

BPB Reports

Report

Serine Protease Inhibitor A3N Expression Increases in the Brain, Liver, and Blood After Cerebral Ischemia in Mice

Saki Egashira,^a Keiichi Irie,^{a,*} Mayuka Morimoto,^b Takafumi Nakano,^c Akiko Manabe,^a Ayuko Masaki,^a Rie Mukai,^d Yoshihiko Nakamura,^e Masato Hosokawa,^f Tomomitsu Satho,^e Kazunori Sano,^a and Kenichi Mishima^a

^aDepartment of Pharmacology, Faculty of Pharmaceutical Sciences, Fukuoka University, Nanakuma 8-19-1, Jonan-ku, Fukuoka, 814-0180, Japan; ^bDepartment of Pharmacy, Almeida Memorial Hospital, Miyazaki 1509-2, Oita, 870-1133, Japan; ^cDepartment of Pharmaceutical and Healthcare Management, Fukuoka University, Nanakuma 8-19-1, Jonan-ku, Fukuoka, 814-0180, Japan; ^dMicrobiology Laboratory, Faculty of Pharmaceutical Sciences, Fukuoka University, Nanakuma 8-19-1, Jonan-ku, Fukuoka, 814-0180, Japan; ^eDepartment of Emergency and Critical Care Medicine, Fukuoka University Hospital, Nanakuma 8-19-1, Jonan-ku, Fukuoka, 814-0180, Japan; ^fDepartment of Immunological and Molecular Pharmacology, Fukuoka University, Nanakuma 8-19-1, Jonan-ku, Fukuoka, 814-0180, Japan

Received September 19, 2025; Accepted February 6, 2026

Objective: There is increasing research interest on the impact of ischemic stroke on organs beyond the central nervous system, and it is now widely recognized that cerebral ischemia induces multiple alterations in peripheral systems. Therefore, it is necessary to elucidate the systemic consequences of cerebral ischemia. Serine protease inhibitor a3 (SERPINA3), a secretory immune-related molecule produced primarily in the liver and brain under normal conditions, is upregulated in response to inflammation. Here, we examined *Serpina3n* gene expression in the brain and liver and evaluated plasma SERPINA3N protein concentrations following cerebral ischemia using a mouse model. **Methods:** We examined changes in SERPINA3N levels in the brain, liver, and blood over time using a mouse model of focal cerebral ischemia induced by middle cerebral artery (MCA) occlusion for 4 h followed by reperfusion. Brain, liver, and blood samples were collected on days 1, 3, and 7 after MCA occlusion (MCAo). *Serpina3n* gene expression levels in the brain and liver were measured by quantitative real-time polymerase chain reaction (qPCR), and plasma SERPINA3N levels were measured by enzyme-linked immunosorbent assay (ELISA). **Results:** *Serpina3n* gene expression levels in the brain and liver were increased on day 1 after MCAo. Plasma SERPINA3N protein levels were increased and peaked on day 1 after MCAo. **Conclusion:** A mouse model of cerebral ischemia showed increased *Serpina3n* gene expression in the liver and SERPINA3N protein level in plasma. This is the first study of the effects of plasma SERPINA3N protein levels using a mouse model of cerebral ischemia.

Key words stroke, cerebral ischemia, serine protease inhibitor a3n

INTRODUCTION

Research on cerebral ischemia has focused primarily on postischemic events within the brain, including excitotoxicity, inflammation, oxidative stress, and disruption of the blood–brain barrier (BBB), ultimately leading to cell death.¹⁾ There is increasing interest in the impact of ischemic stroke on organs beyond the central nervous system, and it is now widely recognized that cerebral ischemia induces multiple alterations in peripheral systems.²⁾ Cerebral ischemia also causes severe injury to peripheral organs, which in turn exacerbates brain damage and influences clinical outcomes.³⁾ Therefore, it is essential to elucidate the systemic consequences induced by cerebral ischemia.

In humans, *SERPINA3* encodes the secretory immune-related molecule anti-protease α 1-antichymotrypsin, which

is produced primarily in the liver and brain under homeostatic conditions and is upregulated in response to systemic inflammation.^{4,5)} *Serpina3n*, the murine homologue, is known to be upregulated in the brain following ischemic stroke, where it attenuates brain injury by inhibiting neuroinflammation.⁶⁾ Therefore, inflammatory responses triggered by cerebral ischemia may also alter the dynamics of SERPINA3N levels in the liver. However, the temporal profile of *Serpina3n* expression in the liver after cerebral ischemia has not been elucidated. Here, we investigated the time course of *Serpina3n* gene expression in the brain and liver using a mouse model of cerebral ischemia.

