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TITLE: Role of RNA-Exosome in PRC2- and NF1-Mutant Malignant Peripheral Nerve Sheath Tumors

PRINCIPAL INVESTIGATOR: Dr. Kunal Rai

CONTRACTING ORGANIZATION: University of Texas, M D Anderson Cancer Center
Houston, TX

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Dr. Kunal Rai

E-Mail:

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13. SUPPLEMENTARY NOTES

14. ABSTRACT:Malignant peripheral nerve sheath tumors (MPNSTs) are highly aggressive malignancies with a significant propensity for local recurrence and metastatic spread. Earlier studies that have characterized the genetic landscape of MPNST have shown that more than 70% of the cases have inactivation of polycomb repressor complex 2 (PRC2) components (SUZ12 and EED loss) in addition to NF1 loss. The rationale for the proposed research is that how loss of H3K27me3-based silencing in PRC2-mutant MPNST tissues leads to increased RNA synthesis from generally r epressed regions that needs to be resolved with enhanced activity of RNA exosome components. The objective of this project is to characterize the dependency of PRC2-mutant and NF1-mutant MPNSTs on RNA-exosome components and determine how depletion of RNA-exosomes leads to accumulation of these toxic RNAs and DNA damage signaling eventually causing cell death and whether this process is aided by mistargeted EZH2 in the absence of functional PRC2 complex. This proposed study will uncover novel biological insights into how RNA transcription and surveillance processes become aberrant in NF-1 and PRC2-mutant MPNSTs. This work will also determine whether inhibiting RNA exosome could be a target in cancer therapeutics.

15. SUBJECT TERMS

NONE LISTED

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1. INTRODUCTION:

Malignant peripheral nerve sheath tumors (MPNSTs) are highly aggressive malignancies with a significant propensity for local recurrence and metastatic spread. Earlier studies that have characterized the genetic landscape of MPNST have shown that more than 70% of the cases have inactivation of polycomb repressor complex 2 (PRC2) components (SUZ12 and EED loss) in addition to NF1 loss. The *rationale* for the proposed research is that the loss of H3K27me3-based silencing in PRC2-mutant MPNST tissues leads to increased RNA synthesis from generally repressed regions that needs to be resolved with enhanced activity of RNA exosome components. The *objective* of this project is to characterize the dependency of PRC2-mutant and NF1-mutant MPNSTs on RNA-exosome components and determine how depletion of RNA-exosomes leads to accumulation of these toxic RNAs and DNA damage signaling eventually causing cell death and whether this process is aided by mistargeted EZH2 in the absence of functional PRC2 complex. This proposed study will uncover novel biological insights into how RNA transcription and surveillance processes become aberrant in NF-1 and PRC2-mutant MPNSTs. This work will also determine whether inhibiting RNA exosome could be a target in cancer therapeutics.

2. KEYWORDS:

MPNST, PRC2, RNA exosome, R loop, PRO-seq

ACCOMPLISHMENTS:

Goals and Accomplishments:

