Targeting O-GlcNAcylation in ischemic stroke

Xuan Li, Wei Yang

Ischemic stroke is a common and often devastating disease that primarily affects the elderly. Treatment currently consists predominantly of acute thrombolyis and/or thrombectomy to restore blood flow. This reperfusion therapy, however, is only accessible to a small fraction of stroke patients, and even among these patients, many still suffer lifelong neurologic deficits. Thus, new stroke therapeutics are urgently needed to improve the quality of life for stroke patients.

During the acute/subacute phase of ischemic stroke, a cascade of detrimental signaling events that lead to brain cell death and infarct formation is rapidly triggered, while during the recovery phase, endogenous neurorestorative processes that contribute to spontaneous recovery of neurologic function are believed to be activated (Turner and Yang, 2021). This 2-phase theory largely guides the current search for therapeutic strategies to improve stroke outcome, i.e., cytoprotection in the acute phase and neurorestoration in the recovery phase. As stroke pathophysiology involves many signaling events, especially during the acute/subacute phase, it has become increasingly appreciated that an effective stroke intervention strategy may be expected to deliver beneficial effects through affecting multiple pathways. Notably, targeting O-linked β-N-acetylglucosamine (O-GlcNAc) modification (O-GlcNAcylation) could be such a strategy. Here, we summarize relevant evidence and discuss the implications of modulating O-GlcNAcylation in stroke therapy.

O-GlcNAcylation, endoplasmic reticulum (ER) stress, and ischemic stroke: O-GlcNAcylation, a post-translational protein modification, refers to the process of adding single O-GlcNAc moieties to serine/threonine residues of target proteins. This modification is executed by 2 enzymes: O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA), which add and remove GlcNAc from proteins, respectively. The sugar donor for O-GlcNAcylation is uridine diphosphate N-acetylglucosamine (UDP-GlcNAc). UDP-GlcNAc is a product of the hexosamine biosynthetic pathway (HBP), a glucose metabolism pathway. More than 1000 proteins have been identified as O-GlcNAcylation substrates, and once modified, their function, stability, or subcellular localization can be altered. Hence, it is not surprising that O-GlcNAcylation plays important roles in numerous cellular processes such as signal transduction, gene transcription, and protein quality control. Notably, expression of both OGT and OGA is high in the brain compared to other organs, suggesting a prominent role for O-GlcNAcylation in brain function and health (Ma et al., 2017). Moreover, mounting evidence demonstrates that an acute increase in O-GlcNAcylation protects cells under various stress conditions including serum or glucose deprivation, ER stress, and oxidative stress (Wang and Yang, 2019).

O-GlcNAcylation has been linked to the unfolded protein response (UPR). The UPR is a conserved stress response that is activated in response to ER stress, a pathologic event that occurs in almost all ischemia/reperfusion injury models (Wang and Yang, 2019). One major component of the UPR pathway is controlled by inositol-requiring enzyme 1 (IRE1)-mediated splicing of spliced X-box binding protein-1 (XBP1s), a transcriptional factor. XBP1s can upregulate the expression of HBP enzymes, including glutamine fructose-6-phosphate aminotransferase (GFAT), a rate-limiting HBP enzyme. Thus, XBP1s couples the UPR to O-GlcNAcylation via the HBP. Together, these pathways form the XBP1s/HBP/O-GlcNAc axis (Figure 1) (Wang et al., 2014; Jiang et al., 2017). This axis has been extensively studied in heart and brain ischemia/reperfusion injury. Wang et al. (2014) showed that after heart ischemia, the IRE1/XBP1s UPR branch is activated, and consequently, O-GlcNAcylation is increased. Blocking this increase worsens ischemic damage to the heart. To understand the role of this axis in brain ischemia, our group has performed several studies using various animal models (Jiang et al., 2017; Li et al., 2021; Wang et al., 2021). It is known that after brain ischemia, the UPR is activated, and XBP1s mRNA levels are increased. Using Xbp1s-TG mice, we demonstrated that neuron-specific overexpression of XBP1s increases expression of HBP enzymes and levels of O-GlcNAcylation in the brain, providing the first evidence that the XBP1/HBP/O-GlcNAc axis is functional in the brain (Jiang et al., 2017). Recently, we developed a novel LC-MS/MS-based approach to analyze UDP-GlcNAc in the brain and found that UDP-GlcNAc levels are significantly higher in the brains of Xbp1s-TG mice than in wild-type mice, further supporting the notion that XBP1s modulates O-GlcNAcylation by increasing UDP-GlcNAc production of the HBP (Wang et al., 2021).