In addition, plasma SERPINA3 levels have been reported to be increased after the onset of ischemic stroke in human patients.⁷⁾ Moreover, increased circulating SERPINA3 levels have been associated with unfavorable outcomes in patients

*To whom correspondence should be addressed. e-mail: kirie@cis.fukuoka-u.ac.jp



with intracerebral hemorrhage.^{8,9)} Therefore, elucidation of the dynamics of circulating SERPINA3 after cerebral ischemia may be helpful for predicting unfavorable outcomes. To our knowledge, this is the first study to investigate the temporal dynamics of SERPINA3N protein levels in the circulation following cerebral ischemia using a mouse model.

We first examined *Serpina3n* gene expression in the brain and liver and subsequently evaluated plasma SERPINA3N protein concentrations following cerebral ischemia.

METHODS

Animals Male ddY mice (6-8 weeks old, body weight 27-38 g; Japan SLC Inc., Shizuoka, Japan) were maintained under a 12-h light/dark cycle (lights on from 7 am to 7 pm) in an air-conditioned room (23°C ± 2°C) with free access to food (CE-2; Clea Japan, Tokyo, Japan) and water. All procedures regarding animal care and use were performed in compliance with the regulations established by the Experimental Animal Care and Use Committee of Fukuoka University. At the end of the experiment, whole blood was collected from mice under anesthesia with 4.0% isoflurane (ViATRIS, Tokyo, Japan), followed by harvesting of the brain and liver.

Focal Cerebral Ischemia A focal cerebral ischemia model was established in male ddY mice according to the method described previously.¹⁰⁾ Briefly, the mice were anesthetized with 4.0% isoflurane using NARCOBIT-E instrument (Natsume Seisakusho Co., Tokyo, Japan) and maintained with 1.5% isoflurane in 70% N₂O and 30% O₂. After making a mid-line neck incision, the left common and external carotid arteries were separated and ligated. An 8-0 nylon monofilament (Echilon; Johnson & Johnson, Tokyo, Japan) coated with silicone resin (Provil novo; Heraeus, Tokyo, Japan) was introduced through a small incision in the common carotid artery and advanced to a position 9 mm distal from the carotid bifurcation for occlusion of the middle cerebral artery (MCA). Rectal temperature was maintained between 36.5°C and 37.5°C. The mice were reanesthetized at 4 h after occlusion and reperfusion was established by withdrawal of the filament. MCA occlusion (MCAo) was confirmed by examining forelimb flexion after awakening from anesthesia, and these animals were referred to as 4-hour MCA occlusion (4hMCAo) model mice.

Evaluation of Brain Damage At 1 day after MCAo, the brain was removed and cut coronally into 4 slices 2 mm thick using a mouse brain matrix. Each slice was stained with 2% 2,3,5-triphenyltetrazolium chloride (TTC; Sigma, St. Louis, MO) and cerebral infarct volume was measured using NIH Image, version 1.63 (National Institutes of Health, Bethesda, MD).

***Serpina3n* mRNA Expression** The levels of *Serpina3n* mRNA expression were measured by quantitative real-time polymerase chain reaction (qPCR). The brain and liver were collected on days 1, 3, and 7 after MCAo. Total RNA was extracted using TRI Reagent (Molecular Research Center Inc., Cincinnati, OH) and purified using an RNeasy Mini kit (Qiagen, Hilden, Germany). cDNA was synthesized from 1 µg of total RNA using ReverTra ACE qPCR RT Master Mix (Toyobo Co., Ltd., Tokyo, Japan). Primers were designed with reference to previous studies^{11,12)} with the following sequences: *Serpina3n*, 5'-tgctactcttgctataaagggg-3' (sense), 5'-atttgcccaatgtctcgaa-3' (antisense); glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*), 5'-aacccatcaccatctccag-3' (sense),

5'-acatactcagcaccagcatcac-3' (antisense). qPCR was performed using THUNDERBIRD SYBR qPCR Mix (Toyobo, Tokyo, Japan) and a LightCycler 96 Instrument (Roche Ltd., Basel, Switzerland). The relative gene expression was calculated using the $\Delta\Delta C_t$ method. Target gene expression was normalized relative to *Gapdh* as an internal control in each sample.

