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INVITED REVIEW

Axon injury induced endoplasmic reticulum stress and neurodegeneration

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Abstract

Injury to central nervous system axons is a common early characteristic of neurodegenerative diseases. Depending on its location and the type of neuron, axon injury often leads to axon degeneration, retrograde neuronal cell death and progressive permanent loss of vital neuronal functions. Although these sequential events are clearly connected, ample evidence indicates that neuronal soma and axon degenerations are active autonomous processes with distinct molecular mechanisms. By exploiting the anatomical and technical advantages of the retinal ganglion cell (RGC)/optic nerve (ON) system, we demonstrated that inhibition of the PERK-eIF2α-CHOP pathway and activation of the X-box binding protein 1 pathway synergistically protect RGC soma and axon, and preserve visual function, in both acute ON traumatic injury and chronic glaucomatous neuropathy. The autonomous endoplasmic reticulum (ER) stress pathway in neurons has been implicated in several other neurodegenerative diseases. In addition to the emerging role of ER morphology in axon maintenance, we propose that ER stress is a common upstream signal for disturbances in axon integrity, and that it leads to a retrograde signal that can subsequently induce neuronal soma death. Therefore manipulation of the ER stress pathway may be a key step toward developing the effective neuroprotectants that are greatly needed in the clinic.

Key Words: *endoplasmic reticulum stress; axonopathy; retinal ganglion cell; optic nerve; neurodegeneration; CHOP; XBP-1*

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Introduction

Axonopathy is a common early characteristic of neurodegenerative diseases in the central nervous system (CNS), including Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), hereditary spastic paraplegia, multiple sclerosis and glaucoma (Conforti et al., 2014). Axon degeneration often leads to retrograde neuronal cell death or atrophy and progressive permanent loss of vital neuronal functions. Deciphering the upstream signals that trigger the neurodegeneration cascades in both neuronal axon and soma is a key step toward developing the effective neuroprotectants that are greatly needed in the clinic. Although neuronal soma and axon degeneration are active autonomous processes with distinct molecular mechanisms (Conforti et al., 2014; Gerdts et al., 2016), they are clearly connected as sequential events (Li et al., 2013). We previously showed that optic nerve (ON) injury induces endoplasmic reticulum (ER) stress in retinal ganglion cells (RGCs), which plays an important role in RGC death in both acute ON traumatic injury and chronic glaucomatous neuropathy (Hu et al., 2012). By exploiting the anatomical and technical advantages of the RGC/ON system and AAV-mediated RGC-specific gene targeting for studies of these two mouse models of optic neuropathies, we also demonstrated that inhibition of the protein kinase RNA-like endoplasmic reticulum kinase (PERK)-eukaryotic initiation factor 2 alpha (eIF2α)-CCAAT/enhancer-binding protein homologous protein (CHOP) pathway and activation of the X-box binding protein 1 (XBP-1) pathway synergistically protect both RGC soma and axon, and preserve visual function (Yang et al., 2016). Therefore we propose that ER stress is a common upstream signaling mechanism for both neuronal axon and soma degeneration and suggest that targeting ER stress molecules is a promising therapeutic strategy for neuroprotection in CNS axonopathies.

ER Stress and Neuronal Cell Death

The neuronal ER network is a continuous membrane system that comprises the nuclear envelope; sheet-like rough ER (rER) decorated with polyribosomes is present predominantly in neuronal perikarya and proximal dendrites; and tubular smooth ER (sER) distributed throughout the axons and distal dendrites. ER physically interacts with, and is functionally coupled with, other cellular organelles and plasma membrane. ER is known for synthesis and proper folding of membrane and secreted proteins in eukaryotic cells. When the ER is overwhelmed by unfolded and misfolded proteins, cells experience ER stress and activate a complex

cascade of adaptive reactions, a process that is called the unfolded protein response (UPR) (Wang and Kaufman, 2016). ER stress occurs in many neurodegenerative diseases; modulating it protects neurons and improves functional recovery (Hetz and Mollereau, 2014; Wang and Kaufman, 2016). Thus unresolved ER stress could be a common mechanism for neurodegeneration in a broad range of neurological diseases.

