

**AWARD NUMBER:** W81XWH-20-1-0803

**TITLE:** Epigenetic Regulation of the Melanoma Microenvironment

**PRINCIPAL INVESTIGATOR:** Emily Bernstein, PhD

**CONTRACTING ORGANIZATION:** Icahn School of Medicine at Mount Sinai, New York, NY

**REPORT DATE:** September 2021

**TYPE OF REPORT:** Annual

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# REPORT DOCUMENTATION PAGE

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<b>14. ABSTRACT</b> Deregulation of epigenetic states promotes melanoma progression. MacroH2A, a histone variant associated with transcriptional repression, is downregulated in melanoma vs. benign nevi, where it suppresses proliferation and metastatic potential. However, its role as a barrier to tumorigenesis has not been investigated <i>in vivo</i> . We found that mice constitutively lacking macroH2A variants exhibit accelerated melanoma growth compared to their wild-type counterparts. MacroH2A-deficient tumors display impaired cytotoxic T cell function and increased monocyte infiltration, consistent with a compromised anti-tumor immune response, as well as upregulation of <i>Ccl2</i> , <i>Cxcl1</i> and <i>Il6</i> , which are myeloid chemo-attractants. Through single-cell transcriptomic profiling of the entire melanoma microenvironment, we identified alterations in the immune cell compartment in macroH2A-deficient tumors. Altogether, our data supports a novel tumor suppressor role for macroH2A through repression of pro-inflammatory signaling within the melanoma microenvironment.					
<b>15. SUBJECT TERMS</b> Melanoma, tumor microenvironment, macroH2A, histone variant, cancer-associated fibroblasts, anti-tumor immunity					
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## 1. INTRODUCTION:

Deregulation of epigenetic states has emerged as a critical driver of aberrant transcriptional programs promoting melanoma development and therapeutic resistance. The Bernstein laboratory showed that macroH2A, a histone variant associated with transcriptional repression, is downregulated in melanoma vs. benign nevi and suppresses melanoma cell proliferation and metastatic potential. However, its role as a barrier to tumorigenesis has not been investigated *in vivo*. We have assessed macroH2A function during the entire process of melanoma development in an animal model where macroH2A is either present (WT) or absent (dKO). We found that melanoma growth was significantly enhanced in dKO animals and the immune cells within these macroH2A-deficient tumors, which normally destroy cancer cells, were abnormal. We thus hypothesized that the absence of macroH2A variants promotes melanoma aggressiveness through a defect in immune cell anti-tumor response. This could stem either from melanoma cells eliciting tolerance by the immune system, from functional deficiencies intrinsic to immune cells, and/or from activation of cancer-associated fibroblasts (CAFs) that can promote an immunosuppressive environment. We proposed to understand the link between macroH2A deficiency and immune tolerance *in vivo* by characterizing how macroH2A loss in melanoma cells promotes their escape from immune surveillance, and how macroH2A regulates the anti-tumor activity of immune cells. This proposal has the potential to highlight macroH2A as a prognostic marker for response to immune checkpoint blockade and/or as a regulator of tractable molecular targets for therapy, and overall, to help refine the clinical benefits of immunotherapy for melanoma patients. Moreover, as the histone variant macroH2A has not been implicated in melanoma immunity, this proposal is innovative and timely given that immunotherapy is currently at center stage of melanoma therapeutics.

## 2. KEYWORDS:

Melanoma, tumor microenvironment, macroH2A, histone variant, cancer-associated fibroblasts, anti-tumor immunity

## 3. ACCOMPLISHMENTS:

### What were the major goals of the project?

Please see attached document (Major Goals)

### What was accomplished under these goals?

Please see attached document (Accomplishments)

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

Seminar presentation by key personnel Dan Filipescu:

**MacroH2A as a Novel Chromatin Regulator of the Melanoma Microenvironment**

Dan Filipescu, Dan Hasson, Navpreet Tung, Étienne Humblin, Matthew Goldberg, Nikki Vyas, Kristin Beaumont, Flávia Ghiraldini, Hélène Salmon, Robert Sebra, Alice Kamphorst, Miriam Merad, Emily Bernstein – Icahn School of Medicine at Mount Sinai Cancer Biology Retreat, April 23, 2021

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

*Nothing to Report.*

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

We plan to continue carrying out the tasks as stated in the Statement of Work.

**4. IMPACT:**

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

*Nothing to Report.*

**What was the impact on other disciplines?**

*Nothing to Report.*

**What was the impact on technology transfer?**

*Nothing to Report.*

**What was the impact on society beyond science and technology?**

*Nothing to Report.*

## 5. CHANGES/PROBLEMS:

### Changes in approach and reasons for change

*Nothing to Report.*

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

We have observed that the BRAF/PTEN/Tyr-Cre melanoma strain on the B6 background develops spontaneous melanomas at an early age and in the absence of experimental induction regardless of macroH2A status. This behavior, which does not occur in our WT and dKO mouse strains on a 129/B6/FVB mixed background, required us to change the diet to one with reduced levels of phytoestrogens, which could be responsible for leaky induction of and at least a third of the mice of this strain must be sacrificed before reaching an age when tumor induction was originally scheduled (8-10 weeks), and therefore future tasks involving these mice could take longer to complete owing to reduced animal availability.

