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

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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.

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

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 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:** *Provide a brief list of keywords (limit to 20 words).*

MPNST, PRC2, RNA exosome, R loop, PROseq

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

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

To characterize the dependence of MPNST cells on various subunits of RNA exosome complex we created exosome depleted MPNST cells using doxycycline inducible sgRNAs for EXOSC3, EXOSC7, and DIS3 in S462 (PRC2 mut) and MPNST724 (PRC2 WT) cell lines. Knockout (KO) of EXOSC3, EXOSC7 and DIS3 was determined by treating the cells with doxycycline (DOX) - 5µg/ml for 7 days and expression of these proteins were determined by immunoblotting. No changes in the expression levels of EXOSC3, EXOCS7 and DIS3 was observed in non-targeting (NT) cells after DOX treatment. For cells transduced with sgRNAs for EXOSC3, EXOCS7 and DIS3, DOX treatment for 7 days led to significant reduction levels in these proteins as shown in **Figure 1A**.

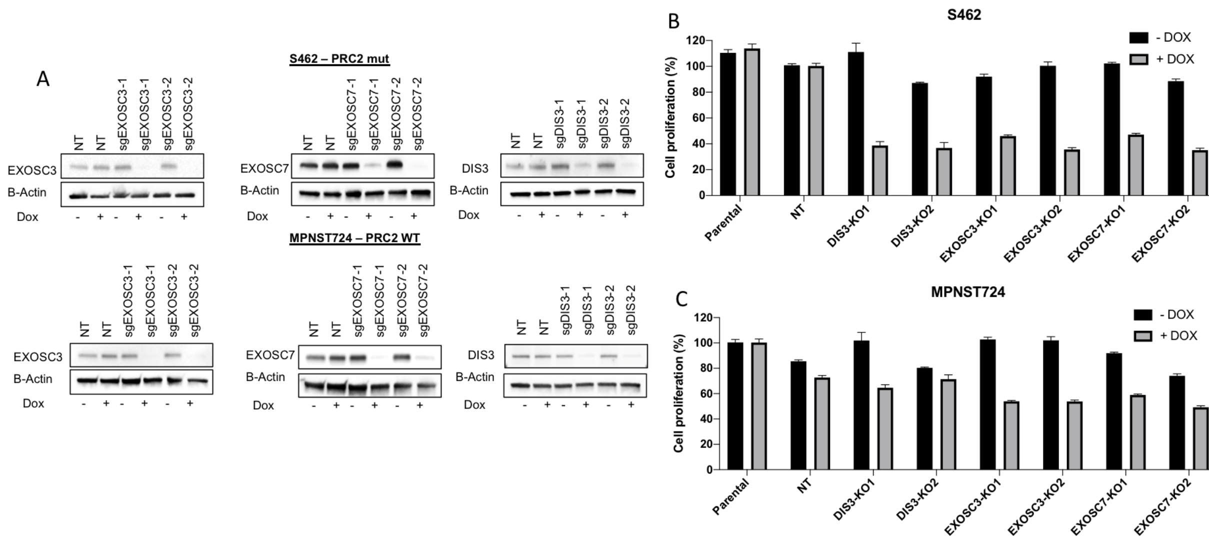

Figure 1: Depletion of RNA exosomes confers dependency to MPNSTs in a PRC2 dependent manner – (A) Immunoblots showing DOX inducible-depletion in levels of EXOSC3, EXOSC7 and DIS3 in MPNST cells after DOX treatment (5µg/ml) for 7 days. **(B)** MTS assay results showing reduced proliferation in S462 cells after DOX inducible-depletion of EXOSC3, EXOSC7 and DIS3. **(C)** MTS assay results showing reduced proliferation in MPNST724 cells after DOX inducible-depletion of EXOSC3, EXOSC7 and DIS3.

