in amino acids 25 through 134 of SEQ ID NO: 2, except for a single amino acid substitution at position 28, and a single amino acid substitution at position 44, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T. In another embodiment, the polypeptide has an amino acid sequence with at least 80 %, 85 %, 90 %, 95 %, 98 % or 99 % identity to any of the polypeptides above, wherein the polypeptide has single amino acid substitution at position 28, and a single amino acid substitution at position 44, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T, and wherein the

polypeptide is capable of binding myostatin, activin A, or GDF-11. In one embodiment, the substitution of the above polypeptides at position 28 is W and the substitution at position 44 is T, wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11.

In another embodiment, the isolated protein comprises a stabilized activin IIB receptor polypeptide, wherein the polypeptide has the sequence set forth in the group consisting of SEQ ID NO: 4, 6, 12 and 14. In another embodiment the protein comprises a polypeptide having at least 80 % sequence identity to SEQ ID NO: 4, 6, 12 or 14, wherein the polypeptide has a W or Y at position 28 and a T at position 44, and wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11. In another embodiment the protein comprises a polypeptide having at least 90 %

the above polypeptides at position 28 is W and the substitution at position 44 is T, wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11.

In a further embodiment, the protein comprises the polypeptides recited above, wherein the amino acid residue at position 64 is alanine.

The present disclosure also provides an isolated nucleic acid molecule comprising a polynucleotide encoding a stabilized ActRIIB polypeptide.

In a fourth aspect, there is provided an isolated nucleic acid molecule comprising a polynucleotide encoding the polypeptide of the third aspect.

In a fifth aspect, there is provided an isolated nucleic acid molecule comprising a polynucleotide selected from the group consisting of:

(a) a polynucleotide encoding a polypeptide consisting of the sequence set forth in the group consisting of SEQ ID NO: 4, 6, 12, and 14;

(b) a polynucleotide encoding a polypeptide having at least 90% sequence identity to (a), wherein the polypeptide has a W or a Y at the position corresponding to position 28 of SEQ ID NO:2 and a T at the position corresponding to position 44 of SEQ ID NO:2; and wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11; and

(c) a polynucleotide encoding a polypeptide having at least 95 % sequence identity to (a), wherein the polypeptide has a W or a Y at the position corresponding to position 28 of SEQ ID NO:2 and a T at the position corresponding to position 44 of SEQ ID NO:2, and wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11.

In one embodiment, the polynucleotide encodes the polypeptide sequence set forth in SEQ ID NO: 2, except for a single amino acid substitution at position 28, and a single amino acid substitution at position 44, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T. In another embodiment, the polynucleotide encodes the polypeptide having the sequence set forth in amino acids 19 through 134 of SEQ ID NO: 2, except for a single amino acid substitution at position 28, and a single amino acid substitution at position 44, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T. In another embodiment, the polynucleotide encodes the polypeptide having the sequence set forth in amino acids 23 through 134 of SEQ ID NO: 2, except for a single amino acid substitution at position 28, and a single amino acid substitution at position 44, wherein the substitution at position 28 is selected from W

or Y, and the substitution at position 44 is T. In another embodiment, the polynucleotide encodes the polypeptide having the sequence set forth in amino acids 25 through 134 of SEQ ID NO: 2, except for a single amino acid substitution at position 28, and a single amino acid substitution at position 44, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T. In another embodiment, the polynucleotide encodes the a polypeptide having an amino acid sequence at least 80 %, 85 %, 90 %, 95 %, 98 % or 99 % identity to any one of the polypeptides above, wherein the polypeptide has single amino acid substitution at position 28, and a single amino acid substitution at position 44, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T, and wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11. In one embodiment, the above polynucleotides encode a polypeptide wherein the substitution at position 28 is W and the substitution at position 44 is T, wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11.

In one embodiment, the nucleic acid molecule comprises a polynucleotide encoding a polypeptide having the sequence set forth in the group consisting of SEQ

capable of binding myostatin, activin A, or GDF-11. In one embodiment, the above polynucleotides encode a polypeptide wherein the substitution at position 28 is W and the substitution at position S44 is T, wherein the encoded polypeptide is capable of binding myostatin, activin A or GDF-11. In a further embodiment, the nucleic acid molecule comprises a polynucleotide having a sequence selected from the group consisting of SEQ ID NO: 7, 9, 15 and 17, or its complement.

