AWARD NUMBER: W81XWH-20-1-0391

TITLE: Molecular and Cellular Functions of LZTR1 Mutations in Schwannomatosis

PRINCIPAL INVESTIGATOR: Dr. Pau Castel

CONTRACTING ORGANIZATION: NEW YORK UNIVERSITY SCHOOL OF MEDICINE
RISE SCHWAB
550 1ST AVE
NEW YORK NY 10016-640

REPORT DATE: NOVEMBER 2023

TYPE OF REPORT: FINAL

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
NOVEMBER 2023	FINAL	1AUG2020 - 31JUL2023

4. TITLE AND SUBTITLE

Molecular and Cellular Functions of LZTR1 Mutations in Schwannomatosis

5a. CONTRACT NUMBER
W81XWH-20-1-0391

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Dr. Pau Castel

E-Mail: pau.castel@nyulangone.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

NEW YORK UNIVERSITY SCHOOL OF MEDICINE

RISE SCHWAB

550 1ST AVE

NEW YORK NY 10016-640

8. PERFORMING ORGANIZATION REPORT NUMBER

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

U.S. Army Medical Research and Development 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

Neurofibromatosis type 3 or Schwannomatosis is a genetic disorder that affects 1 in 40,000 people worldwide. It is characterized by the appearance of several benign tumors arising from the peripheral and spinal nerves called schwannomas. Schwannomas develop from Schwann cells, a type of cell that produces myelin, which is a substance that serves as a sheath for the neuronal axons found in nerves. Patients affected by schwannomatosis are diagnosed due to the high number of schwannomas they develop through their life, because in non-affected individuals this tumor type is rare. The most significant symptom in these patients is chronic pain, which results from the mechanical pressure that schwannomas exert on the surrounding nerves. Schwannomatosis has been linked to familiar and sporadic mutations in a gene termed *LZTR1*, which encodes for a protein of unknown function. Such mutations inactivate the expression of LZTR1, resulting in the loss of the protein in Schwann cells. We have recently uncovered the main function of this protein and showed that it acts as a molecular scaffold that facilitates degradation of a specific substrate. In normal conditions, LZTR1 interacts with such substrate and promotes its degradation, but in the absence of LZTR1, as a result of mutations, the substrate can no longer be degraded and it accumulates in the cells causing an abnormal cell phenotype. However, the consequences of LZTR1 mutations in the context of schwannomatosis have never been explored. In this proposal, I hypothesize that loss of LZTR1 in Schwann cells results in accumulation of its substrate, a protein which causes uncontrolled proliferation and differentiation into schwannomas. Therefore, I will establish different experimental approaches to test this hypothesis, summarized in three aims. In **Aim 1**, I will use primary and immortalized Schwann cells that lack LZTR1 to assess the changes in the biology of these cells, including effects in cell growth and proliferation, migration, and differentiation. In **Aim 2**, I will identify and validate novel substrates of LZTR1 that could be responsible for the phenotype in Schwann cells through different biochemical techniques. In **Aim 3**, I will develop a novel mouse model of schwannoma that will allow us to test the effect of LZTR1 depletion in schwannoma formation. Altogether, the goal of this proposal is to further understand the role of LZTR1 in the formation of schwannomas both at the molecular and cellular level. These results will benefit patients suffering from neurofibromatosis type 3 or schwannomatosis because it will help understand the mechanism of pathogenesis of this incurable disorder. Long term, this important knowledge can be used to identify potential pharmacological treatments that would directly target the genetic source of schwannomas and, ultimately, help these patients.

