

PARP1–FOXO3a–Depp1 signaling axis Drives Blood–Brain Barrier Disruption Under Chronic Cerebral Hypoperfusion by Triggering Excessive Autophagy Initiation

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Abstract

Background

Chronic cerebral hypoperfusion (CCH) is a major contributor to vascular cognitive impairment (VCI), characterized by progressive neurovascular dysfunction, including neuroinflammatory activation and blood–brain barrier (BBB) disruption. Although autophagy dysregulation has been implicated in BBB damage, the upstream molecular mechanisms driving excessive autophagy activation during chronic hypoperfusion remain poorly understood.

Methods/Results

Using a bilateral carotid artery stenosis (BCAS)-induced mouse model of CCH and an oxygen–glucose deprivation/reoxygenation (OGD/R) endothelial cell model, this study investigated the role of poly (ADP-ribose) polymerase-1 (PARP1) in CCH-associated neurovascular injury. PARP1 expression was persistently increased following CCH, and pharmacological inhibition of PARP1 with Olaparib significantly improved cognitive deficits and attenuated neuroinflammatory responses, white matter injury, and BBB hyperpermeability. Mechanistically, this study demonstrated that excessive autophagy initiation, rather than lysosomal dysfunction, was a key driver of tight junction protein degradation and BBB disruption after CCH. Transcriptomic analysis identified FOXO signaling as a potential downstream pathway regulated by PARP1 inhibition. Further investigations revealed that PARP1 promoted FOXO3a activation and transcriptionally upregulated its downstream target Depp1, thereby inducing excessive autophagy initiation and BBB injury. In vitro, Depp1 overexpression abolished the inhibitory effect of Olaparib on autophagy activation, confirming the essential role of Depp1 in the PARP1–FOXO3a–Depp1 signaling axis.

Conclusions

This study identifies a previously unrecognized PARP1–FOXO3a–Depp1 signaling axis that drives excessive autophagy initiation and mediates BBB disruption during CCH. These findings provide new mechanistic insights into CCH-associated neurovascular injury and support the potential repurposing of the FDA-approved PARP1 inhibitor Olaparib as a therapeutic strategy for VCI.

1. Introduction

Vascular cognitive impairment (VCI) encompasses a spectrum of cognitive deficits ranging from mild cognitive impairment to vascular dementia (VaD) [1–3], involving the cumulative effects of microvascular damage, including microinfarcts, microhemorrhages, lacunar infarcts, and white matter lesions [4, 5]. These injuries are frequently associated with hypertension, diabetes, and atherosclerosis [4–7]. Chronic cerebral hypoperfusion (CCH) is a critical driver of SVD initiation and progression [8, 9],

and triggers a cascade of cellular and molecular mechanisms implicated in VCI pathogenesis, including oxidative stress, microvascular injury, excitotoxicity, blood–brain barrier (BBB) dysfunction, and secondary neuroinflammation [10]. For instance, CCH markedly upregulates the expression of matrix metalloproteinases (MMPs) and pro-inflammatory cytokines [11–13]. At the cellular level, the structural and functional integrity of the brain relies on the precise exchange of substances between the blood and brain parenchyma across the BBB. The BBB is composed of continuous cerebral microvascular endothelial cells interconnected by tight junction (TJ) proteins, an intact basement membrane, pericytes, and astrocytic endfeet, and serves as a structural cornerstone for maintaining central nervous system homeostasis. Recent reports suggest that BBB damage is not only one of the early pathological events in CCH, but also continues to play a role in the later stages of disease progression, including white matter lesions, neuronal degeneration and cognitive decline [10, 14]. Studies have shown that under CCH conditions, the expression of TJ proteins is significantly reduced, and the extent of this disruption is closely correlated with cognitive deterioration [15]. Nevertheless, the upstream molecular mechanisms governing BBB structural and functional impairment following CCH remain elusive.

Emerging evidence has demonstrated that CCH induces a marked elevation in autophagy levels within brain parenchymal cells [16]. Under oxygen–glucose deprivation conditions, the autophagic–lysosomal pathway in endothelial cells can specifically degrade TJ proteins at the BBB, suggesting that excessive autophagy activation exerts detrimental effects on BBB integrity [17]. Despite these findings linking autophagy to BBB disruption, the upstream triggers of autophagy overactivation under CCH conditions remain poorly understood.

Poly(ADP-ribose) polymerase-1 (PARP1), the most abundant and well-characterized member of the PARP family, catalyzes the transfer of ADP-ribose moieties (PAR) from NAD⁺ to acceptor proteins such as histones and transcription factors, playing a pivotal role in DNA damage repair and cellular stress responses [18]. PARP1 is tightly regulated by endogenous control mechanisms; its overactivation, however, depletes NAD⁺ and ATP, ultimately culminating in cell death [19]. In neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD), PARP1 participates in disease progression by modulating neuroinflammation, autophagy, and mitochondrial dysfunction, and targeting PARP1 has been shown to confer potential therapeutic benefits [20–22]. With respect to BBB function, previous studies have demonstrated that PARP1 inhibition reduces BBB permeability in animal models of systemic inflammation; in primary human brain microvascular endothelial cells (BMVECs), PARP1 inhibition enhances barrier integrity and upregulates TJ protein expression [23]; moreover, pharmacological inhibition of PARP1 attenuates age-related BBB leakage [24]. Regarding the association between PARP1 and autophagy regulation, recent evidence indicates that PARP1 activation upregulates the expression of autophagy-related genes, thereby overactivating autophagy in a cardiac ischemia–reperfusion injury model [25]. However, whether PARP1 participates in CCH-induced BBB injury through modulation of autophagic activity remains unknown.

Currently, therapeutic strategies for CCH-related brain injury remain largely limited, with no effective molecular-targeted approaches available, and preventing or reversing disease progression remains a

major challenge. Therefore, using a CCH model induced by BCAS and an in vitro OGD/R endothelial cell model, this study aimed to elucidate the regulatory role of PARP1 in CCH-associated BBB injury and the molecular mechanisms by which PARP1 mediates autophagy activation, thereby providing a novel theoretical basis for therapeutic intervention in CCH.

2. Materials and Methods

2.1 Animals

Adult male C57BL/6J mice (25–30 g, 9–12 weeks) were housed under a 12 h light/dark cycle at 24–26°C with ad libitum access to food and water (SPF grade). Animals were randomly assigned to groups using a random number table.

2.2 Establishment of the CCH Model

CCH was induced by BCAS. Mice were anesthetized with 1–1.5% isoflurane, and core body temperature was maintained at $36.5 \pm 0.5^{\circ}\text{C}$ throughout surgery. Microcoils (inner diameter 0.18 mm, SAMINI SPRING CO, Ltd) were applied bilaterally to the common carotid arteries. Sham-operated mice underwent identical surgical procedures without coil placement.

2.3 Drug Treatment

Mice were randomized into five groups: (1) Sham + Vehicle; (2) BCAS + Vehicle; (3) BCAS + Olaparib (5 mg/kg/2 d, HY-10162, MedChemExpress); (4) BCAS + CQ (20 mg/kg/d, HY-17589A, MedChemExpress); (5) BCAS + Olaparib + Rapamycin (RAPA, 3 mg/kg/d, HY-10219, MedChemExpress). All mice were sacrificed at 28 d post-surgery.

2.4 Laser Speckle Contrast Imaging (LSCI)

Cerebral blood flow (CBF) was assessed by LSCI under 1–1.5% isoflurane anesthesia. The scalp was incised along the midline and the skull surface was cleaned with sterile saline. Raw speckle images were acquired with a camera positioned 10 cm above the skull, covering bilateral parietal regions of interest (ROIs). CBF was measured at baseline and at 3 d, 14 d, and 28 d post-surgery, and expressed as a percentage change relative to baseline.

2.5 Behavioral Tests

Y-maze: Spontaneous alternation was assessed over 8 minutes using a Y-maze with three equal arms (120° apart). Animal trajectories were tracked using Labmaze V3.0 software. Spontaneous alternation (%) = $\text{alternations} / (\text{total entries} - 2) \times 100$.

Novel Object Recognition (NOR): After 3 d of habituation, mice were exposed to two identical objects (familiarization phase, 5 min), followed 1 hour later by replacement of one object with a novel object (test phase, 5 min). Recognition index (RI, %) = $\text{novel object explorations} / (\text{novel} + \text{familiar object explorations}) \times 100$. The apparatus was cleaned with 75% ethanol between trials.

2.6 Immunofluorescence

Mice were transcardially perfused with saline followed by ice-cold 4% paraformaldehyde (PFA). Brains were post-fixed overnight at 4°C and cryoprotected in 10%, 20%, and 30% sucrose solutions. Frozen sections (30 µm) were permeabilized with 0.3% Triton X-100 (30 min) and blocked with 5% BSA (60 min). Sections were incubated overnight at 4°C with primary antibodies: anti-Iba-1 (1:500, 019-19741, FUJIFILM Wako), anti-IgG (1:200, 315005003, Jackson ImmunoResearch), or Lectin-488 (1:300, DC-1174, Vector Laboratories). After PBS washes, sections were incubated with Alexa Fluor 488 (1:500, A-11078, Thermo Fisher) or 594-conjugated secondary antibodies (1:500, A-11012, Thermo Fisher) for 2 h at room temperature. Sections were mounted with DAPI-containing anti-fade medium (S2110, Solarbio). Images were acquired using an Olympus BX53 fluorescence microscope (≥ 9 fields per mouse). For quantitative analysis, Iba-1-positive cells were counted as the number of cells, and IgG extravasation was expressed as the percentage of positive area relative to the total image area, using ImageJ software.

2.7 Luxol Fast Blue (LFB) Staining

Frozen sections (30 µm) were dried overnight at 60°C, stained in 0.1% LFB solution at 60°C for 2 h, differentiated in 0.05% lithium carbonate, and dehydrated through graded ethanol and xylene. White matter lesions were graded 0–3: 0 = normal; 1 = disarranged nerve fibers; 2 = prominent vacuolation; 3 = loss of myelinated fibers.

2.8 Western Blotting (WB)

Brain tissues were homogenized in RIPA lysis buffer supplemented with PMSF and protease inhibitors (100:1:1), sonicated on ice, and centrifuged at 12,000 rpm for 15 min at 4°C. Protein concentrations were determined by BCA assay. Equal amounts of protein were separated by SDS-PAGE and transferred to PVDF membrane (0.2 µm, ISEQ00010, Merck). Membranes were blocked with 5% skim milk for 1 h and incubated overnight at 4°C with primary antibodies against PARP1 (1:2000, 66520-1-Ig, Proteintech), β -actin (1:10000, 66009-1-Ig, Proteintech), IL-1 β (1:1000, 16806-1-AP, Proteintech), MMP9 (1:1000, 10375-2-AP, Proteintech), ZO-1 (1:1000, 21773-1-AP, Proteintech), Occludin (1:1000, DF7504, Affinity), LC3B (1:1000, T55992S, Abmart), P62 (1:1000, 18420-1-AP, Proteintech), DEPP (1:1000, 25833-1-AP, Proteintech), or FOXO3A (1:600, WL02891, Wanleibio). After washing, membranes were incubated with HRP-conjugated secondary antibody (1:1500, A0208, Beyotime) for 1 h. Bands were visualized by chemiluminescence and imaged using a ChemiDoc XRS+ system (Bio-Rad). Densitometry was performed with ImageJ, normalized to β -actin.

2.9 Transcriptomic Analysis

Cortical tissues were rapidly frozen in liquid nitrogen and stored at -80°C . RNA sequencing was performed by GeneDenovo Biotechnology Co., Ltd. Total RNA was extracted using TRIzol, and paired-end libraries were sequenced on a high-throughput platform. Differentially expressed genes (DEGs) were identified using thresholds of fold change ≥ 1.5 and $P < 0.05$, followed by GO and KEGG pathway enrichment analyses.

2.10 Cell Culture and OGD Model

Human brain microvascular ECs (hCMEC/D3; QuiCell-H129, Quicell) were cultured in ECM endothelial cell medium (Sciencell, 1001) supplemented with 5% fetal bovine serum (FBS), 1% endothelial cell growth supplement (ECGS), and 1% penicillin–streptomycin, and maintained at 37°C in a humidified atmosphere of 5% CO₂. An oxygen–glucose deprivation/reoxygenation (OGD/R) model was used to mimic hypoxic injury in vitro. For OGD treatment, cells were incubated in glucose-free DMEM (Procell, PM150270) and placed in a sealed modular incubator chamber with an AnaeroPack system (Mitsubishi Gas Chemical Company) to establish hypoxic conditions for 4 h, 6 h, or 8 h. Subsequently, cells were returned to complete medium and subjected to reoxygenation under normoxic conditions for 12 h or 24 h prior to further analyses.

2.11 Cell Drug Treatment and Transfection

Olaparib or equivalent DMSO was co-administered during OGD (4 h) and maintained during reoxygenation (24 h). For overexpression experiments, the Depp1 plasmid (pCMV-EGFP- Depp1 (human)-Neo; Miao Ling Biotechnology) or empty vector (pCMV-EGFP-3×Linker-MCS-Neo; Miao Ling Biotechnology) was transfected using Lipofectamine 3000 (7.5 µg/ 6-well plate, L3000008, Thermo Fisher) according to the manufacturer's protocol. Cells were cultured for 30 h post-transfection before subsequent treatments.

2.12 Cell Viability Assay

Cell viability was assessed using a CCK-8 kit (AC11L054, Life-iLab Biotech). Cells were seeded in 96-well plates, treated as indicated, and incubated with 10 µL CCK-8 solution per well at 37°C for 2 h.

Absorbance was measured at 450 nm.

2.13 Statistical Analysis

Data were analyzed using SPSS 25.0 and visualized with GraphPad Prism 10. Normality was assessed by the Shapiro-Wilk test and homogeneity of variance by Levene's test. Normally distributed data with equal variance are presented as mean ± SD (bar graphs) and compared by two-tailed paired t-test (two groups) or one-way ANOVA with Bonferroni post-hoc test (multiple groups). Non-normally distributed or heteroscedastic data are presented as median with interquartile range (box plots) and compared by Mann-Whitney U test or Kruskal-Wallis test, respectively. $P < 0.05$ was considered statistically significant.

