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SWAP70 alleviates brain damage and neuroinflammation induced by cerebral ischemia-reperfusion by inhibiting autophagy

Yu Shi^{1,*}, Fangfang Hu^{1,*}, Zhengdong Li¹ and Ke Xu²¹ Department of Neurology, Jiangsu University Affiliated Xuzhou Hospital, Xuzhou, Jiangsu, China² Department of Neurology, Xuzhou Central Hospital, Xuzhou, Jiangsu, China

Abstract. Cerebral ischemia-reperfusion (CIR) injury is a leading cause of neurological disability and is characterized by oxidative stress, inflammation, and excessive autophagy, all of which converge to promote neuronal injury and death. Autophagy plays a dual role in this setting by helping to maintain cellular homeostasis under stress, yet it can drive neurodegeneration when overactivated. Switch-associated protein 70 (SWAP70), a Rho GTPase exchange factor that regulates cytoskeletal dynamics and immune responses, has been implicated in neural differentiation and inflammatory regulation; however, its role in CIR injury remains unclear. In this study, using middle cerebral artery occlusion/reperfusion (MCAO/R) mice and oxygen-glucose deprivation/reoxygenation (OGD/R)-treated SH-SY5Y cells, we found that SWAP70 expression was significantly reduced after ischemic injury. Notably, SWAP70 overexpression improved neurological function and reduced infarct size, neuronal damage, and cytokine release in MCAO/R mice. Consistently, in OGD/R-treated SH-SY5Y cells, SWAP70 overexpression restored cell viability, decreased LDH release, and suppressed the expression of autophagy-related proteins. Importantly, rapamycin treatment reversed the protective effects of SWAP70, confirming that SWAP70 alleviates brain injury by inhibiting excessive autophagy. Furthermore, SWAP70 overexpression suppressed NF- κ B pathway activation. In conclusion, SWAP70 mitigates CIR injury by inhibiting excessive autophagy and dampening neuroinflammation, thereby providing a potential therapeutic target for ischemic stroke.

Key words: SWAP70 — Cerebral ischemia-reperfusion — Autophagy — Neuroinflammation — NF- κ B pathway

Introduction

Ischemic stroke is a major cause of disability and death worldwide, arising from the interruption of cerebral blood flow and the subsequent deprivation of oxygen and nutrients within brain tissues (Ding et al. 2022; Ren et al. 2023). Rapid restoration of cerebral circulation is essential to salvage the

ischemic penumbra and improve neurological outcomes; nevertheless, reperfusion itself can aggravate tissue injury, a phenomenon known as cerebral ischemia-reperfusion (CIR) injury. The pathological process of CIR is multifactorial and involves oxidative stress, inflammatory activation, calcium overload, mitochondrial dysfunction, and endoplasmic reticulum stress, which together contribute to neuronal death and blood-brain barrier disruption (Li et al. 2023). Despite advances in mechanical thrombectomy and thrombolytic therapy, effective pharmacological interventions that mitigate reperfusion-induced secondary injury remain limited, highlighting a persistent therapeutic gap. Autophagy, a conserved intracellular degradation process, plays a dual role in CIR injury (Ajooolabady et al. 2021).

* These authors contributed equally to the work and should be considered co-first authors.

Correspondence to: Ke Xu, Department of Neurology, Xuzhou Central Hospital, No. 199, Jiefang South Road, Quanshan District, Xuzhou, Jiangsu, China
E-mail: kexukxx25027@163.com

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Under physiological conditions, moderate autophagy facilitates the clearance of damaged organelles and protein aggregates, thereby maintaining neuronal homeostasis (Tuo et al. 2022). However, when autophagy becomes excessive or dysregulated under ischemic stress, it may shift from an adaptive response to a pathogenic process that promotes neuronal death and exacerbates brain injury. Consistent with this, accumulating evidence indicates that autophagy interacts with apoptosis, necrosis, and inflammatory signaling during ischemic injury, and this crosstalk collectively shapes the extent of neuronal survival (Nixon et al. 2024). Therefore, modulating autophagic activity has been proposed as a promising therapeutic strategy to limit neuronal loss following reperfusion.

Switch-associated protein 70 (SWAP70) is a Rho GTPase exchange factor that regulates cytoskeletal remodeling, cell migration, and immune cell activation (Qian et al. 2022). Beyond its classical role in B-cell signaling and actin dynamics, SWAP70 has recently been implicated in various physiological and pathological contexts, including autoimmunity, cardiac hypertrophy, and neural differentiation (Chen et al. 2022; Qian et al. 2023). Studies have shown that SWAP70 deficiency can limit immune protection, impair cell maturation, and alter the expression of autophagy-related genes (Chandrasekaran et al. 2016). Notably, elevated SWAP70 levels in plasma and cerebrospinal fluid have been associated with a reduced risk of ischemic stroke, suggesting a potential protective role in cerebrovascular disorders. In addition, SWAP70 has been reported to promote cell-cycle exit of neural precursor cells and facilitate oligodendrocyte formation, thereby further supporting its functional relevance to neural homeostasis and regeneration.

Given that autophagy and inflammation are central mediators of neuronal damage after ischemia-reperfusion, the regulatory function of SWAP70 in these processes remains largely unexplored. Preliminary findings suggest that SWAP70 expression decreases following cerebral ischemia, occurring concomitantly with elevated autophagic activity and enhanced neuroinflammatory responses. Therefore, clarifying whether SWAP70 modulates autophagy and inflammation to attenuate neuronal injury may uncover novel molecular mechanisms underlying CIR pathology and help identify potential therapeutic targets.

In this study, we aimed to investigate the role of SWAP70 in CIR injury and to elucidate the underlying mechanisms.

