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LncRNA Snhg3 contributes to dysfunction of cerebral microvascular cells in intracerebral hemorrhage rats by activating the TWEAK/Fn14/STAT3 pathway

Jie Zhang, Buhuai Dong, Jianhong Hao, Shuangqiang Yi, Wenbo Cai, Zhenguo Luo*

Department of Anesthesiology, Honghui Hospital, Xi'an Jiaotong University, Xi'an, 710054, Shannxi province, PR China

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ABSTRACT

LncRNA small nucleolar RNA host gene 3 (Snhg3) has been involved in cell proliferation and migration in malignant cells. However, its role in regulating functions of non-malignant cells has been hardly reported. Here, we found Snhg3 expression was sharply induced in primary brain microvascular endothelial cells (BMVECs) treated with oxygen-and-glucose-deprivation (OGD) plus hemin, an *in vitro* model of intracerebral hemorrhage (ICH). Downregulation of Snhg3 by siRNA transfection improved cell proliferation and migration abilities and reduced cell apoptosis and monolayer permeability in BMVECs under treatment with OGD plus hemin. Snhg3 overexpression suppressed cell proliferation and migration and increased cell apoptosis and monolayer permeability under normal condition. In ICH rats, downregulation of Snhg3 by siRNA injection improved behavioral and histological manifestations, including number of right turns, limb placement score, integrity of blood-brain barrier (BBB), brain water content and cell apoptosis *in vivo*. In the mechanism exploration, we found that, TWEAK and Snhg3 displayed a positive correlation with each other. Snhg3 overexpression increased expression of TWEAK protein and its receptor Fn14, that were also induced by OGD plus hemin, activating the downstream neuroinflammatory pathway STAT3 and enhancing the secretion of MMP-2/9. Finally, the TWEAK-siRNA, the Fn14 inhibitor ATA and the STAT3 blocker AG490 were respectively used to treat BMVECs under treatment with OGD plus hemin. Our results showed either TWEAK downregulation, Fn14 inhibition, or STAT3 blockade, could rescue Snhg3-induced impairment of BMVEC functions. In conclusion, the lncRNA Snhg3 contributes to dysfunction of cerebral microvascular cells in ICH rats by activating the TWEAK/Fn14/STAT3 pathway.

1. Introduction

Intracerebral hemorrhage (ICH) is the second largest type of stroke, accounting for about 15% of all strokes. The incidence of ICH, about 25/100,000 worldwide and about 50/100,000 in Asia, did not decrease during the past several decades [1]. Over the past 25 years, the incidence of primary ICH has declined dramatically, but that of the secondary ICH raised significantly. In the first month after ICH, the general mortality rate was as high as 40%. Secondary ICH, accounting for about 20% of total ICH, was mainly caused by oral anticoagulants and tumors [2]. Damage of ICH can be divided into two successive but inseparable processes, including primary brain damage and secondary brain damage [3]. Primary brain damage, mainly from 0 to 4 h post ICH, refers to the physical damage to brain caused by blood clots after ICH. The enlargement of hematoma in the first few days post ICH increases the intracranial pressure, oppresses the brain-related areas and leads to cerebral ischemia [4,5]. Secondary brain damage, after 4 h post ICH, mainly refers to the cascade pathological reaction of hematoma and coagulation triggered by primary brain injury [4,5].

Intracerebral edema is a typical secondary brain damage of ICH, the severity of which is regarded to be closely related to clinical prognosis of ICH patients [3]. The edema around the hematoma occurred in the hyperacute stage of cerebral hemorrhage. The volume of edema increased significantly within 24 h post ICH and progressed rapidly within 3 days. Rapid development of brain edema led to increased intracranial pressure or even brain hernia. As an important structure to maintain the homeostasis of the central nervous system, vasogenic brain edema caused by the damage of blood brain barrier (BBB) is a key event post ICH [6]. BBB is a dynamic interface between blood and brain, which selectively filters substances into the brain [7]. BBB is composed of continuous capillary endothelium and the tight junction of endothelial cells, subendothelial basement membrane, pericyte and

* Corresponding author. Department of Anesthesiology, Honghui Hospital, Xi'an Jiaotong University, 555 East Youyi, Xi'an, 710054, Shannxi province, PR China.
E-mail address: luo_zhenguo3@163.com (Z. Luo).

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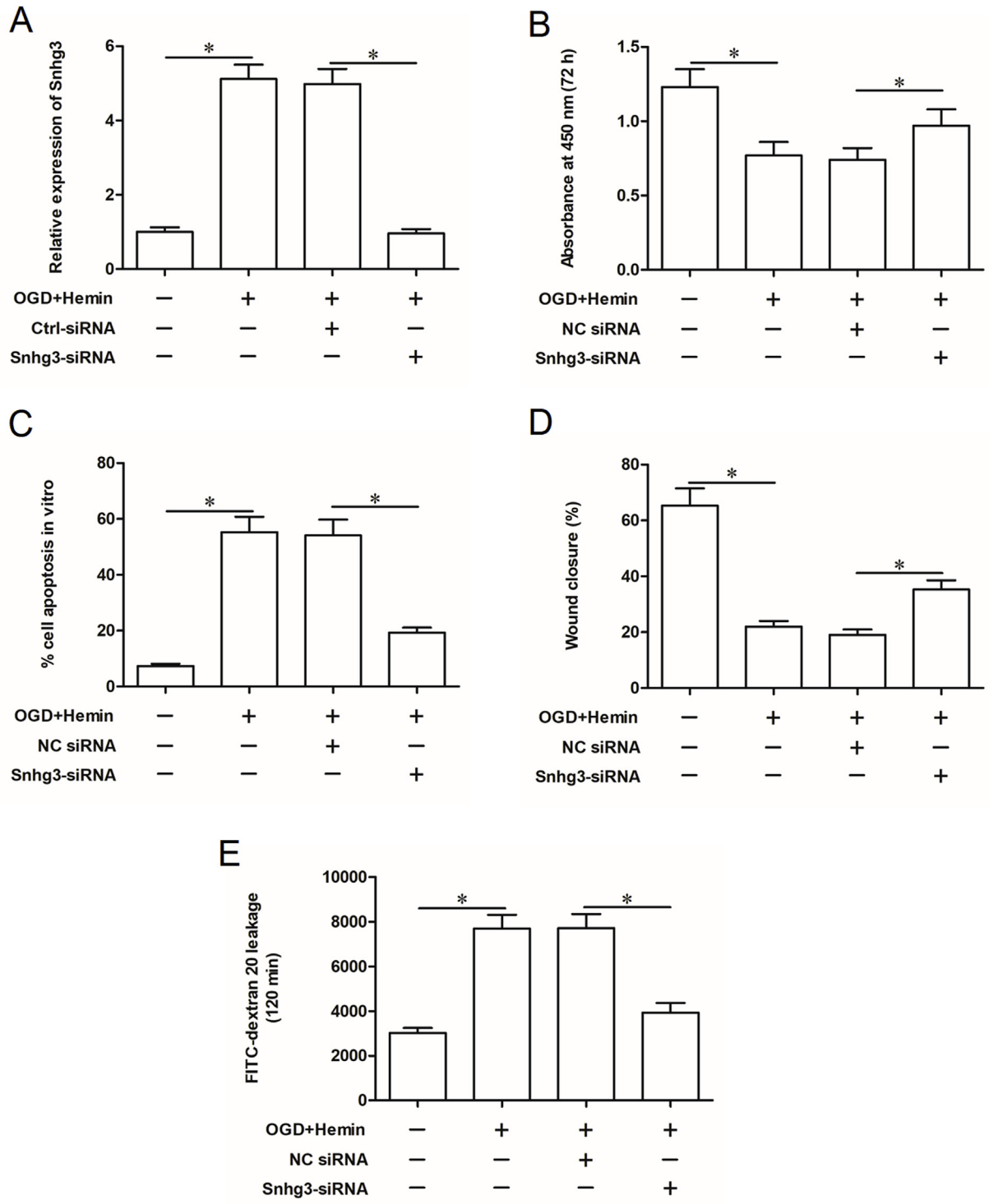