Goals and major activities for Year 2	Percentage of completion & Status
Aim 1: To characterize the dependence of NF1-mutant MPNST cells on RNA exosome complex.	
Subaim 1.1. *To determine if depletion of RNA exosomes confers dependency to NF1-mutant MPNSTs in PRC2-dependent manner.*	***50% completed*** *– RNA exosome components – DIS3 depleted in mouse MPNST cells and effect on proliferation rate determined. SUZ12 overexpressing (OE) generated from two more MPNST cell lines – T265 and MPNST3813E*
Subaim 1.2. *To determine the clinical significance of RNA exosome subunits in NF1- mutant MPNSTs and correlation with Suz12 and EED loss.*	***40% completed –*** *Expression of RNA exosome and rixosome components determined in MPNST cell lines. Optimization of TMA staining conditions for DIS3 done using MPNST PDXs.*
Aim 2. To identify the RNA-species that are RNA-exosomes substrates specifically in NF1- and PRC2- mut MPNSTs.	
Subaim 2.1. *To determine the status of PROMPTs and enhancer RNAs in PRC2-mutant MPNSTs in comparison to PRC2-WT MPNST cells and tissues.*	***70% completed*** *– RNA-Seq and PRO-Seq performed in PRC2 WT and mut MPNST cells and data has been analyzed. PRO-Seq has been performed in the DIS3 KO cells generated from S462 and MPNST724 cells.*
Subaim 2.2. *To determine if RNA-DNA hybrids are increased in PRC2- and NF1-mutant MPNSTs in RNA-exosome dependent fashion.*	***70% completed*** *– Immunofluorescence experiments for RNA-DNA hybrids performed in S462 and MPNST724 as well as EXOSC3/EXOSC7/DIS3 KO cells generated from these lines. DRIP seq performed for R loop mapping in PRC2 mut and WT MPNST cell lines.*
Subaim 2.3. *To assess the contribution of RNA exosome in increased DNA damage signaling in PRC2- and NF1-mutant MPNSTs.*	***30% completed*** *- γ-H2AX and RAD51 staining was performed in MPNST cell lines. Co-staining with RNA-DNA hybrid antibody needs to be optimized and performed.*
Aim 3: To determine if mistargeted EZH2 helps activate RNA-exosome complex through	

SKIV2L2.	
Subaim 3.1. *To determine if EZH2 binds to and methylates SKIV2L2 in PRC2- and NF1-mutant MPNST cells.*	***20% completed*** *– Interaction between SKIV2L2 and EZH2 in S462 and MPNST007 have been determined.*
Subaim 3.2. *To validate the dependency of PRC2- and NF1-mutant MPNST cells on SKIV2L2*	***30% completed*** *– SKIV2L2 KO generated from S462, ST88, MPNST724 and MPNST007 cells and effect on proliferation determined.*
Subaim 3.3. *To determine if RNA-exosomes are dysfunctional in the absence of EZH2 in PRC2- and NF1-mutant MPNSTs.*	***0% completed***

Aim 1: To characterize the dependence of NF1-mutant MPNST cells on RNA exosome complex.

Apart from RNA exosome complex, another RNA-degradation complex known as RNA rixosomes contributes to the silencing of many polycomb targets in human cells. RNA rixosomes play a major role in ribosomal RNA processing and ribosome biogenesis. To characterize the dependence of MPNST cells on various subunits of RNA exosome (EXOSC3, EXOSC7, and DIS3) and rixosome (NOL9, LAS1L, and WDR18) complexes, we determined the expression levels of these components between PRC2 mut and WT MPNST cells as shown in Figure 1A. Of these components tested, higher levels of DIS3 was observed in PRC2 mut cells when compared to WT cells. This was subsequently corroborated by immunofluorescence (IF) staining demonstrating increased DIS3 expression in PRC2 mut MPNST PDX tissues as shown in Figure 2.