3. Results

3.1 BCAS induces cerebral hypoperfusion and cognitive decline in mice at 28 d post-surgery.

LSCI was employed to monitor CBF changes at 3 d, 14 d, and 28 d post-BCAS surgery. Compared with the Sham group, the CCH group exhibited significant time-dependent alterations in CBF. Specifically, CBF

decreased by approximately 31% at 3 d post-BCAS, recovered to about 77% of baseline at 14 d, and reached approximately 80% of baseline by 28 d (Fig. 1A, 1B), a pattern likely attributable to compensatory collateral circulation.

Neurological function was subsequently assessed using the Y-maze and NOR tests. In the Y-maze, the total number of arm entries did not differ significantly among groups, indicating comparable locomotor activity (Fig. 1C, 1D). However, the alternation rate was significantly reduced in the 14 d and 28 d post-BCAS groups compared with the Sham group, suggesting spatial working memory impairment (Fig. 1C, 1E). In the NOR test (Fig. 1F), the discrimination index was significantly lower in the 28 d post-BCAS group than in the Sham group (Fig. 1G, 1H), reflecting diminished exploratory interest in the novel object and indicating recognition memory deficit.

Collectively, these data demonstrate that CCH impairs both spatial working memory and recognition memory in mice. Notably, the progression of cognitive decline did not parallel the partial restoration of CBF; instead, the deficits emerged and progressively worsened with prolonged hypoperfusion, suggesting they may arise from cumulative organic injury during the chronic hypoperfusion phase. Based on this temporal profile, 28 d post-BCAS was selected for subsequent experiments to investigate therapeutic targets and molecular mechanisms underlying CCH-induced cognitive impairment.

3.2 Pharmacological inhibition of PARP1 by Olaparib alleviates cognitive impairment in chronic CCH mice.

The molecular mechanisms underlying CCH-induced cognitive impairment remain unclear, and effective therapeutic targets are urgently needed. PARP1, a central enzyme in DNA damage repair, can be excessively activated under ischemic and hypoxic conditions[25], thereby triggering a series of downstream pathological cascades. To elucidate the role of PARP1 in CCH, PARP1 protein levels in the mouse cerebral cortex were first examined at various time points after BCAS surgery using WB (Fig. 2A, 2C). Compared with the Sham group, PARP1 expression showed no significant changes at 3 d and 14 d post-BCAS, but was markedly elevated by 28 d (Fig. 2B). This temporal pattern suggests that PARP1 overactivation is not an immediate stress response to acute ischemia, but rather a pathological event that accumulates progressively during sustained hypoperfusion.

Olaparib was administered intraperitoneally immediately after BCAS surgery and every other day thereafter, which significantly reduced PARP1 protein levels (Fig. 2D). Given that PARP1 deficiency has been shown to alleviate neurobehavioral deficits in 5XFAD mice[26], whether pharmacological inhibition of PARP1 with Olaparib could rescue cognitive dysfunction in the CCH model at 28 d post-BCAS was further evaluated using the Y-maze and NOR tests. In the Y-maze, the total number of arm entries did not differ significantly across groups, indicating comparable locomotor activity (Fig. 2E, 2F). However, the alternation rate was significantly higher in the Olaparib group than in the vehicle group, suggesting amelioration of spatial working memory deficits (Fig. 2E, 2G). In the NOR test (Fig. 2H), the discrimination index was significantly increased in the Olaparib group compared with the vehicle group

(Fig. 2I, 2J), reflecting greater exploratory interest in the novel object and indicating alleviation of recognition memory impairment.

Collectively, these findings demonstrate that Olaparib treatment effectively ameliorates both working memory and recognition memory deficits induced by CCH in mice at 28 d post-BCAS.

3.3 Olaparib attenuates neuroinflammatory infiltration and white matter demyelination after CCH.

The observed cognitive improvements suggest that Olaparib may exert its therapeutic effects by alleviating pathological damage in brain tissue. Under CCH conditions, sustained microglial activation serves as a central driver of neuroinflammation: activated microglia secrete matrix-degrading enzymes such as MMPs[27], and persistent pathological stress further promotes their transition to a harmful phenotype with increased release of pro-inflammatory cytokines[28]. In turn, elevated inflammatory cytokines upregulate MMP expression, forming a positive feedback loop[29]. Notably, preclinical evidence has strongly implicated IL-1 β as a key driver of the inflammatory response in VaD[30, 31], which upregulates MMP-9 expression; the synergistic action of IL-1 β and MMP-9 perpetuates neuroinflammation and contributes to myelin damage[32]. Given the established association between PARP1 and neuroinflammation[33, 34], the effects of Olaparib on white matter integrity and inflammatory responses following CCH were evaluated, using IL-1 β and MMP-9 as inflammatory markers in conjunction with assessment of microglial infiltration. IL-1 β and MMP-9 protein levels in the cerebral cortex were further examined at 28 d post-BCAS using WB. Compared with the Sham group, BCAS-induced CCH mice exhibited significantly elevated cortical expression of IL-1 β and MMP-9, which was markedly reversed by Olaparib treatment (Fig. 3A–C).

To further investigate the cellular effects of PARP1 inhibition by Olaparib on neuroinflammation following CCH, glial cell activation in the cerebral cortex was assessed using immunofluorescence staining. Compared with the vehicle group, the number of activated microglia/macrophages (Iba-1⁺) was significantly increased in the cerebral cortex of BCAS-induced CCH mice, whereas Olaparib treatment significantly reversed this increase (Fig. 3D, 3E). Moreover, Olaparib treatment significantly attenuated CCH-induced white matter demyelination (Fig. 3F, 3G).

Collectively, these findings demonstrate that Olaparib effectively alleviates CCH-induced neuroinflammation and white matter injury, suggesting that PARP1 inhibition represents a promising therapeutic strategy for CCH that warrants further investigation.

3.4 Olaparib alleviates BBB disruption after CCH.

Given the close association between PARP1 activation and BBB permeability[33, 34], together with our observation of significantly elevated MMP-9 levels following CCH, a key enzyme responsible for degrading TJ proteins, the effects of PARP1 inhibition by Olaparib on BBB integrity following CCH became of interest. To further elucidate the effects of PARP1 inhibition by Olaparib on BBB integrity following CCH, transcriptomic sequencing was performed to analyze the expression profiles of BBB TJ-

related genes and cell adhesion molecules in the cerebral cortex of Sham, BCAS + Vehicle, and Olaparib groups. Heatmap analysis revealed that, compared with the Sham group, the expression of TJ-related genes, including Tjp1, Occludin, and Cldn5, was downregulated at 28 d post-CCH, whereas the expression of the cell adhesion molecules CD44 and ICAM1 was upregulated; these changes were partially reversed by Olaparib treatment (Fig. 4A, 4B).

To validate the transcriptomic findings, we next examined the protein levels of Zo-1 and Occludin in the cerebral cortex of mice at 28 d post-BCAS using WB. Compared with the Sham group, Zo-1 and Occludin protein levels were significantly reduced following CCH, whereas Olaparib treatment partially reversed this reduction (Fig. 4C–E), a finding highly consistent with the transcriptomic data and further supporting the reliability of the RNA-seq results.

To further verify the functional impact of Olaparib on BBB permeability, IgG extravasation in the cerebral cortex was assessed by immunofluorescence staining. Compared with the Sham group, IgG leakage was markedly increased at 28 d post-CCH, whereas Olaparib treatment partially attenuated this increase (Fig. 4F–G).

Collectively, these findings demonstrate that Olaparib effectively alleviates CCH-induced BBB disruption in mice at 28 d post-BCAS.

3.5 Global transcriptomic changes following PARP1 inhibition by Olaparib.

To systematically elucidate the potential molecular mechanisms underlying the multifaceted protective effects of the PARP1 inhibitor Olaparib, enhance the translational relevance of these findings, and identify novel regulatory targets for CCH therapy, RNA sequencing was performed on cerebral cortex samples from the Sham, vehicle, and Olaparib groups. Differential expression analysis revealed that 275 genes were significantly differentially expressed (≥ 1.5 -fold) between the BCAS 28 d group and the Sham group, and 188 genes between the Olaparib group and the BCAS 28 d group, with 88 genes overlapping between the two comparisons (Fig. 5A). Principal component analysis (PCA) based on these differentially expressed genes (DEGs) showed that PC1 and PC2 accounted for 77.4% and 9.66% of the total variance, respectively (Fig. 5B). The Sham group was clearly separated from the bcas28d group, indicating that BCAS surgery induced widespread transcriptomic remodeling; notably, the bcas28d_ola group shifted partially toward the Sham group relative to the bcas28d group, suggesting that Olaparib treatment partially reversed BCAS-induced transcriptional alterations.

GO and KEGG enrichment analyses of the 88 DEGs revealed significant enrichment in biological process categories including "metabolic processes," "response to stress," "immune system processes," "developmental processes," and "regulation of biological processes" (Fig. 5C), indicating that the BCAS model and Olaparib intervention primarily affected biological processes related to inflammatory responses, metabolic adaptation, cellular stress, and neuroplasticity. These transcriptomic findings provide molecular corroboration for the phenotypic results described above, including elevated

inflammatory markers (IL-1 β , MMP-9, Iba-1) and white matter injury. Moreover, KEGG pathway analysis revealed enrichment in the p53 signaling, MAPK signaling, FOXO signaling, and HIF-1 signaling pathways, as well as inflammation-mediated regulation of TRP channels, apoptosis, neuroactive ligand–receptor interactions, and the complement and coagulation cascades (Fig. 5D), suggesting that the BCAS model induces inflammation, apoptosis, hypoxic stress, and neurovascular dysfunction, whereas Olaparib may exert its protective effects through modulation of these pathways.

Among these, the significant enrichment of the FOXO signaling pathway attracted our particular attention. Previous studies have shown that the FOXO transcription factor family, especially FOXO3a, serves as a key upstream regulator of autophagy initiation, and emerging evidence suggests that excessive autophagy activation can compromise BBB integrity[35–37]. This enrichment result raises the possibility that Olaparib's protective effect on the BBB may be partially mediated through FOXO3a-regulated autophagy. Accordingly, attention was next focused on the dynamic changes in autophagy following CCH and its specific contribution to BBB damage.

3.6 PARP1 inhibition by Olaparib alleviates BBB injury by attenuating excessive autophagy initiation after CCH.

The dynamics of autophagy following CCH, the effect of Olaparib intervention on autophagic activity, and the role of autophagy in BBB TJ protein regulation were next evaluated. To characterize the changes in autophagy at different time points after CCH, the protein levels of the autophagic markers LC3-II and p62/SQSTM1 in the cerebral cortex at 3 d, 14 d, and 28 d post-BCAS was examined using WB. LC3 is a critical molecule in the autophagy pathway: during autophagy initiation and autophagosome formation, cytosolic LC3-I undergoes lipidation to generate the membrane-bound form LC3-II, which is incorporated into the expanding autophagosomal membrane. Thus, LC3-II levels are positively correlated with autophagosome abundance and serve as a reliable indicator of autophagosome formation[38, 39]. p62/SQSTM1 binds to LC3 on the autophagosomal membrane and is subsequently degraded via the autophagosome-lysosomal pathway[40, 41]. Our results showed that, compared with the Sham group, LC3-II protein levels remained unchanged at 3 d post-BCAS (Fig. 6A, 6B), whereas p62 levels were significantly reduced (Fig. 6A, 6C), suggesting that autophagic flux was normal or compensatorily enhanced at this stage—autophagic substrates were effectively cleared and lysosomal function remained intact, consistent with adaptive protective autophagy during the early phase of acute ischemia. By 14 d, LC3-II levels remained unaltered (Fig. 6A, 6B), whereas p62 levels had slightly rebounded (Fig. 6A, 6C), indicating that autophagic clearance capacity was beginning to decline. By 28 d, both LC3-II and p62 levels were markedly elevated in parallel (Fig. 6A–C), suggesting that autophagosome formation had far exceeded lysosomal processing capacity, resulting in profound disruption of autophagic flux and massive accumulation of autophagic substrates.

Given the accumulation of autophagic substrates at 28 d post-BCAS, the underlying mechanisms and the effect of PARP1 inhibition by Olaparib on this process were further investigated. Previous studies have shown that PARP1 can activate autophagy. Accordingly, Olaparib, the autophagy inhibitor chloroquine

(CQ), or Olaparib in combination with the autophagy activator rapamycin (RAPA) were administered via intraperitoneal injection, and LC3-II and p62 protein levels in the cerebral cortex were assessed at 28 d post-BCAS using WB.

Compared with the BCAS + Vehicle group, the BCAS + CQ group exhibited significantly increased p62 accumulation, with a slight but non-significant elevation in LC3-II levels (Fig. 6D, 6E). Given that CQ blocks autophagosome–lysosome fusion rather than autophagy initiation, the further accumulation of p62 upon CQ treatment indicates that autophagic substrates were already being generated at an accelerated rate prior to lysosomal blockade, providing indirect evidence that autophagy initiation is enhanced following CCH. Compared with the BCAS + Vehicle group, both LC3-II and p62 levels were significantly reduced following Olaparib treatment (Fig. 6D, 6F), indicating that Olaparib alleviates autophagosome accumulation after CCH. Moreover, co-administration of RAPA attenuated the effect of Olaparib in reducing autophagosome accumulation compared with Olaparib monotherapy, further supporting that autophagy initiation is enhanced at 28 d post-CCH and that Olaparib counteracts this process, thereby mitigating autophagic dysregulation.

To further explore the impact of autophagy on BBB TJ proteins, Zo-1 and Occludin protein levels in the cerebral cortex of each group was examined using WB. Compared with the Olaparib group, the BCAS + Olaparib + RAPA group exhibited significantly reduced Zo-1 and Occludin levels, which were comparable to those observed in the BCAS + Vehicle group (Fig. 6G–I), indicating that the autophagy activator RAPA reversed the protective effect of Olaparib on BBB TJ proteins. Conversely, compared with the BCAS + Vehicle group, Zo-1 and Occludin levels were significantly elevated in the BCAS + CQ group (Fig. 6G–I), suggesting that pharmacological blockade of autophagosome-lysosome fusion with CQ similarly attenuated the loss of Zo-1 and Occludin following CCH.

Collectively, these data demonstrate that autophagy is enhanced at 28 d post-CCH, and that Olaparib inhibits this excessive autophagic response, alleviates autophagic dysregulation, and thereby exerts a protective effect on BBB TJ proteins.

