

AWARD NUMBER: W81XWH-14-1-0428

TITLE: Exploiting Tumor-Activated Testes Proteins To Enhance Efficacy of First-Line Chemotherapeutics in NSCLC

PRINCIPAL INVESTIGATOR: Angelique Whitehurst, PhD

CONTRACTING ORGANIZATION: University of Texas Southwestern Medical Center
Dallas, TX 75390

REPORT DATE: December 2017

TYPE OF REPORT: Final

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE December 2017		2. REPORT TYPE FINAL		3. DATES COVERED 30 Sep 2014 - 29 Sep 2017	
4. TITLE AND SUBTITLE Exploiting Tumor-Activated Testes Proteins To Enhance Efficacy of First-Line Chemotherapeutics in NSCLC				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-14-1-0428	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Angelique Whitehurst, PhD E-Mail: Angelique.Whitehurst@utsouthwestern.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Texas Southwestern Medical Center 5323 Harry Hines Blvd. Dallas, TX 75390-9020				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Cancer Testis Antigens (CTAs) are a group of proteins whose expression is restricted to reproductive tissues (testis and ovaries), but frequently activated in Non Small Cell Lung Cancer (NSCLC). A number of these CTAs are essential for meiosis during spermatogenesis. Their deletion in mice leads to infertility due to an inability to repair DNA Double Strand breaks (DSB) during homologous recombination in meiosis. DSBs frequently occur in the tumorigenic environment due to environmental insults such as hypoxia and reactive oxygen species. We hypothesize that CTAs promote repair of these DSB in NSCLC and are essential for tumor cell survival. To evaluate this hypothesis, we are investigating the biochemical, cell biological and in vivo activity of CTAs in NSCLC. We have identified CTAs that are essential for DNA DB in NSCLC and which are also essential to survival of these cells in vitro. We find that the expression of meiotic CTAs appears to be a marker for the inactivation of metabolic pathways that lead to the generation of DNA-damage species. These findings are under testing in vivo. The physical interaction network of CTAs and identification of additional CTAs that may mediate sensitivity to DNA-damage inducing drugs is under investigation.					
15. SUBJECT TERMS Cancer Testis Antigen (CTA), Fanconia-Anemia (FA), DNA Damage, Genomic Instability, DNA Double Strand Break (DSB)					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	7	19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
1. Introduction.....	4
2. Keywords.....	4
3. Accomplishments.....	
.....	4-6
4. Impact.....	7
5.Changes/Problems.....	7
6. Products.....	7
7. Participants & Other Collaborating Organizations.....	7
8.Special Reporting Requirements.....	None
9. Appendices.....	None

1. **INTRODUCTION:** This proposal is focused on elucidating the function of Cancer-Testes Antigens (CTAs) in Non-Small Cell Lung Cancer. CTAs are genes whose expression is restricted to the testes but frequently reactivated in many tumor types. The purpose of this proposal is to elucidate the contribution of CTAs to the maintenance of genomic integrity at the molecular level and to examine whether CTAs modulate sensitivity to chemotherapy. To do this, a cohort of meiotic CTAs will be examined for their functional contribution to the activation of DNA damage repair pathways. These CTAs, TEX15, HORMAD1 and SYCP1 were implicated as important to NSCLC in preliminary studies. In addition, physical interactions between CTAs and components of the DNA repair machinery will be assessed. In vitro and in vivo studies will be used to assess the contribution of CTAs to tumor cell viability and chemosensitivity. A larger scale analysis of whether additional CTAs contribute to chemotherapeutic sensitivity will also be carried out.

2. **Keywords:**

- Cancer Testis Antigen (CTA)
- Fanconia-Anemia (FA)
- DNA Damage
- Genomic Instability
- DNA Double Strand Break (DSB)

3. **Accomplishments**

- **What were the major goals and objectives of the project?**

0-12 months:

- 1) To analyze the FA pathway in CTA-depleted lung cancer cells (Vaziri Lab)
- 2) Complete Lentiviral-mediated CTA overexpression in HBE cells and analyze the FA pathway. (Vaziri Lab)
- 3) Complete dose curve and transfection optimization for a panel of NSCLC cell lines. (Whitehurst Lab)
- 4) Perform sensitivity assays for TEX15, SYCP1 and HORMAD1. (Whitehurst Lab)
- 5) Obtain IACUC approval for in vivo studies. (Whitehurst Lab)
- 6) Develop shRNA stable cell lines. (Whitehurst Lab)
- 7) Initiate in vivo studies. (Whitehurst Lab)

12-24 months:

- 8) Analyze ectopically-expressed epitope-tagged CTA in lung cancer cell lines. (Vaziri Lab)
- 9) Immunopurification and proteomic analysis of CTA complexes in cancer cells. (Vaziri Lab)
- 10) Screen for CTAs contributing to DNA damage. (Whitehurst Lab)
- 11) Continue in vivo studies. (Whitehurst Lab)

For simplicity in the final report, we are reporting the results from the entire period in paragraph form. We have cited the specific tasks in () that were completed and correspond to the above list.

