AWARD NUMBER: W81XWH-16-1-0730

TITLE: Blocking Breast Cancer Metastasis by Targeting RNA-Binding Protein HuR

PRINCIPAL INVESTIGATOR: Danny Welch, PhD

CONTRACTING ORGANIZATION: University of Kansas Medical Center Research
Shawnee Mission, KS 66205

REPORT DATE: October 2017

TYPE OF REPORT: Annual

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	2. REPORT TYPE	3. DATES COVERED
October 2017	Annual	30 Sept 2016 - 29 Sep 2017

4. TITLE AND SUBTITLE

Blocking Breast Cancer Metastasis by Targeting RNA-Binding Protein HuR

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-16-1-0730

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Welch, D.R.

E-Mail: dwelch@kumc.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

University of Kansas Medical Center Research
4330 SHAWNEE MISSION PKWY
SHAWNEE MISSION KS 66205-2522

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

During this initial reporting period, we completed local and DOD IACUC review and initiated preliminary characterization of the metastasis model to be used for proposed pre-clinical anti-HuR drug testing. Local IACUC was renewed and we are awaiting re-review by DOD.

15. SUBJECT TERMS

HuR, Mouse model, metastasis, IACUC

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	5	19b. TELEPHONE NUMBER *(include area code)*

1. **INTRODUCTION**

Our collaborator, Liang Xu, has identified a molecule (KH-3) which blocks the RNA-binding protein, HuR. Since HuR correlates with increased aggressiveness in breast cancer, the primary objective of this proposal is to assess whether HuR (or analogs) prevent and/or treat metastasis and/or metastasis-associated phenotypes. This project will be accomplished on two campuses, where our group will be primarily responsible for the in vivo pre-clinical testing in experimental and spontaneous metastasis assays. To date, our objective has been to obtain, validate and begin verification of metastatic potential of the luciferase-labeled mammary/breast cell lines. After Dr. Xu's lab determines whether KH-3 affects EMT, identifies HuR signaling pathways blocked by KH-3, and establishes preliminary PK/PD/MTD and corresponding guidance of dosing studies, we will assess KH-3 effects on experimental and spontaneous metastasis.

2. **KEYWORDS**

a. HuR, KH-3, metastasis, anti-metastatic, RNA binding protein, epithelial-mesenchymal transition

3. **ACCOMPLISHMENTS**

a. **What were the major goals of this project?**

i. The ultimate goal of this project is to test whether a novel inhibitor of HuR (KH-3) will prevent metastasis in preclinical models of mammary cancer. In accomplishing that objective, we hope to ascertain a biological, if not molecular, mechanism of action for KH-3 in collaboration with Dr. Xu. Intermediate goals for this reporting period involved validating the origin and authenticity of the cell lines used as well as biologic behaviors.

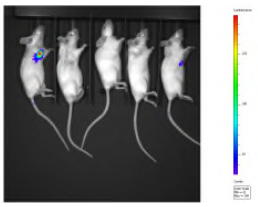
b. **What was accomplished under these goals?**

i. We have verified the species of origin for the cell lines and injected them into mice where we are awaiting determination whether the luciferase-expressing cells still metastasize. Establishment of this baseline is critical for accomplishing the in vivo and in vitro objectives/tasks outlined in the future.

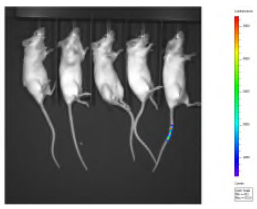
ii. Preliminary results with bioluminescence at two-weeks post-injection

(1) Experimental (i.v.) metastasis

(a) 4T1-Luc pool - 2 out of 5 mice show cells in the lungs. (Figure 1)
(b) 4T1-Luc clone #2 - No lung metastases detected
(c) 4T1-Luc clone #3 - No lung metastases detected
(d) 4T1-Luc clone #4 - No lung metastases detected



4T1Luc Pool at 2 wk post i.v. injection



4T1Luc clone 2 at 2 wk post i.v. injection

(2) Spontaneous (m.f.p.) metastasis

(a) 4T1-Luc pool - 4/5 mice have measureable (>2-3 mm) tumors; 1 mouse has a palpable tumor
(b) 4T1-Luc clone #2 - 2/5 palpable
(c) 4T1-Luc clone #3 - 1/5 measureable; 1/5 palpable
(d) 4T1-Luc clone #4 - 0/5 palpable

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

i. Ms. Vivian completed training as an Animal Resource Coordinator at KUMC.