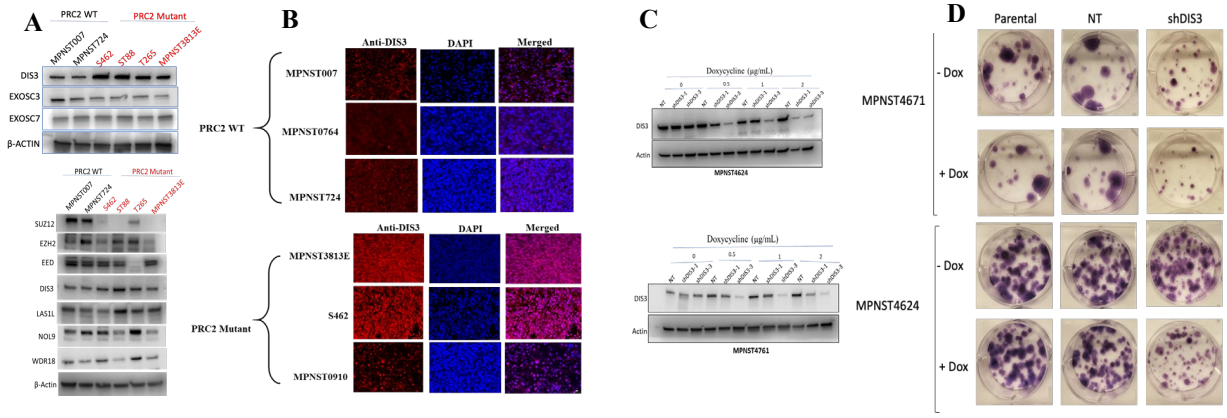

Figure 1: Validation of dependency of PRC2 mut MPNST on RNA exosome components. (A) Immunoblots showing expression levels of RNA exosome and rixosome components in MPNST cells. (B) IF for DIS3 expression in PRC2 mut and WT MPNST PDX tissues. (C) Immunoblots showing downregulation of DIS3 in mouse MPNST cells after induction of DIS3 KD with doxycycline concentrations (0.5. 1 and 2 μg/ml). (D) Clonogenic assays showing effect of DIS3 KD in mouse MPNST cells

To determine the *in vivo* dependency of MPNST cells on DIS3 we first generated inducible DIS3 knockdown cells from two mouse MPNST cell lines (MPNST4624 and MPNST4761). These two cells were generated in our lab from MPNST tumors that developed in B6;129-Trp53^{tm1Tyj} Nf1^{tm1Tyj} Suz12$^{Gt(Betageo)1Khe}$/KcichJ mice. Knockdown (KD) of DIS3 was determined by immunoblotting after treating the cells with different concentrations of doxycycline (DOX) for 7 days (Figure 3). No changes in the expression levels of DIS3 was observed in non-targeting (NT) cells after DOX treatment. However, DOX treatment for 7 days led to significant reduction of DIS3 levels in cells transduced with shRNAs for DIS3. Clonogenic assays showed decreased proliferation of DIS3 KD cells up on DOX induction compared to NT cells (Figure 4).

Aim 2: To identify the RNA-species that are RNA-exosomes substrates specifically in NF1- and PRC2- mut MPNSTs. We performed Precision nuclear run-on sequencing (PRO-Seq) to capture the nascent transcripts throughout the genome. PRO-Seq is a well-recognized nascent transcriptome method [31], which provides a genome-wide snapshot of the position, amount, and orientation of RNA polymerases engaged in transcription. PRO-Seq is perhaps the most sensitive tool to study the transcriptional status of a cell at a given temporal point and is especially well-suited for noncoding RNA transcription. PRO-Seq was performed on two PRC2 WT (MPNST724 and MPNST007) and mut (ST88 and S642) cell lines. To determine these aberrant RNA populations that accumulate in PRC2 mut cells, we performed PRO-Seq to map RNAPII active sites in PRC2 mut and WT cells. We identified the

differential transcripts between PRC2 mut and WT cells by DESeq2 and found that increase in nascent transcription is associated with early stemness genes in PRC2 mutant MPNST as shown in Figure 5.

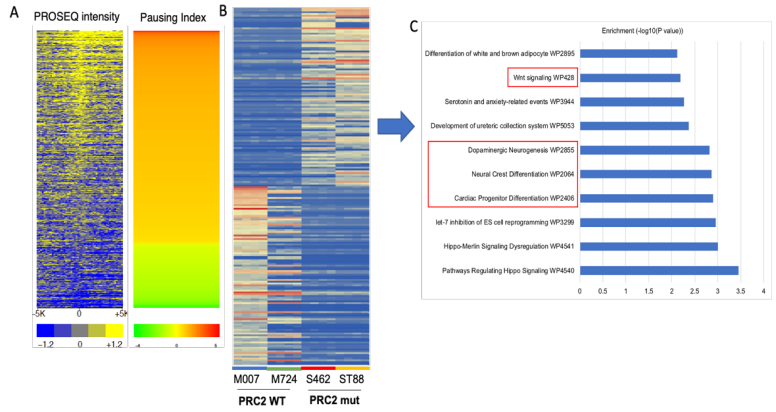

Figure 5: (A) Heatmap of PRO-Seq peak intensity and RNA Pol II pausing index of differential regions between PRC2 mut and WT MPNST cells (B) Differential peaks identified by DESeq2 (C) Pathway analysis of peaks differentially upregulated