Materials and Methods

Animals and experimental design

Male C57BL/6 mice (8–10 weeks old, 22–25 g) were purchased from Beijing Vital River Laboratory Animal Tech-

nology Co., Ltd. (Beijing, China). All animal procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals and were approved by the Ethics and Morality Committee for Laboratory Animals of Xuzhou Medical University. Mice were randomly assigned to the following groups: sham, model (MCAO/R), model+NC, model+SWAP70, and model+SWAP70+rapamycin. The middle cerebral artery occlusion/reperfusion (MCAO/R) model was established as previously described. Briefly, mice were anesthetized with 2% isoflurane, and a monofilament nylon suture (Doccol Corp., USA, 602123PK10) was inserted into the right internal carotid artery to occlude the middle cerebral artery for 60 min, followed by reperfusion for 24 h. Rapamycin (Beyotime, Shanghai, China, S1842) was administered *intraperitoneally* at 2 mg/kg, 30 min before reperfusion. Neurological deficits were scored on a five-point scale by a blinded investigator.

Cell culture and treatment

Human neuroblastoma SH-SY5Y cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS; Gibco, 10099-141) at 37°C in a humidified incubator containing 5% CO₂.

The oxygen-glucose deprivation/reoxygenation (OGD/R) model was used to mimic ischemia-reperfusion injury *in vitro*. Briefly, the cells were incubated in glucose-free DMEM (Gibco, 11966-025) and maintained in a hypoxic chamber (1% O₂, 94% N₂, 5% CO₂) for 6 h, followed by reoxygenation in complete medium for 24 h. Rapamycin (Beyotime, S1842; 200 nM) was added during the reoxygenation period as indicated.

Plasmid construction and lentiviral transduction

A lentiviral vector encoding mouse SWAP70 was purchased from Heyuan Biotechnology Co., Ltd. (Shanghai, China), and a control lentivirus carrying an empty vector (LV-NC) was used as the negative control. For *in vitro* experiments, cells were infected at a multiplicity of infection (MOI) of 20 in the presence of 8 µg/ml polybrene (Beyotime, C0351). After 48 h, cells were harvested for subsequent analyses. For *in vivo* overexpression, 2 × 10⁸ TU of LV-SWAP70 or LV-NC were stereotactically injected into the lateral ventricle of C57BL/6 mice one week before MCAO/R surgery.

Neurological scoring and histological analysis

Neurological deficits were assessed 24 h after reperfusion using a five-point scoring system: 0, no deficit; 1, forelimb weakness; 2, circling to the contralateral side; 3, falling to

the contralateral side; 4, inability to walk spontaneously. For histopathological examination, brains were fixed in 4% paraformaldehyde (Beyotime, P0099), embedded in paraffin, and cut into 5 μ m sections. The sections were then stained with hematoxylin and eosin (H&E; Beyotime, C0105S).

Western blot analysis

Proteins (30 μ g) were separated on 10% SDS-PAGE gels (Beyotime, P0012A) and transferred to PVDF membranes (Millipore, USA, IPVH00010). Membranes were blocked with 5% non-fat milk for 1 h and then incubated overnight at 4°C with the following primary antibodies: SWAP70 (Abcam, Cambridge, UK, ab152178, 1:1000); Beclin 1 (Abcam, ab62557, 1:1000); LC3B (Abcam, ab48394, 1:1000); p62 (Abcam, ab56416, 1:2000); p-p65 (Abcam, ab76302, 1:1000); p65 (Abcam, ab16502, 1:2000); p-I κ B α (Abcam, ab133462, 1:1000); I κ B α (Abcam, ab32518, 1:2000); and GAPDH (Abcam, ab9485, 1:5000). After washing, membranes were incubated with an HRP-conjugated goat anti-rabbit IgG secondary antibody (Abcam, ab6721, 1:5000) for 1 h. The corresponding protein bands were visualized using an ECL chemiluminescence kit (Beyotime, P0018S) and quantified.

Immunohistochemistry (IHC)

Paraffin-embedded brain sections were deparaffinized and rehydrated, followed by antigen retrieval in citrate buffer (pH 6.0; Beyotime, P0083). Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 10 min. Sections were incubated overnight at 4°C with an anti-SWAP70 antibody (Abcam, ab152178, 1:200), followed by an HRP-conjugated secondary antibody (Abcam, ab205718, 1:500). Signal development was performed using a DAB Substrate Kit (Beyotime, P0203), and the sections were counterstained with hematoxylin (Beyotime, C0105S).

Measurement of inflammatory cytokines

Levels of IL-6, IL-1 β , and TNF- α in mouse brain tissue homogenates or cell culture supernatants were measured using enzyme-linked immunosorbent assay (ELISA) kits (Beyotime, PI326, PI301, and PT518, respectively).

Cell viability and cytotoxicity assays

Cell viability was determined using a Cell Counting Kit-8 (CCK-8; Beyotime, C0038). Lactate dehydrogenase (LDH) release was evaluated using an LDH Cytotoxicity Assay Kit (Beyotime, C0016).

Statistical analysis

All experiments were independently repeated at least three times unless otherwise stated. Data are presented as mean $\pm$ standard deviation (SD), and statistical analyses were performed using GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA). For *in vivo* experiments with sufficient sample size, the data distribution was assumed to approximate normality, and comparisons among multiple groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test. For *in vitro* cell-based experiments with a limited number of independent biological replicates ($n = 6$), non-parametric methods were used to avoid assumptions of normal distribution; specifically, comparisons between two groups were analyzed using the Mann-Whitney U test, whereas multiple-group comparisons were analyzed using the Kruskal-Wallis test followed by Dunn's *post hoc* test. A value of $p < 0.05$ was considered statistically significant.

