RESEARCH ARTICLE

LncRNA *SNHG12* ameliorates brain microvascular endothelial cell injury by targeting miR-199a

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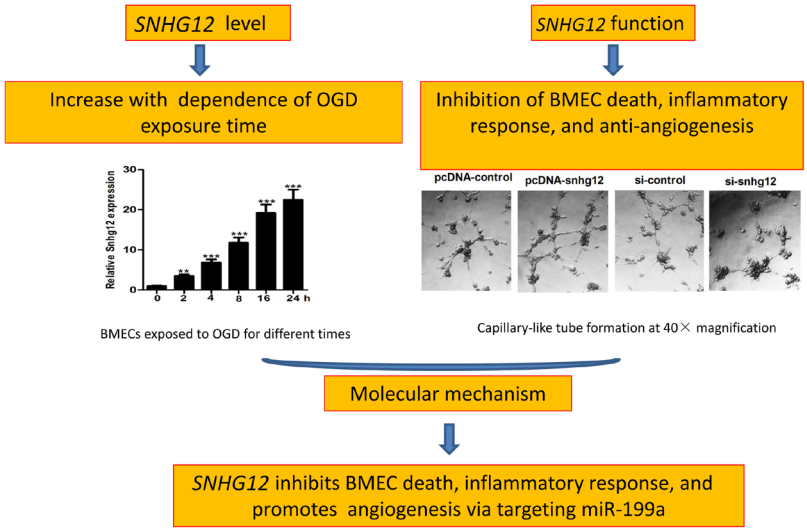
Graphical Abstract

The role and molecular mechanism of lncRNA *SNHG12* in brain microvascular endothelial cell (BMEC) injury induced by oxygen-glucose deprivation (OGD)/reoxygenation



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Abstract

Long non-coding RNAs regulate brain microvascular endothelial cell death, the inflammatory response and angiogenesis during and after ischemia/reperfusion and oxygen-glucose deprivation/reoxygenation (OGD/R) insults. The long non-coding RNA, *SNHG12*, is upregulated after ischemia/reperfusion and OGD/R in microvascular endothelial cells of the mouse brain. However, its role in ischemic stroke has not been studied. We hypothesized that *SNHG12* positively regulates ischemic stroke, and therefore we investigated its mechanism of action. We established an OGD/R mouse cell model to mimic ischemic stroke by exposing brain microvascular endothelial cells to OGD for 0, 2, 4, 8, 16 or 24 hours and reoxygenation for 4 hours. Quantitative real-time polymerase chain reaction showed that *SNHG12* levels in brain microvascular endothelial cells increased with respect to OGD exposure time. Brain microvascular endothelial cells were transfected with pcDNA-control, pcDNA-*SNHG12*, si-control, or si-*SNHG12*. After exposure to OGD for 16 hours, these cells were then analyzed by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide, trypan blue exclusion, western blot, and capillary-like tube formation assays. Overexpression of *SNHG12* inhibited brain microvascular endothelial cell death and the inflammatory response but promoted angiogenesis after OGD/R, while *SNHG12* knockdown had the opposite effects. miR-199a was identified as a target of *SNHG12*, and *SNHG12* overexpression reversed the effect of miR-199a on brain microvascular endothelial cell death, the inflammatory response, and angiogenesis. These findings suggest that *SNHG12* suppresses endothelial cell injury induced by OGD/R by targeting miR-199a.

Key Words: *nerve regeneration; ischemic stroke; microRNA; brain microvascular endothelial cell death; inflammatory response; angiogenesis; oxygen-glucose deprivation/reoxygenation; ischemia/reperfusion; therapeutic targets; neural regeneration; gene regulation; neural regeneration*

Introduction

Ischemic stroke is the primary cause of death and permanent disability in adults worldwide and occurs when blood flow to the brain is blocked, causing immediate deprivation of oxygen and nutrients (Fisher, 2009; Jauch et al., 2013). Despite advances in medical and endovascular recanalization therapy, treatment choices for ischemic stroke are still very limited (Furlan et al., 2003; Lakhan et al., 2009); therefore, novel and effective treatments for ischemic stroke are urgently needed. Undoubtedly, a good understanding of pathogenesis will facilitate the development of such therapies.

Many mechanisms are known to be involved in the pathogenesis of ischemic stroke. Among these, it is increasingly appreciated that brain microvascular endothelial cell (BMEC) death induced by ischemia/reperfusion (I/R) injury is the initial stage of blood-brain barrier disruption and leads to a poor prognosis for ischemic stroke patients (Hawkins and Davis, 2005; Date et al., 2006; Lakhan et al., 2013; Zhang

et al., 2017a). It is also known that endothelial inflammation and subsequent impairment of endothelial function contribute to ischemic brain injury and determine stroke outcome (Anwaar et al., 1998; Stanimirovic and Satoh, 2000; Zoppo et al., 2000; Hassan et al., 2003; Muir et al., 2007; Pei et al., 2015). Angiogenesis after ischemic stroke is also clinically significant; it improves recovery from stroke by promoting neurogenesis and enhancing neuronal and synaptic plasticity (Wang et al., 2012; Liu et al., 2014; Alhusban et al., 2015; Hui et al., 2017; Li et al., 2017).