Integration of PRO-Seq data with ChIP seq data for H3K27Ac showed that an increase in nascent transcription near the transcription start site (TSS) is associated with H3K27Ac gain in PRC2 mut MPNST as shown in Figure 7. Analysis of PRO-Seq data through the NRSA pipeline identified differentially transcribed enhancers in PRC2 WT (36 enhancers) and mut (228 enhancers) cells as shown

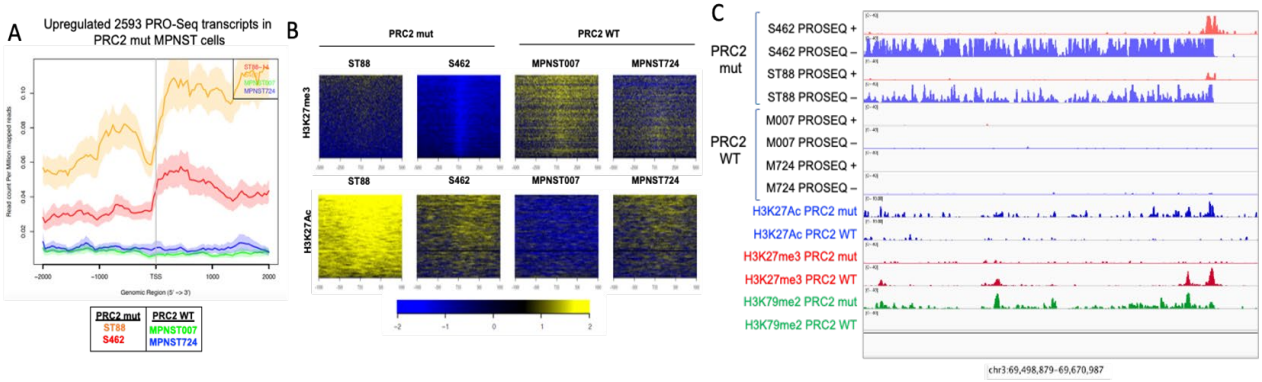

Figure 6: PRC2-mutant cells harbor higher levels of enhancer transcription which is associated with H3K27ac gain. (A-B) Increase in nascent transcription identified by PRO-Seq near TSS is associated with H3K27ac gain and H3K27me loss in PRC2-mutant MPNST. (C) IGV demonstrating differentially transcribed long enhancer

in Figure 6.

To further confirm the role of the PRC2 complex in regulating the nascent transcription levels, SUZ12 was overexpressed in PRC2 mut cell lines (S462, MPNST3813E, T265) after cloning SUZ12 cDNA to PLX303 as shown in Figure 7. Results for the overexpression of SUZ12 in S462 was included in last year's report, whereas the results for the remaining two cell lines are reported here. These cells will be further used perform PRO-Seq experiments. PRO-Seq was also performed in DIS3 KO cells generated from PRC2 mut S462 cells and preliminary analysis indicated that DIS3 KO leads to increase in levels of nascent transcripts from PRC2 target regions in MPNST when integrated

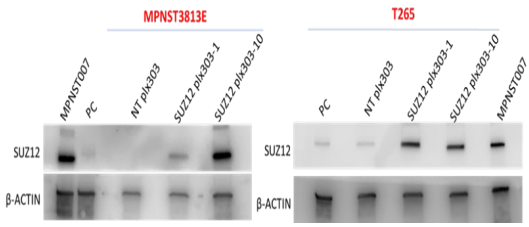

Figure 7: Immunoblots showing overexpression of SUZ12 in PRC2 mut MPNST cells.

with ChIPseq data for H3K27me3 in MPNST cells as shown in Figure 8.

