



## Review Article

# Microglial activation and lysosomal dysfunction in hemorrhagic stroke

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**Submission** : 17-May-2025  
**Revision** : 03-Jun-2025  
**Acceptance** : 12-Jul-2025  
**Web Publication** : 07-Nov-2025

### ABSTRACT

Hemorrhagic stroke, mainly caused by intracerebral hemorrhage (ICH), is a severe neurological condition with high mortality and lasting disability. ICH involves bleeding into the brain parenchyma, hematoma formation, and subsequent edema and tissue damage, triggering inflammatory and degenerative responses that worsen secondary brain injury (SBI). Microglia, the brain's resident immune cells, are key mediators in this process. Their ability to sense, engulf, and clear hematoma-derived debris is essential for controlling neuroinflammation and promoting tissue repair. Central to microglial phagocytosis is lysosomal function. Lysosomes contain hydrolases – proteases, glycosidases, lipases, and nucleases – that degrade proteins, lipids, carbohydrates, and nucleic acids. This coordinated degradation ensures effective recycling of phagocytosed materials and clearance of cellular debris after hemorrhage. However, lysosomal dysfunction impairs microglial clearance capacity, leading to persistent inflammation, aggravated neuronal damage, and poor neurological recovery after ICH. This review focuses on the interplay between microglial activation, lysosomal function, and phagocytosis in hemorrhagic stroke. We examine how lysosomal impairment hinders hematoma resolution, propagates SBI, and delays functional recovery. In addition, we highlight emerging therapeutic strategies targeting the microglia–lysosome axis, such as enhancing lysosomal biogenesis and enzyme activity, as promising approaches to boost hematoma clearance and improve outcomes. Understanding and modulating microglial lysosomal function offers novel therapeutic avenues for ICH management, aiming to mitigate secondary injury and support neurological recovery.

**KEYWORDS:** *Intracerebral hemorrhage, Lysosomal function, Microglial phagocytosis, Neuroinflammation*

## INTRODUCTION

Hemorrhagic stroke, characterized by bleeding into or around the brain parenchyma, remains a devastating neurological disorder with high mortality and morbidity. The primary etiologies include chronic hypertension, cerebral amyloid angiopathy, vascular malformations, and trauma, leading to two major subtypes: intracerebral hemorrhage (ICH), where bleeding occurs within the brain tissue, and subarachnoid hemorrhage (SAH), characterized by bleeding into the subarachnoid space [1]. Despite advances in acute management, effective therapies targeting secondary brain injury (SBI) and promoting functional recovery remain limited, posing significant clinical challenges. Among the endogenous repair mechanisms, microglia, as the resident immune cells of the central nervous system, play a pivotal role in responding to hemorrhagic injury [1]. One of their essential functions is the phagocytic clearance of hematoma components, including

erythrocytes, debris, and toxic byproducts, which is crucial for limiting inflammation and promoting tissue repair [1]. Central to this process is the lysosomal system, which facilitates the degradation and recycling of internalized materials, thereby determining the efficiency of microglial phagocytosis and the resolution of hemorrhagic lesions [2].

This review elucidates the interdependent relationship between lysosomal function, microglial activation, and phagocytosis in hemorrhagic stroke recovery. By integrating current evidence, we highlight how modulating microglial lysosomal pathways could offer novel therapeutic strategies for enhancing hematoma clearance and mitigating SBI.

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**How to cite this article:** Lee CH, Pang CY, Wang MJ, Hu WF, Huang HY, Tsai ST, et al. Microglial activation and lysosomal dysfunction in hemorrhagic stroke. Tzu Chi Med J 2026;38(1):1-10.

Access this article online	
<b>Quick Response Code:</b> 	<b>Website:</b> www.tcmjmed.com
	<b>DOI:</b> 10.4103/tcmj.tcmj_213_25

## HEMORRHAGIC STROKE: PATHOPHYSIOLOGY AND INFLAMMATORY RESPONSE

Hemorrhagic stroke, defined as bleeding in the brain or surrounding tissue due to ruptured blood vessels, is a significant public health problem with high rates of morbidity, mortality, and long-term disability. Among its subtypes, ICH is particularly devastating, with nearly 40% of patients dying within the 1<sup>st</sup> month and two-thirds of survivors experiencing moderate to severe neurological impairment [3].

In Asia, the prevalence of ICH (20%–30%) is notably higher than in Western populations (10%–15%) [4]. In clinical practice, patients presenting with suspected stroke typically undergo MRI or CT imaging to determine the presence and location of ICH. For most cases of mild-to-moderate ICH, medical management is the primary approach to alleviating symptoms while allowing the hematoma to be gradually broken down and absorbed by brain tissue [5]. Surgical intervention is generally reserved for patients with severe hemorrhage and significant bleeding, where hematoma evacuation is necessary to relieve intracranial pressure and minimize neurological damage, ultimately improving survival outcomes. However, complete removal of the hematoma through surgery is often not feasible. Technical limitations and the risk of inducing further bleeding constrain the extent of surgical clearance. Furthermore, there is currently no clinical drug available to address the SBI caused by residual blood clots. According to the 2022 AHA/ASA Guidelines for the Management of Spontaneous ICH, treatment strategies are primarily driven by clinical indications, with no specific therapy targeting clot resolution [6].

