Grant Title: Role of Checkpoint Kinase in Myelin Regeneration
Grant Number: RMM 102516 005
Principal Investigator: Mi-Hyeon Jang, PhD
Project Timeline: 3/1/2017 - 2/28/2019

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 as well as age-related neurodegeneration. Thismay 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. ***Our working hypothesis*** is that reduced BubR1 levels cause defects in oligodendrocyte generation and myelination, and that a sustained increase in BubR1 levels specifically in OPCs can ameliorate deficits in oligodendrocyte generation and myelination. During the past 2 years of the funding period, we have made significant progress, which resulted in publishing two peer reviewed journals. The followings are summary of our findings;

1. A sustained high level of BubR1 reverses hypomyelination and reduced brain size observed in BubR1$^{H/H}$ mice.

Our recent research demonstrates that mutant mice producing low levels of BubR1 (BubR1$^{H/H}$ mice) exhibit smaller brain sizes, which were mainly attributed to hypomyelination, resulting in abnormal corpus callosum formation. To test whether sustained high level of BubR1 can prevent hypomyelination and reduced brain size observed in BubR1$^{H/H}$ mice, we generated double BubR1$^{H/H}$;BubR1 overexpression (OX; BubR1^{T23} line) mice by crossing with BubR1$^{H/+}$ and BubR1$^{T23/+}$ mice. As shown in Fig. 1, a constitutively sustained high level of BubR1 significantly rescues the reduced brain size (Fig. 1A) and hypomyellination (Fig. 1B) observed in BubR1$^{H/H}$ mice. Therefore, our results suggest that myelination and brain development is mediated through BubR1.