In another embodiment, the nucleic acid molecule further comprises polynucleotides encoding the linkers and hinge linkers set forth in the group consisting of SEQ ID NO: 25, 27, 38, 40, 42, 44, 45, 46, 48, 49 and 50.

In a further embodiment, the nucleic acid molecule further comprises a transcriptional or translational regulatory sequence. In another embodiment a recombinant vector comprising a polynucleotide encoding a stabilized ActRIIB protein or polypeptide is provided.

In a sixth aspect, there is provided a recombinant vector comprising the nucleic acid molecule of the fifth aspect.

Also provided are host cells comprising the recombinant vectors, and methods of producing the stabilized ActRIIB proteins and polypeptides by culturing the host cells under conditions promoting expression of the proteins or polypeptides.

In a seventh aspect, there is provided a host cell comprising the recombinant vector of the sixth aspect.

In an eighth aspect, there is provided a method of producing an svActRIIB protein comprising culturing the host cell of the seventh aspect under conditions promoting the expression of the protein, and recovering the protein.

The present disclosure further provides a composition containing at least one stabilized ActRIIB polypeptide or protein of the present disclosure. In one embodiment, the composition is a pharmaceutical composition containing the stabilized ActRIIB polypeptide or protein in admixture with a pharmaceutically acceptable carrier.

In a ninth aspect, there is provided a pharmaceutical composition comprising an effective amount of the protein as defined herein in admixture with a pharmaceutically acceptable carrier.

In a tenth aspect, there is provided the composition of the ninth aspect for use as a medicament.

In another embodiment, the disclosure provides a method of reducing or blocking myostatin, activin A or GDF-11 activity by administering the svActRIIB proteins and polypeptides, or pharmaceutical compositions containing these, to a subject in need of such treatment.

In another embodiment, the disclosure provides a method of increasing lean muscle mass or increasing the ratio of lean muscle mass to fat mass in a subject in need of such treatment by administering an effective amount of the composition or pharmaceutical composition containing svActRIIB proteins or polypeptides to the subject.

In an eleventh aspect, there is provided the use of the protein as defined herein in a method of increasing lean muscle mass or increasing the ratio of lean muscle mass to fat mass, wherein the method is not a method of treatment of the human or animal body by therapy.

In a twelfth aspect, there is provided the composition of the ninth aspect for use in a method of treating muscle-wasting or a metabolic disorder selected from diabetes, obesity, hyperglycemia and bone loss in a subject in need of such treatment.

In another embodiment, the disclosure provides a method of treating or preventing a muscle wasting disease in a subject suffering from such a disorder by administering a therapeutic composition containing an svActRIIB polypeptide or protein to the subject. The muscle wasting disease includes, but is not limited to, the following

conditions: cancer cachexia, muscular dystrophy, amyotrophic lateral sclerosis, congestive obstructive pulmonary disease, chronic heart failure, chemical cachexia, cachexia from HIV/AIDS, renal failure, uremia, rheumatoid arthritis, age-related sarcopenia, age-related frailty, organ atrophy, carpal tunnel syndrome, androgen deprivation, and muscle-wasting due to inactivity from prolonged bed rest, spinal chord injury, stroke, bone fracture, burns, aging, insulin resistance, and other disorders. The muscle wasting may also result from weightlessness due to space flight.

In a thirteenth aspect, there is provided the composition of the ninth aspect for use in a method of treating a disease in which activin is over-expressed in a subject in need of such treatment, and wherein the disease is cancer.

In another embodiment, the present disclosure provides a method of treating conditions in which activin is overexpressed in a subject in need of such treatment, by administering an effective amount of a therapeutic composition containing svActRIIB proteins or polypeptides to the subject. In one embodiment, the disease is cancer. In another embodiment, the present disclosure provides a method of treating a metabolic disorder comprising administering a therapeutic composition containing svActRIIB proteins or polypeptides to a subject in need of such treatment, wherein the metabolic disorder is selected from bone loss, diabetes, obesity, impaired glucose tolerance, hyperglycemia, and metabolic syndrome. In another embodiment, the present disclosure provides a method of gene therapy for treating muscle wasting or metabolic or activin-related disorders comprising administering a vector encoding an svActRIIB polypeptide or protein of the present disclosure to a subject in need thereof, wherein the vector is capable of expressing the svActRIIB protein or polypeptide in the subject.