3.7 PARP1 inhibition by Olaparib suppresses the FOXO3a/Depp1 axis after CCH.

To explore the potential mechanisms by which PARP1 regulates autophagy in BCAS-induced CCH, a total of 88 genes were identified as significantly differentially expressed in both BCAS 28 d versus Sham and Olaparib versus BCAS 28 d comparisons. Among these, six autophagy-related genes were selected for further analysis. Notably, Depp1, a key regulator of autophagy, was significantly upregulated at 28 d post-BCAS, whereas its expression was markedly reduced following Olaparib treatment. Among the six target genes ranked by the magnitude of transcriptional changes, Depp1 was the most significantly upregulated gene in the BCAS 28 d group and ranked second in terms of downregulation following Olaparib treatment (Fig. 7A–D).

In addition, transcriptomic analysis revealed that the FoxO signaling pathway was significantly altered following Olaparib treatment in CCH (Fig. 5D). Given that Depp1 has been identified as a downstream target gene of FOXO3a, we selected Depp1 for in-depth investigation. FOXO3a and Depp1 protein levels in the cerebral cortex of mice at 28 d post-BCAS were next examined using WB. Compared with the Sham group, both FOXO3a and Depp1 expression were significantly elevated at 28 d post-BCAS, whereas Olaparib treatment reversed these changes (Fig. 7E–G).

3.8 Dynamic changes in PARP1, the FOXO3a-Depp1 axis, and autophagy levels in OGD/R-treated endothelial cells at different time points.

Open-access single-cell sequencing data from the Betsholtz laboratory(<http://betsholtzlab.org/VascularSingleCells/Database.html>) was first obtained to verify PARP1 expression in cerebral vascular cells[42, 43]. The results showed that PARP1 is widely expressed in various vascular cell types, including smooth muscle cells, endothelial cells, pericytes, microglia, and fibroblasts (Fig. 8A). Given that the structural basis of the BBB is BMVECs, and that in vivo experiments are confounded by multiple systemic factors that complicate precise dissection of the direct effects of the PARP1-FOXO3a-Depp1 axis in vascular endothelial cells, bEnd.3 cells was utilized as an in vitro endothelial cell model and first determined the optimal parameters for OGD. bEnd.3 cells were subjected to hypoxia for 4, 6, or 8 h, followed by reoxygenation for 12 or 24 h, and PARP1 protein levels under different OGD/R conditions were assessed via WB. The results showed that, compared with the control group, PARP1 expression in bEnd.3 cells was significantly elevated after 4, 6, and 8 h of hypoxia followed by 24 h of reoxygenation (Fig. 8B, 8C). To further investigate changes in autophagy levels under different OGD/R conditions, The protein levels of Depp1 and the autophagic markers LC3-II and p62/SQSTM1 were examined using WB. Compared with the control group, Depp1, LC3-II, and p62/SQSTM1 protein levels in bEnd.3 cells were significantly elevated after 4, 6, and 8 h of hypoxia followed by 24 h of reoxygenation (Fig. 8D–G). Notably, the expression trend of Depp1 was highly consistent with those of PARP1 and the autophagic markers LC3-II and p62/SQSTM1. In conjunction with the transcriptomic data (Fig. 5), the elevation of Depp1 was hypothesized may represent a key intermediate step in PARP1-mediated autophagy regulation under hypoxic conditions. Given that 4 h of hypoxia was sufficient to stably induce activation of this pathological autophagic pathway, and to avoid non-specific cellular damage that might be introduced by prolonged hypoxia, 4 h of oxygen–glucose deprivation (OGD) followed by 24 h of reoxygenation was selected as the standard condition for subsequent experiments.

3.9 PARP1 regulates autophagy via the FOXO3a-Depp1 axis.

To determine the safe dosage of Olaparib in bEnd.3 cells, OGD/R-treated bEnd.3 cells was exposed to various concentrations of exogenous Olaparib and monitored cell viability using the CCK-8 assay. The results showed that Olaparib did not significantly affect bEnd.3 cell viability within a concentration range of up to 50 μ M (Fig. 9A). To further determine the optimal concentration, PARP1 protein levels following treatment with different concentrations of Olaparib was assessed using WB. Compared with the control

group, PARP1 expression was significantly reduced in OGD/R-treated bEnd.3 cells following exposure to 100 nM, 1 μ M, and 10 μ M Olaparib (Fig. 9B, 9C). Moreover, FOXO3a, Depp1, and p62 levels were also significantly downregulated after Olaparib treatment (Fig. 9B, 9D–F), further suggesting that exogenous Olaparib may modulate the FOXO3a–Depp1–autophagy axis through regulation of PARP1 expression. Considering both cell viability and pathway inhibition, and based on the principle of minimal effective concentration, 100 nM was selected as the Olaparib concentration for subsequent experiments.

To validate the role of Depp1 in PARP1-regulated autophagy, the experimental design is depicted in Fig. 9G: Depp1 was overexpressed in bEnd.3 cells prior to OGD establishment, and cells were then subjected to OGD 4 h/reoxygenation 24 h in the presence of 100 nM Olaparib. The efficacy of plasmid-mediated Depp1 overexpression was verified by WB, which showed that Depp1 protein levels were significantly elevated in the Olaparib + OE-Depp1 group (Fig. 9H, 9J), confirming the effectiveness of the overexpression system. Autophagic activity was next assessed in this context. Compared with the OGD/R group, Olaparib treatment attenuated OGD/R-induced enhancement of autophagy initiation (Fig. 9I, 9K, 9L). Notably, compared with the Olaparib-treated group, both LC3-II and p62 levels were significantly increased in the Olaparib + OE-Depp1 group, with no significant differences compared with the OGD/R group, indicating that the inhibitory effect of Olaparib on autophagy was reversed by Depp1 overexpression (Fig. 9I, 9K, 9L).

Collectively, these findings demonstrate that PARP1 mediates dynamic changes in autophagy through regulation of the FOXO3a–Depp1 axis.

4. Discussion

BBB regulates the exchange of substances between the central nervous system (CNS) and the periphery [44], and protects the CNS from harmful substances [45], playing a pivotal role in maintaining healthy brain function. Previous studies have demonstrated that, following CCH, BBB leakage occurs earlier than other pathological events, including oligodendrocyte precursor cell (OPC) activation, loss of mature oligodendrocytes, astrocyte activation, and microglial activation [46]. However, the upstream molecular regulatory mechanisms driving early BBB leakage following CCH have not been systematically elucidated. Using a CCH model and an in vitro OGD/R endothelial cell model, the present study has, for the first time, revealed a previously uncharacterized mechanism whereby PARP1 drives BBB damage through the excessive activation of pathological autophagy following CCH. Inhibiting autophagy-mediated degradation of the TJ proteins ZO-1 and Occludin preserved the structural and functional integrity of the BBB, alleviated neuroinflammatory responses, and improved white matter damage and cognitive function. This excessive autophagic activation was closely associated with the FOXO3a–Depp1 signaling axis. Crucially, Depp1 overexpression significantly attenuated the inhibitory effect of Olaparib on excessive autophagy initiation following OGD/R, providing strong functional evidence for the role of Depp1 in mediating these protective effects. Given that Olaparib has already secured FDA approval for cancer therapy [47, 48], our findings not only reveal a previously unidentified regulatory

mechanism of BBB injury under CCH, but also provide a strong rationale for its immediate translational evaluation in VCI.

Post-BCAS cerebral blood flow had compensatorily recovered to approximately 77–80% of baseline by 14 d and 28 d via collateral circulation; however, cognitive impairment did not diminish with CBF restoration but instead progressively worsened with prolonged hypoperfusion, culminating in a comprehensive deficit pattern by 28 d. Notably, neither PARP1 expression nor autophagic flux markers (LC3-II, p62) showed significant changes at 14 d; however, by 28 d, concurrent PARP1 overactivation and disrupted autophagy initiation emerged, coinciding precisely with the onset of comprehensive cognitive impairment. This temporal alignment suggests that the PARP1–autophagy axis may specifically drive this critical pathological inflection point in CCH-induced cognitive deterioration. Accordingly, subsequent experiments focused on the 28 d post-CCH time window to systematically investigate the regulatory mechanisms of the PARP1–autophagy axis in BBB damage.

PARP1 is the most abundant member of the PARP family and is primarily localized in the nucleus and mitochondria, where it serves as the first line of cellular defense against DNA damage by initiating repair responses. Its roles in [49, 50], apoptosis [51, 52], inflammatory signaling [33, 34], and gene transcription [53, 54] have been extensively characterized. Notably, PARP1 exerts dose-dependent, context-dependent effects on cell fate: under physiological or mild stress conditions, controlled PARP1 activation promotes DNA repair and confers cytoprotection; under severe pathological conditions, however, excessive PARP1 activation drives neurodegeneration through multiple converging mechanisms, including dysregulation of the autophagy–lysosomal pathway, induction of neuroinflammation, mitochondrial dysfunction, and disruption of BBB integrity [55]. In animal models of stroke, traumatic brain injury, meningitis, and multiple sclerosis, PARP inhibitors have been shown to reduce cerebral edema, preserve endothelial TJ proteins and Occludin expression, suppress ICAM-1 upregulation [22, 56, 57], and attenuate leukocyte infiltration and neuroinflammation [56, 58]. Nevertheless, how PARP1 contributes to pathological BBB disruption under CCH has not been systematically investigated. The present study demonstrates for the first time in a CCH model that cortical PARP1 protein levels were not significantly elevated at 3 d or 14 d post-BCAS, but were markedly upregulated by 28 d. This spatiotemporal pattern indicates that sustained PARP1 overactivation is not an immediate stress response to acute ischemia but rather a delayed pathological event accumulating under prolonged chronic hypoperfusion. The absence of significant changes at earlier time points may reflect acute-phase cellular compensatory mechanisms: PARP1 can limit its own excessive accumulation through rapid PAR polymer turnover to prevent apoptosis, and proteasomal degradation mediated by E3 ubiquitin ligases such as RNF146 may further restrain PARP1 protein levels during the acute phase [20]. However, cumulative DNA damage accruing by 28 d may ultimately overwhelm these compensatory mechanisms, leading to marked PARP1 upregulation. Strikingly, this time point coincides with the emergence of BBB leakage and cognitive impairment, a spatiotemporal parallel that strongly implicates chronic PARP1 overactivation as a core upstream driver of BBB disruption during the progressive phase of CCH. At both the transcriptomic and protein levels, ZO-1 and Occludin were significantly downregulated at 28 d post-CCH, and Olaparib treatment partially

reversed these changes. Functional IgG extravasation assays further confirmed that Olaparib attenuated BBB permeability, providing convergent structural and functional evidence.

Disruption and redistribution of TJ proteins leads to subtle BBB hyperpermeability, allowing fluid and molecules to extravasate from the bloodstream into the CNS, weakened BBB is further susceptible to MMP-9-mediated TJ degradation, which perpetuates structural damage and ultimately permits large-molecule leakage [59, 60]. In the present study, Olaparib reduced microglial activation and suppressed the expression of MMP-9 and IL-1 β following CCH, and attenuated CCH-induced white matter injury, these findings are consistent with those reported in models of metachromatic [61]. Transcriptomic analysis further revealed upregulation of the adhesion molecules CD44 and ICAM1 following CCH, indicating that BBB inflammatory remodeling also contributes to post-CCH pathology. This is consistent with prior reports that PARP1 inhibition in systemic inflammatory models downregulates adhesion molecule expression and thereby suppresses immune cell–endothelial interactions [23]. In addition, Olaparib treatment improved neurological function, further extending the breadth of its protective effects.

Autophagy is an essential intracellular degradation system that plays critical roles under both physiological and pathological conditions [62]. Under physiological or metabolic stress conditions, such as starvation or hypoxia, autophagy clears damaged macromolecules and organelles to sustain cellular homeostasis. However, when cells are subjected to sustained, severe pathological stress, this tightly regulated process becomes dysregulated, and excessive autophagy leads to the over-digestion of essential cellular components and autophagic cell death [62, 63]. The regulation of autophagy in CCH has attracted increasing attention. CCH upregulates autophagy-related proteins, increasing autophagosome formation; elevated levels of Beclin-1, LC3B, and p62 have been observed in the cortex and hippocampus during CCH, correlating with neuronal injury, white matter damage, and amyloid- β deposition, collectively suggesting a close link between autophagy and the major pathological features of CCH [64]. Moderate inhibition of autophagy has been shown to confer significant neuroprotection in chronic cerebral ischemia [16, 64]. For example, the FAAH inhibitor URB597 attenuated cognitive impairment by suppressing autophagy via the mTOR signaling pathway [65], inhibited excessive mitophagy [66], and blocked NLRP3 inflammasome activation [67], thereby promoting neuronal survival in CCH models. Similarly, nimodipine reduced excessive autophagy and provided neuroprotection by inhibiting AMPK phosphorylation [68]. Conversely, resveratrol activated autophagy via the AKT/mTOR pathway to improve cognitive function in CCH rats [69], and environmental enrichment protected synaptic integrity and improved cognitive outcomes through autophagy pathway activation [70], indicating that controlled autophagy induction can also be neuroprotective. These findings collectively establish a "double-edged sword" role for autophagy in CCH, with divergent outcomes likely dependent on the degree of activation, temporal context, and cell type involved.

Although the beneficial and detrimental roles of autophagy in neuronal injury, inflammatory responses, and cognitive dysfunction have been reported in both in vivo CCH models and in vitro OGD/R systems, its specific contribution to chronic hypoperfusion-mediated BBB endothelial barrier disruption remained

unclear. Most prior studies of BBB disruption focused on MMP-mediated TJ degradation; however, autophagy has recently emerged as an additional regulatory mechanism. Silencing of histone deacetylase 9 (HDAC9) in the ischemic hemisphere has been reported to activate autophagy and protect against increased BBB permeability [71], and miRNA-mediated promotion of autophagy has been shown to protect BMVECs subjected to OGD/R injury [17], suggesting that moderate autophagy confers BBB protection. In contrast, OGD-induced autophagy and autophagosome–lysosome activation in brain endothelial cells have been shown to degrade claudin-5 and Occludin [35, 36], and enhanced autophagy during cerebral ischemia in diabetic mice has been identified as a contributor to excessive BBB disruption that can be ameliorated by autophagy inhibition [37], indicating that, under specific conditions, autophagy plays a detrimental role in BBB integrity. Previous studies have demonstrated that PARP1 activation upregulates the expression of autophagy-related genes [25]. However, given the dual roles of autophagy and the fact that research on its involvement in BBB dysfunction is still in its infancy, how PARP1 modulates autophagy to influence BBB integrity in the CCH model remains elusive.