To clarify the importance of this axis in stroke outcome, genetic and pharmacologic tools have been exploited. Data have shown that Xbp1-cKO mice with neuron-specific deletion of Xbp1 exhibit worse acute outcomes after transient or permanent ischemic stroke, while Xbp1s-TG mice have better neurologic performance and smaller infarct volumes (Jiang et al., 2017; Wang et al., 2021). Taken together, these findings indicate that activation of the XBP1s UPR branch is beneficial in ischemic stroke. Similar results were reported in a cardiac arrest mouse model (Li et al., 2021). Concurrent with Xbp1s activation, O-GlcNAcylation is also rapidly increased after stroke, which is particularly evident in neurons located in the penumbra, a potentially salvageable brain tissue. This finding suggests that similar to heart ischemia (Wang et al., 2014), stroke activates the pro-survival XBP1s/HBP/O-GlcNAc axis as an initial effort by the brain to help cells withstand ischemic injury. As expected, when Xbp1s is deleted in neurons, stroke-induced O-GlcNAcylation is severely impaired, suggesting that XBP1-mediated activation of O-GlcNAcylation via the axis is a signaling mechanism that underpins the neuroprotective effect of the XBP1 UPR branch (Jiang et al., 2017). Further substantiating this mechanistic link, our recent study demonstrated that worse stroke outcome in Xbp1-cKO mice is reversed by pharmacologically boosting O-GlcNAcylation (Wang et al., 2021). Another line of evidence that the XBP1/HBP/O-GlcNAc axis is a pro-survival pathway in ischemic stroke, comes from a study showing that neuron-
specific OGT knockout mice have less activation of O-GlcNAcylation in the stroke brain and worse stroke outcome (Gu et al., 2017).

Targeting O-GlcNAcylation in ischemic stroke: As discussed before, XBP1/HBP/O-GlcNAc axis could be a therapeutic target for ischemic stroke. Due to a lack of pharmacologic tools to directly modulate the XBP1s UPR branch in the brain, current studies have focused on targeting the O-GlcNAcylation cycle or the HBP. For this purpose, 2 compounds have been extensively evaluated in stroke: thiamet-G and glucosamine.

Thiamet-G is a specific and potent OGA inhibitor, and by blocking GlcNAc removal from target proteins, it can increase O-GlcNAcylation throughout the organ system, including the brain. We have shown that infarct volumes are significantly smaller in mice after treatment with thiamet-G before transient stroke (pretreatment) or after permanent stroke (post-treatment). Moreover, thiamet-G treatment during the acute/subacute stroke phase can offer long-term beneficial effects on the functional outcomes (Wang et al., 2021). Critically, these results are consistent with 2 studies from other groups (Gu et al., 2017; He et al., 2017).

Notably, the enzymatic activity of OGT is highly sensitive to changes in UDP-GlcNAc levels: a high concentration of UDP-GlcNAc can enhance OGT activity, resulting in an increase in O-GlcNAcylation. Thus, another strategy to increase O-GlcNAcylation is to boost UDP-GlcNAc production by targeting the HBP. To this end, glucosamine appears to be an excellent choice. Glucosamine is an amino monosaccharide that is naturally synthesized in the body. Since glucosamine is mainly found in cartilage and connective tissue, its potential therapeutic effects in human osteoarthritis have been the focus of considerable research efforts for decades. Many people with osteoarthritis obtain glucosamine as over-the-counter supplements in most pharmacies and grocery stores, and take them daily. Importantly, in cells, glucosamine can be phosphorylated to glucosamine-6-phosphate, which enters the HBP, bypassing the GFAT-mediated rate-limiting step. Thus, glucosamine can enhance O-GlcNAc production, and then increase global O-GlcNAcylation. Two experimental stroke studies have demonstrated that glucosamine treatment increases O-GlcNAcylation in the brain, and is beneficial in both transient and permanent stroke models (Gu et al., 2017; Wang et al., 2021). Another earlier study also showed that either pretreatment or post-treatment with glucosamine significantly reduces infarct volumes in rats after transient stroke (Hwang et al., 2010). Thus, glucosamine is a potent cytoprotectant in ischemic stroke.