The severity of brain injury is directly correlated with hematoma size – the larger the hematoma, the more severe the neurological impairment and the poorer the prognosis. A hematoma's coagulation and subsequent lysis initiate a cascade of pathological processes, including thrombin activation, microglial activation, heme degradation, and iron release. These events generate reactive oxygen species, increase oxidative stress, and trigger pronounced neuroinflammation and neuronal death [1]. Therefore, any strategy or therapeutic agent that effectively promotes the clearance of residual hematoma could be a promising intervention for reducing secondary brain damage following ICH.

### Primary and secondary brain injury: Hematoma formation, oxidative stress, and secondary neuroinflammation

The mechanisms of brain injury following ICH are complex and are generally categorized into primary brain injury (PBI) and SBI. PBI arises from the rupture of cerebral blood vessels, leading to hematoma formation that exerts a mass effect on brain tissue. This results in cerebral edema, disruption of the blood–brain barrier (BBB), and increased intracranial pressure, which can reduce cerebral perfusion pressure [7] and, in severe cases, cause brain herniation and death [8]. SBI, considered a critical contributor to brain damage post-ICH, involves the release of toxic substances from the coagulation and breakdown of hematomas, triggering inflammatory responses, immune cell infiltration, hemoglobin and iron release, thrombin activation, microglial activation, and other cytotoxic effects [9]. Hematoma lysis, occurring

over hours to days, exacerbates brain edema, damages the BBB, and induces apoptosis in neurons and glial cells [10-12]. During this process, the activation of thrombin, microglia, and hemoglobin metabolism, along with iron release, generates large amounts of free radicals, increasing oxidative stress, mitochondrial dysfunction, excitotoxicity, calcium influx, and severe neuroinflammation, ultimately leading to neuronal apoptosis and exacerbation of brain injury [13]. The clearance of hematomas and cell debris following ICH is primarily mediated by resident microglia, astrocytes, and macrophages derived from peripheral blood.

## MICROGLIAL ACTIVATION IN HEMORRHAGIC STROKE

Microglia and infiltrating monocytes represent distinct populations of mononuclear phagocytes that play pivotal yet dual roles in ICH pathology, encompassing both “classical activation” (M1 polarization) and “alternative activation” (M2 polarization) [14].

During the acute phase of ICH, microglia primarily adopt a pro-inflammatory M1 phenotype. This is characterized by the activation of toll-like receptors, the NLRP3 inflammasome, and nuclear factor kappa signaling. It is also marked by the release of cytokines, such as tumor necrosis factor- $\alpha$ , interleukin-1 $\beta$  (IL-1 $\beta$ ), and IL-12, as well as cytotoxic mediators, such as free radicals. These factors exacerbate neuronal injury [15]. However, as the injury progresses into the subacute and recovery phases, the microglia shift toward an anti-inflammatory, reparative M2 phenotype. These cells adopt an amoeboid morphology and enhance their phagocytic capacity to clear necrotic debris, resolve hematomas, and promote glial scar formation and neural repair. M2 microglia secrete neurotrophic and anti-inflammatory factors, support angiogenesis, and facilitate tissue remodeling via JAK/STAT signaling and lysosome-dependent pathways. Through interactions with erythrocytes, neurons, and astrocytes, microglia orchestrate injury and repair processes [14]. Although the phenotypic transition from pro-inflammatory to reparative microglia generally facilitates recovery and limits secondary damage, prolonged or unchecked M2-like activation can paradoxically contribute to excessive glial scarring, impaired neuroplasticity, and maladaptive tissue remodeling. Furthermore, recent studies challenge the traditional M1/M2 dichotomy, proposing a spectrum-based model of microglial activation that more accurately reflects their dynamic and context-dependent functions in central nervous system injury and repair [16]. This complexity underscores the therapeutic potential of strategies that temporally and selectively modulate microglial responses to optimize repair and minimize long-term adverse effects [16]. Continued research into the molecular mechanisms and intercellular interactions driving microglial plasticity remains essential for developing targeted therapies.