Plasma SERPINA3N Assay Blood samples were collected at 6 h, 1, 3, and 7 days after MCAo. The mice were anesthetized with isoflurane, and blood samples (0.5-0.9 mL) were collected from the inferior vena cava. Plasma was obtained after centrifugation at 5000 rpm for 10 min at 4°C. Plasma SERPINA3N levels were measured by enzyme-linked immunosorbent assay (ELISA) (Ray Biotech, Georgia, USA) according to the manufacturer's instructions.

Statistical Analysis Data are presented as the mean ± standard error of the mean (SEM). The extent of infarct size was compared between the sham surgery and 4hMCAo groups using Student's *t* test. Other data were evaluated by one-way analysis of variance (ANOVA) with the Tukey-Kramer test. In all analyses, *P* < 0.05 was taken to indicate statistical significance.

RESULTS

Infarct Volume After Cerebral Ischemia The infarct volume was significantly greater in 4hMCAo mice on day 1 after MCAo compared to sham operation controls ($t(12) = -13.41$, *P* < 0.001, Student's *t* test) (Fig. 1).

Temporal Dynamics of *Serpina3n* Expression in the Brain and Liver After Cerebral Ischemia There were significant differences in *Serpina3n* gene expression levels in the brain ($F(3, 28) = 14.45$, *P* < 0.01-0.001) (Fig. 2A) and liver ($F(3, 28) = 24.14$, *P* < 0.01-0.001) (Fig. 2B) between 4hMCAo mice and sham operation controls. *Serpina3n* gene expression levels in the brain of 4hMCAo mice were significantly increased on days 1 (*P* < 0.01), 3 (*P* < 0.001), and 7 (*P* < 0.001) after MCAo compared to sham operation controls, with the maximum expression level on day 3. On the other hand, *Serpina3n* gene expression levels in the liver of 4hMCAo mice were significantly increased on days 1 (*P* < 0.001) and 3 (*P* < 0.001) after MCAo compared to sham operation controls, with the maximum expression level on day 1. In the 4hMCAo group, *Serpina3n* expression level in the liver was significantly decreased on day 7 compared to days 1 and

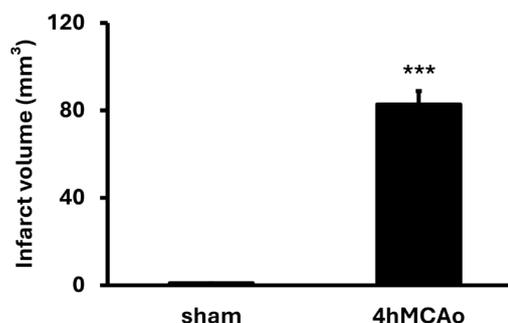


Fig. 1. Infarct Volume in 4-Hour MCA Occlusion Mice

Quantitative analysis of infarct volume in 4-hour MCA occlusion (4hMCAo) and sham operation control groups (*n* = 7 per group). ****P* < 0.001 versus sham. All values are shown as the mean ± SEM.