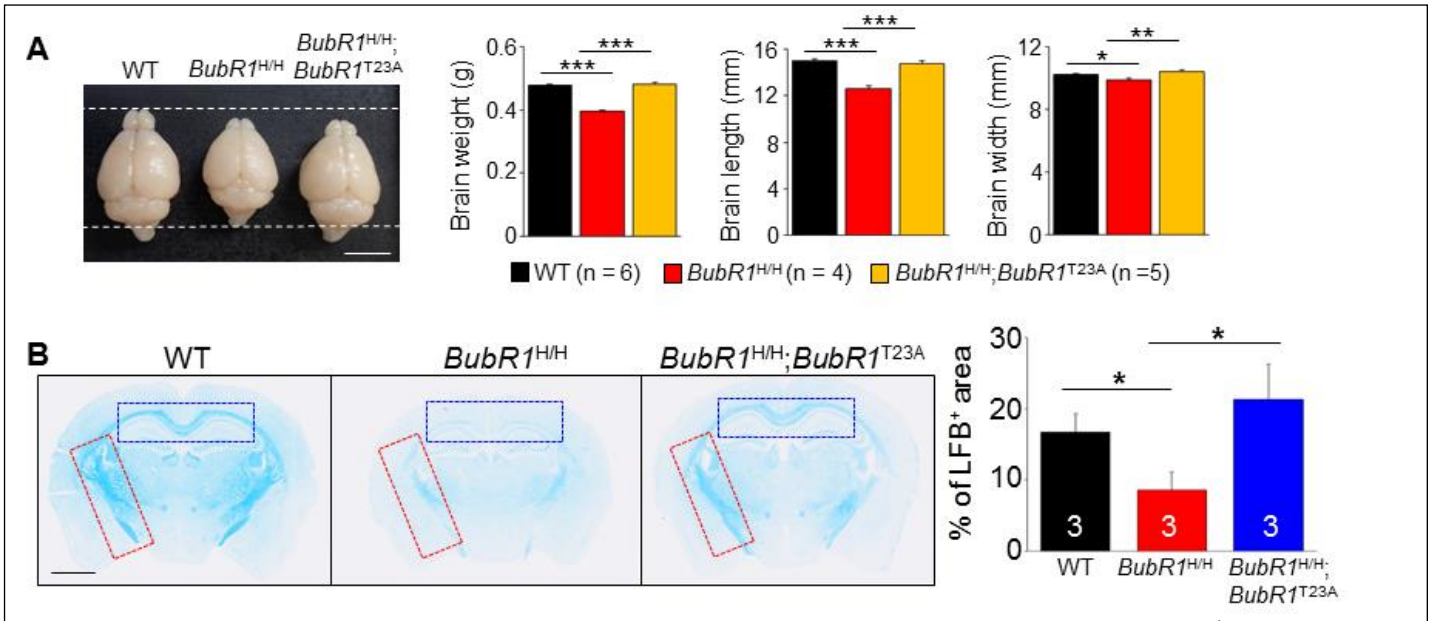

Fig. 1. Increased BubR1 levels reverse reduced brain size and myelination observed in BubR1$^{H/H}$ mice. (A, left) Representative images of brain size of *BubR1*^{WT} mice, *BubR1*$^{H/H}$ mice, and *BubR1*$^{H/H}$;BubR1 overexpression (OX; *BubR1*^{T23} line) mice. Scale bar: 1 cm. **(A, right)** Quantification of brain length in each group. **(B)** Quantification of luxol fast blue (LFB) staining. Note that a globally increased BubR1 level in BubR1$^{H/H}$ mice abolishes impaired brain size and myelination caused by BubR1 insufficiency. Values represents mean ± SEM (**P* < 0.05, ***P* < 0.01, ****P* < 0.001). The number associated with each bar graph represents the number of animals examined. Part of results was published in Aging Cell (Cho et al., 2019).

2. Genetic reduction of sFRP3 rescues hypo myelination and reduced brain/body size observed in BubR1$^{H/H}$ mice.

Due to the critical role of Wnt signaling in oligodendrocyte development, myelination and brain development, we have begun to investigate the relationship between BubR1 and Wnt signaling. In our work, we have focused on determining sFRP3 function, a natural Wnt inhibitor and generated with BubR1$^{H/H}$ and sFRP3 KO double mutant mice (*BubR1*$^{H/H}$;*sfrp3* HET or *BubR1*$^{H/H}$;*sfrp3* KO mice). Remarkably, we show that a genetic reduction of sFRP3 significantly restores reduced brain and body size observed in BubR1$^{H/H}$ mice (Fig. 2). Furthermore, we performed LBF staining and electron microscopy (EM) analysis to measure myelination. We found that the genetic reduction of sFRP3 significantly reverses impaired myelination in BubR1$^{H/H}$ mice (Fig. 3). Taken together, our results suggest that sFRP3 is the key cooperator of BubR1 in regulating myelination and brain development.

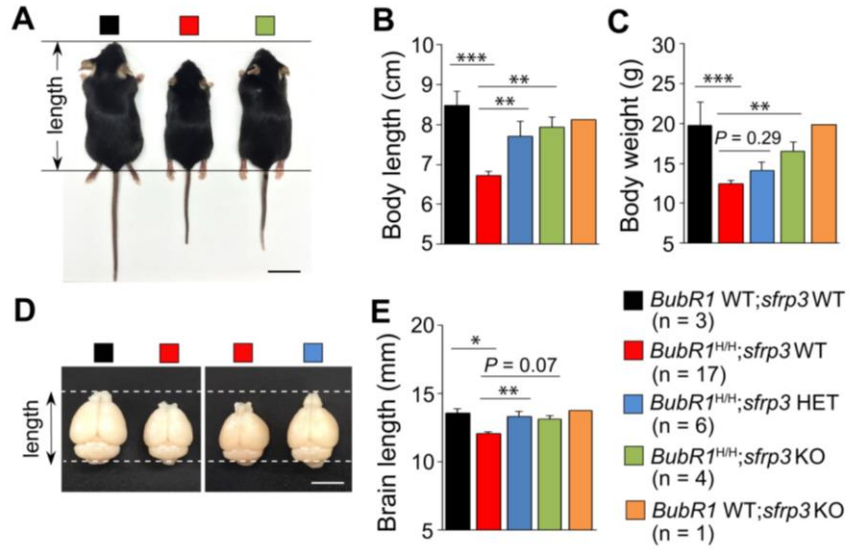

Fig. 2. Genetic inhibition of sFRP3 restores body and brain size of *BubR1*$^{H/H}$ mice (Cho et al., 2019, Aging Cell). (A-C) Representative images and quantification of body size and body weight of each group. Scale bar: 1 cm. KO, knockout; HET, heterozygous. **(D-E)** Representative images and quantification of brain size of each group. Scale bar: 0.5 cm. All values represent mean ± SEM. (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, one-way ANOVA).

