The neuroprotective potential of endoplasmic reticulum chaperones

Diseases of the nervous system are characterized by axon dysfunction, axon degeneration, and neuronal cell death. The roster of neurodegenerative diseases is striking in the selective vulnerability of the affected neuronal populations and the timing of the onset of disease manifestation and functional deficits. We are particularly fascinated by the extreme long term progression of many of these diseases prior to onset of significant functional loss and believe this reveals essential qualities of these diseases and offers considerable therapeutic opportunity. Though a complete mechanistic understanding of axon maintenance and neurodegeneration remains elusive, the primary process of neurodegeneration appears similar (e.g., Wallerian degeneration) across neuronal populations, as does caspase-dependent or -independent apoptotic cell death and likewise other forms of cell death (e.g., necrosis and necroptosis). Thus, it seems probable that the basic mechanisms of neuronal cell death and degeneration can be selectively manipulated through core cell processes, thereby preventing and/or halting the disease onset and progression.

One of the most intriguing of these core processes that determine cell fate and activity is the unfolded protein response (UPR), a highly conserved system in eukaryotes activated when protein folding is interfered with, resulting in an accumulation of misfolded or unfolded proteins in the endoplasmic reticulum (ER). This condition where the ER homeostasis is disrupted can be induced by a variety of stressors including infection, disease, injury, extreme metabolic load, and many others. Three ER transmembrane proteins, inositol-requiring enzyme 1 (IRE1), activating transcription factor 6 (ATF6), and PKR-like endoplasmic reticulum kinase (PERK), essentially act as sensors of ER dishomeostasis by effectively monitoring protein quality within the ER. Under normal healthy conditions, these sensors are bound by the ER chaperone, glucose-regulated protein 78 (GRP78), which is also known as immunoglobulin heavy chain-binding protein (Bip). This association keeps ER stress pathways inactive (Boriushkin et al., 2014; Maly and Papa, 2014). However, in cells under stress or in poor health, misfolded proteins accumulate in the ER leading to GRP78 dissociating from IRE1, ATF6, and PERK, thus elegantly activating the UPR (Figure 1). Once unbound from GRP78, IRE1, ATF6, and PERK activate signaling cascades that initially work to alleviate ER stress. The attempt to return to ER homeostasis involves degrading protein and mRNA as well as attenuating translation of most genes while upregulating genes that increase the capacity of the ER to process protein (Figure 1). If ER stress remains unresolved and homeostasis is irredeemable, the balance of the UPR pathways will tilt toward apoptosis and away from survival (Ma et al., 2014). Generally, the PERK pathway, which is initially crucial to attenuating protein synthesis by phosphorylating eIF2α, advances to promote the transcription and translation of apoptosis-related genes such as C/EBP homologous protein-10 (CHOP). The IRE1 and ATF6 pathways are critical to the expression of ER chaperones and the pro-survival aspects of the UPR (Boriushkin et al., 2014). The balancing act among these three pathways determines the opposed outcomes of survival or death, and is critical to individual cell survival and overall tissue health.

The potential for ER chaperones to act as general neuroprotective factors is intriguing. Neurons typically have high metabolic loads and are thus naturally dependent on ER chaperones. Neurons are also extremely long-lived and thus can be challenged with significant ER stress persistently over years and are not replaced easily or at all. The neural retina is particularly relevant to these ideas as several retinal diseases, including glaucoma, retinal degeneration, and diabetic retinopathy, are directly linked to increases in ER stress pathways and these diseases can have onset in adulthood and aging (e.g., age-related macular degeneration), persist for decades, and have a wide variation in the magnitude of damage (Zhang et al., 2014). Further, ER chaperones are potentially valuable neuroproteoctants because they have relatively specific affects, unlike transcription factors for example, and can act in any cell type.