Non-coding RNAs (ncRNAs) are generally defined as untranslated regulatory RNA molecules. Among ncRNAs, microRNAs (miRNAs, usually 18–25 nucleotides in length) and long ncRNAs (lncRNAs, usually > 200 nucleotides in length) regulate various biological processes, including cell growth, apoptosis, differentiation, the inflammatory response and angiogenesis, and are involved in various diseases (Pillai, 2005; Park et al., 2014; Schaukowitch and Kim, 2014; Asghari et al., 2016; Ninova et al., 2016; Tian et al., 2016; Kondo et al., 2017; Qiu et al., 2017). miRNAs regulate gene expression and function via translation inhibition, mRNA degradation, or both (Pillai, 2005). Accumulated evidence is overwhelming for miRNAs playing a role in stroke. For example, overexpression of circulating miR-223 is associated with the pathogenesis of acute ischemic stroke, inhibition of miR-155 expression facilitates recovery after experimental stroke in mice, and miR-497 modulates neuronal death after transient focal cerebral ischemia in mice (Yin et al., 2010; Wang et al., 2014; Caballero-Garrido et al., 2015).

LncRNAs can modulate gene expression at transcriptional and post-transcriptional levels. Recently, lncRNAs were reported to regulate BMEC survival, the inflammatory response and angiogenesis during and after ischemic stroke. For example, Liu et al. (2016) reported that downregulation of the lncRNA, Meg3, increases angiogenesis after ischemic stroke by activating Notch signaling. Meanwhile, Zhang et al. (2017) reported that the lncRNA, Malat1, is remarkably upregulated after I/R or oxygen-glucose deprivation/reoxygenation (OGD/R) insult and that Malat1 plays a critical role in protecting the cerebral microvasculature by promoting BMEC survival and inhibiting endothelial inflammation after I/R or OGD/R insult. *SNHG12* is also dramatically upregulated by I/R or OGD/R (Liu et al., 2016). In addition, *SNHG12* can promote cell proliferation and angiogenesis in various types of cancer (Ruan et al., 2016; Lan et al., 2017; Wang et al., 2017). However, the function of *SNHG12* in ischemic stroke remains unknown. This study investigated the effects of *SNHG12* on BMEC death, angiogenesis and the inflammatory response after OGD/R, and the molecular mechanisms underlying these effects.

Materials and Methods

Cell culture and OGD/R

Mouse primary BMECs were purchased from Cell Biologics, Inc. (Chicago, IL, USA) and were grown to approximately 90% confluence before use. To mimic OGD ischemia-like conditions *in vitro*, BMECs were cultured in deoxygenated glucose-free Hanks' Balanced Salt Solution (Invitrogen, Carlsbad, CA, USA) gassed with 95% N_2/5% CO_2, in an anaerobic chamber (Forma Scientific, Marietta, OH, USA) for different lengths of time. Control BMECs were not exposed to OGD. For reoxygenation with glucose reintroduction, cells were cultured in standard medium under a humidified 95% air/5% CO_2 atmosphere for 4 hours.

Cell transfection

Plasmid pcDNA3.1, containing *SNHG12* (pcDNA-*SNHG12*), siRNAs targeting *SNHG12* (si-*SNHG12*) and their respective controls (pcDNA-control and si-control), were obtained from Sigma-Aldrich (St. Louis, MO, USA). An miR-199a mimic (miR-199a) and an miR-control were obtained from GenePharma Co., Ltd (Shanghai, China). BMECs were plated in 96-well plates at 2×10^5 cells per well and incubated overnight. Transfection was performed using Lipofectamine 2000 (Invitrogen) in accordance with the manufacturer's instructions. pcDNA-control, pcDNA-*SNHG12*, si-control, si-*SNHG12*, miR-control, and miR-199a groups were established by transection of BMECs with pcDNA-control, pcDNA-*SNHG12*, si-control, si-*SNHG12*, miR-control and miR-199a, respectively. The miR-199a + pcDNA-control group was established by cotransfection of BMECs with miR-199a mimic and pcDNA-control, while the miR-199a + pcDNA-*SNHG12* group was established by cotransfection of BMECs with miR-199a mimic and pcDNA-*SNHG12*.

Quantitative real-time polymerase chain reaction (qRT-PCR)

At 48 hours post-transfection or after exposure to OGD for indicated times, total RNA was extracted from BMECs using Trizol reagent (Invitrogen), and reverse transcribed into cDNA using Taq-Man Reverse Transcription reagents (Applied Biosystems, Foster City, CA, USA). The cDNA was used for qRT-PCR using a SYBR PrimeScript RT-PCR kit (TaKaRa, Dalian, China). GAPDH, a constitutively expressed gene, was used as an internal control. miR-199a expression was detected with a TaqMan microRNA real-time RT-PCR kit (Applied Biosystems), and U6 small nuclear RNA was used as a loading control. Fold change in gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method. Primer sequences are listed in **Table 1**.

Cell growth assay

After exposure to OGD for 16 hours, BMEC viability and death were assessed by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) and trypan blue exclusion assays, respectively, as previously described (Hopkins-Donaldson et al., 2003). Briefly, for the MTT assay, 1×10^5 cells per well in 96-well plates were exposed to OGD for 16 hours, and then cultured in standard medium under a humidified 95% air/5% CO_2 atmosphere for 4 hours, after which 20 μL of MTT (5 mg/ml) solution was added to each well, and the cultures incubated for a further 2 hours. The culture medium was then discarded and 100 μL of dimethyl sulfoxide (Sigma) added per well. The plates were shaken to

Table 1 Primer sequences used for quantitative real-time polymerase chain reaction