In the present study, dynamic profiling of autophagy markers at multiple post-CCH time points revealed that p62 was significantly reduced at 3 d with no change in LC3-II, suggesting that autophagic flux was normal or compensatorily enhanced at this early stage, consistent with adaptive protective autophagy during acute ischemia. By 28 d, however, LC3-II and p62 were both significantly elevated in parallel, indicating profound disruption of autophagic flux and a transition from early compensatory protection to pathological excessive initiation. This time-dependent dynamic shift provides direct evidence supporting the selection of the chronic phase (28 d) as the therapeutic intervention window. Further mechanistic dissection using CQ demonstrated that lysosomal blockade further aggravated p62 accumulation rather than alleviating it, indirectly suggesting that excessive autophagosome generation rather than lysosomal dysfunction, is the primary driver of autophagic flux dysregulation. Co-administration of RAPA reversed the protective effect of Olaparib on BBB TJ proteins, functionally establishing a causal relationship between enhanced autophagy initiation and BBB TJ protein degradation, rather than mere correlation. These findings precisely delineate the "initiation phase" of autophagy as the critical intervention window for BBB protection following CCH, and provide target-specific translational evidence for precision modulation of autophagic rate in the clinical setting. Integrating this temporal framework, we propose that chronic, delayed PARP1 activation at 28 d disrupts the autophagic homeostasis established at 3 d, converting the early protective autophagy into a pathologically excessive initiation in the late phase, ultimately driving TJ protein degradation.

Guided by RNA-seq results highlighting significant enrichment of the FOXO signaling pathway, we focused on FOXO3a as a candidate downstream effector of PARP1. Previous studies in cardiac ischemic injury models have demonstrated that PARP1 activation promotes FOXO3a dephosphorylation and nuclear translocation, thereby enhancing transcriptional activation of autophagy-related genes [25]. Among the differentially expressed genes identified by transcriptomic screening, Depp1 emerged as a candidate of particular interest owing to its established roles in autophagy regulation and its identity as a direct transcriptional target of FOXO3a, which directly binds the Depp1 promoter at transcription initiation to regulate its expression [72]. Depp1 is also a recognized intermediary in autophagy regulation. In

neuroblastoma models, H₂O₂ treatment triggered autophagy induction through FOXO3a-mediated Depp1 expression, and Depp1 knockdown effectively suppressed this process, identifying Depp1 as a functional mediator between FOXO3a and autophagy initiation under oxidative stress [73]. Depp1 expression is also markedly increased in endothelial cells cultured under hypoxic conditions [72], and elevated Depp1 expression has also been observed in malignant glioma cell lines [74], suggesting a role for Depp1 in cellular stress responses. This stress-induced upregulation of Depp1 under hypoxia is highly consistent with the chronic hypoxic microenvironment generated by CCH, providing a plausible pathological basis for the sustained Depp1 upregulation observed in the present study. Importantly, autophagy induction by mTOR inhibition is unaffected by Depp1 loss, indicating that Depp1-mediated autophagy regulation operates independently of the canonical mTOR pathway and represents a non-canonical autophagy initiation route, potentially involving mitochondrial permeability transition pore (MPTP) opening and coordinated mitophagy [75], mechanisms that warrant further investigation. Through transcriptomic profiling and WB validation, Depp1 exhibited a spatiotemporal expression pattern closely paralleling that of PARP1, with significant upregulation at 28 d post-CCH and marked reduction following Olaparib treatment, providing strong correlative evidence for the existence of the PARP1-FOXO3a-Depp1 signaling axis. Crucially, Depp1 overexpression in vitro reversed the inhibitory effect of Olaparib on autophagy initiation, functionally establishing the necessity of Depp1 within this regulatory axis rather than representing a merely passive transcriptional change. This functional evidence corroborates the Depp1-mediated autophagy regulation described above [75]. Nevertheless, the precise mechanism by which PARP1 activates FOXO3a in the context of CCH, specifically whether PARylation influences FOXO3a nuclear localization or transcriptional activity, remains to be elucidated in future studies.

Olaparib is an FDA-approved PARP inhibitor with an established clinical safety and pharmacokinetic profile across multiple oncological indications [47, 48], which substantially lowers the translational barrier for repurposing in neurological disease. This is particularly relevant given that current clinical management of VCI focuses predominantly on vascular risk factor control, with no evidence-based molecular targeted therapies directed at BBB injury. The present findings suggest that the PARP1–FOXO3a–Depp1–autophagy axis represents a novel therapeutic target for VCI intervention, providing a mechanistic rationale for developing precision strategies to protect the BBB. Furthermore, single-cell sequencing data confirming broad PARP1 expression in BMVECs strengthens the rationale for targeting endothelial PARP1 specifically. Future studies should examine this autophagy axis within the multicellular microenvironment of the neurovascular unit (NVU), including endothelial cells, pericytes, and glial cells, to fully resolve the coupling between vascular and neural injury under chronic hypoperfusion.

Despite systematically elucidating the regulatory role of PARP1 in autophagy-mediated BBB injury following CCH, the present study has several limitations. Although in vivo and in vitro intervention experiments established the functional necessity of Depp1, the direct influence of PARP1 activation on FOXO3a nuclear localization and Depp1 promoter binding at the transcriptional level remains to be confirmed by chromatin immunoprecipitation (ChIP) assays or dual-luciferase reporter experiments. The in vitro model relied on the bEnd.3 cell line, whose barrier properties—including transendothelial electrical resistance (TEER)—differ from those of primary human BMVECs; replication of these findings

in primary cells or iPSC-derived cerebrovascular organoids would provide greater clinical relevance. To ensure surgical standardization and consistency, only male mice were used in this study; given the potential modulatory effects of estrogen on autophagic activity and BBB stability, the influence of biological sex on the applicability of this pathway warrants future evaluation. Finally, the systemic administration of Olaparib cannot entirely exclude indirect confounding effects arising from off-target biological activity in peripheral tissues, such as the hematopoietic or gastrointestinal systems. Future studies employing endothelial cell-specific conditional PARP1 knockout mice (TIE2-Cre or Cdh5-Cre) would provide more precise and definitive evidence for vascular endothelial-targeted protection.

5. Conclusion

The present study demonstrates that PARP1 is a critical regulator of BBB disruption following CCH. Sustained PARP1 upregulation in the cerebral cortex after CCH is associated with BBB breakdown, tight junction protein loss, and cognitive impairment. Pharmacological inhibition of PARP1 with Olaparib attenuates neuroinflammatory responses, white matter injury, BBB hyperpermeability, and cognitive deficits. Mechanistically, PARP1 promotes BBB dysfunction through a FOXO3a–Depp1-dependent mechanism, in which enhanced Depp1 expression drives excessive autophagy initiation and subsequent degradation of tight junction proteins, establishing a PARP1–FOXO3a–Depp1–autophagy axis underlying CCH-induced BBB injury.

These findings identify a previously unrecognized upstream regulatory mechanism of CCH-induced BBB disruption and provide preclinical evidence supporting the potential repurposing of the clinically approved PARP inhibitor Olaparib as a therapeutic strategy for vascular cognitive impairment.

Abbreviations

AD

Alzheimer's disease

BBB

blood–brain barrier

BCAS

bilateral carotid artery stenosis

BMVECs

brain microvascular endothelial cells

CCH

chronic cerebral hypoperfusion

CBF

cerebral blood flow

CCK-8

Cell Counting Kit-8

CNS

central nervous system

CQ

chloroquine

DEGs

differentially expressed genes

FOXO3a

forkhead box O3a

GO

Gene Ontology

ICAM1

intercellular adhesion molecule 1

IL-1 β

interleukin-1 beta

KEGG

Kyoto Encyclopedia of Genes and Genomes

LC3

microtubule-associated protein 1 light chain 3

LFB

Luxol Fast Blue

LSCI

laser speckle contrast imaging

MMP9

matrix metalloproteinase-9

NOR

novel object recognition

NVU

neurovascular unit

OGD/R

oxygen–glucose deprivation/reoxygenation

Ocln

occludin

PAR

poly(ADP-ribose)

PARP1

poly(ADP-ribose) polymerase-1

PCA

principal component analysis

PD

Parkinson's disease

PFA

paraformaldehyde

p62/SQSTM1

sequestosome 1

RAPA

rapamycin

RI

recognition index

RNA-seq

RNA sequencing

SDS-PAGE

sodium dodecyl sulfate-polyacrylamide gel electrophoresis

SVD

small vessel disease

TJ

tight junction

VCI

vascular cognitive impairment

WB

western blotting

Zo-1

zonula occludens-1

Depp1

death effector domain-containing protein 1

Declarations

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Author's Contributions

X.Y. Ji, W.J. Hu, and Y.S. Wang contributed equally to this work. Z.X. Zhao, R.H. Sun and J.W. Zhang conceived and designed the study. J.W. Zhang coordinated the overall project. X.Y. Ji, A.Q. Li, M.S. Huang, N.N. Che and X.D. Hao performed the experiments and acquired the data. W.J. Hu analyzed the data and prepared the figures. X.Y. Ji wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All animal procedures were approved by the Animal Ethics Committee of Henan Provincial People's Hospital (Approval No. 77 (2020)) and were conducted in accordance with the ARRIVE guidelines and the principles of Replacement, Reduction, and Refinement (3Rs).

Data availability:

The single-cell RNA-sequencing dataset analyzed in this study is available in the Betsholtz laboratory vascular single-cell database, <http://betsholtzlab.org/VascularSingleCells/database.html>. The database is publicly accessible with no restrictions for non-academic users. Additional datasets generated during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Competing interests

The authors have no competing interests to declare.

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References

1. Rajeev V, Fann DY, Dinh QN, Kim HA, De Silva TM, Jo DG, Drummond GR, Sobey CG, Lai MKP, Chen CL, Arumugam TV. Intermittent Fasting Attenuates Hallmark Vascular and Neuronal Pathologies in a Mouse Model of Vascular Cognitive Impairment. *Int J Biol Sci.* 2022;18:6052–67.
2. Attems J, Jellinger KA. The overlap between vascular disease and Alzheimer's disease—lessons from pathology. *BMC Med.* 2014;12:206.
3. Koizumi K, Hattori Y, Ahn SJ, Buendia I, Ciacciarelli A, Uekawa K, Wang G, Hiller A, Zhao L, Voss HU, et al. Apoε4 disrupts neurovascular regulation and undermines white matter integrity and cognitive function. *Nat Commun.* 2018;9:3816.
4. Coenen M, de Kort FA, Weaver NA, Kuijf HJ, Aben HP, Bae HJ, Bordet R, Chen CP, Dewenter A, Doeven T, et al. Strategic white matter hyperintensity locations associated with post-stroke cognitive impairment: A multicenter study in 1568 stroke patients. *Int J Stroke.* 2024;19:916–24.
5. Wu LY, Chai YL, Cheah IK, Chia RSL, Hilal S, Arumugam TV, Chen CP, Lai MKP. Blood-based biomarkers of cerebral small vessel disease. *Ageing Res Rev.* 2024;95:102247.

6. Biesbroek JM, Coenen M, DeCarli C, Fletcher EM, Maillard PM, Barkhof F, Barnes J, Benke T, Chen C, Dal-Bianco P, et al. Amyloid pathology and vascular risk are associated with distinct patterns of cerebral white matter hyperintensities: A multicenter study in 3132 memory clinic patients. *Alzheimers Dement*. 2024;20:2980–9.
7. Javanshiri K, Waldö ML, Friberg N, Sjövall F, Wickerström K, Haglund M, Englund E. Atherosclerosis, Hypertension, and Diabetes in Alzheimer's Disease, Vascular Dementia, and Mixed Dementia: Prevalence and Presentation. *J Alzheimers Dis*. 2018;65:1247–58.
8. Wong SM, Jansen JFA, Zhang CE, Hoff EI, Staals J, van Oostenbrugge RJ, Backes WH. Blood-brain barrier impairment and hypoperfusion are linked in cerebral small vessel disease. *Neurology*. 2019;92:e1669–77.
9. Sun R, Li W, Ji X, Hu W, Zhao Y, Lu Y, Shang J, Hao X, Zhang J. Simvastatin Ameliorates Lipid Metabolism-Mediated Endothelial Inflammation and Tight Junction Impairment in Chronic Cerebral Hypoperfusion. *Neuromolecular Med*. 2026;28:9.
10. Rajeev V, Fann DY, Dinh QN, Kim HA, De Silva TM, Lai MKP, Chen CL, Drummond GR, Sobey CG, Arumugam TV. Pathophysiology of blood brain barrier dysfunction during chronic cerebral hypoperfusion in vascular cognitive impairment. *Theranostics*. 2022;12:1639–58.
11. Rosenberg GA, Sullivan N, Esiri MM. White matter damage is associated with matrix metalloproteinases in vascular dementia. *Stroke*. 2001;32:1162–8.
12. Matsuyama H, Shindo A, Shimada T, Yata K, Wakita H, Takahashi R, Tomimoto H. Chronic cerebral hypoperfusion activates AIM2 and NLRP3 inflammasome. *Brain Res*. 2020;1736:146779.
13. Ceulemans AG, Zgavc T, Kooijman R, Hachimi-Idrissi S, Sarre S, Michotte Y. The dual role of the neuroinflammatory response after ischemic stroke: modulatory effects of hypothermia. *J Neuroinflammation*. 2010;7:74.
14. Muñoz Maniega S, Chappell FM, Valdés Hernández MC, Armitage PA, Makin SD, Heye AK, Thrippleton MJ, Sakka E, Shuler K, Dennis MS, Wardlaw JM. Integrity of normal-appearing white matter: Influence of age, visible lesion burden and hypertension in patients with small-vessel disease. *J Cereb Blood Flow Metab*. 2017;37:644–56.
15. Yang L, Song J, Nan D, Wan Y, Guo H. Cognitive Impairments and blood-brain Barrier Damage in a Mouse Model of Chronic Cerebral Hypoperfusion. *Neurochem Res*. 2022;47:3817–28.
16. Xu LX, Tang XJ, Yang YY, Li M, Jin MF, Miao P, Ding X, Wang Y, Li YH, Sun B, Feng X. Neuroprotective effects of autophagy inhibition on hippocampal glutamate receptor subunits after hypoxia-ischemia-induced brain damage in newborn rats. *Neural Regen Res*. 2017;12:417–24.
17. Li Z, Li J, Tang N. Long noncoding RNA Malat1 is a potent autophagy inducer protecting brain microvascular endothelial cells against oxygen-glucose deprivation/reoxygenation-induced injury by sponging miR-26b and upregulating ULK2 expression. *Neuroscience*. 2017;354:1–10.
18. Feng FY, de Bono JS, Rubin MA, Knudsen KE. Chromatin to Clinic: The Molecular Rationale for PARP1 Inhibitor Function. *Mol Cell*. 2015;58:925–34.

