AWARD NUMBER: W81XWH-15-1-0016

TITLE: Validation of MECP2 as a new therapeutic target in TNBC

PRINCIPAL INVESTIGATOR: Daniel P. Silver, M.D., Ph.D.

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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.
The purpose of this work is to validate MECP2, an oncogene amplified in 30% of triple negative breast cancer (TNBC) as a useful therapeutic target in TNBC. Established TNBC cell lines and patient-derived xenografts (PDXs) are being tested for their continued dependency on MECP2 expression, and their response to drugs that target MECP2-driven tumors. One very significant event in the first year of funding has been to publish our initial findings that MECP2 is a widely amplified oncogene in human cancers in a high profile journal, Cancer Discovery (Neupane, M. et al. MECP2 Is a Frequently Amplified Oncogene with a Novel Epigenetic Mechanism That Mimics the Role of Activated RAS in Malignancy. Cancer Discovery 6, 45–58 (2016)). This will serve to focus the attention of the cancer research community on the possibility that MECP2 could be a therapeutic target. In addition, we have made progress in terms of identifying PDX and cell line models, refining our MECP2 shRNA lentiviruses, piloting our tissue microarray experiments, and planning a strategy for the construction of the mouse model of MECP2-directed breast cancer.
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ANNUAL TECHNICAL REPORT
Award Number: W81XWH-15-1-0016
Title: Validation of MECP2 as a new therapeutic target in TNBC
Principal Investigator: Daniel Silver, M.D., Ph.D.
Reporting Period: 1 March 2015 – 29 February 2016

1. INTRODUCTION:
Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.
We hypothesized that the growth of a significant percentage of TNBCs is driven by MECP2 overexpression, and that these tumors will be susceptible to less toxic therapies as a result of the unique mechanism of action of MECP2 in tumorigenesis. To investigate this hypothesis, we are assaying a series of TNBC cell lines and primary patient-derived tumor xenografts for MECP2 dependence and response to epigenetic therapies, which are less toxic alternatives to the currently used therapies for TNBC. In addition, we are probing tissue microarrays to establish the prevalence of MECP2 overexpression and amplification in TNBC, and correlating MECP2 levels with clinical features. Lastly, we are creating a mouse model of MECP2-driven mammary cancer to gain additional insight into the role of MECP2 in tumorigenesis and therapy response.

2. KEYWORDS:
Provide a brief list of keywords (limit to 20 words).
MECP2, PDX, Triple negative breast cancer, epigenetics, therapy, HDAC inhibitor, 5-azacytidine, tissue microarrays, oncogene, transgenic mouse

3. ACCOMPLISHMENTS

3a. 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 percentage of completion.
For Specific Aim 1, in the first year, Major Task 1 was to define MECP2 addiction in TNBC cell lines in tissue culture by shRNA directed at MECP2. Another major task was to start to investigate MECP2 addiction in TNBC PDX models (projected to take 18 months). These tasks are about 40% completed.
For Specific Aim 2, in the first year, Task 1 was to begin to Probe a large TNBC tissue microarray with an anti-MECP2 antibody and search for an association of MECP2 overexpression with clinical features. This task is 50% completed and was projected to take 2 years.
For Specific Aim 3, in the first year, Task 1 was to develop a mouse model of MECP2-directed breast cancer. This task is about 30% completed.

3b. What was accomplished under these goals? For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.
For Specific Aim 1, we have made good progress in task 1. We have shown MECP2 addiction in several TNBC cell lines in culture (for an example, see “MECP2 Addiction in TNBC” to the right) and have shown that the shRNA used in this experiment is specific by rescuing the addiction by expressing an shRNA resistant form of MECP2 (See “Rescue of shMECP2 in BT549”). These experiments were done with constitutively active shRNA constructs; for in vivo xenograft experiments, we are developing doxycycline-inducible shRNA constructs, which will enable better experiments. The PDX experiments have been delayed because of the time it took to put a DOD-approved animal care protocol in place. We have defined several suitable PDX models by western blot that overexpress MECP2, and anticipate these experiments starting soon.