Gene	Primer sequences (5′–3′)
SNHG12	Forward: TCT GGT GAT CGA GGA CTT CC
	Reverse: ACC TCC TCA GTA TCA CAC ACT
E-selectin	Forward: CAA CTT CAC CTG TGA GGA AGG C
	Reverse: GAA CTC ACA GCT GGA CCC ATAA
MCP1	Forward: TCA GCC AGA TGC AGT TAA CGC
	Reverse: TGA TCC TCT TGT AGC TCT CCA GC
IL6	Forward: ACA ACC ACG GCC TTC CCT ACT T
	Reverse: CAC GAT TTC CCA GAG AAC ATG TG
VEGFA	Forward: GCC TTG CTG CTC TAC CTC CAC
	Reverse: ATG ATT CTG CCC TCC TCC TTC T
FGFb	Forward: CCG CCC TGC CGG AGG ATG GAG GCA
	Reverse: GCC TTC TGC CCA GGT CCT GT
GAPDH	Forward: TAT GAT ATC AAG AGG GTA GT
	Reverse: TGT ATC CAA ACT CAT TGT CAT AC
miR-199a	Forward: CTC AAC TGG TGT CGT GGA GTC GGC AAT TCA GTT GAG GAA CAG G
	Reverse: ACA CTC CAG CTG GGC CCA GT
U6	Forward: GCT TCG GCA CAT ATA CTA AAA T
	Reverse: CGC TTC ACG AAT TTG CGT GTC AT

MCP1: Monocyte chemotactic protein 1; IL: interleukin; VEGF: vascular endothelial growth factor; FGF: fibroblast growth factor; GAPDH: glyceraldehyde phosphate dehydrogenase. U6 serves as an internal reference to measure the expression of miR-199a.

dissolve the crystals and absorbance was determined with an enzyme-linked immunosorbent assay plate reader (BIOTEK, Winooski, VT, USA) at 570 nm. For the trypan blue exclusion assay, 1×10^6 BMECs per well in six-well plates were exposed to OGD for 16 hours, and then cultured in standard medium for 4 hours. After trypsinizing and washing in PBS, the cells were resuspended in 0.4% trypan blue. Dead and live cells were counted using a hemocytometer, and the percentage of dead cells was calculated.

Western blot assay

After exposure to OGD for 16 hours, western blot assays were performed as described previously (Lou et al., 2012). Briefly, total proteins were extracted from BMECs using lysis buffer. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transfer of proteins to membranes, and immune detection were then sequentially performed. Primary antibodies against vascular endothelial growth factor (VEGF)A (diluted 1:200; Abcam, Cambridge, UK), fibroblast growth factor (FGF) b (diluted 1:500; Abcam), E-selectin (diluted 1:500; Abcam), monocyte chemotactic protein 1 (MCP1) (diluted 1:100; Chemicon, Temecula, CA, USA), interleukin-6 (IL-6) (diluted 1:100; Chemicon), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (diluted 1:1000; Abcam), and horseradish peroxidase-conjugated secondary antibody (diluted 1:5000; Abcam) were employed. Membranes were incubated with primary antibodies for 1 hour at 37°C. After washing, the membranes were incubated with the secondary antibody for 2 hours at room temperature. Protein bands were visualized using a Supersignal West Pico Chemiluminescent Substrate Kit (Pierce, Rockford, IL, USA). Image analysis was performed to quantify the integrated density of the individual bands using Image J software (NIH, Bethesda, USA).

Capillary-like tube formation assay

After exposure to OGD for 16 hours, an in vitro angiogenesis assay was performed as previously described (Haralabopoulos et al., 1994). Briefly, 0.8 mL of growth factor-reduced Matrigel (Becton-Dickinson, San Jose, CA, USA) was added to a 35-mm Petri dish and allowed to polymerize at 37°C for 3 hours. BMECs (2×10^4 cells) were added and cultured in Dulbecco's modified Eagle's medium (Gibco-BRL, Rockville, MD, USA) for 6 hours. To quantify tube formation, five random areas were photographed and the total tube length was measured using cellSens Standard 1.6 image software (Olympus, Napa, CA, USA).

Statistical analysis

All data are presented as the mean ± SEM from at least three independent experiments. All statistical analyses were performed with SPSS 17.0 software (SPSS, Chicago, IL, USA). The differences between groups were analyzed by Student's two-tailed *t*-test or one-way analysis of variance followed by the Bonferroni *post hoc* test. A value of $P < 0.05$ was considered statistically significant.

Results

Upregulated *SNHG12* inhibited BMEC death under OGD/R conditions

After exposure to OGD for 0, 2, 4, 8, 16 or 24 hours and reoxygenation for 4 hours, *SNHG12* levels in BMECs was determined by qRT-PCR. *SNHG12* expression increased with respect to OGD exposure time (**Figure 1A**). To investigate the effect of *SNHG12* on BMEC growth, BMECs were transfected with pcDNA-control, pcDNA-*SNHG12*, si-control, or si-*SNHG12*, and then analyzed by MTT and trypan blue exclusion assays. qRT-PCR assays showed that *SNHG12* levels were higher in the pcDNA-*SNHG12* group than in the pcDNA-control group, and that *SNHG12* levels were lower in the si-*SNHG12* group than in the si-control group, which confirmed successful transfection (**Figure 1B**). The MTT and trypan blue exclusion assays showed that *SNHG12* upregulation increased BMEC viability, and inhibited BMEC death, while *SNHG12* knockdown had the opposite effect under OGD/R conditions (**Figure 1C, D**). Taken together, these results indicate that *SNHG12* is upregulated and inhibits BMEC death under OGD/R conditions.

