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Synergistic effects of brain injury and aging: Common mechanisms of proteostatic dysfunction

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Abstract

The aftermath of TBI is associated with an acute stress response and the accumulation of insoluble protein aggregates. Even after the symptoms of TBI are resolved, insidious molecular processes continue to develop, which often ultimately result in the development of age-associated neurodegenerative disorders. The precise molecular cascades that drive unhealthy brain aging are still largely unknown. In this review, we discuss proteostatic dysfunction as a converging mechanism contributing to accelerated brain aging after TBI. We examine evidence from human tissue and *in vivo* animal models, spanning both the aging and injury contexts. We conclude that TBI has a sustained debilitating effect on the proteostatic machinery, which may contribute to the accelerated pathological and cognitive hallmarks of aging that are observed following injury.

Keywords

Unfolded protein response; heat shock response; ubiquitin-proteasome system; experimental models; cellular stress response; therapeutics

TRAUMATIC BRAIN INJURY: LINKS TO AGING

A traumatic brain injury, or TBI, is caused by a physical insult to the head. Mechanical trauma causes disruptions in normal brain function that can last years following the initial injury, resulting in millions of people worldwide who struggle with lifelong disabilities [1]. Increasing evidence suggests that there is an overlap in the molecular processes that result from TBI, and those that lead to neurodegeneration. A specific neuropathological condition termed chronic traumatic encephalopathy (CTE) has been associated with exposure to repetitive mild TBIs [2]. Multiple epidemiological studies also link TBIs of varying severities to an increased risk of developing neurodegenerative conditions such as Alzheimer's disease (AD), Parkinson's Disease (PD), and Amyotrophic Lateral Sclerosis (ALS) (Box 1) [3]. However, the primary risk factor for most of these diseases is aging.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

Loss of proteostasis that occurs early after TBI as well as during aging favors the formation of toxic insoluble protein deposits like extracellular **amyloid-β** (see Glossary) plaques, intracellular hyperphosphorylated **tau** tangles, **α-synuclein** aggregates, and cytosolic **TAR-DNA binding protein 43 kDa** (TDP-43) aggregates [1, 4]. One hypothesis that associates TBI with neurodegenerative disease suggests that TBI sets in motion a constellation of molecular events that are associated with the normal biological aging process. Common occurrence of these disease-associated aggregates underscores proteostatic dysfunction as a central theme connecting TBI with neurodegenerative disease and aging. A history of TBI appears to hasten the brain along the trajectory of aging, presenting a phenotype of a biologically "older" brain than the chronological age would suggest [4]. Functional imaging indicates that even mild TBIs can cause a reduction in cortical thickness [5] similar to aging, while moderate to severe TBIs cause tissue losses of up to 5% per year [6-8]. A predictive model of brain aging using magnetic resonance imaging found that TBI brains appear between 4.6 and 6 years older than their chronological age [9]. The study also found that cognitive decline is strongly correlated with time since injury, suggesting that the increased biological age is driven by processes that continue to occur in the brain after the initial insult. However, most *in vivo* animal model studies in the TBI field focus on the acute consequences of injury, with a relative paucity of studies that examine the chronic effects of TBI and its impact on aging-related processes.

In this review, we highlight the impact of proteostatic dysfunction as a mechanism contributing to accelerated brain aging after TBI. We focus on the unfolded protein response (UPR), the heat shock response (HSR), and the ubiquitin-proteasome system (UPS), which are three key proteostatic stress response pathways that become dysregulated with age (Figure 1, Key Figure). The current literature provides support for the argument that TBI mobilizes all arms of the proteostatic machinery, but has a sustained debilitating effect on protein homeostasis, as evidenced by accumulation of misfolded aggregates after injury, contributing to unhealthy brain aging. This review also discusses potential therapeutic strategies that have been successfully described in *in vivo* animal models to ameliorate the injury response and improve age-related signs of deterioration, such as cognition and motor function.

MOLECULAR AGING PROCESS

A natural hallmark of the aging process is an attenuated response to stress challenges, due to a compromised ability to effectively invoke pathways that regulate stress resistance [10]. The cellular consequence of the failure of these stress response pathways is the gradual buildup of damage to proteins, lipids, and nucleic acids. Most genetic or pharmacological interventions that influence longevity impinge on various aspects of the initiation and execution of different stress response pathways [11]. Damage that accumulates in biomolecules throughout the organism can be caused by: 1) genotoxic stress brought about by mitochondrial dysfunction and the generation of reactive oxygen species, causing damage to DNA; 2) proteotoxic stress caused by a failure to refold unfolded or misfolded proteins using chaperones or to degrade them efficiently through the proteasome and lysosome functions, leading to aggregate formation [10]. Aging is associated with an imbalance between the damage caused by these stresses and the repair pathways stimulated

by them, culminating in accumulations of age-related pathology and the appearance of disease. We note that stress response pathways, such as the DNA damage response system, oxidative stress response and **autophagy**, also contribute to the synergy between aging and TBI (see [1] and [4]), but are beyond the scope of this review.