The ER chaperone p58IPK is linked to neuronal survival by attenuating ER stress (Boriushkin et al., 2014). Our recent work with the p58IPK knockout (KO) mouse demonstrates that p58IPK is critical to retinal ganglion cell (RGC) ER stress response and survival (Boriushkin et al., 2015). We show that p58IPK is highly expressed in RGCs and inner retinal neurons in mice, consistent with retinal neurons being under high metabolic load and under persistent stress. Adult p58IPK KO mice have fewer RGCs than wild type (WT) counterparts suggesting a critical role for p58IPK in promoting survival over extended time periods in a neuronal population under long term stress. Indeed, p58IPK KO mice are also more sensitive to retinal injury as we demonstrate directly in a glutamate toxicity model (i.e., N-methyl-d-aspartate (NMDA) intravitreal injection) in which we find higher levels of RGC death in p58IPK KO mice than in WT mice as well as increased expression of CHOP (Boriushkin et al., 2015). In vitro, overexpression of p58IPK in stressed R28 cells (a retina-derived cell line) attenuates ER stress as measured by expression of multiple proteins involved in the UPR. For example, both CHOP expression and the activation of caspase-3 are reduced significantly in R28 cells overexpressing p58IPK (Boriushkin et al., 2015). Furthermore, p58IPK overexpression increases cell survival through an increase in anti-apoptotic Bcl-2 expression and a decrease in pro-apoptotic BAX expression, direct indications of the neuroprotective effects of p58IPK (Boriushkin et al., 2015). This work clearly demonstrates that an ER chaperone has a neuroprotective role in an at-risk subpopulation of neurons. As p58IPK is a critical component of the highly conserved UPR, its protective effects would seemingly translate to other systems within, and outside, the nervous system. One verification of this related to the protective effects of p58IPK on cells under oxidative stress (Boriushkin et al., 2015) is that β cell function in p58IPK KO mice is improved with anti-oxidant treatment (Han et al., 2015).

Though we have suggested a critical role for ER chaperones in long-term endogenous management of disease-induced ER stress, acute injury models of retinal damage also indicate the neuroprotective potential of ER chaperones. Acute retinal injury leads to activation of the UPR. In a recent study, overexpression of spliced Xbp1 significantly increased RGC survival after optic
nerve injury (Hu et al., 2012). Xbp1 is an important regulator of ER chaperones, including p58IPK, and is critical to the pro-survival aspects of the UPR (Figure 1). Thus, pushing the balance of the UPR pathways towards pro-survival is relevant to acute injury as well. Furthermore, Hu and associates demonstrated that delayed induction of Xbp1, e.g., 2–3 weeks after chronic RGC injury caused by increased intraocular pressure, still showed significant protective effects on RGCs. Interestingly, these effects appear to be independent of the CHOP-mediated proapoptotic UPR pathway. Future investigation to elucidate whether Xbp1 may exert protection of retinal neurons through its downstream ER chaperones, such as GRP78 and p58IPK, is of interest.

Apart from traditionally defined neurodegenerative diseases, a wide range of retinal disorders such as diabetic retinopathy and age-related macular degeneration are characterized by various vascular abnormalities, including vascular cell death, tight junction damage, vascular leakage, retinal edema, ischemia, and neovascularization, which ultimately lead to retinal neuronal degeneration. One of the most significant recent advances in the treatment of diabetic retinopathy and age-related macular edema with significant success is anti-VEGF therapeutics. VEGF is up-regulated in cells under ER stress and its angiogenic effects in retina can lead to proliferative retinopathy and severe vision loss in diabetics (Ma et al., 2014). Interestingly, p58IPK attenuates the upregulation of VEGF in human retinal capillary endothelial cells (Li et al., 2008), though the mechanisms by which p58IPK reduces VEGF production remain elusive. Thus, a current successful therapeutic avenue, anti-VEGF treatment, is linked directly to UPR pathways in a mechanistically straightforward way involving neuroprotective effects of ER chaperones. Furthermore, studies have also established a critical role of the UPR in regulation of inflammation and immune response and that enhancing the adaptive UPR signaling mediated by Xbp1 effectively prevents TNF-α-induced endothelial cell inflammation and vascular leakage in the retina (Ma et al, 2014). Additionally, p58IPK modulates inflammatory response through regulation of dsRNA-dependent protein kinase (PKR) (Boriushkin et al., 2014). Given the multifaceted functions of the UPR and ER chaperones in regulation of cell death, inflammation, and angiogenic factor production, it is plausible to investigate the potential of enhancing ER chaperones (such as p58IPK) as a novel approach to the treatment of retinal diseases such as diabetic retinopathy through reducing VEGF-mediated vascular lesions.

Figure 1 Accumulation of unfolded and misfolded proteins induces ER stress and activates the UPR.