15. SUBJECT TERMS NONE LISTED

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
U	U	U	UU	11	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. INTRODUCTION:

Schwannomatosis is a very rare type of genetic disorder that shares some clinical features with neurofibromatosis type II (hence, it is also known as type III neurofibromatosis). Schwannomatosis patients often develop multiple schwannomas during their lifetime, which is the major cause of morbidity in this patient population. In contrast to neurofibromatosis type II, schwannomatosis patients can often develop multiple schwannomas and these are mostly localized in the peripheral nerves and cause severe and debilitating pain. Recent clinical genetic work has demonstrated that schwannomatosis is often associated with germline heterozygous loss-of-function mutations in either *SMARCB1* or *LZTR1* genes. Additional somatic mutations in the schwannoma cause the loss of 22q11 (encoding for NF2, SMARCB1, and LZTR1) and singe nucleotide variants in the NF2 gene. Hence, most schwannomas obtained from schwannomatosis patients carry bi-allelic
loss-of-function mutations in both *NF2* and *LZTR1* (or *SMARCB1*). LZTR1 is a protein adaptor for the Cullin-RING E3 ubiquitin ligase 3 (CRL3) that binds to the RIT1 GTPase and promotes the activation of MAPK signaling pathway. In this proposal, we aim to study the role of LZTR1 loss of-function in Schwann and schwannoma cells. The project is focused on identifying novel substrates of the $CRL3^{LZTR1}$ complex that could shed light into the function of LZTR1 in Schwann and schwannoma cells. In addition, this proposal explores the development of a novel mouse model of schwannomatosis by combining CRISPR/Cas9 somatic genetic editing in Schwann cells and classical mouse genetics.

2. KEYWORDS:

LZTR1, schwannomatosis, schwannoma, Schwann cell, CRISPR/Cas9, mouse model, RIT1, Cullin 3, CRL3

3. ACCOMPLISHMENTS:

What were the major goals of the project?

The major goals of this project have been summarized in the statement of work as a % of completed work.

Specific Aim 1: Determining the cellular effects of LZTR1 loss in Schwann cells	**Timeline**	**% Completed**
Major Task 1: Generation of cellular models	Months	
Subtask 1. ACURO review and approval	1-3	100 %
Subtask 2. Breeding of *Lztr1*$^{+/+}$ and *Lztr1*$^{-/-}$ mice	3-7	100 %
Subtask 3. Generation of primary mouse Schwann cells	7-9	0 %
Subtask 4. Generation of immortalized LZTR1 knockout cells	1-3	100 %
Milestones Achieved: Generation of LZTR1 deficient models	1-9	100 %
Major Task 2: Analysis of cellular phenotypes		
Subtask 1. Cell growth and proliferation assays	9-10	100 %
Subtask 2. Cell cycle determination assays	9-10	100 %
Subtask 3. Anchorage independent growth (Matrigel)	9-11	100 %
Subtask 4. Migration assays (Boyden chamber)	9-10	100 %
Milestones Achieved: Performing all the cellular assays to analyze the cellular phenotype of the LZTR1 knockout Schwann cells.	9-11	100 %
Major Task 3: Determination of Schwann cell differentiation		