STRESS RESPONSE: RELATIONSHIP BETWEEN TBI AND AGING

Heat shock response

The heat shock response (HSR) is an evolutionary conserved phenomenon whereby organisms produce a group of proteins, termed the heat shock proteins (HSPs) in response to a wide variety of environmental stressors such as heat, oxidative stress, and injury [12] (Fig 1). The HSR is coordinated by the family of transcription factors termed heat shock factors (HSFs) to organize the rapid induction of HSPs, which act as chaperones to aid in refolding affected proteins [13]. Along with upregulation of factors to promote recovery, there is also a large downregulation of genes regulating translation, cell cycle, and RNA processing [14]. There are five classes of HSPs – HSP100, HSP90, HSP70, HSP60, and small HSPs. Through concerted action, the chaperones broadly assist in refolding stress-denatured proteins to achieve their native state by binding to hydrophobic patches and preventing aggregation [13]. Additionally, when proteins are irreversibly misfolded or under conditions of nutritional stress, HSP70 and its cochaperones target proteins for degradation through the proteasome or autophagy [15]. Upon sufficient increase in cellular concentration, HSP70, HSP90, and HSP40 interact with HSF to repress its transcriptional activity, returning HSF to its monomeric form and attenuating the HSR [16, 17].

Aging is associated with a decline in the efficacy of the organism to maintain proteostasis and mount an effective stress response. The amount of misfolded proteins and aggregates increases with age broadly across organisms and tissues, with a concomitant increase in HSPs to counter these effects [18-20]. However, the stress-induction and chaperoning ability of HSPs also decreases with age [21-23]. Ironically, the increase in HSPs may actually serve to increase the negative regulation of HSF, decreasing the amplitude of the HSR [12, 24]. Other mechanisms of HSF inhibition with age include changes in post-translational modifications affecting its DNA-binding and activation [25-27]. An example of this is the age-dependent decrease in expression and activity of SIRT1, a histone deacetylase that targets and stabilizes HSF, promoting activation of the HSR [28, 29]. Resveratrol, a potent activator of SIRT1, is a prominent anti-aging therapeutic candidate, and one of its mechanisms of action is its ability to induce HSP production [30]. Upregulation of HSPs and HSF to prolong longevity have been demonstrated in multiple model systems like *C. elegans* and *Drosophila* [31-33]. Additionally, the insulin/IGF-1 signaling pathway, which modulates the aging process depending on extracellular growth conditions, has effects on proteostasis and HSF regulation, underscoring the intimate relationship between stress response systems and longevity [33, 34].

The induction of the HSR in response to TBI is well-documented across human studies, as well as in multiple model systems. Encouragingly, genetic, environmental, and pharmaceutical manipulations to increase the HSR have had positive effects on TBI outcome [35-40], while attempts to reduce the HSR through HSP reduction have had deleterious

outcomes [35, 36]. Examination of injured human brain tissue reveals that HSP70 protein and mRNA, but not HSC70 (the constitutively expressed cognate form of the protein) is induced in response to TBI [41-44]. Elevations in HSPs have also been detected early in serum and cerebrospinal fluid (CSF) samples from patients with moderate to severe TBI. Increases in serum HSP70 are observed as early as within 30 min of injury, reaching peak values at 3d post-TBI [45, 46]. Another study demonstrated that serum HSP70 values are an effective predictor of mortality, implying that unresolved cellular stress after injury can have deleterious consequences [47]. Both HSP70 and mitochondrial HSP60 increase in CSF samples, with HSP60 levels independently associated with injury severity as defined by the Glasgow Coma Scale score (a clinical scale for assessing a patient's level of consciousness following a brain injury) [43, 48]. These studies implicate the HSR as an immediate and early response to TBI, but it remains to be studied whether injury has any lasting effects on the ability of patients to mount an effective response to subsequent challenges.

Most of the work examining changes in HSPs following TBI *in vivo* in animal models has been performed in rats using the fluid percussion injury (FPI) model, which applies a brief fluid pulse to the exposed dura and deforms the brain tissue. HSP70 mRNA is increased between 2h and 6h after TBI and returns to baseline by 24h [49-52]. HSP70 protein, on the other hand is only moderately increased at 4h post-TBI and reaches a peak at 24h [50]. HSP60, a chaperone that resides in the mitochondrial matrix and responds to mitochondrial stress, is strongly upregulated at both mRNA and protein levels at 4h and 24h after injury [50]. Another commonly used TBI model is the controlled cortical impact (CCI) approach, in which a piston applies a controlled trauma to the exposed cortex. Studies using CCI indicate similar changes in HSP70 mRNA and protein [53]. The small HSPs are less well-studied, with a few studies indicating that they are induced upon TBI in young animals, but the induction is markedly lower when aged animals are injured [54, 55].

