Award Number: W81XWH-21-1-0466

TITLE: Targeting an Innate Immune Signaling Pathway to Treat TNBC

PRINCIPAL INVESTIGATOR: Jason D. Weber, PhD

CONTRACTING ORGANIZATION: Washington University, St. Louis, MO

REPORT DATE: October 2022

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development 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 2022	Annual	01Sept2021-31Aug2022

4. TITLE AND SUBTITLE

Targeting an Innate Immune Signaling Pathway to Treat TNBC

5a. CONTRACT NUMBER
W81XWH-21-1-0466

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Jason D. Weber, PhD

E-Mail: jweber@wustl.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

Washington University
One Brookings Drive
Campus Box 1054
St. Louis, MO 63130-4862

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Triple negative breast cancer (TNBC) remains a challenge to clinicians, laboratory investigators, and patients due to its disproportionate number of breast cancer deaths and its lack of an established therapeutic target. Numerous studies have identified potential novel mutational gene targets in TNBC, but single-agent therapeutics have lacked substantial impact in TNBC. More recently, immune checkpoint inhibitors gained significant clinical traction in breast cancer. Unfortunately, initial promising results have been subsequently overshadowed with failures, particularly in TNBC. In other solid human tumors, the efficacy of anti-PD-L1 immune checkpoint therapies appeared to be enhanced by stimulating lymphocyte infiltration into the tumor microenvironment with type I IFNs. Here in our first year, we show the ISG signature in a panel of TNBC. Moreover, we determined that these cells are implicitly sensitive to RIG-I protein expression levels.

15. SUBJECT TERMS
ADAR, interferon signaling, RIG-I, triple negative breast cancer

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRDC
U	U	U	UU	7	**19b. TELEPHONE NUMBER** *(include area code)*

Table of Contents

Page

1. Introduction……………………………………………………….4

2. Keywords………………………………………………………….4

3. Accomplishments……….…………………………………………...4-5

4. Impact………………………...……………………………………6

5. Changes/Problems...….……………………………………………6

6. Products…………………………….……….….…………….6-7

7. Participants & Other Collaborating Organizations……………7

1. INTRODUCTION

Triple negative breast cancer (TNBC) remains a challenge to clinicians, laboratory investigators, and patients due to its disproportionate number of breast cancer deaths and its lack of an established therapeutic target. Numerous studies have identified potential novel mutational gene targets in TNBC, but single-agent therapeutics have lacked substantial impact in TNBC. More recently, immune checkpoint inhibitors gained significant clinical traction in breast cancer. Unfortunately, initial promising results have been subsequently overshadowed with failures, particularly in TNBC. In other solid human tumors, the efficacy of anti-PD-L1 immune checkpoint therapies appeared to be enhanced by stimulating lymphocyte infiltration into the tumor microenvironment with type I IFNs. We have now identified a similar IFN-stimulated pathway in TNBC. We show that TNBC cells express high levels of the RIG-I double-stranded RNA sensor and downstream active JAK1/STAT1/INF-β pathway components. Moreover, we show that TNBC cells display an interferon gene signature, suggesting that TNBC cells are primed to respond to type I IFNs. Further stimulation of this pathway would result in enhanced expression of PD-L1, a known transcriptional target of IFN-β, as well as the recruitment of IFN-responsive tumor infiltrating lymphocytes. We now propose to build on these exciting preliminary findings generated from our previous award with a series experiments aimed at determining the clinical utility of hyper activating RIG-I and increasing IFN-β production to sensitize TNBC cells to immune checkpoint therapies.

2. KEYWORDS

ADAR, interferon signaling, RIG-I, triple negative breast cancer

3. ACCOMPLISHMENTS

Major Goals of the Project

There was one major milestone for this first year of the grant proposal: determine the expression levels of IFN-β and ISG signature in each cell line and whether cells are sensitive to RIG-I activation or inhibition.

Goals Accomplished

MAJOR TASK 2: Determine whether the RIG-I pathway is responsible for IFN-β secretion in TNBC.

