According to further features in the described preferred embodiments the at least one modulator of angiogenesis is an endothelin receptor antagonist. The endothelial receptor antagonist can be a dual A-form and B-form endothelin receptor antagonist, or a B-form specific antagonist.

According to yet further features in the described preferred embodiments the B-form specific endothelin receptor antagonist is selected from the group consisting of A192,621; BQ788; Res 701-1 and Ro 46-8443.

According to yet further features in the described preferred embodiments the endothelin receptor antagonist is an endothelin receptor antagonist excluding Bosentan.

Definitions of the specific embodiments of the invention as claimed herein follow.

According to a first embodiment of the invention, there is provided a polynucleotide comprising a conditionally replicating adenovirus genome transcriptionally linked to a pre-proendothelin-1 promoter which selectively directs transcription of said adenovirus in angiogenic endothelial cells and wherein said polynucleotide comprises an adenoviral E1 region consecutive to said pre-proendothelin-1 promoter.

According to a second embodiment of the invention, there is provided a nucleic acid construct comprising the polynucleotide of the first embodiment.

According to a third embodiment of the invention, there is provided a cell expressing the nucleic acid construct of the second embodiment.

According to a fourth embodiment of the invention, there is provided a pharmaceutical composition comprising the nucleic acid construct of the second embodiment and a pharmaceutically acceptable carrier.

According to a fifth embodiment of the invention, there is provided a method of downregulating angiogenesis in a tissue of a subject in need thereof, the method comprising expressing in the tissue the nucleic acid construct of the second embodiment, thereby downregulating angiogenesis in said tissue.

According to a sixth embodiment of the invention, there is provided a method of treating a disease or condition associated with excessive neo-vascularization, the method comprising administering to a subject in need thereof a therapeutically effective amount of the nucleic acid construct of the second embodiment, thereby down-regulating angiogenesis in the tissue and treating the disease or condition associated with excessive neo-

vascularization.

According to a seventh embodiment of the invention, there is provided a method of treating a tumor in a subject, the method comprising administering to a subject in need thereof a therapeutically effective amount of a nucleic acid construct of the second embodiment, thereby down-regulating angiogenesis in the tumor and treating the tumor.

The present invention successfully addresses the shortcomings of the presently known configurations by providing isolated polynucleotide sequences comprising cis regulating elements with novel enhancer elements, and methods of use thereof. The novel enhancer elements can be used to make nucleic acid constructs and pharmaceutical compositions for tissue-specific regulation of transgene expression, and for treating a variety of disorders, diseases and conditions by gene therapy. Specifically, the cis regulatory elements, isolated polynucleotides and pharmaceutical compositions of the present invention can be used, along with selected transgenes, to specifically upregulate and/or downregulate angiogenesis in endothelial cells, thus treating tumors, metastatic disease, and ischemic disease.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only, and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for a fundamental understanding of the

described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims. All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention.

The term 'comprise' and variants of the term such as 'comprises' or 'comprising' are used herein to denote the inclusion of a stated integer or stated integers but not to exclude any other integer or any other integers, unless in the context or usage an exclusive interpretation of the term is required.

Any reference to publications cited in this specification is not an admission that the disclosures constitute common general knowledge in Australia.

WHAT IS CLAIMED IS:

1. ~~Use of a nucleic acid construct comprising an isolated polynucleotide which comprises~~ A polynucleotide comprising a conditionally replicating adenovirus genome transcriptionally linked to ~~an endothelial specific or peri-endothelial specific~~ a pre-proendothelin-1 promoter ~~for the manufacture of a medicament for treating a disease or condition associated with excessive neo-vascularization in a subject in need thereof, wherein said promoter~~ which selectively directs transcription of said adenovirus in angiogenic endothelial cells~~,~~ and wherein said ~~nucleic acid construct further~~ polynucleotide comprises ~~a nucleotide sequence encoding a suicide gene, pro-apoptotic gene or cytotoxic agent~~ an adenoviral E1 region consecutive to said pre-proendothelin-1 promoter.

2. The ~~use~~ polynucleotide of claim 1, wherein ~~said promoter is a pre-proendothelin-1 promoter~~ the conditionally replicating adenovirus is an adenovirus-5 vector.