Fig. 1. Inhibition of Sngh3 increased cell proliferation and migration abilities and reduced cell apoptosis and monolayer permeability in an *in vitro* ICH model. An *in vitro* ICH model was established by treating primary BMVECs with OGD plus hemin. The siRNA specifically against Snhg3 or a negative control siRNA (both 40 nM) was transfected into the cell model. Post transfection for 48 h, A. Expression of Sngh3 was detected by qPCR. Cell functions including (B) cell proliferation, (C) apoptosis, (D) migration, and (E) monolayer permeability were respectively detected with CCK-8, AnnexinV/PI, wound healing and FITC-dextran 20 leakage. N = 6, $^*P < 0.05$.

astrocyte footplate formed by glial membrane [8]. Among them, brain microvascular endothelial cells (BMVECs) got earliest attention of researchers, the number and permeability of which largely determined the function of BBB [9].

Tumor necrosis factor-like weak inducer of apoptosis (TWEAK, also TNFSF12), a member of the tumor necrosis factor superfamily, is well known for its regulatory role in the diseases of central nervous system [10]. TWEAK expression was induced in many inflammatory and degenerative diseases in central nervous system, such as edema, multiple sclerosis and intracerebral ischemia [11]. TWEAK acts on responsive cells by binding to its receptor on cell surface, named fibroblast growth factor-inducible 14 (Fn14), and then activates multiple proinflammatory pathways, such as JAKs/STATs (janus kinases/transducers and activators of transcription) [12,13]. Recently, the TWEAK/Fn14

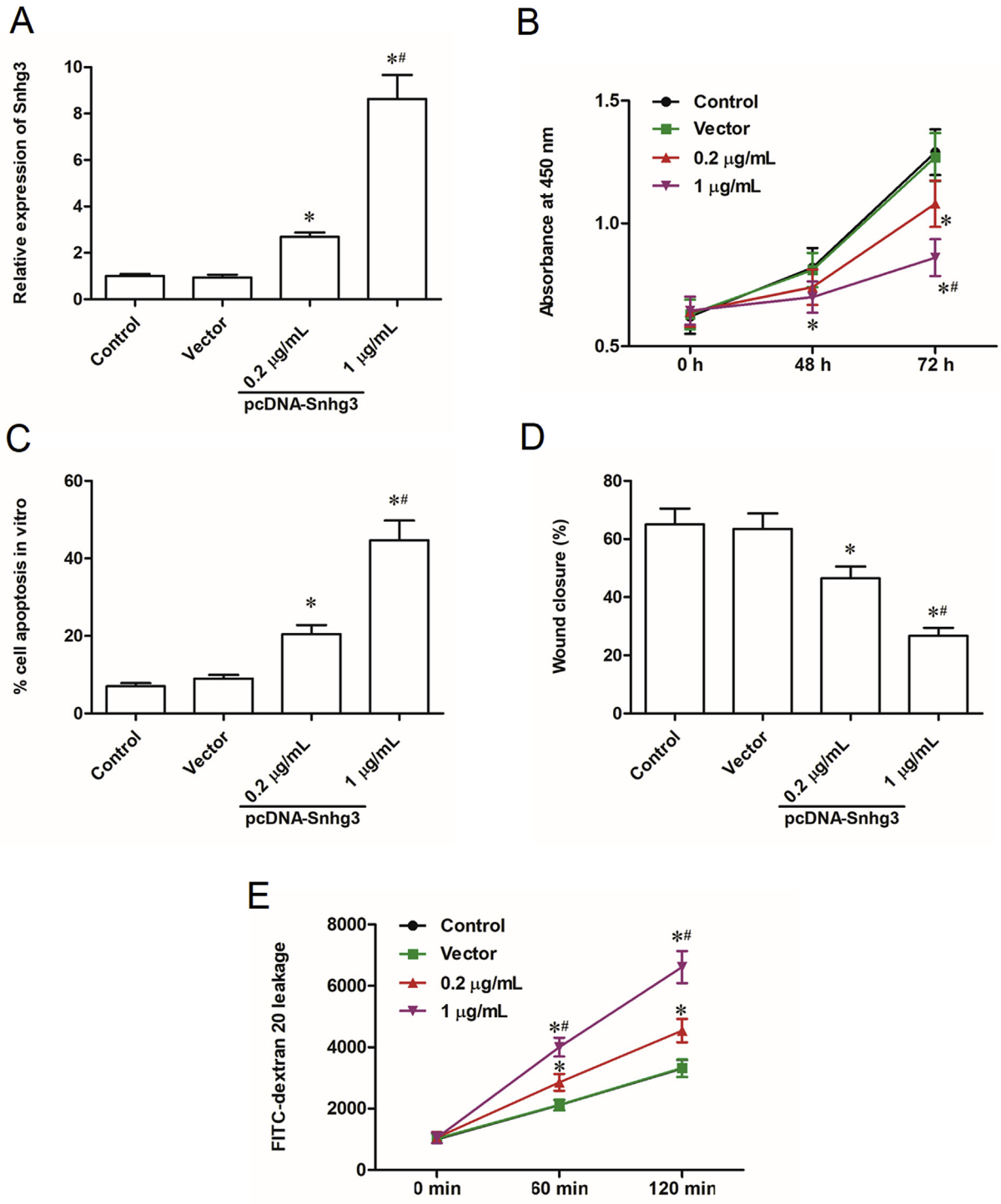

Fig. 2. Overexpression of Sngh3 increased monolayer permeability and apoptosis and suppressed proliferation and migration in primary BMVECs. Different concentrations of pcDNA-Snhg3 expression vectors were transfected into primary BMVECs under normal condition, following transfection for 48 h, A. The overexpression efficiencies were detected with qPCR; Cell functions including (B) cell proliferation, (C) apoptosis, (D) migration, and (E) monolayer permeability were respectively detected. N = 6, $^{*}P < 0.05$.

pathway was reported to increased permeability and secretion of proinflammatory factors in BMVECs *in vitro* [14]. However, it is largely unknown that whether TWEAK regulates function of BMVECs in progression of ICH and how its expression is regulated.