### Hematoma clearance

Microglia play a pivotal role in hematoma clearance following ICH through erythrophagocytosis, a process essential for mitigating SBI by reducing iron-induced

oxidative stress, neuroinflammation, and cerebral edema, thereby promoting tissue repair and functional recovery [17]. This clearance is orchestrated by a complex network of signaling pathways and receptor-ligand interactions, where “find-me” and “eat-me” signals – such as phosphatidylserine exposure and ATP release – attract microglia to injury sites, triggering phagocytosis. Key receptors, including CD36, TREM2, and MerTK, recognize erythrocytes and cellular debris, with CD36-mediated phagocytosis enhanced by IL-10 and PPAR $\gamma$  activation, dampening inflammation [18]. The phenotypic switch from pro-inflammatory M1 to anti-inflammatory M2 microglia, driven by IL-10 and Nrf2 signaling, is critical for effective hematoma resolution and neuroprotection [19]. However, microglial activation presents a therapeutic challenge due to its dual role: While M1 polarization exacerbates neuroinflammation and secondary injury, M2 polarization promotes repair and recovery [19]. Consequently, modulating microglial activity to favor reparative functions – through interventions targeting IL-10, PPAR $\gamma$ , or Nrf2 pathways – emerges as a promising therapeutic strategy [20,21]. Despite these insights, further research is necessary to refine our understanding of microglial regulation and develop targeted therapies that enhance their beneficial roles while minimizing detrimental effects in ICH [17,19,22].

## LYSOSOMAL FUNCTION IN MICROGLIA

Lysosomes are acidic organelles (pH 4.5–6.0) that provide an optimal environment for over 60 hydrolytic enzymes [Table 1] are classified into six main categories based on the type of biomolecule they degrade: proteases, glycosidases, lipases, nucleases, phosphatases, and sulfatases [Table 1]. These hydrolytic enzymes are essential for the breakdown of proteins, carbohydrates, lipids, nucleic acids, phosphates, and sulfated compounds, enabling the efficient degradation of macromolecules and ensuring cellular material turnover, particularly under nutrient deprivation, maintaining cellular homeostasis, and facilitating waste processing within the lysosome. They also serve as vital reservoirs for micronutrients such as iron and calcium, safely storing these cations to prevent cytotoxicity while releasing them in a controlled manner to regulate cellular functions such as membrane fusion, fission, enzymatic reactions, and signaling events [23]. Cellular debris and extracellular materials are delivered to lysosomes for degradation through processes such as phagocytosis, endocytosis, and macropinocytosis. Functionally,

lysosomes interact extensively with other organelles, which are central in coordinating intracellular and extracellular material recycling [24,25]. They also act as cellular sensors, responding to stress stimuli and regulating nutrient and energy homeostasis through pathways involving mammalian target of rapamycin complex 1 and adenosine 5'-monophosphate-activated protein kinase [26]. Damage to lysosomes disrupts their clearance capacity and can result in enzyme leakage, triggering various forms of cell death, including apoptosis [27,28], necrosis [29,30], pyroptosis [31,32], and ferroptosis [33,34], highlighting their critical role in maintaining cellular health. Robust lysosomal quality control (LQC) mechanisms exist to regulate their quantity and preserve their functional capacity to safeguard lysosomal integrity and functionality.

Lysosomal damage primarily manifests as lysosomal membrane permeabilization (LMP), involving alterations or complete rupture of the lysosomal membrane, which poses significant threats to cellular function and survival [35]. Under normal conditions, glycosylation of lysosomal membrane proteins helps maintain membrane stability by preventing degradation by intralysosomal proteases. However, under stress conditions such as oxidative damage, photodamage, or cellular injury, LMP compromises membrane integrity, increasing permeability and releasing cathepsins and hydrolases into the cytoplasm [36,37]. If LMP remains unaddressed, persistent lysosomal rupture can lead to the uncontrolled release of lysosomal contents, cytoplasmic acidification, and extensive hydrolysis of cytoplasmic components, culminating in irreversible cellular damage. To counteract such threats, cells employ robust LQC mechanisms to facilitate lysosomal repair, autophagic degradation of damaged lysosomes, and lysosomal regeneration, ensuring cellular homeostasis and survival under stress.

When LMP occurs, protective mechanisms are activated to repair the damaged membrane and prevent the release of intralysosomal H<sup>+</sup> ions and hydrolases [35]. Recent studies highlight the critical role of the endosomal sorting complexes required for transport (ESCRT) in repairing damaged lysosomal membranes [38,39]. The ESCRT machinery acts early in the damage response by forming small pores on the lysosomal membrane to facilitate repair [39]. While the exact mechanisms of ESCRT-mediated repair remain unclear, it likely involves inducing filamentous helical structures on the membrane surface and constricting pores in the lipid bilayer [38].

**Table 1: Key lysosomal enzymes and their functional classification**

Enzyme group	Specific enzymes	Function
Proteases	Cathepsin A, B, C, D, H, L, S, X	Break down proteins into amino acids
Glycosidases	$\alpha$ -Galactosidase, $\beta$ -Galactosidase, $\alpha$ -Glucosidase, $\beta$ -Glucosidase, Hexosaminidase A/B, Fucosidase, Mannosidase $\alpha/\beta$	Hydrolyze glycosidic bonds in oligosaccharides and polysaccharides
Lipases	Acid lipase, Sphingomyelinase, phospholipase A1/A2	Hydrolyze ester bonds in lipids, releasing glycerol and free fatty acids
Nucleases	DNase II, RNase	Break down nucleic acids (DNA and RNA) into nucleotides
Phosphatases	Acid phosphatase, pyrophosphatase, phosphoprotein phosphatase	Remove phosphate groups from biomolecules
Sulphatases	Arylsulfatase A, B, C; iduronate-2-sulfatase	Hydrolyze sulfate esters in glycosaminoglycans and sulfated biomolecules
Additional enzymes	N-acetylglucosamine-6-sulfatase, $\alpha$ -L-iduronidase, hyaluronidase, elastase	Diverse roles in glycosaminoglycan degradation and extracellular matrix remodeling