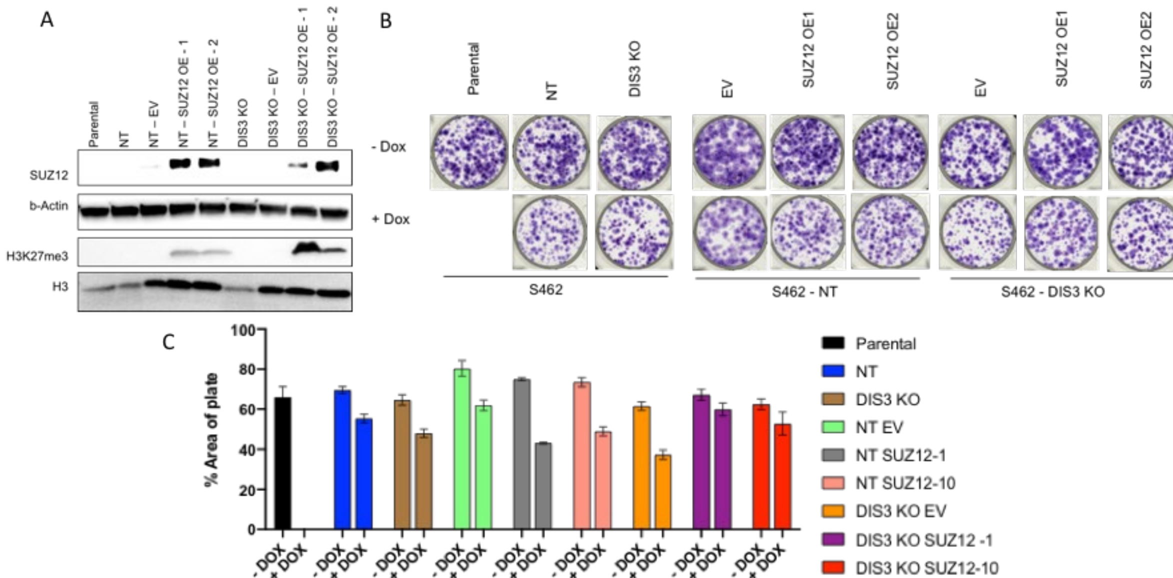

Figure 2: Role of PRC2 in dependency of MPNST cells on RNA-exosome complex : (A) Immunoblots showing over-expression of SUZ12 and reconstitution of H3K27me3 in S462 DIS3 KO and NT cells (PRC2 mut). **(B)** Clonogenic potential of S462 DISKO and NT cells overexpressing empty vector (PLX303) and SUZ12 was performed after plating 500 cells and treating with DOX for 10 days. **(C)** Bar graph showing colony formation and expansion as % area occupied on the plate.

After one week of DOX treatment cells were plated for MTS assay and proliferation was measured after 5 days. Depletion of exosome components in S462 (PRC2 mut) showed a more pronounced effect on cell proliferation compared to exosome depleted MPNST724 cells as shown in **Figure 1B and 1C**. To further determine the role of PRC2 in dependency of MPNST cells on RNA-exosome complex, we generated SUZ12 overexpressing (OE) cells from S462 NT and S462 DIS3

KO cells. SUZ12 cDNA was cloned into PLX303 and cells transduced and blasticidin resistant positive cells were selected. SUZ12 OE and reconstitution on H3K27me3 was determined by immunoblotting as shown in **Figure 2A**. Clonogenic assays using these cells showed that S462 DIS3 KO transduced with empty vector showed less proliferation on DOX induction compared to S462 DIS3 KO cells overexpressing SUZ12 as shown in **Figures 2B and 2C**. This suggested that DIS3 KO had a much more pronounced effect on PRC2 mutant cells than on cells with intact PRC2.

Aim 2: To identify the RNA-species that are RNA-exosomes substrates specifically in NF1- and PRC2- mut MPNSTs.

We wanted to determine whether PRC2 loss is associated with accumulation of aberrant RNA transcripts generated as result of pervasive transcription. To determine these aberrant RNA populations, we performed Precision nuclear run-on sequencing (PRO-Seq) to map RNAPII active sites. The RNA strands extracted were purified by streptavidin pull down, adaptor ligated and reverse transcribed and sequenced to obtain strand-specific data. PRO-Seq experiment showed higher normalized read density of promoter-proximal regions in PRC2 mutant MPNST compared to PRC2 WT MPNST as shown in **Figure 3A**. Pausing index which is defined as ratio of normalized PRO-seq reads in a 1-kb window centered on the TSS to that in the rest of the gene with normalization for gene length was also higher in PRC2 mut cells compared to WT cells as shown in **Figure 3B**. Strand specific analysis of PRO-Seq data showed higher levels of transcription at PRC2 target regions that were determined from H3K27me3 ChIP seq data for MPNST cell lines as shown in **Figure 3C**.