For Specific Aim 2, we have used an MECP2 antibody to successfully probe a tissue microarray, one prepared from ovarian cancers, which has allowed us to work out all technical issues (see “Tissue Microarray Probed for MECP2”). This is in preparation for the TNBC array, and we were able to show that the antibody performed as we expected. We are gathering together the material to proceed with the TNBC microarray experiment.

For Specific Aim 3, we have met with our collaborator, Dr. K. Wong often and have designed the appropriate knock-in constructs to make the transgenic model. Progress here has been delayed while the DOD-approved animal care protocol was put in place.

3c. What opportunities for training and professional development has the project provided? Describe opportunities for training and professional development to anyone who was involved in 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 participation in workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

My postdoctoral fellow, Dr. Manish Neupane, has had the opportunity to learn directly from our collaborator on the transgenic animal aim, Dr. Wong, and learn about cutting edge approaches to construct this model.

3d. How were the results disseminated to communities of interest? 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.

We published our initial findings that MECP2 is a widely amplified oncogene in human cancers in an excellent high profile journal, Cancer Discovery (Neupane, M. et al. MECP2 Is a Frequently Amplified Oncogene with a Novel Epigenetic Mechanism That Mimics the Role of Activated RAS in Malignancy. Cancer Discovery 6, 45–
This should serve to disseminate widely these results and may interest others in developing therapies that target this oncogene.

3e. What do you plan to do during the next reporting period to accomplish these goals? Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

I have recently accepted a job at Jefferson University in Philadelphia. I will be heading a larger laboratory effort there, and my first day there is July 1, 2016. I have contacted my Science Officer Dr. Nicole Williams, about this, and she has forwarded the matter to my Contract Specialist, Ms. Cheryl Lowery, who will facilitate transfer of this grant. I will complete all transfer documents and obtain the appropriate IACUC approvals as quickly as possible. We have made several new inducible shRNA constructs and I am optimistic that one of these will facilitate completion of Aim 1 quickly; if not, we will accomplish these goals with a constitutive shRNA reagent we already have in hand. We are now ready to probe the tissue microarray for Aim 2 after our successful pilot efforts above, and I anticipate this will be accomplished without difficulty. The mouse model work is underway and moving smoothly for Aim 3.

4. IMPACT

4a. 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." Otherwise, 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).

Nothing to report

4b. What was the impact on other disciplines? If there is nothing significant to report during this reporting period, state "Nothing to Report." Otherwise, 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.

Nothing to report

4c. What was the impact on technology transfer? If there is nothing significant to report during this reporting period, state "Nothing to Report." Otherwise, 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

4d. 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

5a. Changes in approach and reasons for change: Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior
approval of the agency.
Nothing to report

5b. 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.
Delays have been incurred related to the time it has taken to obtain IACUC approvals. As discussed above, I am relocating my laboratory to Jefferson University; I am committed to completing all transfer documents and obtaining the appropriate IACUC approvals as quickly as possible.

5c. 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.
None

5d-f. 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.

5d. Significant changes in use or care of human subjects: None
5e. Significant changes in use or care of vertebrate animals: None
5f. Significant changes in use of biohazards and/or select agents: None

6. PRODUCTS

6a. Publications, conference papers, and presentations: Report only the major publication(s) resulting from the work under this award, in the following categories.
   Journal publications: Nothing to report
   Books or other non-periodical, one-time publications: Nothing to report
   Other publications, conference papers, and presentations: Nothing to report

6b. Website or other internet site: If none, state “Nothing to report.” If applicable, 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

6c. Technologies or techniques: If none, state “Nothing to report.” If applicable, identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.
Nothing to report

6d. Inventions, patent applications, and/or licenses: If none, state “Nothing to report.” If applicable, identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number.
Nothing to report

6e. Other Products: If none, state “Nothing to report.” If applicable, 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; biospecimen 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 inventions; new business creation. Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

7a. What individuals have worked on the project? (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period.