In a fourteenth aspect, there is provided the vector of the sixth aspect for use in a method of treating muscle wasting, or a metabolic disorder selected from diabetes, obesity, hyperglycemia and bone loss, or a cancer in which activin is over-expressed in a subject in need of such a treatment, wherein the vector is capable of directing expression of an svActRIIB polypeptide in the subject.

In another embodiment, the present disclosure provides a method of detecting and quantitating myostatin, activin, or GDF-11 by using any of the svActRIIB proteins or polypeptides as capture or binding agents in any number of assays.

CLAIMS

1. An isolated protein comprising a stabilized activin IIB receptor polypeptide, wherein said polypeptide is selected from the group consisting of:

(a) a polypeptide consisting of the sequence set forth in the group consisting of SEQ ID NO: 4, 6, 12 and 14;

(b) a polypeptide having at least 90% sequence identity to (a), wherein the polypeptide has a W or a Y at the position corresponding to position 28 of SEQ ID NO:2 and a T at the position corresponding to position 44 of SEQ ID NO:2, wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11, and

(c) polypeptides having at least 95% sequence identity to (a), wherein the polypeptide has a W or a Y at the position corresponding to position 28 of SEQ ID NO:2 and a T at the position corresponding to position 44 of SEQ ID NO:2, wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11.

2. The protein of claim 1, wherein the polypeptide is connected to at least one heterologous polypeptide.

3. The protein of claim 2, wherein the heterologous polypeptide is an IgG Fc domain.

4. The protein of claim 2, wherein the heterologous polypeptide is connected to the polypeptide by a linker sequence.

5. The protein of claim 4, wherein the linker sequence is selected from the group consisting of the sequences set forth in: SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 49 and SEQ ID NO: 50.

6. The protein of claim 3, wherein the protein comprises a polypeptide selected from the group consisting of:

(a) a polypeptide consisting of the sequence set forth in the group consisting of SEQ ID NO: 8, 10, 16 and 18;

(b) a polypeptide having at least 90% sequence identity to (a), wherein the polypeptide has a W or a Y at the position corresponding to position 28 of SEQ ID NO:2 and a T at the position corresponding to position 44 of SEQ ID NO:2, wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11, and

(c) polypeptides having at least 95% sequence identity to (a), wherein the polypeptide has a W or a Y at the position corresponding to position 28 of SEQ ID NO:2 and a T at the position corresponding to position 44 of SEQ ID NO:2, wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11.

7. An isolated protein consisting of the sequence set forth in SEQ ID NO: 10.

8. An isolated protein comprising a stabilized activin IIB receptor polypeptide (svActRIIB) wherein said polypeptide is selected from the group consisting of:

(a) a polypeptide consisting of the sequence set forth in SEQ ID NO: 2, except for a single amino acid substitution at the position corresponding to position 28 of SEQ ID NO:2, and a single amino acid substitution at the position corresponding to position 44 of SEQ ID NO:2, wherein the substitution at position 28 is selected from the group consisting of W and Y, and the substitution at position 44 is T;

(b) a polypeptide consisting of the sequence set forth in amino acids 19 through 134 of SEQ ID NO:2, except for a single amino acid substitution at the position corresponding to position 28 of SEQ ID NO:2, and a single amino acid substitution at the position corresponding to position 44 of SEQ ID NO:2, wherein the substitution at position 28 is selected from the group consisting of W and Y, and the substitution at position 44 is T;

(c) a polypeptide consisting of the sequence set forth in amino acids 23 through 134 of SEQ ID NO:2, except for a single amino acid substitution at the position corresponding to position 28 of SEQ ID NO:2, and a single amino acid substitution at the position corresponding to position 44 of SEQ ID NO:2, wherein the substitution at position 28 is selected from the group consisting of W and Y, and the substitution at position 44 is T;

(d) a polypeptide consisting of the sequence set forth in amino acids 25 through 134 of SEQ ID NO: 2, except for a single amino acid substitution at the position corresponding to position 28 of SEQ ID NO:2, and a single amino acid substitution at the position corresponding to position 44 of SEQ ID NO:2, wherein the substitution at position 28 is selected from the group consisting of W and Y, and the substitution at position 44 is T;

(e) a polypeptide having at least 80% sequence identity to any one of (a) through (d), except for a single amino acid substitution at the position corresponding to position 28 of SEQ ID NO:2, and a single amino acid substitution at the position corresponding to position 44 of SEQ ID NO:2, wherein the substitution at position 28 is selected from the group consisting of W and Y, and the substitution at position 44 is T, wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11.