The overriding purpose of this proposal was to determine whether testes proteins that are reactivated in tumors can participate in DNA Damage repair pathways to support survival in tumor cells. To this end, we had preliminary data suggesting that TEX15, SYCP1 and HORMAD1 could be important in these pathways and planned an open-ended approach to identify additional regulators. The broad, siRNA-mediated screening analysis was carried out in year 2 (**Task #10**). This screen did not uncover any obvious testes protein whose depletion may sensitize to carboplatin. We retested a few outliers without success during the NCE. We think that the sensitivity of our readout assay (viability) is limited for detecting the subtle changes in growth following DNA damage. We think a replating assay would probably be more sensitive for this analysis, However, given that we are screening over 100 testes proteins, the scale that would be required would be overwhelming. To salvage this portion of the study, we then asked whether any of these testes proteins could predict in vitro sensitivity to any one of a magnitude of synthetic chemicals or natural compounds. This analysis returned an observation that tumor cells that express HORMAD1 are uniquely resistant to the complex I inhibitor, Peiricidin A (PA) (Figure 1). PA activates reactive oxygen species (ROS) in cells, which causes DNA damage. We hypothesized that the HORMAD1 subtype of NSCLC may exhibit resistance to this compound to reduce additional DNA damage.

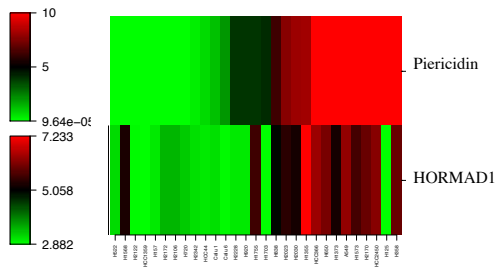


Figure 1: HORMAD1 expression correlates with Piericidin sensitivity in NSCLC.

Importantly, the Vaziri lab had revealed some indication that HORMAD1 may also be required for radiosensitivity and supporting homologous recombination (HR) (Figure 2) (Task #1, Task #3 and #4). This initial work was performed in H1229 NSCLC cells, a squamous cell line. Subsequently, in the second year of this grant, we discovered that HORMAD1, specifically in lung adenocarcinoma, predicts elevated mutational tumor burden and poor survival (Figure 3). This finding caused us to reconsider the cell type context. in which we were performing a number

of the sensitization and HR assays. We assembled a panel of lung adenocarcinoma lines and optimized transfection and sensitivity curves (Tasks #3 and #4). At this point, we also established stable knockdown cells (Task #6). With the advent of CRSPR, we harnessed this new technology to make HORMAD1 CRSPR knockout cells. We repeated the original assays and found that indeed HORMAD1