In this study, we found that lncRNA Snhg3 expression was sharply induced in an *in vitro* ICH model. Moreover, TWEAK and Snhg3 displayed a positive correlation with each other *in vivo*. We investigated the role of Snhg3 in BMVECs *in vitro* and progression of ICH *in vivo*, and explored its regulation on the TWEAK/Fn14/STAT3 pathway.

2. Materials and methods

2.1. Establishment of ICH model

36 male Sprague-Dawley (SD) rats, aging about 8 weeks and weighing around 200 g, were kept at the condition with a room

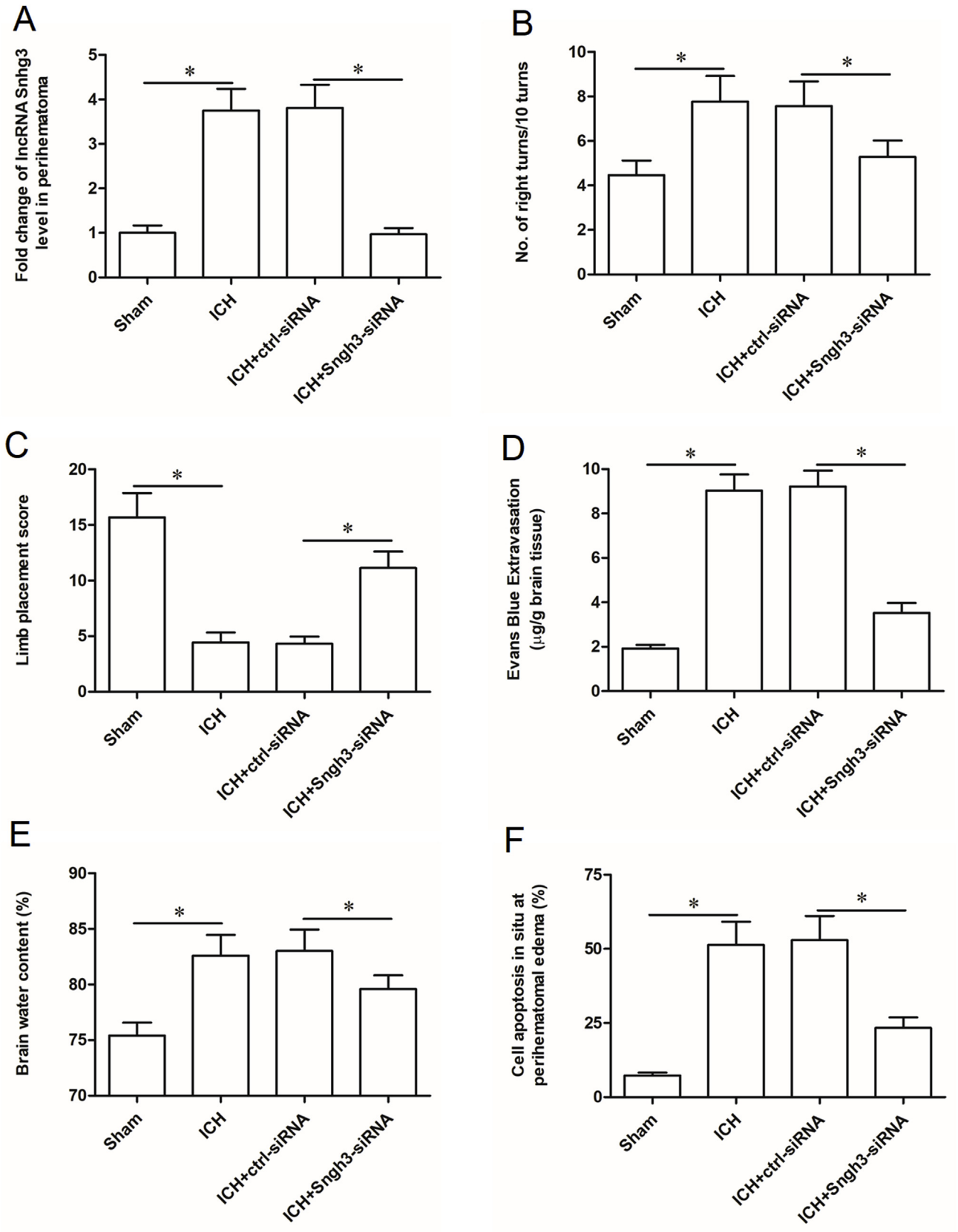

Fig. 3. Inhibition of Sngh3 improved behaviors and BBB disruption in ICH rats. ICH model was established in rats with Col VII injection. After 24 h, Snhg3 siRNA or its negative control (both 40 nM) was injected through the right lateral ventricle every two days at a dosage of 2 mg/kg. At day 7 post ICH induction, (A) the expression of Sngh3 in the brain was detected by qPCR. Behaviors of the rats were evaluated by (B) number of right turns and (C) limb placement. D. Evans blue extravasation assay was used to evaluate BBB integrity. (E) Brain water content and (F) cell apoptosis in situ assay were used to check the damage degree of the brain. N = 9, $^*P < 0.05$.

