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Sema3E/PlexinD1 inhibition is a therapeutic strategy for improving cerebral perfusion and restoring functional loss after stroke in aged rats

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ABSTRACT

Brain tissue survival and functional recovery after ischemic stroke greatly depend on cerebral vessel perfusion and functional collateral circulation in the ischemic area. Semaphorin 3E (Sema3E), one of the class 3 secreted semaphorins, has been demonstrated to be a critical regulator in embryonic and postnatal vascular formation via binding to its receptor PlexinD1. However, whether Sema3E/PlexinD1 signaling is involved in poststroke neovascularization remains unknown. To determine the contribution of Sema3E/PlexinD1 signaling to poststroke recovery, aged rats (18 months) were subjected to a transient middle cerebral artery occlusion. We found that depletion of Sema3E/PlexinD1 signaling with lentivirus-mediated PlexinD1-specific-shRNA improves tissue survival and functional outcome. Sema3E/PlexinD1 inhibition not only increases cortical perfusion but also ameliorates blood-brain barrier damage, as determined by positron emission tomography and magnetic resonance imaging. Mechanistically, we demonstrated that Sema3E suppresses endothelial cell proliferation and angiogenic capacity. More importantly, Sema3E/PlexinD1 signaling inhibits recruitment of pericytes by decreasing production of platelet derived growth factor-BB in endothelial cells. Overall, our study revealed that inhibition of Sema3E/PlexinD1 signaling in the ischemic penumbra, which increases both endothelial angiogenic capacity and recruitment of pericytes, contributed to functional neovascularization and blood-brain barrier integrity in the aged rats. Our findings imply that Sema3E/PlexinD1 signaling is a novel therapeutic target for improving brain tissue survival and functional recovery after ischemic stroke.

1. Introduction

Ischemic stroke, a disease caused by cerebral artery occlusion, persistently ranks among the most common causes of death and disability worldwide, especially in industrialized countries (Feigin et al., 2014; Lloyd-Jones et al., 2009). Current treatment options available for ischemic stroke patients are limited. Thrombolysis therapies induced with intravenous r-tPA or mechanical thrombectomy predominantly using stent retriever devices are currently the major approaches approved by the United States Food and Drug

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Administration. However, these therapies are restricted by a narrow time window for administration and limited efficacy in reversing neurological deficits (Adeoye et al., 2011; Bai and Lyden, 2015).

In patients with acute ischemic stroke, it has been observed that from 6 hours to several days after stroke, perfusion in the ischemic boundary zone, also known as the ischemic penumbra, remains at 25%–50% of preocclusion values (Furlan et al., 1996; Heiss, 2000). The brain tissue in the ischemic penumbra is at risk but not yet dead; neurons in this area are reported to survive for days to weeks (Ferrer and Planas, 2003; Heiss and Zaro Weber, 2017; Kaushal and Schlichter, 2008). An increase in blood flow can switch tissue injury to recovery and prevent further brain function loss due to the decreased death of neurons (Heiss and Zaro Weber, 2017; Lo, 2008). After stroke, collateral circulation reconstruction is the major component in increasing cerebral blood flow and improving cerebral perfusion (Liu et al., 2014). During the subacute and chronic phases in human stroke patients, pathological analysis and cerebral imaging have demonstrated that increased neovascularization in the ischemic penumbra correlates with collateral recruitment and longer survival (Ergul et al., 2012; Krupinski et al., 1994; Liu et al., 2014; Szpak et al., 1999).

However, the naturally occurring neovascularization, which begins between 24 and 48 hours after an ischemic insult, appears only dysfunctionally and transiently in the ischemic cortex (Ergul et al., 2012; Manoonkitiwongsa et al., 2001; Navaratna et al., 2009), especially in senescent brains (Petcu et al., 2010). It remains unclear which endogenous factors are responsible for blocking restorative postischemic angiogenesis. Several endogenous proangiogenic molecules have been reported to stimulate angiogenesis after stroke, including vascular endothelial growth factor (VEGF), fibroblastic growth factor, and brain-derived neurotrophic factor (Ergul et al., 2012). However, simply increasing angiogenesis via proangiogenic factors may lead to immature vasculature and increased permeability, which may exacerbate edema or hemorrhage after stroke (Ergul et al., 2012; Zhang et al., 2000). Recent studies demonstrated that cerebral functional neovascularization depends not only on endothelial cell survival but also on pericyte recruitment and coverage (Dalkara et al., 2011; Hall et al., 2014; Potente et al., 2011). Therefore, identification of new strategies controlling each of these aspects is crucial. Semaphorin 3E (Sema3E), an antiangiogenic mediator, was confirmed to be a typical repulsive guidance cue for cortical neurons and blood vessel networks during natural development (Chauvet et al., 2007; Gu et al., 2005; Kim et al., 2011). During physiological processes, Sema3E regulates vascular patterning by repelling endothelial cells and inhibiting motility, adhesion, and tubulogenesis of endothelial cells (Aghajanian et al., 2014). Sema3E/PlexinD1 signaling is also associated with progression of disease through mediating cell migration and viability under pathological conditions (Casazza et al., 2010; Movassagh et al., 2014). Previous studies indicated that Sema3E is increased by hypoxia (Moriya et al., 2010), suggesting that Sema3E may be upregulated and involved in neovascularization after ischemic stroke. We previously documented a Sema3E-induced impairment of the migration and proliferation of vascular smooth muscle cells during neointimal formation (Wu et al., 2017). However, whether Sema3E/PlexinD1 signaling is associated with cerebral perfusion and functional recovery after CNS (central nervous system) injury is unknown.

Here, we demonstrate for the first time that Sema3E/PlexinD1 inhibition not only improves cerebral perfusion but also reduces blood-brain barrier (BBB) damage in the ischemic penumbra after cerebral ischemia. In addition, we elucidate the molecular mechanisms by which Sema3E governs angiogenic activity and BBB integrity in vivo and in vitro.

2. Materials and methods

2.1. Transient middle cerebral artery occlusion model

All experimental uses of rats for this study were approved by the Institutional Committee of Animal Care and Use and the Medical Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology. Male Sprague-Dawley rats, 18 months old and weighing 300–600 g, were acquired and maintained within the facilities of the Animal Care and Use Committee of Tongji Medical College at Huazhong University of Science and Technology, Wuhan, China. The rats were anesthetized with 1.5% isofluorane in 70% N_2O and 30% O_2. The rectal temperature of the rats was maintained at 37.3 $\pm$ 0.5 °C with a heating pad during the procedures. Transient middle cerebral artery occlusion (tMCAO) was created as previously described (Chen et al., 2003; Hasegawa et al., 2013). In brief, the right common carotid artery, right internal carotid artery, and right external carotid artery of the rats were carefully isolated using a midline incision. Then, the artery branches of the right external carotid artery were ligated. After loosely tying the mobilized external carotid artery stump with a 6-0 nylon suture, a microvascular clip was placed on the origin of the right external carotid artery. Then, a poly-L-lysine-coated 4-0 monofilament nylon suture (Cinontech, Beijing, China) was inserted from the origin of the right external carotid artery and advanced for 18.5–19.5 mm. As a result, the right middle cerebral artery (MCA) is occluded. After 2 hours, MCA blood flow was restored. Sham-operated rats underwent the identical procedure without inserting the filament. The stability of infarction was confirmed by 2% 2,3,5-triphenyltetrazolium chloride staining at 1 day and 3 days after tMCAO. The aged rats involved in this study exhibited similar lesion sizes. The infarct sizes and ischemic boundaries were identified by Nissl staining and NeuN staining. For each animal, 4 brain sections (spaced by 2 mm) were transferred to cresyl violet staining and NeuN staining; the other brain tissues were either used for embedding into OCT compound or stored in liquid nitrogen for Western blot analysis. The perilesion area was identified as ischemic cortex within 1 mm of the ischemic boundary (including the ischemic boundary, region 1 and region 2 were each 0.5 mm) and the core of the infarct area that was devoid of neurons was excluded with a reference section of cresyl violet staining and NeuN staining (Dimitrijevic et al., 2007; Sladojevic et al., 2014). The distant area was identified as being 1 mm outward from the ischemic boundary (region 3 and region 4 were each 0.5 mm). The corresponding regions in the sham group were also analyzed.

2.2. Construction of lentivirus vector and stereotaxic injection

The lentivirus vectors (LVs) that express PlexinD1-shRNA–green fluorescent protein (GFP) (siRNA sequence targeted in PlexinD1: 5′-GAGAGAACATTGA GGCCAA-3′) or GFP alone (control siRNA sequence: 5′-TTCTCCGAACGTGTCACGT-3′) were constructed and purchased from Shanghai Genechem. Three cortical injections of LV-PlexinD1-shRNA-GFP or LV-GFP were administered into the borehole in the right hemisphere with a stereotaxic instrument at the following coordinates from the bregma: point 1 at 1.0 mm anterior, 5 mm lateral, 2.5 mm deep; point 2 at −0.8 mm anterior, 5 mm lateral, 2.5 mm deep; and point 3 at −2.6 mm anterior, 5 mm lateral, 2.5 mm deep. Next, 10 μL of lentivirus suspension containing 1×10^9 TU ml^{-1} was injected at a rate of 1 μL min^{-1}. The needle was withdrawn over the course of 10 minutes. At 5 days after injection, rats were euthanized with a lethal dosage of pentobarbital sodium and the brains were removed for protein extraction and cryosection. The transfection efficiency of shRNA was assessed by