Genetic-manipulation studies in mice have underscored the importance of HSPs in TBI. Mice that lack *HSP70* or *HSP110* have worsened neurological and physiological outcomes following TBI, whereas transgenic mice overexpressing *HSP70* have improved outcomes [35, 36]. As noted earlier, most animal models studies on TBI examine acute effects, and a relatively small number of studies have examined long-term effects. A study in rats showed that HSP70 is chronically downregulated 12 months after injury, suggesting heightened vulnerability to other stresses [56]. The study also observed that the expression levels of genes related to aging are transiently increased after injury, while the expression levels of genes associated with neuroprotection, such as the antiapoptotic factor Bcl-2 and the antioxidant glutathione peroxidase-1, are increased at 12 months in the survivors. These results suggest that TBI may have a sustained debilitating effect on some cellular stress responses such as the HSR, while other systems such as apoptosis and the antioxidant defense are upregulated for maintaining homeostasis.

A number of therapeutic inducers of the HSR have been tested for their potential to protect against TBI-induced deficits in rodent models. 17-AAG, Celastrol, geranylgeranylacetone (GGA), and BGP-15 are potent pharmacological inducers of HSF, either acting on HSP90 to lift its inhibition of HSF or altering the post-transcriptional modifications of HSF to allow increased activity [57]. Administration of 17-AAG either before or at the time of

TBI in rodents induces an increase in HSP70 production that lasts up to 3d post-TBI and results in improved lesion size, neuronal survival, sensorimotor performance, and a reduction in pro-inflammatory cytokines [37, 38]. Celastrol and BGP-15 have protective effects comparable to those of 17-AAG when administered in two doses within 24h of injury or five doses up to one week after TBI in mice [36]. Similarly, GGA confers protection when administered either before or after TBI in mice [58]. Other compounds have been shown to increase HSP70 through other pathways, such as tert-butylhydroquinone which acts on Nrf2, and dexmedetomidine, a sedative. Both these compounds have beneficial effects on edema formation, memory loss, and apoptosis after TBI in mice [59, 60]. Environmental interventions such as exercise have also been shown to have a favorable effect in rats through stimulation of HSP70 and HSP20 production [61, 62]. An interesting environmental modulation of the HSR that was tested in rodent models is based on manipulating the environmental temperature. Specifically, rats reared for one month at a temperature slightly elevated (34°C) compared to typical rearing environments fared better than normothermic animals when subjected to TBI [39]. These heat-acclimated animals were also shown to have improved blood-brain barrier integrity, edema formation, and neurobehavior scores. The animals further displayed increased neurogenesis, anti-apoptotic and antioxidant capacity, and an immune system that was better primed to handle the TBI insult [63-66]. A key mechanism of protection achieved through heat acclimation involves the signaling pathway hypoxia-inducible factor 1 (HIF1) [67, 68]. HIF1 is activated during the adaptation response to ischemia, to increase oxygen delivery. A protective effect similar to the heat-acclimation observed in rodents was shown in a *Drosophila* model of TBI where pretreatment with an acute 30 min heat shock to 37°C, from normal rearing temperature of 25°C, extends lifespan and mitigates brain degeneration in injured animals [40].

Overall, it is evident that the HSR plays a key protective role in re-establishing protein homeostasis after injury as well as during the aging process. However, further work is needed for exploring the long-term effects of TBI on chaperone availability and the functionality of the HSR. Therapeutic modulations of the HSR that increase longevity in animal models are also successful in mitigating the deleterious effects of TBI, arguing that chaperone regulation is a fundamental link between the aging and injury processes. Future efforts are needed to determine whether alleviation of protein misfolding in the immediate aftermath of a TBI will have a lasting effect in preventing chronic post-traumatic neurodegenerative processes.

Unfolded Protein Response (UPR)

The UPR is instigated by endoplasmic reticulum (ER) stress when there is an accumulation of misfolded proteins. The UPR upregulates chaperones to help regulate protein folding, temporarily halts protein synthesis, targets misfolded proteins for proteasomal degradation in the event that they cannot be refolded, and finally, promotes apoptosis in cases of severe chronic stress [69]. These events are orchestrated by the three arms of the UPR: PKR-like ER kinase (PERK), inositol requiring enzyme-1 (IRE-1), and activating transcription factor-6 (ATF-6) [69] (Fig 1). The three leader proteins are normally sequestered in an inactive form in the ER membrane by the stress-sensing chaperone Ig binding protein (BiP)/glucose-regulated protein 78 (GRP78) [69]. Under ER stress, BiP/GRP78 dissociates from

the three proteins, initiating the UPR. The integrated stress response (ISR) is mediated by the phosphorylation of the eukaryotic translation initiation factor 2 alpha (eIF2α) by PERK, resulting in the global repression of translation and the transcription of a few select genes, such as ATF-4 that directs the upregulation of stress response genes, including antioxidant pathways [70]. Upon severe stress, ATF-4 is also responsible for activating C/EBP homologous protein (CHOP), a transcription factor that mediates apoptosis. The second arm of the UPR, IRE-1, facilitates the splicing of X-box binding protein (XBP-1), which regulates genes related to chaperones and the ER association degradation pathway (ERAD) [71]. The IRE-1 arm also activates the Jun N-terminal kinase (JNK) pathway, leading to apoptosis. The third arm of the UPR, ATF-6, translocates to the Golgi upon activation and is processed to form a transcription factor (ATF-6s) that stimulates the transcription of several chaperones, ERAD genes, and XBP-1 [72]. Together, the three arms of the UPR serve to restore homeostasis, mobilize the protein-folding machinery, target misfolded proteins for degradation, and in the event of extreme stress, trigger cell death.