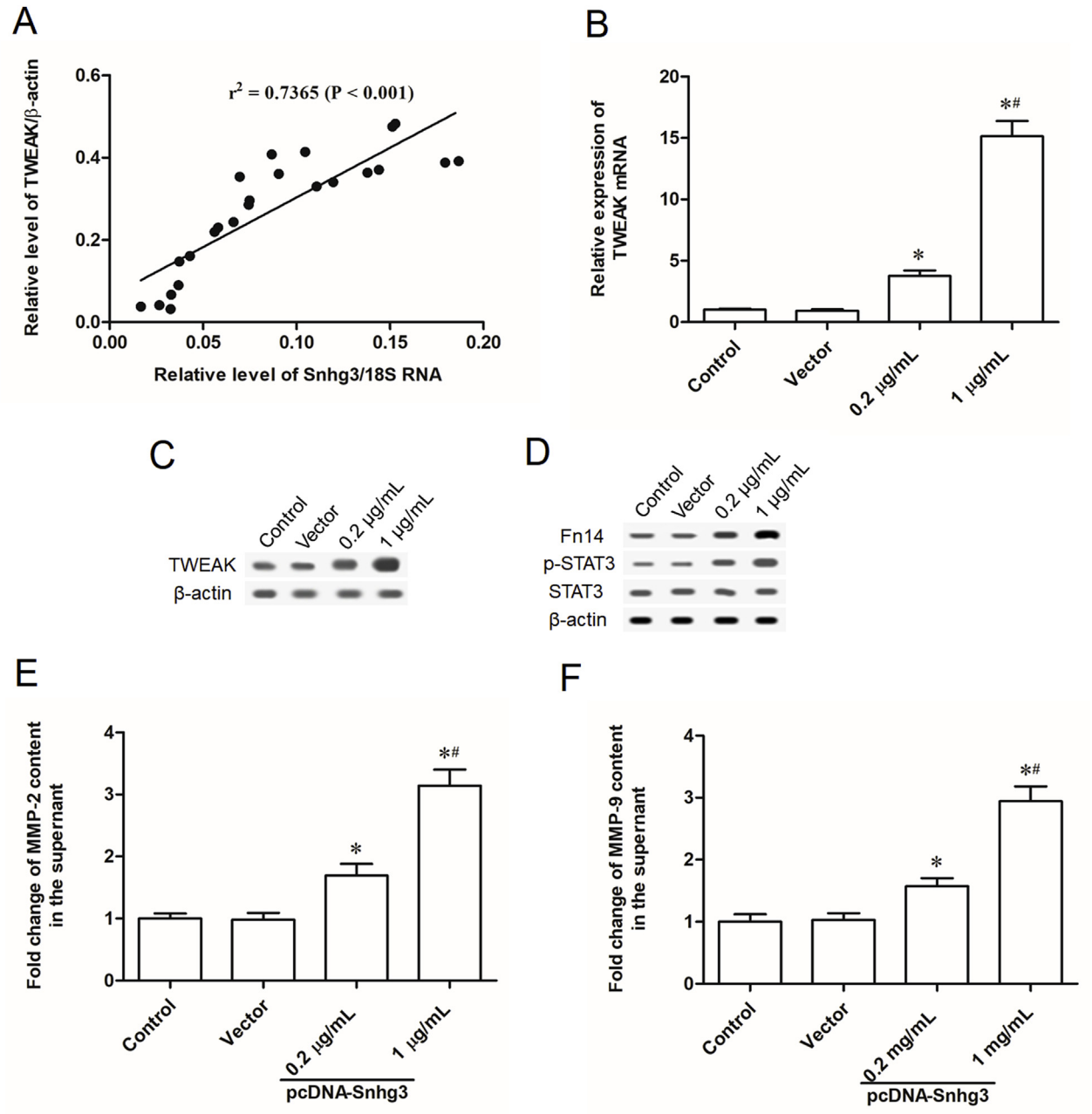

Fig. 4. Snhg3 positively regulated the TWEAK/Fn14/STAT3 pathway. A. Correlation between Snhg3 and TWEAK levels was analyzed with Pearson Correlation test. Then, different concentrations of pcDNA-Sngh3 expression vectors were transfected into primary BMVECs under normal condition, following transfection for 48 h, B and C. Expression of TWEAK mRNA and protein was respectively detected. D. Expression of Fn14 and activation of STAT3 were evaluated with Western blotting. E and F. Secretions of MMP-2 and MMP-9 in the supernatant were detected with ELISA. N = 6, $^{*}P < 0.05$, compared with control; $^{\#}P < 0.05$ compared with 0.2 μg/mL.

temperature and a 12-h light-dark cycle, allowed free access to water and food. The animal protocols were approved by the Ethics Committees of Honghui Hospital, Xi'an Jiaotong University (Xi'an, China). For ICH model establishment, rats were anesthetized with pentobarbital at a dose of 65 mg/kg by the intraperitoneal injection. 2.5 μL of collagenase VII in normal saline was slowly injected into the right globus pallidus (0.1 mm anterior, 3.5 mm lateral, and 6.0 mm ventral to the bregma). After the infusion, the needle was kept for 5 min. For the sham group, the same operation was performed but without collagenase VII infusion. The rats were euthanized after 7 days post ICH induction, and the brain ipsilateral to the hematoma was obtained for following examinations.

2.2. Administration of the Snhg3 siRNA in vivo

After induction of ICH (at 24 h), the Snhg3 siRNA (2 mg/kg) or NC siRNA (2 mg/kg) were packaged by the Active Carrier System (BELED, Guangzhou, China) and injected into the right lateral ventricle of the rats gently every two days. The stereotaxic coordinates were 1.5 mm posterior, 1.0 mm lateral, and 3.2 mm below the horizontal plane of the bregma. An equal volume of the transfection reagent was injected as a control.