Three distinct ER-resident proteins sense stress and initiate the UPR pathways: activating transcription factor-6α (ATF6α), inositol-requiring protein-1 (IRE1α) and PERK. ATF6α is cleaved sequentially by site-1 protease (S1P) and site-2 protease (S2P) in Golgi apparatus to generate a cytosolic fragment which acts as an active transcription factor (ATF6f). Similar to the IRE1α pathway, ATF6f induces expression of ER chaperones to promote protein folding and it activates genes that are involved in ER-associated protein degradation (ERAD), which is generally considered cytoprotective. Interestingly, mutations of ATF6α have been identified recently in degenerative retinal disease, and ATF6α deletion mice develop photoreceptor dysfunction with ageing (Kohl et al., 2015). IRE1α, a bi-functional enzyme that contains both a Ser/Thr kinase domain and an endoribonuclease (RNase) domain, initiates this protective UPR pathway by mediating the splicing of XBP-1 mRNA to generate an active (spliced) form of the transcription factor, XBP-1s. The IRE1α-XBP-1s pathway targets genes that function in increasing ER protein-folding capacity and facilitating degradation of misfolded proteins through ERAD. In addition to its transcriptional function, XBP-1 directly interacts with Forkhead box O1 (FOXO1) to assist its degradation through the proteasome system (Zhou et al., 2011), which blocks FOXO-dependent apoptosis. We detected transient increase of XBP-1s after ON injury and found that sustained overexpression of XBP-1s significantly promotes RGC survival (Hu et al., 2012). XBP-1 activation has been shown to protect neurons in models of both AD and PD, and to improve locomotor recovery after experimental spinal cord injury (Hetz and Mollereau, 2014).

A critical feature of UPR dynamics in chronic ER stress, which may contribute to the pathology of many diseases, is the early and transient, but protective, IRE1α-XBP-1 and ATF6α activation versus the late and persistent, but pro-apoptotic, PERK-CHOP activation (Lin et al., 2007; Hu et al., 2012). PERK phosphorylates and inactivates eIF2α to attenuate global mRNA translation and therefore reduce protein load on the ER. However, phosphorylated eIF2α (eIF2α-P) induces expression of a pro-apoptotic molecule, CHOP, by selectively activating translation of ATF4. As a negative feedback mechanism, ATF4 and CHOP induce expression of growth arrest and DNA-damage-inducible protein 34 (GADD34) to facilitate dephosphorylation of eIF2α-P and resume global mRNA translation. ATF4 and CHOP can form heterodimers to upregulate protein synthesis directly and induce oxidative stress, which causes cell death (Wang and Kaufman, 2016). CHOP has been associated with apoptosis downstream of ER stress through down-regulating anti-apoptotic Bcl2, upregulating pro-apoptotic BH-3 only molecules Bim and PUMA, activating death receptor 5 (DR5). Conversely, deletion of CHOP is beneficial in many non-neuronal disease models (Wang and Kaufman, 2016). Consistent with the theme that sustained activation of the PERK-CHOP pathway and diminished activation of IRE1α-XBP-1 are detrimental for cell survival in prolonged ER stress, we found that deletion of CHOP and activation of XBP-1 synergistically protect RGC and preserve visual function in mouse optic neuropathies (Hu et al., 2012; Yang et al., 2016).

Unlike CHOP, phosphorylation of eIF2α by PERK seems to be a double-edge sword. On one hand, eIF2α-P inhibits cap-dependent mRNA translation, which counteracts ER stress and enables the cell to achieve a new homeostasis and survive by reducing the protein workload of ER. For example, activation of the PERK-eIF2α-P pathway is essential for pancreatic β cell survival, and sustained eIF2α-P by inhibition of eIF2α-P phosphatase enables motor neuron survival in mouse disease models (Hetz and Mollereau, 2014; Wang and Kaufman, 2016). On the other hand, emerging evidence indicates that eIF2α-P also leads to neuronal cell death and dysfunction in AD and prion disease, and that downregulation of eIF2α-P by a PERK inhibitor or de-repression of protein translation by small molecules results in potent neuroprotection (Baleriola et al., 2014; Hetz and Mollereau, 2014; Halliday et al., 2015). We found that genetic blocking of eIF2α-P significantly increases RGC survival (Yang et al., 2016). However, blocking eIF2α-P produces significantly greater RGC protection than CHOP deletion. This disparity in neuroprotection between CHOP inhibition and eIF2α-P inhibition highlights the CHOP-independent role of eIF2α-P in neurodegeneration. Future experiments will determine whether CHOP deletion and eIF2α-P blockade act synergistically to promote more potent neuroprotection. It will be of great interest to decipher the downstream mechanism by which eIF2α acts independently of blocking CHOP to contribute to neuroprotection. Resumed protein synthesis after blocking eIF2α-P may increase cell survival proteins. Intriguingly, X-linked inhibitor of apoptosis (XIAP) is a candidate since eIF2α-P downregulates its translation in a CHOP-independent manner, which contributes to cell death induced by chronic ER stress (Hiramatsu et al., 2014).