3. The ~~use of claim 2~~ polynucleotide of claim 1 or claim 2, wherein ~~said~~ the promoter is a PPE-1-3X promoter.

4. The ~~use of any one of claims 1-3~~ polynucleotide of claim 1 or claim 2, wherein ~~said isolated polynucleotide further includes~~ the pre-proendothelin-1 promoter comprises a polynucleotide having ~~at least one copy of~~ the sequence set forth in ~~SEQ ID NO: 6~~ SEQ ID NO: 8 or the complementary sequence thereof.

5. The ~~use~~ polynucleotide of any one of claims 1 to 4 3, ~~wherein said isolated polynucleotide further includes~~ further comprising at least one copy of the sequence set forth in ~~SEQ ID NO:8~~ SEQ ID NO: 6 or the complementary sequence thereof.

6. The ~~use~~ polynucleotide of any one of claims ~~2 to 5~~ 1 to 3, wherein ~~said~~ the promoter ~~comprises~~ further includes at least ~~one copy~~ two copies of the sequence set forth in ~~SEQ ID NO:1~~ SEQ ID NO: 6 of the complementary sequence thereof.

7. The ~~use~~ polynucleotide of any one of claims 1 to ~~6~~ 3, ~~wherein said isolated polynucleotide~~ further ~~comprises a polynucleotide sequence as set forth in SEQ ID NO: 7~~ comprising at least one copy of the sequence set forth in SEQ ID NO: 1 or a fragment thereof.

8. The ~~use~~ polynucleotide of any one of claims 1 to ~~7~~ 3, ~~wherein said conditionally replicating adenovirus comprises an adenoviral E1 region~~ further comprising at least one hypoxia response element having the sequence as set forth in SEQ ID NO: 5.

9. The ~~use~~ polynucleotide of any one of claims 1 to ~~8~~ 3, ~~wherein said conditionally replicating adenovirus is an adenovirus 5 vector~~ further comprising a polynucleotide sequence as set forth in SEQ ID NO: 7 or a complementary sequence thereof.

10. The ~~use~~ polynucleotide of any one of claims 1 to 9, ~~which construct further comprises at least one hypoxia responsive element having a sequence as set forth in SEQ ID NO:5~~ being devoid of reporter gene sequences.

11. ~~The use of~~ A nucleic acid construct comprising the polynucleotide of any one of claims 1-10~~, wherein said cytotoxic agent is a chimeric protein comprising the extracellular region of TNFR1 and the trans-membrane and intracellular regions of Fas~~.

12. ~~The use of any one of claims 1 to 11, wherein said nucleic acid construct is further devoid of reporter gene sequences~~ A cell expressing the nucleic acid construct of claim 11.

13. ~~The use of any one of claims 1 to 12, wherein said subject is being treated with a corticosteroid and/or N-acetyl cysteine~~ A pharmaceutical composition comprising the nucleic acid construct of claim 11 and a pharmaceutically acceptable carrier.

14. ~~The use of any one of claims 1 to 12, wherein said subject is being treated with an endothelin receptor antagonist selected capable of further potentiating activity of said promoter in a synergic manner~~ Use of the nucleic acid construct of claim 11 for the manufacture of a medicament for downregulating angiogenesis in a tissue of a subject in need thereof, wherein expression of the nucleic acid construct in the tissue downregulates angiogenesis in the tissue.

15. ~~The use of claim 14, wherein said endothelial receptor antagonist is a dual A-form and B-form endothelin receptor antagonist or a B-form specific antagonist~~ Use of the nucleic acid construct of claim 11 for the manufacture of a medicament for treating a disease or condition associated with excessive neo-vascularization in a subject in need thereof, wherein a therapeutically effective amount of the nucleic acid construct is administered to the subject and administration of the nucleic acid construct to the subject treats the disease or condition associated with excessive neo-vascularization.

16. The use of claim 15, wherein said ~~antagonist is a B-form specific endothelin receptor antagonist selected from the group consisting of A192,621; BQ788; Res 701-1 and Ro 46-8443~~ disease or condition associated with excessive neovascularization is selected from the group consisting of cancer, metastatic disease, diabetic retinopathy, psoriasis, arthritis, hemangioma, tumor growth and atherosclerosis.