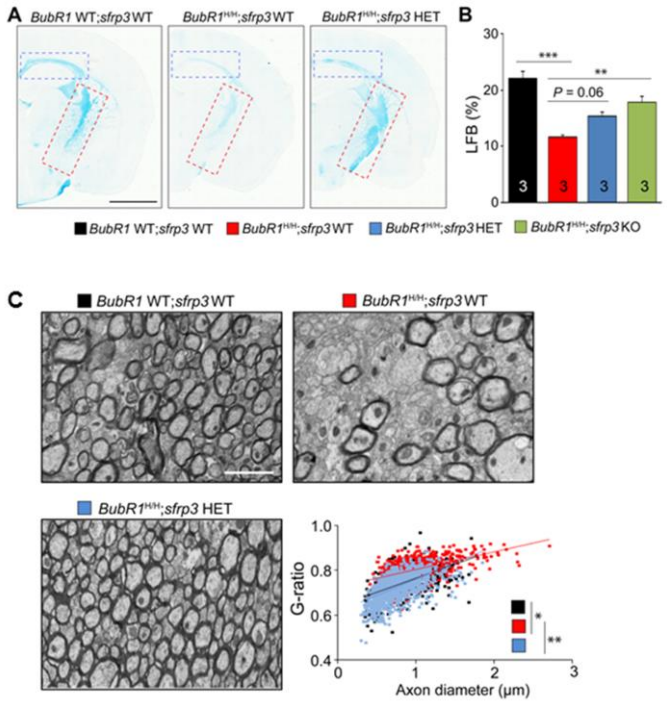

Fig. 3. Genetic inhibition of sFRP3 ameliorates deficits in myelination observed in *BubR1*$^{H/H}$ mice (Cho et al., 2019; Aging Cell). (**A-B**) Luxol fast blue (LFB) staining analysis. (**A**) Representative images of LFB staining in each group. Scale bar: 2 mm. (**B**) Quantification of LFB area in coronal brain sections of 8-week-old *BubR1*$^{H/H}$ mice exhibit a profound reduction in myelin density in the corpus callosum (blue dashed box), and internal capsule (red dashed box). (**C**) Electron microscopy (EM) analysis of the corpus callosum of each conditional genotype and quantification of G-ratio. Scale bar: 2 μm. All values represent mean ± SEM (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, one-way ANOVA for A and B, two-way ANOVA for C).

3. Genetic deletion of sFRP3 promotes myelin-related genes.

How sFRP3 inhibition restores reduced brain size observed in *BubR1*$^{H/H}$ mice is unknown. Notably, we found that genetic deletion of sFRP3 significantly promotes several genes critical for myelin production including myelin basic protein (MBP) (~ 40 fold), Oligo1, and myelin and lymphocyte (MAL), suggesting that sFRP3 negatively regulates expression levels of myelin-related genes (Fig. 4). Since *BubR1*$^{H/H}$ mice show dramatic reduction in myelin-related genes including MBP and PLP1, leading to hypomyelination, increased levels of myelin genes by sFRP3 deletion may compensate the reduced level of myelin-related genes observed in *BubR1*$^{H/H}$ mice. This may be a potential explanation why sFRP3 inhibition normalizes the observed hypomyelination.

