AWARD NUMBER: W81XWH-15-1-0592

TITLE: Role of Snf5 Mutations in Schwannomatosis Pain

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REPORT DATE: October 2016

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 0704-0188*



| 1. REPORT DATE | 2. REPORT TYPE | 3. DATES COVERED |
|---|---|---|
| October 2016 | Annual | 30 Sep 2015 - 29 Sep 2016 |

**4. TITLE AND SUBTITLE**

Role of Snf5 Mutations in Schwannomatosis Pain

**5a. CONTRACT NUMBER**

**5b. GRANT NUMBER**
W81XWH-15-1-0592

**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHOR(S)**
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**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**
Oregon Health & Science University
Oregon National Primate Research Center
505 NW 185th Avenue
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**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

**10. SPONSOR/MONITOR'S ACRONYM(S)**

**11. SPONSOR/MONITOR'S REPORT NUMBER(S)**

**12. DISTRIBUTION / AVAILABILITY STATEMENT**

Approved for Public Release; Distribution Unlimited

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**
The overarching goal of this proposal is to test the hypothesis that Snf5 gene (also called SMARCB1) mutations that occur in schwannomatosis patients contribute to the unique, untreatable pain experienced by these patients. During the first year of this project, we found that mice with Schwann cell-targeted Snf5 mutations demonstrated increased TRPV1 and CGRP expression, two factors linked to pain phenotype, expression in both large and small diameter sensory neurons and were typically co-expressed by these cells. We have also completed a proteomic screen of Snf5-mutant Schwann cell conditioned medium and identified several factors that may contribute to schwannomatosis pain phenotypes.

**15. SUBJECT TERMS**
schwannomatosis, SNF5, SMNACRB1, Schwann cells, TRPV1, CCL1, pain

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
|---|---|---|---|---|---|
| a. REPORT | b. ABSTRACT | c. THIS PAGE | | | USAMRMC |
| Unclassified | Unclassified | Unclassified | Unclassified | 9 | 19b. TELEPHONE NUMBER *(include area code)* |

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## 1. INTRODUCTION

Schwannomatosis is a clinically and genetically distinct form of neurofibromatosis that affects 1 in 40,000 individuals worldwide. The disease is characterized by multiple peripheral nerve tumors, called schwannomas, and a predisposition to other nervous system tumors including meningiomas. In addition, patients with schwannomatosis overwhelmingly present with intractable pain. Clinical findings suggest that the pain afflicting schwannomatosis patients is not strictly linked to tumor growth or mechanical nerve compression by schwannomas. Mutations in the *SNF5* (also called *SMARCB1*) gene (as well as the neurofibromatosis 2 (*NF2)* gene) are linked to schwannomatosis. To test the role of *SNF5* loss in schwannomatosis, we generated and characterized mice in which the *Snf5* gene was knocked-out in Schwann cells. We found that these mice had increased capsaicin sensitivity and elevated levels of the pain mediators, TRPV1 and calcitonin gene-related peptide (CGRP) in sensory neurons. These phenotypes are induced by a factor or factors released by *Snf5*-mutant Schwann cells. The goal of this study is to fully characterize the cells induced to express ectopic TRPV1 and CGRP, identify the factor or factors that induce TRPV1 and CGRP in sensory neurons, determine how TRPV1 activity and elevated CGRP are related and their contributions to schwannomatosis pain, and whether altering the activities of factors released from *Snf5* mutant Schwann cells can reverse pain phenotypes in *Snf5*-mutant mice. All together, these studies will help determine whether *SNF5*/*SMARCB1* mutations in Schwann cells lead to pain, and have the potential to define potential targets for the treatment of schwannomatosis pain.