There is strong evidence that the functional components and stimulation of the UPR gradually decline with age. The levels and enzymatic activity of multiple resident ER chaperones, such as BiP/GRP78 decrease in many tissues with age throughout the body, including the brain [73-76]. Most studies show that when the aged organism is challenged by a stress, the ER fails to effectively stimulate the UPR, triggering pro-apoptotic pathways of cell death rather than survival [76-79]. Studies from nematodes and *Drosophila* indicate that deletion of integral UPR proteins like IRE-1 and XBP-1 decrease lifespan [80, 81], while expressing the spliced form of XBP-1 is sufficient to extend lifespan and improve stress resistance [82].

Most of what we know about the activation of the UPR after TBI comes from preclinical rodent models. The only related study in humans, to our knowledge, examined two postmortem CTE brains and reported increases in markers of all three arms of the UPR, which colocalized with hyperphosphorylated tau tangles [83], suggesting that sustained ER stress is a chronic consequence of TBI.

Signs of UPR activation in rodents, notably increases in the phosphorylated form of eIF2α, BiP/GRP78, processed XBP-1 mRNA, and CHOP have been observed as early as 4h after injury and lasting until 48-72h, accompanied by a reduction in protein synthesis up to 48h [84-86]. An *in vitro* model of compressive neuronal injury demonstrated a gradual increase in ER stress accompanied by a release of intracellular calcium stores from the ER [87]. Assessment of a population of dying neurons after TBI in rats showed that the neurons had increased expression of ER stress genes compared to the population of healthy neurons from the same brain area, indicating that TBI can trigger both the protective and apoptotic arms of the UPR cascade [88]. The induction of the UPR occurs not only in neurons, but also in glial cells, to promote cell survival and astrogliosis, as shown in a mouse model of TBI [89]. There is also evidence to suggest that activation of the UPR is a chronic response to TBI in mice and rats, with levels of the phosphorylated form of eIF2α and ATF-4 being increased up to a month post-injury [90, 91]. From these studies, it is evident that the UPR is triggered early in response to TBI and is sustained for months and perhaps years after the injury.

Significant effort has been put into investigating means of reducing ER stress in rodent models as a therapeutic avenue for TBI [92, 93]. In particular, compounds to modulate phosphorylation of eIF2α have been widely studied. Phosphatase inhibitors that prevent de-phosphorylation of eIF2α can enhance the protective PERK arm of the UPR, prolonging the repression of protein translation and decreasing the protein load entering the ER. The inhibitors salubrinal and guanabenz have been shown to inhibit CHOP upregulation and apoptosis, alleviate ER and oxidative stress, in addition to rescuing lesion volume, neuroinflammation, and memory deficits [94-102]. Contrarily, blocking PERK activation using drugs or a kinase dead mutant, which would prevent eIF2α phosphorylation, also seems to have beneficial effects. However, these effects are possibly mediated through the compound's effect on cAMP response element binding protein (a transcription factor binding to the cAMP element and regulating genes related to neuronal plasticity) and may be independent of eIF2α [103, 104]. Similarly, treatment with ISRIB, an inhibitor of the ISR that blocks eIF2α phosphorylation, corrects chronic memory deficits even when administered weeks after injury in mice [90, 105]. Docosahexaenoic acid (DHA), a polyunsaturated fatty acid that is enriched in neuronal cell membranes and essential for normal brain function, has also demonstrated potential in mitigating ER stress, rescuing white matter damage and reducing accumulations of ubiquitinated material, amyloid and phosphorylated tau deposits [91, 106, 107]. Other therapeutic treatments, like dexmedetomidine (an α2-adrenoreceptor agonist), polydatin (a resveratrol precursor), hypothermia, curcumin, and tauroursodeoxycholic acid (a hydrophilic bile acid) show beneficial effects in part by impinging on the UPR and alleviating ER stress [108-112].

These studies indicate the role of the UPR in TBI pathophysiology is complex, as demonstrated by therapeutic strategies that are successful through both inhibition and promotion of translation. Thus, the type of treatment may depend on various factors that determine the prosurvival vs. the apoptotic state of the UPR after injury, which may be mediated by several factors including injury type and severity. Many studies examining UPR perturbation as a therapeutic strategy have used short-term functional studies as endpoint assays, while relatively few studies have scrutinized changes in misfolded protein accumulation. Furthermore, given the paucity of UPR studies in post-mortem human tissues, the long-term influence of TBI on ER stress in the context of human disease is still largely unknown.

Ubiquitin-proteasome system (UPS)

The UPS is the primary cellular system responsible for the selective degradation of proteins involved in a broad range of cellular activities to maintain homeostatic levels, as well as clearing misfolded or damaged proteins [113]. Proteins targeted for degradation are marked with ubiquitin, a short polypeptide, through a series of enzymatic reactions by E1, E2 and E3 enzymes to form a polyubiquitin chain [114] (Fig 1). Such tagged proteins are directed to the proteasome for proteolytic degradation. The proteasome is a massive multi-subunit assembly comprised of two main components – the 19S and 20S particles – to form the complete 26S structure [113]. The 19S is the regulatory portion of the proteasome that recognizes, unfolds, and translocates the ubiquitinated proteins to the catalytic 20S subunit for enzymatic cleavage [115].

Many studies that examine the contribution of the UPS to aging indicate that proteasome function is compromised with age, across multiple tissues and species [116-118]. Age-associated decline of proteasome activity occurs due to a loss in subunit expression levels, a decrease in the enzymatic activity of the proteases, or an overwhelming of the UPS by damaged proteins and aggregates that it is unable to resolve. Studies in rats demonstrated a reduction in proteasomal activity within cortical areas, hippocampus, and spinal cord, but not the cerebellum and brainstem [119, 120]. Other studies across various species report similar findings of proteasomal inhibition with age in rodents, nematodes, and *Drosophila* [121-125]. Intriguingly, genetic upregulation of different proteasomal subunits has demonstrated not only increased proteasomal activity, but also increased longevity and protection from oxidative challenges in *Drosophila* and human cellular senescent models [123, 126, 127]. In *C. elegans*, other means to extend lifespan, such as caloric or dietary restriction, have been shown to act on specific E2 and E3 ligases to offset the anticipated decline in proteostasis with age [128, 129].

In contrast to age, the number of reports studying changes in UPS activity after TBI is relatively sparse, both in humans and in animal models. A survey of human brain tissue from patients who died after a severe TBI revealed that the K48 ubiquitin linkage (the most prevalent form of polyubiquitin signal that targets proteins to the proteasome) increases within an hour of injury in both neurons and glia [130]. This is observed even in cases when the injury had happened 7 months earlier, indicating that the UPS is likely active chronically after severe TBI [130]. Elevated UPS activity is exhibited in circulating plasma following even mild injury, and the duration of this increase lasts until resolution of the symptoms of concussion [131]. Ubiquitin immunoreactivity is also detected in CSF samples after TBI, with levels becoming progressively higher until death [132]. These studies implicate the association of a dysfunctional proteasome and accumulation of degradation-targeted proteins with adverse outcomes such as death and possibly unhealthy aging.

The accumulation of ubiquitinated material acutely after TBI has been reported in multiple animal models [91, 133-135]. The increase in ubiquitin-conjugated proteins is accompanied by a depletion in free ubiquitin, suggesting a disruption in the tightly-regulated protein turnover process [136]. Similar to aged animals, injured rats exhibit a decrease in the protease activity of the proteasome as well as changes in its subunit composition [137]. Modulation of the UPS does not yield straightforward results. For instance, increase of ubiquitin carboxy-terminal hydrolase L1 (UCHL1), a brain-specific deubiquitinating enzyme that cleaves amino acids from ubiquitin to generate free monomeric ubiquitin, has a therapeutic effect in attenuating neuron death and axon injury, and reducing the ubiquitinated protein load in mice [138]. Treatment with proteasome inhibitors MG-132 and lactacystin has deleterious consequences in *in vitro* models of axon injury (both inhibitors) and *in vivo* models of TBI (only MG-132), while bortezomib, another inhibitor, yields improved physiological consequences [134, 139, 140]. Bortezomib is thought to derive its potent anti-inflammatory activity by preventing the proteasome-mediated degradation of inhibitor of NFκB, thus reducing its activation after trauma [141].

As with the other components of the proteostatic machinery, there is evidence that UPS function gradually declines with age, and promotion of UPS activity is associated with

longevity. However, from studies of injured human tissue, prolonged activation of the UPS appears to be associated with detrimental outcomes, although this has not been verified in more controlled *in vivo* studies.

CONCLUDING REMARKS

Here we have covered key aspects of the three central proteostatic stress response pathways – the HSR, UPR, and UPS – and their integration into the study of TBI and aging, as well as some of the therapeutic approaches and opportunities they offer. There is clear evidence to demonstrate that stress sensitivity is a key phenotype of the aged organism. The pathophysiology of TBI encompasses various molecular events that mobilize many of the stress response pathways. Depending on the nature and severity of the TBI, some of the responses may become dysfunctional, as evidenced by inefficient proteasomal degradation [134] and heightened activation of the apoptotic, rather than survival, mode of the UPR [88].