***SNHG12* inhibited the inflammatory response under OGD/R conditions**

To investigate the effect of *SNHG12* on the inflammatory response of BMECs, BMECs transfected with pcDNA-control, pcDNA-*SNHG12*, si-control or si-*SNHG12* were exposed to OGD for 16 hours and subsequently cultured in standard medium for 4 hours. qRT-PCR and western blot assays were then performed. *SNHG12* upregulation decreased mRNA

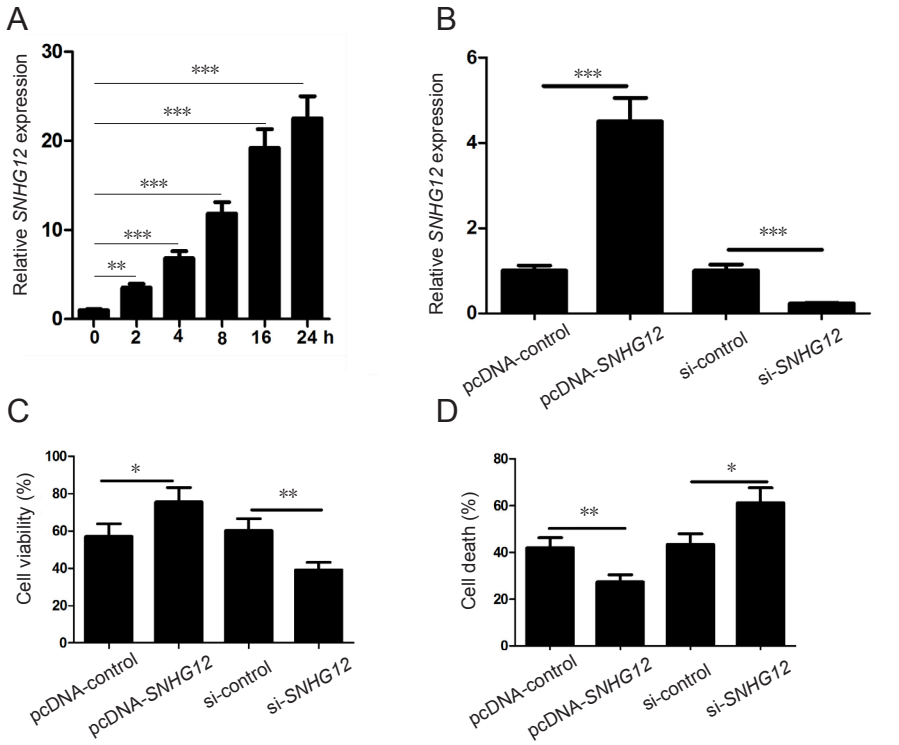

Figure 1 *SNHG1* was upregulated and inhibited BMEC death under OGD/R conditions.
(A) Quantitative real-time polymerase chain reaction assay showed *SNGH1* levels in BMECs exposed to OGD for 0, 2, 4, 8, 16 or 24 h. (B) *SNGH1* levels in BMECs transfected with pcDNA-control, pcDNA-*SNHG12*, si-control or si-*SNHG12* after exposure to OGD for 16 h. (C) 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide assay showed the viability of BMECs transfected with pcDNA-control, pcDNA-*SNHG12*, si-control or si-*SNHG12* after exposure to OGD for 16 h. (D) Trypan blue exclusion assays showed the death rate of BMECs transfected with pcDNA-control, pcDNA-*SNHG12*, si-control or si-*SNHG12* after exposure to OGD for 16 h. All data are expressed as the mean ± SEM (one-way analysis of variance followed by Bonferroni *post hoc* test). All experiments were performed in triplicate. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. BMEC: Brain microvascular endothelial cell; OGD/R: oxygen-glucose deprivation/reoxygenation; h: hours.