Subtask 1. Immunoblot and qPCR analysis of known differentiation Schwann cell markers.	9-12	100 %
Subtask 2. Gene expression analysis by RNA sequencing	9-15	100 %
Milestones Achieved: Assessment of cell differentiation by analysis of the molecular markers and gene expression signatures.	9-15	100 %
Specific Aim 2: Identification of novel substrates of LZTR1	**Timeline**	**Site 1**
Major Task 1: Validation of novel LZTR1 substrates identified in the mass spectrometry screening		
Subtask 1. Immunoblot analysis in mouse embryonic fibroblasts and Schwann cells.	1-2	100 %
Subtask 2. Data-independent acquisition mass spectrometry	2-4	100 %
Milestones Achieved: Validation of hits from MS screening	1-4	100 %
Major Task 2: Biochemical characterization of novel LZTR1 substrates identified by mass spectrometry		
Subtask 1. Cloning and co-immunoprecipitation assays	4-12	100 %
Subtask 2. Degradation assays by co-expression	6-9	100 %
Subtask 3. Ubiquitination assays using Tandem Ubiquitin Binding Elements in cell extracts	6-9	100 %
Milestone(s) Achieved: Identification of substrates that co-immunoprecipitate with LZTR1, promote degradation, and ubiquitination.	4-12	100 %
Specific Aim 3: Modeling LZTR1 loss-of-function *in vivo*	**Timeline**	**Site 1**
Major Task 1: Obtaining animal cohorts and primary Schwann cells for lentiviral transduction		
Subtask 1. ACURO review and approval	1-3	100 %
Subtask 2. Breeding Lztr1 and LSL-Cas9 alleles to obtain desired experimental cohorts	3-7	100 %
Subtask 3. Generation of primary Schwann cell lines	7-9	0 %
Milestones Achieved: Generation of Schwann cell lines with $Lztr1^{f/f}/Cas9^{LSL}$ and $Lztr1^{+/+}/Cas9^{LSL}$ alleles	1-9	0 %
Major Task 2: Genomic editing of Schwann cells using lentivirus		
Subtask 1. Generation of lentiviral particles in 293T cells	9-10	100 %
Subtask 2. Lentiviral transduction and selection of Schwann cells	9-10	0 %
Subtask 3. Analysis of gene edited primary cells by immunoblotting and genomic sequencing using targeted Sanger sequencing	10-12	0 %
Milestones Achieved: Generation and validation of gene edited Schwann cells that are knockout for *Nf2* and *Lztr1* alleles.	9-12	0 %
Major Task 3: Allograft injection schwannoma mouse model		
Subtask 1. Expansion of cells and allograft injection	12-15	0 %
Subtask 2. Monitoring tumors and measurement	13-19	0 %
Subtask 3. Collection of tumors and histological analysis	19-20	0 %
Subtask 4. Immunoblot and mRNA analysis of LZTR1 substrates	20-24	0 %
Milestones Achieved: Tumor engraftment, measurement, and histological and biochemical analysis.	12-24	0 %

What was accomplished under these goals?

A major aim of our project was to identify potential additional substrates of the CRL3^{LZTR1} complex; this is particularly important in the context of schwannomatosis, because we hypothesized that Schwann-cell specific substrates of this complex could be present in this cell lineage, explaining why individuals with this condition develop schwannomas. In order to achieve this, we took advantage of mass spectrometry approaches that were also part of the career development activities of the PI. We initially identified a novel interactor of the CRL3^{LZTR1} complex termed ARIH1 (ariadne RBR E3 ubiquitin protein ligase 1). ARIH1 is a ubiquitin ligase that uses the E2 conjugating enzyme UbcH7 and it has been shown to promote ubiquitination of different substrates. Our initial work tried to validate ARIH1 as a substrate of the CRL3^{LZTR1} complex; however, we found out that ARIH1 is likely a modifier of the activity of the CRL3^{LZTR1} complex. Previous work by others has shown that ARIH1 binds to many Cullin RING ligase complexes and promotes the linear extension of the ubiquitin chain (Scott et al, 2016, Cell). Taking this work into consideration, we demonstrated that ARIH1 can associate with the CRL3^{LZTR1} complex. Inhibition of ARIH1 in mammalian cells is lethal and, therefore, we could not generate knockout cells to test the effect in the regulation of the CRL3^{LZTR1} complex substrate RIT1. However, Scott and colleagues had developed dominant negative mutants of ARIH1 that can be expressed in cells and compete with the function of the endogenous protein. Using these, we were able to demonstrate that inhibition of ARIH1 leads to the accumulation of the substrate RIT1 to a similar extend as other previously reported substrates of CRL complex (such as p21^{CIP}). We also showed that expression of these mutants leads to a change in the ubiquitination chain associated to RIT1. For instance, ARIH1 inhibition promotes the accumulation of mono-ubiquitinated RIT1, suggesting that ARIH1 might be necessary to extend the ubiquitin chains and that CRL3^{LZTR1} complex only promotes mono-ubiquitination of substrates. We have also developed in vitro assays to assess ubiquitination of CRL3^{LZTR1} complex and assess the role of ARIH1. We have been able to obtain these proteins recombinantly from E. coli, although LZTR1 remains difficult to express and we have adapted a mammalian cell purification protocol to obtain this protein for our assays. An unexpected discovery was that in our *in vitro* reconstitution experiments, we observed that in the presence of ARIH1, LZTR1 itself can also be ubiquitinated. This could be due to the ability of some CRL complexes to promote their own ubiquitination as a mechanism to terminate the ubiquitination of the substrate. In this case, it seems that ARIH1 (only when complexed with the E2 UbcH7) can promote polyubiquitination of LZTR1.