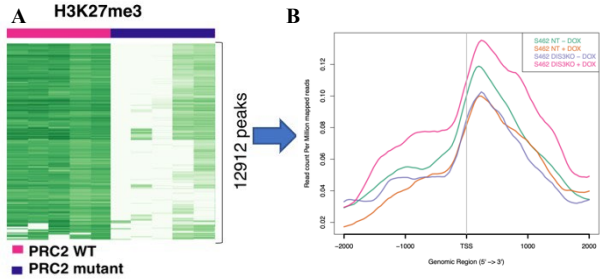

Figure 8. RNA exosome loss leads to increased nascent transcripts at PRC2 binding sites. (A) Heatmap showing loss of H3k27me3 on polycomb binding sites in PRC2-WT versus -mut MPNST cells. (B) average intensity plot for RNA transcript read counts on these polycomb binding sites in S462 cells (PRC2-mutant) that have DIS3 KO upon dox induction. Duplicates are shown in same color line.

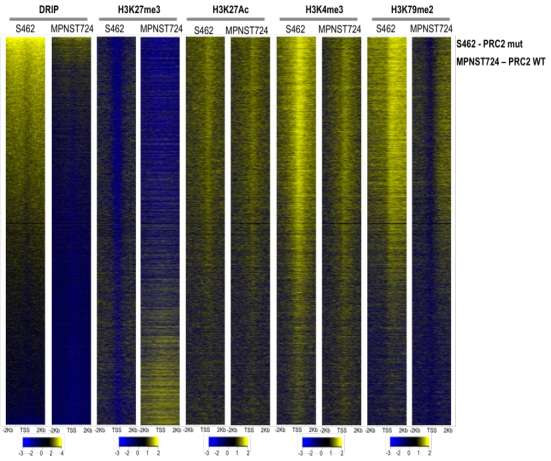

Figure 9: Heatmaps showing integration of DRIP seq peaks with H3K27me3, H3K27Ac, H3K4me3 and H3K79me2 peaks

Since we observed increased RNA-DNA hybrids in PRC2 mut MPNSTs as reported last year, we further performed DNA-RNA hybrid immunoprecipitation (DRIP-Seq) for global R -loop mapping in PRC2 mut (S462) and WT (MPNST724). We found that epigenetically poised regions for transcription in PRC2 mut MPNSTs cells accumulate R loop structures as shown in Figure 9.

To determine whether the expressions of DIS3 (RNA exosome component) and LAS1L (RNA rixosome component) correlates with loss of H3K27me3 and whether it is increased in MPNSTs compared to neurofibroma, we decided to perform immunohistochemical analysis in a TMA consisting of 162 MPNST and 92 Neurofibroma samples. As part of optimization of the experimental conditions for TMA, staining was performed in PRC2 mut and WT MPNST PDXs as shown in Figure 10.

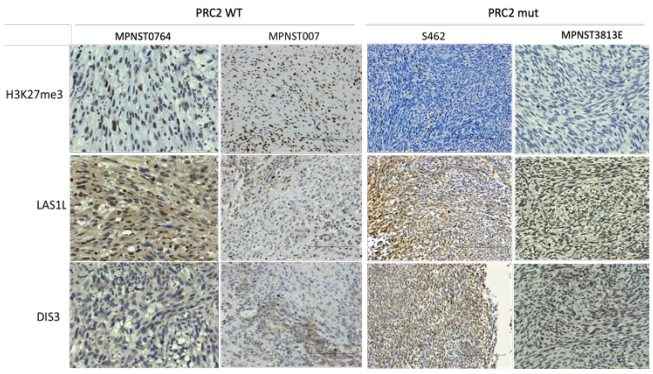

Figure 10: IHC analysis for LAS1L and DIS3 in PRC2 WT and mut MPNST

In aim 2.3 we wanted to assess the contribution of RNA exosome in increased DNA damage signaling in PRC2- and NF1-mutant MPNSTs. Phosphorylation of the histone H2AX at serine 139 is an early event of DNA damage response,