ER Stress and Axon Degeneration

We found that CHOP deletion and XBP-1 activation also synergistically promote RGC axon survival (Yang et al., 2016), consistent with previous findings that CHOP inhibition protects motor neuron axons (Li et al., 2013). The axon protection is either secondary to rescue of the RGC soma or an axonal autonomous effect of ER stress manipulation. We favor an axonal autonomous mechanism because we found similar axon protection when the ON was cut, and RGC somata in retina completely separated from their axons (Yang et al., 2016). Optic neuropathies are likely to have mechanisms in common with other neurodegenerative diseases, such as ALS, AD, PD and MS. Thus axon injury-induced neuronal ER stress could be a common mechanism for both neuronal soma and axon degeneration, suggesting an intra-axonal site of action (Li et al., 2013). That axon degeneration is an actively regulated axon autonomous process is supported by the identification of several key molecules that are involved in Wallerian degeneration through regulation of axonal nicotinamide adenine dinucleotide (NAD^+) metabolism (Conforti et al., 2014; Gerdts et al., 2016). It will be important to determine whether ER stress manipulation affects the NAD^+ levels in axons after axon injury. Additional molecules have recently been found to be critical for axonal degeneration, including SCG10 (superior cervical ganglion 10) and mitogen-activated protein kinase (MAPK) cascade (Yang et al., 2015; Gerdts et al., 2016). It will be of great interest to determine whether there is crosstalk between these pathways and ER stress and

whether modulations of ER stress act synergistically with these pathways to provide more potent neuroprotection.

How is ER Stress Initiated in Axotomized Neurons?

The significant unanswered question is how ER stress is activated in response to disrupted axonal integrity. There is no evidence showing accumulation of misfolded proteins in neuronal ER after axon injury. It is plausible that ER stress in the neuronal soma is either induced indirectly by a retrograde injury signal from the axon, or that axonal ER stress is initiated first and subsequently translocated to the cell body. A wave of intra-axonal Ca^{2+} elevation after axon injury propagates retrogradely to the cell body, where it may disturb Ca^{2+} homeostasis and induce neuronal soma ER stress that results in excitotoxicity and apoptosis (Mattson et al., 2000). Interestingly, traumatic ON injury induces Ca^{2+} influx in both axon and soma, which has been implicated in RGC death (Prilloff et al., 2007). There is also evidence that the UPR pathways can be activated locally in axons. For example, it has been shown that XBP-1 mRNA splicing occurs initially in neurites and that the XBP-1s is transported back to the neuronal soma after BDNF stimulation (Hayashi et al., 2007). Similarly, elevated levels of eIF2α-P and ATF4 have been detected in the axons of cultured neurons treated with $A\beta_{1-42}$ (Baleriola et al., 2014).

But how does axotomy induce ER stress in axons? The critical role of Ca^{2+} in ER function makes it the leading candidate as the messenger connecting axon injury, ER stress, apoptosis and axon degeneration. sER is the major intracellular Ca^{2+} store and maintains a much higher calcium concentration (10–100 μM) than cytoplasm (100–300 nM). The gradient depends on the inward Ca^{2+} pump in the ER membrane, SERCA (sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase), and the ER Ca^{2+} releasing channels, the inositol 1,4,5-triphosphate (IP3) receptors (IP_3Rs) and the ryanodine receptors (RyRs) (Mattson et al., 2000; Stirling and Stys, 2010). Thapsigargin, a SERCA inhibitor, is the most commonly used experimental ER stress inducer, because ER is extremely sensitive to Ca^{2+} depletion. Elevation of intra-axonal Ca^{2+} is a characteristic neuronal response to axon injury that correlates with axon degeneration. It is due to both Ca^{2+} efflux from ER through IP_3Rs and RyRs and Ca^{2+} influx through the plasma membrane (Stirling and Stys, 2010). It is therefore possible that perturbation of intra-axonal Ca^{2+} homeostasis by axon injury stimulates ER stress in axons. Indeed, ER Ca^{2+} depletion can activate PERK, which in turn activates calcineurin, a Ca^{2+}/calmodulin-dependent protein phosphatase, to dephosphorylate calnexin and restore ER Ca^{2+} by activating SERCA (Wang et al., 2013).

Summary

There is increasing evidence that neuronal ER stress and the UPR pathways play a causal role in neurodegeneration. Our recent results reveal axon injury-induced ER stress to be a new link between axonopathy and neurodegeneration. The findings indicate that neuronal ER stress serves as a general upstream signal for both neuron apoptosis and axon autonomous degeneration, and that modulation of the UPR molecules represents a novel and promising strategy for neuroprotection. A more thorough understanding of how ER stress is activated and transported in neurons, and how UPR pathways crosstalk with other key signaling pathways to determine the fate of neuronal somata and axons, is a prerequisite for developing relevant treatment. As a working hypothesis we propose that local axonal ER stress is involved in axon degeneration first, and that it leads to a retrograde signal that can induce neuronal soma death at a later time. What that signal is and the mechanisms by which it is transmitted are intriguing questions for future studies.

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