Unfolded Protein Response in Ischemic Stroke

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Many pathologic states can lead to the accumulation of unfolded/misfolded proteins in cells. This causes endoplasmic reticulum (ER) stress and triggers the unfolded protein response (UPR), which encompasses three main adaptive branches. One of these UPR branches is mediated by protein kinase RNA-like ER kinase (PERK), an ER stress sensor. The primary consequence of PERK activation is the suppression of global protein synthesis, which reduces ER workload and facilitates the recovery of ER function. Ischemic stroke induces ER stress and activates the UPR.

Keywords: endoplasmic reticulum (ER) stress ; unfolded protein response (UPR) ; ischemia

1. Introduction

Ischemic stroke, the most prevalent stroke type, is associated with a high risk of mortality and long-term disabilities [1]. Apart from acute reperfusion therapy, treatment options for stroke patients are limited. This underscores the urgency of developing new and effective stroke therapeutics that can mitigate brain damage and improve functional recovery. To this end, a thorough understanding of stroke pathophysiology is key.

After ischemic stroke, massive cell death and blood–brain barrier (BBB) damage lead to a profound change in the extracellular environment of the affected brain regions. In response to this change, many pathways are activated in brain cells, including both pro-survival and pro-death pathways, which represent potential therapeutic targets for stroke treatment. One such pathway is the unfolded protein response (UPR), which is initiated in the endoplasmic reticulum (ER). The ER is the primary site in cells for protein translation, modification, folding, and maturation, as well as calcium buffering [2]. Thus, ER function is critical for cellular protein homeostasis (proteostasis) [2][3]. After stroke, the adverse environment disrupts calcium homeostasis and exposes cells to toxic reactive oxygen species (ROS). Coupled with reduced energy availability, ER function related to proteostasis is impaired after stroke, leading to the accumulation of misfolded or unfolded proteins in the ER—a state known as ER stress [4][5][6]. To restore ER function and reestablish cellular proteostasis, mammalian cells have three major adaptive response pathways mediated by ER stress sensors: ATF6 (activating transcription factor 6), IRE1 (inositol-requiring enzyme 1), and PERK (protein kinase RNA-like ER kinase) [2]. These pathways are branches of the UPR.

A wealth of evidence indicates that stroke causes ER stress and activates the UPR in the brain [4][5][6]. However, the effect of UPR activation in stroke has not been fully established, as data supporting both its detrimental and beneficial roles have been reported. This discrepancy is likely attributed to the interpretation of stroke data related to the PERK pathway. Unlike the ATF6 and IRE1 UPR branches, which are predominantly considered protective in cells under ER stress, the PERK UPR branch can either restore cellular homeostasis or activate cell death processes [7][8][9][10]. In the early phase of ER stress, activation of all three UPR branches leads to the suppression of general protein synthesis, the improvement of ER capacity for protein folding, and enhanced clearance of unfolded proteins, collectively mitigating ER stress and promoting cell survival. However, if ER stress persists and the activated UPR fails to restore ER function, cell death processes ensue, and PERK signaling plays a major role in these processes [7]. Notably, the PERK pathway has been increasingly studied as a therapeutic target for aging-related cognitive decline and neurodegenerative diseases [11][12]. Thus, a better understanding of the role of PERK in stroke outcomes is critically important to the stroke field.

2. General Overview of the Unfolded Protein Response in Ischemic Stroke

UPR activation exerts its effects by modulating both transcriptional and translational programs in cells [2]. Upon sensing ER stress, ATF6 translocates from the ER to the Golgi where it is cleaved to generate the short-form ATF6 (sATF6), while IRE1 becomes an endoribonuclease that mediates the non-conventional splicing of X-box binding protein 1 (XBP1) mRNA, subsequently generating the active XBP1s protein. Both sATF6 and XBP1s are potent transcriptional factors and, once in the nucleus, upregulate expression of many genes related to protein folding, maturation, and degradation, such as ER chaperones GRP78 (or BiP), GRP94, and ERAD-related proteins [2]. Notably, XBP1s can also upregulate expression

of the enzymes involved in the hexosamine biosynthetic pathway (HBP) and thus increase global O-GlcNAcylation [13][14][15]. This post-translational modification has been demonstrated to be a pro-survival response under various stress conditions [16].

