Oligodendrocyte responses after spinal cord injury.

George Zach Wei

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OLIGODENDROCYTE RESPONSES AFTER SPINAL CORD INJURY

By

George Zach Wei
B.A. Rutgers, The State University of New Jersey, 2014

A Dissertation
Submitted to the Faculty of the
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In Partial Fulfillment of the Requirements
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in Pharmacology and Toxicology

Department of Pharmacology and Toxicology
University of Louisville
Louisville, Kentucky

August 2021
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A Dissertation Approved on

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DEDICATION

This dissertation is dedicated to my beloved family
for their constant love, support, and encouragement in all my endeavors,
and

to all my previous teachers and professors for teaching me patience,
for inspiring hope, and, above all, the courage to fail.

.
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ABSTRACT

OLIGODENDROCYTE RESPONSES AFTER SPINAL CORD INJURY

George Z. Wei

April 27, 2021

Recent studies demonstrate that neuroprotection strategies targeting the proteostasis network and components of its effector signaling pathways improve cell survival and motor recovery outcomes in several models of neuronal injury and degeneration. However, the individual contributions of these signaling pathways to the pathogenesis of spinal cord injury (SCI), white matter damage, and motor recovery have not yet been determined. Here, I explored the role of the HIF prolyl hydroxylase domain proteins (PHD/EGLN), effectors that can modulate stress responses activated by the proteostasis network, on motor function recovery after SCI. Furthermore, I identified previously unknown candidate mechanisms in an unbiased manner that may regulate oligodendrocyte death and survival in the context of SCI using RiboTag technology.

Chapter one presents background information on the SCI pathogenesis, the types of cell death involved in SCI, oligodendrocytes function and death, the proteostasis network as a global target for neuroprotection in SCI, and finally introduces tools that can be used to study OL-specific translatomes after SCI. Chapter two examines the effects of HIF PHD/EGLN inhibition on a mouse model of moderate T9 contusive SCI.
Pharmacological inhibition of PHDs using adaptaquin moderately lowers acute induction of activating transcription (Atf4) and C/EBP homologous protein (Chop/Ddit3) mRNAs and prevents the acute decline of oligodendrocyte (OL) lineage mRNAs, but does not improve long-term recovery of hindlimb locomotion or increase chronic white matter sparing. Furthermore, conditional genetic ablation of all three PHD isoenzymes in OLs did not affect Atf4, Chop or OL mRNAs expression levels, locomotor recovery, and white matter sparing after SCI.

Chapter three uses RiboTag technology to study the translatome of OLs at different time points after SCI. Using mouse genetics, I tagged ribosomes specifically in OLs, and immuno-purified mRNA associated ribosomal complexes. I identified genes that were upregulated in OLs with a maximum increase at 2 days post-injury (dpi). Genes that may induce oxidative stress (Chac1, Steap3), inhibit survival signaling kinases (Spred3, Spry4, Parvb), and directly contribute to OL death (Runx1) were highly upregulated after SCI. Potential pro-survival genes include Sphk1, Aldh18a1, and Gdnf.

My results show that SCI activates multiple signaling pathways that may affect locomotor recovery, cellular homeostasis, and survival. Although PHDs are involved in modulating responses involved in the proteostasis network, PHD inhibition and/or deletion alone is not sufficient to protect white matter after SCI. These observations are in contrast to those from various CNS injury models with primary effects on the grey matter. PHD inhibitors are currently used in 27 clinical trials treating anemia, and currently show potential for neuroprotection in stroke. I show that PHDs may not be suitable targets to improve outcomes in traumatic CNS pathologies that involve acute white matter injury. Finally, I show using RiboTag technology that OLs upregulate genes
that may increase oxidative stress and inactivate survival signaling that may collectively contribute to cell death after SCI. Taken together, the work established in this dissertation shows limited involvement of the PHD-ATF4-CHOP pathway in SCI, and identifies novel candidate mediators of OL death/survival after SCI.
**TABLE OF CONTENTS**

<table>
<thead>
<tr>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIGNATURE PAGE ............................................................... ii</td>
</tr>
<tr>
<td>DEDICATION ........................................................................ iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS .............................................................. iv</td>
</tr>
<tr>
<td>ABSTRACT ................................................................................ v</td>
</tr>
<tr>
<td>LIST OF FIGURES ................................................................. x</td>
</tr>
<tr>
<td>LIST OF TABLES ................................................................. xii</td>
</tr>
<tr>
<td>CHAPTER 1 ........................................................................... 1</td>
</tr>
<tr>
<td>SCI Incidence and Pathology ................................................... 1</td>
</tr>
<tr>
<td>Types of cell death involved in SCI ........................................ 6</td>
</tr>
<tr>
<td>Oligodendrocytes ................................................................. 13</td>
</tr>
<tr>
<td>The Proteostasis Network: A global target for neuroprotection in SCI ...... 18</td>
</tr>
<tr>
<td>OL-specific translational gene expression after SCI .......... 35</td>
</tr>
<tr>
<td>CHAPTER 2 ................................................................. 46</td>
</tr>
<tr>
<td>Introduction ................................................................. 46</td>
</tr>
<tr>
<td>Materials and Methods ......................................................... 48</td>
</tr>
<tr>
<td>Results .............................................................................. 53</td>
</tr>
<tr>
<td>Discussion .......................................................................... 55</td>
</tr>
<tr>
<td>Chapter</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>REFERENCES</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
</tr>
<tr>
<td>CURRICULUM VITA</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1. Injury progression after SCI</td>
<td>5</td>
</tr>
<tr>
<td>Figure 2. Types of Cell Death</td>
<td>7</td>
</tr>
<tr>
<td>Figure 3. Proteostasis Network and Stress Response Pathways</td>
<td>20</td>
</tr>
<tr>
<td>Figure 4. Stress responses activated after SCI</td>
<td>21</td>
</tr>
<tr>
<td>Figure 5. The Integrated Stress Response</td>
<td>28</td>
</tr>
<tr>
<td>Figure 6. Schematic of RiboTag SCI mouse model and experimental workflow</td>
<td>45</td>
</tr>
<tr>
<td>Figure 7. After SCI, AQ attenuates the acute loss of OL lineage mRNAs, moderately reduces acute ATF4 and CHOP activation, but does not improve chronic functional locomotor recovery</td>
<td>59</td>
</tr>
<tr>
<td>Figure 8. Effects of HIF-PHDs deletion in OL lineage cells after SCI</td>
<td>60</td>
</tr>
<tr>
<td>Figure 9. $^{1}$H NMR spectrum for the AQ batch used in the current SCI study</td>
<td>62</td>
</tr>
<tr>
<td>Figure 10. Validation of biological activity for the AQ batch (AQ/KY) used in the current SCI study</td>
<td>63</td>
</tr>
<tr>
<td>Figure 11. Effects of OL-specific HIF-PHDs deletion in the OL-lacking liver tissue</td>
<td>64</td>
</tr>
<tr>
<td>Figure 12. Isolation and validation of OL-specific mRNAs from $P{l}^{p}{C}^{r}{r}^{e}{E}{r}:R{p}{l}^{2}{2}{H}{a}{m}{i}{c}{e}{s}$</td>
<td>87</td>
</tr>
</tbody>
</table>
Figure 13. Optimizing isolation of OL-specific mRNAs from $Plp^{CreER}\cdot Rpl22^{HA}$ mice.................................................................................................................. 89

Figure 14. OLs at naïve, 2, 10, and 42 dpi display distinct clustering and different expression of highly enriched genes.................................................................................. 90

Figure 15. GO analysis of enrichment of genes................................................................. 91

Figure 16. OL-specific translatome changes after SCI. ..................................................... 92

Figure 17. GO analysis of acute 2 dpi downregulated clusters....................................... 93

Figure 18. GO analysis of acute 2 dpi upregulated clusters............................................. 94

Figure 19. Filtering steps performed to sort differentially expressed genes...................... 95

Figure 20. GO analysis of downregulated genes identified through manual filter......... 96

Figure 21. The untrimmed image of the western blot image shown in Fig. 19c............. 97

Figure 22. The untrimmed image of the western blot image shown in Fig. 19d........... 98
LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1. Experimental design</td>
<td>65</td>
</tr>
<tr>
<td>Table 2. List of qPCR primers</td>
<td>66</td>
</tr>
<tr>
<td>Table 3. Raw BMS score data for individual mice</td>
<td>67</td>
</tr>
<tr>
<td>Table 4. List of qPCR primers</td>
<td>99</td>
</tr>
<tr>
<td>Table 5. Isolation yields and conditions</td>
<td>101</td>
</tr>
<tr>
<td>Table 6. Summary of initial sequence analysis</td>
<td>102</td>
</tr>
<tr>
<td>Table 7. Patterns and number of genes contained in the 23 proposed clusters</td>
<td>103</td>
</tr>
</tbody>
</table>
CHAPTER 1
INTRODUCTION

SCI Incidence and Pathology

Traumatic spinal cord injury (SCI) is a debilitating injury to the central nervous system (CNS) which leads to severe neurological impairments. It is the second leading cause of paralysis, only behind stroke, in the United States (Stats about Paralysis. www.christopherreeve.org/living-with-paralysis/stats-about-paralysis). Each year >17,000 people (50 cases per one million individuals) in the U.S. suffer a traumatic SCI, with male cases (80%) vastly outnumbering female cases (20%) (National Spinal Cord Injury Statistical Center, Facts and Figures at a Glance. Birmingham, AL: University of Alabama at Birmingham, 2016.). The average age at the time of SCI follows a bimodal distribution with the one peak occurring between 15-29 years old and the second peak at >50 years old. Injury to the cervical spine level is most common (~60%), followed by thoracic (32%), and lumbosacral (9%). Finally, automobile traffic accidents remain the leading cause of SCI (38%), followed by falls (30%), acts of violence (14%), and finally sports related injuries (9%). The estimated direct cost for patients with SCI is estimated to be at $1.5–4.6 million over their lifetime (National Spinal Cord Injury Statistical Center, Facts and Figures at a Glance. Birmingham, AL: University of Alabama at Birmingham, 2016.). Finally, SCI has far reaching and devastating social and
psychological sequela for the patient which drastically decreases the quality of life for the affected patient. Injury to the spinal cord disrupts the spinal circuitry that connects the brain to muscle/sensory systems and leads to many complications including loss of motor control. According to the American Spinal Injury Association (ASIA), patients in approximately 55% of SCI cases have varying degrees of motor or sensory activity below the level of injury, while in the remaining 45% there is a complete lack of motor or sensory activity in the S4-5 segments (https://asia-spinalinjury.org/isncsci-2019-revision-released/). These symptoms can be directly attributed to effects of SCI on motor neuroaxis. Currently, there is no acute therapeutic modality that has proved to be substantively efficacious in enhancing motor function recovery.

The pathophysiology of SCI is thought to be biphasic and can be divided into a primary injury and secondary injury cascade that progresses hours to months post-SCI (Fig. 1). The initial spinal cord contusion (primary injury) is caused by the mechanical insult to the vertebral column which can compress or transect the spinal cord tissue resulting in immediate death of all impacted CNS cell types, disruption of blood vessels and cellular membranes, accumulation of neurotransmitters, and demyelination (Almad et al., 2011; Oyinbo, 2011). Secondary injury, characterized by a prolonged temporal progression of cellular death, follows the initial injury and spreads rostral and/or caudal to the initial focal SCI lesion (Almad et al., 2011). For instance, Wallerian degeneration of axons contributes to prolonged oligodendrocyte (OL) apoptosis over the course of days or weeks that may result in chronic demyelination (Beattie et al., 2000; Crowe et al., 1997; Shuman et al., 1997; Warden et al., 2001). However, mechanisms mediating OL-death are understudied, but thought to be mediated by inflammatory cytokines (Beattie et
al., 2002a; Beattie, 2004; Casha et al., 2005; Shuman et al., 1997) and/or loss of survival signals from intact axons (Barres et al., 1999). Death of OLs and other cells can also occur through several mechanisms in response to disruptions in protein homeostasis (the proteostasis network) which are induced by hypoxia, bioenergetic failure, excitotoxicity, oxidative stress, and neuro-inflammation (Dumont et al., 2001; Myers et al., 2019; Ohri et al., 2013; Ohri et al., 2020b; Ohri et al., 2012; Ohri et al., 2011; Ohri et al., 2014; Park et al., 2004; Saraswat Ohri et al., 2018a; Saraswat Ohri et al., 2020). For instance, disruption of the neuro-vasculature results in the ischemia and the hemorrhage with extravagated blood components are directly cytotoxic in the CNS (Robinson et al., 2009; Sahinkaya et al., 2014). While ischemia has effects on both the gray matter and the white matter, the re-establishment of blood flow can lead to reperfusion injuries which are characterized by the formation of free radicals that generate oxidative damage to lipids and membranes that further elicit an inflammatory response (Jia et al., 2012). Disruption of the blood spinal cord barrier is an important pathogenic event in the secondary injury cascade and is a major mechanism behind the activation of the neuro-inflammatory response after SCI (Hausmann, 2003; Whetstone et al., 2003; Zhang et al., 2012b). The inflammatory response occurs within hours of the injury with polymorphic nuclear cells infiltrating first, followed then by macrophages (Pukos et al., 2019). Tumor necrosis factor α (TNF-α) levels, although not fully understood, rise within an hour after SCI and have neuroprotective properties, but also induce edema and enhance leukocyte migration (Sharma et al., 2003; Yune et al., 2003). Finally, these cascades of injuries culminate in the formation of a glial scar that is comprised of multiple cellular and extracellular
components that may further limit tissue repair and recovery after SCI (Yuan et al., 2013).

The mechanisms of secondary injury affect multiple neural cell types and are closely interwoven together in a self-propagating cycle that collectively contributes to a toxic environment that promotes prolonged cellular death, demyelination of surviving axons, and inhibition axonal regeneration that ultimately result in neurological impairment (Oyinbo, 2011). Therefore, understanding the various types of death cell that occur after SCI may be of paramount importance when developing neuroprotective therapeutics that are based on targeted mechanisms.
Figure 1. Injury progression after SCI

This diagram illustrates the different events after SCI and is divided into phases. In the acute time points, there is bleeding, as well as glutamate release. Neurons die immediately, while OL death is prolonged and lasts until the subacute time period. In the subacute time monocyte and macrophages infiltrate and activate.
Types of cell death involved in SCI

Traumatic SCI is a complex pathology characterized by a dynamic time-dependent injury cascade with distinct molecular changes occurring at different stages of injury that may result in death of various cells types that ultimately contribute to functional deficits. The balance between cell survival, proliferation, differentiation, and death is a critical parameter in the maintenance of organismal homeostasis. After SCI, various cytotoxic events including hypoxia, hemorrhage, bioenergetics failure, oxidative stress, endoplasmic reticulum (ER) stress, and neuroinflammation contribute to secondary injury (Pukos et al., 2019). Importantly, these events occur at different time points with varying duration of insult, and therefore may differentially contribute to cell death. For instance, hemorrhage, oxidative stress, and excitotoxicity may contribute to acute phase cell death as compared to immune cell infiltration, expression of pro-death cytokines, and Wallerian degeneration that occur in the subacute phase (Pukos et al., 2019). Here, I briefly touch upon a few, but not limited to, types of cellular death that occur after SCI (Fig. 2).
Figure 2. Types of Cell death

Diagram illustrating the different classification of cell death type and major mediators that initiates death.
Apoptosis is perhaps one of the best characterized forms of programmed cell death and involved a series of well-regulated events that result in morphological and molecular changes that include cell shrinkage, membrane blebbing, and DNA fragmentation (Coleman et al., 2001). The three major signaling pathways that mediate apoptotic death are the granzyme/perforin pathway, the extrinsic pathways involving death receptors, and the intrinsic pathway involving the mitochondria. The intrinsic pathway can be initiated by a variety of stressors like DNA damage, ER stress, replication stress, and oxidative stress (Coleman et al., 2001). A key stage for intrinsic apoptosis is the permeabilization of the mitochondrial outer membrane which is regulated by the BCL2 family of proteins (Coleman et al., 2001). Bcl-2 antagonist/killer 1 (BAK) and Bcl-2 associated X (BAX) are two key pro-apoptotic members of the Bcl-2 proteins that have the ability to create pores in the outer mitochondrial membrane (Chipuk et al., 2006). Other pro-apoptotic members of the BCL2 family of proteins (those that contain a single BH3 domain) include BCL2 binding component 3 (PUMA), BCL2 like 11 (BIM), phorbol-12-myristate-13-acetate-induced protein 1 (NOXA), BH3-only harakiri (HRK), and BH3 interacting domain death agonist (BID) can collectively interact with BAX and/or BAK to initiate mitochondrial permeability (Elmore, 2007; Inohara et al., 1997). Increased mitochondrial permeability promotes the release of cytochrome c, second mitochondria-derived activator of caspase (SMAC), apoptosis inducing factor (AIF), and high temperature requirement protein-A2 (Omi/HTRA2) (Elmore, 2007). Cytochrome c can bind to apoptotic protease activating factor-1 (APAF1) to activate Procaspase-9. Caspase-9 cleaves Procaspase-3 to form active Caspase-3 that cleaves DNA fragmentation factor Inhibitor of Caspase-Activated Dnase (ICAD) to stimulate the
DNase activity resulting in DNA fragmentation (Elmore, 2007). In the receptor mediated extrinsic pathway, death receptors play a critical role in transmitting apoptotic signals by specific ligands such as Fas and TNF receptor-1 (TNFR1) (Elmore, 2007). The Fas Ligand (FasL) ligand binds, causes oligomerization of the receptor and through of a series of steps activate Pro-caspase-8 that results in the activation of Caspase-3 and -7 that results in apoptosis (Elmore, 2007). Importantly, apoptotic cell death in both neurons and OLs can be observed in spinal cords obtained from deceased patients after SCI using DNA fragmentation and caspase activation assay (Emery et al., 1998).

Autophagic cell death represents starvation response and may occur in conjunction with other forms of cellular death like apoptosis, ferroptosis, and necroptosis (Glick et al., 2010). It is characterized by membrane blebbing, depletion of cytoplasm organelles, and swelling of intracellular vesicles in the absence of chromatin condensation. It functions to degrade long-lived proteins, cytoplasmic components, and organelles for lysosomal pathway degradation (Glick et al., 2010). Autophagy is regulated by unc-52-like kinase (ULK), Atg 13, and FIP200 under the control of mammalian target of rapamycin complex 1 (mTORC1) (Glick et al., 2010). For instance autophagy can be initiated when mTORC1 is inhibited using rapamycin which results in phosphorylation and activation of Atg13 and FIP200 (Glick et al., 2010). This leads to the elongation of the phagophore around products marked for degradation. Atg12, Atg5, Atg16L complex and LC3 then assists in the formation of the autophagosome (Glick et al., 2010). LC3 is then cleaved, conjugated to phosphatidylethanolamine to form LC3-II, and remain associated with the autophagosome until lysosomal fusion. Several studies have shown changes in markers of autophagy after SCI (Sekiguchi et al., 2012, Kanno et
The role of autophagy is currently protective after SCI. Autophagy marker increases after SCI, and therefore reflects inhibition rather than its activation (Lipinski et al., 2015). For instance, deletion of autophagic gene Atg5 in oligodendrocytes exacerbates and correlates with worse functional recovery after SCI and greater myelin loss suggesting that autophagy serves as protective response in OLs (Ohri et al., 2018). Moreover, autophagy reduces neuronal damage by inhibition of apoptosis after SCI (Tang et al., 2014).

Necrosis is a form of non-programmed cell death that can be caused by toxins, infections, ischemia and trauma that ultimately results in cell membrane rupture, loss of intracellular organelles, and degraded DNA (D'Arcy, 2019). Importantly, this process is energy independent and does not exhibit condensation of chromatin, which is a hallmark observed in apoptosis. Instead, damage to the cell perturbs its function and causes swelling that result in cell membrane rupture and leaking of cellular components into surrounding areas which may result in further inflammation or damage (D'Arcy, 2019). Necrosis has been shown to occur after SCI (Beattie et al., 2002c). Myelotomy that remove necrotic tissue were functionally beneficial in dogs (Ducker et al., 1971; Hu et al., 2015).

Necroptosis is a form as programmed necrosis that is characterized by the activation of receptor-interacting protein kinases (RIPKs) and is similar with the necrotic morphology in sharing membrane rupture and organelle loss (Frank et al., 2019). Necroptosis can be triggered by toll-like receptors (TLRs), death receptors (DRs), and cytosolic DNA (indicative of viral infection). RIPK3 is first activated by RIPK1 and subsequently phosphorylates mixed lineage kinase domain-like protein (MLKL) to
induce MLKL oligomerization (Frank et al., 2019). Oligomerized MLKL then inserts into cellular membranes permeabilizing them and ultimately resulting in cell death. Notably, after SCI, RIPK1, RIPK3, and MLKL have been shown to rapidly accumulate due to lysosomal dysfunction, and rapamycin treatment to stimulate lysosomal function attenuated cell death (Liu et al., 2018). After SCI, necroptosis inhibitors promotes cell protection and function recovery (Kanno et al., 2015; Rojas-Rivera et al., 2017; Wang et al., 2017; Wang et al., 2019; Wang et al., 2014; Wang et al., 2018).

Ferroptosis is a form of iron-dependent cell death and is characterized morphologically as appearing to be normal without cell membrane defects and the absence of chromatin condensation (Dixon et al., 2012). Free iron can generate oxidative stress by reacting with lipids to form lipid peroxidation products. The glutathione-dependent antioxidant defense system involves the Xc⁻ transporter which brings cysteine into the cell that is then converted into cysteine and used for glutathione synthesis (Dixon et al., 2012). The glutathione peroxidase 4 (GPX4) then uses glutathione to protect cells against membrane lipid peroxidation (Dixon et al., 2012). Defects or depletion in system Xc⁻, GPX4, and glutathione synthesis can lead to excessive lipid peroxide accumulation and initiate ferroptosis (Dixon et al., 2012). Studies have shown that treatment with molecular inhibitors of ferroptosis can improve motor function recovery in rodents after SCI (Feng et al., 2019, Zhang et al., 2019).