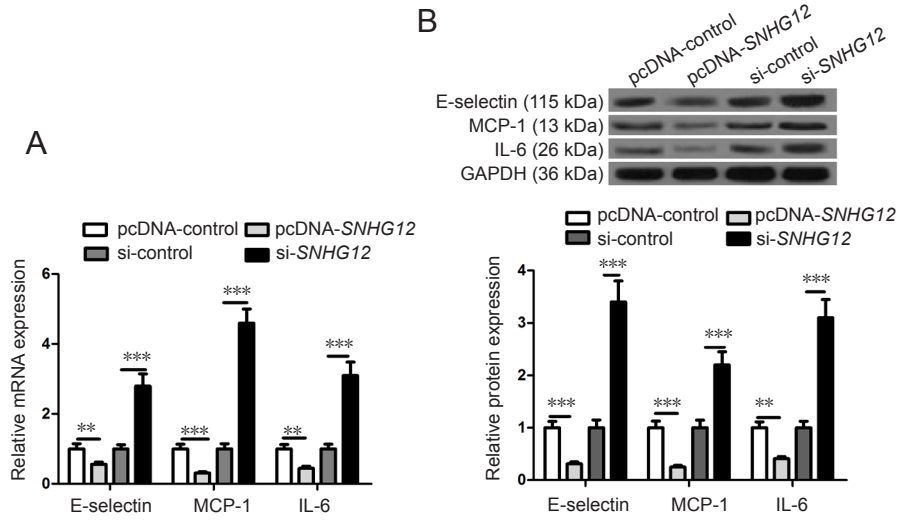

Figure 2 *SNHG12* inhibited the inflammatory response under OGD/R conditions.
(A) Quantitative real-time polymerase chain reaction assays showed the mRNA levels of proinflammatory cytokines-E-selectin, MCP1, and IL-6 in BMECs transfected with pcDNA-control, pcDNA-*SNHG12*, si-control or si-*SNHG12* after exposure to OGD for 16 h. (B) Western blot assays showed the levels of E-selectin, MCP1, and IL-6 proteins in BMECs transfected with pcDNA-control, pcDNA-*SNHG12*, si-control or si-*SNHG12* after exposure to OGD for 16 h. All data are expressed as the mean ± SEM (Student's two-tailed *t*-test and one-way analysis of variance followed by Bonferroni *post hoc* test). All experiments were performed in triplicate. $**P < 0.01$, $***P < 0.001$. OGD/R: Oxygen-glucose deprivation/reoxygenation; BMECs: brain microvascular endothelial cells; MCP1: monocyte chemotactic protein 1; IL: interleukin; GAPDH: glyceraldehyde phosphate dehydrogenase; h: hours.

and protein levels of the proinflammatory cytokines-E-selectin, MCP1, and IL-6, and *SNHG12* knockdown increased mRNA and protein levels of these proinflammatory cytokines under OGD/R conditions (**Figure 2A, B**). Taken together, these results indicate that *SNHG12* plays an anti-inflammatory role in BMECs under OGD/R conditions.

SNHG12 promoted BMEC angiogenesis after OGD insult

To investigate the effects of *SNHG12* on angiogenesis, we detected mRNA and protein levels of the proangiogenic factors, VEGFA and FGFb, under OGD/R conditions, and the formation of capillary-like tubes after OGD/R insult. *SNHG12* upregulation increased levels of VEGFA and FGFb mRNA and protein, while *SNHG12* knockdown decreased VEGFA and FGFb mRNA and protein levels under OGD/R conditions (**Figure 3A, B**). The capillary-like tube formation assay showed that *SNHG12* upregulation promoted capillary-like tube formation, and that *SNHG12* knockdown inhibited capillary-like tube formation (**Figure 3C**). Taken together, these data indicate that *SNHG12* promotes BMEC angiogenesis.

SNHG12 directly targeted miR-199a in BMECs

To further investigate the molecular mechanism of *SNHG12* on BMEC death, angiogenesis, and the inflammatory response, bioinformatic analysis of miRNA recognition sequences in *SNHG12* was performed using miRcode. miR-199a was identified as being capable of binding to complementary sequences in *SNHG12* (**Figure 4A**). miR-199a levels were determined in BMECs exposed to OGD for 0, 2, 4, 8, 16 or 24 hours. and were shown to decrease with respect to OGD exposure time, which was opposite to *SNHG12* expression (**Figure 4B**). In addition, the regulatory effect of *SNHG12* on