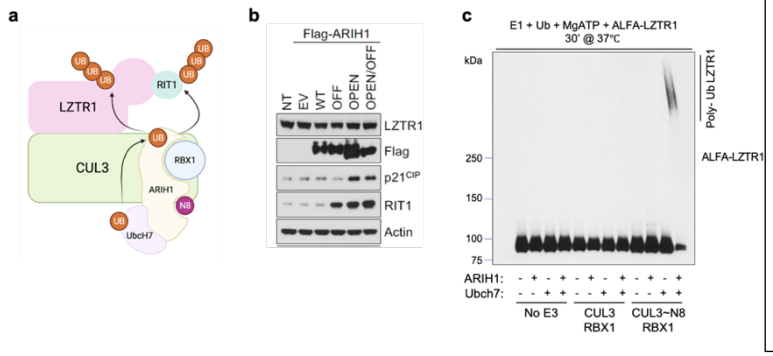

Figure 1. Role of ARIH1 in regulating the CRL3^{LZTR1} complex
(a) Proposed function of ARIH1 in the CRL3^{LZTR1} complex, depicting a canonical role in mediating polyubiquitination of the substrate RIT1 and a non-canonical function in polyubiquitinating LZTR1 described in our system. **(b)** Overexpression of different dominant negative mutants of ARIH1 in 293T cells leads to the inactivation of CRL3^{LZTR1} and rapid accumulation of RIT1 levels. These mutants were previously described and interfere with catalytic activity (OFF) or autoinhibitory conformation (OPEN). P21 was used as a positive control. **(c)** *In vitro* ubiquitination assay using recombinant ALFA-tagged LZTR1 and the indicated components, including E3 ligase Cullin 3 (without or with NEDD8 required for ARIH1 binding), ARIH1, and the E2 conjugating enzyme Ubch7. Note LZTR1 polyubiquitination.

To identify additional substrates of the CRL3^{LZTR1} complex, we have also adapted different mass spectrometry approaches in our lab. For instance, our preliminary screening in *Lztr1* wild type and knockout mouse fibroblasts had revealed that the only substrates in this lineage are RIT1 and MRAS; other proteins enriched in the *Lztr1* knockout cells were not confirmed to be substrates of the CRL3^{LZTR1} complex. In Schwann cells, we decided to use the HEI-193 schwannoma cell line, which was kindly provided by Dr. Giovanini. This *NF2* deleted cell line, was isolated from a vestibular schwannoma and we used CRISPR/Cas9 to genetically delete LZTR1. In this cell line, we found that response to growth factor stimulation was increased, a phenotype that was likely linked to RIT1 and MRAS upregulation, consistent

with our previous data. To assess potential substrates upregulated in this cell line, we undertook two complementary mass spectrometry approaches, the first using Data-Independent acquisition and the second termed NeuCode. The advantage of the NeuCode technology is that it allows to metabolically label cells (in contrast to labelling after protein extraction and digestion like TMT) similar to SILAC, but with the ability to multiplex up to 4 protein samples. Therefore, we have undertaken labelling of two HEI-193 WT biological replicates and two HEI-193 LZTR1 KO biological replicates. Our analysis revealed that in LZTR1 KO cells, there was a significant enrichment of RIT1, as we had previously described. In addition, we found that other proteins like CBL-B and CEP350 were significantly enriched in these cells. However, when validating these targets in cells, we could not find evidence that these were truly substrates, except for RIT1. These results, together with work from other projects in which we have analyzed substrates of $CRL3^{LZTR1}$ complex in different cell lines and lineages, has revealed that RIT1 is probably a universal substrate of the complex and we do not anticipate that other substrates would play an important role. Other laboratories have published potential substrates of the $CRL3^{LZTR1}$ complex, such as EGFR or AXL, but we did not find evidence of upregulation of these proteins in our knockout cells. Therefore, we have accomplished all the experiments proposed in Aim 2, although we only found robust evidence of RIT1 being a substrate.