DNase: Deoxyribonuclease, RNase: Ribonuclease

If lysosomal damage becomes irreversible, selective autophagy of damaged lysosomes, known as lysophagy, is triggered to ensure adequate clearance [35]. Like other forms of selective autophagy, the ubiquitination of damaged lysosomes is a key process driving and regulating lysophagy [40]. Since lysophagy is a lysosome-dependent pathway, timely replenishment of lysosome numbers is essential to maintain lysosomal function and overall cellular homeostasis. When lysosomes are damaged, the process of lysosomal regeneration is initiated. Cells monitor lysosomal status through the Coordinated Lysosomal Expression and Regulation (CLEAR) network, which adjusts the expression of lysosome-related genes as needed [41,42]. Transcription Factor EB (TFEB) directly binds to CLEAR elements, driving lysosomal renewal [43]. Research indicates that TFEB enhances the expression of genes critical for lysosomal function and those involved in exocytosis, phagocytosis, endocytosis, and autophagy [41]. For instance, inhibition of mTOR triggers TFEB dephosphorylation and nuclear translocation, increasing the expression of lysosomal protein genes such as V-ATPase, lysosomal transmembrane proteins, and hydrolases [44]. Lysosomal repair and survival mechanisms, including LQC, involve rapid responses to lysosomal damage, engaging multiple processes to restore functionality. Understanding the molecular mechanisms of LQC and its connection to the pathogenesis of clinical diseases is crucial for developing therapeutic interventions.

The phagocytosis and autophagy processes of microglia rely on proper lysosomal acidification to facilitate cellular degradation and recycling systems [45-47]. Lysosomal acidification is regulated by membrane-bound vacuolar (H<sup>+</sup>)-ATPase (V-ATPase) and ion channels [48,49]. A defect in lysosomal acidification within microglia, marked by increased lysosomal pH, can lead to neuroinflammation and neurodegeneration. When lysosomal acidification is impaired in activated microglia, they produce and release elevated levels of pro-inflammatory cytokines, attracting and activating immune cells, thereby exacerbating neuroinflammation [46,50]. Furthermore, dysfunctional microglia with impaired lysosomal acidification exhibit increased LMP, which promotes the secretion of inflammatory cytokines and induces neuronal death through mechanisms like necroptosis [51,52]. In addition, these compromised microglia show reduced capacity for phagocytosis and autophagy, hindering the clearance of damaged organelles (e.g., mitochondria) [53,54], toxic protein aggregates [55], myelin debris [56], and synaptic pruning [57]. This ultimately contributes to neuronal death.

Restoring microglial autophagic and phagocytic functions is critical for mitigating neuroinflammation [58]. However, the exact mechanisms underlying lysosomal acidification impairment and the impact of lysosomal pH regulation on microglial function and activation remain unclear, warranting further investigation [59].

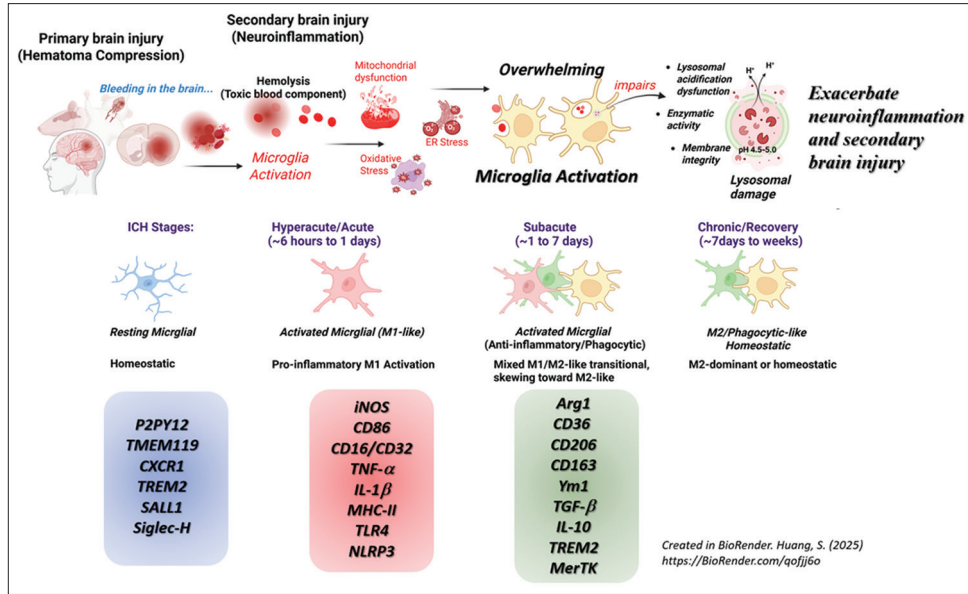
### **Lysosomal dysfunction in hemorrhagic stroke**