2.3. Cell culture and transfection

Primary BMVECs were isolated from 6 neonatal SD rats and cultured

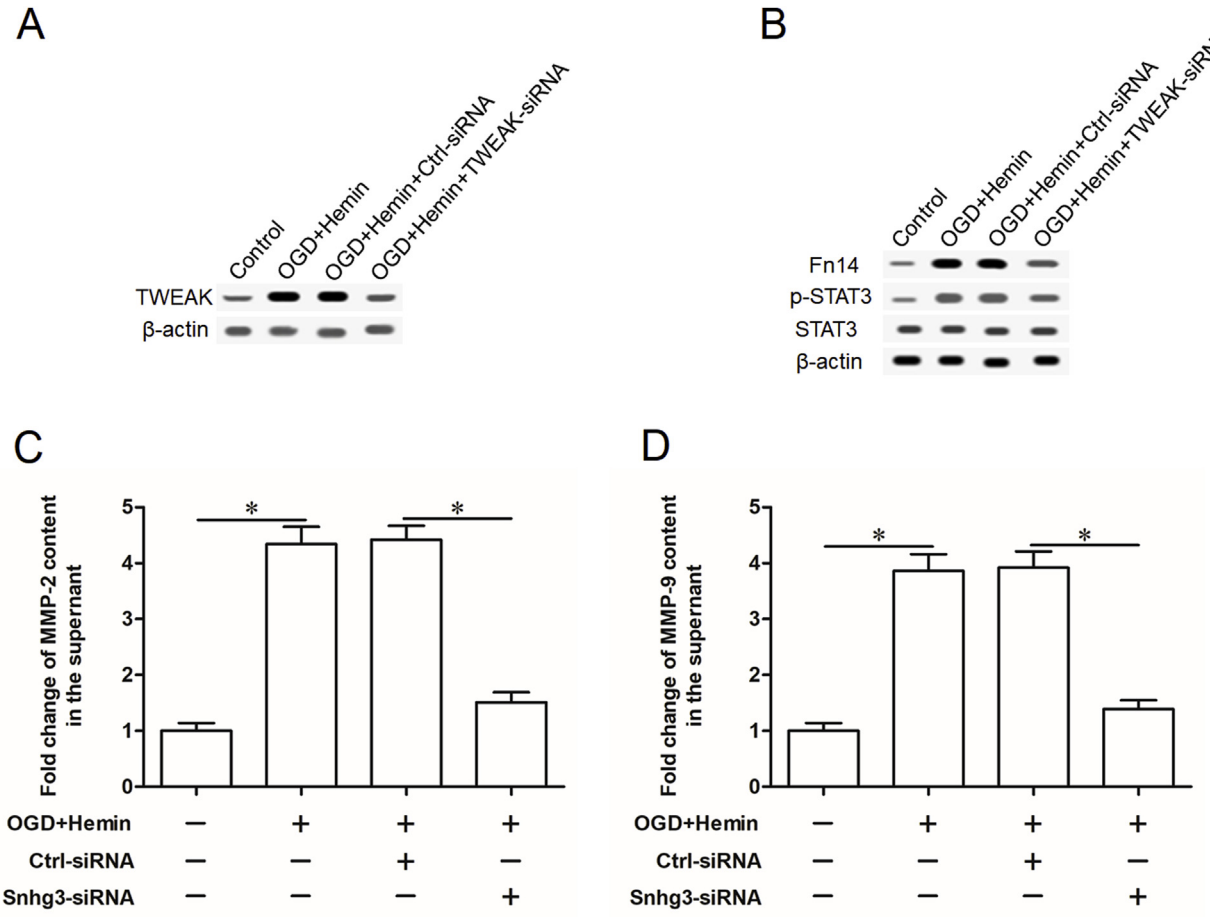

Fig. 5. The TWEAK/Fn14/STAT3 pathway was activated in ICH *in vitro* model. *In vitro* ICH model was established in primary BMVECs, and the TWEAK-siRNA (40 nM) or ctrl-siRNA (40 nM) was transfected into the *in vitro* cell model. Following transfection for 48 h, A and B. Expression of TWEAK, Fn14 and activation of STAT3 were evaluated with Western blotting. C and D. Secretions of MMP-2 and MMP-9 in the supernatant of the culture were detected. N = 6, $^*P < 0.05$.

according to the method reported previously [15]. Vectors and oligos were transfected into BMVECs by using the Lipofectamine 3000 reagents (Invitrogen) according to the manufacturer's instructions.

2.4. Quantitative polymerase chain reaction (qPCR)

SuperScript III Reverse Transcriptase (Invitrogen, Grand Island, NY) was used to reversely transcribe total RNA to obtain cDNA according to the manufacturer's instructions. RNA abundances were detected in a final volume of 25-μL reaction system in triple wells by using a SYBR ExScript qPCR kit (Takara, Dalian, China) in an IQ^5 system (Bio-Rad). The reaction conditions were as follows: 95 °C for 3 min, followed by denaturation at 94 °C for 20 s, annealing at 55 °C for 30 s and extension at 72 °C for 10 s for 35 cycles. RNA abundances were analyzed with the $2^{-\Delta\Delta CT}$ method, using 18S RNA as the internal control.

2.5. Western blotting

Total Protein Extraction Kit (Promega) was used to extract total protein. Twenty-five micrograms of protein samples were separated by polyacrylamide gel at 130 V for 2 h and then electro-transferred onto polyvinylidene fluoride membrane (Millipore, Boston, MA). Primary antibodies including anti-TWEAK (1:400 dilution; Abcam, Cambridge, UK), anti-Fn14 (1:400 dilution; Abcam), anti-p-STAT3 (1:200 dilution; Cell Signaling Technology, Boston, MA), anti-STAT3 (1:500 dilution; Cell Signaling Technology), and β-actin (1:600 dilution; Abcam) were respectively used to incubate with the protein at 4 °C overnight. After incubation with proper horseradish peroxidase (HRP)-conjugated IgG at 37 °C for 1 h, the abundances of the proteins were detected in a ChemiDoc XRS Imaging System (Bio-Rad) after elution.

2.6. Enzyme-linked immunosorbent assays (ELISA)

The concentrations of MMP-2 and MMP-9 in the culture supernatants were detected respectively with Rat MMP-2 ELISA kit (ab213910, Abcam, Cambridge, UK) and Rat MMP-9 ELISA kit (9008-77-7, JPinChem, Shanghai, China) according to the manufacturer's instructions. The measured concentrations were normalized to the control.

2.7. Cell apoptosis in vivo and in vitro

Cell apoptosis in the perihematomal zone *in vivo* were characterized using an *in-situ* cell death detection kit (Roche, Basel, Switzerland).

Cell apoptosis *in vitro* was detected with an Annexin V-PI Cell Apoptosis Kit (Sigma).

2.8. Cell proliferation assay

Cell proliferation was detected with a Cell counting kit-8 (CCK-8) according to the manufacturer's instructions.

2.9. BMVEC monolayer permeability assay

BMVECs were seeded at a density of 2.5×10^4 cells/Transwell chamber (Corning, NY). On reaching monolayer confluence, 0.01%