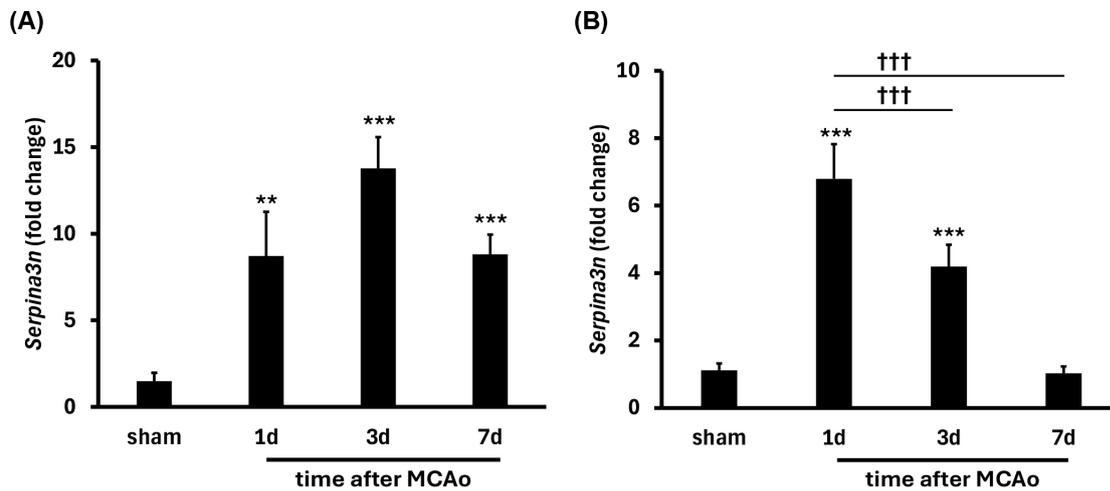


Fig. 2. *Serpina3n* Gene Expression Levels in the Brain and Liver Were Increased After MCAo

(A) *Serpina3n* gene expression levels in the brain of sham operation control and 4hMCAo groups ($n = 8$ per group). ** $P < 0.01$, *** $P < 0.001$ versus sham. All values are shown as the mean \pm SEM. (B) *Serpina3n* gene expression levels in the liver of 4hMCAo and sham operation control groups ($n = 8$ per group). *** $P < 0.001$ versus sham. ††† $P < 0.001$ versus day 1. All values are shown as the mean \pm SEM.

3 after MCAo (both $P < 0.001$). By day 7 after MCAo, *Serpina3n* gene expression levels in the liver in 4hMCAo mice had returned to levels comparable to those in sham operation controls.

Temporal Dynamics of Systemic SERPINA3N Levels After Cerebral Ischemia There were significant differences in SERPINA3N protein levels in the blood between 4hMCAo mice and sham operation controls ($F(4, 25) = 8.88$, $P < 0.05-0.001$) (Fig. 3). Plasma SERPINA3N protein levels in 4hMCAo mice were significantly increased on days 1 ($P < 0.001$) and 3 ($P < 0.05$) after MCAo compared to sham operation controls, with the maximum expression level on day 1. Plasma SERPINA3N protein levels in 4hMCAo mice were significantly decreased at 6 hours ($P < 0.01$) and 7 days ($P < 0.05$) after MCAo compared to day 1 of 4hMCAo mice.

DISCUSSION

We investigated the temporal dynamics of *Serpina3n* gene expression in the brain and liver, as well as plasma SERPINA3N protein levels following cerebral ischemia using a mouse model. The results showed that *Serpina3n* gene expression in the brain and liver, together with plasma SERPINA3N protein levels, increased from day 1 after cerebral ischemia and subsequently showed temporal fluctuations. To our knowledge, this is the first report to demonstrate that cerebral ischemia induces coordinated changes in *Serpina3n* expression in both the brain and liver, accompanied by dynamic alterations in plasma SERPINA3N protein concentrations.

In a mouse model of focal cerebral ischemia with 1 h of MCAo, Zhang *et al.* reported that *Serpina3n* gene expression in the brain was increased on day 1 after cerebral ischemia, decreased on day 3 compared with day 1, and returned to the level in sham operation controls by day 7.⁶ Consistent with their findings, we also observed an increase in *Serpina3n* gene expression in the brain from day 1 after cerebral ischemia. SERPINA3, also known as α -1-antichymotrypsin, is a serine protease inhibitor mainly produced in the liver and brain in response to inflammation.³ Therefore, the induction

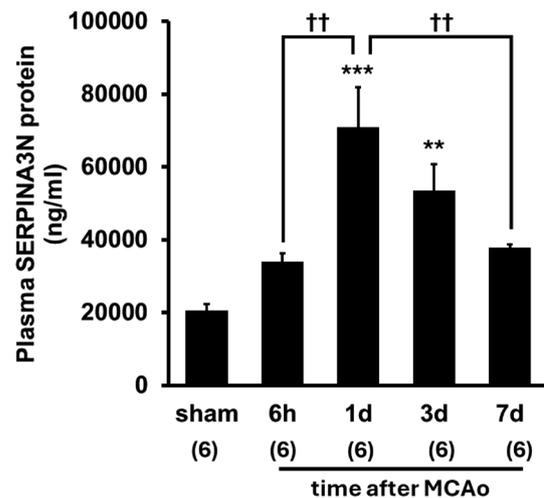


Fig. 3. SERPINA3N protein level in the blood was increased after MCAo

Quantitative analysis of SERPINA3N protein levels in 4hMCAo and sham operation control groups at 6 h, 1, 3, and 7 days after MCAo ($n = 6$ per group). ** $P < 0.01$, *** $P < 0.001$ versus sham. †† $P < 0.01$ versus day 1. All values are shown as the mean \pm SEM