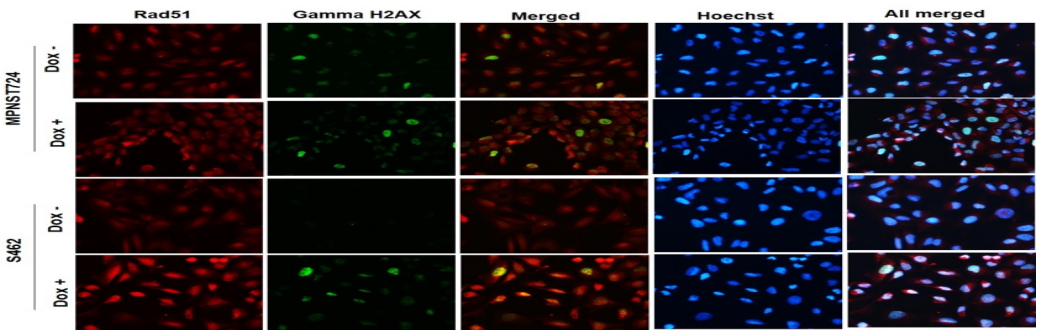

Figure. 11: IF for the detection of foci formed by DNA damage sensor and repair proteins: PRC2-mutant MPNST cells (S462) demonstrate increased Rad51 and γ-H2AX foci following exposure to Dox when compared to PRC2-WT MPNST cells (MPNST724)

while Rad51 is a marker for successful homologous recombination and a vital component of the DNA repair pathway. Our preliminary data demonstrates a significant increase in Rad51 and γ-H2AX expression following treatment with Doxorubicin in PRC2-mutant cells as shown in Figure 11.

Aim 3: To determine if mistargeted EZH2 helps activate RNA-exosome complex through SKIV2L2.

Nothing to report this year.

In the year 1, we had noted SKIV2L2 dependency and interaction in both PRC2 WT and Mutant setting suggesting SKIV2L2 dependent RNA exosome targeting may not be specific to PRC2-mutants which is in contradiction to our original hypothesis. Hence, we are planning on changing the hypothesis of this aim based on our results with RNA rixosome which is known to be targeted by PRC2 complex based on a recent study (Zhou et al Nature 2022). This is also the reason for the additional work on testing dependency of PRC2-mutant NF1-mutant MPNST cells on RNA rixosome in Aim 1.

Key outcomes and achievements in Year 2:

- Characterization of the dependency of PRC2 mut MPNST on RNA exosome and the determination of the expression levels of RNA exosome and rixosome components between PRC2 mut and WT MPNSTs.
- Characterization of nascent transcription and accumulations of PROMPTs and eRNAs in the absence of PRC2 complex in MPNST by PRO-Seq
- R loop mapping in MPNST.

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

Post-doctoral fellow: The project is supporting Dr. Biji Chatterjee, a postdoctoral fellow. She is gaining further experience in molecular and epigenomic techniques and their application to MPNST biology. She is gaining extensive knowledge in how aberrations in RNA processing impact MPNST and how it can be targeted for therapeutic purposes. Dr. Rai meets with Dr. Chatterjee on a weekly basis, reviews the data and generates hypothesis, thus training her toward independence.

The subaward to the University of Texas, Health Science Center Houston is supporting a two graduate student: Lanxin Bei and Ruoyu Wang are gaining further experience in GRO-Seq and knowledge in how this approach can be applied in cancer systems. Dr. Li meets with both on a weekly basis, reviews the data and generates hypothesis, and mentors on building the project.

How were the results disseminated to communities of interest?

Nothing to report

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

We plan to perform experiments as listed in the original SoW.

SubAim 1.1: We plan to perform in vivo experiments, at least for the tumorigenesis model and if possible for the metastasis model, in the presence or absence of RNA exosome components as proposed on SoW.

Sub Aim 1.2: We will perform the proposed TMA analysis for the RNA exosome subunits.

SubAim 2.1: We will perform Pro-Seq in the human MPNST cell lines with SUZ12 overexpression/knockdown.

Sub Aim 2.2: We will perform experiments testing the status of RNA-DNA hybrid in PRC2 WT and mutant cells upon knockdown of RNA-exosome subunits as proposed.