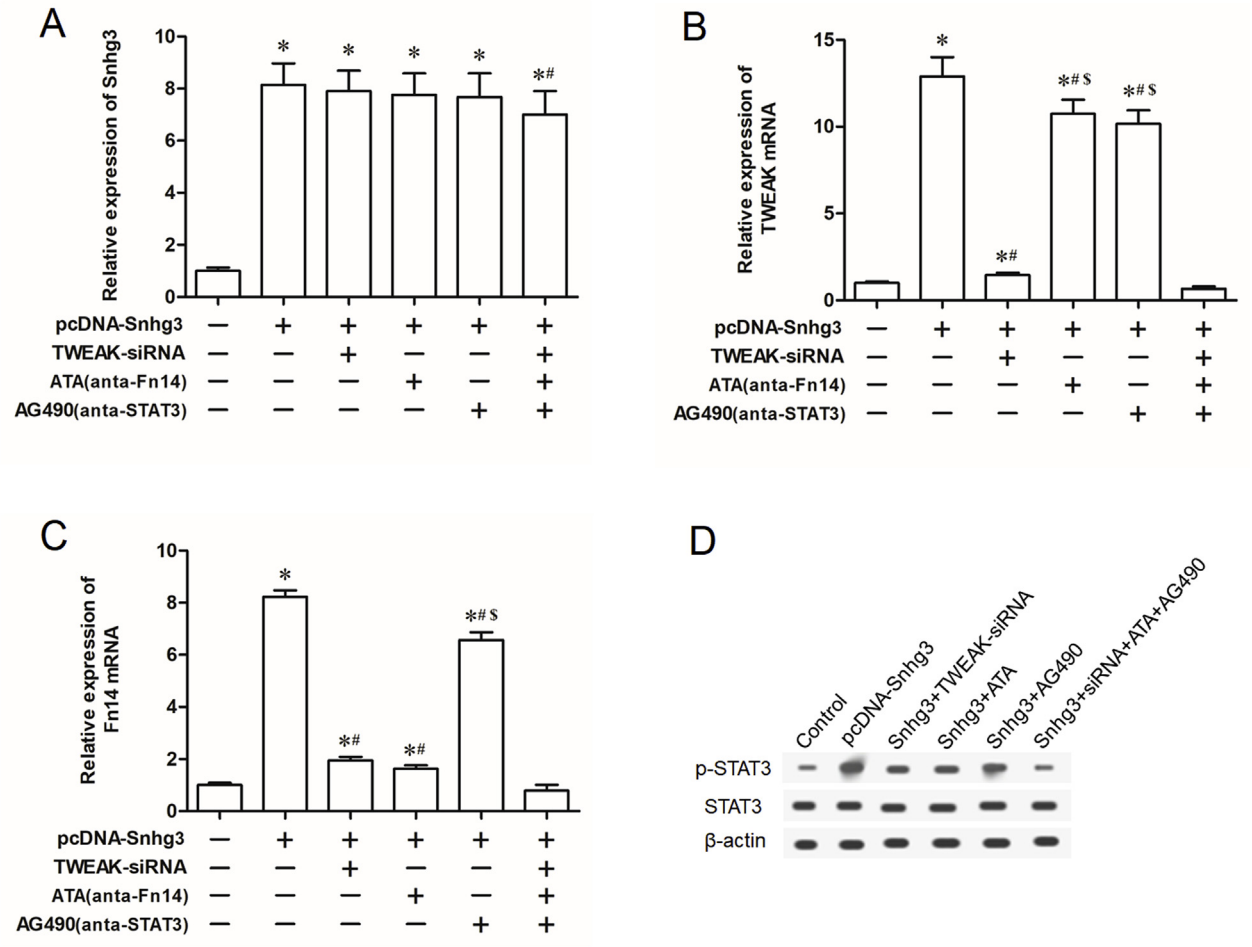

Fig. 6. The TWEAK/Fn14/STAT3 pathway was activated by Snhg3 overexpression. The TWEAK-siRNA (40 nM), ATA (10 nmol), and AG490 (5 μM) were respectively used to treat BMVECs together with OGD + hemin. The RNA levels of (A) Snhg3, (B) TWEAK and (C) Fn14 were detected with qPCR, and D. activation of STAT3 was evaluated with Western blotting. N = 6, $^{*}P < 0.05$ compared with control; $^{\#}P < 0.05$ compared with pcDNA-Snhg3, $^{\$}P < 0.05$ compared with TWEAK-siRNA or ATA.

FITC-dextran 20 (TdB, Uppsala, Sweden) was added into the medium in the upper chamber, and the medium in the lower chamber was collected at different time points. The fluorescence intensity was measured by a microplate reader.

2.10. Wound healing assay

Following transfection for 24 h, BMVECs were grown to a confluent monolayer. A scratch was evenly generated by dragging a 100-μL pipette tip across the cell monolayer. The cells were washed and cultured with serum-free medium for 24 h at normal condition. The cells were photographed at 0 h and 24 h post-scratching, and the percentage of wound closure was calculated.

2.11. Behavioral tests

Behavioral tests were performed at day 7 post ICH induction. The rats were placed in a 30° corner and allowed to turn left or right freely to exit the corner. The non-ischemic mouse turns either left or right, but the ischemic mouse preferentially turns toward the non-impaired, ipsilateral (right) side [16]. Number of right turns was recorded for 15 trials per rat, by an experienced researcher who was blinded to group designation. The scoring criteria of limb placement are as follows: 0 for no placing, 1 for incomplete and/or delayed (> 2 s) placing, and 2 for immediate and correct placing.

2.12. Brain tissue collection and in vivo histological tests

Brain tissues were obtained after the rats were euthanized, divided into ipsilateral and contralateral hemispheres of ICH. For measurement of brain water content, the ipsilateral hemisphere of the brain was weighed (wet weight) and then dried at 100 °C for 24 h to obtain the dry weight. Brain water content = (wet weight - dry weight)/wet weight × 100%.

Evans blue extravasation assay was applied to assess BBB integrity. Evans blue dye (2%, 2 mL/kg; Solarbio, Beijing, China) was injected by the tail vein at 24 h post ICH induction. The rats were carefully perfused with saline to flush out the intravascular dye. Then, the brains were obtained and then incubated with formamide at 37 °C for 24 h. Finally, the extracted Evans blue was centrifuged at 2000 *g* for 10 min, and the optical density of the supernatants was detected at 632 nm.

2.13. Statistical analysis

Each measurement was expressed as the mean ± standard error of mean (SEM). The behavior test results were analyzed by Kruskal-Wallis test with post hoc Dunn's test, and continuous variables were analyzed by analysis of variance with post hoc Newman-Keuls test. Correlation between Snhg3 and TWEAK was analyzed with Pearson Correlation test. Statistical significance difference was set at $P < 0.05$.