Lysosomal dysfunction has emerged as a central mechanism in SBI following ICH, driven by impaired cellular waste clearance, excessive neuroinflammation, oxidative stress, and neuronal

apoptosis [60]. Post-ICH, autophagy – a lysosome-dependent degradation pathway – becomes dysregulated, leading to either neuroprotection or damage depending on its modulation, while impaired mitophagy worsens mitochondrial dysfunction and oxidative injury [61]. LMP releases cathepsins, triggering apoptotic pathways, and activates the NLRP3 inflammasome, amplifying neuroinflammation [62,63]. Defective lysosomal acidification further hinders autophagic flux by impairing enzyme activity and autophagosome–lysosome fusion, accumulating cellular debris and autophagic vacuoles [64]. Iron overload and lipofuscin accumulation exacerbate lysosomal dysfunction through enhanced ROS production and lipid peroxidation, fueling neurodegeneration [65]. A detrimental feedback loop between lysosomal stress and endoplasmic reticulum stress also aggravates neuronal damage [66]. Chaperone-mediated autophagy (CMA), particularly via Lamp2a regulation, has been identified as a potential compensatory and neuroprotective mechanism [67]. Consequently, therapeutic strategies are increasingly focused on lysosome-centered interventions, aiming to restore lysosomal acidification, stabilize lysosomal membranes, enhance autophagy–lysosome fusion, and mitigate iron-induced oxidative stress, offering promising avenues for reducing ICH-induced SBI. These approaches highlight the potential of targeting lysosomal function to improve outcomes after ICH, as restoring autophagic flux could mitigate neuronal damage and promote recovery.

### **Crosstalk between microglial activation and lysosomal dysfunction in intracerebral hemorrhage**

The interplay between microglial activation and lysosomal dysfunction plays a critical role in ICH pathophysiology [Figure 1]. Following ICH, the rapid accumulation of blood components, such as hemoglobin and iron, triggers robust microglial activation. While this response is essential for hematoma clearance and tissue repair, excessive or dysregulated microglial activation can exacerbate neuroinflammation and SBI [68]. Lysosomes are central to microglial phagocytic function, facilitating the degradation of erythrocytes, cellular debris, and toxic metabolites after hemorrhage. However, the overwhelming burden of hemorrhagic byproducts can impair lysosomal acidification, enzymatic activity, and membrane integrity, leading to lysosomal dysfunction [68]. This dysfunction hampers microglial clearance capacity and promotes the release of pro-inflammatory mediators, perpetuating a cycle of neuroinflammation and neuronal damage. Emerging evidence suggests restoring lysosomal function in microglia can enhance erythrophagocytosis and limit inflammation, thereby mitigating secondary injury after ICH [62]. Targeting key pathways involved in lysosomal biogenesis, autophagy–lysosome flux, and membrane stabilization holds promise as a therapeutic strategy. Furthermore, elucidating the molecular mechanisms that link lysosomal stress to microglial activation – such as lysosomal rupture-induced inflammasome activation – may reveal novel intervention points for modulating microglial responses in the context of ICH [62]. In summary, addressing lysosomal dysfunction is crucial for rebalancing microglial activity after ICH, with the potential to improve hematoma resolution, attenuate neuroinflammation, and ultimately enhance neurological recovery.



**Figure 1:** The progression of an intracerebral hemorrhage (ICH) and its associated immune response, specifically focusing on microglial activation and lysosomal dysfunction

**Overview of experimental methods for assessing lysosomal function and integrity**

A diverse array of experimental methods allows for comprehensive lysosomal function and integrity assessment [Table 2]. Lysosomal pH can be precisely measured using pH-sensitive fluorescent probes, providing insights into the organelle’s acidification capacity, a key indicator of its functional state [69]. The degradative efficiency of lysosomes is often assessed through cathepsin activity assays, which quantify the enzymatic function of essential lysosomal proteases [70]. Proteomic analyses provide a broader perspective by identifying alterations in the lysosomal protein composition and uncovering potential deficiencies or dysfunctions in key enzymes [71]. Immunofluorescence microscopy allows for the visualization of lysosomal morphology and the accumulation of undigested substrates by targeting specific lysosomal-associated proteins [72]. The dynamic process of autophagy and the lysosomal contribution to it can be assessed using autophagic flux assays [73]. Lysosomal membrane integrity, crucial for preventing the leakage of harmful enzymes, can be evaluated using specific dyes or galectin-3 assays [74]. Live-cell imaging with fluorescent dyes allows tracking changes in lysosomal distribution and size [75]. Genetic and molecular analyses can pinpoint gene mutations underlying lysosomal dysfunction [76]. Electron microscopy provides high-resolution ultrastructural details, revealing abnormalities like lysosomal aggregation [77]. Biochemical assays directly quantify the activity of specific lysosomal enzymes, which are vital for diagnosing lysosomal storage diseases [78]. Finally, the accumulation of undigested substrates, a hallmark of lysosomal dysfunction, can be quantified using fluorometric or HPLC techniques [79]. Conventionally, lysosome isolation relies on density gradient centrifugation, which is prone to contamination from mitochondria and peroxisomes. This method requires expensive ultracentrifuges, large sample volumes, and multiple lengthy centrifugation

steps (e.g., 141,000 × g for 90 min), often leading to lysosomal rupture and loss of labile components. Recently, the Lyso-IP method has emerged as an efficient alternative, enabling specific immunoprecipitation of intact lysosomes by tagging the cytoplasmic domain of TMEM192 with a triple hemagglutinin (3 × HA) epitope [80].

Together, these methods offer a multifaceted approach to investigate lysosomal health in various experimental settings.

**Therapeutic implications and opportunities**

Therapeutic strategies that target the modulation of microglia are gaining traction as a means to reduce secondary neuroinflammation and improve neurological recovery after ICH [Table 3]. One such strategy is the intranasal administration of gene-edited microglial exosomes (Exo-124) [81], which has been shown to be effective in preclinical models by reducing peripheral immune cell infiltration and promoting neuroprotection. Edaravone, a well-characterized free radical scavenger, exerts neuroprotective effects by inhibiting the activation of the NLRP3 inflammasome in microglia, thereby attenuating cerebral edema and improving functional outcomes [82]. Similarly, the transplantation of induced neural stem cells has been shown to suppress microglial pyroptosis and reprogram microglial polarization toward an anti-inflammatory phenotype [83]. Inhibiting the colony-stimulating factor 1 receptor with GW2580 reduces pathological microglial proliferation and enhances the infiltration of regulatory CD8<sup>+</sup> CD122<sup>+</sup> T cells into the brain. This contributes to a more balanced immune microenvironment [84]. Transforming growth factor-beta 1 (TGF- $\beta$ 1), an endogenous immunomodulator, has been shown to downregulate pro-inflammatory mediators, such as IL-6. Both experimental and clinical data indicate a correlation between elevated TGF- $\beta$ 1 levels and favorable neurological outcomes [85]. Additional pharmacological agents, including fingolimod (an S1P receptor agonist) and etifoxine (a translocator protein ligand), further promote polarization

**Table 2: Overview of experimental methods for assessing lysosomal function and integrity**

Method	Technique/principle	Key markers/indicators	Application/focus	Reference
Lysosomal pH measurement	Use of pH-sensitive fluorescent probes (e.g., lysosensor) to measure lysosomal acidification	Altered pH levels	Assess acidification and lysosomal integrity	[69]
Cathepsin activity assay	Enzyme activity assays using fluorogenic substrates specific for cathepsin B, D, and L	Enzymatic activity of cathepsins	Monitor lysosomal degradation efficiency	[70]
Proteomic analysis	Mass spectrometry to detect changes in lysosomal protein composition	Altered lysosomal enzyme profiles	Identify defective or deficient lysosomal enzymes	[71]
Immunofluorescence microscopy	Detection of lysosomal proteins (e.g., LAMP1 and LAMP2) and substrates using fluorescent antibodies	Lysosome-associated proteins (e.g., LAMPs)	Visualize lysosomal morphology and substrate accumulation	[72]
Autophagic flux assay	Measures the dynamic process of autophagy using inhibitors like chloroquine or bafilomycin A1	LC3-II, p62, lysosome-autophagosome fusion	Evaluate lysosomal contribution to autophagy	[73]
Lysosomal permeability assessment	Measures LMP using specific dyes like acridine orange or galectin-3 assays	Lysosomal membrane integrity	Detect lysosomal rupture in cell death pathways	[74]
Fluorescence-based lysosomal tracking	Live-cell imaging using dyes like lysotracker to stain acidic compartments	Changes in lysosomal distribution and size	Assess lysosomal localization and morphological alterations	[75]
Genetic and molecular analysis	Gene editing (e.g., CRISPR) or transcriptomics to identify lysosomal dysfunction-causing mutations	Gene mutations in lysosomal enzymes	Elucidate genetic contributions to lysosomal dysfunction	[76]
EM	High-resolution imaging to observe ultrastructural features of lysosomes	Lysosome size, autophagic vacuoles	Detect lysosomal aggregation and abnormal morphology	[77]
Biochemical assays	Quantification of lysosomal enzyme activity in biological samples (e.g., plasma, tissue extracts)	Beta-glucocerebrosidase, alpha-galactosidase	Diagnose LSDs such as Gaucher or Fabry diseases	[78]
Lysosomal substrate accumulation test	Use of fluorometric or HPLC techniques to quantify lysosomal substrate buildup	Accumulated substrates (e.g., sphingolipids, glycosaminoglycans)	Evaluate lysosomal storage and degradation pathways	[79]
Lyso-IP method	Enabling specific IP of intact lysosomes by tagging the cytoplasmic domain of TMEM192 with a (3× HA) epitope	3× HA epitope tag fused to the cytoplasmic domain of the lysosomal membrane protein TMEM192	Isolated intact lysosomes	[80]

LSDs: Lysosomal storage diseases, LAMPs: Lysosome-associated membrane proteins, IP: Immunoprecipitation, TMEM192: Transmembrane protein 192, EM: Electron microscopy, LMP: Lysosomal membrane permeabilization, 3× HA: Triple hemagglutinin, HPLC: High-performance liquid chromatography, CRISPR: Clustered regularly interspaced short palindromic repeats

of microglia toward a reparative M2 phenotype and reduce perihematomal damage in animal models of ICH [97,98]. Notably, the targeted delivery of interleukin-10 (IL-10) through phosphatidylserine liposomes provides a cell-specific immunomodulatory approach that enhances localized anti-inflammatory responses and facilitates tissue repair [88]. Together, these approaches constitute an emerging paradigm in ICH therapy. This paradigm leverages precise modulation of microglial phenotypes to counteract neuroinflammatory injury and improve clinical outcomes.

The diverse therapeutic strategies outlined in the table illuminate promising avenues for addressing lysosomal dysfunction across various neurological conditions. Modulating autophagy through pathways such as Nfk6/mTOR and BAG3 regulation presents opportunities for mitigating damage in cerebral ischemia–reperfusion injury, traumatic brain injury, and Alzheimer’s disease by enhancing the clearance of cellular debris and pathological proteins like tau [89]. While CMA holds potential in brain injuries, careful consideration of its context-dependent effects is warranted [90]. Enzyme replacement therapy remains a cornerstone for lysosomal storage disorders, directly addressing the root cause of enzyme deficiency [86]. Emerging strategies such as exosome-mediated

delivery of microRNAs and mesenchymal stem cell-derived extracellular vesicles offer targeted approaches to modulate neuroinflammation, mitophagy, and mitochondrial homeostasis in conditions such as SAH and hypoxia–ischemia brain damage [87]. Furthermore, pharmacological interventions using Glycogen synthase kinase (GSK) inhibitors [93] and osmundacetone demonstrate the potential to amplify endogenous lysosomal pathways and directly target disease-specific pathology in Parkinson’s and Alzheimer’s-like conditions [94]. Finally, levacetylleucine and small-molecule drug C381 highlight the possibility of improving specific disease mechanisms in Niemann–Pick disease type C and repetitive mild traumatic brain injury by addressing cholesterol transport and regulating autophagy/lysosomal function [95]. However, critical considerations regarding the timing of intervention, effective delivery methods, and the heterogeneity of patient responses will be crucial for translating these therapeutic opportunities into successful clinical outcomes.

## CONCLUSION

Microglial phagocytosis is vital in resolving ICH, facilitating hematoma clearance, and mitigating SBI. However, this beneficial process is often hampered by lysosomal dysfunction, which represents a critical bottleneck

**Table 3: Comparison of therapeutic strategies targeting microglial or lysosomal dysfunction**

Therapeutic strategy	Mechanism	Clinical proximity/target condition	Limitation	Reference
Exo-124	Intranasal delivery of exo-124 reduces neuroinflammation by decreasing immune cell infiltration into the brain	Preclinical; promising but no human trials yet/ICH	Delivery via intranasal route may face scalability and reproducibility issues; gene editing raises biosafety and immunogenicity concerns	[81]
Edaravone	Suppresses the NLRP3 inflammasome in microglia, reducing brain edema and improving neurological function	Clinically approved (e.g., for ALS and stroke in some countries); off-label ICH use feasible/ICH-induced oxidative neuroinflammation	Limited microglial specificity; systemic administration may have off-target antioxidant effects	[82]
iNSCs	Suppresses microglial pyroptosis and promotes anti-inflammatory microglial polarization	Preclinical/experimental; stem cell therapies have ongoing trials in CNS injury but not specifically for ICH/ICH-associated neuroinflammation	Risks include immune rejection, tumorigenicity, and complex transplantation logistics	[83]
CSF1R inhibition (GW2580)	Reduces microglial proliferation and enhances CD8 <sup>+</sup> and CD122 <sup>+</sup> regulatory T-cell infiltration	Preclinical; CSF1R inhibitors (e.g., pexidartinib) approved for non-CNS use/Post-ICH neuroinflammation	Potential for global microglial depletion, impaired immune surveillance, and off-target effects	[84]
TGF-β1	Modulates microglial activation, suppresses IL-6 expression, and supports neurofunctional recovery	Translational/biomarker-supported; clinical data link levels to outcome but not yet used therapeutically/ICH (biomarker-linked therapeutic)	Dual roles in inflammation and fibrosis; systemic modulation may lead to adverse immune or vascular effects	[85]
Fingolimod	S1PR agonist; activates M2-type microglia, reducing edema and neuronal apoptosis	Clinically approved for multiple sclerosis; early ICH studies ongoing/ICH-associated secondary injury	Risk of immunosuppression, bradycardia, and infection; not selective for microglia	[86]
Etifoxine	TSPO ligand that inhibits microglial activation and pro-inflammatory cytokine production	Clinically used (in some countries) for anxiety; being repurposed for CNS injury/ICH-induced perihematomal edema	Off-target benzodiazepine-like effects; TSPO expression is not exclusive to microglia	[87]
IL-10 via phosphatidylserine liposomes	Targeted cytokine delivery to microglia/macrophages modulates inflammatory response	Preclinical; nanocarrier strategies under investigation in neuroinflammation/ICH – enhanced anti-inflammatory therapy	Stability and targeting efficiency of liposomes; risk of systemic immunosuppression	[88]
Nek6 regulation via mTOR pathway	Modulates autophagy to alleviate lysosomal dysfunction	Preclinical/cerebral ischemia-reperfusion injury	Targeting Nek6 selectively in CNS remains a challenge; systemic mTOR modulation has multiple metabolic effects	[89]
CMA	Enhances lysosomal degradation of damaged proteins, though with potential double-edged effects	Mechanistically supported but no targeted clinical therapies yet/brain injuries	CMA enhancement may have unintended degradation of essential proteins; context-dependent efficacy	[90]
BAG3 regulation of autophagy	Reduces tau hyperphosphorylation, synaptic dysfunction, and cognitive deficits	Preclinical; mainly in models of TBI and Alzheimer's/TBI, AD pathology	Lack of delivery method specificity; limited understanding of long-term modulation effects	[91]
ERT	Replaces deficient enzymes to prevent lysosomal substrate accumulation	Clinically established for LSDs like Pompe disease/lysosomal storage disorders (e.g., Pompe)	High cost; BBB penetration is limited; not directly applicable to acquired brain injuries	[86]
Exosome-mediated delivery of miR-486-3p	Reduces neuroinflammation and promotes mitophagy regulation	Preclinical/SAH	miRNA stability and off-target gene silencing remain challenges; exosome production is complex	[87]
Mesenchymal stem cell-derived EV therapy	Enhances mitochondrial homeostasis via delivery of Nrf2-enhanced EVs	Early-phase clinical interest in various CNS injuries/hypoxia-ischemia brain damage	Heterogeneity of EV cargo, large-scale GMP-grade production, and functional consistency need refinement	[92]
GSK-3β inhibitor	Amplifies autophagy-lysosomal pathways by regulating TFEB	Preclinical/early clinical trials in neurodegeneration/Parkinson's disease	Broad kinase effects; potential off-target toxicity and tumorigenesis; autophagy overactivation risks	[93]
Osmundacetone treatment	Promotes lysosomal degradation of β-amyloid plaques, reducing oxidative stress and inflammation	Experimental/natural compound with limited translational progress/Alzheimer's-like pathologies	Bioavailability, BBB permeability, and specificity are unclear	[94]
Levacetylleucine	Improves neurological decline caused by Niemann-Pick disease type C through cholesterol transport	Clinically used (e.g., in Europe) for Niemann-Pick type C/Niemann-Pick disease type C	Narrow indication; not directly applicable to ICH or microglial regulation; unclear generalizability	[95]

Contd...

**Table 3: Contd...**

Therapeutic strategy	Mechanism	Clinical proximity/target condition	Limitation	Reference
Small molecule drug C381	Attenuates vascular damage and regulates autophagy and lysosomal function	Preclinical; proposed for traumatic brain injury models/repetitive mild traumatic brain injury	Unknown safety profile in humans; dual targeting of vascular and lysosomal pathways needs clarification	[96]

SAH: Subarachnoid hemorrhage, IL-10: Interleukin-10, TSPO: Translocator protein, AD: Alzheimer's disease, mTOR: Mammalian target of rapamycin complex 1, LSDs: Lysosomal storage diseases, CNS: Central nervous system, EV: Extracellular vesicle, iNSCs: Induced neural stem cells, CMA: Chaperone-mediated autophagy, TBI: Traumatic brain injury, ERT: Enzyme replacement therapy, TFEB: Transcription factor EB, TGF- $\beta$ 1: Transforming growth factor-beta 1, ICH: Intracerebral hemorrhage, BBB: Blood-brain barrier, CSF1R: Colony-stimulating factor 1 receptor, Exo-124: Gene-edited microglial exosomes, GSK: Glycogen synthase kinase, GMP: Good manufacturing practice, BAG3: Bcl-2-associated athanogene 3, ALS: Amyotrophic lateral sclerosis

limiting the degradative capacity of microglia. Consequently, therapeutic strategies to restore lysosomal function and enhance microglial phagocytosis hold significant promise for improving hematoma clearance and promoting neurological recovery following ICH. Targeting this microglia-lysosome axis offers a compelling avenue for developing novel interventions to address the unmet clinical need in ICH treatment.

#### Data availability statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

#### Financial support and sponsorship

Nil.

#### Conflicts of interest

There are no conflicts of interest.

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