17. ~~The use of claim 14, wherein said endothelin receptor antagonist is an endothelin receptor antagonist excluding Bosentan~~ Use of the nucleic acid construct of claim 11 for the manufacture of a medicament for treating a tumor in a subject in need thereof, wherein a therapeutically effective amount of the nucleic acid construct is administered to the subject and administration of the nucleic acid construct to the subject treats the tumor.

18. The use of any one of claims ~~1~~ 14 to 17, ~~wherein said disease or condition associated with excessive neovascularization is selected from the group consisting of cancer, metastatic disease, diabetic retinopathy, psoriasis, arthritis, hemangioma, tumor growth and metastases~~ further comprising administering corticosteroid and/or N-acetyl cysteine.

19. The use of any one of claims ~~1 to 18~~ 14 to 17, ~~wherein a therapeutically effective amount of said nucleic acid construct is about $5X10^{11}$ to about $5X10^{14}$ virus particles per kilogram body weight~~ further comprising administering a B-form or non-selective endothelin receptor antagonist which enhances transcription from said pre-proendothelin-1 promoter in a synergistic manner.

20. The use of ~~any one of claims 1 to 15~~ claim 19, wherein ~~a therapeutically effective amount of said nucleic acid construct is about $5X10^{10}$ to about 10^{12} virus particles per kilogram body weight~~ said B-form specific endothelin receptor antagonist is selected from the group consisting of A192,621; BQ788; Res 701-1 and Ro 46-8443.

21. The use of any one of claims ~~1-15~~ 14 to 20, wherein ~~said medicament is formulated for treating a disease or condition in a human subject, and said therapeutically effective amount of said nucleic acid construct is about $1X10^{10}$ to $1X10^{14}$ virus particles~~ a therapeutically effective amount of said nucleic acid construct is about $5Xl0^{9}$ to about $5X10^{14}$ virus particles per kilogram body.

22. The use of claim 21, wherein a therapeutically effective amount of said nucleic acid construct is about 10^{11} to about 10^{13} virus particles.

According to further features in the described preferred embodiments the at least one modulator of angiogenesis is an endothelin receptor antagonist. The endothelial receptor antagonist can be a dual A-form and B-form endothelin receptor antagonist, or a B-form specific antagonist.

According to yet further features in the described preferred embodiments the B-form specific endothelin receptor antagonist is selected from the group consisting of A192,621; BQ788; Res 701-1 and Ro 46-8443.

According to yet further features in the described preferred embodiments the endothelin receptor antagonist is an endothelin receptor antagonist excluding Bosentan.

Definitions of the specific embodiments of the invention as claimed herein follow.

According to a first embodiment of the invention, there is provided a polynucleotide comprising a conditionally replicating adenovirus genome transcriptionally linked to a pre-proendothelin-1 promoter which selectively directs transcription of said adenovirus in angiogenic endothelial cells and wherein said polynucleotide comprises an adenoviral El region consecutive to said pre-proendothelin-1 promoter.

According to a second embodiment of the invention, there is provided a nucleic acid construct comprising the polynucleotide of the first embodiment.

According to a third embodiment of the invention, there is provided a cell expressing the nucleic acid construct of the second embodiment.

According to a fourth embodiment of the invention, there is provided a pharmaceutical composition comprising the nucleic acid construct of the second embodiment and a pharmaceutically acceptable carrier.

According to a fifth embodiment of the invention, there is provided a method of downregulating angiogenesis in a tissue of a subject in need thereof, the method comprising expressing in the tissue the nucleic acid construct of the second embodiment, thereby downregulating angiogenesis in said tissue.

According to a sixth embodiment of the invention, there is provided a method of treating a disease or condition associated with excessive neo-vascularization, the method comprising administering to a subject in need thereof a therapeutically effective amount of the nucleic acid construct of the second embodiment, thereby down-regulating angiogenesis in the tissue and treating the disease or condition associated with excessive neo-vascularization.

According to a seventh embodiment of the invention, there is provided a method of treating a tumor in a subject, the method comprising administering to a subject in need thereof a therapeutically effective amount of a nucleic acid construct of the second embodiment, thereby down-regulating angiogenesis in the tumor and treating the tumor.

The present invention successfully addresses the shortcomings of the presently known configurations by providing isolated polynucleotide sequences comprising cis regulating elements with novel enhancer elements, and methods of use thereof. The novel enhancer elements can be used to make nucleic acid constructs and pharmaceutical compositions for tissue-specific regulation of transgene expression, and for treating a variety of disorders, diseases and conditions by gene therapy. Specifically, the cis regulatory elements, isolated polynucleotides and pharmaceutical compositions of the present invention can be used, along with selected transgenes, to specifically upregulate and/or downregulate angiogenesis in endothelial cells, thus treating tumors, metastatic disease, and ischemic disease.

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described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination.

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Any reference to publications cited in this specification is not an admission that the disclosures constitute common general knowledge in Australia.

WHAT IS CLAIMED IS:

1. A polynucleotide comprising a conditionally replicating adenovirus genome transcriptionally linked to a pre-proendothelin-1 promoter which selectively directs transcription of said adenovirus in angiogenic endothelial cells and wherein said polynucleotide comprises an adenoviral E1 region consecutive to said pre-proendothelin-1 promoter.

2. The polynucleotide of claim 1, wherein the conditionally replicating adenovirus is an adenovirus-5 vector.

3. The polynucleotide of claim 1 or claim 2, wherein the promoter is a PPE-1-3X promoter.

4. The polynucleotide of claim 1 or claim 2, wherein the pre-proendothelin-1 promoter comprises a polynucleotide having the sequence set forth in SEQ ID NO: 8 or the complementary sequence thereof.

5. The polynucleotide of any one of claims 1 to 3, further comprising at least one copy of the sequence set forth in SEQ ID NO: 6 or the complementary sequence thereof.

6. The polynucleotide of any one of claims 1 to 3, wherein the promoter further includes at least two copies of the sequence set forth in SEQ ID NO: 6 of the complementary sequence thereof.

7. The polynucleotide of any one of claims 1 to 3, further comprising at least one copy of the sequence set forth in SEQ ID NO: 1 or a fragment thereof.

8. The polynucleotide of any one of claims 1 to 3, further comprising at least one hypoxia response element having the sequence as set forth in SEQ ID NO: 5.

9. The polynucleotide of any one of claims 1 to 3, further comprising a polynucleotide sequence as set forth in SEQ ID NO: 7 or a complementary sequence thereof.

10. The polynucleotide of any one of claims 1 to 9, being devoid of reporter gene sequences.

11. A nucleic acid construct comprising the polynucleotide of any one of claims 1-10.

12. A cell expressing the nucleic acid construct of claim 11.

13. A pharmaceutical composition comprising the nucleic acid construct of claim 11 and a pharmaceutically acceptable carrier.

14. Use of the nucleic acid construct of claim 11 for the manufacture of a medicament for downregulating angiogenesis in a tissue of a subject in need thereof, wherein expression of the nucleic acid construct in the tissue downregulates angiogenesis in the tissue.

15. Use of the nucleic acid construct of claim 11 for the manufacture of a medicament for treating a disease or condition associated with excessive neo-vascularization in a subject in need thereof, wherein a therapeutically effective amount of the nucleic acid construct is administered to the subject and administration of the nucleic acid construct to the subject treats the disease or condition associated with excessive neo-vascularization.

16. The use of claim 15, wherein said disease or condition associated with excessive neovascularization is selected from the group consisting of cancer, metastatic disease, diabetic retinopathy, psoriasis, arthritis, hemangioma, tumor growth and atherosclerosis.

17. Use of the nucleic acid construct of claim 11 for the manufacture of a medicament for treating a tumor in a subject in need thereof, wherein a therapeutically effective amount of the nucleic acid construct is administered to the subject and administration of the nucleic acid construct to the subject treats the tumor.

18. The use of any one of claims 14 to 17, further comprising administering corticosteroid and/or N-acetyl cysteine.

19. The use of any one of claims 14 to 17, further comprising administering a B-form or non-selective endothelin receptor antagonist which enhances transcription from said pre-proendothelin-1 promoter in a synergistic manner.

20. The use of claim 19, wherein said B-form specific endothelin receptor antagonist is selected from the group consisting of A192,621; BQ788; Res 701-1 and Ro 46-8443.

21. The use of any one of claims 14 to 20, wherein a therapeutically effective amount of said nucleic acid construct is about $5X10^9$ to about $5X10^{14}$ virus particles per kilogram body.

22. The use of claim 21, wherein a therapeutically effective amount of said nucleic acid construct is about 10^{11} to about 10^{13} virus particles.