In this first year, we have worked exclusively on understanding the role of RIG-I in activating an interferon-beta response in TNBC cells. This work was entirely proposed in a panel of established breast cancer cell lines, a mix of both TNBC and non-TNBC lines. As shown in **Figure 1**, we were successfully able to knock down RIG-I expression in all cell lines.

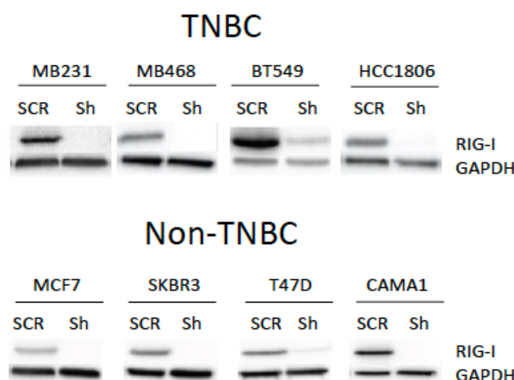

Figure 1. Knock down of RIG-I in established breast cancer cell lines. The indicated cell lines were infected with lentiviruses encoding shRNAs against either scrambled (SCR) or RIG-I (Sh) sequences. Lysates were harvested and immunoblotted with the indicated antibodies.

Depletion of RIG-I did not affect the overall proliferation or apoptosis rates of either TNBC or non-TNBC cells, as predicted. Also, knockdown of RIG-I did not change the migration of any cells tested nor did it alter the amount of secreted IFN-beta in the media. Thus, we next sought to determine the role of RIG-I activation in the proliferation, migration, and IFN-beta production of TNBC and non-TNBC cells.

To directly test this, we treated established breast cancer cells with 3pRNA, a known agonist of RIG-I in cells. Cells were treated for 24 hours and then proliferation was measured using the cell titer glo assay. **Figure 2** shows that non-TNBC cells treated with 3pRNA did not significantly alter their cell proliferation. However, all four TNBC cell lines exhibited significant decreases in cell proliferation following 3pRNA treatment and activation of RIG-I indicating that activation of RIG-I in TNBC cells results in an inhibition of cell proliferation. Because of this cell cycle arrest and some increased cellular apoptosis, we were unable to accurately measure cell motility after 3pRNA treatment. We have plans to measure cell migration with either a lower dose of 3pRNA or at an earlier timepoint, one that precedes arrest.

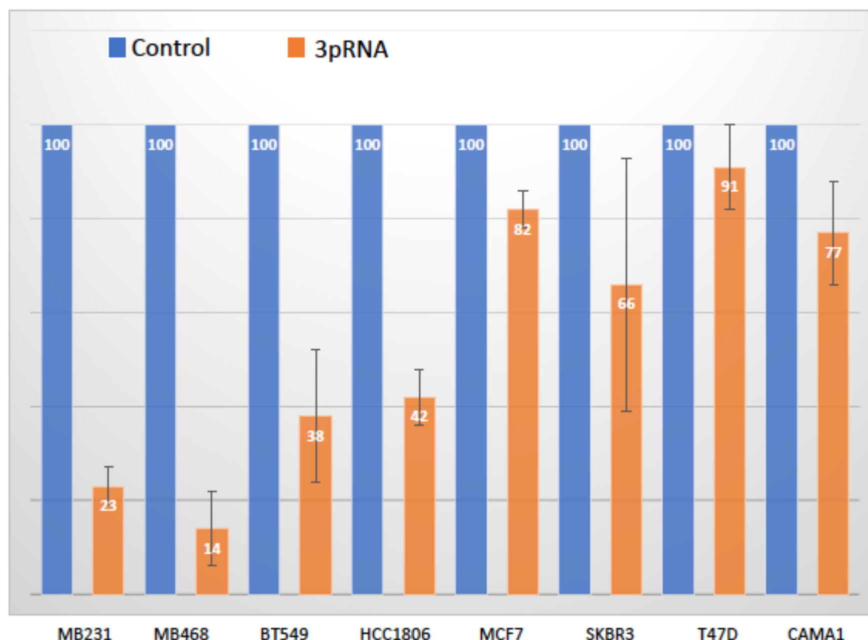

Figure 2. Activation of RIG-I inhibits TNBC proliferation. The indicated cells were treated with control or 3pRNA to activate endogenous RIG-I. Cells were analyzed after 24 hours via cell titer glo to mark proliferating cells. Each cell was done in triplicate for n=3.