Results

Reduced SWAP70 expression and activation of autophagy following cerebral ischemia-reperfusion

To clarify the potential involvement of SWAP70 in CIR injury, we first examined its expression together with autophagic activity in the mouse MCAO/R model. Neurological deficit scoring showed a significant increase in neurological dysfunction in the model group compared with the sham group (Fig. 1A). Western blot analysis revealed that SWAP70 protein levels were markedly reduced after ischemia-reperfusion, whereas the autophagy-related proteins Beclin 1 and LC3II/I were increased, and the autophagy substrate p62 was decreased (Fig. 1B). Immunohistochemical staining further demonstrated that SWAP70-positive cells were abundantly distributed in the cerebral cortex of sham mice but were sparsely present in model mice (Fig. 1C). Taken together, these concordant findings from protein detection and tissue staining indicate that ischemia-reperfusion injury is accompanied by reduced SWAP70 expression and excessive activation of autophagy. Collectively, these results suggest that SWAP70 downregulation may contribute to heightened autophagic activity following CIR.

SWAP70 overexpression alleviates brain injury and suppresses autophagy *in vivo*

To assess whether SWAP70 plays a protective role *in vivo*, we next overexpressed SWAP70 in mice subjected to MCAO/R and evaluated its effects on autophagy and neuroinflammation. Western blot analysis demonstrated that SWAP70

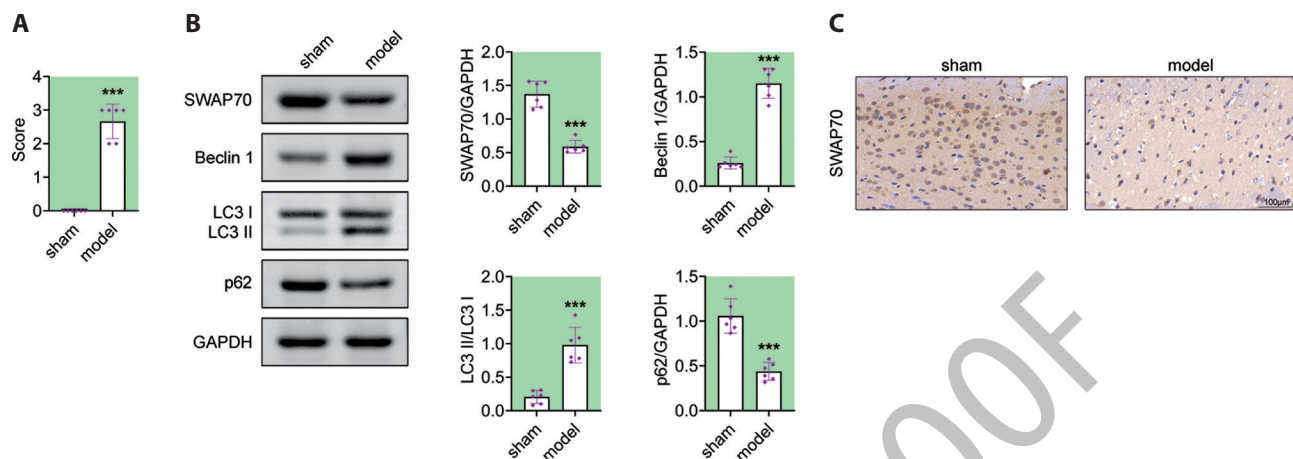


Figure 1. SWAP70 expression is downregulated while autophagy is activated in mouse cerebral ischemia-reperfusion model. **A.** Neurological deficit scores of mice in the sham and model (MCAO/R) groups. **B.** Western blot analysis of SWAP70, Beclin 1, LC3 I/II, and p62 expression in brain tissues from sham and model groups, with quantitative densitometry normalized to GAPDH. **C.** Immunohistochemical staining showing SWAP70 localization in brain tissues from sham and model groups (scale bar = 100 μm). *n* = 6 in each group. Data are expressed as mean ± SD; *** *p* < 0.001.

overexpression markedly increased SWAP70 protein levels, decreased Beclin 1 expression and LC3II/I ratios, and increased p62 accumulation compared with the model and model+NC groups; notably, these effects were abolished by rapamycin, an autophagy activator (Fig. 2A). Consistent with these molecular changes, neurological deficit scores were significantly lower in SWAP70-overexpressing mice than in model mice, whereas rapamycin treatment again aggravated neurological dysfunction (Fig. 2B). Histopathological evaluation by H&E staining further revealed reduced neuronal loss and vacuolization in the model+SWAP70 group compared with the model or model+NC groups (Fig. 2C). In parallel, ELISA showed that the levels of inflammatory cytokines IL-6, IL-1β, and TNF-α were markedly decreased after SWAP70 overexpression, while rapamycin treatment partially reversed these reductions (Fig. 2D). Collectively, these results suggest that SWAP70 protects against CIR-induced brain injury, at least in part, by suppressing excessive autophagy and dampening neuroinflammatory responses.

SWAP70 overexpression protects SH-SY5Y cells from OGD/R-induced injury by suppressing autophagy

To further verify these findings *in vitro*, we used SH-SY5Y neuronal cells subjected to oxygen-glucose deprivation/reoxygenation (OGD/R) as a cellular model of ischemia-reperfusion injury. As shown in Figure 3A, OGD/R markedly increased both yellow (autophagosomes) and red-only (autolysosomes) LC3 puncta compared with the control group, indicating enhanced autophagic flux following

ischemia-reperfusion *in vitro*. Notably, SWAP70 overexpression significantly reduced the number of LC3 puncta, supporting the notion that SWAP70 suppresses excessive autophagy activation under OGD/R conditions. In contrast, treatment with the autophagy activator rapamycin restored autophagic flux and partially abolished the inhibitory effect of SWAP70 (Fig. 3A). Consistent with these imaging results, Western blotting revealed that OGD/R stimulation significantly downregulated SWAP70 expression, increased Beclin 1 levels and LC3II/I ratios, and decreased p62 levels, whereas SWAP70 overexpression largely reversed these changes. By contrast, rapamycin reactivated autophagy and diminished the inhibitory effects of SWAP70 on autophagy-related markers (Fig. 3B). Functional assays demonstrated that SWAP70 overexpression significantly improved neuronal survival (Fig. 3C) and reduced LDH release (Fig. 3D), indicating decreased cytotoxicity. ELISA further confirmed that the levels of IL-6, IL-1β, and TNF-α were markedly decreased by SWAP70 overexpression, but elevated again following rapamycin treatment (Fig. 3E). Collectively, these data demonstrate that SWAP70 confers protection against OGD/R-induced injury in SH-SY5Y cells by restraining autophagic hyperactivation while concurrently suppressing proinflammatory cytokine production.