4. Globally reduced level of BubR1 increases anxiety- and depressive-like behaviors:

Defects in myelination lead to deficits in motor-related and cognitive function by impairing signal conduction in affected nerves. In our previous study, we showed that reduction of BubR1 levels leads to abnormal motor-related behavior tests. Subsequently, to test if BubR1 regulates emotional behaviors, *BubR1*$^{H/H}$ mice and WT littermate controls were assessed for anxiety with the elevated plus maze (EPM) and light dark (LD) test. As depicted in Fig. 5 (left), we revealed that *BubR1*$^{H/H}$ mice spent significantly less time in the open arms of the EPM, while increasing the amount of time spent in the closed arms. Interestingly, we also found that the number of open arm entries were significantly lower when compared to WT controls (Fig. 5A, right). We then assessed anxiety with an alternative anxiety assay, the LD test, and found that relative to WT controls, *BubR1*$^{H/H}$ mice spent less time in the lighted, exposed compartment of the LD chamber (Fig. 5B). Although our LD test result did not reach statistical significance ($P = 0.07$), when considered in conjunction with increased anxiety in the EPM, it suggests that BubR1 may play a role in regulation of anxiety. Next, we investigated the involvement of BubR1 in depressive-like behavior utilizing the tail suspension test (TST), and found that *BubR1*$^{H/H}$ mice exhibited increased time spent immobile, suggesting a role for BubR1 insufficiency in depressive-like behavior (Fig. 5C). Next, to determine the involvement of BubR1 in recognition memory, we carried out the novel objective recognition (NOR) test (Fig. 5D), a well characterized assay known to be strongly affected by hippocampal impairment. As displayed on Fig. 5E, our results show that in the NOR familiarization phase (Day 2), both WT and *BubR1*$^{H/H}$ mice did not display differences in time spent exploring the two familiar objects (F1 and F2), indicating a lack of location preference. However, introduction of the novel object (test phase, Day 3), *BubR1*$^{H/H}$ mice spent a similar amount of time exploring the novel object (N) and the familiar object (F1). This is in contrast with WT littermates, which as expected, spent significantly more time exploring the novel object (N). Therefore, our findings strongly suggest that a reduced level of BubR1 impairs mood and recognition memory function.

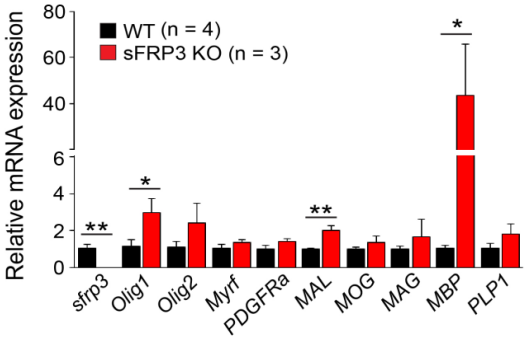

Fig. 4. Genetic deletion of sFRP3 promotes myelin-related genes (Cho et al., 2019, Aging Cell). Quantification of hippocampal mRNA expression of selected genes important for myelination in 2-month-old male sFRP3 KO and their WT littermates. Myelin basic protein (MBP), Olig1, and myelin and lymphocyte (MAL) were significantly increased in sFRP3 KO mice relative to their WT littermates. All values represent mean ± SEM (*: $P < 0.05$, **: $P < 0.01$, student's *t*-test).