of *Serpina3n* gene expression after cerebral ischemia is likely attributable to postischemic inflammatory responses. However, the duration of increased *Serpina3n* expression in the brain differed between studies: persisting for at least 7 days in our study but only 3 days in the study by Zhang *et al.* The duration of arterial occlusion in models of cerebral ischemia is known to be correlated with infarct volume and thus with the severity of ischemic stroke.¹³ Moreover, ischemic stroke severity has been suggested to be associated with the magnitude of the inflammatory response.¹⁴ In the present study, the occlusion time was 4 h, which was longer than that in Zhang's model, suggesting that the inflammatory response may have been more pronounced. Taken together, these findings suggested that the duration of *Serpina3n* gene upregulation in the

brain after cerebral ischemia may depend on ischemic stroke severity, including the period of arterial occlusion and infarct size. Furthermore, *Serpina3n* reduces mRNA expression of the inflammatory cytokines Interleukin-6 (IL-6) and Tumor Necrosis Factor- α (TNF- α) in the brain, thereby mitigating brain injury.¹⁵⁾ In addition, *Serpina3n* deficiency increases infarct volume and enhances BBB disruption.¹⁶⁾ Therefore, in this study, *Serpina3n*, whose expression of which increases following cerebral infarction treatment, may possess a function that suppresses damage caused by cerebral infarction.

SERPINA3 is known to be primarily produced in the liver and brain in humans.³⁾ In the present study, we examined the temporal dynamics of *Serpina3n* gene expression in the liver, a peripheral organ outside the primary site of cerebral ischemia. We found a significant increase in *Serpina3n* gene expression in the liver beginning on day 1 following cerebral ischemia. Recent studies have demonstrated that brain injury following cerebral ischemia affects peripheral organs, and that brain–liver interactions influence stroke outcomes.^{17,18)} Consistent with these findings, the observed increase in *Serpina3n* gene expression in the liver suggested that liver-derived SERPINA3N may play a role in mediating systemic responses induced by cerebral ischemia.

It has been reported that plasma SERPINA3N protein levels are increased in conditions such as spinal cord injury¹⁹⁾ and steroid-dependent muscular dystrophy.²⁰⁾ Therefore, it is conceivable that plasma SERPINA3N protein levels are also increased in diseases originating from the central nervous system. We demonstrated here that plasma SERPINA3N protein levels increased from day 1 after ischemia and remained elevated up to day 3 in a mouse model of cerebral ischemia. This finding supports clinical observations of increased plasma SERPINA3 levels in patients with ischemic stroke.⁷⁾ SERPINA3 is known to be induced in response to inflammatory stimuli.²¹⁾ Inflammatory cytokines in cerebral infarction reach peak expression levels in the blood and brain within hours to 24 hours after onset.²²⁾ Our study showed that plasma SERPINA3N protein levels were not increased at 6 hours post-cerebral ischemia but peaked on day 1. Therefore, the observed increase in plasma SERPINA3N protein levels in this study was likely a secondary response to inflammation following cerebral ischemia. Furthermore, *Serpina3n* mRNA is scarcely detected in blood,²³⁾ and SERPINA3 is known to be primarily produced in the liver.²¹⁾ Furthermore, SERPINA3 produced in hepatocytes has been reported to be secreted into the blood in response to inflammatory stimuli or tissue injury.²⁴⁾ Therefore, the increased *Serpina3n* expression in the liver observed in this study may have contributed to the elevated plasma SERPINA3N protein levels. Furthermore, following cerebral ischemia, neuronal cell death and disruption of the BBB occur, leading to the leakage of various brain substances and cytokines into the periphery.²⁵⁾ The increase in *Serpina3n* gene expression in the brain observed in this study may have contributed to the elevated plasma SERPINA3N protein levels. Therefore, the observed increase in plasma SERPINA3N protein levels in this study was likely due to contributions from both the liver and brain expression induction. Moreover, elevated plasma SERPINA3 level in patients with intracerebral hemorrhage has been shown to be associated with poor outcomes, suggesting its potential as a prognostic monitoring marker.^{8,9)} Taken together, these findings suggested that dynamic changes in plasma SERPINA3N protein lev-

els after cerebral ischemia may serve as a potential biomarker for unfavorable outcomes.

