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Harnessing the integrated stress response for the treatment of multiple sclerosis

Sharon W. Way and **Brian Popko***

Department of Neurology, The University of Chicago Center for Peripheral Neuropathy, The University of Chicago, Chicago, IL 60637, USA

Summary

Multiple sclerosis (MS) is a chronic demyelinating autoimmune disease of the central nervous system (CNS) with no known cure. Though a dozen immunomodulatory therapies exist, their impact on progression of disease appears limited. The field has hence focused on alternate strategies for treatment such as enhancing remyelination and CNS repair. Recent progress has been made on a third complimentary treatment approach that involves protecting oligodendrocytes, and the myelin they generate and maintain, from inflammatory-mediated death via enhancement of the integrated stress response (ISR). Studies in cells and mouse models of MS have demonstrated that the ISR, an innate protective pathway that maintains proteostasis, may be effectively harnessed to aid in the protection of oligodendrocytes and myelin during inflammation. With one ISR-modifying drug already in clinical trial and a number of potential future therapies under investigation, this approach may offer an important component in halting the progression of multiple sclerosis.

I. Introduction

In 1993, the Food and Drug Administration (FDA) approved the first drug for the treatment of multiple sclerosis (MS), the immunosuppressive cytokine IFN-β1b[1,2]. Currently, more than two decades later, clinicians have a choice of 12 drugs – all immunomodulatory in nature – from which to devise therapeutic strategies[3], an unprecedented advance for a

*Please direct all correspondence to: Brian Popko, Ph.D., Department of Neurology, MC 2030, The University of Chicago, 5841 S. Maryland Ave, Chicago, IL 60637, 773-702-4953, bpopko@neurology.bsd.uchicago.edu.

Search strategy and selection criteria

We searched PubMed for articles in English published from Jan 1, 1990 to November 8, 2015 using the search terms “multiple sclerosis AND cellular stress”, “integrated stress response” and “unfolded protein response”. Additional references were identified from the reference lists of articles deemed most pertinent to this review. The final reference list was generated on the basis of applicability to the themes discussed in this review.

Contributors

SWW and BP contributed equally to all aspects of manuscript preparation, including the writing of the manuscript, the preparation of the figures and tables, and the search for appropriate literature references.

Declaration of interests

We declare no competing interests.

disease that was first defined in the mid-19th century[4]. Nonetheless, while the available therapeutics have been found to be effective at delaying or alleviating relapses with varying degrees of success, it has now become apparent that strategies that provide CNS protection and/or CNS repair are needed for a comprehensive therapeutic approach to MS[5–7]. This realization has encouraged examination into non-immune strategies by which MS may be targeted.

Though the etiology of MS is unknown, it is primarily recognized as an autoimmune disease that is characterized by inflammation, oligodendrocyte loss, demyelination, and axonal degeneration[8]. Recent studies strongly suggest that the progression of disease is driven by irreparable axon loss[9–11]. Attention in the field has thus focused on CNS reparative approaches to prevent this degeneration. Oligodendrocytes, which not only produce and maintain myelin but also provide trophic support to axons[12,13], have hence entered the spotlight as the primary target cell for potential new MS therapies[5]. These complimentary approaches may be grouped into three categories. In MS, the loss of mature, myelin-maintaining oligodendrocytes and pre-myelinating oligodendrocyte precursor cells (OPCs) during inflammation has been well-documented. Nonetheless, unaffected OPCs have been observed to quickly proliferate, migrate to the areas of damage, and undergo differentiation in order to remyelinate unprotected axons as needed[14–16]. The remission of symptoms in early-stage relapsing-remitting MS patients has been attributed in large part to this effective repair process[17]. As inflammatory events accumulate, however, remissions become less common and less reparative[18]. The first two therapeutic strategies therefore seek to address the finding that the increasing disability in MS patients is correlated with the gradual inability of OPCs, despite their presence in damaged areas, to differentiate and properly remyelinate the demyelinated axons. In these two related approaches, pathways relevant to these events are tested for their ability to force OPCs to differentiate or to enhance the ability of differentiating OPCs to remyelinate axons after inflammatory events (reviewed in [6]).

The subject of this review is the third approach, which seeks to protect mature oligodendrocytes and OPCs, and thus myelin and axons, from inflammatory-mediated cell death altogether. Current work in this area has centered on an innate protective mechanism called the integrated stress response (ISR). Manipulations of the ISR in preclinical models of MS have indicated that the pathway plays an important role in the survival of oligodendrocytes during inflammation[19]. Here, we review the progress that has been made in this field and future work that may contribute to the establishment of an effective therapeutic that enhances the ISR to protect oligodendrocytes in MS.