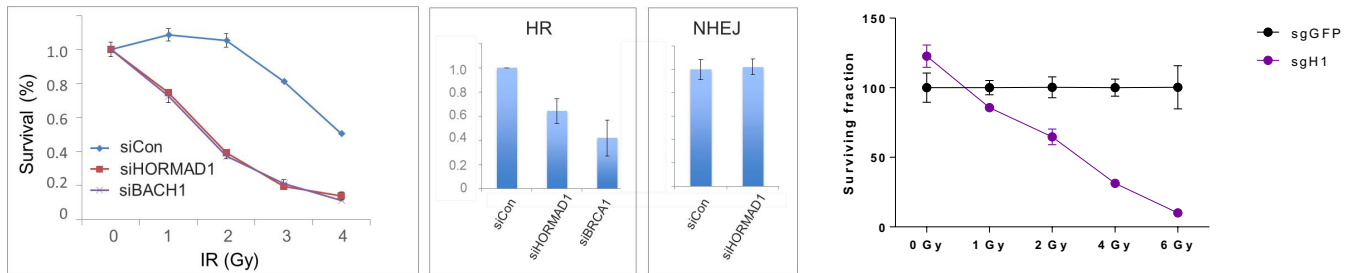
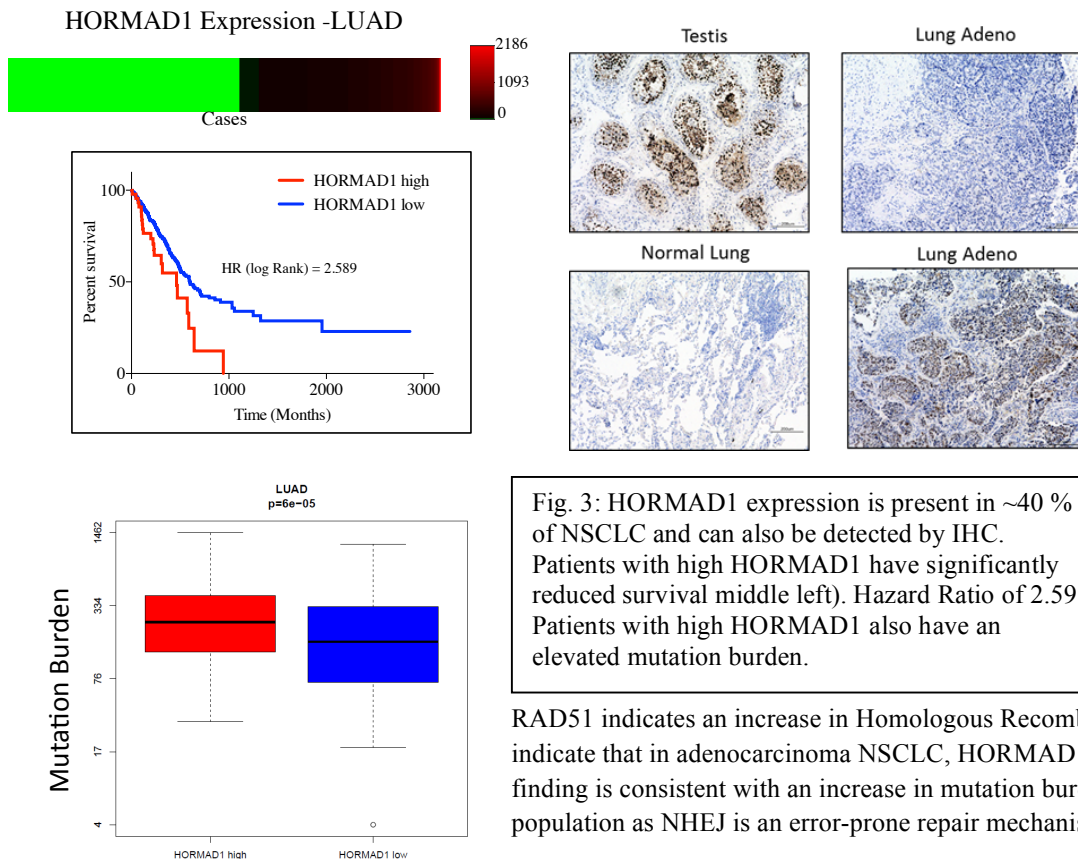


Figure 2: Radiosensitivity (left) and reduction of homologous recombination (middle) in NSCLC cells depleted of HORMAD1. Radio sensitization in NSCLC with sgHORMAD2.



depletion vastly reduced sensitivity to irradiation (Task #1, #4). In addition, we discovered that depletion of HORMAD1 led to an elevation in 53BP1 foci and a decrease in RAD51 foci, a marker of Homologous Recombination (HR) (Figure 4). The significance of these

findings is the following: reduction in 53BP1 indicated a loss of Non-Homologous End Joining Activity (NHEJ) and elevated

Fig. 3: HORMAD1 expression is present in ~40 % of NSCLC and can also be detected by IHC. Patients with high HORMAD1 have significantly reduced survival middle left). Hazard Ratio of 2.59. Patients with high HORMAD1 also have an elevated mutation burden.

RAD51 indicates an increase in Homologous Recombination. Thus, these studies indicate that in adenocarcinoma NSCLC, HORMAD1 alters HR activity. This finding is consistent with an increase in mutation burden among this patient population as NHEJ is an error-prone repair mechanism.