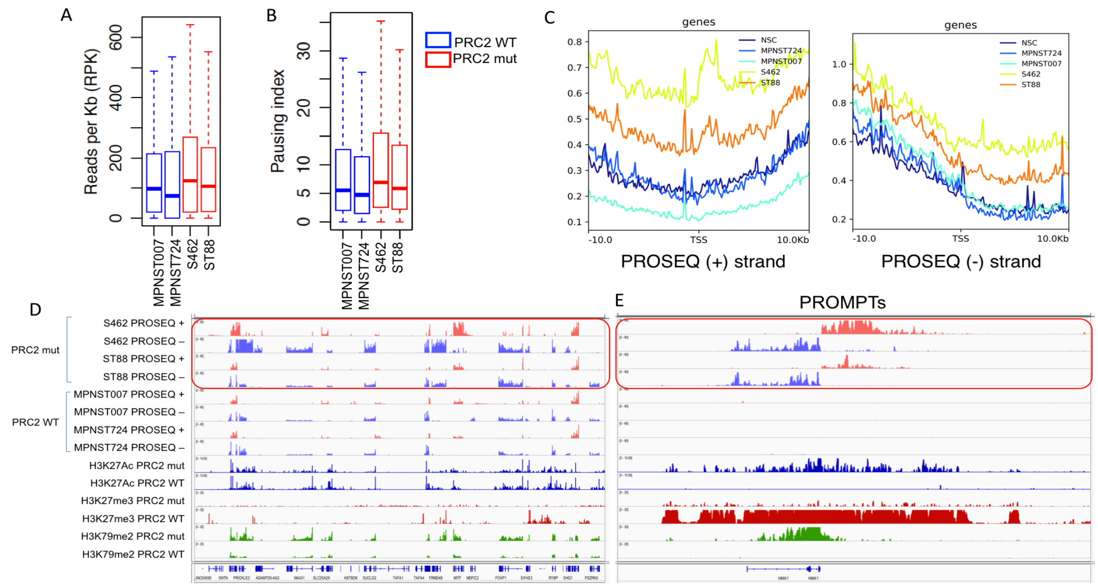

Figure 3: PRC2 mutant cells have higher levels of nascent transcription – (A) Increased normalized read density at regions proximal to promoter **(B)** Pausing Index of RNAP is higher in PRC2 mut cells compared to WT cells. **(C)** Transcription levels at PRC2 target regions determined by stand specific analysis in PRO-Seq. **(D)** Increased transcription from large chunks of chromosome 3. **(E)** Generation of unique PROMPTs in PRC2 mutant cells compared to WT cells.

Further analysis of PRO-Seq data showed higher levels of transcription from large chunks of chromosomal regions in PRC2 mut cells as shown in **Figure 3D**. Higher transcription in these PRC2 target regions was also corroborated by increased H3K79me2 levels and decreased H3K27me3 levels. Higher transcription from promoter regions also suggested the possibility of increased generation and accumulation of promoter upstream transcripts (PROMPTs) that are divergently transcribed in the reverse direction of mRNAs from promoters and an example is shown in **Figure 3E**. We also determined whether the

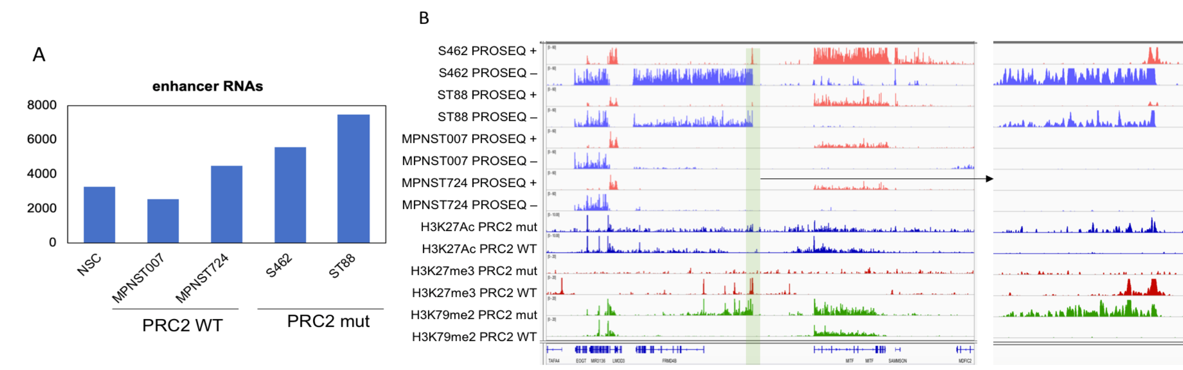

Figure 4: PRC2 mut MPNST cells exhibit higher levels of enhancer transcription – (A) Comparison of eRNAs among MPNST cell lines and NSCs. **(B)** IGV browser view of unique eRNA transcription from non-coding regions of genome. Transcripts were identified by PRO-Seq and the enrichment of histone marks – H3K27Ac, H3K27me3 and H3K79me2 were determined by ChIP-Seq.

increased transcription is also associated with higher transcription of enhancer RNAs (eRNAs). For this we overlapped the PRO-Seq peaks with H3K27Ac peaks to define transcribed eRNAs and found that their levels were higher in PRC2 mut cells compared to WT cells as shown in **Figure 4A** and several unique eRNA transcripts were observed in PRC2 mutant cells as they gained enhancers (H3K27Ac) at PRC2 target sites **(Figure 4B)**.

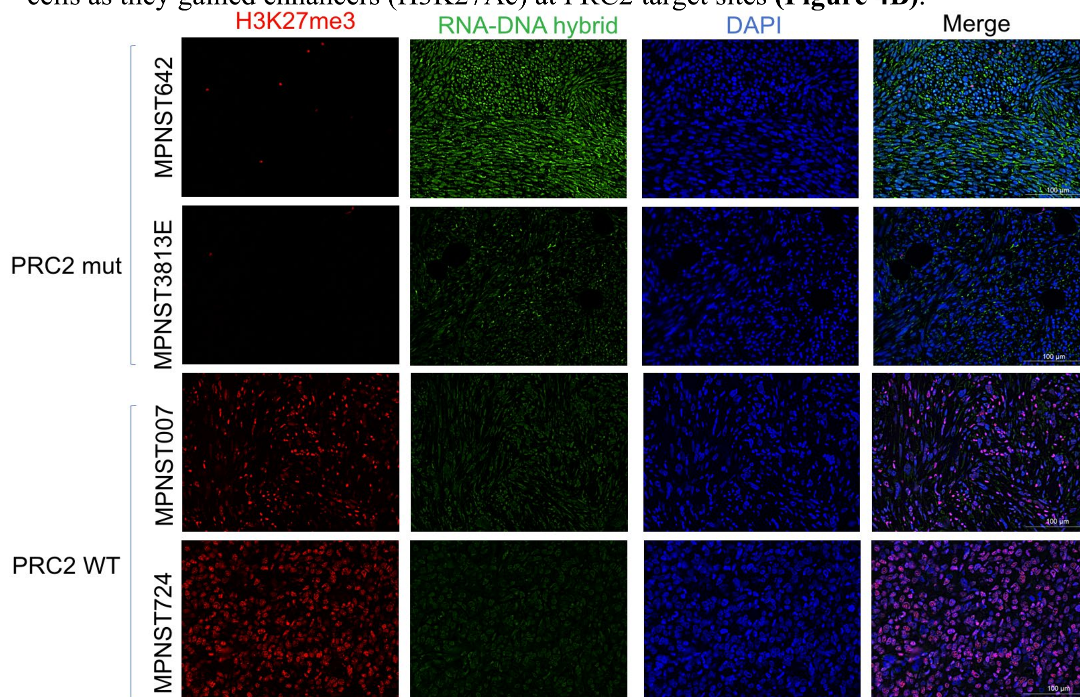

Figure 5: Higher R loop formation in PRC2 mut MPNST PDXs – Staining was performed in PRC2 mut PDXs – MPNST3813E and MPNST642 and PRC2 WT PDXs – MPNST007 and MPNST724 for H3K27me3 (AF594/red) and RNA-DNA hybrids (AF488/green). Images shown are of 20X magnification.

We hypothesized that increase RNA transcription in PRC2 mut MPNSTs leads to higher accumulation of RNA-DNA hybrids that form R loop structures which can result in DNA damage if these remain unresolved. RNA exosome is very critical to resolving these R loop structures and hence PRC2 mut MPNST cells can be more dependent on this function of RNA exosome. Immunofluorescent staining for H3K27me3 and RNA-DNA hybrids in MPNST PDXs showed that RNA-DNA hybrids were higher in PRC2 mut PDXs – MPNST3813E and MPNST642 compared to PRC2 WT PDXs – MPNST007 and MPNST724 as shown in **Figure 5**. Consistent with these results we also found higher levels of γ-H2Ax suggesting increased DNA damage in PRC2 mut PDXs compared to PRC2 WT PDXs as shown in **Figure 6**.

To determine if RNA-DNA hybrids are increased in MPNSTs in RNA-exosome depleted MPNST cells, we performed immunofluorescence experiments to detect RNA-DNA hybrids in S462 (PRC2 mut) and MPNST724 (PRC2 WT) cells which are individually depleted for EXOSC3, EXOSC7 and DIS3 as shown in **Figure 7A**. R loop structures in nuclei were quantified and was found to be higher in EXOSC3 and DIS3 KO cells generated from S462 cells compared to those from MPNST724 (**Figure 7B**).

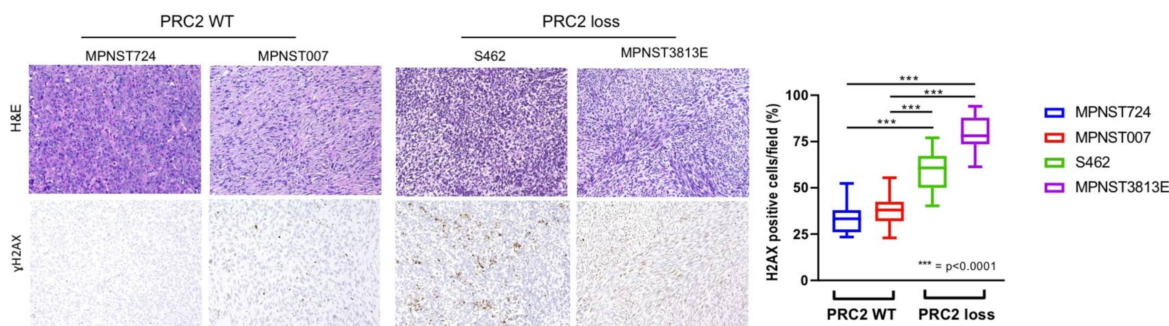

Figure 6: γ-H2Ax as a marker for DNA damage in MPNST – H and E images of MPNST PDXs. γ-H2Ax staining in PRC2 WT and mut PDXs. Images acquired in 20X magnification. γ-H2Ax positive cells were quantified and compared between PRC2 WT and mut PDXs. *** denotes P <

To determine whether RNA-DNA hybrid formation correlates with loss of H3K27me3 and whether it is increased in MPNSTs compared to neurofibroma, we performed RNA-DNA hybrid staining in a tissue microarray that has been previously created with pathologically confirmed neurofibroma (n=26)/MPNST (n=92) samples. Comparison of R-loop^{high}(%) cells showed that it was higher in MPNST samples compared to neurofibroma as shown in

Figure 8A. Among the MPNST samples, primary and recurrent MPNST samples had significantly higher R-loop^{high}(%) compared to neurofibroma and metastatic MPNST tissues as shown in **Figure 8B**. No significant correlation was found between H3K27me3 positivity and R loop positivity in the TMA analysis. RNA-DNA hybrid staining would be performed in another TMA that we have to further increase the sample number to improve statistical analysis.

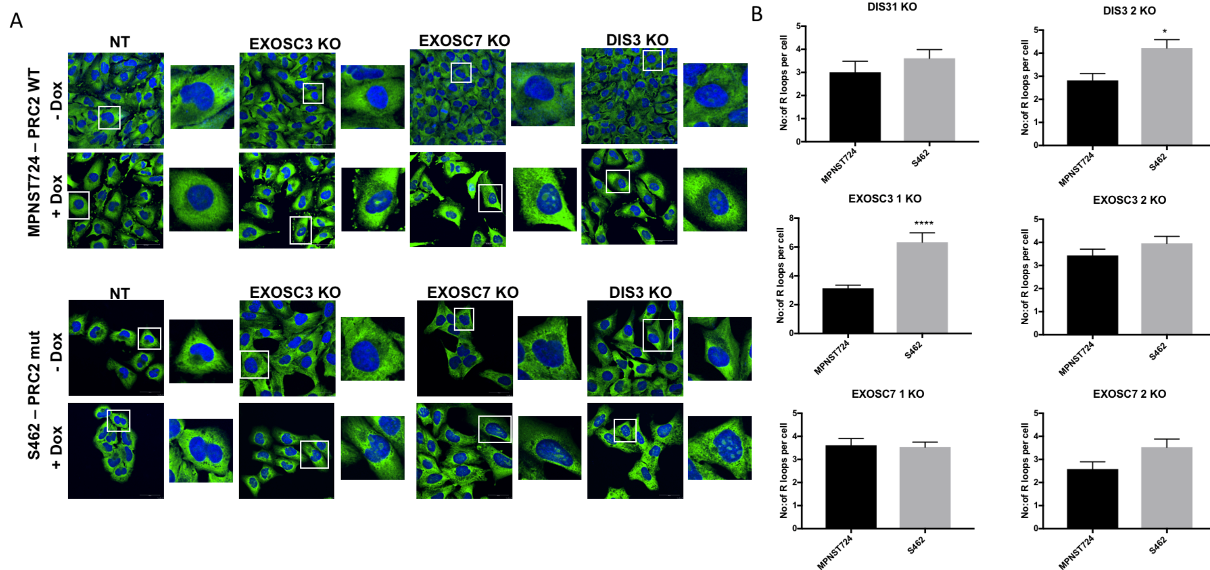

Figure 7 – Exosome depletion leads to accumulation of RNA-DNA hybrids in MPNST cells – (A) Immunofluorescence staining for RNA-DNA hybrid (AF488/green) in EXOCS3, EXOSC7 and DIS3 KO cells generated from S462 (PRC2 mut) and MPNST724 (PRC2 WT) cells. High magnification confocal images were acquired at 63X magnification. **(B)** Quantification of R loops in the nuclei of cells from 5 different fields was performed. Comparison of R loop structures between exosome depleted cells generated was done. from S462 and MPNST762. * denotes P < 0.05. **** denotes P < 0.0001.

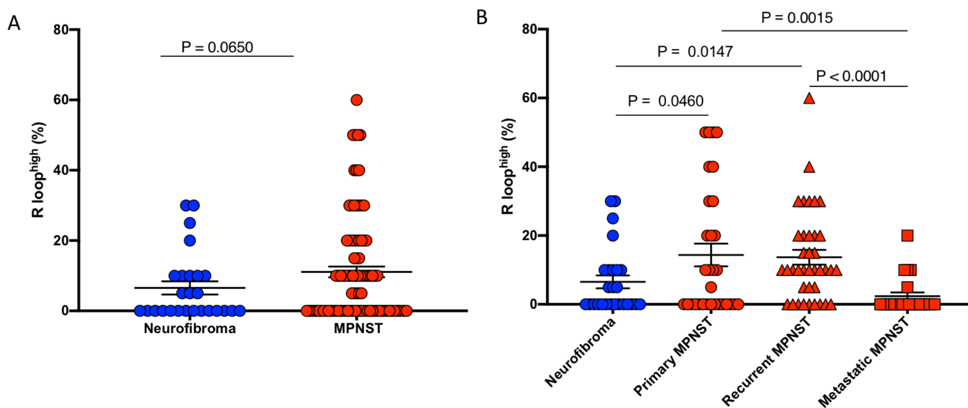

Figure 8: R loop analysis in neurofibroma and MPNST samples – (A) Comparison R-loop^{high}(%) between neurofibroma and MPNST samples. **(B)** Comparison R-loop^{high}(%) between neurofibroma and MPNST – primary, recurrent and metastatic samples.