II. The ISR

The ISR is a cytoprotective mechanism that maintains cellular proteostasis in response to stress conditions such as oxygen-glucose deprivation, hypoxia/ischemia, viral infection, amino acid starvation, and endoplasmic reticulum (ER) stress, which occurs when there is an accumulation of misfolded or unfolded proteins in the ER[20] (Figure 1). Activation of the ISR occurs when the eukaryotic translation initiation factor 2 alpha (eIF2α) is phosphorylated, which diminishes global translation while selectively upregulating

translation of chaperone and protective proteins such as the transcription factor ATF4 (Figure 2). These steps seek to restore proteostasis by decreasing the influx of proteins while increasing the chaperone to protein ratio. As part of a tight negative feedback loop that guarantees that translational activity is restored once stress is relieved, ATF4 also upregulates the transcription factor C/EBP homologous protein (CHOP), which in turn increases expression of growth arrest and DNA-damage inducible 34 (GADD34), an essential non-enzymatic cofactor of protein phosphatase 1c (PP1c) that dephosphorylates p-eIF2α in a stress-dependent manner. Further protection of this key translational pathway exists in the form of a constitutive, stress-independent eIF2α phosphatase, CReP. The existence of two phosphatase complexes is particularly notable as it allows specificity in the control of this pathway, such that it is possible to only manipulate stressed cells. Importantly, a final mechanism is in place should efforts to restore proteostasis fail: the protein CHOP has been found to activate the transcription of genes involved in apoptosis. The ISR therefore attempts to protect the cell by encouraging proteostasis and, if this is not successful, resorts to destroying the damaged cell (reviewed in [21]).

III. The ISR in MS and Experimental Autoimmune Encephalomyelitis (EAE)

The highly protective role of the ISR during ER stress indicated that it could play an important role in the survival of oligodendrocytes, which are required to produce a vast amount of membrane protein in a short time span. Other “secretory cells”, such as pancreatic beta cells, have been found to be particularly susceptible to ER stress due to their large protein production requirements[22, 23]. Thus, it was hypothesized that oligodendrocyte loss in MS might be due to the additional stress created by the MS inflammatory environment, which increases demands on protein production and the secretory pathway, thereby overwhelming the already-stressed cell (Figure 3). For example, the T cell-derived inflammatory cytokine interferon-gamma (IFN-γ) causes oligodendrocytes to dramatically increase the expression of the antigen-presenting MHC Class I molecules, which are assembled in the secretory pathway and have been shown to be deleterious to oligodendrocyte function and viability[24,25].

A number of cytotoxic factors that activate the ISR have been implicated as pathogenic effectors in MS (Table 1), suggesting that the ISR might represent a critical response in MS lesions. This speculation was supported when key markers of the ISR were found to be upregulated in human MS tissues[47]. In post-mortem human MS samples, immunohistochemical analysis demonstrated an increase in CHOP[48], notably at the edge of chronic active MS lesions. Using laser capture microdissection, transcript levels of CHOP and ATF4 were also found to be significantly increased in the perilesions of chronic plaques, and in the case of ATF4, active plaques of biopsied patient brain samples from primary progressive and relapsing-remitting patients[49]. Interestingly, in these samples the authors also detected significant increases in levels of CHOP and ATF4 mRNA in normal appearing white-matter (NAWM), which they suggest may indicate “pre-lesional” activity, similar to that found by several other groups[50,51]. Likewise, in a frequently used mouse model of MS, experimental autoimmune encephalomyelitis (EAE), upregulation of ISR proteins have been detected during disease in lesioned tissue[52,53]. By finding increased expression of key ISR proteins at the center and edge of actively demyelinating lesions, these studies provide

evidence that the ISR may participate in oligodendrocyte loss and myelin destruction in response to inflammation. It is worthy to note, however, that this well-documented expression of ISR proteins in MS tissue has been predominantly found in macrophages, microglia, and astrocytes. Nonetheless, as the majority of affected oligodendrocytes have already undergone cell death in the active lesions in which these studies have been conducted, direct detection of stressed oligodendrocytes may require the analysis of lesions at earlier disease stages.

IV. The ISR protects oligodendrocytes and myelin during inflammation

Investigations into the role of the ISR in protecting oligodendrocytes and myelin first required a method of modeling the inflammatory environment found in MS. As a pleiotropic cytokine that plays a key deleterious role in MS and EAE, IFN-γ was found to be an ideal candidate. Secreted by activated T cells, IFN-γ levels are normally undetectable in the CNS but rise considerably during the symptomatic phase of MS[54]. Addition of IFN-γ to differentiated oligodendrocytes *in vitro* resulted in cell death that was associated with activation of the ISR[55]. Moreover, CNS-specific expression of IFN-γ in young transgenic mice led to upregulation of the ISR in oligodendrocytes, mild hypomyelination, ataxia and tremor, and eventually oligodendrocyte apoptosis[55]. This *in vivo* model, called the *GFAP/tTA; TRE/IFN*-γ transgenic mouse, was achieved using a tetracycline-regulated approach, in which the transcriptional control region of the glial fibrillary acidic protein (GFAP) gene drives expression of the tTA protein, which in turn activates the tetracycline regulatory element (TRE) that drives IFN-γ expression specifically in the CNS[56]. This mouse model system thus provides a means of delivering IFN-γ to the CNS, via astrocyte expression, in a tetracycline-controlled manner. The evidence that the presence of IFN-γ leads to oligodendrocyte apoptosis both in culture and in mice confirmed its use as a method of modeling inflammation.

