Grant Title: Role of Checkpoint Kinase in Myelin Regeneration

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Progress to Date:

Myelin is formed by oligodendrocytes that arise from oligodendrocyte precursors (OPCs) in the central nervous system (CNS). It insulates neuronal axons and is essential for rapid and efficient transmission of action potentials. Deficits in myelination are major pathological hallmarks of myelin disorders such as multiple sclerosis, and may result from inadequate OPC maturation and oligodendrocyte generation that underlies neuronal damage, ultimately leading to dysfunction in movement, motor coordination, balance, as well as cognition. Therefore, our *main goal* is to identify the molecular pathway that promotes oligodendrocyte generation involved in myelin regeneration, providing potential targets for effective therapeutic strategies for myelin disorders.

In an effort to identify molecular targets, our laboratory recently identified the mitotic checkpoint kinase BubR1 as a key regulator in oligodendrocyte generation, myelination, and motor function. However, while our findings strongly suggest a crucial role of BubR1 in oligodendrocyte generation and myelination, whether increased BubR1 levels may prevent myelin deficits and/or promote remyelination and thus represent a novel therapeutic target for myelin disorders, remains a key open question. To address this, we propose to test the following two Specific Aims;

Aim 1: Determine whether Sirt2 is a key binding partner of the BubR1 regulating oligodendrocyte generation and myelination *in vivo*.

Aim 2: Evaluate whether high BubR1 levels in OPCs mitigate deficits in oligodendrocyte generation and myelination after brain damage *in vivo*.

During the first year of award period, we have studied new molecular mechanisms of BubR1 regulation of oligodendrocyte generation and myelination proposed in Aim 1. Based on our RNA sequencing and biochemical analysis, we have identified one gene that significantly altered by BubR1 insufficiency was NAD⁺-dependent deacetylase sirtuin-2 (*Sirt2*). We found high levels of *Sirt2* level in the adult hippocampus compared to other sirtuin members (Sirt1, 3, 4, 5, 6, and 7). Notably, only *Sirt2* levels were significantly reduced by BubR1 insufficiency, while other sirtuin members are unchanged, suggesting a unique expression pattern of *Sirt2* governed by BubR1 insufficiency in the adult hippocampus. To test the hypothesis that an increased Sirt2 level can recover reductions in oligodendrocyte generation and myelination observed in BubR1 insufficient mice, we have crossed BubR1^{H/H} with *Sirt2* overexpression (OX) mice. We first compared gross morphology and brain size *in vivo*, but found that double BubR1^{H/H} and *Sirt2* OX mice did not recover reduced size in body and brain compared to those in single BubR1^{H/H} mice at 7 weeks of age. In addition, we also did not see improved myelination as analyzed by

luxol fast blue (LFB) staining in double BubR1^{H/H} and *Sirt2* OX mice compared to single BubR1^{H/H} mice. Therefore, these observations suggest that an increased Sirt2 level did not recover defects observed in BubR1^{H/H} mice, and *Sirt2* is not a downstream target molecule of BubR1.

To identify an alternative downstream molecular target of BubR1, we have focused on the What signaling pathway for the following reasons. First, a dysregulation of What signaling has been implicated in the pathogenesis of multiple sclerosis and other autoimmune diseases. Second, previous research demonstrates that fibroblasts from mosaic variegated aneuploidy syndrome (MVA) patients with BubR1 mutations alter Wnt signaling through the downstream Wnt effector Dishevelled. Third, our pilot in situ hybridization data shows an increased natural Wnt inhibitor, secreted frizzled related protein 3 (*sfrp3*) expression in the BubR1^{H/H} mouse hippocampus compared to WT mice. Lastly, our previously published study demonstrated that the genetic deletion of sFRP3, which leads to increased Wnt signaling activity, has robust neuroregenerative potential. Based on these reasons, we have begun to investigate the relationship between BubR1 and sFRP3. To test this, we have generated double sfrp3 knockout (KO) and BubR1^{H/H} mice and compared brain and body size. Remarkably, we found that the genetic deletion of sFRP3 significantly restores reduced brain and body size observed in BubR1^{H/H} mice. Furthermore, to test if genetic deletion of sFRP3 could reverse myelination defects observed in BubR1^{H/H} mice, we performed LBF staining and electron microscopy (EM) analysis to measure myelination. We found that the genetic deletion of sFRP3 significantly reverses impaired myelination in BubR1^{H/H} mice, indicating that sFRP3 is the key downstream target molecule of BubR1 in regulating myelination, and brain growth.

As a future plan, we aim to determine whether a high level of BubR1 mitigates deficits in oligodendrocyte generation and myelination in a mouse model of demyelination *in vivo*, proposed in Aim 2.

Please list any of the following that have resulted from the Minnesota Regenerative Medicine grant funding:

Publications and/or manuscripts submitted for publication: None Disclosures/patents: None Grant applications and/or awards: One pending R01 grant

Budget Update:

Year 1 budget has been spent, and Year 2 budget will be fully spent by the end of the project period to test the hypothesis proposed in Aim 2.

Reporting to all Minnesotans:

Myelin related disorders including multiple sclerosis are devastating human neurological and autoimmune illnesses. Despite the increasing prevalence rate of myelin disorders, no effective current therapies exist due to our limited understanding of the causative pathology mediating demyelination. Therefore, there is critical need to identify the molecular factors in preventing demyelination and/or promoting myelin regeneration, so that effective therapeutic strategies can be realized. Aided by Regenerative Medicine Minnesota, we have identified the molecular pathway that promotes myelin regeneration and reverses abnormality in brain growth. Given that myelin defects are also associated with a broad range of neurodegenerative disorders

including Alzheimer's disease as well as traumatic spinal cord injury, our findings will broadly cover other myelin-related disorders.