19. Swindall AF, Stanley JA, Yang ES. PARP-1: Friend or Foe of DNA Damage and Repair in Tumorigenesis? *Cancers (Basel)*. 2013;5:943–58.
20. Mao K, Zhang G. The role of PARP1 in neurodegenerative diseases and aging. *Febs j*. 2022;289:2013–24.
21. Koh SH, Park Y, Song CW, Kim JG, Kim K, Kim J, Kim MH, Lee SR, Kim DW, Yu HJ, et al. The effect of PARP inhibitor on ischaemic cell death, its related inflammation and survival signals. *Eur J Neurosci*. 2004;20:1461–72.
22. Lescot T, Fulla-Oller L, Palmier B, Po C, Beziaud T, Puybasset L, Plotkine M, Gillet B, Meric P, Marchand-Leroux C. Effect of acute poly(ADP-ribose) polymerase inhibition by 3-AB on blood-brain barrier permeability and edema formation after focal traumatic brain injury in rats. *J Neurotrauma*. 2010;27:1069–79.
23. Rom S, Zuluaga-Ramirez V, Dykstra H, Reichenbach NL, Ramirez SH, Persidsky Y. Poly(ADP-ribose) polymerase-1 inhibition in brain endothelium protects the blood-brain barrier under physiologic and neuroinflammatory conditions. *J Cereb Blood Flow Metab*. 2015;35:28–36.
24. Zhan R, Meng X, Tian D, Xu J, Cui H, Yang J, Xu Y, Shi M, Xue J, Yu W, et al. NAD(+) rescues aging-induced blood-brain barrier damage via the CX43-PARP1 axis. *Neuron*. 2023;111:3634–e36493637.
25. Wang C, Xu W, Zhang Y, Zhang F, Huang K. PARP1 promote autophagy in cardiomyocytes via modulating FoxO3a transcription. *Cell Death Dis*. 2018;9:1047.
26. Jhaldiyal A, Kumari M, Guttman LC, Tripathi T, Khan MR, Wang J, Biswas D, Pasupuleti A, Aggarwal A, Pandya S, et al. PARP1 deficiency mitigates amyloid pathology, neurodegeneration, and cognitive decline in a familial Alzheimer's disease model. *Proc Natl Acad Sci U S A*. 2026;123:e2525028123.
27. Lively S, Schlichter LC. The microglial activation state regulates migration and roles of matrix-dissolving enzymes for invasion. *J Neuroinflammation*. 2013;10:75.
28. Haruwaka K, Ikegami A, Tachibana Y, Ohno N, Konishi H, Hashimoto A, Matsumoto M, Kato D, Ono R, Kiyama H, et al. Dual microglia effects on blood brain barrier permeability induced by systemic inflammation. *Nat Commun*. 2019;10:5816.
29. Feng D, Zhou J, Liu H, Wu X, Li F, Zhao J, Zhang Y, Wang L, Chao M, Wang Q, et al. Astrocytic NDRG2-PPM1A interaction exacerbates blood-brain barrier disruption after subarachnoid hemorrhage. *Sci Adv*. 2022;8:eabq2423.
30. Poh L, Fann DY, Wong P, Lim HM, Foo SL, Kang SW, Rajeev V, Selvaraji S, Iyer VR, Parathy N, et al. AIM2 inflammasome mediates hallmark neuropathological alterations and cognitive impairment in a mouse model of vascular dementia. *Mol Psychiatry*. 2021;26:4544–60.
31. Wang R, Yin YX, Mahmood Q, Wang XJ, Gao YP, Gou GJ, Ahmed MM, Kohji F, Du YZ, Han F. Calmodulin inhibitor ameliorates cognitive dysfunction via inhibiting nitrosative stress and NLRP3 signaling in mice with bilateral carotid artery stenosis. *CNS Neurosci Ther*. 2017;23:818–26.
32. Shao Q, Chen S, Zheng Y, Xu W, Chen J, Shao W, Wang Q, Li C, Wang X. Crucial role of microglia-mediated myelin sheath damage in vascular dementia: Antecedents and consequences. *Neural Regen Res*. 2026;21:1000–12.

33. Arroyo AB, Bernal-Carrión M, Cantón-Sandoval J, Cabas I, Corbalán-Vélez R, Martínez-Menchón T, Ferri B, Cayuela ML, García-Moreno D, Mulero V. NAMPT and PARylation Are Involved in the Pathogenesis of Atopic Dermatitis. *Int J Mol Sci* 2023, 24.
34. Vitali R, Mancuso AB, Palone F, Pioli C, Cesi V, Negroni A, Cucchiara S, Oliva S, Carissimi C, Laudadio I, Stronati L. PARP1 Activation Induces HMGB1 Secretion Promoting Intestinal Inflammation in Mice and Human Intestinal Organoids. *Int J Mol Sci* 2023, 24.
35. Liu J, Weaver J, Jin X, Zhang Y, Xu J, Liu KJ, Li W, Liu W. Nitric Oxide Interacts with Caveolin-1 to Facilitate Autophagy-Lysosome-Mediated Claudin-5 Degradation in Oxygen-Glucose Deprivation-Treated Endothelial Cells. *Mol Neurobiol*. 2016;53:5935–47.
36. Kim KA, Kim D, Kim JH, Shin YJ, Kim ES, Akram M, Kim EH, Majid A, Baek SH, Bae ON. Autophagy-mediated occludin degradation contributes to blood-brain barrier disruption during ischemia in bEnd.3 brain endothelial cells and rat ischemic stroke models. *Fluids Barriers CNS*. 2020;17:21.
37. Wei N, Yu SP, Gu XH, Chen DD, Whalin MK, Xu GL, Liu XF, Wei L. The involvement of autophagy pathway in exaggerated ischemic brain damage in diabetic mice. *CNS Neurosci Ther*. 2013;19:753–63.
38. Chueh KS, Lu JH, Juan TJ, Chuang SM, Juan YS. The Molecular Mechanism and Therapeutic Application of Autophagy for Urological Disease. *Int J Mol Sci* 2023, 24.
39. Wang H, Feng N, Liu C, Xie Y, Zhou Z, Zhao H, Xiao G, Yang D. Inhibition of CSPG-PTP σ Activates Autophagy Flux and Lysosome Fusion, Aids Axon and Synaptic Reorganization in Spinal Cord Injury. *Mol Neurobiol*. 2025;62:773–85.
40. Liu J, Wu Y, Meng S, Xu P, Li S, Li Y, Hu X, Ouyang L, Wang G. Selective autophagy in cancer: mechanisms, therapeutic implications, and future perspectives. *Mol Cancer*. 2024;23:22.
41. Alcober-Boquet L, Zang T, Pietsch L, Suess E, Hartmann M, Proschak E, Gross LZ, Sacerdoti M, Zeuzem S, Rogov VV, et al. The PB1 and the ZZ domain of the autophagy receptor p62/SQSTM1 regulate the interaction of p62/SQSTM1 with the autophagosome protein LC3B. *Protein Sci*. 2024;33:e4840.
42. He L, Vanlandewijck M, Mäe MA, Andrae J, Ando K, Del Gaudio F, Nahar K, Lebouvier T, Laviña B, Gouveia L, et al. Single-cell RNA sequencing of mouse brain and lung vascular and vessel-associated cell types. *Sci Data*. 2018;5:180160.
43. Vanlandewijck M, He L, Mäe MA, Andrae J, Ando K, Del Gaudio F, Nahar K, Lebouvier T, Laviña B, Gouveia L, et al. A molecular atlas of cell types and zonation in the brain vasculature. *Nature*. 2018;554:475–80.
44. Xiong R, Zhou XG, Tang Y, Wu JM, Sun YS, Teng JF, Pan R, Law BY, Zhao Y, Qiu WQ, et al. Lychee seed polyphenol protects the blood-brain barrier through inhibiting A β (25–35)-induced NLRP3 inflammasome activation via the AMPK/mTOR/ULK1-mediated autophagy in bEnd.3 cells and APP/PS1 mice. *Phytother Res*. 2021;35:954–73.
45. Rhea EM, Banks WA. Role of the Blood-Brain Barrier in Central Nervous System Insulin Resistance. *Front Neurosci*. 2019;13:521.

46. Sun Z, Gao C, Gao D, Sun R, Li W, Wang F, Wang Y, Cao H, Zhou G, Zhang J, Shang J. Reduction in pericyte coverage leads to blood-brain barrier dysfunction via endothelial transcytosis following chronic cerebral hypoperfusion. *Fluids Barriers CNS*. 2021;18:21.
47. Shah M, Chen TY, Ison G, Fiero MH, Zhang H, Gao X, Neilson M, Goldberg KB, Nair A, Ricks TK, et al. Overall Survival and the Evolving Benefit-Risk Assessment for Poly (ADP-ribose) Polymerase Inhibitors in Advanced Ovarian Cancer. *J Clin Oncol*. 2025;43:2218–27.
48. Saeidi H, Sarafbidabad M. PARP inhibitors in prostate cancer: clinical applications. *Mol Biol Rep*. 2024;51:1103.
49. Hottiger MO, Hassa PO, Lüscher B, Schüler H, Koch-Nolte F. Toward a unified nomenclature for mammalian ADP-ribosyltransferases. *Trends Biochem Sci*. 2010;35:208–19.
50. Brunyanszki A, Szczesny B, Virág L, Szabo C. Mitochondrial poly(ADP-ribose) polymerase: The Wizard of Oz at work. *Free Radic Biol Med*. 2016;100:257–70.
51. Suskiewicz MJ, Zobel F, Ogden TEH, Fontana P, Ariza A, Yang JC, Zhu K, Bracken L, Hawthorne WJ, Ahel D, et al. HPF1 completes the PARP active site for DNA damage-induced ADP-ribosylation. *Nature*. 2020;579:598–602.
52. Yang J, Wan SY, Song QY, Xie YH, Wan J, Zhou YH, Zhang ZT, Xiao YS, Li X, Chen H, et al. Angiopoietin-like protein 8 directs DNA damage responses towards apoptosis by stabilizing PARP1-DNA condensates. *Cell Death Differ*. 2025;32:672–88.
53. Qin Y, Dong X, Lu M, Jing L, Chen Q, Guan F, Xiang Z, Huang J, Yang C, He X, et al. PARP1 interacts with WDR5 to enhance target gene recognition and facilitate tumorigenesis. *Cancer Lett*. 2024;593:216952.
54. Fu H, Liu R, Jia Z, Li R, Zhu F, Zhu W, Shao Y, Jin Y, Xue Y, Huang J, et al. Poly(ADP-ribosylation) of P-TEFb by PARP1 disrupts phase separation to inhibit global transcription after DNA damage. *Nat Cell Biol*. 2022;24:513–25.
55. Hu ML, Pan YR, Yong YY, Liu Y, Yu L, Qin DL, Qiao G, Law BY, Wu JM, Zhou XG, Wu AG. Poly (ADP-ribose) polymerase 1 and neurodegenerative diseases: Past, present, and future. *Ageing Res Rev*. 2023;91:102078.
56. Lenzser G, Kis B, Snipes JA, Gáspár T, Sándor P, Komjáti K, Szabó C, Busija DW. Contribution of poly(ADP-ribose) polymerase to postischemic blood-brain barrier damage in rats. *J Cereb Blood Flow Metab*. 2007;27:1318–26.
57. Farez MF, Quintana FJ, Gandhi R, Izquierdo G, Lucas M, Weiner HL. Toll-like receptor 2 and poly(ADP-ribose) polymerase 1 promote central nervous system neuroinflammation in progressive EAE. *Nat Immunol*. 2009;10:958–64.
58. Zhang C, Yang J, Jennings LK. Attenuation of neointima formation through the inhibition of DNA repair enzyme PARP-1 in balloon-injured rat carotid artery. *Am J Physiol Heart Circ Physiol*. 2004;287:H659–666.
59. Kaya M, Ahishali B. Assessment of permeability in barrier type of endothelium in brain using tracers: Evans blue, sodium fluorescein, and horseradish peroxidase. *Methods Mol Biol*. 2011;763:369–82.

60. Gong S, Cao G, Li F, Chen Z, Pan X, Ma H, Zhang Y, Yu B, Kou J. Endothelial Conditional Knockdown of NMMHC IIA (Nonmuscle Myosin Heavy Chain IIA) Attenuates Blood-Brain Barrier Damage During Ischemia-Reperfusion Injury. *Stroke*. 2021;52:1053–64.
61. Mekhaeil M, Conroy MJ, Dev KK. Olaparib Attenuates Demyelination and Neuroinflammation in an Organotypic Slice Culture Model of Metachromatic Leukodystrophy. *Neurotherapeutics*. 2023;20:1347–68.
62. Mizushima N, Levine B. Autophagy in Human Diseases. *N Engl J Med*. 2020;383:1564–76.
63. Kim KA, Shin D, Kim JH, Shin YJ, Rajanikant GK, Majid A, Baek SH, Bae ON. Role of Autophagy in Endothelial Damage and Blood-Brain Barrier Disruption in Ischemic Stroke. *Stroke*. 2018;49:1571–9.
64. Zou W, Song Y, Li Y, Du Y, Zhang X, Fu J. The Role of Autophagy in the Correlation Between Neuron Damage and Cognitive Impairment in Rat Chronic Cerebral Hypoperfusion. *Mol Neurobiol*. 2018;55:776–91.
65. Wang D, Lin Q, Su S, Liu K, Wu Y, Hai J. URB597 improves cognitive impairment induced by chronic cerebral hypoperfusion by inhibiting mTOR-dependent autophagy. *Neuroscience*. 2017;344:293–304.
66. Su SH, Wu YF, Wang DP, Hai J. Inhibition of excessive autophagy and mitophagy mediates neuroprotective effects of URB597 against chronic cerebral hypoperfusion. *Cell Death Dis*. 2018;9:733.
67. Su SH, Wu YF, Lin Q, Wang DP, Hai J. URB597 protects against NLRP3 inflammasome activation by inhibiting autophagy dysfunction in a rat model of chronic cerebral hypoperfusion. *J Neuroinflammation*. 2019;16:260.
68. Chen Y, Guo Z, Peng X, Xie W, Chen L, Tan Z. Nimodipine represses AMPK phosphorylation and excessive autophagy after chronic cerebral hypoperfusion in rats. *Brain Res Bull*. 2018;140:88–96.
69. Wang N, He J, Pan C, Wang J, Ma M, Shi X, Xu Z. Resveratrol Activates Autophagy via the AKT/mTOR Signaling Pathway to Improve Cognitive Dysfunction in Rats With Chronic Cerebral Hypoperfusion. *Front Neurosci*. 2019;13:859.
70. Xu L, Qu C, Liu Y, Liu H. The environmental enrichment ameliorates chronic cerebral hypoperfusion-induced cognitive impairment by activating autophagy signaling pathway and improving synaptic function in hippocampus. *Brain Res Bull*. 2023;204:110798.
71. Shi W, Wei X, Wang Z, Han H, Fu Y, Liu J, Zhang Y, Guo J, Dong C, Zhou D, et al. HDAC9 exacerbates endothelial injury in cerebral ischaemia/reperfusion injury. *J Cell Mol Med*. 2016;20:1139–49.
72. Chen S, Gai J, Wang Y, Li H. FoxO regulates expression of decidual protein induced by progesterone (DEPP) in human endothelial cells. *FEBS Lett*. 2011;585:1796–800.
73. Salcher S, Hagenbuchner J, Geiger K, Seiter MA, Rainer J, Kofler R, Hermann M, Kiechl-Kohlendorfer U, Ausserlechner MJ, Obexer P. C10ORF10/DEPP, a transcriptional target of FOXO3, regulates ROS-sensitivity in human neuroblastoma. *Mol Cancer*. 2014;13:224.
74. Ragel BT, Couldwell WT, Gillespie DL, Jensen RL. Identification of hypoxia-induced genes in a malignant glioma cell line (U-251) by cDNA microarray analysis. *Neurosurg Rev*. 2007;30:181–7.

discussion 187.