SWAP70 inhibits NF-κB pathway activation after cerebral ischemia-reperfusion

To elucidate downstream mechanisms through which SWAP70 may regulate inflammatory responses, we next examined NF-κB signaling in brain tissues from the differ-

ent groups. Western blot analysis revealed that ischemia-reperfusion markedly increased phosphorylation of p65 and I κ B- α , whereas total I κ B- α expression was reduced compared with the sham group. SWAP70 overexpression significantly decreased p-p65 and p-I κ B- α levels and restored I κ B- α expression, while the model+NC group showed no apparent difference from the untreated model

group (Fig. 4). These findings suggest that SWAP70 negatively regulates NF- κ B pathway activation after ischemia-reperfusion, thereby limiting inflammatory signaling. Taken together, these results indicate that SWAP70 ameliorates ischemia-reperfusion-induced neuroinflammation and excessive autophagy, at least in part, through inhibition of the NF- κ B signaling pathway.

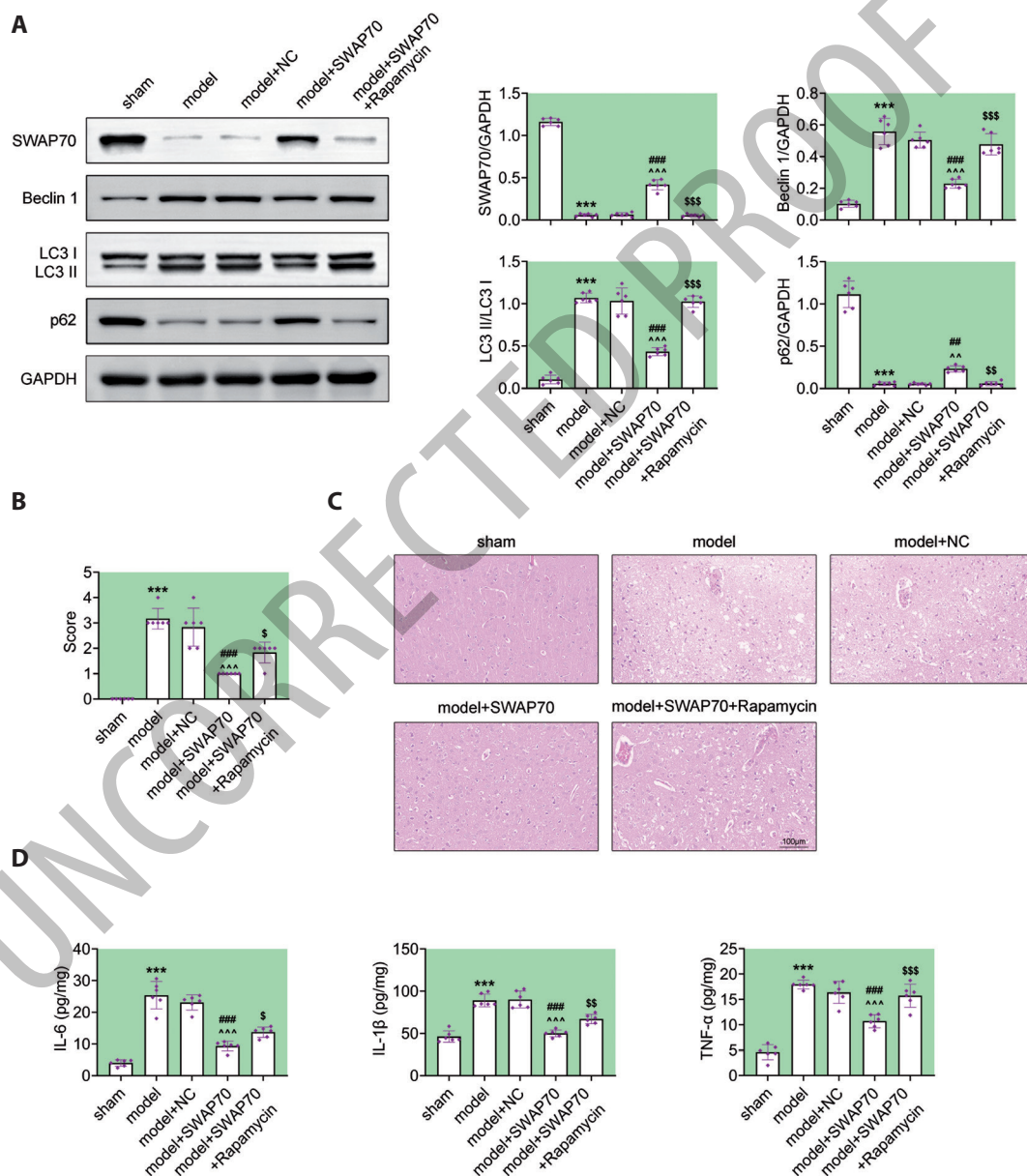


Figure 2. Overexpression of SWAP70 alleviates cerebral ischemia-reperfusion-induced brain injury by inhibiting autophagy *in vivo*. **A.** Western blot analysis of SWAP70, Beclin 1, LC3 I/II, and p62 expression in brain tissues from the sham, model, model+NC, model+SWAP70, and model+SWAP70+Rapamycin groups. **B.** Neurological deficit scores of mice from each group. **C.** Representative hematoxylin and eosin (H&E) staining of brain tissues from different groups (scale bar = 100 μ m). **D.** ELISA analysis of IL-6, IL-1 β , and TNF- α levels in brain tissues from each group. $n = 6$ in each group. Data are presented as mean $\pm$ SD; *** $p < 0.001$ vs. sham; ### $p < 0.001$ vs. model; ^^ $p < 0.01$, ^^^ $p < 0.001$ vs. Model+NC; \$ $p < 0.05$, \$\$ $p < 0.01$, \$\$\$ $p < 0.001$ vs. model+SWAP70.