Pyroptosis is a form of programmed cell death that is characterized by chromatin condensation, membrane permeabilization and fragmentation (Bergsbaken et al., 2009). It is triggered by processes related to the innate immunity such as pathogen invasion (Bergsbaken et al., 2009). After encountering a pathogen, a inflammasome is formed to
induce inflammation and pyroptosis (Xue et al., 2019). Pyroptosis has been shown to involve activation of human and mouse caspase-1, human caspase-4 and caspase-5, or mouse caspase-11, and is often initiated in response to pro-inflammatory sections IL-1β and IL18 (Bergsbaken et al., 2009; Man et al., 2017). After SCI, P2X4 receptors influence inflammasome activation (de Rivero Vaccari et al., 2012). Importantly, P2X(4) knock-out mice have impaired inflammasome signaling associated with decreased levels of IL-1β and reduced neutrophil/macrophage infiltration resulting in significant improvements in functional recovery (de Rivero Vaccari et al., 2012).

Neutrophil extracellular trap-associated cell death (NETosis) is a form of cell death that commonly occurs in immune cells like neutrophils that encounter pathogens, although exact molecular mechanisms are still unknown (de Bont et al., 2019). However, it is thought that NADPH oxidases generate intracellular ROS that triggers the release of neutrophil elastase (NE) and myeloperoxidase from granules (de Bont et al., 2019). Elastases then catalyze the proteolysis of F-actin and promote histone degradation (de Bont et al., 2019). This ultimately leads to cell death and the extrusion of chromatin fibers mixed with cytoplasmic components known as the neutrophil extracellular trap (NET). After SCI, neutrophil elastase has been shown to be released at the injury site and disrupt vascular endothelium (Kumar et al., 2018). In the same study, sivelstat, a neutrophil elastase inhibitor, attenuated levels of inflammatory cytokines, reduced glial formation, increased blood vessel formation, and improved locomotor recovery after SCI (Kumar et al., 2018).

Taken together, traumatic SCI is complex pathology associated with a variety of different stressors that induce distinct molecular changes in the CNS that collectively
result in cell death. The balance between cell survival and death is a critical parameter in the maintenance of homeostasis and function recovery following SCI. Importantly, in the rodent mid-thoracic contusive SCI models, white matter loss and not neuronal loss, is the principal driver of impaired hindlimb motor function suggesting that reducing OL loss and/or protecting axonal integrity by attenuating secondary injury cascades improves hindlimb locomotor functional recovery (Hadi et al., 2000; Magnuson et al., 2005; Magnuson et al., 1999).

**Oligodendrocytes**

**Oligodendrocytes: Function**

Oligodendrocytes (OLs) are a type of glial cell found in the CNS that differentiates from oligodendrocyte precursor cells (OPCs) and perform several functions. Non-myelinating functions of OLs include a supportive, immunomodulatory, and regulatory role for neurons, including the secretion of neurotrophic factors such as glial- and brain-derived neurotrophic factor (GDNF and BDNF) which supports axonal functionality and modulate neurite outgrowth (Simons et al., 2015; Wilkins et al., 2003). However, OLs are also the main myelin forming cells in the CNS and have a number of processes that contacts segments of axons and wraps them in concentric layers of membrane to form the myelin sheath that insulates axons and potentiates fast axonal conduction (Bradl et al., 2010). The sheath is comprised of lipids (70% dry weight) and several different proteins, but myelin basic protein (MBP, 30%) and proteolipid protein (PLP, 50%) make up approximately 80% of the total protein fraction (Baumann et al., 2001; Duncan et al., 2016; Gudz et al., 2002). These proteins are synthesized by ribosomes located on the rough endoplasmic reticulum (RER), while the lipid
components of myelin are synthesized in the smooth ER. It has been estimated that a single OL can synthesize as much as $20 \times 10^5 \, \mu m^2$ in surface area of myelin making it one of the most prolific membrane producing cells, but also placing a substantial burden on the ER of myelinating OLs (Pfeiffer et al., 1993). The myelinated axons contain gaps between myelin segments, the nodes of Ranvier, which allow for faster propagation of action potentials through the axon by salutatory conduction. The formation of myelin requires an elevated metabolism that is coupled to a high protein translation capacity along with increased demand for iron which serves as a cofactor for enzymes involved in making essential components of myelin such as cholesterol and glycolipid (Lin et al., 2009). Moreover, in the rodent CNS, OLs accumulate highest levels of iron and that the highest period of iron uptake coincides with demand for peak myelination (Connor et al., 1996; Morath et al., 2001; Taylor et al., 1990). While essential for these cellular processes, iron can be highly reactive by generating oxidative radicals that directly damage proteins, lipids, and DNA (Koskenkorva-Frank et al., 2013). Despite the requirement for high iron concentrations, OLs are more readily damaged by free radicals than other glial cells due to lower production of glutathione (Thorburne et al., 1996). Collectively, these unique OL characteristics make them particularly sensitive to oxidative and metabolic stressors. Indeed, loss of OLs has been implicated in contributing to several CNS diseases such as Pelizaeus-Merzbacher disease, multiple sclerosis, Alzheimer’s disease, cerebral palsy, stroke, and SCI (Goldman et al., 2015; Lucchinetti et al., 2000; Nasrabady et al., 2018; Nobuta et al., 2019).
**OL-specific death and dysfunction in disease**

Multiple sclerosis (MS) is a chronic multifocal neuro-inflammatory disorder that causes functional deficits in walking, vision, and cognition. It is characterized by immune cell infiltration that induces demyelination, causing axonal damage and loss of neuronal synapses (Ghasemi et al., 2017). Specifically, the inflammatory and immune systems attack components of myelin or OLs resulting in OL death (Cudrici et al., 2006). In early MS lesions, OPCs accumulate while OLs are still detected (Boyd et al., 2013). However, as the lesion progresses chronically, both OPC and OL numbers drop. Despite the loss in OPC/OLs numbers, they are still detected in chronic demyelinated lesions, indicating that deficiencies may occur in OPC maturation and/or OL remyelination (Caprariello et al., 2012). These deficiencies have been hypothesized to be caused by the elevated levels of inflammatory mediators such as immune cytokines and reactive oxygen species that contribute to an inflammatory environment which leads to the activation of cellular stress responses (Peferoen et al., 2014). Although previous therapies targeted the immune response, more recently studies show the therapeutic potential of modulating OL stress response that regulates the proteostasis network such as the unfolded protein response (UPR) (Naughton et al., 2016; Peferoen et al., 2014).

Pelizaeus-Merzbacher disease (PMD) is an X-linked leukodystrophy that is caused by mutations in proteolipid protein 1 (PLP1) and the inability to form functional myelin (Gencic et al., 1989). The clinical presentations of PMD range and is thought to be dependent on the mutation in PLP1, however severe mutations can result in developmental delay and lethality (Hoffman-Zacharska et al., 2013). Some PMD-associated mutant PLP proteins accumulate in the ER lumen, which causes ER stress and
UPR activation resulting in death of OLs (Inoue, 2017). Recent studies suggest that targeting the ferroptosis may be therapeutically beneficial. Nobuta and colleagues showed that PLP1 mutations can cause iron-induced OL death that may be rescued by iron chelators or lipophilic antioxidants (Nobuta et al., 2019). However, they also showed that certain PLP1 mutations, namely exon 6 deletion mutations that have more upregulation of the UPR when compared to other mutants cannot be rescued by iron chelation, suggesting that various PLP1 mutations have different mechanisms of cytotoxicity.

Myelin breakdown has also been implicated in neurodegenerative diseases such as AD. The white matter of patients with AD shows an increase of beta-amyloid accumulation along with decreases in MBP, PLP, and OLs indicating a role for white matter degeneration and impaired cortical processing (Desai et al., 2010). Indeed, beta-amyloid aggregates induce death of OLs in vitro (Lee et al., 2004). Moreover, diffusion tensor imaging (DTI) MRI detected disturbances to myelin integrity as evidenced by white matter hyper-intensities in AD patients (Heo et al., 2009).

Finally, in SCI, OLs undergo several modes of cell death, including necrosis and apoptosis. In the rodent SCI model, neuron and OL numbers decrease immediately. By 7 dpi, OLs at the injury site are reduced by 93% (McTigue et al., 2001). However, OLs continuously die over nearly 3 weeks post-injury which further contributes to the loss of myelin and impede axonal function (Pukos et al., 2019). Numerous factors contribute to mechanisms of OL loss including immediate excitotoxicity, disruption of the proteostasis network, ischemia, and oxidative stress associated with reactive oxygen species (ROS) formation generated from reperfusion (Anthes et al., 1996; Benton et al., 2011;
Fassbender et al., 2011; Pukos et al., 2019; Wallace et al., 1986). Oxidative stress can damage ceramides which are a family of waxy lipid molecules involved in the formation of sphingosine and used in cellular membranes (Novgorodov et al., 2018). Ceramides are released by sphingomyelinase activity and can activate pro-apoptotic signaling cascades that lead to OL death (German et al., 2006). Interestingly, ceramides accompanied by ROS accumulation induce death that did not exhibit apoptotic characteristics, and perhaps mediated by ferroptosis (Yang et al., 2016). In addition to ischemia, Ca$^{2+}$ influx, and secretions from inflammatory cells have been show to play a role (Shultz et al., 2017). Infiltrating mononuclear (macrophages, neutrophils) cells peak at early time points within the lesion and secrete products including free radicals and pro-inflammatory cytokine such as TNFα and interleukin-1α (IL-1α), IL-1β, and IL-6 which stay elevated for several days after injury (Jurewicz et al., 2005; Nakamura et al., 2005; Sato et al., 2012; Uindreaj et al., 2016). TNFα induces OL death by acting through TNF receptor p55 (TNFR-p55) (Jurewicz et al., 2005). Upon ligand binding, apoptosis-inducing factor (AIF) translocates from mitochondria translocates to the nucleus triggering cell death (Jurewicz et al., 2005). Disruption of AIF prevents this TNFα-induced cell death in human OLs (Jurewicz et al., 2005). Macrophages and neutrophils also generate ROS causing further oxidative damage. Furthermore, OLs express AMPA-type glutamate receptors (Gallo et al., 1996), NMDA-type glutamate receptors (Salter et al., 2005), and the ATP receptor P2X7 (Matute et al., 2007) making them vulnerable to glutamate and ATP-induced excitotoxicity. Compromised blood-spinal cord barrier leaks components of blood into the CNS which can induce apoptosis and inhibit OPC proliferation (Sahinkaya et al., 2014; Zhang et al., 2012b). Finally, OLs may also initiate apoptotic mechanisms through
FAS and p75 receptor activation (Beattie et al., 2002b). After SCI, OLs that express p75 receptor bind proNGF and activate apoptosis (Beattie et al., 2002). In the same study, p75−/− mice that underwent SCI had a 33% reduction in apoptotic OLs. The mechanisms of OL death after a complex injury like contusive SCI are diverse multiple, but several studies have shown that preventing OL death-induced demyelination and/or promoting remyelination represents a key therapeutic avenue in treating acute SCI (Dong et al., 2003). Importantly, therapeutic effects of any pharmacological treatment on OPCs/OLs responses can extend to all cell types undergoing these similar responses. Targeting the proteostasis network and its effector signaling pathways is a potential global therapeutic approach to facilitate neuroprotection in acute SCI.

The Proteostasis Network: A global target for neuroprotection in SCI

The proteostasis network is comprised of all the elements that are necessary to maintain biologically functional and relevant levels of protein within a cell (Fig 3.). Its effectors include chaperone proteins which help to ensure that newly synthesized proteins are folded correctly to their final native conformation at precise times as dictated by cellular demands (Hartl et al., 2011). Additionally, the proteostasis network removes the accumulation of misfolded or cytotoxic aggregated proteins through either the ubiquitin proteasome system (UPS) or autophagy. The UPS is a highly regulated proteolytic system that controls intracellular protein degradation and turnover through a series of concerted actions by enzymes that link ubiquitin to targeted proteins. The UPS degrades 90% of all cellular proteins (Shen et al., 2013). Aging and cellular stress responses can disrupt proteostasis resulting in an increase of protein misfolding, aggregation, and degradation (Kurtishi et al., 2019). To counteract these types of stresses, the cell utilizes
key stress response pathways that are integral to proteostasis network: the heat shock response (HSR), endoplasmic reticulum stress response (ERSR), integrated stress response (ISR), and the unfolded protein response (UPR) which collectively determines if cellular homeostasis is restored or apoptosis is initiated (Costa-Mattioli et al., 2020). Importantly, proteostasis is critical for normal maintenance of the nervous system and its dysregulation may contribute to several different neurodegenerative pathologies as mentioned above (Lottes et al., 2020). Notably, it also represents a major pathway in mediating secondary injury after SCI (Fig. 4.). Because it is a system that all cells utilize globally, understanding the contribution of each component of the proteostasis network to cell death is critical when designing a rational therapeutic strategy. Such strategies should consider the potential deleterious consequences of proteostasis interventions on inflammation as studies have shown that manipulation of the ISR can lead to enhanced inflammasome activation which may potentially cause more damages (de Rivero Vaccari et al., 2012; Ravindran et al., 2016). Neuroprotective strategies that target global responses such as the proteostasis network after SCI will likely prevent and limit the progression of secondary injury induced neural cell death on all cell types undergoing these responses after SCI and therefore may reduce neurological impairment.
Figure 3. Proteostasis Network and Stress Response Pathways.

The proteostasis network promotes the folding of newly synthesized proteins. Chaperones are involved to help proteins fold into final conformational form. Misfolded or protein aggregation is removed through the proteasome system or though autophagy by proteolytic degradation. Disruptions in proteostasis network can lead to the activation of stress response pathways that maintain homeostasis or initiate cell death (Figure from Whittemore, Saraswat Ohri, Forston, Wei, Hetman, unpublished)
Figure 4. Stress responses activated after SCI.

Cellular stress after SCI ER stress, nutrient deprivation, and oxidative stress all lead to elevated phosphorylation of eIF2α through the PERK, PKR, GCN2, and HRI kinases, respectively. Phosphorylation of eIF2α inhibits global translation of most mRNAs and results in a decline of protein synthesis. The translation of the transcription factor ATF4 is increased. The HIF-PHDs can directly stimulate ATF4 activity independent of eIF2α phosphorylation (Figure from Whittemore, Saraswat Ohri, Forston, Wei, Hetman, unpublished).
Heat shock response (HSR)

The HSR is triggered when cells are exposed to stresses such as oxidation, heavy metals, ischemia, inflammation, infection, and sudden increases in temperatures that interfere with protein homeostasis (Richter et al., 2010a). Cells respond rapidly through a collective group of heat shock transcription factors that bind to promoter regions of various heat shock genes that encode for the heat shock proteins (HSP), molecular chaperones, and other effectors involved in the proteostasis network (Morimoto et al., 1997). For instance, heat shock factor 1 (HSF1) is a well-known transcription factor whose function is regulated by HSP90 and HSP70 (Craig, 1985). While it is known to upregulate production of HSP molecular chaperone proteins that assist in folding, refolding, or degrading misfolded proteins, HSF 1 has diverse mechanisms that can modulate the ERSR, UPS, autophagy, cellular differentiation, survival, and apoptosis.

The endoplasmic reticulum stress response (ERSR)

The endoplasmic reticulum (ER) is a key component of the proteostasis network and performs a variety of different cellular functions that include the synthesis, folding, and maturation of proteins before they form components of cellular membranes or are excreted extracellularly. Additionally, the ER lumen serves as a storage site for cellular calcium (Ashby et al., 2001). The ER can further be divided into the rough ER and smooth ER. Proteins that are synthesized by ribosomes studded on the cytosolic surface of the rough ER are modified and folded inside the ER lumen. The cytosolic surface of the smooth ER is the site of cholesterol, lipids, and steroid synthesis. The protein folding capacity of the ER is dependent on cell type and cellular function. When protein demand exceeds folding capacity, unfolded or misfolded proteins that accumulate in the ER
lumen cause ER stress that triggers the ER stress response (ERSR) (Hetz, 2012). Examples of pathophysiological process that can induce ER stress include ischemia, reperfusion injury, and viral infections (Dara et al., 2011). The ERSR is comprised of both a transcriptional and translational response that attempts to alleviate ER stress by increasing folding capacity, inhibiting general protein translation, and degrading misfolded proteins (Ron, 2002). The transcriptional upregulation of ER components and chaperones proteins help facilitate protein folding and is described as the UPR (Schroder et al., 2005). The UPR signaling pathways regulate and monitor protein folding capacity within the ER to minimize improperly folded forms, and degrade cytotoxic misfolded proteins (Ron, 2002). Unfolded or misfolded proteins that accumulate in the ER lumen are detected by UPR sensors endoribonuclease inositol-requiring enzyme 1-α (IRE1α), protein kinase RNA-like endoplasmic reticulum kinase (PERK) and activating transcription factor 6 (ATF6α), and partly requires the assistance of ER chaperone protein GRP78 (Safra et al., 2013). In the absence of ER stressed conditions, GRP78 binds to the luminal domains of the three sensors and prevents their activation (Chen et al., 2013b). When misfolded proteins begin to accumulate, GRP78 dissociates from the UPR sensors and preferentially binds to regions within the misfolded proteins (Gardner et al., 2013). This allows for IRE1α and PERK to dimerize and become active through autophosphorylation. Following autophosphorylation, IRE1α undergoes a conformational change that activates an RNase domain that excises a 26 nucleotide segment of X-Box binding protein 1 (Xbp1) mRNA that shifts the reading frame (XBP1 splicing) and generates the XBP1s transcription factor which activates genes involved in protein folding and secretion (Lee et al., 2003). Although initially, IRE1 promotes restoration of
homeostasis, in the presence of persistent ER stress IRE1 can directly activate the pro-apoptotic ASK1-JNK signaling cascade and shift from favoring homeostasis to the induction of cell death (Brown et al., 2016). Finally, in the presence of ER stress, the transcription factor ATF6α is cleaved by site-1 protease (S1P) and site-2 protease (S2), exported from the ER and translocated to the nucleus to activate adaptive programs including genes that help eliminate misfolded or unassembled proteins from the ER (Lee et al., 2002). Activated PERK phosphorylates eukaryotic initiation factor 2 α (eIF2α) which transiently inhibits global cellular synthesis of de novo proteins by transiently pausing mRNA translation in an attempt to reduce extra protein load the ER needs to process (Teske et al., 2011). Additionally, peIF2α increases translation of ATF4, which enhances chaperone protein synthesis but also stimulates expression of the pro-death transcription factor C/EBP Homologous Protein (CHOP) and growth-arrest DNA damage gene 34 (GADD34) which contains a protein phosphatase 1 (PP1) and provides negative feedback in PERK-eIF2α axis through GADD-mediated dephosphorylation of eIF2α (Marciniak et al., 2004; Ohoka et al., 2005; Teske et al., 2011). The ultimate biological aim of the ERSR is to restore proteostasis, but if chronic ER stress fails to resolve, persistent activation of the ERSR results in cellular death through various mechanisms some of which well-characterized, and others currently unknown (Johnson et al., 2011).

In *Atf6*<sup>−/−</sup> mice, there was no enhanced recovery after contusive SCI (Saraswat Ohri et al., 2018b). Moreover, in *Xbp1-Nestin<sup>Cre</sup>* mice, there was worse locomotor recovery after a T12 hemisection SCI (Valenzuela et al., 2012). Likewise, *Plp-cre<sup>ERT2/+;Xbp1<sup>fl/fl</sup></sup>* mice that have OL/OPC-specific deletion of *Xbp1* also show a reduced locomotor recovery and less spared white matter after contusive thoracic SCI (Ohri et al., 2020a). SCI injured
mice treated with salubrinal, a pharmacological agent that disrupts GADD34 (R15A, ER-stress induced) and CReP (R15B, a constitutive homolog of GADD34) interactions with protein phosphatase 1c (PP1c) therefore prevents dephosphorylation of peIF2α, acutely (0, 24, and 48 hours post-SCI) show increases in the level of peIF2α and decreases the levels of ATF4, GADD34, and BiP protein in the injury epicenter (Ohri et al., 2013). Transient with salubrinal treatment attenuates OL loss, enhances hindlimb locomotor function, and white matter damage in the epicenter, yet global genetic deletion of GADD34 using Ppp1r15a/Gadd34−/− mice shows no effects on behavioral recovery (Ohri et al., 2014). Similarly, treatment with guanabenz, inhibitor of ER-stress induced GADD34 (R15A) and not CreP (R15B), does not affect functional recovery after SCI (Ohri et al., 2014). Salubrinal disrupts PP1c binding of either regulator (Choy et al., 2015). This suggests temporally and/or substrate specific inhibition of pS51-eIF2α dephosphorylation may be required to reduce SCI-associated white matter damage.

**Integrated Stress Response (ISR)**

The Integrated Stress Response (ISR) is a conserved translational and transcriptional signaling program that is activated in response to a various stress stimuli such as amino acid deprivation, hypoxia, glucose deprivation, oxidative damage, viral infection, and ER stress (Fig. 5). The four independent stress sensing kinases (PERK, PKR, HRI, GCN2) involved in ISR signaling all converge on phosphorylation of eIF2α which, as detailed above, causes global attenuation protein synthesis, but allows for the translation of specific genes that aid in cell survival and recovery such as ATF4 (Pakos-Zebrucka et al., 2016). PERK is predominately activated by ER stress, but also by hypoxia-ischemia, oxidative stress, and oxygen-glucose deprivation (Pakos-Zebrucka et
When unfolded or misfolded proteins begin to accumulate in the ER lumen, glucose-regulated protein (GRP78) dissociates from PERK resulting in autophosphorylation and activation (Wang et al., 2009a). PKR is mainly activated in the presence of double stranded RNA which usually indicates a viral infection, but can also be activated by oxidative stress, ER stress, and bacterial infections (Pakos-Zebrucka et al., 2016). In the presence of double stranded viral RNA, PRK dimerizes which results in autophosphorylation and activation of its kinase activity to phosphorylate eIF2α leading to a shutdown of global protein synthesis in an attempt to inhibit viral protein synthesis (Pakos-Zebrucka et al., 2016). The exact mechanism by which HRI is activated is not well defined, although it seems to play a major role in regulating erythrocyte globin protein levels in the presence of an iron deficiency (Chen, 2014). In the presence of heme, a component needed for the production of hemoglobin, HRI kinase activity is inhibited, but when heme is absent, HRI forms a dimer and becomes active (Zhang et al., 2019). Other stress stimuli including oxidative stress, osmotic stress, heat shock, cytosolic protein aggregates during inflammasome formation, and proteasome inhibition also activate HRI (Zhang et al., 2019). Finally, the GCN2 kinase is activated in response to amino acid starvation, glucose deprivation, and UV irradiation (Pakos-Zebrucka et al., 2016). Although these kinases are activated by distinct pathophysiological stimuli, they can all be activated by oxidative stress. Furthermore, the various signals from each response can differentially summate and converge on key signaling molecules or pathways allowing for a fine tuned control on cellular response to stresses. In general, the ISR is considered to be a pro-survival response, however if stresses are prolonged or
severe enough to devastate the adaptive capacity of the ISR response, ISR may trigger
cell death (Pakos-Zebrucka et al., 2016).
Figure 5. The Integrated Stress Response.