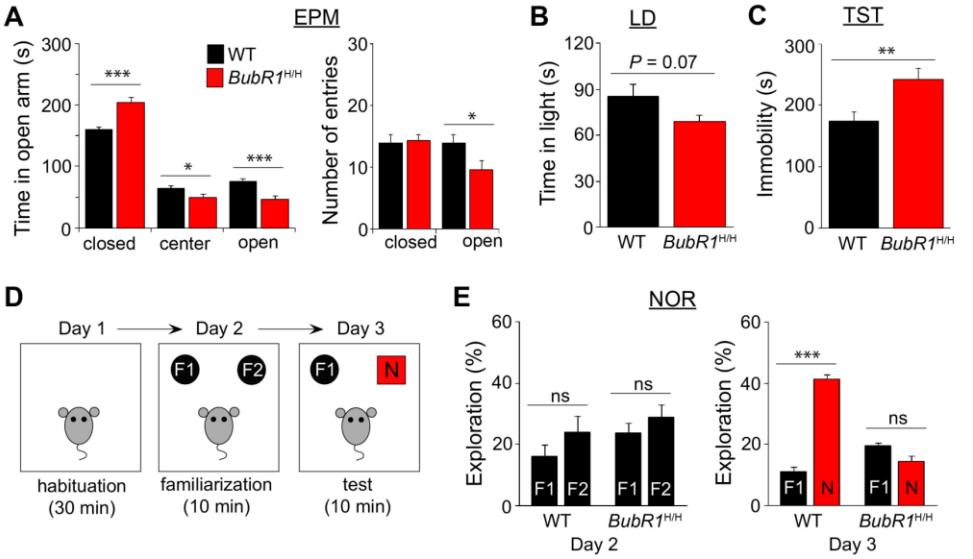

Fig. 5. *BubR1*$^{H/H}$ mice exhibit increased anxiety, depressive-like behaviors and impaired memory function (Cho et al., 2018, Int Neurourol J). (A) Elevated plus maze (EPM) test: **Left** is a summary of time spent in close, center and open arms. **Right** is a summary of number of entries into open or closed arm. *BubR1*$^{H/H}$ mice spent increased time in the closed arms and a decreased time in the open arms relative to WT mice, suggesting increased anxiety in *BubR1*$^{H/H}$ mice. **(B)** Light-Dark (LD) test. **(C)** Tail suspension test (TST): *BubR1*$^{H/H}$ mice display increased immobility time. **(D-E)** Novel objective recognition (NOR) test: **(D)** Schematic diagram of NOR. **(E)** In the NOR familiarization phase on Day 2, both *BubR1*$^{H/H}$ and WT mice showed no differences in time spent exploring the two familiar objects, indicating a lack of location preference. In test phase Day 3, *BubR1*$^{H/H}$ mice spent less time exploring the novel object, relative to WT littermates, suggesting impaired recognition memory in *BubR1*$^{H/H}$ mice. All values represent mean ± SEM (n = 17 *BubR1*$^{H/H}$ mice and 12 WT mice; **, $P < 0.01$; ***, $P < 0.001$; ns, no significance; Student *t*-test).

5. Dentate gyrus-specific BubR1 reduction increases anxiety- and depressive-like behaviors:

Our previous research demonstrated that *BubR1*$^{H/H}$ mice develop significant motor and movement abnormalities at 2-3 months of age. Therefore, we speculated, whether the observed affective and memory behavior impairments in *BubR1*$^{H/H}$ mice could be due to confounding deficits in motor function. To rule out these possibilities, we generated shRNA-*BubR1* lentiviral (LV) constructs to selectively knockdown endogenous BubR1 levels within adult WT mouse dentate gyrus (DG) in a non-cell autonomous manner. Four weeks after lentiviral injection, mice were underwent anxiety, depressive-like, and memory tests as described above. As shown in Fig. 6A, we confirmed wide-spread lentiviral-mediated transgene expression across the septal-temporal axis of the DG. Similar to *BubR1*$^{H/H}$ mice, hippocampal DG specific knockdown of BubR1 led to higher levels of anxiety in the EPM (Fig. 6B) and LD test (Fig. 6C), increased depressive-like behavior in the FST test (Fig. 6D), and impaired memory function in NOR test (Fig. 6E). Taken together, these results indicate the critical DG-specific role of BubR1 in regulation of emotional and memory-related function.