a. Genetic PERK models

Initial studies to establish the role of the ISR in oligodendrocyte and myelin protection centered on experiments with protein kinase RNA-like ER kinase (PERK). PERK is the earliest responder to ER stress, undergoing dimerization and autophosphorylation when deleterious accumulation of misfolded or unfolded proteins threaten to overwhelm the cell. Once activated as p-PERK, the kinase is able to phosphorylate eIF2α[57]. Loss of one allele of PERK has been shown to dramatically increase the sensitivity of cells to ER stress[58] (Figure 4a, b). Developing *GFAP/tTA; TRE/IFN*-γ transgenic mice that were haploinsufficient for PERK displayed a severely exacerbated phenotype such that they were runted, demonstrated severe tremor and ataxia, and exhibited significant hypomyelination and a large loss of mature oligodendrocytes. As expected, the oligodendrocytes in these mice also had lower levels of p-eIF2α compared to mice with both copies of PERK intact[55]. Further characterization of the importance of the ISR in oligodendrocytes was accomplished *in vivo* by eliminating both alleles of PERK specifically in mature oligodendrocytes in mice. Although oligodendrocyte and myelin development occurred normally in these mice when unperturbed, they experienced an exacerbated disease course and increased loss of oligodendrocytes, myelin, and axons when immunized with EAE[59]. Together, these findings

show that when the ability to activate the ISR by phosphorylating eIF2α is diminished during stress, oligodendrocyte survival and myelin health is severely compromised, indicating that the ISR normally provides protection to oligodendroctyes against inflammation.

This conclusion is further supported by the results of converse experiments in which upregulation of PERK activity was shown to be protective of oligodendrocytes and myelin. When adult *GFAP/tTA; TRE/IFN*-γ transgenic mice that were immunized with EAE expressed IFN-γ prior to EAE symptom onset, p-PERK and p-eIF2α levels were found to be increased specifically in oligodendrocytes[60]. This enhancement of ISR activity prior to EAE symptom onset protected oligodendrocytes and myelin, resulting in a milder disease course compared to transgenic mice that did not express IFN-γ. Loss of one allele of PERK in these transgenic mice removed the protection seen[60]. This ISR-mediated protection of oligodendrocytes was found to occur despite the lack of innate or adaptive immune system involvement[55, 60, 61]. Indeed, further studies demonstrated that phosphorylation of eIF2α in oligodendrocytes, independent of ER stress or other cellular stressors, was sufficient to confer oligodendrocyte and myelin protection from immune attack during EAE, resulting in attenuated EAE symptoms[61]. This was achieved by the generation of an artificial PERK derivative that activated PERK kinase activity upon addition of a chemical dimerizer[61]. The protection of oligodendrocytes and myelin from inflammatory loss by PERK activation in these experiments further attest to the important role the ISR plays in the survival of oligodendrocytes and myelin.

b. Genetic CHOP model

The transcription factor CHOP is an immediate downstream target of ATF4 activity in stressed cells. CHOP in turn activates the expression of a number of pro-apoptotic genes, suggesting that it regulates a maladaptive response to cellular stress[62, 63]. Consistent with this possibility, a number of studies have shown that mutant cells in which the CHOP gene is inactivated display increased resistance to cytotoxic insults (reviewed in [64]). In preliminary data from our laboratory we have observed enhanced survival of CHOP mutant oligodendrocytes in models of CNS inflammation (Dzhashiashvili et al., unpublished observations). Nevertheless, in a mouse model of the childhood leukodystrophy Pelizaeus-Merzbacher disease (PMD), which is caused by mutations in the abundant myelin membrane protein proteolipid protein (PLP), CHOP expression has been shown to be protective to the mutant oligodendrocytes[65]. These contrasting results suggest that the role of CHOP in oligodendrocytes may be context-dependent, which will require further analyses for our complete understanding and potential therapeutic exploitation.

c. Genetic GADD34 model

Additional insights into the protective role of the ISR were generated by manipulating the major phosphatase responsible for dephosphorylating eIF2α. GADD34 is an essential non-enzymatic cofactor of protein phosphatase 1 (PP1), which is expressed upon p-eIF2α-driven upregulation of ATF4. It is thus a critical component of the negative feedback loop that regulates stress-induced, ISR-controlled translation. Prolonged phosphorylation of eIF2α increases GADD34 expression, and upon forming a complex with PP1, GADD34-PP1 is

able to dephosphorylate eIF2α and thus moderate the ISR protective response[66]. Upregulation of GADD34 is thus significant during stress-induced ISR activity: *GFAP/tTA; TRE/IFN*-γ transgenic mice that express IFN-γ in the CNS display a significant increase in GADD34 levels in oligodendrocytes[67]. In an experiment that demonstrated that inhibiting dephosphorylation of eIF2α had a similar effect to enhancing its phosphorylation, loss of both alleles of GADD34 in the *GFAP/tTA; TRE/IFN*-γ transgenic mice significantly diminished oligodendrocyte loss and hypomyelination, resulting in a much milder phenotype without affecting IFN-γ levels[67] (Figure 4c). These results further demonstrated that the genetic enhancement of the ISR has the potential to provide protection to oligodendrocytes against inflammation.

d. Pharmacological enhancement of the ISR

The genetic mouse models involving PERK and GADD34 established the proof of principle that enhancing the ISR results in protection of oligodendrocytes and myelin during inflammation, laying the foundation for testing this hypothesis with potential therapeutics. Salubrinal, a small chemical compound, was found to inhibit the dephosphorylation of p-eIF2α, thus protecting cells from ER stress[68]. Addition of salubrinal to developing rat hippocampal slice cultures concomitantly with IFN-γ decreased the hypomyelination and oligodendrocyte loss that resulted from addition of IFN-γ alone[67]. Although these results were promising, the use of the compound as a therapeutic was found to be limited as salubrinal inhibited stress-induced as well as constitutive p-eIF2α dephosphorylation, resulting in translational inhibition that could not be mitigated.