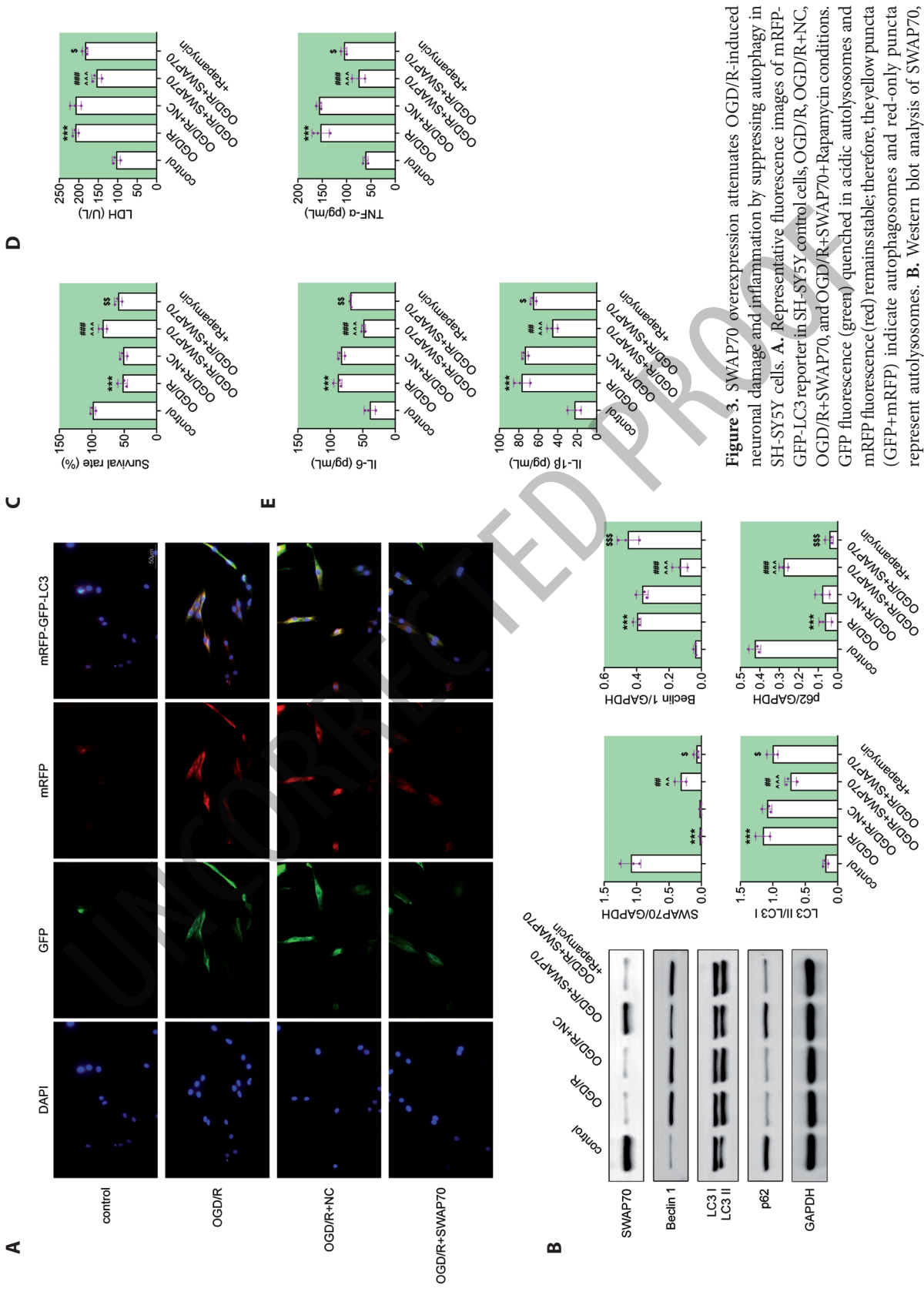


Figure 3. SWAP70 overexpression attenuates OGD/R-induced neuronal damage and inflammation by suppressing autophagy in SH-SY5Y cells. **A.** Representative fluorescence images of mRFP-GFP-LC3 reporter in SH-SY5Y control cells, OGD/R, OGD/R+NC, OGD/R+SWAP70, and OGD/R+SWAP70+Rapamycin conditions. GFP fluorescence (green) quenched in acidic autolysosomes and mRFP fluorescence (red) remains stable; therefore, the yellow puncta (GFP+mRFP) indicate autophagosomes and red-only puncta represent autolysosomes. **B.** Western blot analysis of SWAP70, Beclin 1, LC3 I/II, and p62 expression in SH-SY5Y cells from the control, OGD/R, OGD/R+NC, OGD/R+SWAP70, and OGD/R+SWAP70+Rapamycin groups. **C.** Cell survival rates determined by CCK-8 assay in each group. **D.** Measurement of LDH release levels in cell culture supernatants. **E.** ELISA detection of inflammatory cytokines IL-6, IL-1β, and TNF-α in different groups. $n = 6$ in each group. Data are expressed as mean $\pm$ SD; *** $p < 0.001$ vs. control; ## $p < 0.01$, ### $p < 0.001$ vs. OGD/R; ^^^ $p < 0.001$ vs. OGD/R+NC; \$ $p < 0.05$, \$\$ $p < 0.01$, \$\$\$ $p < 0.001$ vs. OGD/R+SWAP70. (See online version for color figure.)