Name: Daniel P. Silver  
Project Role: PD/PI  
Researcher Identifier (ORCID): None  
Nearest person month worked: 4  
Contribution to project: Design, planning, direction and oversight of the project overall.  
Funding support: N/A (this award)

Name: Thanh Von  
Project Role: Technician  
Researcher Identifier (ORCID): None  
Nearest person month worked: 1  
Contribution to project: Assist with propagation and orthopic implantation of PDX models.  
Funding support: N/A (this award)

Name: Manish Neupane  
Project Role: Postdoctoral Fellow  
Researcher Identifier (ORCID): None  
Nearest person month worked: 11  
Contribution to project: Performance of majority of experiments including lentiviral packaging, infections, tissue culture, microarray probing, and transgenic construction.  
Funding support: N/A (this award)

Name: Allison Clark  
Project Role: Postdoctoral Fellow  
Researcher Identifier (ORCID): None  
Nearest person month worked: 2  
Contribution to project: Construction of DNA clones for the MECP2 transgenic.  
Funding support: N/A (this award)

Name: Katherine Dunn  
Project Role: Postdoctoral Fellow  
Researcher Identifier (ORCID): None  
Nearest person month worked: 2  
Contribution to project: Provide expertise and technical assistance in designing, deriving and using the MECP2 transgenic model.  
Funding support: N/A (this award)

7b. Has there been a change in the active other support of the PD/PI or senior/key personnel since the last reporting period?
Dr. Silver’s award from the DF/HCC Men’s Collaborative to Cure Women’s Cancers has been extended. His current active other support is as follows:

5 P50 CA168504-03 (Winer – PD) 09/21/13 – 09/20/18 15% effort
NIH (Annual Direct Costs – Silver portion) Dana-Farber/
Harvard SPORE in Breast Cancer – Project 4 and Administrative Core

Goals: The overarching goal of the DF/HCC SPORE in Breast Cancer is to promote translational research derived from fundamental discoveries in the laboratory that can lead to tangible clinical benefit.

Specific Aims: 1) To identify underlying mechanisms of resistance to therapy and explore novel approaches to overcome these resistance mechanisms (Projects 1, 2, 3, 4) 2) To develop and test new rationally based therapies for breast cancer in clinical trials (Projects 2, 3) and/or identify promising approaches for future studies (Projects 1, 2, 3, 4) 3) To understand the mechanisms of breast cancer risk, development and progression (Project 1 – risk, development and progression; Projects 2, 3, 4 – progression)

Funding Agency Contact: Igor Kuzmin, PhD, NCI, NIH, 9609 Medical Center Drive, Rm 3W112, Bethesda, MD 20892, kuzmini@mail.nih.gov
Overlap: None.

(Silver – PI) 07/01/13 – 11/30/16 20% effort
The Men’s Collaborative to Cure Women’s Cancers (Annual Direct Costs, in no-cost extension) Dana-
Farber/Harvard Cancer Center

Goals: We propose to provide proof of principle of the effectiveness of therapy targeted at this oncogene in ovarian cancer, once this project is completed successfully, a clinical trial can be started without delay.

Specific Aims: 1. To identify MECP2-overexpressing human ovarian cancer lines and primary xenografts and test them for MECP2 addiction and response to epigenetic therapies 2. To develop an antibody to characterize MECP2 expression in paraffin embedded, formalin fixed clinical material and use this antibody to probe tissue microarrays.

Funding Agency Contact: Deborah Goff, Dana-Farber/Harvard Cancer Center, deborah_goff@dfci.harvard.edu
Overlap: None.

7c. What other organizations were involved as partners?
None

8. SPECIAL REPORTING REQUIREMENTS
N/A

9. APPENDICES N/A