The few reports that study the long-term consequences of TBI suggest that select stress pathways, such as the UPR, HSR, UPS, and autophagy are chronically affected after an injury [56, 90, 91, 130, 135]. This implies that TBI, a super-stressor in itself, may have a sustained debilitating impact on the stress response, contributing to the accelerated pathological and cognitive hallmarks of aging that are observed after injury. Furthermore, it is known that age too, has a detrimental effect on the response to TBI [142]. Aged animals that are injured have a much higher mortality rate, elicit a far less robust stress response, and have worsened pathological outcomes [55, 143-145]. The confluence of TBI and aging needs more preclinical research, to investigate and define in molecular terms both the long-term effects of TBI on the normal pathways of the aging process, as well as the response of the aged organism to TBI (see Outstanding Questions).

Animal model studies indicate that modulation of different stress response pathways could offer a notable therapeutic strategy in overcoming the noxious effects of age, TBI, and neurodegenerative disease. Increasing chaperone availability through mechanisms that include activation of HSF extends lifespan, protects from degenerative TBI pathology, and serves a protective role in disease by inhibiting aggregation and targeting proteins for degradation through chaperone-mediated autophagy [35, 40, 146, 147]. Among the signaling pathways that merit attention in this regard is mTOR. The mTOR signaling cascade monitors environmental cues of nutrient availability to control cellular growth and metabolism through protein synthesis and autophagy, thus impinging on key processes of protein homeostasis [148]. The mTOR pathway is involved in several age-associated mechanisms, including the immune response, stem cell regulation, cellular senescence, and mitochondrial function. Inhibition of the mTOR pathway with rapamycin has been widely used to achieve longevity in animal models and is also being considered as a therapeutic in neurodegeneration, dementia and brain injury [148]. Another compound to be noted in this context is docosahexaenoic acid (DHA). DHA has been extensively studied as a neuroprotective agent that inhibits neuroinflammation, ER, and oxidative stress in the context of TBI, although a definitive mechanistic explanation for these protective effects is currently lacking. DHA also reduces age-associated cognitive decline. Lastly, manipulation of the proteasome through peptide- or small molecule-based activators, although less well-

studied, remains an attractive avenue for ameliorating the toxicity associated with protein accumulation in aging, disease and injury [149].

Much work remains for elucidating the biology of TBI, particularly when taking into account the variability presented by the type and severity of injury. Addressing this variability is further complicated by the various modes for infliction of injury in experimental settings, although this variety of approaches also confers some advantages. Further research is also needed regarding the long-term repercussions of TBI: what are the molecular players involved in perpetuating the degenerative pathologies that define and characterize the injury response and its outcomes?

Invertebrate model organisms, such as *Drosophila* and nematodes, with their short lifespan compared to mammals, have been extensively used in longevity studies, and have helped inform about basic cellular pathways of the aging process. Recent adaptations of such model systems to TBI research [40, 135, 143, 150] carry significant potential for identifying fundamental aspects of injury pathways and risk factors. These models also offer opportunities for efficient large-scale screens of genetic and therapeutic interventions, as a basis for subsequent testing in more expensive and complex animal model systems of TBI. Integrating detailed understanding of impacts on various cellular pathways of age and disease promises to further define the molecular players and pathways that confer the biological and pathological effects of TBI. Hopefully, this understanding will lead to approaches for interfering with the detrimental actions of these cellular pathways or even preventing them to achieve better outcomes after TBI, and perhaps also for promoting healthy brain aging.

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Glossary:

Autophagy
A highly regulated process of degradation of damaged or unnecessary cytosolic contents that is necessary to maintain cellular homeostasis.

Amyloid-β
The main component of extracellular plaques found in AD. It is formed from amyloid precursor protein through cleavage to yield the peptide that can aggregate and form oligomers that can misfold and be toxic.

Tau
A microtubule-binding protein whose physiological function is to bind and stabilize microtubules. Hyperphosphorylated forms of the protein aggregate and form the intracellular neurofibrillary tangles characteristic of AD and other tauopathies. Intracellular tau tangles are the predominant feature in CTE brains and used to diagnose the disease, while the localization pattern of the tangles is used to stage CTE severity.

TAR-DNA binding protein 43 kDa (TDP-43)

A DNA/RNA-binding protein that regulates RNA splicing, mRNA stabilization, and transcriptional regulation. Although a predominantly nuclear protein, it becomes enriched in the cytoplasm in diseases like AD, ALS, CTE and FTD. Under these conditions, it is abnormally phosphorylated and ubiquitinated, and forms aggregates that can label with stress granule components.

α-synuclein

A presynaptic neuronal protein that regulates synaptic vesicle trafficking, neurotransmitter release, and fatty acid uptake. In its pathological state, it is misfolded and aggregates into oligomers that are the main constituent of Lewy bodies, a pathological hallmark of PD. Lewy body disease appears as a common co-morbidity in CTE patients.

