receptor activation, which may contribute to tumor resistance to anti-VEGF therapy [11]. Therefore, EC metabolism has been identified as a new target for anti-angiogenic therapy, particularly through inhibition of energy metabolism and glycosylation.

SUMMARY

[0005] The present disclosure provides pharmaceutical compositions and methods for treating an ischemic condition in a subject. In ~~embodiments~~examples, a composition for treating an ischemic condition in a subject includes hexosamine D-mannosamine (ManN). In ~~embodiments~~examples, a method for treating an ischemic condition in a subject includes administering to the subject in need thereof an effective amount of hexosamine D-mannosamine (ManN).

[0005a] In one aspect, the present disclosure provides use of hexosamine D-mannosamine (ManN) in the manufacture of a medicament for treating an ischemic condition in a subject.

[0006] In ~~embodiments~~examples, the administration is effective to promote endothelial cell proliferation and angiogenesis in the subject. In ~~embodiments~~examples, the method further includes administering to the subject in need thereof an effective amount of an N-glycosylation inhibitor. In ~~embodiments~~examples, the method further includes administering to the subject in need thereof an effective amount of VEGF.

[0007] In ~~embodiments~~examples, the ischemic condition is caused by a disease or a trauma. In ~~embodiments~~examples, the administration is intravenous, intraperitoneal, or intravitreal.

[0008] ~~In embodiments, the~~The present disclosure provides pharmaceutical compositions and methods for inducing angiogenesis in a subject, including administering to the subject in need thereof an effective amount of hexosamine D-mannosamine (ManN).

[0008a] In another aspect, the present disclosure provides hexosamine D-mannosamine (ManN) for use in inducing angiogenesis in a subject.

[0009] In ~~embodiments~~examples, the administration is effective to reduce ischemia in the subject. In ~~embodiments~~examples, the method further includes administering to the subject in need thereof an effective amount of an N-glycosylation inhibitor. In ~~embodiments~~examples, the method further includes administering to the subject in need thereof an effective amount of VEGF.

[0010] In ~~embodiments~~examples, the subject is in need of inducing angiogenesis due to an ischemic condition is caused by a disease or a trauma.

[0011] In ~~embodiments~~examples, the administration is intravenous, intraperitoneal, or intravitreal.

[0012] ~~In embodiments, the~~The present disclosure provides pharmaceutical compositions and methods for inhibiting protein glycosylation in a cell, including administering to the cell an effective amount of hexosamine D-mannosamine (ManN).

[0012a] In another aspect, the present disclosure provides an in vitro method of inhibiting protein glycosylation in a cell, the method comprising administering to the cell an effective amount of hexosamine D-mannosamine (ManN).

[0013] In ~~embodiments~~examples, the administration is *in vivo*. In ~~embodiments~~examples, the administration is *ex vivo*. In ~~embodiments~~examples, the administration is effective to stimulate EC proliferation and angiogenesis. In ~~embodiments~~examples, the administration is effective to activate JNK

and an unfolded protein response caused by ER stress. In ~~embodiments~~examples, the administration is effective to induce changes in N-glycan and O-glycan profiles.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] FIGS. 1A to 1F represent examples showing the effects of Mannosamine (ManN) on bovine choroidal microvascular endothelial cells (BCEC) proliferation. FIG. 1A is an image showing crystal-violet stained BCEC samples treated with ManN, in the presence or absence of VEGF. BCECs were treated with various concentrations of ManN ranging from 0.5 µM to 1 mM for 5-6 days, with or without 5ng/ml VEGF. At the end of the experiment, cells were fixed and stained with crystal violet. Cell-covered areas in various treatment groups were quantified by ImageJ software. FIG. 1B is a chart showing the effect of ManN in the presence or absence of VEGF on cell numbers. Cell numbers were quantified by adding AlamarBlue and fluorescence was measured at 530 nm/590 nm. n=3 independent samples were used. FIG. 1C is a chart showing the effect of ManN on bovine retinal microvascular endothelial cells (BREC) proliferation. n=3 biologically independent samples were used. FIG. 1D is an image showing effects of hexosamines other than ManN on BCEC proliferation in samples. Each treatment group was tested in duplicate. FIG. 1E is a chart and images showing effect of ManN on wounded BCEC samples. BCEC confluent monolayers were scratched with 1ml pipet tip, washed and then incubated for 40 hours in low glucose DMEM containing 1% FBS. n=3 independent samples were used. Scale bar= 400 µm. Images were taken and gaps between leading wound front were quantified using AxioVision LE Rel.4.4 software. Representative images from crystal violet staining are shown. FIG. 1F is a chart showing effects of ManN in BCEC transwell migration assay. n=4 independent samples were used. Asterisks indicate a significant difference compared with control. When statistical analysis was done using a different control, a line was used between specific groups. A representative experiment is shown from 2 independent studies. Data are means +/- SD, Statistical analysis was done by 2-tailed, two-sample unequal variance *t* test. * $p<0.05$, ** $p<0.01$.