The PKR, PERK, GCN2 and HRI kinases react to various forms of stresses and increase peIF2α. This leads to the inhibition of GEF activity of eIF2B and therefore, mRNA translation initiation. Although there is inhibition on global mRNA translation, the increased levels of peIF2α facilitate the translation of select mRNAs containing that contain uORFs in their 5′UTR, such as mRNA for *Atf4* that plays a key role in determining cell fate. peIF2α is negatively regulated by the CreP/PP1 phosphatase and GADD34/PP1 phosphatase complexes. (modified from Pakos-Zebrucka et al., 2016)
**Activating transcription factor 4 (ATF4)**

The ISR and ERSR contain overlapping signaling pathways that converge to transiently phosphorylate eIF2α and upregulate expression of ATF4. ATF4 modulates several signaling pathways including oxidative stress, inflammation, autophagy, and translation. The outcome on cellular fate can be either pro-survival or pro-death, dependent on the severity of stress, the duration of eIF2a phosphorylation, and translation of ATF4 (Han et al., 2013; Pihan et al., 2017). ATF4 is a transcription factor that belongs to the cAMP response element-binding protein (CREB)-2 family of proteins. It regulates gene transcriptional programs that are influenced upon interactions with its various partners and is tightly regulated at the transcriptional, translational, and post-translational level as it plays a major role determining cell fate in response to stress (Blais et al., 2004).

In response to stress, cells inhibit protein synthesis by phosphorylating eIF2α (Harding et al., 2003). However, despite the transient pause of protein synthesis there is an increase in protein levels of ATF4 that is modulated by translational control (Vattem et al., 2004). *Atf4* mRNA contains two upstream open reading frames (uORF) (Vattem et al., 2004). uORF1 is a positive-acting element, while uORF2 is an inhibitory element that blocks ATF4 expression (Vattem et al., 2004). Under homeostatic conditions, uORF1 facilitates ribosomal scanning and re-initiates at uORF2 that is out-of-frame and overlaps with the coding sequence of ATF4 (Vattem et al., 2004). Thus, ATF4 is not synthesized. However, when eIF2α is phosphorylated under conditions of stress, the accompanied reduction in levels of eIF2-GTP prolongs the duration required for scanning ribosomes to reinitiate translation, and allows ribosomes to scan past uORF2 (Vattem et al., 2004). This allows re-initiation at the ATF4 coding region and the synthesis of ATF4 under
conditions of stress. ATF4 protein stability and activity can further be regulated as it contains multiple sites for post-translational modification that includes ubiquitination, phosphorylation, hydroxylation, SUMOylation, and acetylation (Wortel et al., 2017). For instance, HIF-PHDs hydroxylate proline residues located in the ATF4 protein sequence and regulate its activity (Koditz et al., 2007). ATF4 plays several important roles in regulating neural plasticity, mitochondrial stress, basal metabolic processes such as glucose homeostasis and energy expenditure, and is a key transcription factor during activation of stress responses (Vattem et al., 2004). In response to stress, ATF4 facilitates transcriptional upregulation of gene programs that are involved in the restoration of cellular homeostasis including components of anti-oxidant defense systems, nutrient uptake, and amino acid synthesis and translation (Vattem et al., 2004). Additionally, ATF4 can regulate mitochondrial stress by activating cytoprotective genes that reprogram cellular metabolism via the ISR that results in the attenuation of mitochondrial function, mitochondrial ribosomal proteins, and inhibition of mitochondrial translation (Quiros et al., 2017). In global Atf4−/− mice that received a lateral hemisection SCI, there was worse functional recovery (Valenzuela et al., 2012). This may be attributed to dependence of the hemisection lesion on plasticity for recovery, and suggests ATF4’s role as a positive regulator of neural plasticity (Chen et al., 2003; Corona et al., 2018).

**C/EBP homologous protein (CHOP)**

ATF4 induces additional transcription factors like CHOP/DDIT3 which can induce apoptosis (Rozpedek et al., 2016). While ATF4 itself can promote cellular death by activating pro-apoptotic BCL-2 family member PMAIP1B, one of the best characterized mechanisms of ATF4-induced cell death is through activation of CHOP.
CHOP is a 29 kD protein that belongs to the family of CCAAT/enhancer binding proteins (C/EBPs) and is involved in the regulation of genes that control proliferation, differentiation, and metabolism (Yoneshima et al., 2016). It is comprised an N-terminal transcriptional activation domain and a C-terminal basic-leucine zipper (bZIP) domain. In ER stress, CHOP can function both as a transcriptional repressor and activator. It selectively enhances expression BAK and BAX, pro-apoptotic members of BCL2, which can result in the release of apoptotic molecules like cytochrome c (Cyt-C) and AIF (Hu et al., 2018; McCullough et al., 2001). Deletion of the bZIP domain demonstrates a critical role in CHOP-induced apoptosis (Ubeda et al., 1999). However, CHOP-deficient cells are only partially resistant to ER stress-induced cell death (Song et al., 2008). Moreover, CHOP overexpression alone is insufficient to induce cell death (Oyadomari et al., 2004; Song et al., 2008; Southwood et al., 2016). In the moderate mid-thoracic contusive SCI model, global Chop−/− mice exhibited decreases in acute expression levels of ERSR effector mRNAs (Xbp1, Atf4, Gadd34, and BiP) in the injury epicenter (Ohri et al., 2011). More importantly, at 6 week post-SCI, CHOP deletion attenuates OL apoptosis and leads to an improvement in hind limb locomotor accompanied by an increase of white matter sparing (WMS) at the epicenter (Ohri et al., 2011). Likewise, treatment with valproic acid, an ER stress and pan HDAC inhibitor, reduces CHOP induction, OL, and myelin loss in the injury epicenter after SCI resulting in greater functional recovery (Penas et al., 2011). However, in a severe thoracic contusive SCI model, the same global Chop−/− mice did not have any enhanced functional recovery, suggesting that additional unidentified mediators of secondary tissue damage maybe involved in such injury or that the secondary injury cascade contributions after...
severe injury are diminutive (Ohri et al., 2012). CHOP mediated cell-death is thought to occur through several mechanisms. For instance, CHOP expression of pro-death genes including BH3-only members of the BCL-2 family members, BCL2L11, and BBC3, and DR5 contributes to cell death under ER stress conditions (Li et al., 2014). CHOP can also cooperate and form interactions with other transcription factors including ATF4 to further stimulate the preferential expression of ATF3, GADD34, Trib3, and ER-associated protein synthesis that can lead to additional oxidative stress and mitochondrial damage (Ohoka et al., 2005). CHOP and ATF4 together can regulate expression of 218 genes, while ATF4 alone regulates expression of 254 genes (Pitale et al., 2017). TRB3, an intracellular pseudokinase, is upregulated by ATF4-CHOP when cells are under hypoxia and ER stress conditions (Rozpedek et al., 2016). TRB3 expression inhibits the anti-apoptotic activity of AKT, and increases activation of caspase-3 (Eyers et al., 2017). Expression of CHOP increases ERO1α, an ER reductase that catalyzes the oxidation of protein disulfide isomerase (PDI), causes a highly oxidized environment within the ER resulting in the production of ROS (Muller et al., 2013). The production of ROS within the ER activates a cascade of events serves as positive feedback and further increases CHOP transcription which ultimately results in cell death (Muller et al., 2013; Rozpedek et al., 2016). Finally, ATF4/CHOP-mediated increase in protein synthesis leads to excessive generation of reactive oxygen species (ROS) in the ER that can damage mitochondria and lead to mitochondrial permeabilization induced cell death (Rozpedek et al., 2016).
Hypoxia-inducible factor prolyl hydroxylases (HIF-PHDs)

The HIF-PHD proteins are iron-dependent dioxygenases and exist in three isoforms: PHD1 (EGLN1), PHD2 (EGLN2), and PHD3 (EGLN3). They are a class of metalloenzymes oxygen sensors that hydroxylate proline residues of the hypoxia-inducible factor 1α (HIF-1α) to stimulate its proteasomal degradation under normoxic conditions (Ratcliffe et al., 1998; Semenza, 2001). Under hypoxic conditions, PHDs are inactive so that HIF-1α accumulates and activates adaptive genes that protect against hypoxia (Semenza, 2001, 2007). Furthermore, they are transcriptionally upregulated by HIF-1 and are required for feedback inhibition during persistent hypoxia (Ratcliffe et al., 1998; Semenza, 2001, 2007). The PHDs may also function as a sensor in cellular metabolism of iron (Nandal et al., 2011). Because proline hydroxylation requires the decarboxylation of 2-oxoglutarate, PHDs are inhibited by high concentrations of Krebs cycle intermediates (Ratcliffe et al., 1998; Semenza, 2001, 2007, 2012). The poly (rC) binding protein 1 (PCBP1) functions as an iron chaperone by transferring iron from ferritin and delivering it to the PHDs (Nandal et al., 2011). Under conditions of iron deficiency, loss of PCBP1 leads to diminished hydroxylation of HIF1 and downregulation of HIF target genes (Nandal et al., 2011). However, under conditions of iron deficiency followed by incubation of PCBP1 loaded with iron, PHD activity was restored, suggesting that PCBP1 is able to mediate changes in iron levels into PHD activity (Nandal et al., 2011). Therefore in conditions of iron overload, free iron is scavenged by PCBP1 complexes where it then loads the PHDs thereby increasing its hydroxylation activity (Nandal et al., 2011). Although PHDs are best characterized for their ability to hydroxylate HIF-1α (Fong et al., 2008), they have also been shown to
target other proteins such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) (D'Ignazio et al., 2016), mitogen-activated protein kinase 6 (MAPK6) (Strowitzki et al., 2019), nuclear factor kappa-light-chain-enhancer of activated B cells p100 (NF-κB2/p52) (D'Ignazio et al., 2016), and ATF4 (Cummins et al., 2006). Recent studies have shown that some HIF-PHD isoforms can hydroxylate ATF4 directly on prolines (P156, P162, P164, P167, and P174) (Karuppagounder et al., 2016). In neurons, proline-to-alanine mutations in ATF4 prevent proline hydroxylation and reduce ATF4-dependent cell death (Karuppagounder et al., 2016). Pharmacological PHD inhibition using adaptaquin (AQ) or genetic deletion of the Egln1-3 genes protect neurons from oxidative death due to blood lysis-derived free iron and improves functional outcome after experimental intracerebral hemorrhage (ICH) (Karuppagounder et al., 2016). Moreover, in models of Parkinson's disease, PHD inhibition by AQ directly suppressed Atf4 and/or Chop mRNA as well as the induction of pro-death Trib3 mRNA (Aime et al., 2020). Those beneficial effects appear to be mediated by attenuation of the ATF4-driven cytotoxic gene expression program that is activated in neurons under oxidative stress (Aime et al., 2020; Karuppagounder et al., 2016). One possible explanation is that inhibition of proline hydroxylation functions to reduce ATF4 transcriptional activity, and prevents ATF4 from being recruited to the promoters of pro-death genes such as Trib3 or Chop in response to oxidative stress (Aime et al., 2020; Karuppagounder et al., 2016). Furthermore, the extent of protection of neurons by AQ in individual mice correlated strongly with the improvement of motor function (Karuppagounder et al., 2016). Importantly, these studies demonstrate that ATF4 activity can be upregulated by the PHDs independent of the mediators involved in the proteostasis network after ICH and
represents a potential target for neuroprotection. However, PHD inhibitors have not been evaluated for their therapeutic potential in protecting white matter against acute injuries such as thoracic contusive SCI where the ATF4 target gene *Chop* also plays a major pathogenic role. Furthermore, the role of PHDs in ATF4-CHOP activation after SCI is unknown.

**OL-specific translational gene expression after SCI**

Recent studies demonstrate the involvement of the ERSR in OLs after SCI (Matsuyama et al., 2014; Ohri et al., 2013; Ohri et al., 2011; Saraswat Ohri et al., 2021; Valenzuela et al., 2012). The ERSR employs extensive translational regulation and several of its effector mechanisms are controlled by master transcription factors (TFs) including ATF4, CHOP, XBP1, ATF6 and HSF (Han et al., 2013). Manipulation of the ERSR-associated translational regulation as well as the ERSR-regulated transcription factor CHOP affects both white matter loss and locomotor outcome of SCI (Ohri et al., 2011). Taken together, although it is likely that there are unidentified transcriptional and translational programs that regulate OL responses, little is known about the OL gene expression in the setting of SCI.

Previous transcriptomic studies using homogenized fragments of whole spinal cord provide unprecedented and unbiased systematic knowledge about global transcriptional responses after SCI (Chen et al., 2013a; Li et al., 2019b). However, the various cellular compositions of the spinal cord that undergoes pathological insult after SCI limit cell-specific interpretation of such gene expression data and confound mechanistic insight on OL-specific injury responses. Furthermore, there is no consideration for the extensive post-transcriptional regulation of gene expression that is
employed in cells that are under stress. For instance, SCI induces altered expression of thousands of mRNAs in the contused spinal cord, but a much smaller number of proteins were affected (Chen et al., 2013a; Li et al., 2019b; Squair et al., 2018). Therefore, an unbiased transcriptome-wide approach of OLs in the setting of SCI may provide direct insight of key unknown mechanisms and drivers of OL injury responses that can be targeted to reduce white matter damage and/or facilitate its repair. As detailed further below, the RiboTag methodology can be utilized to immunoprecipitate and capture cell-specific hemagglutinin (HA)-tagged ribosome-associated mRNA complexes in tissues consisting of different cell types.

**Mechanisms of translational regulation**

The ribosome serves as the site of translation and produces proteins that are needed for cell function and survival (Genuth et al., 2018). Gene expression of a cell is regulated at the epigenetic, transcriptional, translational, and post-translational levels (Genuth et al., 2018). Although transcriptional mechanisms can control gene expression through master regulators such as transcription factors and alternative splicing, the processes are complex and slow. Translation control is a rapid and highly dynamic process that can be regulated by cell responses to stress (Hershey et al., 2012). For instance, under conditions of nutrient deficiency such as a lack of amino acid or glucose availability, the cell’s primary response is to regulate overall protein synthesis which through a series of cascading reactions, can reduce ATP use nearly 50% and conserve energy for other critical tasks (Rolfe et al., 1997). However, certain genes during increased cellular stress can resist translational inhibition through different mechanisms such as decreased reliance on ribosomal initiating proteins and upstream open reading
Translation plays a critical role in responding to exogenous and endogenous stressors by selectively enhancing and targeting the production of proteins that exert protective functions. The structural composition of genes influences how the mechanisms of translational control are accomplished (Hershey et al., 2012). During translation, ribosomal initiation is considered to be the rate-limiting step of protein synthesis, which means that in m7G(5')ppp(5')A cap-dependent ribosomal translation initiation, the critical step of 5’ cap recognition of the ribosome with the help of initiation factors is a target for translation regulation (Hershey et al., 2012). Translational regulation of initiation involves the phosphorylation of subunits in initiation factors that induce changes in conformation that affect its ability to recognize mRNA (Hershey et al., 2012). For instance, cap-mediated inhibition can be mediated through phosphorylation of the initiation factor eIF4F complex (eIF4E, eIF4A, RNA helicase, eIF4G) (Hershey et al., 2012). Under normal conditions, 4EBPs are phosphorylated and lowers affinity to bind to eIF4E, but under stress, 4EBPs become dephosphorylated and bind eIF4E, which reduces the complex’s ability to bind to the mRNA and diminishes cap-dependent translation initiation (Carroll et al., 2006). Stress-induced phosphorylation of the eIF2α decreases GDP-GTP exchange and alters formation of the initiation complex, thereby transiently pausing translation (Clemens, 2004). Translational elongation can also be inhibited and controlled through phosphorylation of eukaryotic elongation factor 2 (eEF2) (Hershey et al., 2012). It is targeted by multiple growth-related regulatory pathways triggered by stressors like hypoxia and starvation.
mRNA concentrations and protein abundance correlate significantly, however the variation in mRNA expression only accounts for \(~25\text{-}30\%\) of the variation in protein abundance for human Daoy medulloblastoma cell line (Vogel et al., 2010). Similarly, another study shows gene-to-gene changes in protein synthesis rates contributes to \(~55\%\) of final protein levels, while mRNA abundance contributes \(~40\%\) (Schwanhausser et al., 2011). Additionally, only a fraction of total mRNAs produced in a cell are actively being translated. This is especially true when the proteostasis stress responses are activated, such that specific genes that launch adaptive responses are selectively and preferentially translated. Ribosome concentrations are in excess to mRNAs levels with a total of \(2 \times 10^5\) ribosomes (von der Haar, 2008; Warner, 1999) and \(6 \times 10^4\) mRNAs (Zenklusen et al., 2008) estimated to be in a cell at any one time. Ribosome occupancy (mRNA engaged in translation) among expressed genes ranges from \(41\%\) to \(84\%\) (Lackner et al., 2007; Picard et al., 2012) suggesting that \(20\%\) to \(60\%\) (gene dependent) of total gene-specific mRNA transcripts are freely diffusing and not bound to ribosomes. Using ribosome profiling of the 2,300 total genes in the \(Lactococcus lactis\) genome, 1948 genes were expressed at mRNA level, while ribosomes were associated to 1619 genes, demonstrating that \(83\%\) of genes expressed have ribosomes bound (Picard et al., 2012). Furthermore, during oxidative stress in \(Saccharomyces cerevisiae\), only \(15\%\) of genes involved in the translational response showed changes at the mRNA levels (Shenton et al., 2006). In cells that require rapid induction for function such as cells of the immune system that produce cytokines and chemokines to mediate the inflammatory response, transcriptional and post-transcriptional controls are tightly regulated (Schott et al., 2014). In macrophages (one of the first lines of defense against infections) stimulated with LPS
(lipopolysaccharide), 34.4% of translationally up-regulated mRNAs accounted for 7.8% of total mRNA pool (Schott et al., 2014). Additionally, this response is so potent that genes that are transcriptionally upregulated >3,000 fold, can still be inhibited as demonstrated by the lack of protein product detection (Schott et al., 2014). Furthermore, after LPS stimulation, nearly 40% of genes exhibit a change in translation efficiency using ribosome profiling combined with RNA-Seq (Ribo-Seq), suggesting that immune cells like macrophages extensively use translational regulatory networks to shift from homeostatic responses and synthesize proteins that are critical to launching the inflammatory response in conditions of stress (Zhang et al., 2017).

The mTOR (mammalian target of rapamycin) pathway is a good example of a translational regulatory network that enhances translation and involves two distinct serine/threonine kinases: mTORC1 affects growth, proliferation, protein synthesis and metabolism, while mTORC2 targets cellular survival (Carroll et al., 2006). mTORC1 can directly modulate protein synthesis through interactions with eIF4G, eIF4E, eIF4A helicase, and ribosomal s6 kinase (S6K1) (Holz et al., 2005). For instance, mTORC1 may activate S6 kinase that phosphorylates PDCD4, an eIF4a binding protein, which triggers its ubiquitination by E3 ubiquitin ligase and subsequent degradation, and therefore increasing eIF4a activity. S6K1 can also regulate translation initiation by phosphorylating the cap binding complex component eIF4B at S422 (Shahbazian et al., 2006). Finally, S6K1 can phosphorylate translation initiation factor 4B (eIF4B) which recruits eIF4B to eukaryotic initiation factor 4A (eIF4A) at the translation initiation complex to function as a cofactor of eIF4A that ultimately enhances processivity. The MAPK-ERK pathway and the RAS-RAF pathway can also target eIF4 through kinases MNK1 and MNK2.
(Shahbazian et al., 2006). MNK1/2 can phosphorylate Ser209 on eIF4E, and increase translation of selective survival-related genes (Wendel et al., 2007).

The ISR serves as another pathway that can modulate translation through phosphorylation of eIF2 and eIF2α and its network of kinases and phosphatases (Pakos-Zebrucka et al., 2016). Nutrient deprivation, viral infection, iron deficiency, and ER stress can cause GCN2, PKR, HRI and PERK to phosphorylate eIF2α and trigger an adaptive response that preferentially translates ATF4, while decreasing relative global translation (Pakos-Zebrucka et al., 2016). The dysregulation of these networks that regulate translation is common to different pathologies involving oxidative stress, ischemia, aberrant cellular proliferation, and protein expression in multiple cells types (Costa-Mattioli et al., 2020). Therefore, the proteostasis network and its signaling pathways represents potential global therapeutic target (Oakes, 2017). However, little is known about the OL-specific post-transcriptional regulation of gene expression in the spinal cord that occurs under SCI stress. While it is likely that both transcriptional and translational reprogramming regulate critical components of the injury response in a cell type-specific manner, previous studies of spinal cord transcriptomics or translatomics only provide a global view of spinal cord tissue response to injury (Zhang et al., 2020).

**Limitations in bulk RNAseq after spinal cord injury**

RNA sequencing and microarrays are usually two high-throughput methods that are utilized for identifying genome-wide mRNA transcripts (Kukurba et al., 2015). These methods offer an unprecedented insight and information about changes in the transcriptome and cellular processes. Microarrays can offer information about the relative expression of thousands of defined genes that are detected by chosen probes fixed to an
array matrix chip that bind and hybridize to complementary sequences in the mRNAs of target genes (Kukurba et al., 2015). However, the hybridization-based detection has poor sensitivity, low specificity, and a limited dynamic range. RNA-seq determines sequences of all RNA fragments in a sample and is therefore is truly non-biased and highly quantitative (Evans et al., 2018). This method is able to detect sequence variations including single nucleotide polymorphisms (SNPs), alternative splicing, and non-coding miRNAs. RNA-seq using next-generation sequencing (NGS) by Illumina allows for short reads in parallel rather than individual fragments of DNA and uses the sequencing by synthesis method (Evans et al., 2018). A full description of how RNAseq is performed can be found in the Illumina handbook (https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/samplepreps_truseq/truseq-stranded-total-rna-workflow/truseq-stranded-total-rna-workflow-reference-1000000040499-00.pdf). Briefly, isolated RNA is first reverse transcribed to produce cDNA, which is then fragmented using enzyme digestion or mechanical shearing. Fragments of 200 base pairs in size are recommended (Wang et al., 2009b). After fragmentation, a poly-A tail is ligated at the 3’ end, while a phosphate group is attached at the 5’ end, which contributes to cDNA stability and facilitates ligation of adapters that are used in sequencing. These adapters may contain short barcode sequences so that several samples can be sequenced on the same flow cell. After adapter ligation, cDNA libraries undergo an amplification process that uses the adapter sequences as primers in a PCR reaction so that products with ligated adapters are enriched (Head et al., 2014). In the sequencing by synthesis method, the adapters bind to complementary
oligonucleotides that are fixed to the flow cell. Every fragment that is captured undergoes a process called bridge amplification, while adapters at the unbound end of the fragments bind to nearby oligonucleotides that are complementary (Head et al., 2014). A parallel strand is synthesized and this process is repeated so that identical fragments clusters are formed in a process known clonal amplification (Wang et al., 2009b). Reverse strands are washed from the flow cell leaving behind the forward strands so that a primer can attach to the adapter primer binding site. Polymerase adds a single fluorescently labeled dNTP to the DNA strand. Since each of the four bases are labeled with a unique fluorescent label, a detector then images the flow cell and can identify the dNTP that is incorporated by wavelength of light emitted by the fluorophore, after laser excitation. The number of base pairs sequenced from a DNA fragment determines the read length and is directly correlated to the number of cycles, while the number of clusters determines the number of reads. These metrics are then input into base-calling algorithms to determine the accuracy of the read. NGS pipeline produces raw data in the form of millions of reads of nucleotides 50-200 nucleotides in length along with an associated quality score (Wang et al., 2009b). Low quality reads are then removed in a quality control step using FastQC so that reads can better align to a selected genome in the next set. Genome alignment accounts for introns that are removed during mature mRNA synthesis and can performed using RNAseq aligners such as TopHat2 or STAR. Finally, the aligned reads can be quantified using HTSeq or Cufflinks prior to downstream analysis of differentially expressed genes between samples using DESeq2 or cuffdiff. Prior RNASeq studies that evaluate differentially expressed genes after SCI utilized total tissue homogenates, and therefore report global mRNA changes that occur (Chen et al., 2013a; Li et al., 2019b;
Shi et al., 2017). However, in those experiments, genes identified were overrepresented with functions that are involved with the inflammatory response after SCI (Chen et al., 2013a; Li et al., 2019b; Shi et al., 2017). For instance, biological processes (BP) associated with the immune response, neutrophil chemotaxis, innate immune response, regulation of cell proliferation, and positive regulation of cell migration dominate the top groups of GO (gene ontology) enrichments after SCI (Chen et al., 2013a; Li et al., 2019b; Shi et al., 2017). These genes are likely due to the massive influx of immune system cells after SCI, and can therefore obscure the detection of critical responses in various other cell types that undergo significant cell death post injury.

**RiboTag allows for OL-specific translatome profiling after SCI**

There are several methods for studying the translatome (King et al., 2016). Polysome profiling using sucrose density-gradient methods separated mRNAs that were associated to polysomes from free RNA and ribosomal subunits by mass and density. This fractionation method also separated out polysomes by the number of bound ribosomes per mRNA and allowed for direct determination of translation efficiencies (Panda et al., 2017). Recent techniques for polysome profiling include translating ribosome affinity purification (TRAP) and RiboTag. Both are based on affinity purification of genetically labeled ribosomes (Heiman et al., 2014; Sanz et al., 2019). Tags are introduced to core ribosomal proteins such as EGFP to RPL10a, in TRAP or HA to RPL22, in RiboTag. This allows for the effective selection of mRNAs undergoing translation from a cellular homogenate (Heiman et al., 2008). Furthermore, by using cell-type specific promoters, these methods can be utilized to drive expression of tagged ribosomal proteins in specific cell populations of transgenic animals and avoid the
possible contamination issues that plagued fractionation techniques caused by polysome bound mRNA complexes that were also generated from spatially touching cells in complex tissues (Haimon et al., 2018). These mRNA-associated tagged ribosome complexes can then be immunoprecipitated using magnetic beads coated in antibodies against the anti-EGFP or anti-HA antibodies so that mRNAs can be selectively isolated and coupled together with RNA-Seq. Comparison of purified immuno-precipitated mRNA (IP) to input RNA (IN), which is comprised of both bound and unbound mRNAs enables measuring of ribosome bound mRNA enrichment and can identify cell specific translational gene expression (Ramanathan et al., 2019). Indeed, such an approach was utilized to study gene expression changes in OLs during remyelination that revealed cholesterol homeostasis a therapeutic target in a MS model (Itoh et al., 2018). Another study using RiboTag labeled macrophages and showed that lipid catabolic pathways could be therapeutically targeted after SCI (Zhu et al., 2017). However, there are currently no studies that look at OL-specific responses in the setting of SCI. Therefore, the RiboTag can be applied to study SCI-mediated gene expression in OPC/OLs (Fig. 6).
Figure 6. Schematic of RiboTag SCI mouse model and experimental workflow.

An illustration that highlights RiboTag technology. A RiboTag mouse is crossed to a Cre mouse under the control of the Plp1 promoter. Oligodendrocytes that express Cre recombinase will delete WT exon 4 so that HA-tagged exon 4 is now expressed. Translating mRNA ribosome complexes are extracted from the spinal cord homogenate and immunoprecipitated using anti-HA antibodies and magnetic beads. After overnight incubation, magnetic beads are washed with a high salt buffer; RNAs are then purified and used in RNA-Seq analysis.
CHAPTER 2
HYPOXIA-INDUCIBLE FACTOR PROLYL HYDROXYLASE DOMAIN (PHD) INHIBITION AFTER CONTUSIVE SPINAL CORD INJURY DOES NOT IMPROVE LOCOMOTOR RECOVERY

Introduction

The pathophysiology of spinal cord injury (SCI) consists of a primary injury phase which occurs as the direct result of mechanical insult to the spinal cord and a secondary injury phase that involves multiple pathophysiological mechanisms including inflammation, vascular disruption, blood hemorrhage and ischemia. These mechanisms further exacerbate the initial injury and lead to greater functional loss (Ahuja et al., 2017; O'Shea et al., 2017). The proteostasis network includes all proteins with a role in protein synthesis, folding, disaggregation, or degradation (Labbadia et al., 2015). Integral to proteostasis are the heat shock response (HSR), the endoplasmic reticulum stress response (ERSR), the integrated stress response (ISR), and the unfolded protein response (UPR) pathways which further attempt to restore cellular homeostasis and if unsuccessful initiate apoptosis and cell death. We and others have shown that the ERSR is activated acutely after SCI (Ohri et al., 2013; Ohri et al., 2020b; Ohri et al., 2012; Ohri et al., 2011; Penas et al., 2007; Valenzuela et al., 2012). The ERSR initially promotes cell survival by reducing global protein synthesis and upregulating chaperones that assist in protein synthesis, folding, and disposal.
folding. However, excessive or sustained ER stress that cannot be resolved initiates cellular death and contributes to the secondary injury cascade (Lin et al., 2008; Ohri et al., 2011; Ohri et al., 2014; Penas et al., 2007). Interventions that target or alleviate the ERSR after SCI reduce oligodendrocyte (OL) death, protect white matter, and improve locomotor recovery (Myers et al., 2019; Ohri et al., 2013; Ohri et al., 2012; Ohri et al., 2011; Valenzuela et al., 2012).

The ERSR drives expression of activating transcription factor 4 (ATF4) and its target and partner CCAAT-enhancer-binding protein homologous protein (CHOP/DDIT3). Upon activation of the ERSR, these transcription factors (TFs) drive a gene expression program that supports amino acid and protein synthesis in an attempt to restore ER homeostasis. However, if ER stress is unresolved, ATF4/CHOP-mediated gene expression becomes cytotoxic by causing ER protein overload, ER-induced oxidative stress, mitochondrial damage, and ultimately apoptosis (Han et al., 2013).

The HIF prolyl hydroxylase domain proteins PHD1/EGLN1, PHD2/EGLN2, and PHD3/EGLN3, encoded by the $Egln1$, $Egln2$, and $Egln3$ genes, are a class of metalloenzymes oxygen sensors that, under normoxic conditions, hydroxylate proline residues of hypoxia-inducible factor 1α (HIF-1α) to stimulate its proteasomal degradation (Fong et al., 2008). Under hypoxic conditions, PHDs are inactive so that HIF-1α accumulates and activates adaptive genes that protect against hypoxia. However, pharmacological PHD inhibition using adaptaquin (AQ) or genetic deletion of the $Egln1$-$3$ genes protect neurons from oxidative death due to blood lysis-derived free iron and improves functional outcome after experimental intracerebral hemorrhage (ICH) (Karuppagounder et al., 2016). Those beneficial effects appear to be mediated by
attenuation of the ATF4-driven cytotoxic gene expression program that is activated in neurons under oxidative stress (Karuppagounder et al., 2016). PHD-mediated hydroxylation of several ATF4 proline residues is a likely mechanism that promotes cytotoxic activity of ATF4 (Karuppagounder et al., 2016).

Recent studies further demonstrate the protective role of pharmacological PHD inhibition by reducing ATF4-CHOP-mediated neuronal death and protecting against oxidative damage in models of neurodegeneration including Parkinson's and Alzheimer's disease (Aime et al., 2020; Li et al., 2019a; Li et al., 2016; Neitemeier et al., 2016; Niatsetskaya et al., 2010), where neuronal loss is paramount. However, PHD inhibitors have not been evaluated for their therapeutic potential in protecting white matter against acute injuries such as thoracic contusive SCI where the ATF4 target gene Chop plays a major pathogenic role (Ohri et al., 2011). Furthermore, the role of PHDs in ATF4-CHOP activation after SCI is unknown. The current study was undertaken to determine the contributions of PHDs to ATF4-CHOP activation and white matter loss after SCI.

Materials and Methods

Animals

All animal procedures were approved by the University of Louisville Institutional Animal Care and Use Committee and the Institutional Biosafety Committee, in accordance with guidelines from the Public Health Service Policy on Humane Care and Use of Laboratory Animals, Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council, 1996), and strictly adhered to NIH guidelines on use of experimental animals. AQ experiments were performed on wild type (WT) 8-10 week-old C57BL/6 female mice (Envigo, Indianapolis, IN) fed a
standard ad libitum chow and housed under 12h dark/light cycle. Females are predominantly used in rodent SCI literature due to lower incidence of post-operative complications and, therefore, have better survival as compared to males (Stewart et al., 2020). Moreover, while sex-specific drug effects are possible, locomotor recovery in SCI rodents is not significantly affected by sex (Fukutoku et al., 2020; Luchetti et al., 2010; Walker et al., 2019). Therefore, AQ studies were performed in females to reduce the animal number needed for adequately powered data. Plp-creERT2 (proteolipid protein) (Plp-cre-B6.Cg-Tg (Plp1-Cre/ERT) 3Pop/J; catalog #5975) and Egln1/2/3fl/fl (Egln1tm2Fong Egln2tm2Fong Egln3tm2Fong/J; Stock No: 028097) mice were acquired from The Jackson Laboratory (Bar Harbor, ME). Those lines were crossed to generate experimental subjects (Egln1/2/3fl/fl:Plp-CreERT2), which, due to limited availability of animals with the desired genotype, included both males and females. Those mice were treated with tamoxifen to induce Egln1/2/3 knockout primarily in CNS myelinating OLs (Doerflinger et al., 2003; Fuss et al., 2001; Wight et al., 1993) (see the Drug treatments paragraph for more details). Controls included male and female Egln1/2/3fl/fl:Plp-CreERT2 that were treated with vehicle and WT mice that were treated with tamoxifen. Importantly, PLP is expressed earlier than myelin basic protein (MBP) during development, beginning during embryonic life and at early developmental stages of OPC differentiation (Mallon et al., 2002; Wight et al., 1993), so in tamoxifen-induced Egln1/2/3fl/fl:Plp-CreERT2 mice, some OPC recombination may be expected as well. Sex unbalanced groups emerged in studies using Egln1/2/3fl/fl:Plp-CreERT2 mice due to limited availability of males of comparable age and peri- and/or post-operative loss of animals (Table 1 for more details).
**Spinal cord injury**

Prior to surgery, mice were anesthetized using an intraperitoneal injection of 0.4 mg/g body weight Avertin (2,2,2-tribromoethanol in 0.02 ml of 1.25% 2-methyl-2-butanol in saline, Sigma-Aldrich, St. Louis, MO). The back of the mice was shaved and disinfected using a 4% chlorohexidine solution. Lacri-Lube ophthalmic ointment (Allergen, Madison, NJ) was used to prevent drying of the eyes. A dorsal laminectomy was done at the T9 vertebrae and positioned under the Infinite Horizons (IH) Impactor as previously described (Ohri et al., 2011; Scheff et al., 2003). A moderate contusion injury (50 kdyn force/400–600 μm displacement) was delivered and mice were immediately placed onto a temperature-controlled 37°C heating pad until sternal time. Mice were administered buprenorphine twice daily for the following 2 days. Gentamycin (50 mg/kg; Boehringer Ingelheim, Ridgefield, CT) was administered subcutaneously to reduce infection. Controls included sham animals that received T9 laminectomy only. All surgeries were performed without knowledge of a group assignment or genotype. For each study, surgeries for all groups were performed on the same day or two consecutive days with random sequence of animals from various groups/genotypes.

**Drug treatments**

Tamoxifen was dissolved in corn oil (Sigma, C8267) at 20 mg/mL and administered intraperitoneally (1 mg/day) beginning 21 days prior to SCI and continuing for 8 consecutive days as previously described (Ohri et al., 2018). Adaptaquin (AQ), obtained from Dr. Rajiv Ratan and Dr. Saravanan Karuppagounder (Burke Neurological Institute, Weill Medical College of Cornell University), was dissolved in a solution of 0.03% DMSO and olive oil. Fresh aliquots were prepared daily for treatment. AQ was
administered by intraperitoneal injections (0.1 cc/injection 30 mg/kg) first, within 1 hour after SCI, and then, daily for either 3 days (the experiment to collect tissues at 3 days post injury; the last AQ injection administered 2h before euthanasia) or 7 days (the experiment to determine effects of AQ on locomotor recovery) of treatment. The AQ dosing was based on the previous ICH study which confirmed blood-brain barrier penetration and anti-ATF4 activity in the brain (Karuppagounder et al., 2016). Proton nuclear magnetic resonance spectroscopy ($^1$H NMR) was used to confirm the molecular integrity of AQ (Fig. 9).

**Behavioral Assessment**

Animals were habituated to human interaction and handling twice a day for 5 consecutive days, 1 week prior to SCI/Sham. Baseline Basso Mouse Scale (BMS) locomotor scores were obtained prior to injury for every individual animal and weekly following SCI for 6 weeks (Basso et al., 2006; Ohri et al., 2011). Raters were trained by Dr. Basso and colleagues at the Ohio State University and were blinded to the animal genotype and treatment groups. The order of animal analysis was random.

**RNA extraction and analysis**

Total RNA was extracted from spinal cord tissue at the injury epicenter (5 mm segment spanning the injury site) using Trizol (Invitrogen) according to the manufacturer’s guidelines. RNA was quantified by ultraviolet spectroscopy (NanoDrop2000, Thermo Scientific, Waltham, MA). cDNA synthesis was performed with random hexamers using 500 ng of total RNA using the Invitrogen SuperScript IV VILO Master Mix I (Thermo Fisher) in a 20 μL reaction volume. All cDNAs were diluted 10x with water before using as a template for quantitative real time RT-PCR.
qPCR was performed using ViiA 7 system (Applied Biosystems, Foster City, CA). Briefly, diluted cDNAs were added to TaqMan universal PCR master mix or SYBR Green master mix (Applied Biosystems) and run in triplicate. Primer sets are listed in Table 2. For qPCR analysis of PHD family mRNAs, protocols and primers were used as previously described (Takeda et al., 2006; Tojo et al., 2015). RNA levels were quantified using the ΔΔCT method with Gapdh as a reference gene. Transcript levels were normalized to their respective levels in sham or vehicle controls and expressed as fold-changes.

**White matter sparing (WMS)**

WMS was evaluated as described previously (Magnuson et al., 2005; Ohri et al., 2011). Six weeks post-SCI, mice were anesthetized with avertin, followed by thoracotomy and transcardial perfusion with ice-cold PBS and 4% paraformaldehyde (PFA). Spinal cords were dissected and submerged in 4% PFA overnight at 4°C. They were then transferred to 30% sucrose for at least 7 days at 4°C, blocked in Tissue Freezing Media (Cat # 72592, Electron Microscopy Science, Hatfield, PA) and stored at -20°C. Spinal cords were serially cut (20 μm) in transverse sections spanning 5 mm rostral and caudal to the injury epicenter, and were stained for myelin using iron eriochrome cyanine (EC) with an alkali differentiator (Stefanovic et al., 2015). Images were captured using a Nikon Eclipse Ti inverted microscope (Nikon Instruments Inc., Melville NY), and white matter was traced using Nikon Elements software. The epicenter of each injury was identified visually based on the section with the least amount of spared white matter. Data were normalized to spared white matter in corresponding non-injured sections. All imaging and analyses were performed blinded to ensure unbiased quantification.
**ODD-luciferase assay**

The biological activity of AQ used in animal studies was confirmed on cultured SH-SY5Y cells using the HIF-1α oxygen degradation domain (ODD)-luciferase reporter assay (Fig. 10) as previously described (Karuppagounder et al., 2016; Smirnova et al., 2010).

**Statistical analysis**

All qPCR or image analysis data (WMS) were analyzed using the non-parametric Mann-Whitney test ($u$-test, single sided). Repeated-measures ANOVA (RM-ANOVA) followed by Tukey post hoc tests were used for analyzing BMS locomotor recovery data. A priori power calculations were performed for analysis of locomotor recovery. Power analysis based on BBB/BMS variance in published rodent SCI studies with standard deviations between 1.5-2.3 shows that the ability to detect a significant difference of 10% in BMS with at least 90% power in a sample size of 8/group. Hence, all locomotor recovery assessments were adequately powered. Data are reported as mean ± SD. Statistical analyses were performed using SPSS, version 25 (IBM).

**Results**

At 3 days post-SCI, there were similar declines in neuron-specific enolase ($Nse/Eno2$) mRNA and increases in astrocyte-specific mRNAs ($Gfap, Glul$) in vehicle- and AQ-treated animals suggesting no effects of AQ on either cell type (Fig. 7a). However, there were increased OL-specific transcripts ($Olig2, Mbp$), along with decreased mRNAs of $Atf4, Chop$, and the ATF4/CHOP target gene $Trib3$ in AQ-treated animals (Fig. 7b). Moreover, $Map2$ mRNA, which is expressed mainly in neurons, but also detected in OLs (LoPresti et al., 1995), was increased. These results suggest reduced
acute loss of OLs and attenuated activation of the ATF4-CHOP pathway. However, as expression of the ER stress-associated ATF4/CHOP targets Gadd34/Ppp1r15a and Slc7a11 was unaffected, the anti-ATF4 effects of AQ appear to be target gene-specific. Finally, AQ treatment did not modify ERSR-associated induction of Grp78 and Xbp1 mRNAs suggesting no general attenuation of the ERSR.

To determine the effects of AQ treatment on chronic locomotor recovery, mice received vehicle or 30 mg/kg AQ immediately after injury and then daily for 7 days. Hindlimb function was evaluated using the BMS for 6 weeks post-SCI. Comparison of BMS scores between vehicle- and AQ-treated mice revealed no significant differences (Fig. 7c). Consistent with no AQ effects on locomotor recovery, a similar extent of white matter sparing was detected in AQ- and vehicle-treated groups (Fig. 7d, e). These data show that despite transient improvement in OL survival acutely after contusive SCI, pharmacological inhibition of PHDs using AQ did not increase chronic white matter sparing or locomotor recovery.

To determine if the genetic deletion of the PHDs in OLs affects outcome after SCI, we used OL-specific Plp-creERT2+/+:Egln1/2/3fl/fl mice to conditionally remove all 3 PHD isoenzymes in OLs following treatment with tamoxifen. Fourteen days after the completion of tamoxifen or vehicle treatment, we confirmed that tamoxifen treatment resulted in lower Phd/Egln mRNA levels in uninjured spinal cords of Plp-creERT2+/+:Egln1/2/3fl/fl mice (Fig. 8a). Consistent downregulation of Phd/Egln transcripts ranged from 23 to 25% of control values. These findings suggest that (i) gene deletion efficiency was similar for each isoform and (ii) the deletion occurred in most PLP-positive, mature OLs as their estimated spinal cord content is 20-25% (Dawson et al.,
2003; Sathyamurthy et al., 2018; Valerio-Gomes et al., 2018) and at least the maximally expressed Phd2/Egln2 appears to be ubiquitously present in all spinal cord cells (Lein et al., 2007). In addition, Phd/Egln mRNAs were downregulated by 25-32% in the OL-rich tissue of the optic nerve (Fig. 8a), but not the OL-lacking liver of tamoxifen-treated Plp-cre<sup>ERT2</sup>+/+:Egln1/2/3<sup>fl/fl</sup> mice (Fig. 11). The OL-specific loss of Phd/Egln genes was biologically relevant as the established HIF target genes Vegf and Epo were upregulated, including 27% increase of Vegf or 37-39% increases of Vegf and Epo transcripts in the spinal cord or the optic nerve, respectively. Such upregulation is expected as a result of reduced negative regulation of HIF due to PHD deficiency (Karuppagounder et al., 2016; Ramakrishnan et al., 2014). The lesser response in the spinal cord than the optic nerve is likely caused by high basal level spinal neuron expression of Epo and Vegf mRNAs (Lein et al., 2007) which can potentially dilute the OL induction of those genes after Phd/Egln deletion. Thus, our data suggest OL-specific knockout of PHD1/2/3 isoforms.

At 72 h after SCI, tamoxifen- and vehicle-treated Plp-cre<sup>ERT2</sup>+/+:Egln1/2/3<sup>fl/fl</sup> mice had similar neural cell mRNA levels, suggesting unaffected acute loss of neurons and OLs (Fig. 8b). No effects on the expression of Atf4, Chop, Gadd34 or Grp78 mRNAs were observed (Fig. 8c). Moreover, comparison of 6-week BMS scores for tamoxifen- and vehicle-treated Plp-cre<sup>ERT2</sup>+/+:Egln1/2/3<sup>fl/fl</sup> mice revealed no significant differences in locomotor recovery (Fig. 8d). Likewise, no differences in chronic hindlimb locomotor recovery were observed when tamoxifen-induced OL-PHD knockouts were compared to WT mice that received identical tamoxifen treatment (Fig. 8d). Therefore, OL-selective deletion of HIF-PHD does not affect SCI-associated acute OL loss, ATF4-CHOP signaling or chronic locomotor recovery.
Discussion

Adaptaquin (AQ), a hydroxyquinoline-based inhibitor of PHDs, abrogates ATF4-CHOP-dependent neuronal death and improves functional outcomes in mouse models of ICH and Parkinson’s disease (Aime et al., 2020; Karuppagounder et al., 2016). While these studies implicate PHDs in ATF4-CHOP-mediated neuronal death, their involvement in OL death and white matter damage after SCI remains unknown. Acutely after SCI, the pro-apoptotic, ERSR-activated transcription factors ATF4 and CHOP are upregulated in neurons and OLs (Ohri et al., 2013; Ohri et al., 2012; Ohri et al., 2011; Penas et al., 2007; Valenzuela et al., 2012). Our previous study showed improved behavioral outcome after thoracic contusive SCI in Chop−/− mice that was associated with increased WMS and attenuated acute loss of OLs (Ohri et al., 2011). Likewise, pharmacological interventions targeting proteostasis attenuated SCI-associated activation of ATF4-CHOP while improving functional recovery and WMS (Ohri et al., 2013). Therefore, we hypothesized that pharmacological and/or genetic suppression of ATF4-CHOP signaling after thoracic contusive SCI would similarly restore proteostasis and prevent chronic OL loss, leading to increased spared white matter and improved functional outcomes. However, AQ-mediated attenuation of acute OL loss and ATF4-CHOP signaling did not improve chronic locomotor recovery or WMS. Importantly, although AQ reduced Atf4, Chop, and Trib3 mRNA levels, there was no effect on three other ERSR transcripts, Gadd34, Grp78, and Xbp1 (Fig. 7b). In previous studies, where increased acute OL/OPC mRNA levels were accompanied by increases in chronic locomotor recovery, there was reduced activation of not only Atf4 and Chop, but also
other ERSR transcripts (Myers et al., 2019; Ohri et al., 2018; Ohri et al., 2013; Ohri et al., 2011; Saraswat Ohri et al., 2020). It is likely that AQ inhibits ATF4-CHOP signaling that is distinct from their involvement in the ERSR and therefore limits the effects of AQ’s effects on SCI outcome. Indeed, in the ICH model, AQ was proposed to inhibit the ATF4-mediated iron-dependent ferroptosis, but not the cytotoxic ER stress (Karuppagounder et al., 2016; Ratan, 2020). Hence, our AQ studies suggest that although PHDs contribute to ATF4-CHOP signaling in the contused spinal cord, the cytotoxic ERSR is the dominant signaling pathway that drives chronic white matter damage and functional deficits (Ohri et al., 2020b; Ohri et al., 2011). This interpretation is further supported by results from SCI mice with OL/OPC-specific PHD deletion (Fig. 8). OLs are uniquely sensitive to injury-induced ERSR because of their high protein translation requirements (D'Antonio et al., 2009; Lin et al., 2009; Ohri et al., 2011; Pennuto et al., 2008; Southwood et al., 2002). However, no effects on thoracic contusive SCI-associated increases of ATF4 and CHOP signaling were observed in the OL-specific Phd/Egln1,2,3−/− mice. Likewise, acute OL loss and locomotor recovery were unaffected.

These observations are in contrast to those from various CNS injury models with primary effects on the grey matter (Aime et al., 2020; Li et al., 2019a; Li et al., 2016; Neitemeier et al., 2016; Niatsetskaya et al., 2010). Those data suggest that PHD inhibition might improve functional outcomes in lumbar or cervical models of SCI, where neuronal death is paramount to loss of function (Hadi et al., 2000; Magnuson et al., 1999; Nishida et al., 2015). The differences in the effects of AQ on ICH and thoracic contusive SCI highlight the complexities of the proteostasis network (Labbadia et al., 2015). Following CNS trauma, multiple proteostasis signaling pathways are differentially
activated by distinct pathophysiological stimuli. Depending on the extent and duration of those stimuli, the various pro-homeostatic and pro-apoptotic signals differentially summate. Moreover, there are both overlapping and diverse aspects of the respective arms of proteostasis signaling (Adamson et al., 2016; Lee et al., 2003; Pakos-Zebrucka et al., 2016; Shoulders et al., 2013). Unfortunately, there is currently no consensus on how best to globally target proteostasis effectors for treating CNS trauma. While it is likely to be therapeutically beneficial, it will have to be empirically determined for each type of injury.
Figure 7. After SCI, AQ attenuates the acute loss of OL lineage mRNAs, moderately reduces acute ATF4 and CHOP activation, but does not improve chronic functional locomotor recovery. (a) neural cell-specific and (b) ISR/ERSR and ATF4-regulated gene mRNA levels 72 hours-post SCI. Transcript levels (normalized to Gapdh) are expressed as fold change sham controls. Data in (a, b) are the mean ± SD (n = 8, *p<0.05; **p<0.01; ns, p>0.05, u-test). (c) BMS analysis of hindlimb locomotion revealed no significant differences in hindlimb locomotor recovery between vehicle (Veh)- (n=6) and AQ-treated 30 mg/kg (n=7) mice (repeated measure two-way ANOVA showed significant effects of time after injury /F_{5,21} = 12.34; p<0.001/ but no significant effects of treatment /F_{1,59} = 0.03; p>0.05/ or the interaction between time after injury and treatment /F_{5,21} = 0.97, p>0.05/; see Table 3 for raw BMS data). (d) Representative images of the injury epicenter stained with EC to identify myelin (6 weeks post-SCI). (e) Quantitative analysis of EC-stained sections through the injury epicenter shows similar extent of spared white matter. Data are the mean ± SD (n=4, p>0.05, u-test).
Figure 8. Effects of HIF-PHDs deletion in OL lineage cells after SCI. (a) Tamoxifen (Tam)-treated Plp-cre\textsuperscript{ERT2+/+}:Egln1/2/3\textsuperscript{fl/fl} mice (n=10, M:F=4:6) show reduced Phd1/Egln1, Phd2/Egln2, and Phd3/Egln3 mRNAs in the spinal cord (controls received vehicle /Veh/, n=9, M:F=4:5). Similarly, Tamoxifen (Tam)-treated Plp-cre\textsuperscript{ERT2+/+}:Egln1/2/3\textsuperscript{fl/fl} mice (n=4, M:F=2:2) show reduced Phd1/Egln1, Phd2/Egln2, and Phd3/Egln3 in the optic nerve (controls received vehicle /Veh/, n=3, M:F=2:1).

Confirming biological relevance of the OL-specific PHD1/2/3 deficiency, established HIF target genes including Vegf or Vegf and Epo were upregulated in OL-containing spinal cord and optic nerve, respectively. (b) At 72 h after injury, SCI-associated declines of neuronal- or OL mRNAs are unaffected by Tam-mediated OL-selective deletion of PHD1/2/3. (c) Likewise, Tam-mediated knockout of OL-PHD1/2/3 did not attenuate SCI-associated induction of ERSR transcripts including Atf4 and Chop. Transcript levels (normalized to Gapdh) are expressed as fold changes of Veh control (a) or sham Plp-
$cre^{ERT2+/+}:Egln1/2/3^{fl/fl}$ controls (b,c). Data (b,c) are the mean ± SD (n=4, M:F=2:2 *p<0.05; ns, p>0.05, u-test). (d) BMS analysis of hindlimb locomotion revealed no significant differences in recovery between Veh- (n=8, M:F=2:6), Tam-treated $Plp-cre^{ERT2+/+}:Egln1/2/3^{fl/fl}$ mice (n=12, M:F=5:7), and Tam-treated WT C57BL/6 mice (n=10, M:F=4:6). When comparing Veh- and Tam-treated $Plp-cre^{ERT2+/+}:Egln1/2/3^{fl/fl}$ mice, repeated measure two-way ANOVA showed significant effects of time after injury ($F_{5,56} = 11.89$, p<0.001), but no significant effects of Tam treatment ($F_{2,95} = 2.6$, p>0.05), or the interaction between the time after injury and Tam treatment ($F_{10,56} = 0.99$, p>0.05); see Supplementary S3Table for raw BMS data.
Figure 9. $^1$H NMR spectrum for the AQ batch used in the current SCI study.

$^1$H NMR (DMSO-$d_6$, 500 MHz) δ 9.84 (s, 1H), 8.84 (dd, 1H), 8.2-8.4 (m, 1H), 7.66 (d, 1H), 7.54 (dd, 1H), 7.3-7.5 (m, 6H), 6.8-6.9 (m, 2H), 6.4-6.5 (m, 2H) matches the spectra of AQ as previously described (Thinnes et al., 2015). Data were recorded on a Varian Unity Inova 500 MHz and chemical shifts are reported in ppm using the solvent as an internal standard (DMSO-$d_6$ at 2.5 ppm).
Figure 10. Validation of biological activity for the AQ batch (AQ /KY/) used in the current SCI study. SH-SY5Y ODD-Luc cells were treated with increasing concentrations of AQ (0.1 µM-10 µM) for 3 h. ODD-luciferase activity was determined by luminometry. AQ inhibits HIF prolyl hydroxylase activity and stabilize luciferase fused to the oxygen degradation domain (ODD).
Figure 11. Effects of OL-specific HIF-PHDs deletion in the OL-lacking liver tissue.

Tamoxifen (Tam)-treated Plp-cre$^{ERT2^{+/+}:Egln1/2/3^{0/0}}$ mice (n=4, M:F=2:2) show no changes in Phd1/Egln1, Phd2/Egln2, Phd3/Egln3, Epo, and Vegf mRNAs (controls received vehicle /Veh/, n=3, M:F=2:1)
Table 1. Experimental design.

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<th>Study (figure panel)</th>
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<th>Number of animals lost to peri- or post-operative mortality (M:F)</th>
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<td>Analyzing effects of AQ on locomotor recovery (Fig 1c)</td>
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<td>8 (0:7)</td>
<td>2 (0:2) &lt;sup&gt;a&lt;/sup&gt; 1 (0:1) &lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Analyzing effects of AQ on WMS (Fig 1d, e)</td>
<td>WT SCI Veh @ dpi 42, WT SCI AQ @ dpi 42</td>
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<td>None</td>
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<td>Analysis of HIF-PHDs deletion on HIF-dependent gene mRNA levels (Fig 2a)</td>
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<td>4 (2:2) 4 (2:2) 4 (2:2)</td>
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<td>11 (5:6) 14 (7:7) &lt;sup&gt;a&lt;/sup&gt; 12 (6:6) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>2 (0:2) 2 (0:2) 2 (0:2)</td>
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<sup>a</sup> Unbalanced gender ratios were caused by limited availability of males of comparable age

<sup>b</sup> In cases where animals were lost before completion of all behavioral assessments, their individual scores were excluded from group analyses
Table 2. List of qPCR primers.

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Gene Primer sequences used for SYBR Green reactions

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<th>Primer sequences used for SYBR Green reactions</th>
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| *Phd1* | For: 5'- ATGGCTACGTGAGCGACCTA-3'  
Rev: 5'- CATTGCTCTGATAACGCCAC-3' |
| *Phd2* | For: 5'- TAAACGGCGCAACGAAAGC-3'  
Rev: 5'- GGTTATCAACGTCGAGGACA-3' |
| *Phd3* | For: 5'- CTATGTCAAGGAGCGTGCTCAA-3'  
Rev: 5'- GTCCACATGGCGAAACATAAACC-3' |
| *Epo* | For: 5'- CATCTGCACAGTGAGTCTGT-3'  
Rev: 5'- CACAAACCATCGTGACATTTTC-3' |
| *Vegfa* | For: 5'- CTGCTGAAACAGATGACCCCTG-3'  
Rev: 5'- GCTGTAGGAAGGCCTCTCC-3' |
Table 3. Raw BMS score data for individual mice.

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*5 davs post injury
CHAPTER 3

THE TRANSCRIPTOMIC LANDSCAPE OF OLIGODENDROCYTES RESPONSES TO SCI – SIGNATURES OF CELL DEATH

Introduction

The pathogenesis of spinal cord injury (SCI) results in temporal components that include primary and secondary injuries as well as post-injury remodeling and plasticity (Norenberg et al., 2004; Rowland et al., 2008). In thoracic contusive SCI, functional deficits are primarily driven by white matter (WM) damage (Magnuson et al., 2005; Magnuson et al., 1999). Acute loss of axons and oligodendrocyte (OL) death are major contributors to the injury-induced white matter damage (Pukos et al., 2019) and is likely caused by a combination of cytotoxic events that contribute to the secondary injury cascade. Increased levels of glutamate (Xu et al., 2004), proinflammatory cytokine interleukin-1α (IL-1α), tumor necrosis factor-α (TNFα) (Donnelly et al., 2008), and proNGF (Beattie et al., 2002b) after SCI have been reported to mediate OL death. Recent studies demonstrate the involvement of the ERSR in OLs as a major cause of secondary injury induced death after SCI (Myers et al., 2019; Ohri et al., 2013; Ohri et al., 2011; Valenzuela et al., 2012). The ERSR employs extensive translational regulation and transcriptional changes with several of its effector mechanisms controlled by master transcription factors (TFs) such as ATF4, CHOP, XBP1, and ATF6 (Boyce et al., 2006).
Manipulation of the ERSR-associated translational regulation through eIF2α and the ERSR-regulated transcription factor CHOP decreases white matter loss and improves locomotor outcome of SCI (Myers et al., 2019; Ohri et al., 2013; Ohri et al., 2011; Valenzuela et al., 2012). Aside from these characterized mechanisms, very little is known about transcriptional and translational events that regulate time-dependent OL responses in SCI.

Previous transcriptomic studies using homogenized fragments of whole spinal cord that contain heterogeneous cell populations limit cell-specific interpretation of gene expression and confound mechanistic insight on OL-specific injury responses (Chen et al., 2013a; Shi et al., 2017). Furthermore, post-transcriptional regulation of gene expression by cells under stress is overlooked. RiboTag technology can overcome these limitations and obtain cell-specific information from complex tissues (Sanz et al., 2009). Notably, RiboTag showed that despite widely believed negative effects of astrocytic scar on post-SCI regeneration/structural plasticity, astrocytes upregulate of many genes that are conducive to axonal growth (Anderson et al., 2016). Another study that utilized RiboTag identified CD36 as a regulator of macrophage lipid metabolism to drive a post-SCI switch from inflammation to lipid catabolism (Zhu et al., 2017). Interestingly, deleting CD36 was previously shown to increase locomotor recovery after SCI in the context of alleviating ERSR, inflammation, and microvascular dysfunction (Myers et al., 2014). Hence, RiboTag is a powerful method that can not only be used to obtain insight to reveal novel functions or pathways, but also to integrate various mechanisms reported from previous literature and build a more complete understanding of SCI pathogenesis.
Here, we utilized RiboTag technology to obtain OL-specific mRNAs from $Plp^{CreER}:Rpl22^{HA}$ mice and compared translational gene expression changes at 2 (peak of OL cell death, acute), 10 (delayed OL cell death/remyelination, subacute), and 42 (chronic phase) dpi to identify translatomic responses that may contribute to OL cell death. Our data show that at 2 days dpi, OLs upregulate genes that may induce oxidative stress ($Chac1$, $Steap3$), inhibit survival signaling kinases ($Spred3$, $Spry4$, $Parvb$), and contribute directly to death ($Runx1$). Pro-survival genes upregulated included $Sphk1$, $Aldh18a1$, $Gdnf$. These data represent the first study to use the RiboTag approach in studying the OL-specific translational profile after SCI and defines cellular mechanisms that are activated so that therapeutic strategies can be better target response after SCI.

**Materials and Methods**

**Animals**

All animal procedures were approved by the University of Louisville Institutional Animal Care and Use Committee and the Institutional Biosafety Committee, in accordance with guidelines from the Public Health Service Policy on Humane Care and Use of Laboratory Animals, Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council, 1996), and strictly adhered to NIH guidelines on use of experimental animals. $Plp$-cre$^{ERT2}$ (The Jackson Laboratory, Plp-cre-B6.Cg-Tg (Plp1-Cre/ERT)3Pop/J; Stock No: 005975) and $Rpl22^{HA}fl/fl$ (The Jackson Laboratory, B6J.129(Cg)-$Rpl22^{m1.1Psam}$/SjJ; Stock No: 029977) mice were crossed to generate experimental subjects ($Plp$-cre$^{ERT2+}$,$Rpl22^{HA}fl^{wt}$). Those mice were treated with tamoxifen to induce expression of $Rpl22^{HA}$ primarily in CNS.
myelinating OLs. Controls included female $Plp-Cre^{ERT2+/wt}:Rpl22(HA)fl^{wt}$ that were treated with vehicle.

**Spinal cord injury**

Prior to surgery, 10 week old female mice $Plp-Cre^{ERT2+/wt}:Rpl22(HA)fl^{wt}$ were anesthetized using an intraperitoneal injection of 0.4 mg/g body weight Avertin (2,2,2-tribromoethanol in 0.02 ml of 1.25% 2-methyl-2-butanol in saline, Sigma-Aldrich, St. Louis, MO). The back of the mice was shaved and disinfected using a 4% chlorohexidine solution. Lacri-Lube ophthalmic ointment (Allergen, Madison, NJ) was used to prevent drying of the eyes. A dorsal laminectomy was done at the T9 vertebrae and positioned under the Infinite Horizons (IH) Impactor as previously described (Ohri et al., 2011; Scheff et al., 2003). A moderate contusion injury (50 kdyn force/400–600 μm displacement) was delivered and mice were immediately placed onto a temperature-controlled 37°C heating pad until sternal time. Mice were administered buprenorphine twice daily for the following 2 days. Gentamycin (50 mg/kg; Boehringer Ingelheim, Ridgefield, CT) was administered subcutaneously to reduce infection. Controls included sham animals that received T9 laminectomy only. All surgeries were performed without knowledge of group assignment or genotype.

**Drug treatments**

Tamoxifen was dissolved in corn oil (Sigma, C8267) at 20 mg/mL and administered intraperitoneally (1 mg/day) beginning 35 days prior to SCI and continuing for 8 consecutive days as previously described (Saraswat Ohri et al., 2018a).
RNA Isolation and high throughput sequencing

The RiboTag protocol for immunoprecipitation of RNA was followed (Sanz et al., 2019; Sanz et al., 2009). Briefly, frozen tissues from SCI and naïve animals (5 mm spanning the SCI epicenter) at 2, 10, and 42 day post-SCI (2 animals were pooled together to produce one biological replicate, n=3 per time point injured, n=1 per time point naïve) were homogenized with a pre-chilled Dounce homogenizer in cold homogenization buffer on ice [50 mM Tris (Sigma), pH 7.5, 100 mM KCl (Sigma), 12 mM MgCl₂ (Sigma), 1% Nonidet P-40 (Sigma), 200 U/mL RNAsin (Promega, catalog #N2115), 1 mM DTT (Sigma-Aldrich), proteinase inhibitors (Roche), 1 mg/mL heparin (Sigma-Aldrich), 0.1 mg/mL cyclohexamide (Sigma-Aldrich)]. Sample homogenates were then centrifuged at 10,000xg for 10 minutes at 4 °C. Monoclonal mouse anti-HA antibody (HA.11 Clone 16B12, Biolegend) was added to the supernatant (1000 ml), and the mixture was incubated for 4 h in a 4 °C cold room. Prewashed protein A/G magnetic beads (Pierce) in homogenizing buffer were added to mixture and further incubated at 4 °C overnight with rotation. The following day, samples were placed in a rack containing magnets pre-chilled on ice to remove supernatants. Magnetized beads were washed 3 times using a high salt buffer (50 mM Tris, pH 7.5, 300 mM KCl, 12 mM MgCl₂, 1% Nonidet P-40, 1 mM DTT, 100 g/mL cycloheximide). Input and immunoprecipitated mRNA was purified by using RNeasy Mini, RNA isolation kit (Qiagen, 74104 ) and Arcturus PicoPure RNA Isolation Kit (Applied Biosystems, LSKIT0204) according to manufacturer’s protocol, respectively. RNA was quantified with a NanoDrop 1,000 spectrophotometer (Thermo Scientific), the Qubit Fluorometer (Thermo Scientific), and RNA integrity was assessed by a Bioanalyzer (Agilent Technologies). Libraries were
prepared using the Universal Plus mRNA-Seq (NuGEN Cat# 0508) and consisted of Poly (A) selection, RNA fragmentation, cDNA synthesis, cDNA purification, end repair, adaptor ligation, strand selection, strand selection purification, library amplification, and library purification. Sequencing was performed on the University of Louisville Center for Genetics and Molecular Medicine’s (CGeMM) Illumina NextSeq 500 using the NextSeq 500/550 75 cycle High Output Kit v2.5 (20024906). These procedures were performed at the University of Louisville Genomics Core Facility while analysis was performed by the Kentucky Biomedical Research Infrastructure Network Bioinformatics Core.

Total RNA was extracted from spinal cord tissue at the injury epicenter (5 mm segment spanning the injury epicenter) using Trizol (Invitrogen) according to the manufacturer’s guidelines. RNA was quantified by ultraviolet spectroscopy (NanoDrop2000, Thermo Scientific, Waltham, MA).