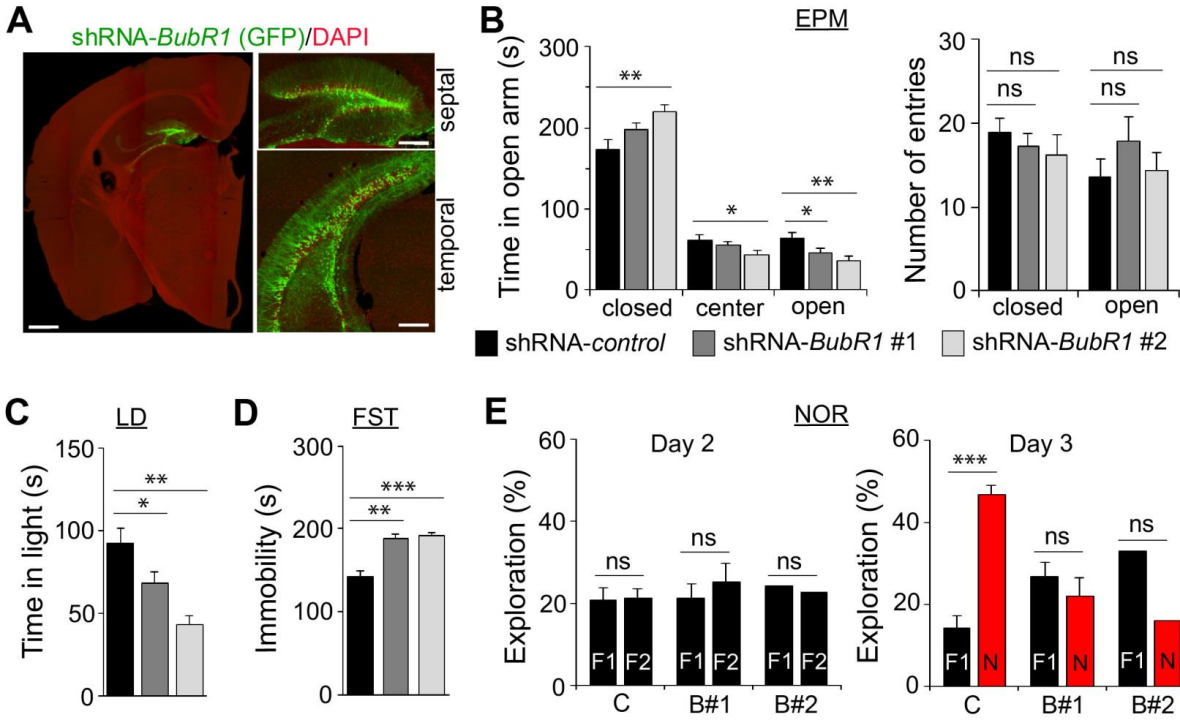

Fig. 6. Dentate gyrus-specific *BubR1* knockdown in mouse hippocampus show increased anxiety, depressive-like behaviors and impaired memory function (Cho et al., 2018, Int Neurourol J). (A) Representative images of lentiviral infected dentate gyrus (DG) section. Note the wide spread of lentiviral-mediated transgene expression across the septo-temporal axis of the whole DG. Left: an enlarged view (Scale bar; 1 mm). Right: a high-magnification view (Scale bar; 200 μm). **(B)** Elevated plus maze (EPM) test: **Left** is a summary of time spent in close, center and open arms. **Right** is a summary of number of entries into open or closed arm. Similar to *BubR1*$^{H/H}$ mice, BubR1 knockdown mice spend an increased amount of time in the closed arms and a decreased in the open arms relative to WT mice, indicating an increased anxiety in *BubR1*$^{H/H}$ mice. **(C)** Light-dark (LD) test: A summary of time spent in light chamber. **(D)** Forced swimming test (FST): Both shRNA-*BubR1* #1 and #2 knockdown mice display increased immobility time. **(E)** Novel objective recognition (NOR) test: **(D)** Diagram of NOR test. **(E)** In the NOR familiarization phase on Day 2, all three groups of mice showed no differences in the percentage of distance exploring the two familiar objects, indicating a lack of location preference. However, in the test phase on Day 3, both shRNA-*BubR1* #1 and #2 knockdown mice spent less time exploring the novel object, relative to BubR1 control mice, suggesting BubR1 knockdown mice show memory dysfunction. All values represent mean ± SEM (n = 10 mice each group; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, no significance, Student *t*-test).

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

Publications:

1. Cho CH, Yoo KH, Oliveros A, Paulson S, Hussaini SMQ, van Deursen JM, **Jang MH**. sFRP3 inhibition improves age-related cellular changes in BubR1 progeroid mice. *Aging Cell* 2019 18(2):e12899. doi: 10.1111/acel.12899 (PMID: 30609266).

2. Cho CH, Yang Z, Yoo KH, Oliveros A, **Jang MH**. BubR1 insufficiency impairs affective behavior. Int Neurourol J. 2018 Oct;22 (Suppl 3):S122-130. doi: 10.5213/inj.1836218.109 (PMID: 30396261).

3. Hussaini SMQ, **Jang MH**. New roles for old glue: Astrocyte function in synaptic plasticity and neurological disorders. Int Neurourol J. 2018 Oct;22(Suppl 3):S106-114. doi: 10.5213/inj.1836214.107 (PMID: 30396259).

Grant applications and/or awards:

Active

R01AG058560 Jang (PI) 3/1/2018 - 12/31/2022
NIH/NIA
Title: Role of BubR1 as a juvenile protective factor in hippocampal aging.
The major goal is to determine the role of BubR1 in age-related declines in neurogenesis, synaptic plasticity and cognitive function.
Role: PI

Discovery Science Award from Regenerative Medicine Minnesota Jang (PI) 4/1/2019 - 3/31/2021
Title: Targeting Adora2a as a novel regenerative therapy for chemobrain.
The major goal is to determine the role of adenosine A_{2a} receptor (Adora2a) in chemotherapy-induced cognitive dysfunction.
Role: PI

Disclosures/patents: None

Budget Update:

Years 1 and 2 budget has been fully spent.

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.