Discussion

Ischemic stroke remains one of the leading causes of death and long-term disability worldwide, primarily resulting from the abrupt interruption of cerebral blood flow followed by reperfusion. Although early restoration of blood flow is the most effective therapeutic strategy, reperfusion itself may aggravate neuronal injury through oxidative stress, calcium overload, mitochondrial dysfunction, and inflammatory activation, a constellation of events collectively known as CIR injury (Campbell et al. 2020). Multiple cell death mechanisms, including apoptosis, necrosis, ferroptosis, and autophagy-related neuronal loss, have been implicated in this complex pathological cascade (Chen et al. 2024). Among these processes, excessive autophagy together with heightened neuroinflammation appears particularly detrimental, contributing to secondary neuronal degeneration and blood-brain barrier disruption. In this study, using both the MCAO/R mouse model and the OGD/R cellular model that reliably reproduce key pathophysiological features of CIR, we observed a significant decrease in SWAP70 expression accompanied by enhanced autophagic activity and an intensified inflammatory response, suggesting that SWAP70 participates in the progression of ischemic brain injury.

Neuroinflammation plays a pivotal role in amplifying tissue damage after ischemic insult. Activated microglia, infiltrating leukocytes, and pro-inflammatory cytokines such as IL-6, IL-1 β , and TNF- α can trigger a self-perpetuating inflammatory cascade that worsens neuronal injury and impairs neurological recovery (Candelario-Jalil et al. 2022). Therefore, controlling inflammatory signaling is recognized as a promising therapeutic approach for mitigating CIR injury. In the present study, SWAP70 overexpression significantly reduced the levels of these cytokines in both MCAO/R mice and OGD/R-treated SH-SY5Y cells, whereas rapamycin administration partially reversed this effect (Zou et al. 2024). These findings indicate that the role of SWAP70 is closely linked to its capacity to attenuate neuroinflammatory responses. By suppressing excessive inflammation, secondary neuronal loss may be reduced and functional recovery may be supported, thereby further highlighting the therapeutic potential of targeting inflammatory pathways in ischemic stroke.

SWAP70 is a multifunctional Rho GTPase exchange factor known to regulate actin cytoskeleton remodeling, cell migration, and immune cell signaling (Fang et al. 2025). It has been implicated in various physiological and pathological contexts, including immune tolerance, cardiac hypertrophy, and neural differentiation (Takada et al. 2011; He et al. 2018; Qian Hu 2023). Accumulating evidence suggests that SWAP70 can modulate immune cell activation and the expression of autophagy-related genes, thereby influencing

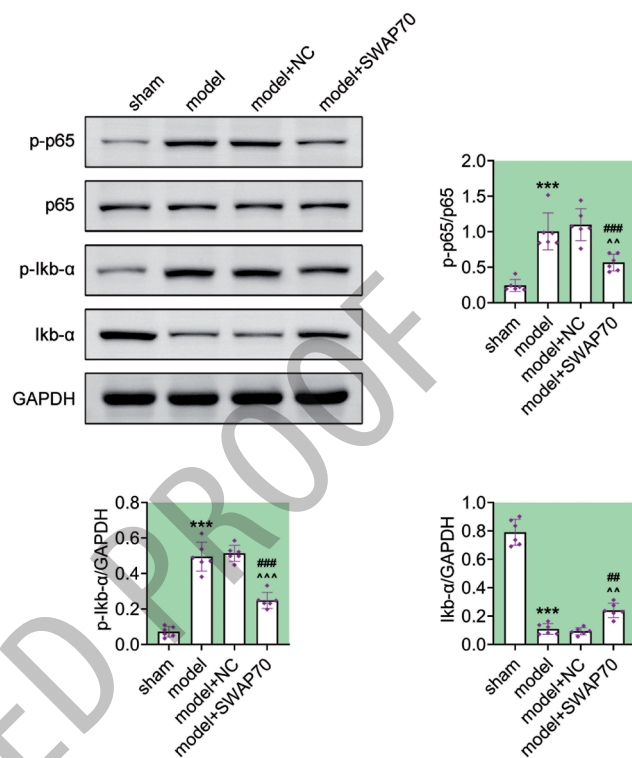


Figure 4. SWAP70 inhibits the NF- κ B pathway activation to reduce neuroinflammatory signaling after cerebral ischemia-reperfusion. Western blot analysis of p-p65, p65, p-I κ B- α , and I κ B- α expression in brain tissues from the sham, model, model+NC, and model+SWAP70 groups, with densitometric quantification normalized to GAPDH. $n = 6$ in each group. Data are presented as mean $\pm$ SD; *** $p < 0.001$ vs. sham; ### $p < 0.001$ vs. model; ^^ $p < 0.01$ vs. model+NC.

inflammatory diseases (Bahaie et al. 2012; Ricker et al. 2021). In autoimmune models, loss of SWAP70 alters regulatory T-cell function and enhances systemic inflammation (Chopin et al. 2010). Consistent with these findings, our study demonstrated that SWAP70 downregulation after ischemia-reperfusion is associated with increased autophagic activity and inflammatory responses, whereas SWAP70 overexpression alleviates both. Together, these results extend existing understanding on the biological relevance of SWAP70 beyond immune regulation and suggest that it may act as an intrinsic modulator of neuroinflammation and neuronal survival during ischemic injury.

Targeting inflammatory signaling cascades, particularly the NF- κ B pathway, represents a promising strategy for reducing neuroinflammation and tissue injury after stroke (Zhang et al. 2025). NF- κ B is a central transcription factor regulating the production of pro-inflammatory mediators and cell-survival genes, and its activation is initiated by phosphorylation of p65 and I κ B- α , which promotes the nu-

clear translocation of p65 and the subsequent induction of downstream cytokines (Wang et al. 2020). In our MCAO/R mouse model, ischemia-reperfusion markedly enhanced NF- κ B pathway activation, whereas SWAP70 overexpression significantly suppressed p-p65 and p-I κ B- α expression and restored I κ B- α levels. Similar regulatory effects were observed in OGD/R-treated neurons, indicating that SWAP70 acts as a negative modulator of NF- κ B signaling under both in vivo and in vitro conditions. These findings suggest that the anti-inflammatory effect of SWAP70 is at least partly mediated through inhibition of NF- κ B activation, thereby reducing cytokine production and mitigating neuronal damage.