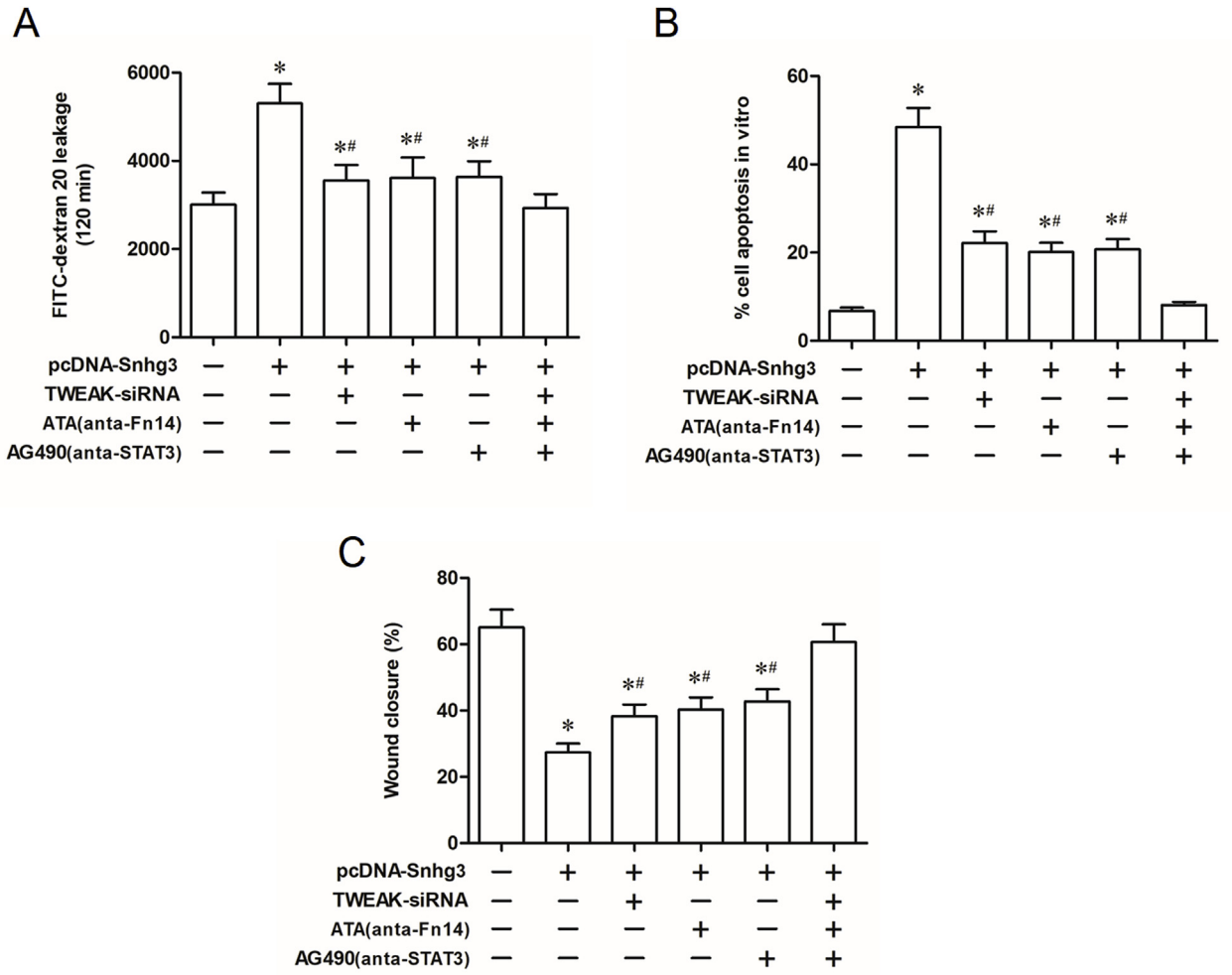

Fig. 7. TWEAK downregulation, Fn14 inhibition, or STAT3 blockade rescued the impairment of BMVEC functions by pcDNA-Snhg3. The TWEAK-siRNA (40 nM), ATA (10 nmol), and AG490 (5 μM) were respectively used to treat BMVECs together with OGD + hemin. Cell functions including (A) monolayer permeability (B) apoptosis, and (C) migration, were respectively detected. N = 6, $^{*}P < 0.05$ compared with control; $^{\#}P < 0.05$ compared with pcDNA-Snhg3.

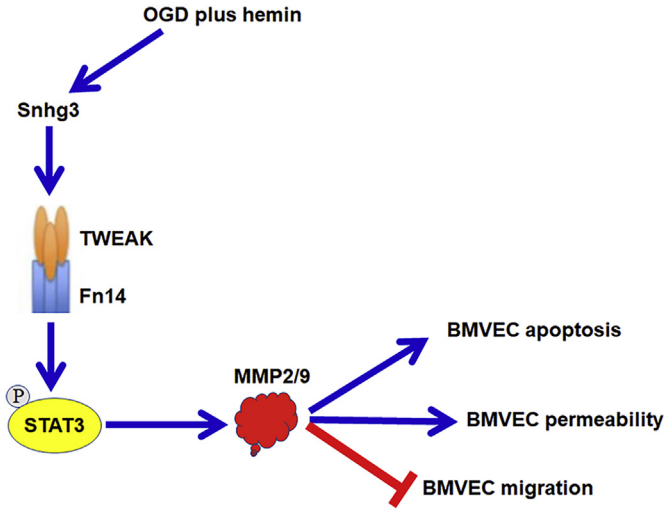

Fig. 8. Diagrammatic sketch of the pathways linked with Shng3 in regulating BMVEC functions.

3. Results

3.1. LncRNA Snhg3 expression was sharply increased in an in vitro ICH model, and inhibition of Snhg3 improved functions of BMVECs

Snhg3 has been involved in cell proliferation and migration in malignant cells. However, the role of Snhg3 in regulating functions of non-malignant cells has been hardly reported. Our previous study suggested that Snhg3 expression was dysregulated in central nervous system of individuals with neuroinflammation. To investigate the role of Snhg3 in progression of ICH, an *in vitro* ICH model was established by treating primary BMVECs with oxygen-and-glucose-deprivation (OGD) plus hemin, and the expression of Snhg3 was detected with qPCR. The results showed that Snhg3 expression was sharply induced in the *in vitro* ICH model (Fig. 1A), accompanied by decreased abilities of cell proliferation and migration and increased apoptosis and monolayer permeability (Fig. 1B–E). Simultaneously, the siRNA specifically against Snhg3 was used to inhibit Snhg3 expression in OGD/hemin-treated BMVECs. Inhibition of Snhg3 improved cell proliferation and migration abilities and reduced cell apoptosis and monolayer permeability (Fig. 1B–E).

3.2. Overexpression of Snhg3 impaired BMVEC function under normal condition

To further validate the role of Snhg3 in progression of ICH *in vitro*,

the pcDNA-Snhg3 expression vectors at different concentrations were respectively transfected into primary BMVECs under normal condition. Overexpression efficiency detected by qPCR revealed that pcDNA-Snhg3 was successfully expressed in BMVECs (Fig. 2A). Then, BMVEC functions were detected with the methods as same as above. The results showed that Snhg3 suppressed the proliferation and migration and increased apoptosis and monolayer permeability in a dose-dependent manner (Fig. 2B–E).

3.3. Inhibition of Snhg3 improved behaviors and reduced histological injuries in ICH rats

The above findings revealed that Snhg3 upregulation could impair functions of BMVECs under normal condition and its downregulation improved BMVEC functions in the *in vitro* ICH model. We then investigated whether Snhg3 was involved in ICH progression *in vivo*. ICH rat model was established, and then injected with Snhg3-siRNA and negative control siRNA (Ctrl-siRNA or NC siRNA) respectively. Snhg3 expression was significantly induced in the brain of ICH rats, and Snhg3 could be suppressed by siRNA injection (Fig. 3A). ICH rats, compared with the sham group, displaying abnormal behaviors and histological characteristics, including, more right turns (Fig. 3B), less limb placement (Fig. 3C), much higher Evans blue extravasation (Fig. 3D), obvious edema and hematoma (Supplementary Figs. 1A and B), more brain water content (Fig. 3E) and higher cell apoptosis level (Fig. 3F). Inhibition of Snhg3 by siRNA injection significantly improved ICH rats' behaviors and histological characteristics (Fig. 3B–F). These findings indicated that Snhg3 was involved in ICH progression *in vivo* and inhibition of Snhg3 could improve ICH rats' behaviors and histological characteristics.