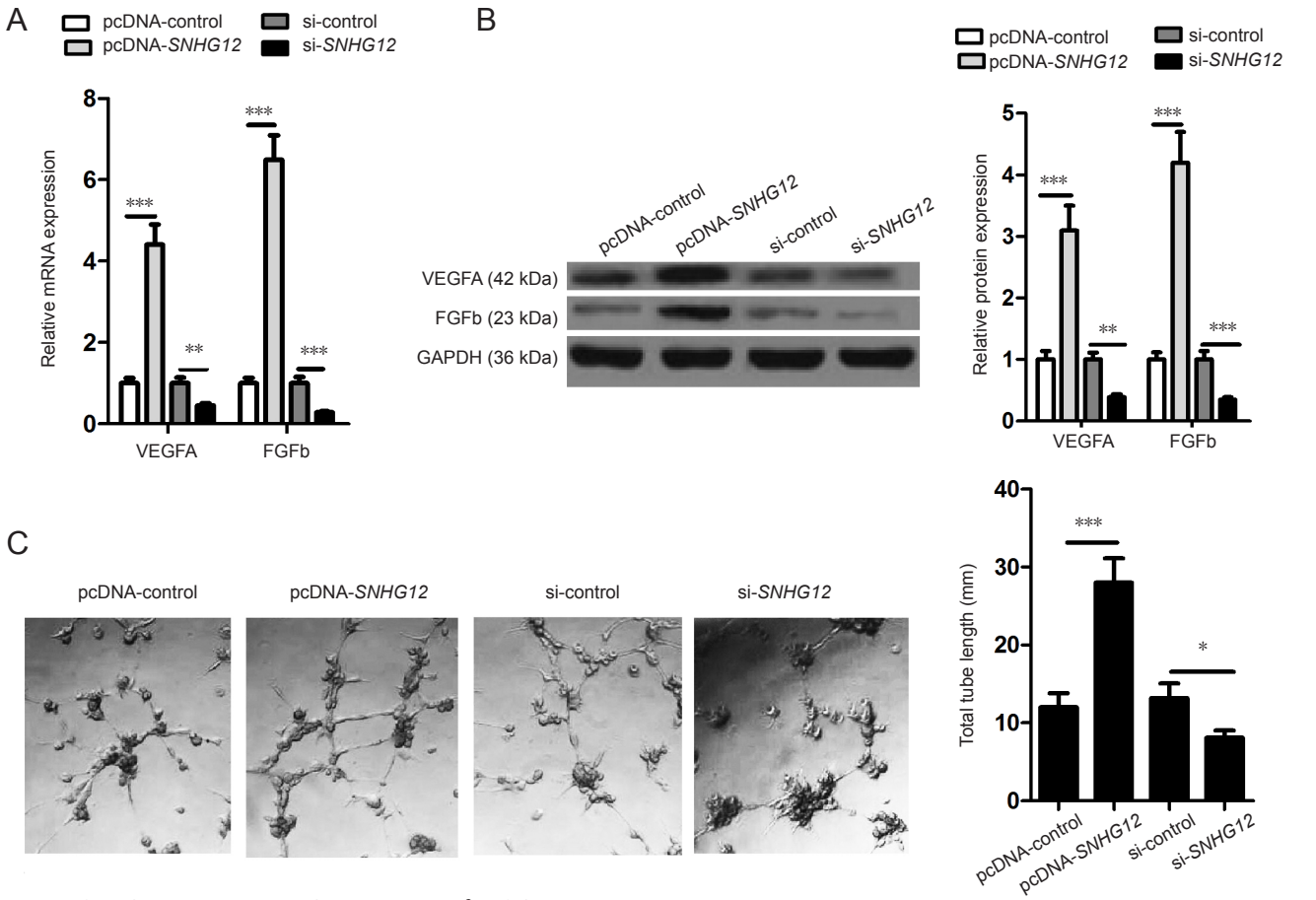

Figure 3 *SNHG12* promotes BMEC angiogenesis after OGD.
(A) Quantitative real-time polymerase chain reaction assays showed mRNA levels of the proangiogenic factors, VEGFA and FGFb, in BMECs transfected with pcDNA-control, pcDNA-*SNHG12*, si-control or si-*SNHG12* after exposure to OGD for 16 h. (B) Western blot assays show VEGFA and FGFb protein levels in BMECs transfected with pcDNA-control, pcDNA-*SNHG12*, si-control or si-*SNHG12* after exposure to OGD for 16 h. (C) Capillary-like tube formation of BMECs transfected with pcDNA-control, pcDNA-*SNHG12*, si-control or si-*SNHG12* (inverted phase contrast microscopy images; original magnification, 40×) after exposure to OGD for 16 h. All data are expressed as the mean ± SEM. All experiments were performed in triplicate. Student's two-tailed *t*-test and one-way analysis of variance followed by Bonferroni *post hoc* test were used for comparison between groups. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. BMECs: Brain microvascular endothelial cells; OGD: oxygen-glucose deprivation; VEGF: vascular endothelial growth factor; FGF: fibroblast growth factor; GAPDH: glyceraldehyde phosphate dehydrogenase; h: hours.

miR-199a expression was also determined. *SNHG12* upregulation decreased miR-199a expression, and *SNHG12* knockdown increased miR-199a expression (**Figure 4C**). Taken together, these data indicate that *SNHG12* inhibits miR-199a expression by directly targeting miR-199a.

SNHG12 inhibited BMEC death, the inflammatory response, and anti-angiogenesis by targeting miR-199a

Considering the inhibitory action of *SNHG12* on miR-199a expression in BMECs, we further explored whether *SNHG12* inhibited the function of miR-199a. Cell growth, the inflammatory response, and angiogenesis assays were performed. The transfection of miR-199a mimic decreased BMEC viability, and promoted BMEC death, while cotransfection of miR-199a + pcDNA-*SNHG12* abolished the effects induced by miR-199a (**Figure 5A, B**). In the inflammatory response assay, transfection of the miR-199a mimic promoted mRNA and protein expression of E-selectin, MCP1, and IL-6, and the cotransfection of miR-199a + pcDNA-*SNHG12* reversed the effects induced by miR-199a (**Figure 5C, D**). The angiogenesis assay showed that transfection of the miR-199a mimic inhibited mRNA and protein expression of VEGFA and FGFb, and capillary-like tube formation, while cotransfection of miR-199a + pcDNA-*SNHG12* mitigated the effect induced by miR-199a (**Figure 5C–E**). These data indicate that *SNHG12* targets miR-199a to inhibit BMEC death and the inflammatory response, and to promote angiogenesis.

Discussion

Ischemic stroke begins with serious focal hypoperfusion, and results in excitotoxicity and oxidative damage, which in turn cause microvascular damage, blood-brain barrier dysfunction and the initiation of inflammation. These events can further worsen the initial damage, and result in permanent brain damage and even death (Lo et al., 2003; Sandoval and Witt, 2008; Brouns and De Deyn, 2009; Lakhan et al., 2009). Therefore, it is essential for the development of effective ischemic stroke therapies to understand the molecular mechanism of microvascular damage, blood-brain barrier dysfunction and initiation of inflammation after ischemia.

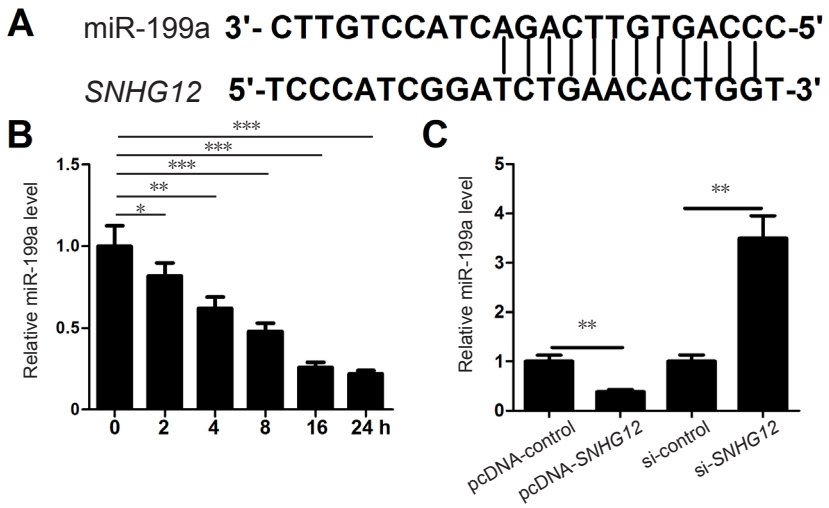

Figure 4 *SNHG12* directly targeted miR-199a in BMECs.
(A) Bioinformatic analysis showed putative binding sites between *SNHG12* and miR-199a. (B) Quantitative real-time polymerase chain reaction assays showed miR-199a levels in BMECs exposed to oxygen-glucose deprivation for 0, 2, 4, 8, 16 or 24 h. (C) The effects of *SNHG12* downregulation or upregulation on miR-199a expression. All data are expressed as the mean ± SEM (Student's two-tailed *t*-test and one-way analysis of variance followed by Bonferroni *post hoc* test). All experiments were performed in triplicate. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. BMECs: Brain microvascular endothelial cells; h: hours.

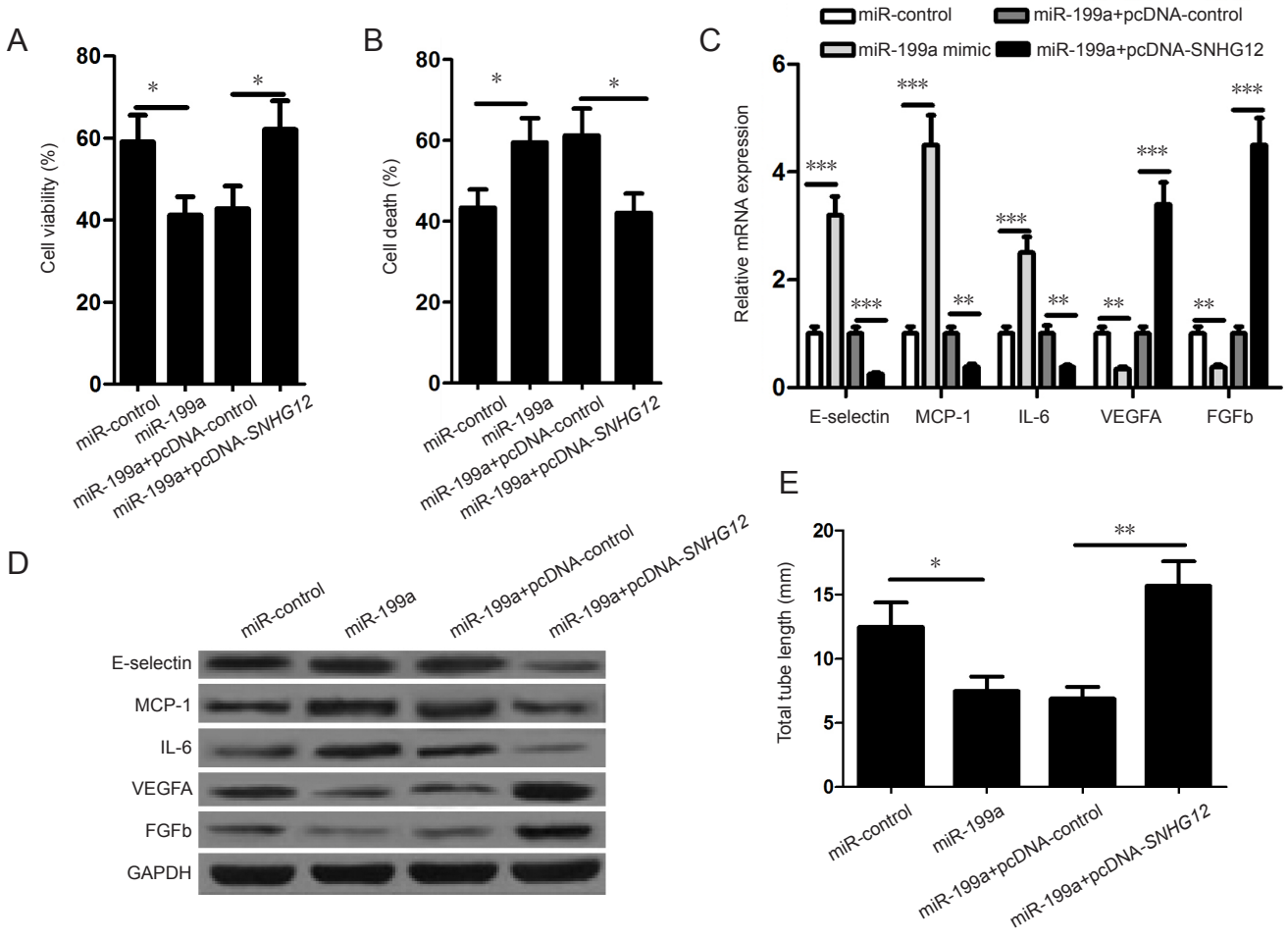

Figure 5 *SNHG12* inhibited BMEC death, the inflammatory response, and promoted angiogenesis by targeting miR-199a.
(A, B) 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide and trypan blue exclusion assays show the viability and death of BMECs transfected with control, miR-199a mimic, miR-199a + pcDNA-*SNHG12*, or miR-199a + pcDNA-control after exposure to OGD for 16 h. (C, D) Quantitative real-time polymerase chain reaction and western blot assays show the mRNA and protein levels of E-selectin, MCP1, IL6, VEGFA and FGFb in BMECs transfected with control, miR-199a mimic, miR-199a + pcDNA-*SNHG12*, or miR-199a + pcDNA-control after exposure to OGD for 16 h. (E) Capillary-like tube formation assays show capillary-like tube formation by BMECs transfected with control, miR-199a mimic, miR-199a + pcDNA-*SNHG12*, or miR-199a + pcDNA-control after exposure to OGD for 16 h. All data are expressed as the mean ± SEM (Student's two tailed *t*-test and one-way analysis of variance followed by Bonferroni *post hoc* tests). All experiments were performed in triplicate. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. BMEC: Brain microvascular endothelial cell; OGD: oxygen-glucose deprivation; MCP1: monocyte chemotactic protein 1; IL: interleukin; VEGF: vascular endothelial growth factor; FGF: fibroblast growth factor; GAPDH: glyceraldehyde phosphate dehydrogenase; h: hours.