9. An isolated nucleic acid molecule comprising a polynucleotide encoding the polypeptide of claim 8.

10. An isolated nucleic acid molecule comprising a polynucleotide selected from the group consisting of:

(a) a polynucleotide encoding a polypeptide consisting of the sequence set forth in the group consisting of SEQ ID NO: 4, 6, 12, and 14;

(b) a polynucleotide encoding a polypeptide having at least 90% sequence identity to (a), wherein the polypeptide has a W or a Y at the position corresponding to position 28 of SEQ ID NO:2 and a T at the position corresponding to position 44 of SEQ ID NO:2; and wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11; and

(c) a polynucleotide encoding a polypeptide having at least 95 % sequence identity to (a), wherein the polypeptide has a W or a Y at the position corresponding to position 28 of SEQ ID NO:2 and a T at the position corresponding to position 44 of SEQ ID NO:2, and wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11.

11. The nucleic acid molecule of claim 10, wherein the polynucleotide has the sequence selected from the group consisting of the sequence set forth in SEQ ID NO: 3, 5, 11 and 13 or its complement.

12. The nucleic acid molecule of claim of claim 10, wherein the polynucleotide further comprises a polynucleotide encoding at least one heterologous protein.

13. The nucleic acid molecule of claim 12, comprising a polynucleotide selected from the group consisting of:

(a) a polynucleotide encoding a polypeptide consisting of the sequence set forth in the group consisting of SEQ ID NO: 8, 10, 16, and 18;

(b) a polynucleotide encoding a polypeptide having at least 90% sequence identity to (a), wherein the polypeptide has a W or a Y at the position corresponding to position 28 of SEQ ID NO:2 and a T at the position corresponding to position 44 of SEQ ID NO:2, and wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11; and

(c) a polynucleotide encoding a polypeptide having at least 95% sequence identity to (a), wherein the polypeptide has a W or a Y at the position corresponding to position 28 of SEQ ID NO:2 and a T at the position corresponding to position 44 of SEQ ID NO:2, and wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11.

14. The nucleic acid molecule of claim 13, wherein the polynucleotide has the sequence selected from the group consisting of the sequence set forth in SEQ ID NO: 7, 9, 15 and 17, or its complement.

15. The nucleic acid molecule of claim 10, wherein the polynucleotide is operably linked to a transcriptional or translational regulatory sequence.

16. A recombinant vector comprising the nucleic acid molecule of claim 10.

17. A host cell comprising the recombinant vector of claim 16.

18. The host cell of claim 17, wherein the host cell is a mammalian cell.

19. A method of producing an svActRIIB protein comprising culturing the host cell of claim 17 under conditions promoting the expression of the protein, and recovering the protein.

20. A pharmaceutical composition comprising an effective amount of the protein of any one of claims 1-8 in admixture with a pharmaceutically acceptable carrier.

21. The composition of claim 20 for use as a medicament.

22. The composition of claim 20 for use in a method of treating muscle-wasting or a metabolic disorder selected from diabetes, obesity, hyperglycemia and bone loss in a subject in need of such treatment.

23. The composition for use according to claim 22, wherein the muscle-wasting is due to a muscle-wasting disease selected from muscular dystrophy, amyotrophic lateral sclerosis, congestive obstructive pulmonary disease, chronic heart failure, cancer cachexia, AIDS, renal failure, uremia, rheumatoid arthritis, age-related sarcopenia, organ atrophy, carpal tunnel syndrome, androgen deprivation, burn injury, diabetes, and muscle-wasting due to prolonged bed rest, spinal chord injury, stroke, bone fracture, aging or exposure to micro-gravity.

24. The composition of claim 20 for use in a method of treating a disease in which activin is over-expressed in a subject in need of such treatment, and wherein the disease is cancer.

25. The vector of claim 16 for use in a method of treating muscle wasting, or a metabolic disorder selected from diabetes, obesity, hyperglycemia and bone loss, or a cancer in which activin is over-expressed in a subject in need of such a treatment, wherein the vector is capable of directing expression of an svActRIIB polypeptide in the subject.

26. The composition for use according to claim 24 or the vector for use according to claim 25, wherein the cancer is ovarian cancer.

27. Use of the protein of any one of claims 1 to 8 in a method of increasing lean muscle mass or increasing the ratio of lean muscle mass to fat mass, wherein the method is not a method of treatment of the human or animal body by therapy.