**RNA-seq data analysis**

RNA-seq data analysis RNA sequencing produced ~960,000,000 reads across the 24 samples (Table 6). The sequence read quality was assessed using FastQC v.0.10.127, which indicated that quality was considered to be good so that no sequence trimming was necessary. The sequences were then directly aligned to the *Mus musculus* reference genome assembly using the STAR aligner v.2.628. Raw read counts were obtained from the STAR aligned bam format files using HTSeq version 0.10.0. The raw counts were normalized using the Relative Log Expression (RLE) method and then filtered to exclude genes with fewer than 10 counts across the samples. UCSC Genome Browser tracks were created to easily explore expression across the tested sites. A principal component analysis (PCA) was performed using the plotPCA function in DESeq2. Differential
expression was performed using DESeq2, which computes expected gene counts and log2 fold changes based on a negative binomial regression model. This study design included two independent variables of interest, group (IN, IP) and dpi (naïve, 2dpi, 10dpi, 42dpi). Group and dpi were expected to interact with each other across many genes. Therefore, an interaction term (group x dpi) was added to the DESeq2 negative binomial regression model to identify genes for which IP vs IN changed over time. There were 12 animals across 24 samples with each animal sampled once for IP and once for IN. To identify genes with a group-specific effect (IP vs IN) at one or more time points (2x4 interactions), the full regression model was compared to a reduced model using a likelihood ratio test.

**Quantitative real-time PCR**

To validate the OL specific immunopurified mRNAs prior to RNA sequencing, cDNA synthesis was performed with random hexamers using 50-100 ng of total RNA using the Invitrogen SuperScript IV VILO Master Mix I (Thermo Fisher) in a 20 μL reaction volume. qPCR was performed using ViiA 7 system (Applied Biosystems, Foster City, CA). Briefly, cDNAs were added to Custom TaqMan Gene Expression Array Cards (Thermo Fisher, 4342249) Taqman Fast Advanced Master Mix (Thermo Fisher, 4444557). Primer sets are listed Table 4. RNA levels were quantified using the ΔΔCT method with Gapdh as a reference gene. IPs were normalized to their respective inputs and expressed as fold changes (enrichment).

**Western Blot Analyses**

Protein lysates were prepared from 5 mm contused spinal cord tissue isolated from sham and injury epicenter of WT mice at 2, 10, and 42 days post-injury (dpi) in
protein lysis solution (20 mM Tris, pH-6.8, 137 mM NaCl, 25 mM β-glycerophosphate, 2 mM NaAPP, 2 mM EDTA, 1 mM Na$_3$VO$_4$, 1% Triton X-100, 10% glycerol, protease inhibitor, 0.5 mM DTT, 1 mM PMSF). The BCA kit (Pierce) was used to quantify the protein lysates. Equal concentrations of proteins were separated on SDS-PAGE gels and transferred to PVDF membrane (Amersham Hybond) and probed with RUNX1 (sc-522308, Santa Cruz Biotechnology, 1/1000, Santa Cruz, CA), GDNF (sc-13147, Santa Cruz Biotechnology, 1/1000, Santa Cruz, CA), PARVB (sc-374581, Santa Cruz Biotechnology, 1/1000, Santa Cruz, CA), and GAPDH (Chemicon, 1/5000, Temecula, CA) antibodies.

**Histology**

Mice were anesthetized with avertin, followed by thoracotomy and transcardial perfusion with ice-cold PBS for 5 minutes and 4% paraformaldehyde (PFA). Spinal cords were dissected and submerged in 4% PFA overnight at 4°C, then transferred to 30% sucrose for at least 7 days at 4°C. Tissue was blocked in Tissue Freezing Media (Cat # 72592, Electron Microscopy Science, Hatfield, PA) and stored at -20°C. Spinal cords were cryosectioned (20 µm) at -22°C in transverse sections.

**Results**

**Isolation of OL-specific mRNA**

The strategy we utilized to isolate mRNA specifically from OLs is outlined in Fig. 12a. To validate the OL-specific expression of HA labeling in $\text{Plp}^{\text{CreER}}:\text{Rpl22}^{\text{HA}}$ mice, we performed immunohistochemistry and quantitative RT-PCR (qPCR) on uninjured spinal cord isolated from $\text{Plp}^{\text{CreER}}:\text{Rpl22}^{\text{HA}}$ mice treated with tamoxifen (Tam) or vehicle (Veh). In Tam-treated animals, double immunofluorescence showed that co-localization of the
mature OL marker CC1 with HA was ~60% (Fig. 12b,c). Conversely, very little HA expression was detected in Veh-treated animals (~5%). We performed immunoprecipitation (IP) isolations in accordance to the RiboTag procedure with a few modifications. To optimize OL-specific IP mRNA yields, we homogenized spinal cord tissues in varying volumes of supplemented homogenizing buffer and established that a 0.05% weight:volume ratio produced the highest and most consistent yields (Fig. 13, Table 5). We then utilized qPCR to confirm enrichment (IP-HA RNA/Input RNA) of OL-specific mRNAs (Mbp, Plp1, Mog, Mag, Sox10) and de-enrichment of astrocyte (Aldh111, Hgf), neuron (Reln, Snap25), and microglia (Cd68, Tmem19) mRNAs (Fig. 12d). These data validate that OL-specific RNAs can be isolated from the spinal cord in Plp\textsuperscript{CreER}:Rpl22\textsuperscript{HA} mice using the RiboTag approach.

**RNA-Seq of OL-specific mRNA after SCI**

A moderate T9 contusion SCI was performed on Plp\textsuperscript{CreER}:Rpl22\textsuperscript{HA} mice treated with tamoxifen. Spinal cords were isolated at 2 (peak of OL death, acute), 10 (sub-acute), and 42 (chronic phase) dpi. A 5 mm segment of the epicenter was homogenized, followed by immunoprecipitation of RNA associated HA-labeled ribosome complexes using anti-HA antibody. RNA extraction was performed and sequenced. We obtained >37 million pair-end reads that aligned >97% with the mouse reference genome for every individual sample (Table 6). Principal component analysis (PCA) was performed and showed sufficient separation among groups across all time points (Fig. 14a). PC1 accounts for 70% of the variance which is attributed to the difference between the total RNA samples and the OL-specific RNA samples, while PC2 accounts for 19% of the variance across due to differences between naïve and post-injury samples. Similarly, pairwise comparison
between IP and input (IN) at all time points show that OL-specific genes are enriched, while astrocyte, neuron, and microglia genes are de-enriched in IP samples. (Fig. 14b). Additionally, comparison of our naïve uninjured IP-HA mRNA data set to a previously published transcriptomic data set that focused specifically on neural cells of the cerebral cortex (Zhang et al. 2014), indicates that the IP mRNAs are predominately isolated from mature OLs and to a lesser extent from newly formed OLs (Fig. 14c). These results demonstrate effective RiboTag isolation of OL-enriched mRNAs from the spinal cord.

Differential gene expression using DESeq2 was obtained. Importantly, we normalized IP-HA samples with their respective IN samples to reduce background noise and potential contamination of RNA from non-OL-specific cells that may produce falsely detected differently expressed genes (DEGs) as commonly reported from RiboTag affinity purification isolations (Gregory et al., 2020; Haimon et al., 2018; Jambusaria et al., 2020; Kang et al., 2018). When IN-normalized OL gene expression was analyzed using the interaction function of DESeq2, a total of 3,757 differentially expressed genes (2,015 upregulated, 1,742 downregulated) were identified as having a significant interaction in the 2x4 (group x dpi, i.e. a significant OL gene expression change over the entire time course of analysis) interaction term model as determined by q-value cutoff ≤ 0.05 with log2FC > 0. A 2x2 (group x dpi, i.e. a significant OL gene expression change over an individual time point of analysis, q-value cutoff ≤ 0.05 with log2FC > 1) interaction was then used to identify individual genes that were upregulated at a given individual time point. In summation, only genes that were significant across all time, and a particular chosen individual time point were identified. Gene ontology (GO) enrichment of input normalized IP-HA DEGs identified from the previous filtering steps show OL
DEGs enriched in annotated functions associated with bioenergetic metabolism across all time points (Fig. 15a). Conversely, GO analyses of input injury versus input naïve show a strong enrichment of biological processes that are related to inflammation or immune-associated responses after SCI which are not observed in the input normalized IP-HA injury samples further suggesting that our data set is OL-specific across all time points.

To evaluate how these genes change at different stages after injury, we used K-means clustering to sort the 3,757 DEGs into 23 clusters according to significant upregulation (assigned a value of 1), significant downregulation (assigned a value of -1) or no significant change (assigned a value of 0) at 2, 10, and 42 dpi (Fig. 16a). The total number of genes associated with each cluster ranged from 947 to 1 (Table 7). Clusters that contain fewer numbers of genes were likely to not reach the threshold required for many biological processes and therefore received a limited set of annotations (Fig 17, 18). Therefore, we combined smaller clusters with larger clusters of similar patterns as defined by upregulated (U), downregulated (D), or no change (N) when compared to naïve animals. For instance Clusters 2 contained DEGs that are downregulated at 2 and 10 dpi, but upregulated or no change at 42 dpi when compared to control naïve uninjured animals, and hence assigned D/D/N (Table 7). Similarly, clusters 4, 15, and 17 follow a similar trajectory as time passes, namely downregulation at 2 dpi then gradual up regulation. Clusters 5, 9, 12, and 19 were combined as they were classified as U at 2 dpi, U/D/N at 10 dpi, and D/N at 42 dpi. GO enrichment (q-value cutoff ≤ 0.05 and |log2FC| > 1, dpi 2 IP-HA vs naïve) was performed on the combined clusters classified (2, 4, 15, 17) and (5,9,12,19) (Fig. 16b,c). Consistent with secondary injury induced damage after
SCI and OL function, amino-acid biosynthesis, regulation of neuron death, cell junction organization, regulation of membrane potential, and synaptic transmission processes were some of the most enriched. We then identified genes associated with the regulation of neuron death and extract a limited set of genes (Fig. 16b). Therefore, we manually filtered the upregulated DEGs to establish a list of 98 candidate gene list according to these criterion: 1) log2FC >1; q<0.05 for input normalized IP-HA injury versus input normalized IP-HA naïve 2) >2 fold (q≤0.05) at 2 dpi when input normalized IP-HA injury was compared to input normalized IP-HA naïve at 42 dpi, and therefore likely to represent acute OL response that include regulation of the death and/or survival 3) log2FC >1; q<0.05 for IP-HA injury verses IP-HA naïve to avoid false detection of scenarios where variations in input levels lead to apparent changes in expression (Fig 19a). We utilized PUBMED to classify candidate genes as either pro-survival or pro-death (Fig. 19b). Similarly, we repeated the process for acutely downregulated genes and establish a list of 38 (>2 fold at 2 dpi when compared to naïve or 42 dpi, q≤0.05).

However, the majorities of genes identified were associated with synaptic functions and likely represented OL-specific transcriptional responses to neuron loss (Fig. 20). In total, 136 genes (98 upregulated, 38 downregulated) were identified using these criteria. Given that MAPK signaling pathways are involved in both pro-survival and pro-death responses after SCI (Genovese et al., 2008), we identified the sprouty related EVH1 domain containing 3 (Sprad3) and sprouty RTK signaling antagonist 4 (Spry4) as inhibitors of MAPK-associated pro-survival pathways. Similarly, activation of RUNX family transcription factor 1 (RUNX1) was identified as a response to DNA-damage that by interaction with p53 is able initiate cell death (Satoh et al., 2012). Additionally we
identified six-transmembrane epithelial antigen of prostate (Steap3) and parvin beta (Parvb) as potential contributors to oxidative stress and the inhibition of the pro-survival Integrin pathway, respectively. Pro-survival genes included Sphingosine-1-p Sphk1 and glial cell line-derived neurotrophic Gdnf possesses diverse processes that regulate several molecular events underlying cellular homeostasis and viability (Maceyka et al., 2012; Wilkins et al., 2003). In mouse models of Huntington’s and Parkinson’s, stimulation of SPHK1 using K6PC-5, pramipexole, or fingolimod proves to be neuroprotective by reducing toxic aggregate protein and stimulating Akt kinase (Di Pardo et al., 2019; Motyl et al., 2018). Another target that was significantly induced after SCI nearly ~20 fold is aldehyde dehydrogenase 18 family member A1 (Aldh18a1) which encodes a protein (P5C) that catalyzes the reduction of glutamate to delta1-pyrroline-5-carboxylate, a critical step in the de novo biosynthesis of proline, ornithine and arginine. Importantly, proline mediates anti-oxidant defenses and overexpression of this enzyme was shown to be protective against oxidative stress (Krishnan et al., 2008).

**Up-Regulation of potential death/survival regulating genes**

To confirm these changes in total protein expression in the spinal cord 2 dpi, western blot analysis showed ~3 (a 55kDa isoform) to 13.8 (a - 28 kDa isoform) fold increase in RUNX1 protein levels (Fig. 19c) and ~7.5 fold increase in GDNF protein levels (Fig. 19d) of sham controls. These changes have a correlation with log2FC values of ~11.5 for Gdnf and 5.8 for Runx1 obtained from dpi 2 IP-HA versus dpi 2 IP-naïve samples. For Runx1, the demonstrated upregulation may come from both OL and non-OL cells, as Ribotag data indicated that non-OL cells are the predominant source of Runx1 mRNA at dpi 2 (IP/IN dpi 2, ~.62 fold, q<0.05). For GDNF, the change is likely
mediated by OLs as *Gdnf* mRNA is enriched in IP samples at dpi 2 (IP/IN, ~4.37 fold, q<0.05).

**Discussion**

OL death and white matter damage contribute to functional deficits after traumatic SCI, but OL specific-mechanisms that are activated after SCI remain largely unknown. Although previous transcriptomic studies provide systematic knowledge about global transcriptional responses after SCI (Chen et al., 2013a; Shi et al., 2017), a major challenge has been identifying OL-specific responses apart from responses that occur in other cell populations in at the injury epicenter. Using RiboTag to profile the OL-specific translatome, our data indicate that OLs are enriched in upregulated genes that are associated with pro-survival signaling pathways and cell death. We identified *Steap3*, *Chac1*, *Spry4*, *Spred3*, *Parvβ*, and *Runx1* as potential candidates that directly contribute to cell death and oxidative stress or inactivate survival pathways after SCI. Potential pro-survival genes included *Sphk1*, *Aldh18a1*, and *Gdnf*.

Although, RiboTag is an extremely powerful tool to recover and examine cell type specific translomes from complex tissues, a technical challenge is to maximize recovery of cell-specific mRNA from myelin rich tissues. The large quantities of myelin debris, inherent in spinal cord tissue, that is generated during the homogenizing process can aggregate with polysomes and subsequently settle with the pellet after centrifugation to significantly reduce isolation yields (Fig. 13, Table 5). There were negligible RNA yields when following the standard RiboTag isolation protocol. We systematically modified individual components of the isolation protocol to optimize conditions relevant to our model. Adjusting antibody concentration, antibody incubation duration, and tissue
amount slightly increased recovery but also increased non-specific RNA binding. However, adjusting the volume of the homogenizing buffer greatly improved our yields. We reasoned that a larger volume permits for polysomes to more freely diffuse in solution and kinetically avoid interactions with myelin contaminants. Decreasing the percent weight to volume ratio to 0.5% improved recovery OL-specific mRNA by ~10 fold, and may be considered for polysome isolation from tissues rich in myelin without a Percoll debris removal step (Whittemore et al., 1993) which may introduce unwanted isolation bias.

Another technical challenge is to avoid contamination from other cells. For instance, since macrophages begin to infiltrate and accumulate within the epicenter acutely, Cd68, specific to macrophages, was highly expressed in our initial raw data set of IP-HA samples despite having a ~20 fold depletion (IP/IN, data not shown) using RiboTag immunopurification. Therefore, we normalized our IP-HA samples with their respective IN samples and utilized an additional interaction term to identify genes that have a changing relationship between IP and IN across time points (i.e. identifying mRNAs whose input-normalized expression in OL IP samples changes, see Materials and Methods). This process filters out genes that do not have significant temporal interactions as determined by our selection criteria, and also excludes potential contaminate genes such as CD14 (macrophage), CD44 (neutrophil), CDH5, and VWF (endothelial). Despite this filtering process some T-lymphocyte genes including CD52, CD81, and H2-T23 were still detected as significantly changing their OL expression (data not shown). However, given the absence of classical T-cell markers such as CD2, CD3, CD4, CD28, and CTLA-4, wild scale T-lymphocyte contamination is highly unlikely. Furthermore, as
proliferating astrocytes comprise of a substantial amount of cells at the injury epicenter, the lack of *Gfap, Aldh1l1, Apod, Mt1, and Lgals3bp*, all of which are highly translated in astrocytes, further supports a notion that our filtered data represent OLs without a major contamination by other cell types. It is possible that *CD52* and *CD81* are expressed, as OLs have recently been shown to upregulate antigen processing/presentation genes (*H2-K1, H2-D1, H2-T23*) and participate in immunomodulation in MS (Falcao et al., 2018; Kirby et al., 2020). Therefore, to account for potential contamination we used a filtering criterion to exclude a significant portion of genes during quantitative data analysis and show that processes involved in myelin synthesis (Fig. 15a) are enriched acutely after SCI confirming that our data set is OL-specific.

Our goal was to define responses by mature OLs after SCI. Therefore the inducible Plp-Cre mouse line was selected to label those OLs and exclude OPCs. While *Plp* is expressed by OPCs, only a few single OPCs were Cre\textsuperscript{ERT2}-positive in this mouse system (Doerflinger et al. 2002). The majority of HA expression is restricted to CC1\textsuperscript{+} cells following tamoxifen treatment (Fig. 12 a). When comparing OL-specific IP data to a previously published neural cell transcriptome (Zhang et al., 2014), we observe very strong correlation (0.96-1) with myelinating OLs (Schober et al., 2018) (Fig. 14c). In contrast, they weakly correlate with OPC (0.28-0.39), neurons (0.3-0.33), and astrocyte (0.23-0.33) equally (Patrick et al. 2018). After SCI, OPCs immediately being to proliferate and differentiate into new OLs within 3 days (Hesp et al., 2015; McTigue et al., 2001; Zai et al., 2005). Our data do not show processes involving OPC proliferation enriched at 2 DPI (Fig. 15a). Therefore, the majority of our dataset is represented by more mature OLs after SCI.
Gene ontology performed on combined cluster sets revealed several processes upregulated including amino acid biosynthetic process (*Bcat1, Bcat2, Asns, Aldh18a1, Pycr1*) and regulation of neuron death (*Hrk*), while downregulated processes were associated with synaptic function processes (Fig. 16c). Recent studies report induction of the ER stress response (ERSR) can lead to increased ATF4 transcription/translation that results in the upregulation of genes involved in amino acid synthesis in OLs after SCI (Myers et al., 2019; Ohri et al., 2013; Ohri et al., 2011; Valenzuela et al., 2012). This is consistent with our finding of upregulated amino acid biosynthesis genes (*Bcat1, Bcat2, Asns, Aldh18a1, Pycr1*), all of which are ATF4-target genes. Additionally, previous work has shown that *Hrk* activation contributes to OL apoptosis in the contused spinal cord (Yin et al., 2005). Taken together, the confirmation of these findings gave us confidence that our OL-specific data accuracy reflects the acute pathology of SCI.

There were no obvious OL-intrinsic cell death mechanisms uncovered by gene ontology (Fig. 16, 17, 18). Thus, we manually filtered our data set to account for biologically relevant processes that occur after SCI such as peak of OL-death and characterized every individual gene as either pro-survival or pro-death (Fig. 19a). We decided to first focus our efforts on our upregulated data set which contained more pro-death/survival genes, and as the majority of genes identified in the downregulated dataset using the same approach were related to OL-specific synaptic functions. Several OL-specific intrinsic pro-death genes (*Spry4, Spred3, Parvb*) that emerged from the upregulated dataset were ones that inactivated survival pathways. Others (*Runx1, Chac1, Steap3*) found were likely to directly contribute to cell death, while pro-survival genes included *Sphk1, Aldh18a1*, and *Gdnf*. With the exception of *Gdnf*, none of these genes
have reported roles in OLs. For instance, previous reports demonstrate that the MAPK signaling pathway and its stress activated protein kinases mediate secondary injury on a global scale after SCI (Genovese et al., 2008; Stirling et al., 2008), but OL-specific molecular mechanisms have never before been studied. Our finding may suggest that Spred3 and Spry4 can modulate the MAPK, so that extrinsic survival signals are subdued while pro-apoptotic stress activated pathways are amplified (pro-death Runx1 downstream of MAPK) and drive OLs to cell death. Additionally, OLs have long been known to be uniquely sensitive to SCI-induced oxidative death due to their high iron content and metabolic requirements (Thorburne et al., 1996). The upregulation of Chac1, Runx1, and Steap3 identified in our data set may contribute to this process and compliments that finding, but also introduces potential new OL-specific cellular targets. Conversely, OL-intrinsic pro-survival mechanisms (Aldh18a1, Gdnf, Sphk1) that can reduce oxidative stress and/or prevent cell death may be stimulated to increase oxidative stress tolerance. Importantly, western blot analysis confirms increased total protein levels of GDNF and RUNX1 and further validates our RiboTag approach (Fig. 19c,d).

However, because total homogenate lysates were used for western blot analysis, changes in protein levels include those from other cell types. Therefore, a more sensitive quantitative method with single cell resolution such as immunohistochemistry (protein quantification) or RNAscope (RNA quantification) is required for definitive validation of our data set. OL survival depends on extrinsic signals such as neurotrophic factors supplied by axons (Barres et al., 1993, 1999). After SCI, neurons and OLs begin to die immediately. Neuron loss tapers off at 2 dpi, while OLs death continues nearly 2 week post-injury (Pukos et al., 2019). In the context of SCI, this area of OL death is
understudied and OL-intrinsic mechanisms that occur after oligo-axonal synapse loss are unknown. Genes identified in our downregulated data set is the first report of OL-specific synapse-related transcriptional changes that occur when oligo-axonal junction are disrupted and can provide mechanistic insight about axon loss induced OL death after SCI. Collectively, our RiboTag data accurately reflect previously reported pathophysiological processes that are associated with SCI-induced death, and therefore can be used to find relevant OL-specific cell death/cell survival regulators. Furthermore, these data can serve as a guide to integrate seemingly unrelated cytotoxic events and provide a broad mechanistic understanding of OL-specific responses to SCI.