Using mice with global deletion of *Atf6* (*Atf6*$^{-/-}$), a previous study showed that these mice exhibited worse brain damage after stroke [17]. Researchers' group has developed a conditional and tamoxifen-inducible sATF6 knock-in mouse line, sATF6-MER [18]. After generating sATF6-MER;Emx1-Cre (sATF6-KI^{Neuron}) mice with neuron-specific expression of sATF6 in the brain, researchers subjected these mice to transient filament middle cerebral artery occlusion (tMCAO), a stroke model, and found that forced activation of the ATF6 branch in neurons significantly reduced infarct volumes and improved neurologic function [18]. Recently, researchers examined sATF6-KI^{Neuron} mice in permanent stroke using photothrombotic (PT) and transcranial MCAO models and observed improved short- and long-term stroke outcomes in these mice [19]. Together, the current data support the notion that activation of the ATF6 branch is protective in ischemic stroke.

Leveraging a gain-of-function mouse model (TRE-XBP1s;Camk2a-tTA [XBP1s-TG^{Neuron}]) and a loss-of-function mouse model (*Xbp1*$^{fl/fl}$;Emx1-Cre [Xbp1-cKO^{Neuron}]), researchers' group has provided the first evidence that activation of XBP1 signaling in neurons is neuroprotective in both transient and permanent ischemic stroke models, and that activation of the XBP1/HBP/O-GlcNAc axis is a critical mechanism that underpins XBP1-mediated neuroprotection [14][15]. In support of this mechanism, researchers also found that pharmacologically boosting O-GlcNAcylation with thiamet-G partially reversed worse stroke outcomes observed in Xbp1-cKO^{Neuron} mice [15]. Consistently, it has been shown that *Xbp1s* overexpression in cardiomyocytes protects the heart from ischemia/reperfusion injury, reducing the infarct area by nearly 50% [13]. Of note, however, a recent study reported that sustained overactivation of XBP1s signaling in neurons can cause seizure and animal death [20]. Thus, careful consideration is needed for long-term treatments that target IRE1/XBP1 signaling.

Under ER stress, activated PERK phosphorylates serine 51 of the α subunit of eukaryotic translation initiation factor 2 (eIF2) which leads to attenuation of global protein synthesis, as detailed below. The consequence is a reduction in ER workload, which facilitates the restoration of ER homeostasis. An increase in phosphorylated eIF2α (p-eIF2α) and protein synthesis inhibition (PSI) has been found in the ischemic brain [21][22][23]. However, this observation alone does not necessarily indicate that the PERK branch is the cause of stroke-induced PSI, because four kinases—GCN2, PKR, HRI, and PERK—have been identified as eIF2α kinases [8]. These kinases are differentially activated according to stress types: GCN2 responds to nutrient deprivation, PKR to double-stranded RNA, HRI to low heme concentration, and PERK to ER stress. Using global *Perk* knockout mice, an early study suggested that PERK is responsible for post-ischemic eIF2α phosphorylation [24]. Further supportive data regarding the involvement of the PERK pathway in stroke-induced PSI come from experiments on *Perk*$^{f/f}$;Camk2a-Cre (PERK-cKO^{Neuron}) mice in which *Perk* is specifically deleted in neurons [21]. In these conditional knockout mice, ischemia-induced p-eIF2α is markedly suppressed, and levels of protein synthesis in the brain are higher in PERK-cKO^{Neuron} vs. control mice. Moreover, salubrinal, an inhibitor of p-eIF2α de-phosphorylation, can largely reverse the elevated protein synthesis observed in PERK-cKO^{Neuron} mice [21]. All these data together support a mechanistic link between PERK, p-eIF2α activation, and PSI in the ischemic brain. Moreover, PERK-cKO^{Neuron} mice have worse acute stroke outcomes [21]. However, it must be noted that although the current data indicate that a protein synthesis-related mechanism drives the effects of PERK signaling on stroke outcomes, other PERK-controlled downstream pathways/processes may also be involved.

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