The discovery in 2011 that the drug guanabenz selectively inhibited GADD34-PP1 stress-induced dephosphorylation[69] (Figure 4d) while sparing CReP-PP1 constitutive dephosphorylation was therefore welcome news. Guanabenz, an orally administered small molecule that is FDA-approved for hypertension, was also able to protect oligodendrocytes and myelin from IFN-γ-mediated loss in slice cultures[70]. In addition, guanabenz treatment of the IFN-γ-expressing *GFAP/tTA; TRE/IFN*-γ transgenic mice resulted in significantly decreased oligodendrocyte and myelin loss compared to untreated mice. Treatment of wild type chronic EAE mice with guanabenz immediately prior to disease onset not only ameliorated but also delayed clinical disease, accompanied by an increase in p-eIF2α levels in oligodendrocytes. Likewise, guanabenz treatment in relapsing-remitting EAE mice after onset of disease was found to alleviate relapse, demonstrating that the drug was effective in a situation more similar to a clinical setting[70]. As EAE is a T cell driven model of MS[71], however, efforts were made to determine whether guanabenz might be directly affecting the immune system rather than protecting oligodendrocytes in the chronic EAE mice, with results indicating that either means of impact, if not both, might be working in this model system[70]. The uncertainty lies in the observation that oligodendrocyte protection has an indirect dampening effect on the CNS immune response likely mediated by limiting CNS myelin antigen presentation[72,73]. Nevertheless, the body of work using guanabenz treatments both in culture and in several mouse models of MS indicate that pharmacological enhancement of the ISR can clearly confer oligodendrocyte and myelin protection against inflammation.

These encouraging results have inspired the initiation of a Phase I clinical trial at the National Institutes of Health (NIH) to determine at what dosage guanabenz can be tolerated in MS patients[74]. In this trial, relapsing-remitting MS patients that have been on glatimer acetate for a minimum of one year are being given gradually escalating doses of guanabenz in a combinatorial treatment to determine the maximum tolerated dose (MTD) in MS patients as a primary outcome. Participants will be screened to have either active inflammation, defined as the development of new T2 hyperintense or contrast enhancing lesions by MRI during a two month screening period, or stable inflammation, defined as no documentation of MS relapse within the year prior to enrollment, no development of new T2 hyperintense or contrast enhancing lesions by MRI during the screening period, and no development of more than two lesions per year relative to an MRI performed at least one year prior to screening. Patients will also be evaluated throughout the five-month study using MRI, patient-reported outcomes and clinical assessments. If the drug is well-tolerated, a larger Phase II efficacy trial is planned in relapsing-remitting MS patients.

V. The ISR and remyelination

It is evident from these studies that the ISR plays a significant role in the survival of oligodendrocytes and myelin during inflammation. In fact, the ISR may show even greater promise: recent studies have indicated that enhancement of the ISR may also promote remyelination. Accumulating evidence has made it clear that remyelination failure is in large part due to the insufficient repopulation of oligodendrocytes[6, 75]. As remyelinating oligodendrocytes appear to be more sensitive to the deleterious effects of IFN-γ compared to developing or mature oligodendrocytes[76], it stands to reason that a strategy to protect remyelinating oligodendrocytes from the inflammatory environment, such as enhancement of the ISR, would be beneficial.

This hypothesis has been tested in cuprizone-fed and EAE mice. Cuprizone feeding is often used as a method of studying remyelination in mice, as consumption of the copper chelator induces massive demyelination followed by robust remyelination[77]. Cuprizone-fed *GFAP/tTA; TRE/IFN*-γ transgenic mice that express IFN-γ were found to have slower remyelination capabilities compared to wild type control mice on cuprizone[76]. To determine what impact the ISR might have on remyelination, haploinsufficient PERK mice were studied in this remyelination paradigm. These ISR-deficient, IFN-γ-expressing mice demonstrated even slower remyelination capabilities than those with two functioning alleles of PERK[76]. Once it was clearly established that the ISR did play a role in remyelination, a converse experiment was conducted using EAE mice. Mice immunized with EAE were allowed to achieve high clinical scores such that they were at the peak of clinical disease, a timepoint where demyelination and oligodendrocyte loss is typically near its highest. PERK was then activated in the mice using the aforementioned chemical dimerizer approach. Excitingly, these EAE mice displayed significantly enhanced remyelination and higher mature oligodendrocyte numbers[78], further emphasizing that a therapy that involves ISR enhancement will benefit the treatment of MS.

VI. Next steps

While treatments involving pathways important in protein translation are often met with pause, the highly cell-specific activity of the ISR makes it an ideal candidate for therapeutic targeting. Unstressed cells typically express low levels of p-eIF2α, such that healthy cells should be minimally impacted by the inhibition of GADD34-mediated eIF2α dephosphorylation through drugs such as guanabenz. Unstressed cells treated with guanabenz, for example, have normal p-eIF2α levels *in vivo*[70] and display normal translational activity *in vitro*[69]. Indeed, the complete deletion of GADD34 in mutant mice, which are unaffected clinically, resulted in normal p-eIF2α levels both in vitro[79] and *in vivo*[70], indicating that a therapy involving the ISR should selectively affect only those cells experiencing a cytotoxic event and hence have minimal side effects.