One frequently criticized issue in experimental stroke research is the predominant use of young animals. Indeed, age is the most common non-modifiable risk factor for stroke, and the majority of stroke patients are elderly. Importantly, there are many functional and structural differences between young and aged brains. For example, aging is associated with a decline in the brain’s capacity to restore cellular homeostasis after ischemic stress (Wang and Yang, 2019). It is therefore conceivable that a promising cytoprotective strategy that can be promoted in aged animals after stroke will have a substantially higher likelihood of translational success in the clinic. Thus, it is crucial that new therapeutic targets for stroke be evaluated in aged animals.

Interestingly, activation of O-GlcNAcylation, as seen in young post-ischemic brains, is largely absent in aged brains after both global and focal brain ischemia (Jiang et al., 2017; Wang and Yang, 2019), suggesting compromised ability of aged brains to activate the XBP1/HBP/O-GlcNAc pro-survival axis. One of the major reasons could be diminished availability of basal levels of GlcNAc in the aged brain appears to be stable in rats after maturation till 2 years (Liu et al., 2012). Mechanistically, UDP-GlcNAc availability is diminished in the aged brain, which may account for impaired O-GlcNAcylation activation in the aged brain after ischemia (Wang et al., 2021). These findings again illustrate that experimental stroke studies in young animals do not mimic the aging-related pathologic milieu associated with ischemic stress in aged brains. They also suggest that pharmacologic interventions that can reverse aging-related impairments may be effective in mitigating injury to the aged brain after stroke. Indeed, post-treatment with thiamet-G or glucosamine to reverse impaired O-GlcNAcylation activation in aged mice significantly reduces infarct sizes and improves neurologic function after stroke (Jiang et al., 2017; Wang et al., 2021). Further, a long-term study in which aged rats were subjected to permanent stroke showed that functional outcome was significantly improved at 28 days post-stroke after thiamet-G treatment (Wang et al., 2021).

Perspectives: Many lines of evidence indicate that boosting O-GlcNAcylation can confer organ protection under ischemic conditions. For example, increasing O-GlcNAcylation attenuates both Aβ and tau pathologies in Alzheimer’s disease progression (Ma et al., 2017). Studies have also demonstrated that an increase in O-GlcNAcylation remarkably protects the heart from ischemia/reperfusion injury (Ng et al., 2021). Importantly, we and others have provided strong evidence that pharmacologically increasing O-GlcNAcylation is a promising cytoprotective strategy to improve functional outcomes after ischemic stroke, even in aged animals. Such activation of O-GlcNAc enzyme, glucosamine, deserves further pursuit. First, studies have shown that glucosamine can attenuate inflammation, oxidative stress, ER stress, loss of mitochondrial membrane potential, and apoptosis, all of which occur after ischemic stroke. Second, the beneficial effects of glucosamine on both short- and long-term outcomes after stroke have been demonstrated in young and aged mice or rats. Lastly, glucosamine is safe for human use. Human studies did not find any major adverse effects of glucosamine, even with intravenous infusion. Further, a prospective study reported that people who used supplemental glucosamine habitually had a lower risk for stroke events (Ma et al., 2019). Taken together, these findings indicate that glucosamine represents a promising translational cytoprotectant in stroke therapy. If further confirmed in rigorous preclinical testing (e.g., multicenter preclinical studies), glucosamine treatment may be rapidly advanced to clinical trials.

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Xuan Li, Wei Yang Center for Perioperative Organ Protection, Department of Anesthesiology, Duke University Medical Center, Durham, NC, USA *Correspondence to: Wei Yang, PhD, wei.yang@duke.edu. https://orcid.org/0000-0001-5719-4393 (Wei Yang) Date of submission: September 13, 2021 Date of decision: October 23, 2021 Date of acceptance: November 15, 2021 Date of web publication: April 1, 2022

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