## 2. KEYWORDS:

Schwannomatosis, Schwann cells, Snf5, SMARCB1, TRPV1, pain, calcitonin gene-related peptide, proteomics, CCL2

## 3. ACCOMPLISHMENTS:

**What were the major goals of the project?**

Our specific aims are:

1. *To identify factors secreted by Snf5-/- Schwann cells that induce increased TRPV1 and CGRP expression in sensory neurons.* We are further characterizing the effects of *Snf5*-/- Schwann cell conditioned medium (CM) on TRPV1 and CGRP expression in sensory neurons by defining which cells express TRPV1 and CGRP and determining the time it takes for TRPV1 and CGRP expression to increase. We are also determining the profile of secreted proteins in Snf5-/- vs. Snf5+/+ Schwann cell CM using a novel and sensitive proteomic strategy. Finally, we are determining if factors identified in our proteomic and earlier DNA microarray screens are expressed by human schwannomatosis patient schwannoma cells *in vitro* and *in situ*, and whether these factors are sufficient to induce TRPV1 and CGRP expression in sensory neurons.
2. *To test if TRPV1 elevation is sufficient to induce increased pain sensitivity in mice with Schwann cell-targeted Snf5 mutations*. Given that numerous genes demonstrate altered expression in *Snf5*-null Schwann cells, it is possible that pain sensitivity in our *Snf5* mutant mice may be linked to alterations in multiple proteins in sensory neurons. Here, we will assess (in year 2) whether elevated TRPV1 expression is necessary and sufficient for the elevated pain sensitivity in our mice by crossing our B6.Cg-Tg(Plp1-cre/ERT)3Pop/J +/-; Snf-fl/fl mice into a *Trpv1*-null background. We will then perform a battery of pain

sensitivity assays. Because both TRPV1 activity and expression can be influenced by inflammation, we will also test if inducing inflammation in our *Snf5*-mutant mice (in both the *Trpv1*+/+ and *Trpv1*-/- backgrounds) alters pain sensitivity and whether these effects are TRPV1-dependent.

3. *To test how CGRP influences pain sensitivity in mice with Schwann cell targeted loss of Snf5.* Because TRPV1 can influence CGRP expression, we will test (in year 3) if *Snf5*-/- Schwann cell CM induces CGRP in *Trpv1*-/- DRG neurons and test if CGRP expression is elevated in the DRG of our *Snf5*-mutant mice in the *Trpv1*-/- background. We will also use a pharmacological inhibitor of CGRP receptors to determine the contribution of CGRP to pain phenotypes in our *Snf5* mutant mice under both basal and inflammatory conditions.

**What was accomplished under these goals?**

We have confirmed that the vast majority of cells that express elevated TRPV1 in the dorsal root ganglia of *Snf5* mutant mice also express CGRP, and that both small and large diameter neurons are double positive. We have also confirmed that the effects of *Snf5* null Schwann cell conditioned medium on wild type sensory neuron TRPV1 expression can be observed in as little as an hour following exposure suggesting that the effect may not require transcriptional upregulation of the *TRPV1* gene. We see similar effects when using conditioned media from schwannomatosis patient schwannoma cell CM.

We have now completed the first round of our proteomics screen to identify proteins secreted by Snf5 mutant Schwann cells. Several proteins were significantly elevated as shown in Figure 1.

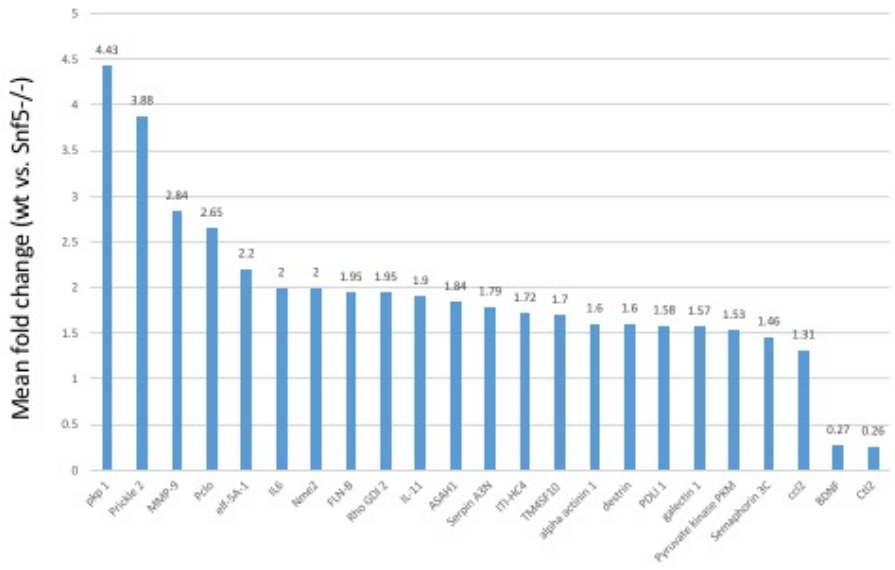


Figure 1: Proteins elevated in Snf5-/- Schwann cell conditioned media compared to conditioned media from wild type Schwann cells.

Although there are potential roles for some of these proteins in pain and in the regulation of TRPV1 (e.g. IL-6), one of the most interesting with a direct potential role in pain is CCL2 (C-C motif ligand 2). CCL2 has been implicated in neuropathic pain following peripheral nerve injury, and activation of spinal TRPV1 receptors plays an important role in the modulation of nociceptive signaling induced by CCL2 (Spicarova et al., Neuropharmacology. 2014 81:75-84). We therefore chose to further examine CCL2 expression in Snf5-/- Schwann cells. We find that CCL2 mRNA is 2 times higher in *Snf5* mutant Schwann cells than in wild type Schwann cells (Fig. 2). We are currently examining the changes in CCL2 protein expression in mouse *Snf5*

null Schwann cells and in human schwannoma cells from schwannomatosis patients with confirmed *SMARCB1* mutations.

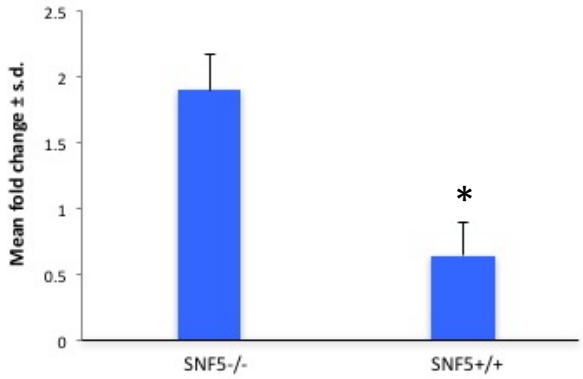


Figure 2: Quantitative PCR analysis of CCL2 transcripts in wild type (SNF5+/+) and mutant (SNF5-/-) Schwann cells. Cyclophilin A was used as the reference gene for data normalization. *p<0.001

**What opportunities for training and professional development has the project provided?**
Nothing to Report.

**How were the results disseminated to communities of interest?**
Some of these data were presented in an oral presentation by Dr. Sherman at the 2016 International Neurofibromatosis Conference in Austin, Texas.

**What do you plan to do during the next reporting period to accomplish the goals?**
We will continue to validate novel targets from our proteomic screen both in mouse and human tissue and CM samples, and test if any of these targets can reverse the TRPV1 and CGRP phenotypes as well as the pain phenotypes in the *Snf5* mutant mice. We will also continue breeding our conditional *Snf5* mutant mice into the TRPV1 background to perform the experiments outlined in aims 2 and 3.

4. **IMPACT:**
**What was the impact on the development of the principal discipline(s) of the project?**
To date, this project has demonstrated that the loss of SNF5/SMARCB1 in Schwann cells, two genes that are commonly mutated in the Schwann cells of patients with schwannomatosis, leads to increased levels of CCL2. This is potentially highly significant given the known roles of CCL2 in neuropathic pain. The project has also identified a number of other proteins that are elevated in *SNF5* mutant Schwann cells. Future work will demonstrate if CCL2 alone or in combination with these other proteins contribute to schwannomatosis pain, and whether targeting these proteins could be a way to relieve schwannomatosis pain.

**What was the impact on other disciplines?**
Nothing to Report.

**What was the impact on technology transfer?**
Nothing to Report.

**What was the impact on society beyond science and technology?**
Nothing to Report.

5. **CHANGES/PROBLEMS:**

**Changes in approach and reasons for change**

Nothing to report.

**Actual or anticipated problems or delays and actions or plans to resolve them**

**Changes that had a significant impact on expenditures**

Nothing to report.

**Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

Nothing to report.

6. **PRODUCTS:**

**Publications, conference papers, and presentations**

**Journal publications.**

Nothing to report

**Books or other non-periodical, one-time publications.**

Nothing to report

**Other publications, conference papers, and presentations.**

Oral presentation by Dr. Sherman at the 2016 International Neurofibromatosis Conference in Austin, Texas.

**Website(s) or other Internet site(s)**

Nothing to Report

**Technologies or techniques**

Nothing to Report

**Inventions, patent applications, and/or licenses**

Nothing to report.

**Other Products**

Nothing to report.

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

- **What individuals have worked on the project?**

| | |
|---|---|
| • Name: | • *Larry Sherman* |
| • Project Role: | • *PI* |
| • Researcher Identifier (e.g. ORCID ID): | • 0000-0001-6098-6551 |
| • Nearest person month worked: | • *2.4 calendar months* |
| • Contribution to Project: | • *Dr. Sherman served as PI of the project, coordinating all of the research projects, interpreting data, presenting data at meetings, and authoring reports.* |

| | |
|---|---|
| • Funding Support: | • *NIH, National Multiple Sclerosis Society* |

| | |
|---|---|
| • Name: | • *Steven Matsumoto* |
| • Project Role: | • *Co-Investigator* |
| • Researcher Identifier (e.g. ORCID ID): | • 0000–0002–3352–8077 |
| • Nearest person month worked: | • *1.2 calendar months* |
| • Contribution to Project: | • *Dr. Matsumoto prepared primary cultures of Schwann cells and dorsal root ganglion neurons, and performed and analyzed all immunohistochemistry experiments.* |
| • Funding Support: | • *National Multiple Sclerosis Society* |
| • Name: | • *Fatima Banine* |
| • Project Role: | • Staff Scientist |
| • Researcher Identifier (e.g. ORCID ID): | • |
| • Nearest person month worked: | • 7.2 calendar months |
| • Contribution to Project: | • Dr. Banine performed all of the proteomics experiments and the subsequent data analysis and validation assays (e.g. qPCR) |
| • Funding Support: | • National Multiple Sclerosis Society |
| • Name: | • Scott Foster |
| • Project Role: | • Research Assistant |
| • Researcher Identifier (e.g. ORCID ID): | • |
| • Nearest person month worked: | • 4.2 calendar months |
| • Contribution to Project: | • Mr Foster assisted Dr. Sherman, Dr. Matsumoto and Dr. Banine with all aspects of the project. He maintained the mouse colony, arranged for timed matings, prepared reagents, and maintained cell cultures. |
| • Funding Support: | • NIH, National Multiple Sclerosis Society |

| | |
|---|---|
| • Name: | • Cristina Fernandez-Valle |
| • Project Role: | • Co-Investigator |
| • Researcher Identifier (e.g. ORCID ID): | • 0000–0002–6718–1243 |
| • Nearest person month worked: | • 0.6 calendar months |
| • Contribution to Project: | • Dr. Fernandez-Valle organized the acquisition of human tissues, prepared human schwannoma cell cultures, and arranged for shipments of media and tissues to the Sherman lab |
| • Funding Support: | • DOD, NIH |
| • Name: | • Stephanie Klingman-Plati |
| • Project Role: | • Research Assistant |
| • Researcher Identifier (e.g. ORCID ID): | • |
| • Nearest person month worked: | • 1.2 calendar months |
| • Contribution to Project: | • Ms. Klingman-Plati assisted Dr. Fernandez-Valle, maintained cell cultures, and assisted with the preparation of tissues and conditioned media. |
| • Funding Support: | • DOD, NIH |
| | |

**Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
No

**What other organizations were involved as partners?**
Dr. Fernandez-Valle and Ms. Klingman-Plati are located at the University of Central Florida. Otherwise, no other institutions were involved to date.