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

EZH2 has been shown to have non-canonical interacting partners in several cancers. In the absence of functional PRC2 complex EZH2 has been found to interact with other proteins in a previous mass spectrometric analysis performed in MPNST cell lines. On among the interactions was with SKIV2L2 which is a component of NEXT complex that activates RNA exosomes. This interaction was further validated by co-immunoprecipitation experiments. EZH2 was found to interact with SKIV2L2 and DIS3 of RNA exosome complex in S462 (PRC2 mut) MPNST cells, while the interaction was absent in MPNST007 (PRC2 WT) cells as shown in **Figure 9.**

Figure 9: EZH2 interacts with SKIV2L2 and DIS3 in PRC2 mut MPNST cells – Immunoprecipitation was performed for EZH2 and then interaction with SKIV2L2 and DIS3 was determined by immunoblotting in S462 and MPNST 007 cells.

To validate the dependency of MPNST cells on SKIV2L2, we generated DOX inducible SKIV2L2 KO cells from PRC2 mut cells (S462 and ST88) and PRC2 WT cells (MPNST007 and MPNST724). Cell were treated with DOX (2, 5, 10 µg/ml) for 5 days which led to loss of SKIV2L2 expression as shown in **Figure 10A**. SKIV2L2 KO led to reduced clonogenic potential in PRC2 mut and PRC2 WT cells validating the MPNST dependency on SKIV2L2 as shown in **Figure 10B and 10C**. PROseq experiments will be performed in these cells to check the status of eRNA transcripts or nascent transcripts.
We have also generated EZH2 KOs from these MPNST cell lines. PROSeq experiments will be conducted in these cells too to determine whether EZH2 loss can inhibit the RNA exosome activation by SKIV2L2.

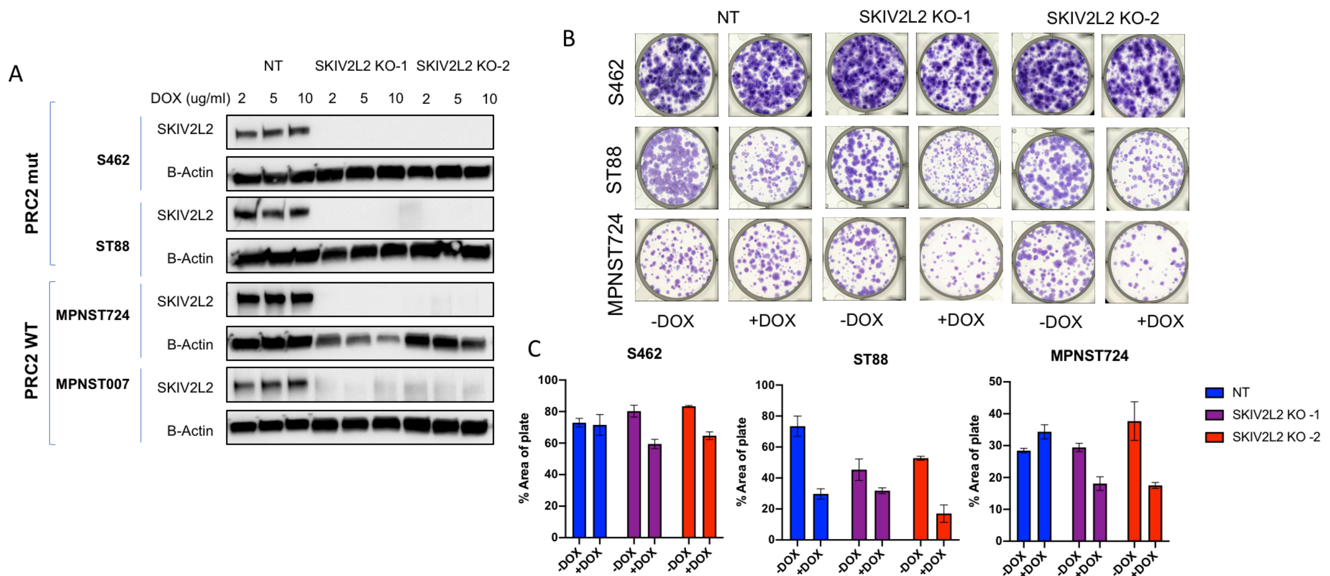

Figure 10: Dependency of MPNST cells on SKIV2L2 – (A) Immunoblots showing loss of SKIV2L2 expressions in DOX inducible SKIV2L2 KO cells generated from S462, ST88, MPNST007 and MPNST724 after treatment for 5 days. **(B)** Clonogenicity assay performed in DOX inducible SKIV2L2 KO cells treated with doxycycline for 10 days after plating 500 cells/plate. **(C)** Clonogenic potential of SKIV2L2 KO cells was quantified showing that SKIV2L2 reduces cell proliferation in MPNST after DOX treatment.

Key outcomes and achievements in Year 1:

- Characterization of dependency of PRC2 mut MPNST on RNA exosome and SKIV2L2.
- Characterization of nascent transcription and accumulations of PROMPTs and eRNAs in the absence of PRC2 complex in MPNST by PROSeq
- Determining the role of RNA exosome in resolving R loop structures in MPNST.