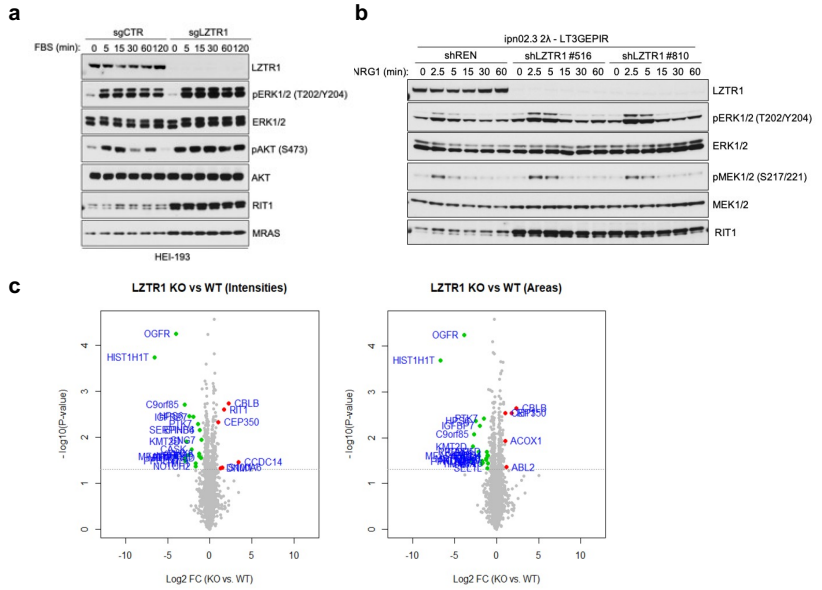

Figure 2. RIT1 is the main substrate of the $CRL3^{LZTR1}$ complex in Schwann and schwannoma cells
(a) Generation of LZTR1 knockout cells in the schwannoma cell line HEI-193. In these cells, we observe high levels of RIT1 and MRAS and exacerbation of MAPK and PI3K pathways in response to FBS stimulation. **(b)** Similarly, the immortalized human Schwann cell line ipn02.3 in which LZTR1 has been knocked down using RNAi, shows increase in RIT1 levels and increased MAPK response to NRG1 stimulation. (c) Volcano plot of HEI-193 cells LZTR1 WT or KO analyzed using NeuCode mass spectrometry show RIT1 upregulation in the knockout cells. Other proteins are enriched, but could not be confirmed by immunoblot

In our original proposal, we hypothesized that deletion of LZTR1 and NF2 by CRISPR/Cas9 editing in primary Schwann cells isolated form our conditional *Lztr1*^{flox} mice, would promote their transformation and grow *in vivo* in nude mice. Our attempts to obtain primary Schwann cells from our Lztr1/Cas9 conditional mice have not succeeded, after trying different published protocols. The low yield and purity of these cells from mice was a well-documented limitation of the approach and alternative options were presented as part of the proposal. Therefore, we decided to freeze this mouse line and undertake an alternative approach by deletion of both *Lztr1* and *Nf2* in the Schwann cells, using the Mpz-Cre driver line, which targets Cre recombinase under the control of the Myelin Protein Zero promoter. However, because these animal experiments are not covered under our project proposal and ACURO protocol, these experiments will be undertaken in the future with funding from other sources. Alternatively, we have developed a robust and efficient experimental framework to genetically edit the immortalized human Schwann cell line ipn02.3 using transient transfection of Cas9 and sgRNA targeting any gene. Using this approach, we now are able to knockout any gene in this cell line, which is highly relevant for the research related to NF and schwannomatosis. Using our protocol, we generated several single-cell derived clones and pools of cells in which *LZTR1*, *NF2* or both genes were deleted. Similar to LZTR1-related schwannomatosis, our double knockout cells lack expression of *LZTR1* and *NF2*. The cells with different genotypes have been validated by immunoblot and lack the expression of the indicated proteins.