ii. Dr. Marquez, a postdoctoral fellow in Dr. Xu's laboratory, completed work on a Susan Komen postdoctoral fellowship with Dr. Welch as a co-mentor. She now works as a Medical Science Liaison at Eli Lilly & Co.

iii. Dr. Manley is learning how to perform breast cancer work, focusing on metastasis-related assays. Her PhD training was in the area of liver disease and autophagy; so, all of the work performed as part of this proposal is new to her.

d. **How were the results disseminated to communities of interest?**

i. Not applicable | Nothing to report

e. **What do you plan to do during the next reporting period to accomplish these goals?**

i. We will closely coordinate with Dr. Xu to assure that all of the cell lines being utilized for our respective component tasks are the same ones.

ii. Dr. Xu's and Welch's labs have participated in periodic (approximately quarterly) teleconferences to exchange progress reports/notes

4. **IMPACT**

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

i. Nothing to report

b. **What was the impact on other disciplines?**
   i. Nothing to report

c. **What was the impact on technology transfer?**
   i. Nothing to report

d. **What was the impact on society beyond science and technology?**
   i. Nothing to report

5. **CHANGES/PROBLEMS**

a. **Changes in approach and reasons for change**
   i. Nothing to report

b. **Actual or anticipated problems or delays and actions or plans to resolve them**
   i. Actual
      (1) Re-review of animal use took much longer than anticipated. We are now awaiting re-review of already internally reviewed and renewed IACUC by ACURO so that the second round of cell line validations can begin.
      (2) Although not directly related to progress on this contract, Dr. Xu (PI: Site 1) experienced significant delays in obtaining IRB and IACUC approvals. Those delays extended well past the time when we already had approvals. Since much of our work depends upon cells and reagents developed in the Xu lab, some delays have been experienced. However, we do not presently believe that we will be slowed greatly.
   ii. Anticipated
      (1) The only concern is that the luciferase-expressing cell line variants which were proposed for use will not demonstrate a baseline metastatic potential which is sufficient for follow-up. This is possible if non-metastatic clones had a selective advantage. To resolve the issue, we will work with Dr. Xu to re-transduce and select metastatic variants.

c. **Changes that had a significant impact on expenditures**
   i. None

d. **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
   i. Significant changes in use or care of human subjects
      (1) Not applicable
   ii. Significant changes in use or care of vertebrate animals
      (1) None
   iii. Significant changes in use or care of biohazards and/or select agents
      (1) None

6. **PRODUCTS**

a. **Publications, conference papers and presentations**
   i. **Journal publications**
      (1) Nothing to report
   ii. **Books or other non-periodical, one-time publications**
      (1) Nothing to report
   iii. **Other publications, conference papers and presentations**
      (1) Nothing to report
   iv. **Website(s) or other Internet site(s)**
      (1) Nothing to report
   v. **Technologies or techniques**
      (1) Although not unique to this grant, we became trained and proved proficient with bioluminescence imaging, as needed for this project.
   vi. **Inventions, patent applications, and/or licenses**
      (1) Nothing to report
   vii. **Other products**
      (1) Nothing to report

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

a. What individuals have worked on the project?

Name:	**Danny R. Welch, PhD**
Project Role	PI
ORCID	0000-0002-1951-4947
Nearest person month worked	1.8

Contribution to project	Oversaw research, prepared IACUC/ACURO paperwork, injected mice
Funding Support	N/A

Name:	**Carolyn J. Vivian**
Project Role	Lab Manager
ORCID	0000-0001-9598-0109
Nearest person month worked	9
Contribution to project	Obtained cells from Dr. Xu, performed Mycoplasma testing and validated murine origin of the cells, injected and monitoring mice bearing tumor cells, assisted in preparation of IACUC/ACURO paperwork
Funding Support	N/A

Name:	**Sharon Manley, PhD**
Project Role	Postdoctoral Fellow
ORCID	0000-0002-2411-9706
Nearest person month worked	3.6
Contribution to project	Assisted Ms. Vivian with animal injections and imaging, assisted in preparation of IACUC/ACURO paperwork
Funding Support	N/A

b. **Has there been a change in the active other support of the PD/PI or senior/key personnel since the last reporting period?**
   i. No significant changes (grants renewed)
c. **What other organizations were involved as partners?**
   i. University of Kansas at Lawrence, 1200 Sunnyside Ave, Lawrence, KS 66045

8. **SPECIAL REPORTING REQUIREMENTS**
   a. **Collaborative Awards**
      i. Not Applicable
   b. **Quad Charts**
      i. Not applicable

9. **APPENDICES**