### Changes that had a significant impact on expenditures

*Nothing to Report.*

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

*Nothing to Report.*

### 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:

- **Publications, conference papers, and presentations**

### **Journal publications.**

*Nothing to Report.*

### **Books or other non-periodical, one-time publications.**

*Nothing to Report.*

### **.Other publications, conference papers and presentations.**

Seminar presentation by key personnel Dan Filipescu:

#### **MacroH2A as a Novel Chromatin Regulator of the Melanoma Microenvironment**

Dan Filipescu, Dan Hasson, Navpreet Tung, Étienne Humblin, Matthew Goldberg, Nikki Vyas, Kristin Beaumont, Flávia Ghiraldini, Hélène Salmon, Robert Sebra, Alice Kamphorst, Miriam Merad, Emily Bernstein – Icahn School of Medicine at Mount Sinai Cancer Biology Retreat, April 23, 2021

Seminar presentations by Emily Bernstein:

May, 2021	University of Arkansas for Medical Sciences, Winthrop P. Rockefeller Cancer Institute, Virtual Event.
April, 2021	Fred Hutchinson Cancer Research Center, Virtual Event.
March, 2021	Department of Biochemistry and Biophysics, University of Pennsylvania, Virtual Event.
February, 2021	Human Oncology and Pathogenesis Program Research Seminar Series, MSKCC, Virtual Event.
January, 2021	Cold Spring Harbor Laboratory seminar series, Virtual Event.
December, 2020	Department of Genetics and Development, Columbia University, Virtual Event.
November, 2020	UPMC Hillman Cancer Center Pittsburgh, Virtual Event.

- **Website(s) or other Internet site(s)**

*Nothing to Report.*

- **Technologies or techniques**

*Nothing to Report.*

- **Inventions, patent applications, and/or licenses**

*Nothing to Report.*

- **Other Products**

*Nothing to Report.*

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

### What individuals have worked on the project?

Name:	<b>Dan Hasson, PhD</b>
Project Role:	Scientist
Researcher Identifier:	Commons ID: HASSON
Nearest person month worked:	3
Contribution to Project:	Dr. Hasson has performed work to support all genomics wet lab and computational analyses.
Funding Support:	NIH/NCI, NIH/NIAID, NIH/NIDDK
Name:	<b>Dan Filipescu, PhD</b>
Project Role:	Postdoctoral Fellow (months 1-2), Instructor (months 3-12)
Researcher Identifier:	<a href="https://orcid.org/0000-0001-6381-2557">https://orcid.org/0000-0001-6381-2557</a>
Nearest person month worked:	2
Contribution to Project:	Dr. Filipescu performed experiments, collected and analyzed data, interpreted results and prepared them for presentations and future publications; worked closely with the teams of Drs. Kamphorst, Merad, and Sebra on immune profiling and scRNA-seq studies.
Funding Support:	NIH/NCI, Melanoma Research Alliance

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

New projects:

**Dissecting lysine methylation signaling in melanoma pathobiology**

**Principal Investigator:** Bernstein, Levy

**Funding Agency:** Binational Science Foundation

**Goal:** In this proposal, we will combine classical biochemical and cellular approaches with cutting-edge genomic and proteomic tools to elucidate the role of lysine methylation of non-histone proteins in melanoma.

**Project Dates:** 10/01/2020-09/30/2024

**Total Costs:**

**Percent Effort:** 1%

**Solving Indolent Neuroblastoma**

**Principal Investigator:** Maris

**Funding Agency:** Solving Kids Cancer/Children's Hospital of Philadelphia

**Goal:** Dr. Bernstein will contribute to Aims 2 and 3 by providing isogenic systems to study ATRX IFFs.

**Role:** Co-Investigator

**Project Dates:** 05/01/2021-04/30/2023

**Total Costs:**

**Percent Effort:** 5%

Completed projects:

**Toward Therapeutic Manipulation of Endothelial to Mesenchymal Transition**

**Principal Investigator:** Kovacic

**Funding Agency:** NIH/NHLBI; R01HL130423

**Role:** Co-Investigator

**Project Dates:** 01/25/2016-12/31/2020

**Percent Effort:** 2%

**What other organizations were involved as partners?**

*Nothing to Report.*

## 8. SPECIAL REPORTING REQUIREMENTS

**COLLABORATIVE AWARDS:**

**QUAD CHARTS:**

## 9. APPENDICES:

*Nothing to Report.*