What opportunities for training and professional development has the project provided?

There have been many opportunities for training and development during the grant period, both for the PI and for the different people that have participated in the work. For instance, the award funded some of the work of the PI as a postdoctoral fellow and through the transition to an independent investigator. The support of the DOD NFRP was very important to facilitate this transition and allow that the project is completed. Both as a postdoctoral fellow and as an assistant Professor, Dr. Castel has been able to supervise different postdocs and technicians. In agreement with the scientific program officer and after the transfer of the award to NYU, a proportion of the funds were used to cover the salary of a lab manager that has been undertaking some of these experiments and learning the techniques necessary for the work carried out in the lab. Dr. Castel has supervised her experimental work and helped finalize the experiments from the proposal. In addition, the PI has been able to put preliminary data that resulted in a career enhancement award from the NCI Developmental and Hyperactive Ras Tumor (DHART) SPORE, which has allowed the PI to meet many investigators from the Neurofibromatosis field. The data obtained through both this program and the SPORE Career Enhancement Award will now be used to apply to the DOD NFRP New Investigator Award, so that the PI can continue developing projects in the context of neurofibromatosis research. Moreover, one of the postdocs in the lab (Dr. Stephanie Mo) mentored by Dr. Castel was able to put a competitive proposal for the Early Investigator Award of the DOD CDMRP Neurofibromatosis Research Program, that was recently funded. In addition, Dr. Castel has now established collaborations with Drs. Kaleb Yohay and Devorah Segal at the Neurofibromatosis Center of NYU Langone. As part of the PI's commitment to neurofibromatosis, a second postdoc has been encouraged to work on this field and has put together a proposal for the Tumor's Children Foundation Young Investigator Award. Both PI and postdocs in the lab had the opportunity to attend the 2023 NF conference to present their work and establish new collaboration with labs in the field.

How were the results disseminated to communities of interest?

Most of the results that have resulted from this project have been presented in two important conferences that bring together NF and schwannomatosis scientists, physicians, and patients. First, the PI presented in the 2023 NF Conference as an invited speaker, as well as a poster presented by a postdoc in the lab. Second, the PI presented at the 8th International RASopathies conference as an invited speaker. In addition, some of the publications that have been recently published in the lab have included work related to this application.

What do you plan to do during the next reporting period to accomplish the goals?

Nothing to report.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?

During the duration of the project, our laboratory has made significant progress in the understanding of LZTR1 function and mechanisms. The impact in the field of schwannomatosis is important, because LZTR1 loss is found in at least half of the cases diagnosed with this disorder. Given that LZTR1 and its function were discovered recently, our project was aimed at studying the role of LZTR1 in Schwann cells. For this purpose, we have developed novel cellular models to study the function of LZTR1 in this cell lineage. This has resulted in a new protocol in our laboratory that allows the efficient and rapid gene editing of the immortalized Schwann cell line ipn02.3 using CRISPR/Cas9. In addition, using this reliable protocol, we have now been able to generate genotypes that mirror the genetic alterations found in LZTR1-related

schwannomatosis patients. Using these cell models, we have been able to analyze the changes observed in cells upon deletion of LZTR1, as well as the combination with NF2 loss. Our biochemical experiments also revealed that LZTR1, when part of a complex with the ubiquitin ligase 3 ($CRL3^{LZTR1}$), mostly targets RIT1 and MRAS as a substrate, and no other Schwann cell-specific substrates. We have also identified ARIH1 as a protein that associates with the $CRL3^{LZTR1}$ complex and have studied the function of this protein in the formation of the ubiquitin chains in its substrates.