Sub Aim 2.3: We will perform experiments testing the status of DNA damage in PRC2 WT and mutant cells upon knockdown of RNA-exosome subunits as proposed.

Sub Aim 3.1: We will perform biochemical experiments to determine if EZH2 methylates SKIV2L2.

Sub Aim 3.2 and 3.3: We will perform PRO-Seq experiments upon knockout of EZH2 and

4. **IMPACT: What was the impact on the development of the principal discipline(s) of the project?**

- Our findings have validated that RNA-exosome is a dependency in PRC2 mutant NF1-mutant MPNST. This has identified RNA-exosome as a novel target in MPNST.
- Our findings are also beginning to implicate a new RNA surveillance complex, RNA rixosome, in NF-1 mediated MPNST genesis.
- Our findings stress the need for in-depth study of the field of RNA surveillance issues in the NF1- mutant MPNSTs.

What was the impact on other disciplines?

- Our findings suggest deeper need of investigation on RNA exosomes when considering PRC2 biology during normal development or other diseases.
- Our findings also implicate future study in the subgroup of patients with defective PRC2 complex across all cancer types. This set of patients could be targeted using RNA exosome inhibitors.
- Our data also suggest need for development of potent inhibitors of RNA exosome complex.

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

In the year 1, we had noted SKIV2L2 dependency and interaction in both PRC2 WT and Mutant setting suggesting SKIV2L2 dependent RNA exosome targeting may not be specific to PRC2-mutants which is in contradiction to our original hypothesis. Hence, we are planning on changing the hypothesis of this aim based on our results with RNA rixosome which is known to be targeted by PRC2 complex based on a recent study (Zhou et al Nature 2022). This is also the reason for the additional work on testing dependency of PRC2-mutant NF1-mutant MPNST cells on RNA rixosome in Aim 1.

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

Nothing to report.

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

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.

- Kochat V, Raman AT, Landers SM, Tang M, Schulz J, Terranova C, Landry JP, Bhalla AD, Beird HC, Wu CC, Jiang Y, Mao X, Lazcano R, Gite S, Ingram DR, Yi M, Zhang J, Keung EZ, Scally CP, Roland CL, Hunt KK, Feig BW, Futreal PA, Hwu P, Wang WL, Lazar AJ, Slopis JM, Wilson-Robles H, Wiener DJ, McCutcheon IE, Wustefeld-Janssens B, **Rai K*** and Torres KE* (2021). Enhancer reprogramming in PRC2-deficient malignant peripheral nerve sheath tumors induces a targetable de-differentiated state. **Acta Neuropathol.** 2021 Sep;142(3):565-590. doi: 10.1007/s00401-021-02341-z. *Co-corresponding authors.

- Bhalla AD, Landers SM, Singh AK, Landry JP, Yeagley MG, Myerson GSB, Delgado-Baez CB, Dunnand S, Nguyen T, Ma X, Bolshakov S, Menegaz BA, Lamhamedi-Cherradi SE, Mao X, Song X, Lazar AJ, McCutcheon IE, Slopis JM, Ludwig JA, Lev DC, **Rai K**, Torres KE. Experimental models of undifferentiated pleomorphic sarcoma and malignant peripheral nerve sheath tumor. **Lab Invest.** 2022 Jun;102(6):658-666. doi: 10.1038/s41374-022-00734-6. Epub 2022 Feb 28.PMID: 35228656

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

Nothing to report

Other publications, conference papers and presentations.

Nothing to report

- **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:	*Rai Kunal*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	*https://orcid.org/0000-0003-2321-6894*
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Rai is a PI of the project.*
Funding Support:	*In addition to this DoD, Dr. Rai is supported by multiple NIH, CPRIT and private foundation grants*

Name:	*Lanxin Bei*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*NA*
Nearest person month worked:	*1*
Contribution to Project:	*Lanxin has contributed to the work of GRO-Seq in this project.*
Funding Support:	*In addition to this DoD, Feng Xiong is also supported by Wenbo Li's CPRIT grant.)*