Autophagy, another crucial component of ischemia-reperfusion injury, plays dual roles depending on the severity and duration of stress (Ajoalabady et al. 2021). Moderate autophagy contributes to cellular repair and survival, whereas excessive or uncontrolled autophagy can promote neuronal death. Our findings demonstrate that SWAP70 overexpression reduced the autophagy-related markers Beclin 1 and LC3II/I and increased p62 accumulation in both animal and cellular models, while rapamycin reversed these effects.

These results indicate that SWAP70 protects neurons from ischemia-reperfusion-induced injury, at least in part, by suppressing autophagic overactivation. The concomitant reduction in inflammatory cytokines further supports the view that autophagy and inflammation are functionally interconnected processes, and that their coordinated regulation by SWAP70 may represent a key mechanism for limiting secondary injury after stroke. An important observation in this study is that rapamycin treatment partially attenuated the protective effects of SWAP70 overexpression and was accompanied by a relative reduction in SWAP70 protein levels. It should be emphasized that rapamycin is a canonical downstream activator of autophagy by inhibiting mTOR signaling and does not directly regulate SWAP70 transcription or translation. Therefore, the ability of rapamycin to counteract SWAP70-mediated autophagy suppression likely reflects a hierarchical relationship in which strong mTOR inhibition functionally overrides upstream modulatory signals. The observed decrease in SWAP70 expression under rapamycin treatment may represent a secondary feedback response to enhanced autophagic flux, potentially through altered protein turnover or compensatory regulatory mechanisms triggered by excessive autophagy activation.

Although autophagy has been widely recognized as a cytoprotective mechanism under physiological conditions or mild stress, accumulating evidence indicates that excessive or dysregulated autophagy contributes to neuronal damage during CIR injury. The seemingly contradictory roles of autophagy largely depend on the intensity of activation, the temporal stage of injury, and the cellular context. In the present study, we observed a pronounced downregulation of

SWAP70 accompanied by marked activation of autophagic flux following ischemia-reperfusion, which suggests that loss of SWAP70 may permit maladaptive autophagy overactivation. Importantly, restoration of SWAP70 expression did not abolish autophagy; instead, it normalized autophagy-related protein levels, supporting a modulatory rather than purely suppressive role. Notably, previous studies reporting neuroprotection through autophagy activation often emphasize early or moderate autophagy induction, which facilitates the clearance of damaged organelles and protein aggregates. In contrast, sustained ischemic stress and subsequent reperfusion can trigger excessive autophagy that may exceed lysosomal degradative capacity, ultimately promoting neuronal death. Our findings support the concept that SWAP70 functions as an upstream, stress-responsive regulator that fine-tunes autophagic activity under pathological conditions. Accordingly, variations in SWAP70 expression under different ischemic intensities or durations may influence whether autophagy exerts protective or detrimental effects on neuronal function.

Despite these promising findings, several limitations should be acknowledged. First, our study mainly relied on overexpression systems, and loss-of-function approaches, including conditional knockout models, are needed to further establish a causal relationship between SWAP70 and ischemic injury. Second, the precise molecular interactions between SWAP70 and components of the NF- κ B pathway or autophagy-related proteins remain to be clarified, and mechanistic experiments such as co-immunoprecipitation and confocal colocalization will be important to define these links. Finally, translational studies using human brain tissue or relevant clinical samples would strengthen the clinical relevance of our observations and provide a clearer foundation for therapeutic development. Future work is needed to determine upstream regulators of SWAP70 and to explore its potential as a biomarker or therapeutic target for ischemic cerebrovascular disease.

In conclusion, this study demonstrates that SWAP70 expression is suppressed after CIR, and that restoration of SWAP70 attenuates neuronal injury by inhibiting excessive autophagy and dampening NF- κ B-mediated inflammation.

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Conflicts of interest. The authors state that there are no conflicts of interest to disclose.

Ethics approval. Ethical approval was obtained from the Ethics and Morality Committee for Laboratory Animals of Xuzhou Medical University.

Data availability. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Contribution of authors. YS, FH designed the study and carried them out, prepared the manuscript for publication and reviewed the draft of the manuscript; YS, FH, ZL, KX supervised the data collection, analyzed the data, interpreted the data. All authors have read and approved the manuscript.