Nonetheless, any potential therapies involving manipulation of such a central pathway must be carefully considered. While enhancement of the ISR with guanabenz, for example, appears to be specific to only stressed oligodendrocytes, how it might affect other cells in which the ISR is a key regulator has yet to be addressed. Moreover, the presence of concomitant diseases would likely complicate use of this treatment scheme - in most cancers, for example, driving the ISR towards a pro-apoptotic condition may be beneficial[80]. Indeed, considerations also exist regarding the potential disadvantages of the basic premise of enhancing neuroprotection in MS. While ISR upregulation clearly confers protection to mature and developing oligodendrocytes, it is thought that death of mature oligodendrocytes may be a driving factor for subsequent oligodendrocyte precursor cell proliferation and differentiation[81] to replenish the lost cells. Would ISR-mediated protection of oligodendrocytes hence stunt this crucial process? This point is particularly important as studies have suggested that mature, myelin-maintaining oligodendrocytes are incapable of producing new myelin[82]. If this is the case, then any successful therapy must protect both the oligodendrocyte and its myelin to be beneficial.

Though further studies are warranted to address these and additional considerations, multiple avenues are quickly becoming available that might realize the goal of an ISR-enhancing treatment more safely and effectively (Table 2). While guanabenz is clearly able to enhance ISR protection of oligodendrocytes and myelin *in vitro* and *in vivo* during inflammation[70], the drug also functions as an α2-adrenergic agonist[84], an anti-hypertensive role that has been shown to have side effects such as drowsiness and dry mouth in patients. In addition, though its FDA-approved status will significantly accelerate the use of guanabenz in patients, its post-patent standing makes it less desirable for drug companies to pursue. Recent reports have demonstrated, however, that these concerns are already being addressed: one group has synthesized and characterized a guanabenz derivative, Sephin1, which was found to retain the GADD34-PP1 specific inhibition displayed by guanabenz *in vitro* and *in vivo* while minimizing its anti-hypertension side effects[83], while another group has identified and found similar characteristics in an additional two more guanabenz derivatives[85]. Thus, second-generation small molecule inhibitors of eIF2α dephosphorylation will likely be soon tested in inflammatory demyelinating disorders.

Transplantation of neural stem cells as a regenerational therapy method for MS has recently gained traction (reviewed in [86], [87]). Eventually, this technique may be routine enough to begin tests with genetically modified neural stem cells that display increased resistance to inflammatory insults. As mice[70] and cells[79] lacking GADD34 have been found to have normal levels of p-eIF2α until stressed, transplantation of stem cells in which GADD34 is inactivated, for example, would theoretically allow regenerated areas to withstand additional inflammatory attack via ISR enhancement without the side effects of constitutive translational inhibition.

Meanwhile, genetic therapies are rapidly becoming more reliable, indicating that somatic cell genetic manipulation techniques to enhance the ISR may eventually be realized in patients. Similar to the effect of modified stem cells, gene knockdown of GADD34 using RNA interference (RNAi) may prove useful. For example, short hairpin RNA (shRNA) knockdown in mice of cFLIP, a caspase 3 inhibitor that is upregulated by IFN-γ, has been shown to increase oligodendrocyte and myelin loss[88], demonstrating that *in vivo* RNAi is able to affect oligodendrocyte and myelin outcomes. The use of antisense oligonucleotides (ASOs) to modulate gene expression has also recently been receiving increased attention as a therapeutic approach with a number of clinical trials ongoing[89]. Advances in ASO chemistry have resulted in ASOs with dramatically extended half-lives, resulting in long-lived modulation of gene expression[90]. The use of ASOs to enhance the ISR is particularly exciting since this approach would limit the effect on gene expression to the CNS. Moreover, the increasingly widespread and effective use in research of highly specific gene editing tools such as the CRISPR/Cas9 system suggests that therapies that use such methods may be available soon[91]. The *in vivo* genetic inactivation of GADD34 in oligodendrocyte lineage cells, for example, would likely provide increased protection to these cells against inflammation.

VII. Conclusion

The demonstration that enhancement of the ISR is capable of protecting oligodendrocytes and myelin from inflammatory loss provides an additional key approach to a comprehensive treatment that might finally be capable of stopping MS disease activity. In combination with immunomodulatory drugs, which minimize the innate inflammation that occurs in an autoimmune disease, and upcoming reparative drugs, which enhance oligodendrocyte differentiation and remyelination capabilities, ISR-mediated protective therapies may ensure that the majority, if not complete, spectrum of the disease will finally be adequately addressed. At minimum, use of such combinatory therapies should improve the overall efficacy of treatments, allowing the option of bypassing current anti-inflammatory drugs with greater side effects, such as Tysabri, in favor of milder alternatives. Results to date are encouraging, such that enhancement of the ISR appears to be a promising addition to the growing therapeutic options for MS.

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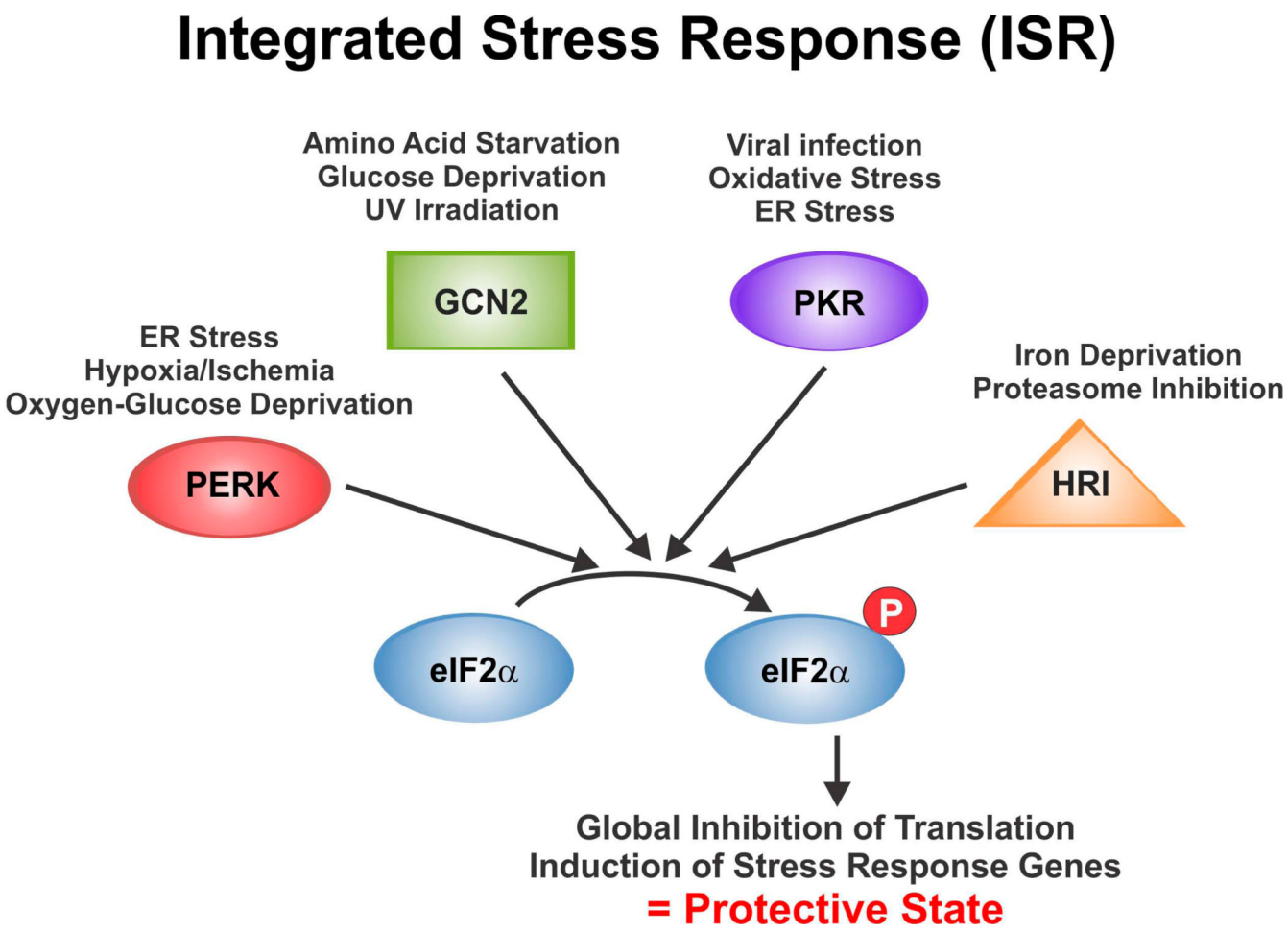

Figure 1. The integrated stress response (ISR) is sensitive to numerous stress conditions

Activation of the kinases PERK, GCN2, PKR, or HRI in response to various stressors leads to phosphorylation of eIF2α, resulting in inhibition of mRNA translation and upregulation of cytoprotective genes to restore proteostasis.

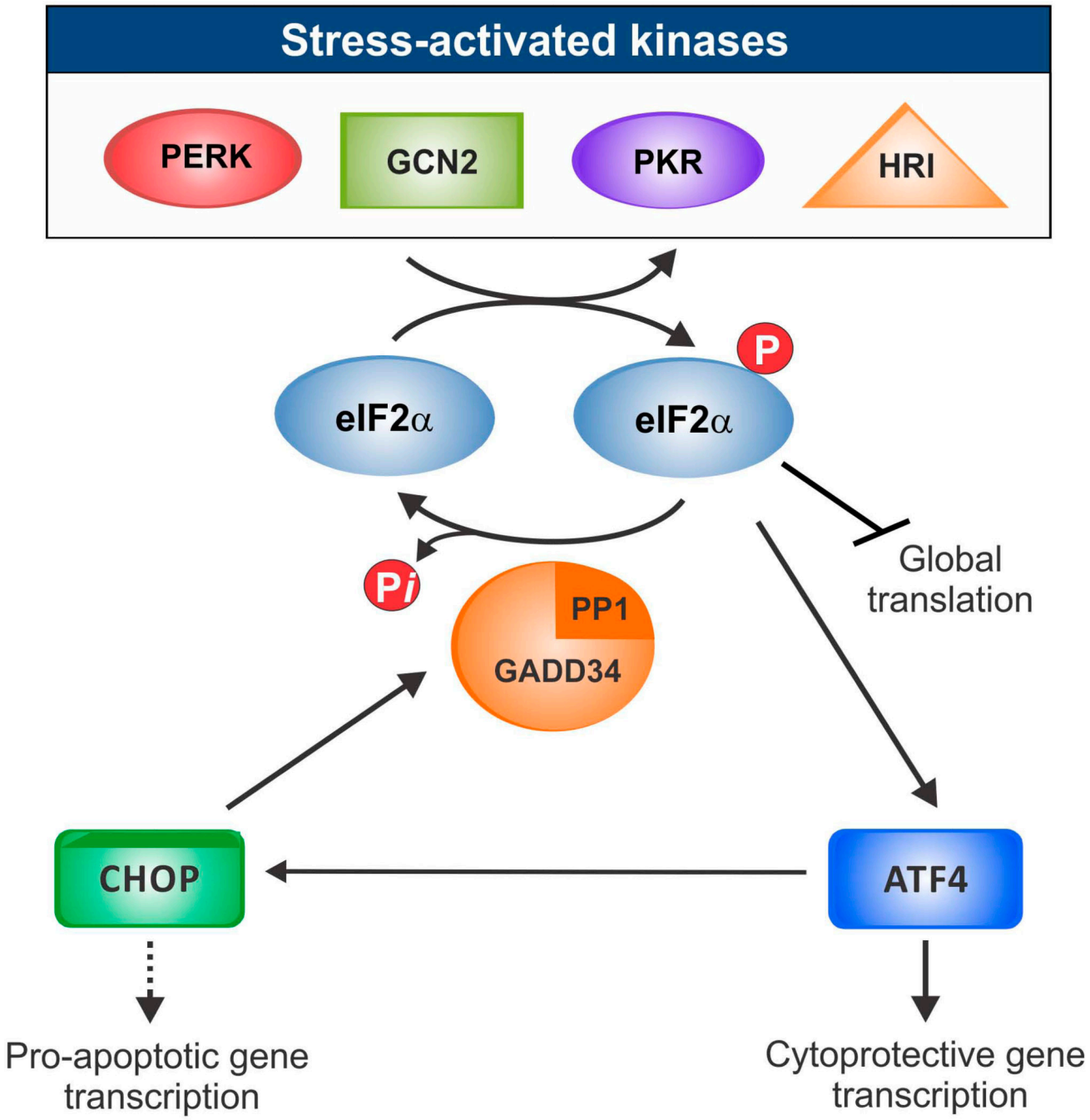

Figure 2. Translational control and cellular protection by the ISR

Translational inhibition to restore proteostasis through the phosphorylation of eIF2α is delicately controlled by a tight negative feedback loop: activation of the transcription factor ATF4 upregulates the transcription factor CHOP, which in turn drives GADD34 expression. Binding of GADD34 to the phosphatase PP1 allows the complex to dephosphorylate eIF2α and hence restore global translation. When proteostasis cannot be achieved, CHOP increases the transcription of pro-apoptotic genes in the cell.

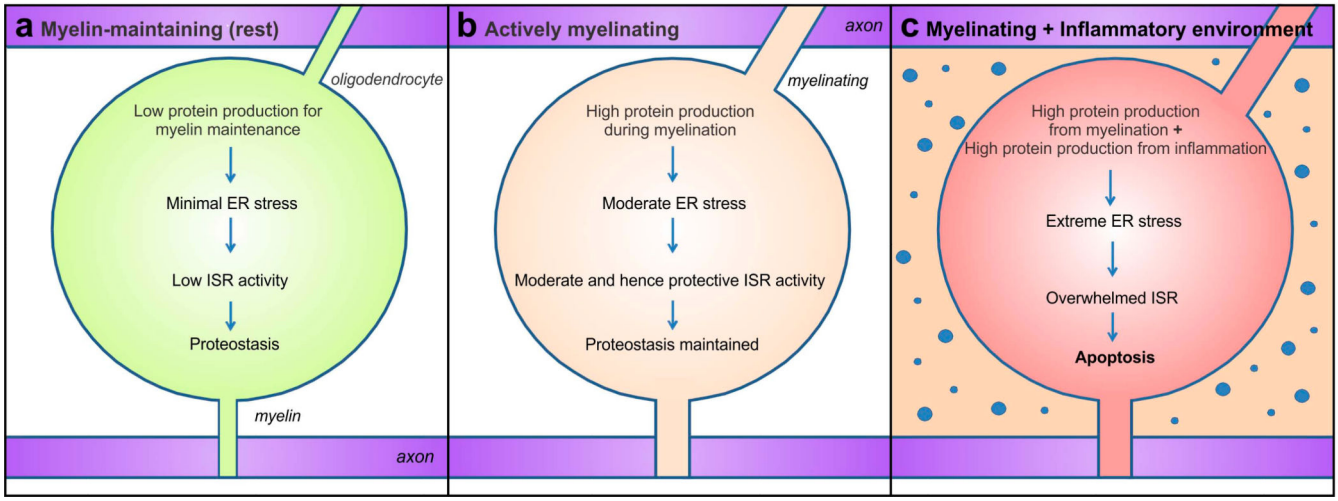

Figure 3. Oligodendrocyte loss in the MS inflammatory environment

(a) A myelin-maintaining oligodendrocyte experiences relatively little ER stress, such that minimal ISR activity is required. **(b)** During myelination, oligodendrocytes produce extraordinary amounts of protein and hence display moderate ER stress, which a fully activated ISR is able to effectively counter to maintain proteostasis. **(c)** The inflammatory MS environment, which dramatically increases protein production to meet demands from immune cell proliferation, differentiation, and related events, combined with the ER stress already experienced by a myelinating oligodendrocyte, often overwhelms the ISR, sending it towards an apoptotic fate.