Cells in homeostasis use ER chaperones (pink) to assist in processing newly made protein (maroon) through the ER. The ER chaperone, GRP78 (black), associates with ATF6 (dark blue), PERK (green), and IRE1 (purple) thereby keeping these three sensors inactive. ER stress develops when unfolded and misfolded proteins accumulate and sequester GRP78. GRP78 becomes unbound from ATF6, PERK, and IRE1, thereby activating them and the UPR. Activated ATF6 translocates to the Golgi and is cleaved into an active transcription factor enhancing the transcription of ER chaperones and XBP1. IRE1 and PERK become phosphorylated (red) and activate signaling cascades. IRE1 induces the splicing of XBP1 full length mRNA into spliced XBP1 mRNA, which is translated into XBP1 protein (light purple). XBP1s upregulates the expression of ER chaperones such as p58IPK in an attempt to return the cell to homeostasis. PERK activation leads to phosphorylation of eIF2α which inhibits general protein translation in an attempt to reduce ER stress. However, activated eIF2α allows translation of ATF4 (light blue), which increases expression of proapoptotic genes, including CHOP. The balance of these three pathways of the UPR determines whether the cell returns to homeostasis or undergoes cell death. ER: Endoplasmic reticulum; UPR: unfolded protein response; GRP78: chaperone, glucose-regulated protein 78; ATF: activating transcription factor; PERK: PKR-like endoplasmic reticulum kinase; IRE1: inositol-requiring enzyme 1; CHOP: C/EBP homologous protein; eIF2α: eukaryotic translation initiation factor-2α.
while protecting retinal neurons and restoring retinal function.

The same concept is applicable to other diseases in the central and peripheral nervous systems. Because stress from many disease conditions like diabetes can persist for decades consistently threatening retinal function through multiple avenues, the balancing act of maintaining the (as of now) irreplaceable retinal neurons is constant and critical. Similar challenges are presented in neurodegenerative diseases such as Alzheimer’s disease, Parkinson’s disease, and Huntington’s disease, all of which are characterized by the accumulation of misfolded proteins (Maly and Papa, 2014) and protein misfolding has long been directly and causally linked to neurodegeneration (Uehara et al., 2006). Clearly, attenuating the accumulation of misfolded proteins is potentially directly addressed by ER chaperones. At the cellular level, diabetes and neurodegenerative diseases are essentially diseases of small cellular imbalances. Over weeks, months, years, and even decades these imbalances are managed by endogenous cellular responses such as the UPR. Although some damage incurred is due to secondary issues, for example, the breakdown of the blood-retina-barrier in diabetes can lead to eventual neuronal loss, the primary deficits can still be addressed at the cellular level. Further, the long term progression of many of these diseases without obvious functional deficits suggests that each cell lost is relatively inconsequential, but the sum total over time can be catastrophic. In this regard, the potential of chaperones is exciting in that they directly address cellular imbalance (ER stress, protein aggregation, etc.) and do so continuously and effectively over extended time periods. Moreover, since chaperones are highly conserved and ubiquitous in the organism, every cell type can potentially be treated. Additionally, molecular chaperones such as GRP78 and p58\(^{IPK}\) are thought to prevent protein aggregations by binding to exposed hydrophobic residues (Tao and Sha, 2011), providing an additional potential avenue for neuroprotection with ER chaperones.

In summary, molecular chaperones and the manipulation of the UPR pathways have exciting therapeutic potential as neuroprotectors at the cellular level for a variety of neurodegenerative diseases. Additionally, the importance of ER chaperones in regulating the production and homeostasis of neurotrophic factors through autocrine, paracrine, and endocrine signaling is worth pursuing. Furthermore, the implications of ER chaperone manipulation in the development and regeneration of neurons in the retina as well as other neural systems is potentially a very promising area for future study. All in all, in-depth understanding of the functions, in particular the neuroprotective potential, of ER chaperones in healthy and stressed neural tissues may provide an intriguing approach in managing multiple disease types to preserve long-term functionality.

This work is supported, in part, by NIH/NEI grants EY019949 and EY025061, American Diabetes Association Research Grant #7-11-BS-182, and an Unrestricted Grant to the Department of Ophthalmology, SUNY-Buffalo, from Research to Prevent Blindness.

Todd McLaughlin, Sarah X. Zhang*

Departments of Ophthalmology and Biochemistry, Ross Eye Institute, University at Buffalo, State University of New York, Buffalo, NY, USA; SUNY Eye Institute, State University of New York, Buffalo, NY, USA

*Correspondence to: Sarah X. Zhang, M.D., xzhang38@buffalo.edu.
Accepted: 2015-06-27
doi:10.4103/1673-5374.162696 http://www.nrronline.org/


References