LncRNAs were initially regarded as products generated from transcriptional background noise (Hung et al., 2011; Hudson and Ortlund, 2014). However, it has become increasingly clear that lncRNAs are key regulators of physiological and pathological responses, and are involved in various biological processes, such as cell death, the inflammation response, and angiogenesis. For example, lncRNA GAS5 regulates prostate cancer cell apoptosis (Pickard et al., 2013). LncRNA IL7R knockdown reduces trimethylation of histone H3 and is involved in the regulation of the inflammatory response (Cui et al., 2014) and lncRNA MIAT knockdown remarkably ameliorates diabetes mellitus-induced retinal microvascular dysfunction *in vivo* (Yan et al., 2015). In recent years, the involvement of lncRNAs in cerebrovascular pathophysiology, such as stroke, has been increasingly investigated. For example, lncRNA N1LR inhibits p53 phosphorylation and enhances neuroprotection against ischemic stroke (Wu et al., 2016). LncRNA TUG1 promotes neuronal apoptosis by upregulating Bcl2l11 expression under ischemia (Chen et al., 2017). LncRNA FosDT increases ischemic brain injury by interaction with REST-associated chromatin-modifying proteins (Mehta et al., 2015). In addition, recent studies report that upregulation of Malat1 and downregulation of Meg3 after I/R and OGD/R insults can regulate BMEC death and the inflammatory response, and angiogenesis, respectively (Liu et al., 2016; Zhang et al., 2017). LncRNA *SNHG12* is also upregulated in BMECs after I/R or OGD/R insult. However, its role remains unknown. In this study, *SNHG12* inhibited BMEC death and the inflammatory response under OGD/R conditions and promoted BMEC angiogenesis after OGD/R by suppressing miR-199a expression. This study provides new clues for understanding the molecular mechanism of microvascular damage, blood-brain barrier dysfunction and the initiation of inflammation after ischemic stroke.

SNHG12 was initially discovered as an oncogene that promotes the initiation and development of many tumors. Ruan et al. (2016) found that *SNHG12* promotes human osteosarcoma cell proliferation and migration by increasing angiomotin gene expression. Wang et al. (2017) found that overexpression of *SNHG12* promotes cell proliferation and migration and inhibits apoptosis in triple-negative breast cancer. Lan et al. (2017) found that *SNHG12* promotes carcinogenesis in hepatocellular carcinoma by targeting miR-199a/b-5p. However, to date, the role of *SNHG12* upregulation in BMECs after I/R or OGD/R insult has not been studied. Here, a series of *in vitro* cell-based experiments were performed to investigate the role of *SNHG12* during and after OGD/R insult. *SNHG12* inhibited BMEC death and the expression of the proinflammatory cytokines, E-selectin, MCP1, and IL6, and promoted the expression of proangiogenic factors, VEGFA and FGFb under OGD/R conditions. In addition, *SNHG12* also promoted capillary-like tube formation after OGD/R. Capillary-like tube formation is the reorganization stage of angiogenesis, involving cell adhesion, migration, differentiation, and growth. These findings indicate that *SNHG12* inhibits BMEC death and the inflammatory response under OGD/R conditions and promotes BMEC angiogenesis after OGD/R insult. Enhancing *SNHG12* expression may, therefore, improve outcomes for ischemic stroke patients.

The molecular mechanism by which *SNHG12* functions in BMECs was further investigated. miR-199a was identified as a potential target of *SNHG12* by bioinformatic prediction using miRcode software. miR-199a, a cancer-related miRNA, regulates cell growth, the inflammatory response, and angiogenesis. Xu et al. (2012a) found that miR-199a increases the inhibition of cell proliferation induced by cisplatin. Zhang et al. (2015) found that downregulation of miRNA-199a-5p reduces hyper-inflammation by normalizing CAV1 mRNA levels in cystic fibrosis macrophages. Raimondi et al. (2014) found that miR-199a suppresses myeloma-related angiogenesis *in vitro* and *in vivo*. Decreased miR-199a expression was recently reported to play a neuroprotective role in ischemic tolerance induced by 3-nitropropionic acid in rat brain (Xu et al., 2012b). The above findings prompted our further explorations into miR-199a. Our results confirmed that *SNHG12* can directly target miR-199a, and that the effects of miR-199a overexpression on BMEC death, the inflammatory response, and angiogenesis can be mitigated by *SNHG12*. These results indicate that *SNHG12* exerts its function on BMECs by suppressing miR-199a.

In summary, our study, shows that *SNHG12* plays a protective role in BMECs during and after OGD by suppressing miR-199a. These findings inform our understand of ischemic stroke pathogenesis and will facilitate the development of effective therapies for this deadly disease. In addition, future endeavors are needed to determine whether *SNHG12*/miR-199a has a function *in vivo*.

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