What was the impact on other disciplines?

LZTR1 mutations can also be found in the RASopathy Noonan syndrome and somatically in some tumors, including brain tumors like glioblastoma that are often associated with NF. Our results can be extended to these conditions and provides novel mechanistic insights into the function of this disease-associated gene.

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

The main problems that we have encountered during the project related to the generation of primary Schwann cells from our mouse model that would have allowed to perform CRISPR/Cas9 gene editing in those cells and assess tumorigenesis. However, as previously reported in our reports, we found that most protocols for isolation of Schwann cells from mouse are not reliable and yield low numbers of cells.

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

To overcome this problem, we decided to not pursue this approach and instead developed a CRISPR/Cas9-based experimental framework to be able to generate these cells in the human cell line ipn02.3 2λ. In addition, we now know that alternative approaches to model schwannomatosis in the mouse can be done using this cell line and this will be part of future projects not funded through this award.

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.

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

Castel, P. 2022. Defective protein degradation in genetic disorders. Biochim Biophys Acta Mol Basis Dis. 1868(5):166366.

Cuevas-Navarro, A., Rodriguez-Muñoz, L., Grego-Bessa, J., Cheng, A., Rauen, K.A., Urisman, A., McCormick, F., Jimenez, G., Castel, P#. 2022. Cross-species analysis of LZTR1 loss-of-function mutants demonstrates dependency to RIT1 orthologs. Elife. 11:e76495. doi: 10.7554/eLife.76495. #Corresponding author.

Chen, S., Vedula, R.S., Cuevas-Navarro, A., Lu, B., Hogg, S.J., Wang, E., Benbarche, S., Knorr, K., Kim, W.J., Stanley, R.F., Cho, H., Erickson, C., Singer, M., Cui, D., Tittley, S., Durham, B.H., Pavletich, T.S., Fiala, E., Walsh, M.F., Inoue, D., Monette, S., Taylor, J., Rosen, N., McCormick, F., Lindsley, R.C., Castel, P#., Abdel-Wahab#, O. 2022. Impaired proteolysis of non-canonical RAS proteins drives clonal hematopoietic transformation. Cancer Discov. doi: 10.1158/2159-8290.CD-21-1631. #Corresponding author.

Cuevas-Navarro, A., Wagner, M., Van, R., Swain, M., Allison, M.R., Cheng, A., Messing, S., Simanshu, D.K., Sale, M.J., McCormick, F., Stephen, A.G#., Castel, P#. 2022. Ras-dependent RAF-MAPK hyperactivation by pathogenic RIT1 is a therapeutic target in Noonan syndrome-associated cardiac hypertrophy. Science Advances. doi: 10.1126/sciadv.adf4766 #Corresponding author.

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

Mo, S., Castel, P. 2023. The non-canonical RAS/MAPK pathway and the RASopathies. Book: The RASopathies. Editorial: Springer. In press.

Other publications, conference papers and presentations.

Castel, P. Molecular mechanisms and potential therapeutic strategies for RIT1-driven Noonan syndrome. Invited speaker and session chair at RASopathies Network 8th International Conference. July 2023.

Castel, P. Molecular Mechanisms of LZTR1 in RASopathies and Schwannomatosis. Invited speaker and session chair at CTF 2023 Neurofibromatosis Conference. June 2023.

- **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?

Pau Castel (PI): 3 months
Martha Vega (technician): 9 months
Frank McCormick (mentor/supervisor): as requested
Katherine Rauen (mentor/supervisor): as requested
Anatoly Urisman (collaborator): as requested
Monte Winslow (collaborator): as requested

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

What other organizations were involved as partners?

University of California San Francisco
NYU Grossman School of Medicine

8. SPECIAL REPORTING REQUIREMENTS

N/A

9. APPENDICES:

Nothing to report