Serpina3n gene expression levels were increased in both the brain and liver following cerebral ischemia. In addition, plasma SERPINA3N protein levels were also elevated after ischemia. These findings provided insights into the dynamics and potential roles of SERPINA3N in the post-ischemic period. Moreover, plasma SERPINA3N protein level may reflect postischemic changes in the brain and liver and could serve as a predictive biomarker for ischemic stroke outcomes.

Acknowledgments We thank Dolphin (<https://www.dolphin-tr.com/>) for editing a draft of this manuscript. S.E. received Nagai Memorial Research Scholarship from the Pharmaceutical Society of Japan (N- 246902) and Tsukushi Fellowship and Research Foundation.

Conflict of interest The authors declare no conflict of interest.

REFERENCES

- 1) Belov Kirdajova D, Kriska J, Tureckova J, Anderova M. Ischemia-Triggered Glutamate Excitotoxicity From the Perspective of Glial Cells. *Front. Cell. Neurosci.*, **14**, 51 (2020).
- 2) Wesley UV, Bhute VJ, Hatcher JF, Palecek SP, Dempsey RJ. Local and systemic metabolic alterations in brain, plasma, and liver of rats in response to aging and ischemic stroke, as detected by nuclear magnetic resonance (NMR) spectroscopy. *Neurochem. Int.*, **127**, 113–124 (2019).
- 3) Wang J, Zhang J, Ye Y, Xu Q, Li Y, Feng S, Xiong X, Jian Z, Gu L. Peripheral Organ Injury After Stroke. *Front. Immunol.*, **13**, 901209 (2022).
- 4) Sergi D, Campbell FM, Grant C, Morris AC, Bachmair EM, Koch C, McLean FH, Muller A, Hoggard N, de Roos B, Porteiro B, Boeschoten MV, McGillicuddy FC, Kahn D, Nicol P, Benzler J, Mayer CD, Drew JE, Roche HM, Muller M, Nogueiras R, Dieguez C, Tups A, Williams LM. *Serpina3N* is a novel hypothalamic gene upregulated by a high-fat diet and leptin in mice. *Genes Nutr.*, **13**, 28 (2018).
- 5) Zhu M, Wang Y, Park J, Titus A, Guo F. Dispensable regulation of brain development and myelination by the immune-related protein *Serpina3n*. *J. Neurochem.*, **169**, e16250 (2025).
- 6) Zhang Y, Chen Q, Chen D, Zhao W, Wang H, Yang M, Xiang Z, Yuan H. *Serpina3N* attenuates ischemic stroke injury by reducing apoptosis and neuroinflammation. *CNS Neurosci. Ther.*, **28**, 566–579 (2022).
- 7) Hu X, Xiao ZS, Shen YQ, Yang WS, Wang P, Li PZ, Wang ZJ, Pu MJ, Zhao LB, Xie P, Li Q. SERPINA3: A novel inflammatory biomarker associated with cerebral small vessel disease burden in ischemic stroke. *CNS Neurosci. Ther.*, **30**, e14472 (2024).
- 8) Shen Y, Yang W, Xiong X, Li X, Xiao Z, Yu J, Liu F, Gui S, Xie X, Lv F, Zhao L, Hu L, Manaenko A, Xie P, Li Q. Integrated Multiomics Analysis Identifies a Novel Biomarker Associated with Prognosis in Intracerebral Hemorrhage. *Oxid. Med. Cell. Longev.*, **2021**, 2510847 (2021).
- 9) Zheng P, Qi Z, Gao B, Yao Y, Chen J, Cong H, Huang Y, Shi FD. SERPINA3 predicts long-term neurological outcomes and mortality in patients with intracerebral hemorrhage. *Cell Death Dis.*, **16**, 218 (2025).
- 10) Nakano T, Tagashira Y, Egashira S, Morimoto M, Irie K, Hosokawa M, Hayashi T, Egawa T, Hayakawa K, Mishima K. Therapeutic effect of anti-HMGB1 antibody in a mouse model of 4-h middle cerebral artery occlusion: comparison with tissue plasminogen activator. *Neuroreport*, **33**, 297–303 (2022).
- 11) Al-Ani M, Elemam NM, Hachim IY, Raju TK, Muhammad JS, Hachim MY, Bendardaf R, Maghazachi AA. Molecular Examination of Differentially Expressed Genes in the Brains of Experimental Autoimmune Encephalomyelitis Mice Post Herceptin Treatment. *J. Inflamm. Res.*, **14**, 2601–2617 (2021).