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Goals and major activities for Year 1	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.*	***30% completed*** *– RNA exosome components – EXOSC3, EXOSC7 and DIS3 depleted in S462 (PRC2 mut) and MPNST724 (PRC2 WT) MPNSTs and S462 cells overexpressing (OE) SUZ12 and effect on proliferation rate determined.*
Subaim 1.2. *To determine the clinical significance of RNA exosome subunits in NF1- mutant MPNSTs and correlation with Suz12 and EED loss.*	***0% completed***
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.*	***50% 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, MPNST724 and S462 SUZ12 OE cells. Data analysis is pending.*
Subaim 2.2. *To determine if RNA-DNA hybrids are increased in PRC2- and NF1-mutant MPNSTs in RNA-exosome dependent fashion.*	***50% completed*** *– Immunofluorescence experiments for RNA-DNA hybrids performed in S462 and MPNST724 as well as EXOSC3/EXOSC7/DIS3 KO cells generated from these lines.* *Tissue microarray for RNA-DNA hybrids was performed in one MPNST TMA slide. This needs to be performed for another TMA slide too.*
Subaim 2.3. *To assess the contribution of RNA*	***10% completed*** *- γ-H2AX staining was*

exosome in increased DNA damage signaling in PRC2- and NF1-mutant MPNSTs.	*performed in MPNST tissues and 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***

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Post-doctoral fellow: The project is supporting Dr. Veena Kochat, a postdoctoral fellow.
She is gaining further experience in epigenomic approaches 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. Kochat 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 postdoctoral fellow and a graduate student: Dr. Xiong Feng, a postdoctoral fellow at Dr. Li's lab. Dr. Feng is gaining further experience in GRO-Seq and knowledge in how this approach can be applied in cancer systems. Dr. Li meets with the post-doctoral fellow on a weekly basis, reviews the data and generates hypothesis, thus training Dr. Feng toward independence.

Mr. Ruoyo Wang, a graduate student at Dr. Li's lab. Dr. Feng is gaining further experience in the area of nascent RNA-seq such as GRO-Seq and some analysis in this project. Dr. Li meets with him on a weekly basis, reviews data and mentors on building the project.

How were the results disseminated to communities of interest?

If there is nothing significant to report during this reporting period, state "Nothing to Report." Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."
Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

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 tumors.

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: We will test dependency of PRC2 WT and mutant cells on SKIV2L2 in vitro experiments as outlined in the SoW.

Sub Aim 3.2 and 3.3: We will perform PRO-Seq experiments upon knockout of EZH2 and SKIV2L2 in PRC2 WT and mutant cells.

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?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

- 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 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?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an 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 patient 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?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

- Progress during the first half of the year was impacted due to Covid19 mediated lockdown. The institution was under lockdown between March end to July, followed by gradual opening with limited capacity until Sep.

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

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

- Since Sep, we put extra effort to make sufficient progress to meet with the goals of the project.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Starting September 2020, Dr. Rai received promotion from Assistant to Associate Professor, followed with salary increase of 60%. This salary increase resulted with big jump in the personnel expenses of the project, and to offset the impact we needed to reduced Dr. Rai's effort from 10% to 8%. This effort reduction doesn't have any impact on Dr. Rai's commitment to achieve all of the objectives of this project.

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

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

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:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Some data generated as part of this grant were reported in the following manuscript.

  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**, Torres KE. Enhancer reprogramming in PRC2-deficient malignant peripheral nerve sheath tumors induces a targetable de-differentiated state. Acta Neuropathologica. 2021 Jul 20. doi: 10.1007/s00401-021-02341-z. PMID: 34283254

  or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report

- **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

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: *Xiong Feng*
Project Role: *Postdoctoral Fellow*
Researcher Identifier (e.g. ORCID ID): *NA*
Nearest person month worked: *1.2*
Contribution to Project: *Dr. Xiong 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. Xiong 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. Landers is support by an institutional program.*

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

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Dr. Rai completed the following grants:

Title: Epigenetic effectors of responses to immune checkpoint blockade agents
Funding Agency: Melanoma Research Alliance
Performance Period: 05/15/2017-05/14/2021
Funding Level: $225,000
Role: PI
Goals: The major goal of this project is to define epigenetic basis of resistance to immune checkpoint blockade therapy.

Title: Epigenetic Effectors of Tumor Response to Immune Checkpoint Inhibitors
Funding Agency: Department of Defense
Performance Period: 08/01/2017-07/31/2021
Funding Level: $576,000
Role: PI
Goals: The major goal of this project is to determine functional chromatin state changes during resistance to immune checkpoint agents, test combination strategies in pre-clinical models as guided by current knowledge of chromatin state changes associated with resistant to anti-PD1 therapy.

Title: Co-operative roles for YAP1 and UCHL5 in cancer progression and therapy
Funding Agency: NIH/NCI
Performance Period: 07/16/2018-06/30/2021
Funding Level: 399,500
Role: PI
Goals: The goal of this proposal is to determine the functional outcome and mechanistic basis of cooperation between YAP1 and UCHL5 as well as assess the therapeutic potential of this observation.