[0015] FIGS. 2A to 2C are western blot images showing activation of ERK, AKT, mTOR, AMPKα, CREB, ACC, and eNOS is not unique to ManN. Enhanced activation of ERK (Thr 202/Tyr 204), AKT (Ser 473) and CREB (Ser 133) in BCECs following treatment with ManN together with VEGF for various times (FIG. 2A) or following pre-treatment with ManN for 8 hours, followed by VEGF stimulation for 15 minutes (FIG. 2B). For the samples shown in FIG. 2C, BCECs were treated with 40 µM ManN, ManNAc or mannose for various times. Total mTOR, ACC, eNOS, AMPKα, ERK, AKT, CREB, as well as phosphorylation of

CLAIMS

What is claimed is:

1. ~~A method of treating an ischemic condition in a subject, the method comprising administering to the subject in need thereof an effective amount of~~Use of hexosamine D-mannosamine (ManN) in the manufacture of a medicament for treating an ischemic condition in a subject.
2. The ~~method~~ use of claim 1, wherein the ~~administering is effective~~medicament is to be administered in an effective amount to promote endothelial cell proliferation and angiogenesis in the subject.
3. The ~~method~~ use of claim 1, wherein ~~the method further comprises administering to the subject in need thereof~~ an effective amount of an N-glycosylation inhibitor is to be further administered to the subject in need thereof.
4. The ~~method~~ use of claim 1, wherein ~~the method further comprises administering~~ an effective amount of VEGF is to be further administered to the subject in need thereof.
5. The ~~method~~ use of claim 1, wherein the ischemic condition is caused by a disease or a wound.
6. The ~~method~~ use of claim 1, wherein ~~the administration is~~the medicament is to be administered intravenously, intraperitoneally, topically, or intravitreally.
7. ~~A method of inducing angiogenesis in a subject, the method comprising administering to the subject in need thereof an effective amount of hexosamine~~Hexosamine D-mannosamine (ManN) for use in inducing angiogenesis in a subject.
8. The ~~method~~ ManN for use of claim 7, wherein the ManN is to be administered in an effective amount ~~administering is effective~~ to reduce ischemia in the subject.
9. The ~~method~~ ManN for use of claim 7, wherein ~~the method further comprises administering to the subject in need thereof~~ an effective amount of an N-glycosylation inhibitor is to be further administered to the subject in need thereof.
10. The ~~method~~ ManN for use of claim 7, wherein ~~the method further comprises administering~~ an effective amount of VEGF is to be further administered to the subject in need thereof.

11. The ~~method~~ ManN for use of claim 7, wherein the ~~subject is in need of induction of angiogenesis is an~~ ManN is for use in treating an ischemic condition due to a disease or trauma, and the ManN is to be administered to induce angiogenesis.

12. The ~~method~~ ManN for use of claim 7, wherein the ~~administration is~~ ManN is to be administered intravenously, intraperitoneally, topically, or intravitreally.

13. An in vitro method of inhibiting protein glycosylation in a cell, the method comprising administering to the cell an effective amount of hexosamine D-mannosamine (ManN).

~~14. The method of claim 13, wherein the administration is in vivo.~~

~~15. The method of claim 13, wherein the administration is in vitro.~~

~~16.~~14. The in vitro method of claim 13, wherein the administration is effective to stimulate EC proliferation and angiogenesis.

~~17.~~15. The in vitro method of claim 13, wherein the administering is effective to activate JNK and an unfolded protein response caused by ER stress.

~~18.~~16. The in vitro method of claim 13, wherein the administering is effective to induce a change in the N-glycan and O-glycan profile.