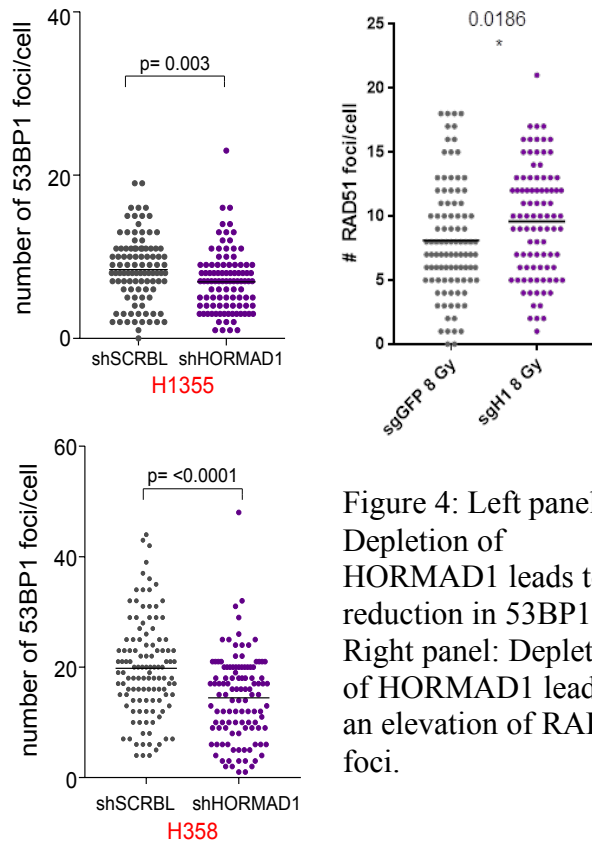


Figure 4: Left panel: Depletion of HORMAD1 leads to a reduction in 53BP1. Right panel: Depletion of HORMAD1 leads to an elevation of RAD51 foci.

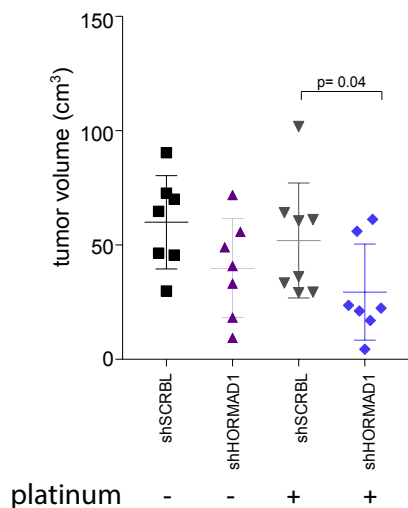


Figure 5: Results of in vivo experiments. NSCLC depleted of HORMAD1 were grown subcutaneously in mice. We observed subtle defects in tumor growth yet a significant sensitization to DNA damage

As our findings indicate that HORMAD1 is essential for radiosensitivity possibly through alteration of HR, we asked whether overexpression of HORMAD1 was sufficient to induce transformative phenotypes in normal cells (**Task #2**). In this analysis, we found no evidence of HORMAD1 driving alterations in the DNA repair machinery. We subsequently analyzed the effect of HORMAD1 overexpression in normal colonic epithelial cells with RAS, and observed no increase in soft agar formation (**Task #2**).

To begin to develop the underlying molecular mechanism for HORMAD1's function in lung cancer, we engineered a series of lung cancer cell lines with epitope tagged HORMAD1 (**Task #8**). We validated we could immunopurify these complexes. We next spent ~ 2 years (the 2nd year and the NCE period) attempting to identify protein interactors for HORMAD1. These IP-mass spectrometry studies were unsuccessful despite our efforts to change expression levels, treat cells with radiation, synchronize cells, etc (**Task #9**). One explanation is that the interactions are so transient in nature that we cannot detect them. We have taken on a number of directed interactions including testing for BRCA1, which was unsuccessful. We are continuing to work on these studies

using more complex methods to investigate HORMAD1's interactome and using add-back experiments into our CRISPR knockout cells.

To establish whether the depletion or inhibition of HORMAD1 decreases the viability or sensitive of tumor cells to DNA damage agents, we initiated in vivo studies (**Task #5 and #11**). We obtained appropriate approvals for these studies and performed a series of experiments where we monitored growth of tumors and their response to a platinum agent. Here, we found that depletion of HORMAD1 and exposure to platinum reagents, led to a subtle reduction in tumor growth (Figure 5) (**Task #11**). This finding is consistent with our in vitro findings and supports a role for HORMAD1 in DNA damage repair in tumors and possibly as a marker for resistance.

In summary, this funding has allowed us to identify a novel subtype of lung adenocarcinoma. This subtype expressed HORMAD1 and is characterized by elevated mutation burden and decreased survival time. We hypothesize that HORMAD1 is supporting resistance to platinum and radiation agents (used in first line treatments), which may contribute to the poor outcome of these patients. Our studies indicate that HORMAD1 influences homologous recombination, potentially in an antagonistic manner. Our goal going forward is to publish these initial results (2018) and continue to dissect the molecular mechanisms by which HORMAD1 functions in NSCLC.

How were the results disseminated to communities of interest?

These studies have been presented at local meetings. Preparation for publication is under way.

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

N/A

4. **Impact**
Nothing to report.

5. **CHANGES/PROBLEMS**
Nothing to report.

6. **PRODUCTS:**
Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

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).

NONE for the NCE

Has there been a change in the active other support of the PD/PIs since the last reporting period?
Nothing to Report

What other organizations were involved as partners?
University of North Carolina at Chapel Hill
Chapel Hill, North Carolina

Partner's Contribution to the project: Collaboration

8. **SPECIAL REPORTING REQUIREMENTS:** None

9. **APPENDICES:**NONE