- 12) Yamashita Y, Irie K, Kochi A, Kimura N, Hayashi T, Matsuo K, Myose T, Sano K, Nakano T, Takase Y, Nakamura Y, Satho T, Mishima K, Mishima K. Involvement of Charcot-Marie-Tooth disease gene mitofusin 2 expression in paclitaxel-induced mechanical allodynia in rats. *Neurosci. Lett.*, **653**, 337–340 (2017).
- 13) Knab F, Koch SP, Major S, Farr TD, Mueller S, Euskirchen P, Eggers M, Kuffner MTC, Walter J, Berchtold D, Knauss S, Dreier JP, Meisel A, Endres M, Dirnagl U, Wenger N, Hoffmann CJ, Boehm-Sturm P, Harms C. Prediction of Stroke Outcome in Mice Based on Noninvasive MRI and Behavioral Testing. *Stroke*, **54**, 2895–2905 (2023).
- 14) Tarkowski E, Rosengren L, Blomstrand C, Wickelsö C, Jensen C, Ekholm S, Tarkowski A. Early intrathecal production of interleukin-6 predicts the size of brain lesion in stroke. *Stroke*, **26**, 1393–1398 (1995).
- 15) Zhang Y, Chen Q, Chen D, Zhao W, Wang H, Yang M, Xiang Z, Yuan H. SerpinA3N attenuates ischemic stroke injury by reducing apoptosis and neuroinflammation. *CNS Neurosci. Ther.*, **28**, 566–579 (2022).
- 16) Li F, Zhang Y, Li R, Li Y, Ding S, Zhou J, Huang T, Chen C, Lu B, Yu W, Boltze J, Li P, Wan J. Neuronal Serpina3n is an endogenous protector against blood brain barrier damage following cerebral ischemic stroke. *J. Cereb. Blood Flow Metab.*, **43**, 241–257 (2023).
- 17) Ma S, Zhao H, Ji X, Luo Y. Peripheral to central: organ interactions in stroke pathophysiology. *Exp. Neurol.*, **272**, 41–49 (2015).
- 18) Duan H, Ding Y, Cheng Z, Cai L, Tong Y, Che F, Han Z, Li F, Wang Q, Geng X. Low serum alanine aminotransferase (ALT) levels are associated with poor outcomes in acute ischemic stroke patients regardless of age. *Brain Res.*, **1842**, 149130 (2024).
- 19) Chen H, Wu L, Zhang Y, Ding W, Xiaofan Y. Steroid inhibited Serpina3n expression which was positively correlated with the degrees of spinal cord injury. *Heliyon*, **10**, e26649 (2024).
- 20) Tjondrokoesoemo A, Schips T, Kanisicak O, Sargent MA, Molkentin JD. Genetic overexpression of Serpina3n attenuates muscular dystrophy in mice. *Hum. Mol. Genet.*, **25**, 1192–1202 (2016).
- 21) Zhu M, Lan Z, Park J, Gong S, Wang Y, Guo F. Regulation of CNS pathology by Serpina3n/SERPINA3: the knowns and the puzzles. *Neuropathol. Appl. Neurobiol.*, **50**, e12980 (2024).
- 22) Simats A, Liesz A. Systemic inflammation after stroke: implications for post-stroke comorbidities. *EMBO Mol. Med.*, **14**, e16269 (2022).
- 23) Vanni S, Moda F, Zattoni M, Bistaffa E, De Cecco E, Rossi M, Giaccone G, Tagliavini F, Haïk S, Deslys JP, Zanusso G, Ironside JW, Ferrer I, Kovacs GG, Legname G. Differential overexpression of SERPINA3 in human prion diseases. *Sci. Rep.*, **7**, 15637 (2017).
- 24) de Mezer M, Rogaliński J, Przewoźny S, Chojnicki M, Niepolski L, Sobieska M, Przysiańska A. SERPINA3: Stimulator or Inhibitor of Pathological Changes. *Biomedicines*, **11**, 156 (2023).
- 25) Banks WA, Hansen KM, Erickson MA, Crews FT. High-mobility group box 1 (HMGB1) crosses the BBB bidirectionally. *Brain Behav. Immun.*, **111**, 386–394 (2023).