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BOX 1 -

TRAUMATIC BRAIN INJURY: A LINK TO NEURODEGENERATIVE DISEASE

As the aging population increases across the world, the silent epidemic of TBI will prove to have enormous implications for public health. While the mechanistic aspects of the association between TBI and neurodegeneration are still under intense investigation, it is widely agreed that a history of head trauma can trigger pernicious processes that increase the overall risk of developing a neurodegenerative disorder. Following from clinical and pathological observations of pugilists in the early 20th century, and more recently athletes and military veterans, a specific neurological condition termed chronic traumatic encephalopathy (CTE) has been associated with exposure to repetitive mild TBIs [2]. Tentatively defined, CTE is a tauopathy that can only be diagnosed post-mortem by its pathognomonic lesions of perivascular accumulations of tau around neurons and astrocytes in the depths of cortical sulci [151]. However, around 40% of CTE cases studied to date have presented with other comorbid degenerative diseases such as AD, Lewy body disease, frontotemporal lobar degeneration (FTD) and motor neuron diseases like ALS [152]. Independent of CTE, there is evidence from multiple epidemiological studies for links between TBI of varying severities and neurodegenerative conditions [3]. Two large cohort studies conducted by the US Veterans Health Administration found that one or more instances of moderate to severe TBIs as well as mild TBIs were associated with a higher risk of developing dementia and PD [153-155]. Other large cohort studies have also found marginally increased associations between a history of a single severe or mild TBI and dementia [156, 157]. When neuropathological confirmation of AD was available, a study found that dementia onset was 3.6 years earlier when a history of TBI was involved [158]. These studies provide epidemiological weight to the hypothesis that TBI precipitates the aging process, causing an earlier advent of typically late-onset neurodegenerative processes.

Aggregation of disease proteins is the best studied link between TBI and the development of neurodegenerative disorders. TBI stimulates rapid induction of amyloid precursor protein, amyloid-β peptides, hyperphosphorylated tau, physiological TDP-43, and α-synuclein, within hours to days of the insult [4, 159]. It is unknown how these early disruptions in proteostasis eventually culminate in CTE or any other neurodegenerative disease. Therapeutic interventions aimed at halting neurodegenerative processes have been largely unsuccessful, both in the field of age-induced disorders and TBI-induced proteinopathy. This failure calls for a deeper dissection of the pathogenic mechanisms that drive neurodegeneration, to identify key therapeutically targetable processes that significantly impact progression of the disease.

OUTSTANDING QUESTIONS:

- How can aging be successfully incorporated into TBI studies? There are numerous studies that address cellular and molecular mechanisms affected in the acute and subacute phases of TBI. However, due to the lengthy lifespans of mammalian experimental animal models, there is a relative scarcity of *in vivo* studies that examine the long-term repercussions of TBI. With the advent of invertebrate TBI models in *C. elegans* and *Drosophila*, studies that focus on the interrelationship between TBI and aging become more feasible.
- There are inconsistencies in TBI studies about whether augmentation or suppression of a stress response is protective. What is the source of these discrepancies? Many of these stress response systems have the ability to both restore homeostasis and trigger signaling pathways of cell death in cases of severe stress. Further research into the roles of the heat shock response, unfolded protein response, the ubiquitin-proteasome system, and autophagy are required to understand the consequences of modulating these pathways in an injury setting.
- How are other pathomechanisms of the TBI-response associated with aging? The complex acute pathophysiology of TBI includes excitotoxicity, oxidative stress, DNA damage and inflammation. Most of these pathways are also known to become impacted with age, strengthening the link between a history of TBI and advanced aging. Thus, a more thorough understanding of how TBI perpetuates these measures of unhealthy brain aging, and how they can be prevented, is necessary.
- Are there common genetic risk factors between negative TBI outcomes and age-related diseases? The apolipoprotein E4 allele is associated with an increased risk of poor post-traumatic outcome as well as an increased risk for Alzheimer’s disease. A deeper analysis and understanding of such risk factors can help identify individuals who may be more vulnerable to injury-induced, age-associated degenerative disorders.

HIGHLIGHTS

- The aging process represents a gradual deterioration of the various arms of the proteostatic network, with decreased capacity to maintain homeostasis and compromised ability to respond to insults. A similar cellular state of proteostatic disruption is also reflected in the post-injury setting of traumatic brain injury (TBI).
- The accumulation of misfolded, degenerative proteins observed years after TBI indicates that disruption of protein homeostasis is a long-term consequence of injury. However, further research in both human tissue and animal models is warranted to examine the molecular cascades that are chronically active after TBI.
- Genetic and pharmacological modulators of the proteostasis machinery can extend lifespan in animal models, as well as rescue the negative age-associated consequences of TBI, indicating shared mechanisms of therapeutic opportunities.

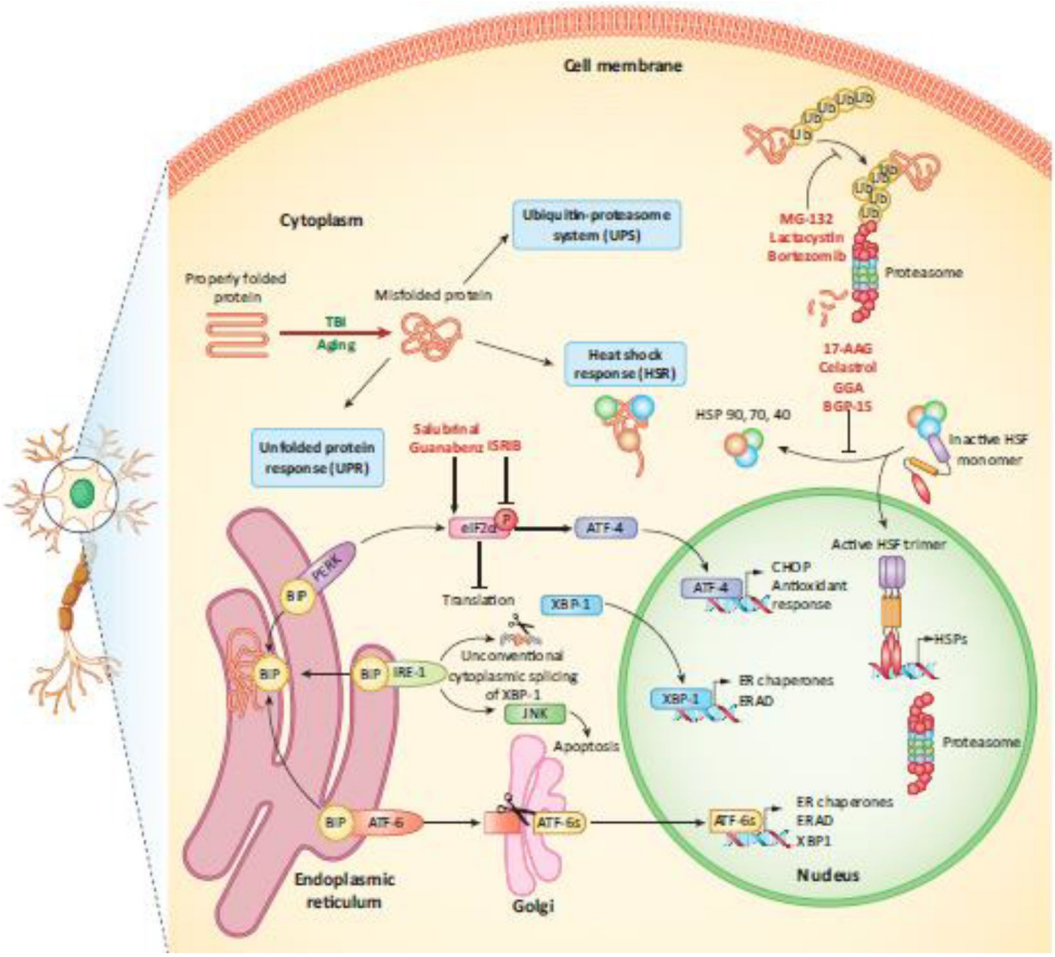

Figure 1 (Key Figure). Stress response pathways dysregulated with age and injury:

Three different stress pathways triggered by misfolded protein in neuronal and glial cells are illustrated – the heat shock response (HSR), the unfolded protein response (UPR), and the ubiquitin proteasome system (UPS). Therapeutic modulators of TBI are shown in red, indicating the pathways they affect. UPR: Misfolded proteins in the ER bind the stress-sensing chaperone, BiP, sequestering it away from PERK, IRE-1 and ATF-6 and activating them. PERK phosphorylates eIF2α leading to global repression of translation and increasing selective translation of ATF-4. IRE-1 triggers the unconventional cytoplasmic splicing of XBP-1, as well as activation of the JNK pathway. ATF-6 translocates to the Golgi apparatus and is processed to form the active transcription factor ATF-6s. Together, these mediators dictate the induction of pathways that attempt to restore normal cellular function by refolding or degrading misfolded proteins, and in the event of prolonged stress, activating apoptotic pathways. UPS: Misfolded proteins are tagged with a polyubiquitin chain and targeted to the cytoplasmic or nuclear proteasome for degradation. HSR: HSPs bind HSF and sequester it in an inactive monomeric form. During cellular stress, HSPs bind misfolded proteins, allowing HSF to trimerize, translocate to the nucleus and trigger the transcription of more HSPs. Abbreviations: BiP, Ig binding protein; PERK, PKR-like ER kinase; IRE-1, inositol requiring enzyme-1; ATF, activating transcription factor; eIF2α, eukaryotic initiation factor 2α; CHOP, C/EBP homologous protein; XBP-1, X-box binding

protein-1; ER, endoplasmic reticulum; ERAD, ER associated degradation; JNK, Jun N-terminal kinase; Ub, ubiquitin; HSP, heat shock protein; HSF, heat shock factor.