75. Wyant GA, Jiang Q, Singh M, Qayyum S, Levrero C, Maron BA, Kaelin WG Jr.. Induction of DEPP1 by HIF Mediates Multiple Hallmarks of Ischemic Cardiomyopathy. *Circulation*. 2024;150:770–86.

Figures

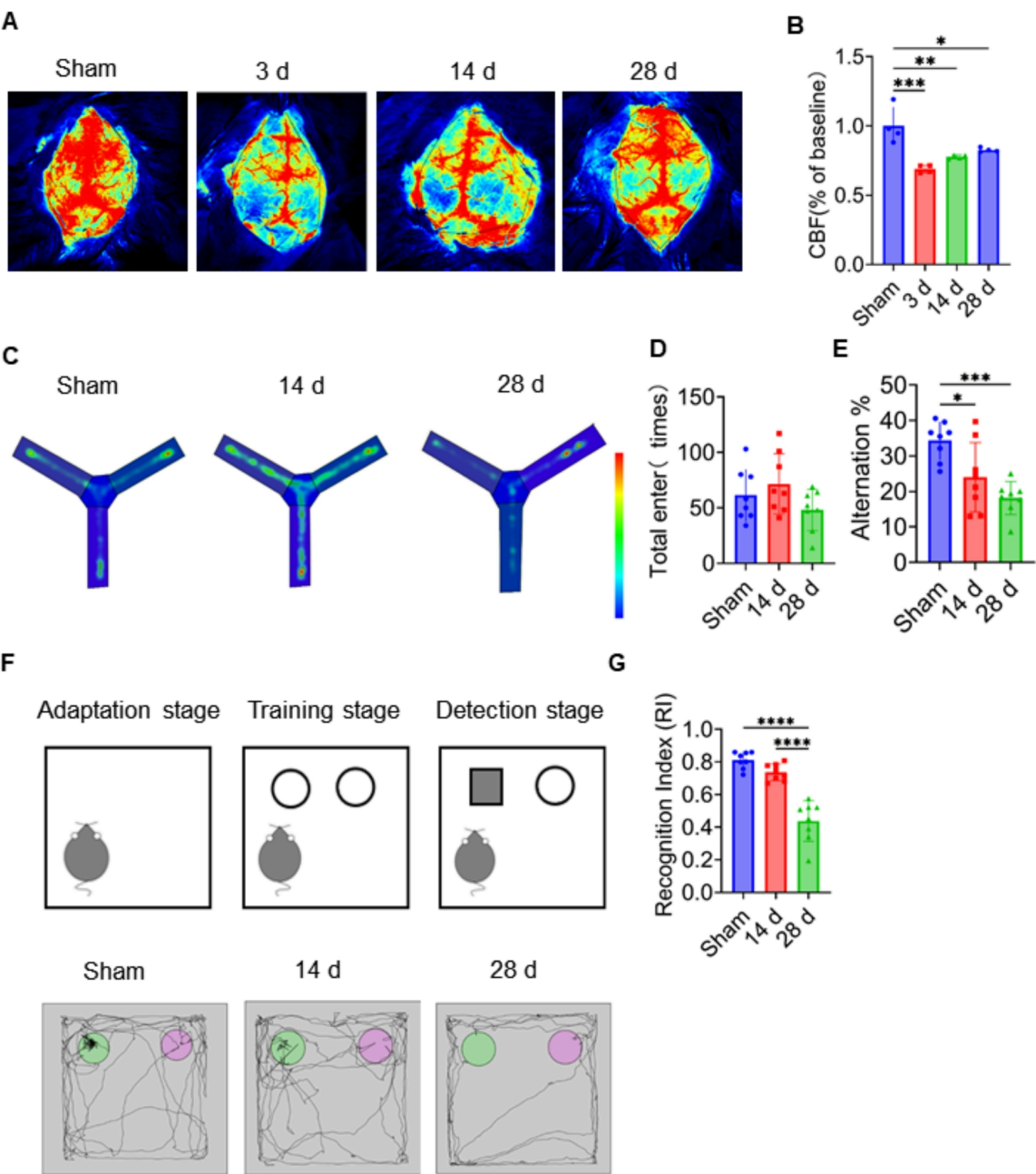


Figure 1

BCAS induces cerebral hypoperfusion and cognitive decline in mice

(A-B) Representative cerebral blood flow (CBF) images obtained by laser speckle contrast imaging and quantitative analysis of relative CBF. One-way ANOVA with Tukey's post hoc test; n = 4 mice/group.

(C-E) Representative heatmaps showing exploratory trajectories of mice in the Y-maze, with quantitative analysis of total arm entries and spontaneous alternation rate. One-way ANOVA with Tukey's post hoc test; n = 8 mice/group.

(F-H) Schematic illustration of the novel object recognition (NOR) test paradigm, with representative trajectory plots showing exploratory paths and quantitative analysis of exploration frequency toward the novel object. One-way ANOVA with Tukey's post hoc test; n = 8 mice/group. *P < 0.05, **P < 0.01, ***P < 0.001.

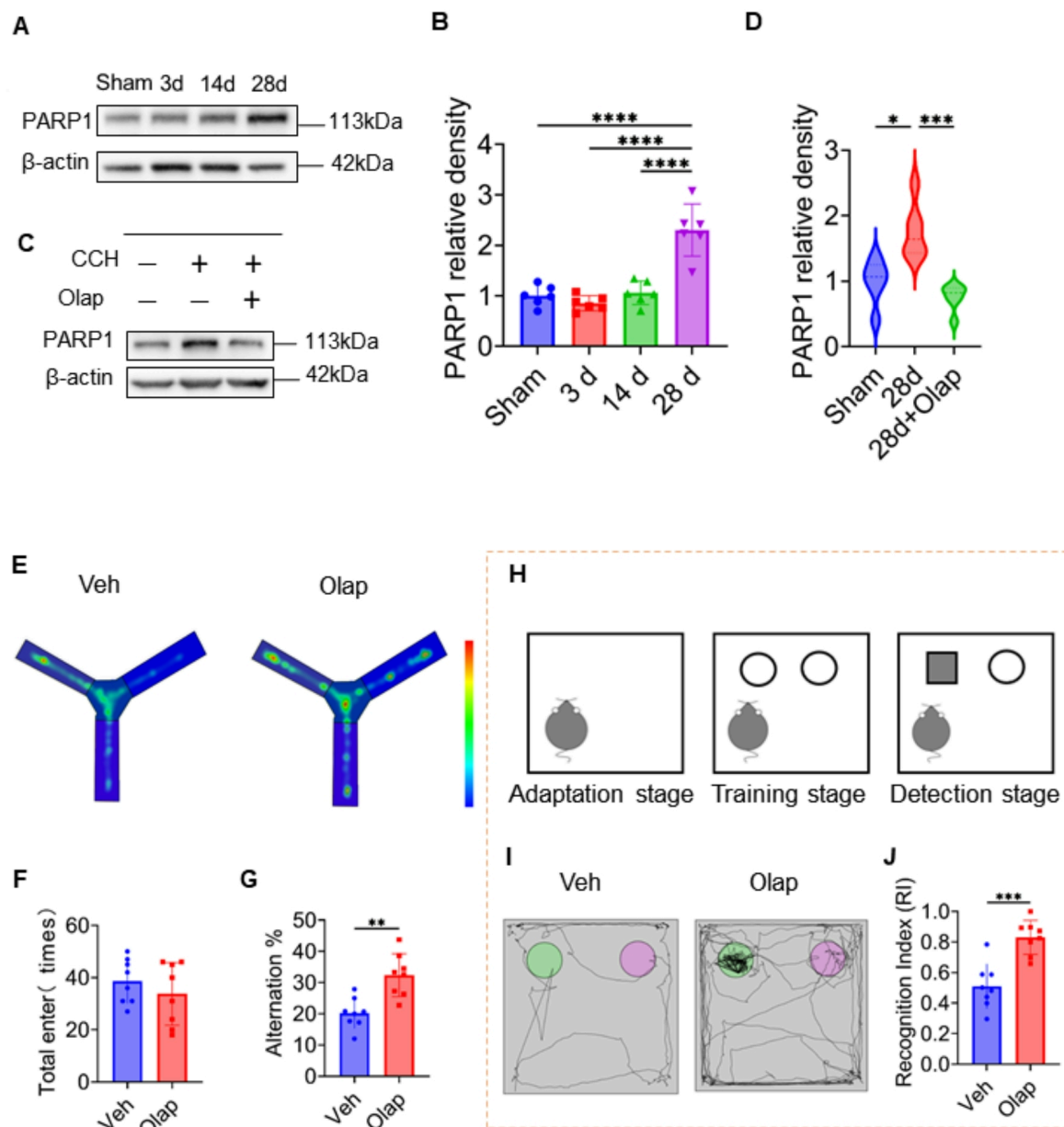


Figure 2

Pharmacological inhibition of PARP1 by Olaparib alleviates cognitive impairment in chronic cerebral hypoperfusion (CCH) mice.

(A-B) Western blot analysis of PARP1 expression in the cerebral cortex of CCH mice at different time points post-BCAS, normalized to β-actin. One-way ANOVA with Tukey's post hoc test; n = 6/group.

(C-D) Western blot analysis of PARP1 expression in the cerebral cortex of CCH mice following Ola treatment, normalized to β -actin. Kruskal-Wallis test; n = 6/group.

(E-G) Representative heatmaps showing exploratory trajectories in the Y-maze, with quantitative analysis of spontaneous alternation rate and total arm entries. Unpaired t-test; n = 8 mice/group.

(H-J) Schematic illustration of the NOR test paradigm, with representative trajectory plots and quantitative analysis of exploration frequency toward the novel object. Unpaired t-test; n = 8 mice/group. *P < 0.05, **P < 0.01, ***P < 0.001.

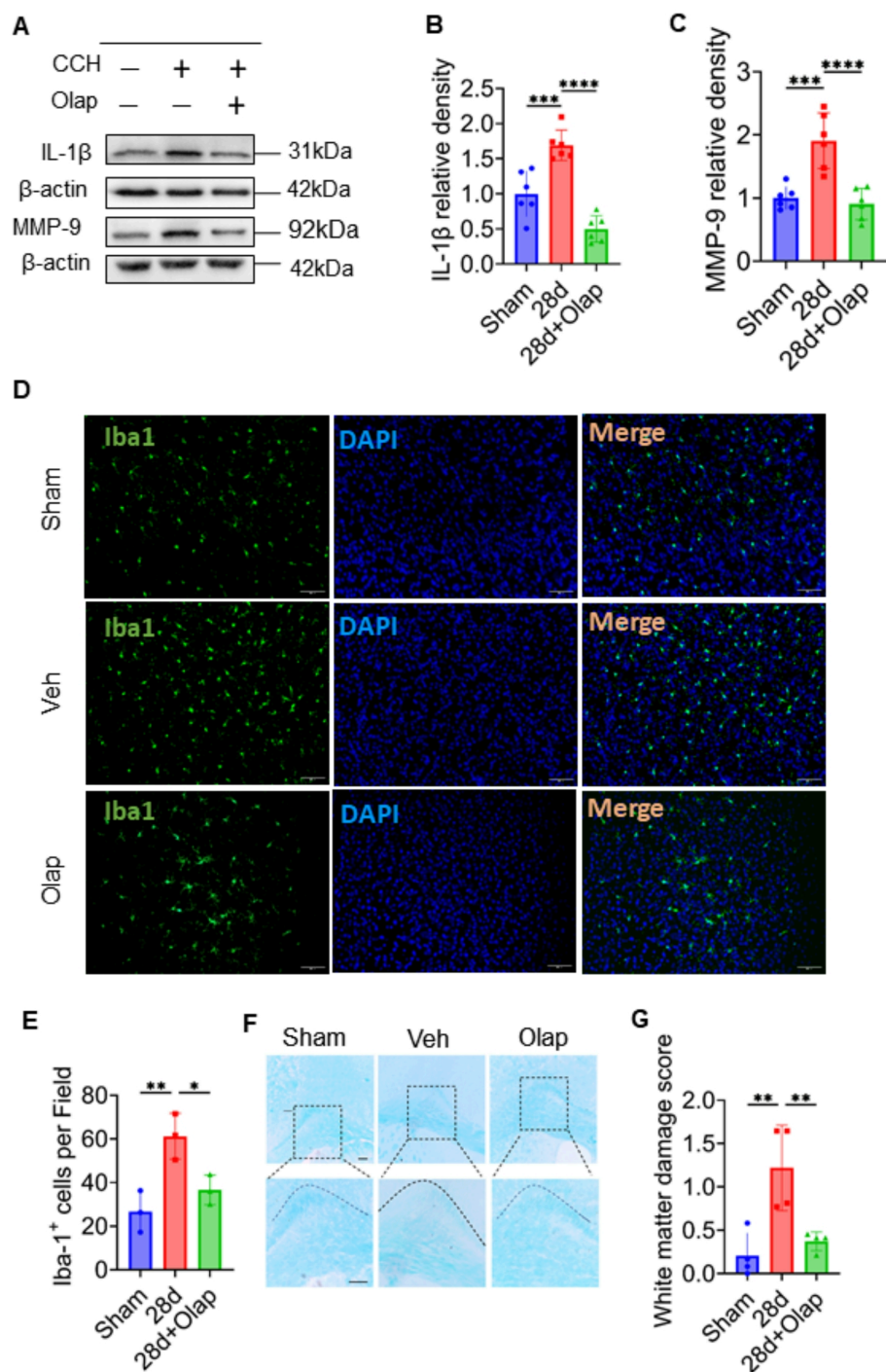


Figure 3

Olaparib attenuates neuroinflammatory infiltration and white matter demyelination after CCH.