Dr. Rai was awarded the following grant:

Title: Contribution of Epigenome to PRKCI-Driven High-Grade Serous Ovarian Cancer
Time Commitment: 0.6 calendar months (5%)
Funding Agency: NIH/NCI
Agency Contact and Address: Anu Sharman
Phone: 240-276-6250
Email: sharmananu@mail.nih.gov
Performance Period: 12/01/2020-11/30/2022
Funding Level: $445,500
Role: PI
Goals: The objective of this proposal is to identify such chromatin states associated with PRKCI or 3q26 amplification and P53 mutation in ovarian cancer, determine their functional nature and determine the role of PRKCI-interacting chromatin regulators in establishing tumorigenesis and chromatin states in this genetic context.
Specific Aims:

- To determine the chromatin states in PRKCI overexpressing and P53 mutant serous ovarian epithelium and tumors.
- To determine the role of SMARCA5 in PRKCI mediated phenotypes in ovarian tumorigenesis.

Overlap: None

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Dr. Lazar completed the following grants:

Title: MD Anderson Melanoma Moonshot
Funding Agency: UTMDACC
Performance Period: 09/01/2014-8/31/2020
Funding Level: $400,000
Role: co-PI
Goals: Overall objectives of this project is to focus on prevention, early detection and treatment of this most deadly and aggressive form of skin cancer.

Title: PDGFRAD842V Mutated Gastrointestinal Stromal Tumor Consortium
Funding Agency: David Foundation
Performance Period: 9/1/2017-4/31/2021
Funding Level: $254,800
Role: PI
Goals: Create an internal repository and clinical database for these tumors to provide resources to an international consortium. Will also develop a circulating DNA based test to detect initial and resistance mutations.

Dr. Li completed the following grants:

Title: Cancer Prevention and Research Institute of Texas (CPRIT) Faculty Recruitment Award-Wenbo Li
Effort:10% (PI) / 1.2 calendar months
Supporting Agency: CPRIT
Performance Period:12/01/2016-11/30/2020
Funding Amount:$2,000,000
Project Goals: Establish a laboratory with strong experimental and bioinformatics expertise to conduct research work on gene transcriptional control in breast and other cancer cells.

Dr. Li was awarded the following grants:

Title: Mechanisms underlying Enhancer RNP mediated gene regulation and genome organization
Time Commitment: 3 calendar months (25%)
Funding Agency: NIH/NIGMS
Agency Contact and Address: Ronald Adkins
Email: ronald.adkins@nih.gov
Performance Period: 09/10/2020 – 08/31/2025
Funding Level: $229,000 to Dr. Li lab / yr
Role: PI
Goals: The goal of this study is to study the roles of RNA binding proteins that locate to enhancers in transcription and genome control.
Specific Aims:

- We plan to focus on two novel eRNA- binding proteins (eRBPs) to characterize their functions in gene transcription and 3D genome interaction in mammalian cells.
- To fully characterize the biochemical basis permitting eRNA:protein interaction and their possible involvement in mediating the formation of transcriptionally-associated sub-nuclear condensates.

Overlap: None

Title: Beyond Gene Dosage: Understanding Down Syndrome via 4D Genome Organization
Time Commitment: 3 calendar months (25%)
Funding Agency: NIH/ NHLBI
Agency Contact and Address: Ronald Adkins
Email: ronald.adkins@nih.gov
Performance Period: 09/18/2020 – 08/31/2025
Funding Level: $268,053 to Dr. Li lab / yr
Role: PI
Goals: The goal of this study is to investigate the chromatin organization changes in Down syndrome patient cells and to understand their roles in deregulating gene expression or cellular phenotypes.
Specific Aims:

- In Aim-1, in multiple pairs of isogenic iPSC cells and their derived neuron/glia cells that contain disomic versus trisomy HSA21, we will conduct assays to systematically characterize their 3D genome (in situ Hi-C and PLAC-Seq), transcriptome and 1D epigenome (PRO-Seq, ATAC-Seq, and histone modification ChIP-Seq).
- In Aim-2, we focus on functionally dissecting the roles of aberrant inter-chromosomal interactions in gene deregulation.

Overlap: None

Dr. Torres completed the following grants:

Title: Characterization of EZH2's pro-oncogenic activity as a transcriptional modulator in NF1-MPNST
Supporting Agency: Department of Defense (DOD) W81XWH-17-1-0219 | NF160026
Performance Period: 08/01/2017-01/31/2021
Level of Funding: $306,194
Role: PI
Brief description of project's goal(s): The major goals of the projects are; 1) Determine the role of EZH2 as a transcriptional repressor/activator in NF1-associated MPNST, 2) Determine the mechanism by which EZH2-dependent transcriptional activation is modulated in NF1-associated MPNST and 3) Determine combination therapies that include EZH2 inhibition and offer superior anti-MPNST effects.
Overlap: None

Title: Targeting DNA damage signaling and epigenetic deregulation as a combination therapy for malignant peripheral nerve sheath tumors
Supporting Agency: Children's Tumor Foundation
Performance Period: 05/10/2019-1/31/2021
Level of Funding: $85,000
Role: PI
Brief description of project's goal(s): The major goals of the projects are; 1) Evaluate inhibition of the inhibitor BMN 673 and I-BET-764 in NF1-MPNST cell lines, 2) Perform pharmacokinetic and pharmacodynamics studies using BMN 673 and I-BET-764

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

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

- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx) should be updated and submitted with attachments.*

9. **APPENDICES:** *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*