SCI is a multifaceted injury with shifting time-dependent components that include various signaling mechanisms and cellular interactions that ultimately result in the loss of white matter. The extent of spared white matter, in part determined by OL cell death, is a large determinant of functional recovery. This study is the first to provide a comprehensive description of in vivo translational gene expression changes that occur in OL-specific responses to SCI over acute, subacute, and chronic time points. Our data reveal the identity of multiple molecular signaling pathways and genes associated with cellular death that occur at 2 dpi. How manipulation of these pathways and genes affect spared white matter, OL survival, and functional recovery after SCI will have to be empirically determined in future studies.
Figure 12. Isolation and validation of OL-specific mRNAs from $Plp^{CreER}:Rpl22^{HA}$ mice. (a) Experimental design to isolate oligodendrocyte-specific RNAs from spinal cord of $Plp^{CreER}:Rpl22^{HA}$ mice. (b) Representative images of T9 thoracic level spinal cords of $Plp^{CreER}:Rpl22^{HA}$ mice treated with tamoxifen showed abundant immunostaining for HA co-localized with mature oligodendrocyte marker CC1/APC, while vehicle treated animals show few HA+/CC1+. (d) Quantitative analysis of immunostaining for HA and mature oligodendrocyte marker CC1/APC at T9 shows significant increases in HA+/CC1+ co-localization when compared to vehicle treatment. Data are the mean ± SD (n=4, p>0.05, $u$-test). (d) qPCR comparison of IP-HA RNA samples vs. input RNA samples of spinal cord isolated from injured $Plp^{CreER}:Rpl22^{HA}$ mice (naïve, 2, 10, 42dpi) treated with tamoxifen mice showed enrichment of oligodendrocyte ($Mbp$, $Plp1$, $Mog$, ...
and Sox10) mRNA and de-enrichment of astrocyte (Aldh1l1, Hgf), neuronal (Reln, Snap25), and microglial (Cd68, Tmem119) mRNA.
Figure 13. Optimizing isolation of OL-specific mRNAs from \( Plp^{CreER} \cdot Rpl22^{HA} \) mice.

Varying amounts of supplemented homogenizing buffer volumes with respect to tissue mass were used in the tissue dissociation and isolation process to optimize recovery of IP-HA RNA yields. As myelin debris can associate with polysomes to form a component of pellet during centrifugation, larger volumes of homogenizing buffer (smaller % weight to volume ratio) can be used to obtain higher IP-HA RNA yields. While increasing volumes increases IP-HA RNA yields, volumes that are too large reduce input RNA recovery yields and inefficiently utilizes valuable reagents.
Figure 14. OLs at naïve, 2, 10, and 42 dpi display distinct clustering and different expression of highly enriched genes. (a) Principal component analysis of all genes in IP-HA and input (n=3 biologic replicates per condition) (b) Volcano plots indicate OL-specific translatome genes are significantly separated from differentially expressed genes (c) Correlation matrix comparing differentially expressed genes obtained from HA-IP normalized to input to reference dataset that focused specifically on neural cells of the cerebral cortex (Zhang et al. 2014). Heatmap showing Spearman’s Rho correlation coefficient (r), indicating very strong correlation (0.96-1) with myelinating OLs and weakly correlating with OPC (0.28-0.39), neurons (0.3-0.33), and astrocyte (0.23-0.33) suggesting that our samples are most likely representative of myelinating OLs.
Figure 15. GO analysis of enrichment of genes. (a) GO analysis of input normalized IP-HA injury as determined by the following filtering steps: 1) q < 0.05 in 2x4 interaction 2) logFC > 1, q < 0.05 in 2x2 interaction for the acute 2 dpi, subacute 10 dpi, and chronic 42 dpi time points, shows enrichment of biological processes related to bioenergetics. (b) GO analyses of input injury versus input naïve (pairwise comparison log2FC > 1) show enrichment of biological processes that are related to immune responses at acute 2 dpi, subacute 10 dpi, and chronic 42 dpi time points after SCI suggesting that our IP-HA samples are OL-specific.
Figure 16. OL-specific translatome changes after SCI. (a) Heat map representing k-means clustering by IP-HA injury versus IP-HA naïve from genes established by DESeq2 group by dpi (2x4) interaction analysis (3,757 genes out of 17,186 detected into 23 clusters) as defined by group (IP-HA, IN) and dpi (naïve, dpi) and significant interaction (q≤0.05, fold change≥0) (b) GO enrichment of genes upregulated acutely at 2 dpi in combined cluster 5,9,12, and 19. Top processes enriched are associated with ISR stress activation (alpha-amino acid biosynthetic process), regulation of neuron death process contain only a few genes, some of which have been previously implicated in mediating cell death after SCI (c) GO enrichment analysis of downregulated genes in cluster 4, 2, 15, and 17 show processes involved in synaptic transmission which may reflect loss of
oligo-axonal synapses, clusters were filtered using the following cut off: \(|\text{Log2FC}| \geq 1, q \leq 0.05\).

**Figure 17. GO analysis of acute down regulated clusters.** GO enrichment analysis of individual cluster 1, 3, and 4 with patterns D/D/D, D/N/D, and D/N/N was performed (q-value cutoff \(\leq 0.05\) and \(|\log2\text{FC}| > 1\), dpi 2 IP-HA vs naive) individually and demonstrated enrichment of biological processes associated with synaptic transmission.
Figure 18. GO analysis of acutely upregulated clusters. GO enrichment analysis of individual cluster 11, 12, and 19 was performed (q-value cutoff ≤ 0.05 and |log2FC| > 1, dpi 2 IP-HA vs naive) and show limited processes are enriched and suggests grouping similar behaving clusters. Cluster 11, 12 and 19 are U/N/D, U/N/N, and U/U/N respectively.
Figure 19. Filtering steps performed to sort differentially expressed genes. (a)
Manually filtered upregulated DEGs established a 98 candidate gene list >2 fold, q ≤0.05 at 2 dpi when compared to 42 dpi to detect acute responses OLs undergo that contribute to death. (136 total genes identified using criterion, 38 downregulated not shown, majority of genes were associated with synaptic function) (b) Log2FC expression changes of (injury IP-HA/naive IP-HA of select pro-death and pro-survival gene identified in (a). (c,d) Western blot analysis reveals increased levels of RUNX1 and GDNF proteins. Untrimmed western blot images are shown in Fig. 21 and 22. RUNX1 western blot densitometry quantification was done on n=3 sham, n=3 2 dpi, one sample from 2 dpi was excluded because of high background. GDNF western blot densitometry quantification was done on all samples shown (n=3 sham, n=4 2dpi). Data are mean ± SD (*p < 0.05; ns, p > 0.05, student t-test.)
Figure 20. GO analysis of downregulated genes identified through manual filter.

Analysis was conducted using <\text{-}2 \text{ fold}, q\leq0.05\text{at 2 dpi when compared to 42 dpi to detect acute downregulated responses. A limited set of functional processes were obtain and justifies our approach with starting our analysis with upregulated genes first.}
Figure 21. The untrimmed image of the western blot image shown in Fig. 19c.

Location of the molecular weight markers is marked. There are bands where the expected size of the detected targets are (mouse RUNX1, 28 kDa isoform, 55 kDa isoform, ~80 kDa uncharacterized band, GAPDH 35.8 kD).
Figure 22. The untrimmed image of the western blot image shown in Fig. 19d.

Location of the molecular weight markers is marked. There are bands where the expected size of the detected targets is (mouse GDNF, 24 kDa, GAPDH 35.8 kD).
Table 4. List of qPCR primers

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<thead>
<tr>
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Table 5. RiboTag isolation yields and conditions

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<th>Weight (mg)</th>
<th>HB Volume (% w/v ratio)</th>
<th>Input RNA yield (ng/uL)</th>
<th>IP-HA RNA yield (ng/uL)</th>
<th>HA Antibody (uL)</th>
<th>Incubation time (h)</th>
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<th>qPCR Validation IP-HA</th>
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<td>4 (IgG)</td>
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Table 6. Summary of initial sequence analysis

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<th>Alignment Rate</th>
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<td>MH_06_S23_RT_neive_IP-280251574</td>
<td>37,133,183</td>
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<td>MH_07_S12_RT_2DPL_IN-280255169</td>
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<td>37,759,958</td>
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Table 7. Patterns and number of genes contained in the 23 proposed clusters.

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<td>DDN</td>
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<td>Cluster 3</td>
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<td>Cluster 4</td>
<td>DNN</td>
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<td>NDD</td>
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<tr>
<td>Cluster 6</td>
<td>NDN</td>
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CHAPTER 4

GENERAL DISCUSSION

Review of findings

As discoveries and technological limitations advance, our knowledge about spinal cord injury (SCI) also evolves to delineate more detailed pathways and mechanisms involved. The research in this dissertation is in part based on the discovery of additional pathways that contribute to the activation of downstream effectors involved in mediating the endoplasmic reticulum stress response (ERSR). The remainder of this research is focused on identifying and defining novel pathways that contribute to the neurological impairment after SCI. This section will provide a review of work conducted, touch upon future work, and finally highlight the clinical implications.

My journey began with the goal of identifying cytotoxic mechanisms that initiate death after SCI. Various cytotoxic events including hypoxia, bioenergetics failure, oxidative stress, endoplasmic reticulum (ER) stress, and hemorrhage occur at different time points with varying duration of insult to differentially contribute to secondary injury induced cell death (Pukos et al., 2019). Apoptosis, necrosis, and autophagy induced cell death have been characterized in secondary injury. Recently, a newly characterized form of iron-dependent cell death named ferroptosis (Dixon et al., 2012), showed involvement in an intracerebral hemorrhage (ICH) injury model (Karuppagounder et al., 2016). The Ratan Lab proposed a mechanism whereby the upregulation of select ATF4-target genes contributes to ferroptosis, and that inhibiting hypoxia-inducible factor prolyl hydroxylase
(HIF-PHD) suppresses the ATF4/CHOP signaling cascade to inhibit ferroptotic cell death (Karuppagounder et al., 2016; Ratan, 2020). Interestingly, our previous work showed that mediators of the ERSR including ATF4/CHOP are upregulated in neurons and oligodendrocytes (OLs), and that CHOP serves as an ERSR specific pro-apoptotic transcription factor after SCI (Ohri et al., 2011). In the same study, Chop null mice showed improved behavioral outcome after thoracic contusive SCI associated with increased WMS and attenuated acute loss of OLs. We therefore investigated the role of HIF-PHDs in ATF4-CHOP activation and white matter loss after SCI. We hypothesized that pharmacological and/or genetic suppression of ATF4-CHOP signaling after thoracic contusive SCI would similarly restore proteostasis and prevent chronic OL loss, leading to increased spared white matter and improved functional outcomes. First, we showed in an acute study that SCI animals treated with AQ for 3 days had lower mRNA levels of Atf4, Chop, and Trib3, but there were no effect on three other ERSR transcripts, Gadd34, Grp78, and Xbp1. Clearly, AQ had a biological effect, so we wanted to examine if there were any long term functional changes and treated SCI animals with AQ for 7 days in a chronic locomotor study. There were no differences in WMS or BMS locomotor outcomes at week 6. We then determined the effects of genetically deleting the HIF-PHDs in OLs by using OL-specific Plp-creERT2+/+;Egln1/2/3fl/fl mice. Tamoxifen treatment resulted in the downregulation of Phd/Egln transcripts in both OL-rich tissues of the spinal cord and optic nerve, but not in OL-lacking liver of tamoxifen-treated Plp-creERT2+/+;Egln1/2/3fl/fl mice. At 72 h after SCI, tamoxifen- and vehicle-treated Plp-creERT2+/+;Egln1/2/3fl/fl mice had similar neural cell mRNA levels, indicating no effect on the acute loss of neurons and OLs. Furthermore, there were no effects on the expression
of Atf4, Chop, Gadd34 or Grp78 mRNAs. Consistent with those findings (Ohri et al., 2021), there were no significant differences in locomotor recovery between 6-week BMS scores for tamoxifen- and vehicle-treated Plp-cre<sup>ERT2+/−;Egln1/2/3<sup>fl/fl</sup> mice.

The work in Chapter 2 provides evidence that HIF-PHDs may not be suitable targets to improve outcomes in traumatic CNS pathologies that involve acute white matter injury like SCI. Our study was the first to evaluate the involvement of HIF-PHDs in ATF4/CHOP signaling after SCI and that pharmacological inhibition of HIF-PHDs using AQ has no effect on spared white matter or locomotor recovery. One possibility of these negative findings is that AQ inhibits the ATF4-CHOP signaling that is distinct from their involvement in ERSR. Indeed, in the ICH model, AQ was proposed to inhibit the ATF4-mediated iron-dependent ferroptosis but not the cytotoxic ERSR (Karuppagounder et al., 2016; Ratan, 2020). ER stress may be a downstream event in ferroptosis that occurs as result of oxidative ER damage (Dixon et al., 2014; Lee et al., 2018) and ER stress (thapsigargin and tunicamycin) induced ATF4 activation appears to be capable of preventing ferroptosis (Hayano et al., 2016; Lange et al., 2008; Lewerenz). This is consistent with our unpublished results which indicate that OPCs pretreated with tunicamycin first, and then with hemin (ferroptosis inducer) show no synergistic effects in cell viability when compared to tunicamycin treatment alone. Furthermore, Hong et al. demonstrated activation of the ERSR and its mediators including CHOP after treatment with the ferroptosis inducers artesunate (ART) and erastin (Hong et al., 2017). It is well established that CHOP induces several pro-apoptotic proteins such as p53 upregulated modulator (PUMA), ER oxidoreductin-1α (ERO1α), growth arrest and DNA damage–inducible protein (GADD34), BIM (Bcl-2-like protein 11), and NOXA (BH3-only
apoptosis protein) (Hu et al., 2018). However, ART only induces PUMA expression, and not NOXA or BIM (Hong et al., 2017). ART-induced PUMA expression does not result in apoptosis (Hong et al., 2017). Taken together, this is consistent with our suggestion that the **ISR and ERSR are predominantly the activators of ATF4 after SCI**.

Apoptosis rather than ferroptosis may be primary cell death mechanism in neurotrauma-challenged OLs after SCI. **Sensitivity to ferroptosis** is associated with several biological processes involving amino acid, iron and polyunsaturated fatty acid (Dixon et al., 2012). Hence, various cell types have different susceptibility to ferroptosis inducing stress. For instance, glutathione depletion can induce ferroptotic death in embryonic cortical neurons, neuroblasts, OLs, and astrocytes (Ratan, 2020). However, primary neurons and immature OLs are more sensitive to ferroptosis. Mature OLs have increased expression of solute carrier family 7 member 11 (SLC7A11), a cystine/glutamate transporter, making them more tolerant to ferroptosis (Hoshino et al., 2020). The difference in sensitivity may account for the lack of recovery in our SCI model where damage is primarily due to white matter loss associated with mature OL death when compared to the ICH model where neuronal loss is the key driver of impairment. Indeed, in animals treated with systemic iron chelator, deferasirox, there was improved gray matter sparing, attenuated neuron loss, but no effects on white matter sparing or OL numbers post injury (Sauerbeck et al., 2013). Additional work could investigate the protective effects of adaptaquin in models of cervical contusion injury or subarachnoid hemorrhage where neuronal death is paramount to loss of function (Magnuson et al., 2005; Magnuson et al., 1999; Sabri et al., 2008).
Finally, Chapter 3 identified transcriptional and translational gene expression changes that regulate OPC/OL responses at different time points after SCI by using RiboTag technology and RNA-sequencing. As expected, the isolated mRNA fraction was enriched for myelin basic protein/proteolipid protein (Mbp/Plp, OLs, ~5-fold) and depleted for hepatocyte growth factor (Hgf, astrocyte), synaptosomal-associated protein (Snap25, neuron), and cluster of differentiation 68 (Cd68, microglia). Finally, we sequenced mRNA from OLs in the epicenter of the injury at 0, 2, 10, and 42 dpi. We identified 98 genes that were upregulated >2 fold in OLs at 2 dpi that when compared to 42 dpi and defined their potential roles according to their functions as currently defined in the literature. Genes that induce oxidative stress (Chac1, Steap3), inhibit MAP kinase (Spred3, Spry4), and inhibit the AKT pathway (Parvb) were highly upregulated. Our working hypothesis is that OLs upregulate genes that may increase oxidative stress and inactivate survival pathways that may collectively contribute to cell death after SCI. This study will be one of the most comprehensive descriptions of OL-specific gene expression after SCI to be reported.

Although RiboTag is an extremely powerful tool to recover and examine cell type specific translatomes from complex tissues, there are limitations to the scope of this study and technical limitations inherent to the RiboTag technology. For instance, our focus was on defining responses by myelinating OLs during SCI. Therefore, we selected the inducible Plp-Cre mouse line to label myelinating cells and exclude OPC responses, including proliferation and differentiation, which have been documented to be extremely active acutely after injury (Hesp et al., 2015; McTigue et al., 2001; Zai et. al., 2005). Indeed, our data do not show proliferation-associated processes at 2 dpi. Hence, this
study is the first description of myelinating OL-specific translational profile after SCI. Although OPC responses are likely excluded, so too are newly formed OLs. For the first two weeks after SCI, OPCs begin to markedly proliferate and differentiate into mature myelinating OLs (Hesp et al., 2015). They begin to myelinate by week 3 acutely, and remarkably they maintain this chronically (Hesp et al., 2015). However, the exact functional implications of this remyelination after SCI are unclear and the identity of the axons they myelinate are unknown (Hesp et al., 2015). When OPC differentiation is blocked, the subsequent myelination response is also inhibited. However, despite no new myelin being formed, there was no decease in motor function behavior (Duncan et al., 2018). This suggests that the endogenous remyelination response is not sufficient to improve recovery. Importantly, given that more than more than half of the OLs were newly formed, we could evaluate this response using our mouse model by starting tamoxifen induction after SCI. Another technical issue to consider is the lack of IP-IgG controls utilized in this study. Studies utilizing the RiboTag system have shown that IP-IgG can immunoprecipitate nonspecific RNAs that contribute to background noise and confound the later analyses (Haimon et al., 2018). However, this issue can be rectified by utilizing stringent selection criteria given that IP-HA specific genes are detected are significantly higher than that of IP-IgG genes (Haimon et al., 2018; Sanz et al., 2019). Nonetheless, genes can be unintentionally eliminated by criteria selection as background noise. Moreover, there is well documented contamination of RNAs (from total pool of RNA, target cell specific and non-specific) that can associate with HA-labeled ribosomes during the isolation and antibody incubation period (Gregory et al., 2020; Haimon et al., 2018; Jambusaria et al., 2020; Kang et al., 2018; Scheckel et al., 2020). Such potential
contamination can at least in part be removed by normalizing to input RNAs and filtering by relative enrichment as demonstrated in this study. While the immunopurification process likely excludes mRNAs that are translationally regulated at the initiation complex formation step, ribosomes that remain attached to mRNAs in cases where protein synthesis or elongation is depressed or stalled due to regulation (Haimon et al., 2018; Sanz et al., 2019) may still be precipitated. Such scenario would inaccurately reflect translatomic gene expression changes and lead to discrepancies between the translatome and the proteome. Finally, when the OL-specific IP mRNAs are normalized to the IN to obtain enrichment, genes that are also transcriptionally upregulated or downregulated by surrounding cells and tissues may be minimized by the normalization procedure and therefore subsequently eliminated by the selection criteria process (Haimon et al., 2018). A common technique is to perform downstream gene analyses by only comparing IP-mRNA without normalization with the assumption that all mRNAs that are obtained through immunopurification process are target cell specific (Itoh et al., 2018; Voskuhl et al., 2019). Using IPs only can avoid apparent enrichment of genes that would be otherwise be detected when IPs are normalized to IN (caused by a reduction of such transcripts in total input mRNAs due to cellular death of non-target cells) is avoided. To address this issue, we examined the use of both IPs that are normalized to the IN and IP only when considering differentially expressed genes between naïve and SCI. Specifically, we used IP normalized to IN and changes with their interactions across our time as defined by $q \leq 0.05$ and $|\log 2 \text{FC}| \geq 0$ to obtain DEGs. Genes defined by this initial criterion were then subjected to k-mean clustering using fold change values obtained from IP only. Consequently, changes that are minimized because of the normalization
process are detected when compared to IPs only, while contamination issues that maybe overlooked when using IPs only are detected.

We focused on genes that were implicated in pro-death pathways that have not characterized in OLs after SCI and therefore represent only a small fraction of overall genes identified. One such gene identified that may contribute to OL cell death was six-transmembrane epithelial antigen of the prostate 3 (STEAP3) which functions to reduce Fe$^{3+}$ to Fe$^{2+}$ (Li et al., 2020). Importantly, Fe$^{2+}$ is highly reactive and forms reactive oxygen species through the Fenton reaction (Lloyd et al., 1997). Increased expression of STEAP3 can increase levels of Fe$^{2+}$ that may lead to the generation of more oxidative damage. For instance, Giacci et al. used nanoscale secondary ion mass spectrometry (NanoSIMS) to quantify oxidative damage after optic nerve transection and show that increases in DNA, protein, and lipid damage occurred in both OPCs and mature OLs at 3 dpi, followed by a decline of OLs at day 7 (Giacci et al. 2018). STEAP3 is upregulated and further increases cellular death when p53 is activated in breast cancer and leukemia cells (Lespagnol et al., 2008), while knockdown results in inhibition of apoptosis (Ohgami et al., 2005). Moreover, aside from general increases of oxidation, it can interact with NIX (comprised of BCL2 and adenovirus E1B 19-kDa interacting protein 3 and BNIP3-IL), members of the BH3-only apoptotic family of proteins, and therefore further intensify the apoptotic effects of STEAP3 (Zhang et al., 2012a). Additionally, STEAP3 interacts with Myt1 kinase which is a regulator of cyclin-dependent kinase activity (Lespagnol et al., 2008). Collectively, these studies suggest that STEAP3 may be directly involved in apoptosis and cell-cycle progression. A natural next step would be to study
the effects of manipulating STEAP3 whether through overexpression or knockdown on survival or proliferation in cultured rat OPCs and ultimately in vivo after injury.

Another group of genes inhibit either the MAP kinase (Spred3, Spry4), or AKT (Parvb) pathways. The mitogen-activated protein kinase (MAPK) and protein kinase B (Akt) pathways serve as a connection that bridges extracellular signals to intracellular responses and mediate the responses that results in cell survival, proliferation, differentiation, and death. Extracellular signal-regulated kinase (ERK) is one component of the MAPK family that can be activated in response to several extracellular ligands through G protein coupled receptors, tyrosine kinase receptor, and the protein kinase C (PKC) (Bhat et al., 1996; Bhat et al., 2007; Guo et al., 2007). The Akt pathway is in part regulated by integrin-linked kinase (ILK) and can be activated by signals including integrins, cytokine receptors, B and T cell receptors, receptor tyrosine kinases (RTKs), and G protein-coupled receptors (GPCRs). ILK-mediated phosphorylation of Akt increases proliferation and survival of cells, while inhibition of ILK inhibits PKB/Akt activation and induces apoptosis (Edwards et al., 2005; Persad et al., 2000; White et al., 2001). Furthermore, ERK and Akt are pro-survival by mediating the cellular growth factors and blocking apoptosis (Bellacosa et al., 1995; Bellacosa et al., 2005; Duronio, 2008; Fresno Vara et al., 2004). The sprouty family of proteins (Spry4, Spred3) acts as negative regulators of ERK activation (Casci et al., 1999; Hacohen et al., 1998; Reich et al., 1999), but have no inhibitory effect on either the JNK or p38 MAPK pathways (Yusoff et al., 2002). Moreover, the sprouty and spred proteins suppress ERK activation induced by fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), vascular endothelial cell growth factor (VEGF), nerve growth factor (NGF), and glial cell
line-derived neurotrophic factor (GDNF), all of which play pro-survival roles in the CNS (Bansal et al., 1997; Cohen et al., 1996; Hu et al., 2008; Wilkins et al., 2003). Finally, β-parvin (PARVB) binds to the integrin-linked kinase (ILK) to inhibit its kinase activity (Mongroo et al., 2004). Expression of PARVB in MDA-MB-231 cells inhibited ILK kinase activity, cell growth and in vitro matrigel invasion (Mongroo et al., 2004). Importantly, these genes have never before been implicated to have a role in OLs after SCI. Upregulated Spred3, Spry4, and ParvB levels may collectively act to modulate the MAPK and Akt pathways, so that extrinsic survival signals are subdued while pro-apoptotic stress activated pathways are unaffected, collectively resulting in OL death.

Given that MAPK and Akt function largely through tyrosine kinase mediated phosphorylation, one possible intervention is the use of phosphatase inhibitors to acutely stimulate pro-survival pathways (Dewang et al., 2005; Heneberg, 2009). Another possible approach to facilitate OL survival is to inhibit the pro-death pathways. Because these cellular mechanisms are widely used in all cells, emphasis should be on pro-proliferating and pro-myelinating responses. Therefore, in the case of phosphatase inhibition, it might be possible that in trying to prevent death of existing myelinating cells, we also stimulate OL function to increase myelination given that ERK MAPK signaling is required to increase myelin thickness (Ishii et al., 2012), but inhibits OPC to OL differentiation (Suo et al., 2019). Similar, inhibitors of JNK or p38 may initially prevent OL death, but halt the differentiation of OPCs into OLs (Bhat et al., 2007). What effect this has on overall functional recovery and spared white matter remains unknown and potential a future direction. Additionally, future work could also include studying genes involved in OPC cell proliferation, differentiation, and myelination after SCI. For instance, a Cre mouse
under the control of the PDGFRα promoter (Duncan et al. 2018) which is expressed
earlier in the OL lineage (Pringle et al., 1992) may be used to specifically study OPC
proliferation, differentiation, and new myelination events after SCI. Finally, our current
mouse model can be used to define OL responses in various other traumatic CNS injuries
such as stroke, TBI or ICH. Defining elements that are common to multiple injury models
may narrow down and help identify mechanisms that can be targeted to reduce white
matter damage and/or stimulate its repair.

**Potential Clinical Implications**

The oldest medical journal known to exist from ancient Egyptian times described
SCI as “an ailment not to be treated” (Whiteneck et al., 2009). Tremendous advances in
technology have accelerated knowledge about pathophysiological mechanisms
underlying SCI, but there are currently still no effective treatments acutely. This is in part
due to our lack of understanding of acute and chronic events that occur at the cellular
level of the injury. Until such progress has been made, we are still very far from being
able to develop clinically effective therapies that can treat SCI. In neurotrauma, the goal
of neuroprotective strategies is to minimize the secondary damage and therefore preserve
functional tissue. Neuroprotective strategies that target an individual component of
secondary injury- induced cellular death like excitotoxicity (Hawryluk et al., 2008) using
gacyclidine (a NMDA receptor antagonist) or nimodipine (a Ca^{2+} channel blocker) allow
other secondary injury associated mechanism to still progress. Importantly, both of these
failed to demonstrate chronic improvements in motor function (Pointillart et al., 2000).

As mentioned in Chapter 1, SCI involves several different cytotoxic mediators of
death which raises 3 important considerations for neuroprotective strategies: 1. the
identification of cell-specific molecular mechanisms that contribute to death after SCI; 2. the selection of adaptive/maladaptive mechanisms to target; 3. the potential consequences target manipulation. Given that the amount of spared white matter after contusive thoracic SCI largely determines the extent of functional recovery (Basso et al., 1995; Blight, 1983a, 1983b; Blight et al., 1986; Bresnahan et al., 1987; Bresnahan et al. 1976; Kelley et al., 2014; Kim et al., 2010; Loy et al., 2007), treatments for SCI should protect OLs as they play a critical role in the formation and maintenance of white matter. The identification of OL-specific mechanisms that occurs over the duration of SCI is perhaps the most challenging, yet the most informative and critical step forward toward clinical significance. Therefore in Chapter 3, we used RiboTag to obtain OL-specific gene expression and identified candidate mechanisms that are activated post SCI that may contribute to cell death or survival.

The regenerative potential of any neuroprotective strategies will likely depend on the severity of injury, and also, its mechanism of action. Additionally, the extent of neurological recovery that is achievable using neuroprotective therapy is unknown. For instance, strategies can be selected to enhance survival and/or reduce death, but until we can make some substantial progress bridging the gap in knowledge about preclinical SCI studies and clinical injury trajectory and treatment, we can only speculate about such approaches. For faster clinical translation, repurposed FDA approved pharmacological agents will have the most potential to reach the largest amount of people the quickest. However, despite all these uncertainties, it is evident that as time progresses post SCI and numerous cytotoxic stressors begin to accumulate, it likely becomes an increasingly difficult task to prevent death, until it reaches a point where it becomes impossible. There
is a brief time frame that begins immediately after SCI when OLs activate responses that help them contemplate between pro-survival and pro-death. Therefore, neuroprotective interventions should coincide with this time frame and be administered quickly, as there is likely a rapidly closing therapeutic window that maximizes cellular protection. Neuroprotective treatments outside of this window will likely not be functionally beneficial. Arguably, some mechanisms may be better to target than others. Of all the options for neuroprotection, this simplest and most logical solution would be to inhibit mechanisms that contribute to cell death or cell death directly. However, there are nearly ~22 different types of cellular death to choose from (Galluzzi et al., 2018) and just as many different cytotoxic stressors that occur after SCI (Pukos et al. 2019). Data from Chapter 3 suggest OLs launch a robust cellular and molecular response to SCI by 2 dpi. How they respond likely depends on the combination of extrinsic, intrinsic, and environmental factors. Because many of these pathways and mechanisms that lead to death are deliberately redundant, it is effortless for the cell to find an alternative way to die if one pathway is targeted and may perhaps be a reason why there hasn’t been any clinical success which this approach (Hawryluk et al., 2008). This however could be viewed as strength as it gives us the opportunity to identify and target mechanisms or stressors that are common in mediating death, and therefore have broad widespread implications. In the case of secondary injury induced death, oxidative stress damages DNA, lipids, proteins, and mitochondria. Research described in the Chapter 3 identified upregulated genes that can contribute to oxidative stress (Steap3) and reduce anti-oxidant (Chac1) levels within the cell. Targeting these proteins using pharmacological inhibitors would likely alleviate overall oxidative stress. A potential side effect of STEAP3 is the
inhibition of myelin synthesis since Fe$^{2+}$ is required for enzymatic activity. RUNX1 (a transcription factor) is another attractive therapeutic target as manipulating this single mechanism leads to extensive transcriptional and translation regulation of multiple signaling molecules in a pathway. However, such broad changes may also have extensive side effects given the wide scale of changes.

Despite fundamental differences in our model and underlying pathogenesis, the cancer field in many regards faced similar hurdles that parallel the SCI neuroprotection field. Thus, there are several key points we can learn from our colleagues who has had remarkable success with manipulating cell death and survival (Baudino, 2015). For instance, the combination of two or more chemotherapy agents is a hallmark of cancer therapy and demonstrates a clear advantage over mono-therapy because of additive and synergistic effects when targeting key mechanisms (Bayat Mokhtari et al., 2017). A clear advantage over mono-therapy is that lower therapeutically relevant dosages can be used to combat off target effects and reduce drug toxicity. A common example is the use of CHOP (cyclophosphamide, hydroxydaunorubicin, oncovicn, and prednisone), not be confused with the pro-apoptotic ERSR effector CHOP, which individually function through different mechanisms when treating lymphoma (Lenz et al., 2005). Therefore, similar to inducing death in malignant cells, a combinatorial approach may perhaps be needed to block various different cytotoxic events and reduce cellular death. In the context of SCI, dual therapy is utilized in strategies that involve enhancing neuroplasticity, such as combining physical therapy with epidural stimulation, but less prevalent in neuroprotection (Harkema et al., 2011). A 2004 study from Mary Bunge’s lab combined a neuroprotective strategy with cell therapy and showed greater motor
improvement in groups with combined treatment when compared to mono-therapy (Pearse et al., 2004). Taken together, the combinatorial approach has been used with different treatment modalities in SCI, yet it is underutilized in the neuroprotective field. Therefore, aside from identifying different mechanisms that may be targeted, a more focused effort should be made on combining different treatments that have demonstrated effectiveness with mono-therapy in animal models, and assess their potential synergistic effects such as combinations of minocycline, gacyclidine, and nimodipine. However, if we consider the same principle where the goal is to block death instead of inducing it as in cancer, such strategies may still be insufficient to restore cellular homeostasis or functionality, and therefore not likely to address the chronic neurological disabilities associated with SCI. Inhibiting cell death mechanisms can prolong the therapeutic window of intervention, but will inevitably lead to death if intrinsic survival mechanisms are not enough to compensate and restore function to overcome secondary injury induced stress. Importantly, this represents another opening for therapeutic intervention where it would be advantageous to manipulate pro-survival mechanisms identified in Chapter 3 and enhance or facilitate endogenous responses that restore homeostasis. For instance, molecular activation of Sphk1 can lead to Akt signaling-induced proliferation of non-post mitotic cells, enhance survival, and directly inhibit apoptosis (Guan et al., 2011; Kapitonov et al., 2009)

As extensively described in Chapter 2, HIF-PHDs are one seemingly perfect target: 1. there are multiple molecular inhibitors currently developed for HIF-PHDs (Joharapurkar et al., 2018) 2. HIF-PHD can target a downstream transcription factor, ATF4, which acts as a downstream effector for several different stress responses
including the ERSR and ISR (Karuppagounder et al., 2016). By manipulating ATF4 there is a potential to not only reduce cell death but also enhance endogenous survival mechanisms that may aim to restore stress-induced aberrant OL behavior (Harding et al., 2000). 3. HIF-PHD inhibition can lead to HIF1α (transcription factor) induction which activates genes involved in angiogenesis, vessel dilatation, and apoptosis inhibition (Ziello et al., 2007). 4. HIF-PHD inhibitors have a protective effect by reducing damage in animal models of intracerebral hemorrhage and hypoxia-ischemia (Karuppagounder et al., 2016; Li et al., 2019a). 5. Currently HIF-PHD inhibitors (FG-4592, GSK1278863, Molidustat and Vadadustat) are being tested in 27 clinical trials involving anemia. In summation, with one pharmacological agent, HIF-PHD inhibition modulates two transcriptional pathways that are heavily involved in mediating endogenous survival/death and therefore likely to restore homeostasis. Furthermore, the majority of literature shows an overwhelming positive role of PHD inhibition, although there have been some reports linking aberrant HIF activation and PHD knockdown to cancer (Semenza, 2010). However, adaptaquin had no effect on WMS or BMS locomotor outcomes after thoracic SCI suggesting that mechanisms of grey matter damage are different from those of white matter damage. Our results show that adaptaquin moderately lowered acute induction of Atf4/Chop mRNAs but this was insufficient to provide enough protection against white matter pathologies. These data suggest that PHD inhibition may improve functional outcomes in lumbar or cervical models of SCI and subarachnoid hemorrhage (SAH), where neuronal death is paramount to loss of function (Hadi et al., 2000; Magnuson et al., 1999; Nishida et al., 2015). Importantly, despite the lack of effect in thoracic SCI, adaptaquin’s multiple potential clinical implications in
various diseases does provide hope that our approach of targeting mechanisms that are central to multiple stress responses may one day be successful in SCI.

The journey to developing a treatment for acute SCI is a long and arduous. Although there is an urgent need to develop clinically relevant translatable therapies, we are severely lacking a fundamental understanding of events that occur at the cellular level after SCI. However, despite these limitations, we are now better equipped than ever before to observe these events and further use a systematic approach that is mechanism driven to identify potential therapeutics.

**Conclusion**

Traumatic SCI is a complex pathology characterized by the loss of gray and white matter that results in permanent neurological impairment. Although research on SCI has been ongoing for over a century (Allen, 1911), the underlying pathophysiology of SCI is still not well understood and therefore hampers our ability to develop clinically meaningful treatments. Currently, there is no treatment for acute SCI that offer consistent neuroprotection. However, rapid advances in technology, neurobiological knowledge, preclinical animal models, and transgenic animals have equipped us with unprecedented new tools to examine and discover events occurring at the cellular level after SCI that may serve as novel targets for neuroprotective strategies. The work in this dissertation was enabled by such progresses, and represents a small but necessary step forward in advancing the field of SCI research. Here, we showed that the pharmacological agent, adaptaquin, which offers neuroprotection in several models of CNS injury, and functions by inhibiting a newly characterized type of cell death, ferroptosis, is ineffective in protecting against white matter loss and improving locomotor outcome after SCI. Next
we generated one of the most comprehensive descriptions of OL-specific translational
gene expression after SCI. Taken together, we are increasing our fundamental knowledge
of SCI mechanisms at remarkable pace, but more work is still needed to identify and fully
comprehend the complex cascade of events that occur after SCI before we are able to
translate these findings into clinical applications for humans. However, the work
presented here sets the contextual framework for others to build up and generate new
hypotheses that can be tested in a more sophisticated manner as technology improves to
ultimately develop a treatment for SCI.
REFERENCES


in patients with the classical form of the Pelizaeus-Merzbacher disease. Med Wieku Rozwoj, 17(4), 293-300.


Luchetti, S., Beck, K. D., Galvan, M. D., Silva, R., Cummings, B. J., & Anderson, A. J. (2010). Comparison of immunopathology and locomotor recovery in C57BL/6,


contribute to ER stress and apoptosis induced by oxidized low density lipoproteins. *Antioxid Redox Signal*, 18(7), 731-742. doi:10.1089/ars.2012.4577


in a Huntington's Disease Pre-clinical Model. *Front Mol Neurosci*, 12, 100. 


doi:10.1016/j.freeradbiomed.2007.10.054

doi:10.1016/j.tcb.2011.09.003


doi:10.1038/leu.2011.202


doi:10.1038/cdd.2017.58

doi:10.1152/physrev.1997.77.3.731


140


White, D. E., Cardiff, R. D., Dedhar, S., & Muller, W. J. (2001). Mammary epithelial-specific expression of the integrin-linked kinase (ILK) results in the induction of
mammary gland hyperplasias and tumors in transgenic mice. *Oncogene*, 20(48), 7064-7072. doi:10.1038/sj.onc.1204910


<table>
<thead>
<tr>
<th>ABBREVIATION</th>
<th>DESCRIPTION</th>
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<tbody>
<tr>
<td>AD</td>
<td>Alzheimer's Disease</td>
</tr>
<tr>
<td>AIF</td>
<td>Apoptosis Inducing Factor</td>
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<tr>
<td>AMPA</td>
<td>α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid</td>
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<tr>
<td>AQ</td>
<td>Adaptaquin</td>
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<tr>
<td>ASIA</td>
<td>American Spinal Injury Association</td>
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<tr>
<td>ATF4</td>
<td>Activating Transcription Factor 4</td>
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<tr>
<td>ATF6α</td>
<td>Activating Transcription Factor 6</td>
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<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
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<tr>
<td>BDNF</td>
<td>Brain Derived Neurotrophic Factor</td>
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<tr>
<td>BiP</td>
<td>Beclin-1/p62</td>
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<tr>
<td>BMS</td>
<td>Basso Mouse Scale</td>
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<tr>
<td>CHAC1</td>
<td>Glutamylcyclotransferase 1</td>
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<tr>
<td>CHOP</td>
<td>C/EBP Homolougous Protein</td>
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<tr>
<td>CNS</td>
<td>Central Nervous System</td>
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<tr>
<td>Dpi</td>
<td>Days Post Injury</td>
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<tr>
<td>DRG</td>
<td>Dorsal Root Ganglion</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>DTI</td>
<td>Diffusion Tensor Imagining</td>
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<tr>
<td>ER</td>
<td>Endoplasmic Reticulum</td>
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<tr>
<td>ERSR</td>
<td>Endoplasmic Reticulum Stress Response</td>
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<tr>
<td>GADD34</td>
<td>Growth-arrest DNA Damage Gene 34</td>
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<tr>
<td>GCN2</td>
<td>General Control Nonderepressible 2</td>
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<tr>
<td>GDNF</td>
<td>Glial Derived Neurotrophic Factor</td>
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<tr>
<td>GO</td>
<td>Gene Ontology</td>
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<tr>
<td>GRP78</td>
<td>78-kDa glucose-regulated protein</td>
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<tr>
<td>HA</td>
<td>Human influenza hemagglutinin</td>
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<tr>
<td>HIF</td>
<td>Hypoxia Inducable Factor</td>
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<tr>
<td>HSF1</td>
<td>Heat Shock Factor 1</td>
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<tr>
<td>HSP</td>
<td>Heat Shock Protein</td>
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<tr>
<td>HSR</td>
<td>Heat Shock Response</td>
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<tr>
<td>ICH</td>
<td>Intracerebral Hemorrhage</td>
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<tr>
<td>IN</td>
<td>Input</td>
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<tr>
<td>IP</td>
<td>Immunoprecipitated</td>
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<tr>
<td>IRE1α</td>
<td>Inositol Requiring Enzyme 1 Alpha</td>
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<tr>
<td>ISR</td>
<td>Integrated Stress Response</td>
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<tr>
<td>JNK</td>
<td>c-Jun N-terminal kinase</td>
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<tr>
<td>MAPK</td>
<td>Mitogen Activated Protein Kinase</td>
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<tr>
<td>MBP</td>
<td>Myelin Basic Protein</td>
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<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<td>MS</td>
<td>Multiple Sclerosis</td>
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<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>NMDA</td>
<td>N-Methyl-D-aspartic acid</td>
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<tr>
<td>NP</td>
<td>Neuropathic Pain</td>
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<tr>
<td>ODD</td>
<td>Oxygen-dependent degradation</td>
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<tr>
<td>OL</td>
<td>Oligodendrocyte</td>
</tr>
<tr>
<td>OPC</td>
<td>Oligodendrocyte Precursor Cell</td>
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<tr>
<td>PARVB</td>
<td>Beta Parvin</td>
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<tr>
<td>PERK</td>
<td>PKR-like ER Kinase</td>
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<tr>
<td>PHD</td>
<td>Prolyl Hydroxylase</td>
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<tr>
<td>PLP</td>
<td>Proteolipid Protein</td>
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<tr>
<td>PMD</td>
<td>Pelizaeus Merzbacher Disease</td>
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<tr>
<td>PP1</td>
<td>Protein Phosphatase 1</td>
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<tr>
<td>RER</td>
<td>Rough Endoplasmic Reticulum</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
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<tr>
<td>S1P</td>
<td>Site-1 Protease</td>
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<tr>
<td>SCI</td>
<td>Spinal Cord Injury</td>
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<tr>
<td>SNPs</td>
<td>Single Nucleotide Polymorphisms</td>
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<tr>
<td>SPRED3</td>
<td>Sprouty Related EVH1 Domain Containing 3</td>
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<td>SPRY4</td>
<td>Sprouty RTK Signaling Antagonist 4</td>
</tr>
<tr>
<td>STEAP3</td>
<td>Six-Transmembrane Epithelial Antigen Of Prostate 3</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumour Necrosis Factor Alpha</td>
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<tr>
<td>TRAP</td>
<td>Translating Ribosome Affinity Purification</td>
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<tr>
<td>uORFs</td>
<td>Upstream Open Reading Frames</td>
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<tr>
<td>UPR</td>
<td>Unfolded Protein Response</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>UPS</td>
<td>Ubiquitin Proteasome System</td>
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<td>WMS</td>
<td>White Matter Spared</td>
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<tr>
<td>XBP1</td>
<td>X-Box Binding Protein 1</td>
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CURRICULUM VITAE

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EDUCATION

Rutgers University, New Brunswick, NJ, Chemistry, B.A. 2014
University of Louisville School of Medicine, Louisville, KY, MD-PhD Present

SCIENTIFIC PAPERS IN REFERRED JOURNALS


ABSTRACTS AND PRESENTATIONS


5. Forston M, Wei G. Using Ribotag technology to identify changes in RNA transcripts in oligodendrocytes after SCI: Oral presentation at Kentucky Spinal Cord Injury Research Center Seminar. April 2019

6. Wei G. ATF4 and Oligodendrocyte Survival After Spinal Cord Injury: Oral presentation at Department of Pharmacology & Toxicology, University of Louisville School of Medicine. March 2020

7. Wei G. Translational profiling of C-fiber DRGS in Bortezomib-induced Neuropathic Pain: Oral presentation at Department of Pharmacology & Toxicology, University of Louisville School of Medicine. August 2020

8. Wei G. Translational profiling of C-fiber DRGS in Neuropathic Pain after Spinal Cord Injury: Oral presentation at Department of Pharmacology & Toxicology, University of Louisville School of Medicine. August 2020