Name:	*Ruoyu Wang*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*NA*
Nearest person month worked:	*1*
Contribution to Project:	*Lanxin has contributed to the work of GRO-Seq in this project.*
Funding Support:	*In addition to this DoD, Feng Xiong is also supported by Wenbo Li's CPRIT grant.)*

Name:	*Khalida Wani*
Project Role:	*Research Scientist*
Researcher Identifier (e.g. ORCID ID):	*NA*
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Wani worked on the pathology aspects of the grant.*
Funding Support:	*In addition to this DoD, Dr. Wani is also supported by other DoD and NIH grants, and institutional accounts.*

Name:	*Sharon Landers*
Project Role:	*Research Scientist,*
Researcher Identifier (e.g. ORCID ID):	*NA*
Nearest person month worked:	*1.2*
Contribution to Project:	*Dr. Landers has contributed to the DNA damaging work of the project*
Funding Support:	*In addition to this DoD, Dr. Landers is support by an institutional program.*

Name:	*Veena Kochat*
Project Role:	*Post-doctoral fellow,*
Researcher Identifier (e.g. ORCID ID):	*NA*
Nearest person month worked:	*2*
Contribution to Project:	*Dr. Kochat She will contributed to the functional experiments pertaining to RNA exosome components in MPNST cells.*
Funding Support:	*In addition to this DoD, Dr. Kochat is support by an institutional program.*

Name:	*Biji Chatterjee*
Project Role:	*Post-doctoral fellow,*
Researcher Identifier (e.g. ORCID ID):	*NA*
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Chatterjee contributed to the functional experiments pertaining to RNA exosome components in MPNST cells.*
Funding Support:	*In addition to this DoD, Dr. Chatterjee is supported by Dr. Torres DoD grant .*

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

Dr. Rai completed the following grant:

Title: Fasting Protects Small Intestinal Stem Cells from Lethal DNA Damage: Mechanistic Insight and Preclinical Translation
Time Commitment: 0.12 calendar months (1%)
Funding Agency: NIH/NCI
Agency Contact and Address: Pat. G. Prasanna, Email: patajeprasanna@mail.nih.gov
Performance Period: 03/14/2017-02/28/2022
Funding Level:
Role: Co-Investigator
Goals: The major goal of this project is to test the hypothesis that the metabolic changes associated with fasting lead to epigenetic changes in SI stem cells, which in turn, leads to expression of genes whose protein products protect SI stem cells from lethal DNA damage. The clinical applications of our findings will be evaluated in a mouse model of pancreatic cancer.

Specific Aims:
1. Determine if βOHB and HDAC inhibitors prevent GI toxicity and protect SI stem cells from high dose chemotherapy and radiotherapy.
2. Explore the feasibility of dose-escalated treatment of pancreatic cancer using fasting.
3. Identify epigenetic and transcriptional changes that occur in small intestinal stem cells of mice.

Dr. Rai was awarded the following grant:
Title: Mechanisms and therapies focused on epigenomic alterations in therapy-resistant prostate cancers
Time Commitment:

Year (YYYY)	Person Months (##.##)
1. 2022	1.8 calendar
2. 2023	1.8 calendar
3. 2024	1.8 calendar

Funding Agency: CPRIT
Agency Contact and Address: Info desk, Phone: , Email: cprit@cprit.texas.gov **Performance Period:** 3/01/2022 – 02/28/2025
Funding Level:
Role: PI
Goals: We hypothesize that inhibition of H3K4me1-marked enhancers along with use of enzalutamide may be an effective therapeutic strategy for therapy-resistant prostate cancers..
Specific Aims:
1. To identify functional enhancer elements, their gene targets and downstream mechanism in specific epigenetic subtypes (EPIC).
2. To determine the molecular mechanism of EPIC-specific patterns of aberrant enhancer activation
3. To determine the efficacy of BRD inhibition alone or in combination with pathway inhibitors for specific CMS subtypes

Overlap: None

What other organizations were involved as partners?

Organization Name: University of Texas Health Science Center Houston
Location of Organization: Houston, TX
Partner's contribution to the project (identify one or more)

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES: