proposed that inhibiting glycosylation may result in suppression of key angiogenesis pathways, including VEGF/VEGFR2 and Notch. Evidence that has emerged in recent years points to glycans as novel angiogenesis regulators due to changes in protein glycosylation. For example, the glycan-binding protein Galectin1 has been reported to interact with VEGFR2, leading to ligand-independent receptor activation, which may contribute to tumor resistance to anti-VEGF therapy. Therefore, EC metabolism has been identified as a new target for anti-angiogenic therapy, particularly through inhibition of energy metabolism and glycosylation.

SUMMARY

[005] There remains a need for effective therapeutics and methods of treatment for stroke. Stroke continues to be a debilitating condition that can lead to extensive rehabilitating periods, to decreases in an ability to conduct activities of daily living, to social and financial hardships, and can be fatal. Preventing a first stroke, preventing a recurrence of a stroke, mitigating the effects of an ongoing stroke, or improving the treatment and recovery from a stroke would all present great benefits to society and improve quality of life for many individuals.

[006] The present disclosure provides pharmaceutical compositions and methods for treating an ischemic condition in a subject caused by a stroke. In some examples, a pharmaceutical composition for treating a stroke comprises an effective amount of an N-glycosylation inhibitor administered to a subject in need thereof. In some examples, the N-glycosylation inhibitor comprises hexosamine D-mannosamine (ManN), Kifunensine (Kif), Castanospermine (Cas), or a combination thereof. In some examples, the N-glycosylation inhibitor comprises ManN. In some examples, the subject has experienced an ischemic stroke. In some examples, the ischemic stroke comprises a thrombotic stroke or an embolic stroke. In some examples, the subject has experienced a hemorrhagic stroke. In some examples, the hemorrhagic stroke comprises a subarachnoid hemorrhage or an intracerebral hemorrhage. In some examples, the subject has experienced a brainstem stroke. In some examples, the subject has experienced a transient ischemic attack. In some examples, the subject has experienced a cryptogenic stroke.

[007] Described herein are pharmaceutical compositions providing an effective amount of an N-glycosylation inhibitor to treat stroke. In some examples, the effective amount of an N-

glycosylation inhibitor comprises a single dosing treatment regimen. In some examples, the single dosing treatment regimen comprises pretreatment, concurrent treatment, or post-treatment in relation to an ischemic event affecting the brain. In some examples, the effective amount of an N-glycosylation inhibitor comprises a multiple dosing treatment regimen. In some examples, the multiple dosing treatment regimen comprises pretreatment, concurrent treatment, and/or post-treatment in relation to an ischemic event affecting the brain. In some examples, the effective amount of an N-glycosylation inhibitor is administered orally, intravenously, intrathecally or intraperitoneally. In some examples, the effective amount of an N-glycosylation inhibitor comprises about 10 mg to about 100 g of the N-glycosylation inhibitor. In some examples, the effective amount of an N-glycosylation inhibitor comprises about 200 mg to about 40 g of the N-glycosylation inhibitor. In some examples, the effective amount of an N-glycosylation inhibitor comprises about 5 g to about 20 g of the N-glycosylation inhibitor.

[008] Described herein are pharmaceutical compositions providing an effective amount of an N-glycosylation inhibitor to treat stroke that may be combined with other factors that modulate angiogenesis. In some examples, pharmaceutical compositions described herein further comprise an effective amount of a pro-angiogenic factor. In some examples, the pro-angiogenic factor comprises vascular endothelial growth factor (VEGF), or a derivative thereof. In some examples, the VEGF is VEGF-A, VEGF-B, VEGF-C, VEGF-D, placental growth factor (PLGF), or a combination thereof. In some examples, the VEGF is a recombinant VEGF. In some examples, the VEGF is administered locally near a site in the brain of a subject affected by an ischemic event.

[009] Described herein are methods for treating stroke comprising administering to a subject in need thereof an effective amount of an N-glycosylation inhibitor. In some examples, the N-glycosylation inhibitor comprises hexosamine D-mannosamine (ManN), Kifunensine (Kif), Castanospermine (Cas), or a combination thereof. In some examples, the N-glycosylation inhibitor comprises ManN. In some examples, the subject has experienced an ischemic stroke. In some examples, the ischemic stroke is a thrombotic stroke or an embolic stroke. In some examples, the subject has experienced a hemorrhagic stroke. In some examples, the hemorrhagic stroke comprises a subarachnoid hemorrhage or an intracerebral hemorrhage. In some examples, the subject has experienced a brainstem stroke. In some examples, the subject has experienced a transient ischemic attack. In some examples, the subject has experienced a cryptogenic stroke.

[0010] Described herein are methods for treating stroke comprising administering to a subject in need thereof an effective amount of an N-glycosylation inhibitor. In some examples, the effective amount of an N-glycosylation inhibitor comprises a single dosing treatment regimen. In some examples, the single dosing treatment regimen comprises pretreatment, concurrent treatment, or post-treatment in relation to an ischemic event affecting the brain. In some examples, the effective amount of an N-glycosylation inhibitor comprises a multiple dosing treatment regimen. In some examples, the multiple dosing treatment regimen comprises pretreatment, concurrent treatment, and/or post-treatment in relation to an ischemic event affecting the brain. In some examples, the effective amount of an N-glycosylation inhibitor is administered orally, intravenously, intrathecally or intraperitoneally. In some examples, the effective amount of an N-glycosylation inhibitor comprises about 10 mg to about 300 g of the N-glycosylation inhibitor. In some examples, the effective amount of an N-glycosylation inhibitor comprises about 200 mg to about 100 g of the N-glycosylation inhibitor. In some examples, the effective amount of an N-glycosylation inhibitor comprises about 5 g to about 40 g of the N-glycosylation inhibitor. In some examples, the single dosing treatment regimen comprises administering a formulation of the N-glycosylation inhibitor at a dosage of about 20mg to about 2000mg/kg body weight of a subject. In some examples, the single dosing treatment regimen comprises administering a formulation of the N-glycosylation inhibitor at a dosage of about 100 mg to about 300mg/kg body weight of a subject. In some examples, the multiple dosing treatment regimen comprises administering a formulation of the N-glycosylation inhibitor at a dosage of about 10mg to about 2000mg/kg body weight of a subject. In some examples, the multiple dosing treatment regimen comprises administering a formulation of the N-glycosylation inhibitor at a dosage of about 100 mg to about 300mg/kg body weight of a subject.

[0011] Described herein are methods of treating stroke comprising administering to a subject in need thereof an effective amount of an N-glycosylation inhibitor that may be combined with other factors that modulate angiogenesis. In some examples, the administering to a subject further comprises administering an effective amount of a pro-angiogenic factor. In some examples, the pro-angiogenic factor comprises vascular endothelial growth factor (VEGF), or a derivative thereof. In some examples, the VEGF is VEGF-A, VEGF-B, VEGF-C, VEGF-D, placental growth factor (PLGF), or a combination thereof. In some

examples, the VEGF is a recombinant VEGF. In some examples, the VEGF is administered locally near a site in the brain of a subject affected by an ischemic event.

[0012] Described herein are methods of treating stroke comprising administering to a subject in need thereof an effective amount of an N-glycosylation inhibitor wherein N-linked glycosylation of proteins is inhibited in an endothelial cell in a subject. Also described herein are methods of treating stroke comprising administering to a subject in need thereof an effective amount of an N-glycosylation inhibitor wherein the administering is effective to stimulate endothelial cell proliferation and angiogenesis of blood vessels near the brain. Also described herein are methods of treating stroke comprising administering to a subject in need thereof an effective amount of an N-glycosylation inhibitor wherein the administering is effective to stimulate brain endothelial cell proliferation and angiogenesis. Also described herein are methods of treating stroke comprising administering to a subject in need thereof an effective amount of an N-glycosylation inhibitor wherein the administering is effective to activate JNK signaling and upregulate an unfolded protein response caused by endoplasmic reticulum stress. Also described herein are methods of treating stroke comprising administering to a subject in need thereof an effective amount of an N-glycosylation inhibitor wherein the subject demonstrates an improvement in one or more symptoms of stroke following the administering. In some examples, the one or more symptoms are selected from the group consisting of: muscle weakness, numbness in the face, paralysis in the face, paralysis in a limb, paralysis on one side of the body, slurred speech, garbled speech, difficulty understanding others, sudden onset blindness in one or both eyes, double vision, vertigo, confusion, lack of mental alertness, loss of balance, loss of coordination, stroke-induced mortality, hypoxic damage to one or more areas of the brain, stroke-induced memory impairment, stroke-induced voluntary movement impairment, stroke-induced language impairment, stroke-induced reduction in cognitive capacity, stroke-induced reduction in mobility. In some examples of methods of treating stroke described herein, the subject demonstrates a significant increase in vascular density in or near a region of stroke-induced brain injury following the administering.

[0013] In some examples, a pharmaceutical composition for treating an ischemic condition in a subject caused by a stroke includes inhibitors of N-glycosylation. In some examples, a pharmaceutical composition for treating an ischemic condition in a subject caused by a stroke includes hexosamine D-mannosamine (ManN). In some examples, a method for treating an ischemic condition in a subject caused by a stroke includes

administering to the subject in need thereof an effective amount of hexosamine D-mannosamine (ManN). The present disclosure also provides pharmaceutical compositions and methods for preventing the occurrence of a stroke in a subject. The present disclosure also provides pharmaceutical compositions and methods for attenuating the effects of an ongoing ischemic event affecting the brain.

[0014] In some examples, the administration is effective to promote endothelial cell proliferation and angiogenesis in the subject. In some examples, the method further includes administering to the subject in need thereof an effective amount of an N-glycosylation inhibitor. In some examples, the method further includes administering to the subject in need thereof an effective amount of VEGF.

[0015] Described herein are methods for treating stroke comprising administering to a subject in need thereof an effective amount of an N-glycosylation inhibitor. In some examples, N-linked glycosylation of proteins is inhibited in an endothelial cell in a subject. In some examples, the administration is effective to stimulate endothelial cell proliferation and angiogenesis of blood vessels near the brain. In some examples, the administration is effective to stimulate brain endothelial cell proliferation and angiogenesis. In some examples, the administration is effective to activate JNK signaling and upregulate an unfolded protein response caused by endoplasmic reticulum stress.

[0016] Described herein are methods of preventing stroke comprising administering to a subject in need thereof an effective amount of an N-glycosylation inhibitor. In some examples, the N-glycosylation inhibitor comprises hexosamine D-mannosamine (ManN), Kifunensine (Kif), Castanospermine (Cas), or a combination thereof. In some examples, the N-glycosylation inhibitor comprises ManN. In some examples, the effective amount of an N-glycosylation inhibitor comprises a single dosing treatment regimen. In some examples, the effective amount of an N-glycosylation inhibitor comprises a multiple dosing treatment regimen. In some examples, the effective amount of an N-glycosylation inhibitor is administered orally, intravenously, intrathecally or intraperitoneally. In some examples, the effective amount of an N-glycosylation inhibitor comprises about 10 mg to about 300 g of the N-glycosylation inhibitor. In some examples, the effective amount of an N-glycosylation inhibitor comprises about 200 mg to about 100 g of the N-glycosylation inhibitor. In some examples, the effective amount of an N-glycosylation inhibitor comprises about 5 g to about 40 g of the N-glycosylation inhibitor.

[0017] Described herein are methods of preventing stroke comprising administering to a subject in need thereof an effective amount of an N-glycosylation inhibitor. In some examples, a risk of recurrent stroke is reduced in a subject. In some examples, a subject has previously experienced an ischemic stroke, a hemorrhagic stroke, a brainstem stroke, a transient ischemic attack, or a cryptogenic stroke. In some examples, the risk of a recurrent stroke comprises a risk of an ischemic stroke, a hemorrhagic stroke, a brainstem stroke, a transient ischemic attack, or a cryptogenic stroke. In some examples, a measurement of C-reactive protein (CRP) in the blood in a subject is reduced compared to a previous measurement of blood CRP level in the subject. In some examples, the measurement of CRP in the blood in a subject is reduced to below about 10 mg/L.

[0018] Described herein are methods of attenuating an ongoing ischemic event affecting the brain comprising administering to a subject in need thereof an effective amount of an N-glycosylation inhibitor. In some examples, the N-glycosylation inhibitor comprises hexosamine D-mannosamine (ManN), Kifunensine (Kif), Castanospermine (Cas), or a combination thereof. In some examples, the N-glycosylation inhibitor comprises ManN. In some examples, the subject is experiencing an ischemic stroke, a hemorrhagic stroke, a brainstem stroke, a transient ischemic attack, or a cryptogenic stroke. In some examples, the effective amount of an N-glycosylation inhibitor is administered orally, intravenously, intrathecally or intraperitoneally. In some examples, the effective amount of an N-glycosylation inhibitor comprises about 10 mg to about 300 g of the N-glycosylation inhibitor. In some examples, the effective amount of an N-glycosylation inhibitor comprises about 5 g to about 40 g of the N-glycosylation inhibitor. In some examples, a neurological assessment of acute stroke in a subject improves compared to a previous assessment. In some examples, blood flow to the brain of a subject is improved. In some examples, the blood flow to the brain is assessed by transcranial doppler ultrasound.

[0019] In some examples, the ischemic condition affecting the brain is caused by a disease or a trauma. In some examples, the subject is in need of inducing angiogenesis due to an ischemic condition is caused by a disease or a trauma. In some examples, the administration is effective to reduce ischemia in the subject. In some examples, the method further includes administering to the subject in need thereof an effective amount of an N-glycosylation inhibitor. In some examples, the method further includes administering to the subject in need thereof an effective amount of VEGF. In some examples, the administration is oral, intravenous, intrathecal, or intraperitoneal.

[0020] 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). 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 Kifunensine (Kif). 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 Castanospermine (Cas).

[0021] In some examples, the administration is *in vivo*. In some examples, the subject in need of administration of a N-glycosylation inhibitor is a mammal. In some examples, the subject is a rodent. In some examples, the subject belongs to the genus *Rattus.* In some examples, the subject belongs to the genus *Mus*. In some examples, the subject belongs to the genus *Canis.* In some examples, the subject belongs to the genus *Felis.* In some examples, the subject belongs to the genus *Equus.* In some examples, the subject is a human. In some examples, the administration of the N-glycosylation inhibitor is effective to stimulate EC proliferation and angiogenesis. In some examples, the administration is effective to activate JNK signaling and an unfolded protein response caused by ER stress. In some examples, the administration is effective to induce changes in N-glycan and O-glycan profiles in endothelial cells.

[0021a] In one aspect, the present disclosure provides use of Hexosamine D-mannosamine (ManN) in the manufacture of a medicament for treating a stroke in a subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] This patent application contains at least one drawing executed in color. Copies of this patent or patent application with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0023] The novel features of the disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments.

[0024] 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

CLAIMS

What is claimed is:

1. Use of Hexosamine D-mannosamine (ManN) in the manufacture of a medicament for treating a stroke in a subject.

2. The use of claim 1, wherein the subject has experienced an ischemic stroke, optionally wherein the ischemic stroke is a thrombotic stroke or an embolic stroke.

3. The use of claim 1, wherein the subject has experienced a hemorrhagic stroke, optionally wherein the hemorrhagic stroke comprises a subarachnoid hemorrhage or an intracerebral hemorrhage.

4. The use of claim 1, wherein the subject has experienced a brainstem stroke, a transient ischemic attack, or a cryptogenic stroke.

5. The use of claim 1, wherein the ManN is to be delivered to the subject via a single dosing treatment regimen or a multiple dosing treatment regimen.

6. The use of claim 5, wherein the single dosing treatment or the multiple dosing treatment regimen comprises pretreatment, concurrent treatment, and/or post-treatment in relation to an ischemic event affecting the brain of the subject.

7. The use of claim 1, wherein the ManN is to be administered orally, intravenously, intrathecally, or intraperitoneally.

8. The use of claim 1, wherein an effective amount of the ManN comprises about 10 mg to about 300 g to be administered per administration.

9. The use of claim 5, wherein the single dosing treatment regimen comprises a formulation of ManN to be administered at a dosage of about 20 mg to about 2000 mg/kg body weight of a subject.

10. The use of claim 1, further comprising a pro-angiogenic factor.

11. The use of claim 10, wherein the pro-angiogenic factor comprises vascular endothelial growth factor (VEGF), or a derivative thereof, optionally wherein the VEGF is VEGF-A, VEGF-B, VEGF-C, VEGF-D, or placental growth factor (PLGF).

12. The use of claim 11, wherein the VEGF is a recombinant VEGF.

13. The use of claim 1, wherein N-glycosylation of proteins is to be inhibited in an endothelial cell of the subject.

14. The use of claim 1, wherein proliferation or angiogenesis of an endothelial cell in the brain of the subject is to be stimulated.

15. The use of claim 1, wherein the subject demonstrates an improvement in one or more symptoms of stroke after being administered the ManN.

16. The use of claim 15, wherein the one or more symptoms are selected from the group consisting of: muscle weakness, numbness in the face, paralysis in the face, paralysis in a limb, paralysis on one side of the body, slurred speech, garbled speech, difficulty understanding others, sudden onset blindness in one or both eyes, double vision, vertigo, confusion, lack of mental alertness, loss of balance, loss of coordination, stroke-induced mortality, hypoxic damage to one or more areas of the brain, stroke-induced memory impairment, stroke-induced voluntary movement impairment, stroke-induced language impairment, stroke-induced reduction in cognitive capacity, stroke-induced reduction in mobility.

17. The use of claim 1, wherein the ManN results in an increase in vascular density in or near a region of stroke-induced brain injury.

18. The use of claim 1, wherein the ManN results in a decrease in a blood C-reactive protein (CRP) level of the subject when compared to a blood CRP level of the subject prior to administration of the medicament.

19. The use of claim 18, wherein the blood CPR level in the subject is reduced to below about 10 mg/L after being administered the ManN.

20. The use of claim 1, wherein blood flow to the brain of the subject is improved, optionally wherein the blood flow to the brain of the subject is assessed by transcranial doppler ultrasound.