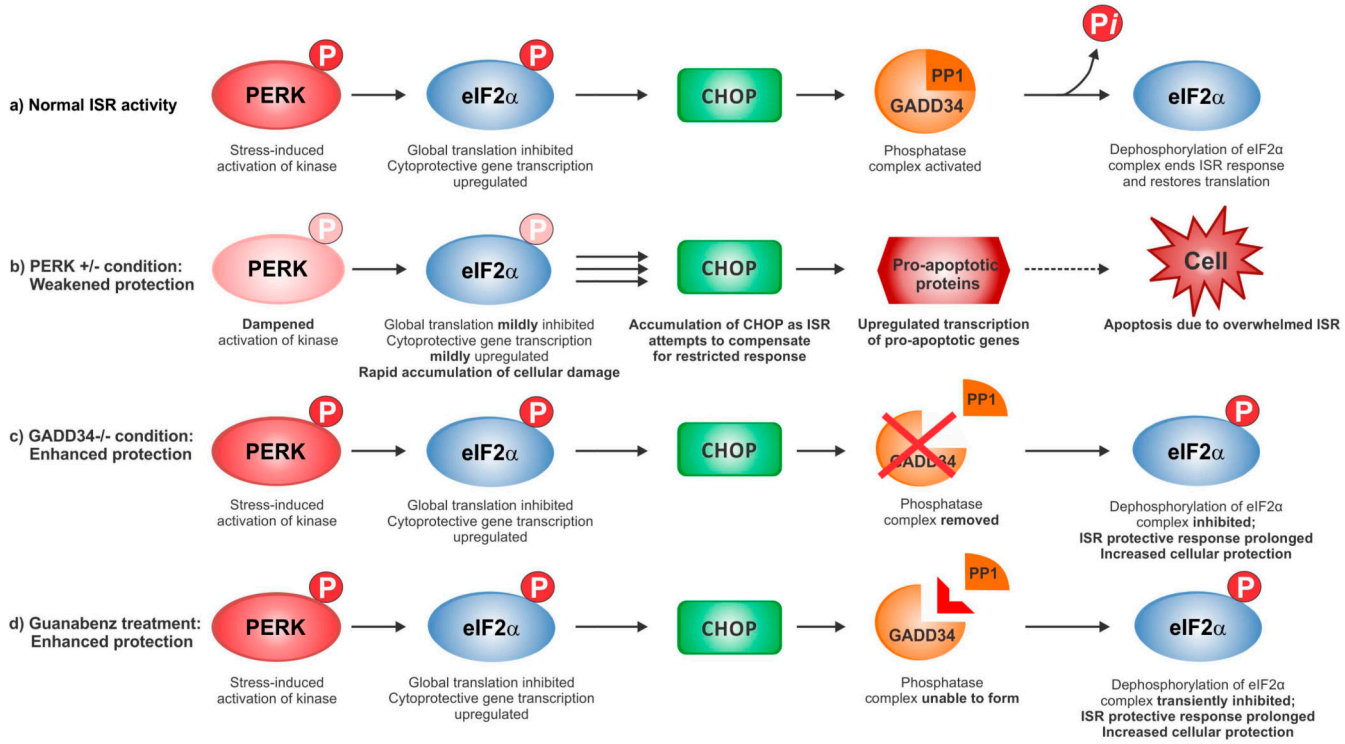

Figure 4. Genetic and pharmaceutical manipulation of the ISR

(a) Normal ISR activity in a mildly stressed cell. **(b)** PERK haploinsufficiency limits the phosphorylation of eIF2α and thus weakens the protective ISR response, resulting in increased oligodendrocyte apoptosis and exacerbated demyelination. **(c)** Lack of GADD34, on the other hand, limits the dephosphorylation of p-eIF2α, thus prolonging and enhancing the protection conferred by the ISR. **(d)** Treatment with guanabenz, which inhibits binding of protein phosphatase 1 (PP1) to its regulator GADD34, similarly enhances ISR protection.

Table 1

MS-associated factors that activate the ISR.

MS-Associated Factor	eIF2-alpha kinase(s)	References
Amino Acid Deficit	GCN2	26, 27
Cytokines	PERK, PKR	28, 29
Growth Factors	PKR	30, 31
Glutamate Excitotoxicity	PERK	32, 33
Hypoxia	PERK	34, 35
Iron Level Dysregulation	HRI	36
Ischemia	PERK	37, 38
Nitric Oxide	PERK, PKR, GCN2, HRI	39, 40
Oxidative Stress	PERK, PKR	41, 42
Unfolded Proteins	PERK, PKR	43, 44
Viral Infection	PKR, GCN2	45, 46

Table 2

Potential therapeutic approaches to enhancing the ISR.

Approach	Examples	Status
Pharmacological	Salubrinal	Animal models only68
	Guanabenz	Clinical trial initiated[(a)],74
	Sephin 1	Animal models83
Genetic	shRNA	Animal models[(b)]
	ASOs	Animal models[(b)]
	CRISPR/Cas9	Cellular models[(b)]
Cellular	Genetically-modified Stem Cell Replacement	In design[(c)]

[(a)] Six patients with relapsing-remitting MS who have previously been on glatimer acetate for one year were recruited for this Phase I single-site, open-label, dose escalation study in which 4–64 mg of guanabenz will be orally administered. Patients will be followed for five months with maximum tolerated dose as a primary outcome measure and patient-reported outcomes, objective clinical and imaging assessments, and pharmacokinetics as secondary outcomes. (clinicaltrials.gov identifier: NCT02423083).

[(b)] Chen et al., unpublished observations.

[(c)] Efforts are underway to genetically alter cells to increase their ISR potential, thereby increasing their resistance to the inflammatory extracellular environment present in MS lesions (Chen et al., unpublished observations).