(A-C) Western blot analysis of IL-1 β and MMP-9 expression in the cerebral cortex following Ola treatment, normalized to β -actin. One-way ANOVA with Tukey's post hoc test; n = 6/group.

(D-E) Representative immunofluorescence images and quantitative analysis of microglial numbers in the cortex. One-way ANOVA with Tukey's post hoc test; n = 3 mice/group. Scale bar = 100 μ m.

(F-G) Representative Luxol fast blue (LFB)-stained images of the corpus callosum with magnified insets, and quantification of LFB staining scores in the Sham, Vehicle (Veh), and Ola groups. Scale bar = 100 μ m (insets, 100 μ m). One-way ANOVA with Tukey's post hoc test; n = 4 mice/group. *P < 0.05, **P < 0.01, ***P < 0.001.

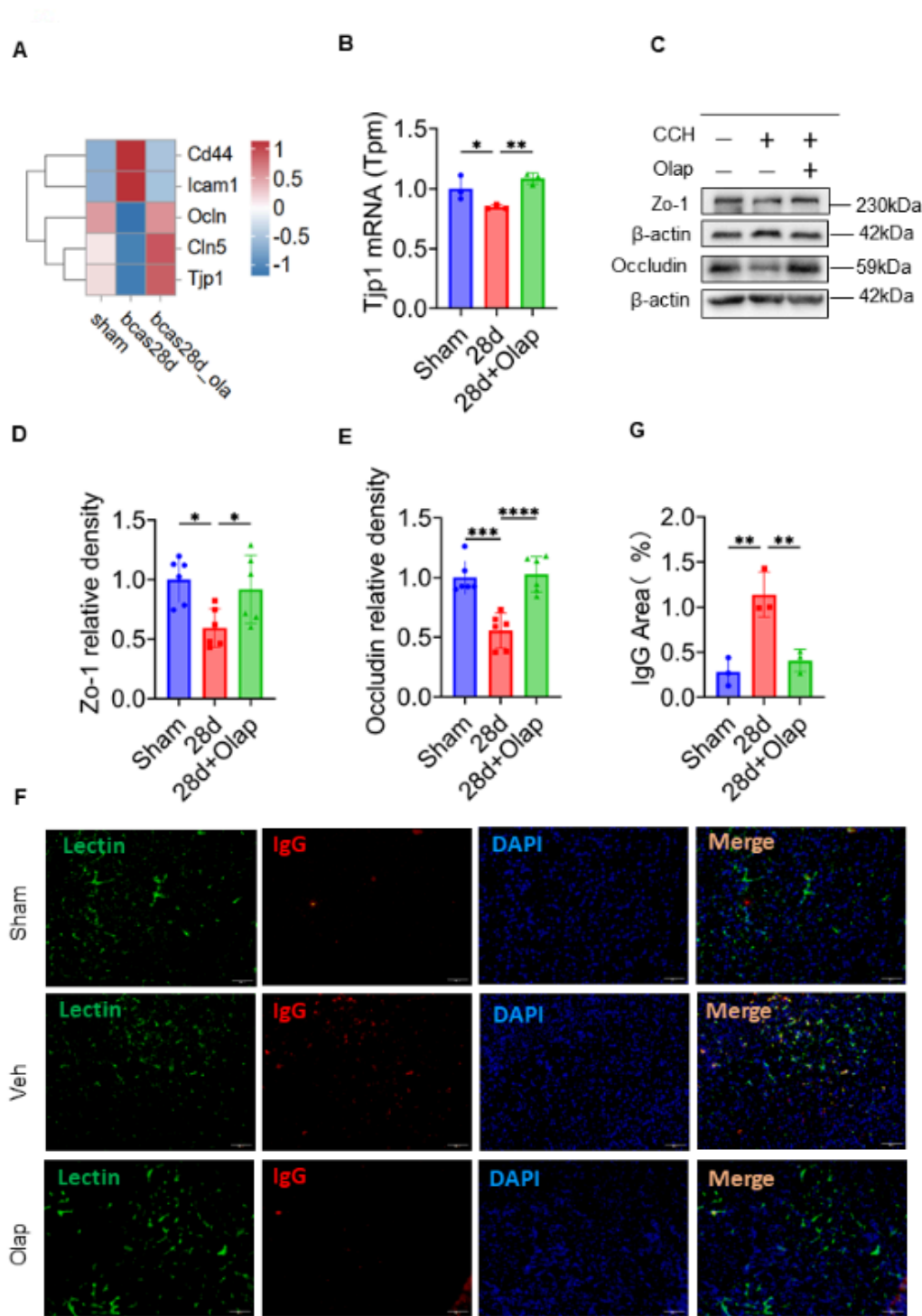


Figure 4

Olaparib alleviates blood-brain barrier (BBB) disruption after CCH.

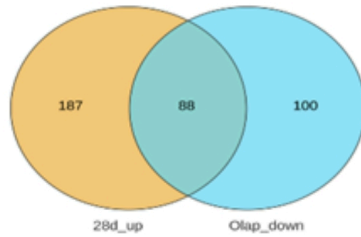
(A) Heatmap showing expression profiles of genes related to BBB tight junctions and adhesion molecules in the Sham, BCAS 28d, and Ola groups.

(B) Fragments per kilobase of transcript per million mapped reads (FPKM) of TJP1 transcripts. One-way ANOVA with Tukey's post hoc test; n = 3/group.

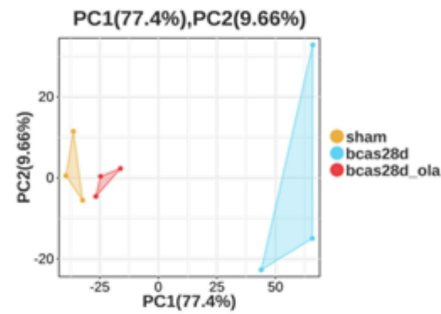
(C-E) Western blot analysis of Zo-1 and Occludin expression in the cerebral cortex following Ola treatment, normalized to β -actin. One-way ANOVA with Tukey's post hoc test; n = 6/group.

(F-G) Representative immunofluorescence images and quantitative analysis of IgG-positive area in the cortex. One-way ANOVA with Tukey's post hoc test; n = 3 mice/group. Scale bar = 100 μ m. *P < 0.05, **P < 0.01, ***P < 0.001.

A

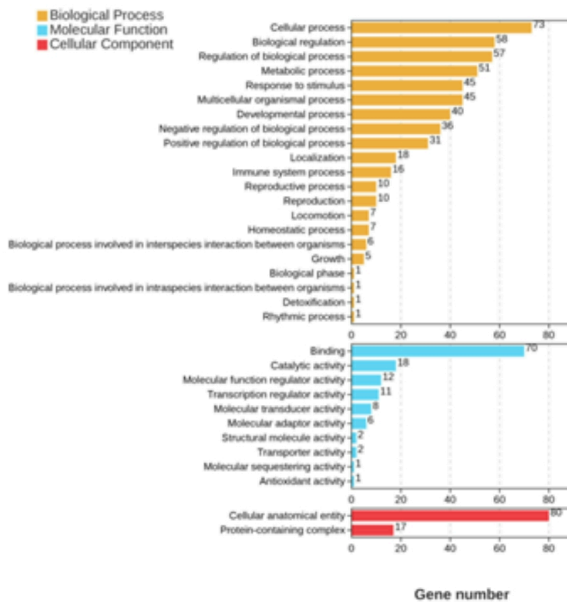


B



C

GO Enrichment



D

Pathway Enrichment

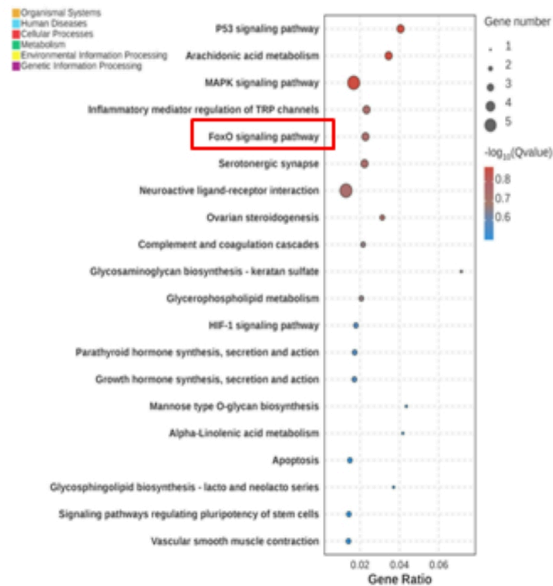


Figure 5

Global transcriptomic changes following PARP1 inhibition by Olaparib

(A) Venn diagram showing the overlap between genes upregulated in the Sham vs. BCAS 28d comparison (275 genes) and genes downregulated in the BCAS 28d vs. BCAS 28d + Ola comparison

(188 genes), with a fold change ≥ 1.5 . The overlapping region indicates 88 shared differentially expressed genes (DEGs).

(B) Principal component analysis (PCA) plot based on the 88 shared DEGs identified in (A). PC1 and PC2 accounted for 77.4% and 9.66% of total variance, respectively.

(C) Bar plot of Gene Ontology (GO) enrichment analysis of the 88 shared DEGs, showing enriched terms across Biological Process (BP), Molecular Function (MF), and Cellular Component (CC) categories. The x-axis represents the number of enriched genes.

(D) Bubble plot of the top 20 KEGG pathway enrichment terms based on the 88 shared DEGs (human disease-related pathways excluded). Bubble size indicates the number of enriched genes; color intensity represents the Q value.

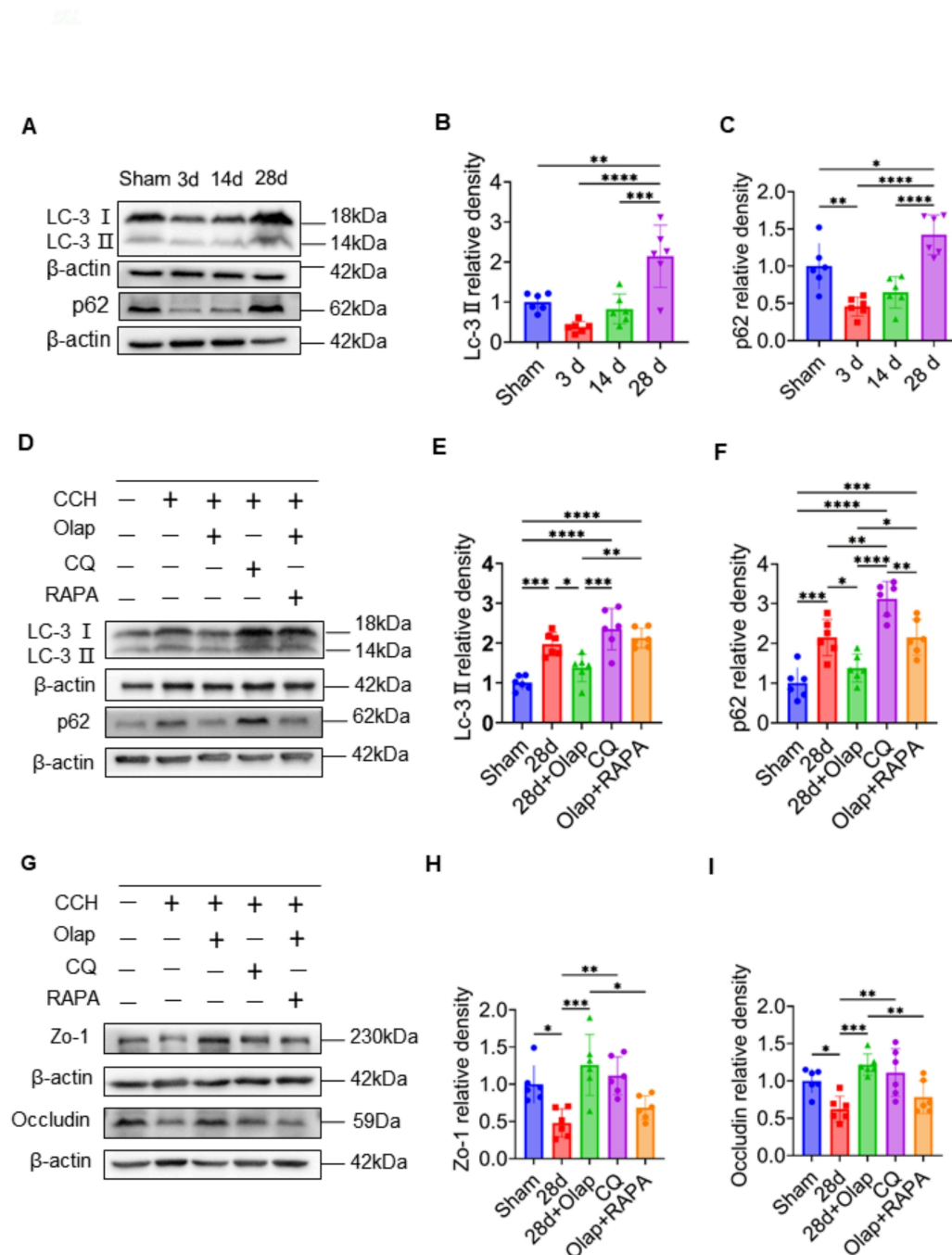


Figure 6

PARP1 inhibition by Olaparib alleviates BBB injury by attenuating excessive autophagy initiation after CCH.

(A-C) Western blot analysis of LC3 and P62 expression in the cerebral cortex of CCH mice at different time points, normalized to β-actin. One-way ANOVA with Tukey's post hoc test; n = 6/group.