3.4. Snhg3 level was positively related with TWEAK protein level and positively regulated the TWEAK/Fn14/STAT3 pathway

Snhg3 was suggested to be potentially related to TNF receptor superfamily members (TNFSFs) [17]. TWEAK is also named TNFSF12, which is frequently activated in multiple central nervous diseases and is famous for its regulatory role in neuroinflammation. Recently, the TWEAK/Fn14 pathway was reported to have increased permeability and secretion of proinflammatory factors in BMVECs *in vitro* [14]. We speculated that there is a relationship between Snhg3 and TWEAK. Pearson Correlation test was used to evaluate the relation between Snhg3 and TWEAK expression levels in the brain of ICH rats. The results showed that there was a strongly positive correlation between Snhg3 and TWEAK expression levels *in vivo* (Fig. 4A). Moreover, in response to overexpression of Snhg3, levels of TWEAK mRNA and protein were significantly increased in BMVECs (Fig. 4B and C). As a result, Fn14, the receptor of TWEAK, was significantly upregulated (Fig. 4D), and the STAT3 pathway, downstream of TWEAK/Fn14, was sharply activated, manifested by increased phosphorylation level of STAT3 protein and enhanced secretion of MMP-2 and MMP-9 (Fig. 4D–F).

3.5. The TWEAK/Fn14/STAT3 pathway was activated in ICH in vitro model

To study the role of TWEAK in progression of ICH, *in vitro* ICH model was established in primary BMVECs by treatment with OGD + hemin. The TWEAK-siRNA or ctrl-siRNA was transfected into the *in vitro* cell model. We found that, similar to the results of Snhg3-siRNA transfection, TWEAK protein was sharply induced by OGD + hemin (Fig. 5A). Downregulation of TWEAK by TWEAK-siRNA transfection suppressed expression of TWEAK protein (Fig. 5A), antagonized the activation of Fn14 and STAT3 pathway (Fig. 5B), and suppressed the secretion of MMP-2 and MMP-9 in BMVECs treated with OGD + hemin (Fig. 5C and D).

3.6. TWEAK downregulation, Fn14 inhibition, or STAT3 blockade, could rescue Snhg3-induced impairment of BMVEC functions

Finally, the TWEAK-siRNA, aurin tricarboxylic acid (ATA, inhibitor of Fn14), and AG490 (blocker of STAT3) were respectively used to treat BMVECs together with OGD + hemin. The results showed that regardless of TWEAK downregulation, Fn14 inhibition, or STAT3 blockade did not affected, but the three treatments applied together slightly reduced the expression of Snhg3 (Fig. 6A) and abolished the activation of the TWEAK/Fn14/STAT3 pathway induced by pcDNA-Snhg3 (Fig. 6B–D). Moreover, cell function detection revealed that TWEAK downregulation, Fn14 inhibition, or STAT3 blockade alone could partially rescue and the three treatments applied together could reverse the impairment of BMVEC functions, including monolayer permeability, apoptosis and migration (Fig. 7A–C).

4. Discussion

Snhg3 was originally reported to be expressed in the brain of individuals with Alzheimer's disease [18]. Not long after that, it was suggested that Snhg3 might be a target gene of activation-induced cytidine deaminase (AID) and involved in AID-induced DNA breakage [19,20]. However, there have been no reports stating the exact role of Snhg3 in any biological processes until 3 years ago. Existing literature indicates that Snhg3 is an oncogene that positively regulates cell proliferation, migration, invasion and energy metabolism in multiple types of solid cancer, including ovarian cancer, colorectal cancer, and glioma etc. [21–23]. However, the role of Snhg3 in regulating functions of non-malignant cells has been hardly reported. In this study, we reported that Snhg3 expression was sharply induced in primary BMVECs under treatment with OGD plus hemin, an *in vitro* model of ICH. Snhg3 contributed to impairment of BMVEC functions, manifested by suppressing cell proliferation and migration and increasing monolayer permeability and cell apoptosis. In ICH rats *in vivo*, downregulation of Snhg3 by siRNA injection improved behavioral and histological manifestations, which might be related to activation of the TWEAK/Fn14/STAT3 pathway. To our knowledge, this is the first report to reveal the exact role of Snhg3 in regulating progression of acute cerebral accidents.

In the mechanism exploration, we found that, TWEAK and Snhg3 displayed a positive correlation with each other. Snhg3 overexpression increased expression of TWEAK protein and its receptor Fn14, that were also induced by OGD plus hemin, activating the downstream neuroinflammatory pathway STAT3 and enhancing the secretion of MMP-2 and MMP-9 (Fig. 8). Moreover, TWEAK downregulation, Fn14 inhibition, or STAT3 blockade, could rescue Snhg3-induced impairment of BMVEC functions. Snhg3 was suggested to be possibly associated with some of TNFSFs [17]. TWEAK is also named TNFSF12, which was frequently activated in multiple central nervous diseases, famous for its regulatory role in neuroinflammation, and reported increasing permeability and secretion of proinflammatory factors in BMVECs *in vitro* [14,24,25]. No existing report reveals and our evaluation of their interaction possibility (data not shown) also suggests that there is no possibility that Snhg3 and TWEAK interact with each other directly. Considering the ways lncRNAs regulate gene expression and the factors activate TWEAK, two possible ways that Snhg3 positively regulates TWEAK expression are Snhg3 sponges a shared miRNA with TWEAK gene or Snhg3 upregulates some factors activating TWERK.

STAT proteins comprise a family of cytoplasmic transcription factors regulating intracellular signals, which are involved in diverse biological events including embryonic development, organogenesis, innate/adaptive immunity programmed cell death, and cell growth among all various higher animals. Generous studies have indicated that STAT3 was activated in neurological diseases, particularly in cerebral ischemic and hemorrhagic stroke, and it is now acknowledged as a critical target in neuroprotective therapies [26]. Previous researches referred that STAT3 activation was closely related to destruction of

blood-brain barrier, for its promotion on production of multiple pro-inflammatory cytokines, peroxidase and hydrolase matrix metalloproteinases, including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), myeloperoxidase, matrix metalloproteinase-2 (MMP-2) and MMP-9 [27,28]. STAT3 activation was usually stimulated by epithelial growth factors or kinase signaling pathways, such as EGFR, AKT and JAK [28–30]. In this study, we also showed that STAT3 was activated in ICH *in vitro* model and stimulated by the TWEAK/Fn14 pathway, and it participated in Snhg3-induced dysfunction of BMVECs, the main cell type constituting the blood-brain barrier. Moreover, blockade of STAT3 could partially rescue Snhg3-induced dysfunction of BMVECs.

In conclusion, Snhg3 expression was sharply induced in ICH, downregulation of Snhg3 improved functions of BMVECs *in vitro* and ICH rat behaviors and integrity of blood-brain barrier *in vivo*. In conclusion, the lncRNA Snhg3 contributes to dysfunction of cerebral microvascular cells in ICH rats by activating the TWEAK/Fn14/STAT3 pathway.

Declaration of competing interest

There is no conflict of interest to be declared by the authors.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lfs.2019.116929.

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