Training Opportunities

Nothing to Report

Results Disseminated to the Community

I participated this past year in disseminating our initial findings to three independent groups of large donors to the American Cancer Society. These donors visited my laboratory at Washington University where I discussed the research in this grant proposal and how our results were moving the field of breast cancer research forward. We engaged in a question-and-answer session where the donors queried me on the clinical impact of this work. I anticipate doing this laboratory tour again next year and have already been asked by the American Cancer Society to do so. I also

presented preliminary data at the AACR Disparities in Cancer Meeting in Philadelphia as an invited speaker.

Plans for Next Reporting Period

In year 2, we will focus exclusively on MAJOR TASKS 4, 5 and 6. Specifically, we will begin experiments aimed at determining whether activation of RIG-I results in increased T cell infiltration into implanted tumors. This will also be done following direct IFN-beta stimulation. We have accumulated our 525 primary TNBC core samples and are now poised to perform immunohistochemical analysis of all tumors for markers of IFN pathway activation and T cell infiltration. Finally, we will begin injecting immune compromised mice with four patient derived TNBC tumors.

4. IMPACT

Impact on Principal Discipline

Our current work will be incredibly impactful for those studying TNBC. We have discovered that

This information will be deposited in a public database for RNA sequencing so that other scientists can view the raw data.

Impact on Other Disciplines

Nothing to Report

Impact on Technology Transfer

Nothing to Report

Impact on Society

We have disseminated the data and ideals from this grant proposal to several groups in the St. Louis community including the American Cancer Society as well as to a general audience at the AACR Disparities in Cancer meeting. They were encouraged by our progress and excited about the future clinical impact our work might provide.

5. CHANGES/PROBLEMS

Changes in Approach

Nothing to Report

Anticipated Problems or Delays

Nothing to Report

Changes in Human, Animal Biohazards and/or Selective Agents

Nothing to Report

6. PRODUCTS

Publications, Conference Papers and Presentations

Nothing to Report

Internet Sites

Nothing to Report

Technologies or Techniques

Nothing to Report

Inventions, Patents and/or Licenses
Nothing to Report

7. PARTICIPANTS
Individuals That Have Worked on Project

Name:	Jason D. Weber
Project Role:	PI
Nearest person month worked:	1.2
Contribution to Project:	Dr. Weber served as the mentor for Drs. Maggi and Cottrell in planning all experiments and overseeing the final data analysis.
Funding Support:	NIH CA262804; W81XWH-21-1-0476; W81XWH-21-1-0391

Name:	Leonard B. Maggi
Project Role:	Co-Investigator
Nearest person month worked:	1.2
Contribution to Project:	Dr. Maggi worked with Dr. Cottrell on all the experiments detailed in year 1.

Name:	Shunqiang Li
Project Role:	Co-Investigator
Nearest person month worked:	0.6
Contribution to Project:	Dr. Li provided expertise on all mouse and patient sample experiments.

Name:	Cynthia Ma
Project Role:	Co-Investigator
Nearest person month worked:	0.3
Contribution to Project:	Dr. Ma provided oncology guidance on all experiments.

Name:	Kyle Cottrell
Project Role:	Postdoctoral Research Associate
Nearest person month worked:	6
Contribution to Project:	Dr. Cottrell worked with Dr. Maggi on all the experiments detailed in year 1.

Changes in Active Other Support for PD/PI
Nothing to Report

Other Organizations Involved as Partners
Nothing to Report