(D-F) Western blot analysis of LC3 and P62 expression in the cerebral cortex of mice from the Sham, Veh, Ola, Chloroquine (CQ), and Ola + Rapamycin (Ola+RAPA) groups, normalized to β -actin. One-way ANOVA with Tukey's post hoc test; $n = 6/\text{group}$.

(G-I) Western blot analysis of Zo-1 and Occludin expression in the cerebral cortex of mice from the Sham, Veh, Ola, CQ, and Ola+RAPA groups, normalized to β -actin. One-way ANOVA with Tukey's post hoc test; $n = 6/\text{group}$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

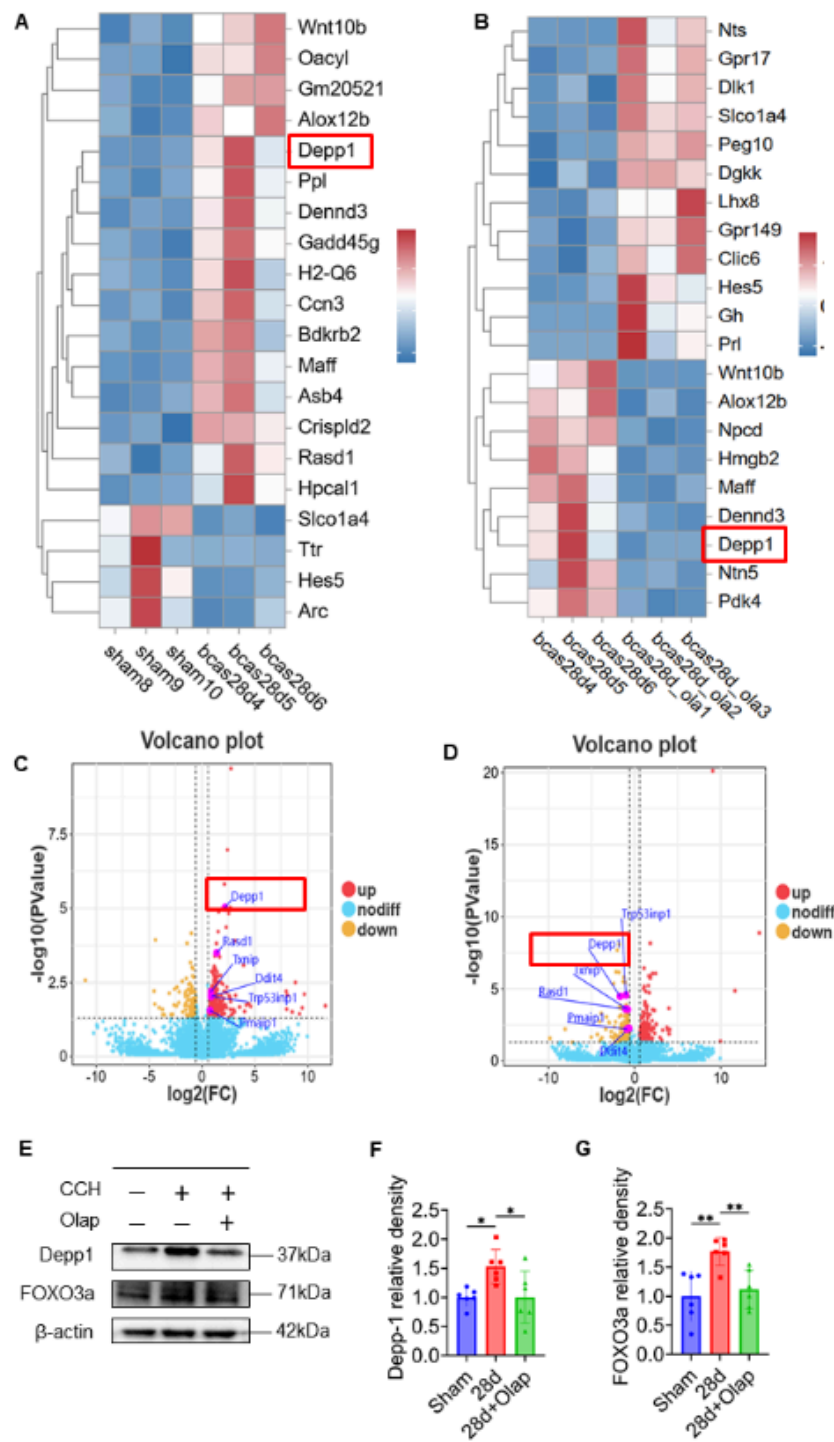


Figure 7

PARP1 inhibition by Olaparib suppresses the FOXO3a/Depp1 axis after CCH.

(A) Hierarchical clustering heatmap of differentially expressed genes between the Sham and CCH 28d groups.

(B) Hierarchical clustering heatmap of differentially expressed genes between the BCAS 28d and Ola groups.

(C) Volcano plot showing transcriptomic differential expression profiles between the Sham and CCH 28d groups.

(D) Volcano plot showing transcriptomic differential expression profiles between the BCAS 28d and Ola groups.

(E-G) Western blot analysis of Depp1 and FOXO3a expression in the cerebral cortex following Ola treatment, normalized to β -actin. One-way ANOVA with Tukey's post hoc test; n = 6/group. *P < 0.05, **P < 0.01, ***P < 0.001.

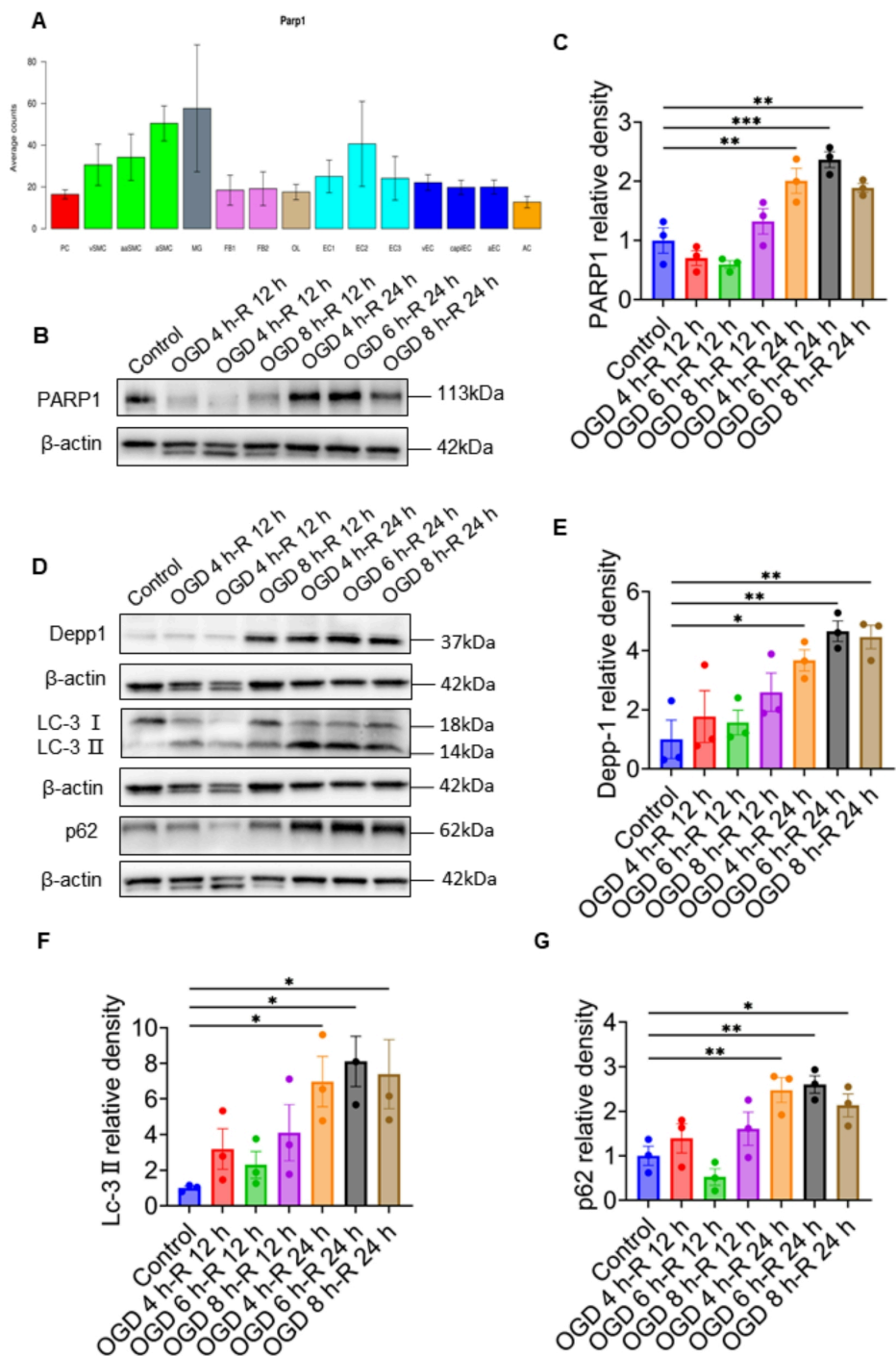


Figure 8

Dynamic changes in the PARP1–FOXO3a–Depp1 axis in OGD/R endothelial cells.

(A) Histogram showing PARP1 read counts derived from single-cell RNA-sequencing of mouse brain. AEC, arterial endothelial cell; AS, astrocyte; CAEC, capillary endothelial cell; EC, endothelial cell; FB,

fibroblast; MG, microglia; OL, oligodendrocyte; PC, pericyte; SMC, smooth muscle cell; VEC, venous endothelial cell.

(B-G) Western blot analysis of PARP1, Depp1, FOXO3a, LC3, and P62 expression in bEnd.3 endothelial cells following OGD/R at different time points, normalized to β -actin. One-way ANOVA with Tukey's post hoc test; n = 3/group. *P < 0.05, **P < 0.01, ***P < 0.001.

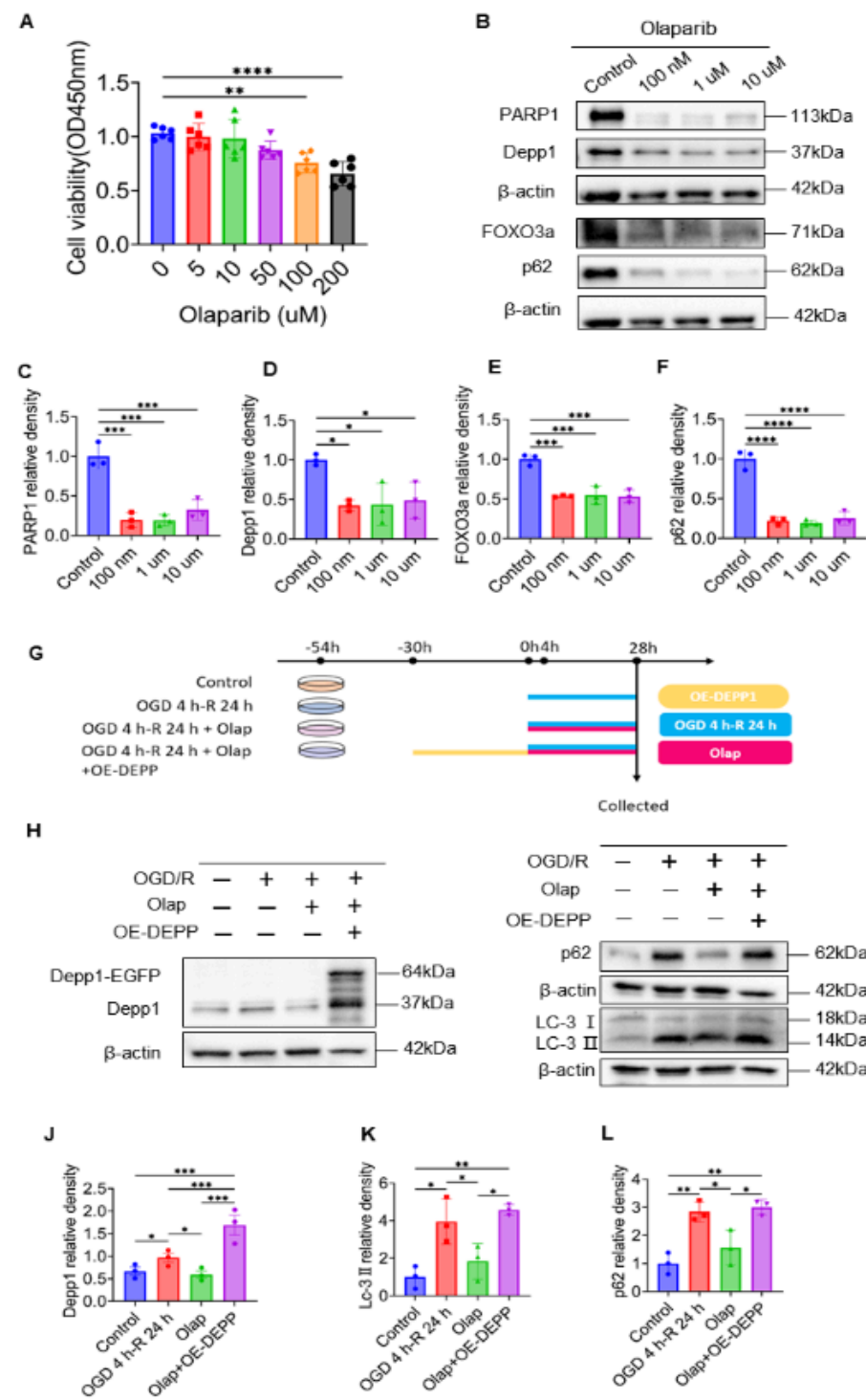


Figure 9

PARP1 regulates autophagy via the FOXO3a-Depp1 axis.

(A) CCK-8 assay showing the effect of different concentrations of Ola on the viability of OGD/R-treated endothelial cells.

(B-F) Western blot analysis of PARP1, Depp1, FOXO3a, and P62 expression in OGD/R-treated endothelial cells following treatment with different concentrations of Ola, normalized to β -actin. One-way ANOVA with Tukey's post hoc test; n = 3/group.

(G) Schematic illustration of the experimental treatment protocol for hCMEC/D3 cells across different groups.

(H-L) Western blot analysis of Depp1, P62, and LC3 expression in OGD/R-treated endothelial cells following different treatments, normalized to β -actin. One-way ANOVA with Tukey's post hoc test; n = 3/group. *P < 0.05, **P < 0.01, ***P < 0.001.

Supplementary Files

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