References

- Ajoolabady A, Wang S, Kroemer G, Penninger JM, Uversky VN, Pratico D, Henninger N, Reiter RJ, Bruno A, Joshipura K et al. (2021): Targeting autophagy in ischemic stroke: From molecular mechanisms to clinical therapeutics. *Pharmacol Ther* **225**, 107848
<https://doi.org/10.1016/j.pharmthera.2021.107848>
- Bahaie NS, Hosseinkhani MR, Ge XN, Kang BN, Ha SG, Blumenthal MS, Jessberger R, Rao SP, Sriramara P (2012): Regulation of eosinophil trafficking by SWAP-70 and its role in allergic airway inflammation. *J Immunol* **188**, 1479-1490
<https://doi.org/10.4049/jimmunol.1102253>
- Campbell BCV, Khatri P (2020): Stroke. *Lancet*. **396**, 129-142
[https://doi.org/10.1016/S0140-6736\(20\)31179-X](https://doi.org/10.1016/S0140-6736(20)31179-X)
- Candelario-Jalil E, Dijkhuizen RM, Magnus T (2022): Neuroinflammation, stroke, blood-brain barrier dysfunction, and imaging modalities. *Stroke* **53**, 1473-1486
<https://doi.org/10.1161/STROKEAHA.122.036946>
- Chandrasekaran U, Yi W, Gupta S, Weng CH, Giannopoulos E, Chinenov Y, Jessberger R, Weaver CT, Bhagat G, Pernis AB (2016): Regulation of effector treg cells in murine lupus. *Arthritis Rheumatol* **68**, 1454-1466
<https://doi.org/10.1002/art.39599>
- Chen H, Malhotra A, Gandhi D (2024): Thrombectomy for large strokes. *N Engl J Med* **391**, 378-379
<https://doi.org/10.1056/NEJMc2406965>
- Chen Z, Flores Castro D, Gupta S, Phalke S, Manni M, Rivera-Correa J, Jessberger R, Zaghoulani H, Giannopoulos E, Pannellini T et al. (2022): Interleukin-13 receptor alpha1-mediated signaling regulates age-associated/autoimmune B cell expansion and lupus pathogenesis. *Arthritis Rheumatol* **74**, 1544-1555
<https://doi.org/10.1002/art.42146>
- Chopin M, Quemeneur L, Ripich T, Jessberger R (2010): SWAP-70 controls formation of the splenic marginal zone through regulating T1B-cell differentiation. *Eur J Immunol* **40**, 3544-3556
<https://doi.org/10.1002/eji.201040556>
- Ding Q, Liu S, Yao Y, Liu H, Cai T, Han L (2022): Global, regional, and national burden of ischemic stroke, 1990-2019. *Neurology* **98**, e279-e290
<https://doi.org/10.1212/WNL.00000000000013115>
- Fang Q, Fan H, Li Q, Zhang M, Zhou Z, Du J, Huang J (2025): Multi-omic insight into the molecular networks in the pathogenesis of coronary artery disease. *J Am Heart Assoc* **14**, e037203
<https://doi.org/10.1161/JAHA.124.037203>
- He Q, Naqvi S, McLellan H, Boevink PC, Champouret N, Hein I, Birch PRJ (2018): Plant pathogen effector utilizes host susceptibility factor NRL1 to degrade the immune regulator SWAP70. *Proc Natl Acad Sci USA* **115**, E7834-E7843
<https://doi.org/10.1073/pnas.1808585115>
- Li J, Xu P, Hong Y, Xie Y, Peng M, Sun R, Guo H, Zhang X, Zhu W, Wang J, Liu X (2023): Lipocalin-2-mediated astrocyte pyroptosis promotes neuroinflammatory injury via NLRP3 inflammasome activation in cerebral ischemia/reperfusion injury. *J Neuroinflammation* **20**, 148
<https://doi.org/10.1186/s12974-023-02819-5>
- Nixon RA, Rubinshtein DC (2024): Mechanisms of autophagy-lysosome dysfunction in neurodegenerative diseases. *Nat Rev Mol Cell Biol* **25**, 926-946
<https://doi.org/10.1038/s41580-024-00757-5>
- Qian Q, Hu F, Yu W, Leng D, Li Y, Shi H, Deng D, Ding K, Liang C, Liu J (2023): SWAP70 overexpression protects against pathological cardiac hypertrophy in a TAK1-dependent manner. *J Am Heart Assoc* **12**, e028628
<https://doi.org/10.1161/JAHA.122.028628>
- Qian Q, Li Y, Fu J, Leng D, Dong Z, Shi J, Shi H, Cao D, Cheng X, Hu Y et al. (2022): Switch-associated protein 70 protects against nonalcoholic fatty liver disease through suppression of TAK1. *Hepatology* **75**, 1507-1522
<https://doi.org/10.1002/hep.32213>
- Ren K, Pei J, Guo Y, Jiao Y, Xing H, Xie Y, Yang Y, Feng Q, Yang J (2023): Regulated necrosis pathways: a potential target for ischemic stroke. *Burns Trauma* **11**, tkad016
<https://doi.org/10.1093/burnst/tkad016>
- Ricker E, Manni M, Flores-Castro D, Jenkins D, Gupta S, Rivera-Correa J, Meng W, Rosenfeld AM, Pannellini T, Bachu M et al. (2021): Altered function and differentiation of age-associated B cells contribute to the female bias in lupus mice. *Nat Commun* **12**, 4813
<https://doi.org/10.1038/s41467-021-25102-8>
- Takada N, Appel B (2011): Swap70 promotes neural precursor cell cycle exit and oligodendrocyte formation. *Mol Cell Neurosci* **48**, 225-235
<https://doi.org/10.1016/j.mcn.2011.08.003>
- Tuo QZ, Zhang ST, Lei P (2022): Mechanisms of neuronal cell death in ischemic stroke and their therapeutic implications. *Med Res Rev* **42**, 259-305
<https://doi.org/10.1002/med.21817>
- Wang D, Liu F, Zhu L, Lin P, Han F, Wang X, Tan X, Lin L, Xiong Y (2020): FGF21 alleviates neuroinflammation following ischemic stroke by modulating the temporal and spatial dynamics of microglia/macrophages. *J Neuroinflammation* **17**, 257
<https://doi.org/10.1186/s12974-020-01921-2>
- Zhang M, Guo B, Zhang X, Han D, Lv L, Yan X, Su C, Chai D, Zhao N, Yan X et al. (2025): IFP35, a novel DAMP, aggravates neuroinflammation following acute ischemic stroke via TLR4/NF-kappaB/NLRP3 signaling. *J Neuroinflammation* **22**, 164
<https://doi.org/10.1186/s12974-025-03492-6>
- Zou X, Wang L, Wang S, Zhang Y, Ma J, Chen L, Li Y, Yao TX, Zhou H, Wu L et al. (2024): Promising therapeutic targets for ischemic stroke identified from plasma and cerebrospinal fluid proteomes: a multicenter Mendelian randomization study. *Int J Surg* **110**, 766-776
<https://doi.org/10.1097/JS9.0000000000000922>

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