

AWARD NUMBER: W81XWH-15-1-0097

TITLE: The Role of Hypoxia in the Tumor Microenvironment: Implications for
Ovarian Cancer Therapy

PRINCIPAL INVESTIGATOR: Erinn B. Rankin

CONTRACTING ORGANIZATION: Stanford University
Palo Alto, CA 94304

REPORT DATE: July 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 July 2017		2. REPORT TYPE Annual		3. DATES COVERED 1 Jul 2016 - 30 Jun 2017	
4. TITLE AND SUBTITLE The Role of Hypoxia in the Tumor Microenvironment: Implications for Ovarian Cancer Therapy				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-15-1-0097	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Erinn B. Rankin E-Mail: erankin@stanford.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Stanford University Palo Alto, CA 94304				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 Hypoxia is a potent microenvironmental factor promoting metastatic progression. A critical step in metastatic tumor progression is the ability of tumor cells to evade immune attack. Tumor cells utilize a complex set of mechanisms that prevent the immune system from mounting effective anti-tumor responses. Moreover, the hypoxic tumor microenvironment plays an important role in immune escape by favoring immune suppression and tumor resistance. Tumor hypoxia is thought to promote the immunosuppressive phenotypes of both tumor cells as well as infiltrating immune cells. However, the mechanisms by which hypoxia promotes immunosuppression in ovarian cancer remains to be elucidated and may have important therapeutic implications in the treatment of metastatic ovarian cancer. We hypothesize that hypoxia through HIF-1 signaling in regulatory T cells promotes angiogenic and immunosuppressive phenotypes, each contributing to metastatic ovarian cancer tumor growth. Here we generated mice to directly assess the functional role of HIF-1 in Treg cells in ovarian cancer metastatic tumor growth, angiogenesis, and immunosuppression.					
15. SUBJECT TERMS Ovarian cancer, hypoxia, tumor microenvironment, immune suppression, angiogenesis					
16. SECURITY CLASSIFICATION OF: U			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 15	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT Unclassified	b. ABSTRACT Unclassified	c. THIS PAGE Unclassified			19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
1. Introduction.....	4
2. Keywords.....	4
3. Accomplishments.....	4
4. Impact.....	6
5. Changes/Problems.....	7
6. Products.....	8
7. Participants & Other Collaborating Organizations.....	8
8. Special Reporting Requirements.....	9
9. Appendices.....	9

INTRODUCTION:

Metastatic disease is the leading cause of death in ovarian cancer patients. Metastasis is a highly complex and dynamic process that involves critical interactions between tumor cells and the microenvironment. Hypoxia is a potent microenvironmental factor promoting metastatic progression. Clinically, hypoxia and the expression of the hypoxia inducible transcription factors HIF-1, and HIF-2 are associated with increased distant metastasis and poor survival in ovarian cancer. A critical step in metastatic tumor progression is the ability of tumor cells to evade immune attack. Tumor cells utilize a complex set of mechanisms that prevent the immune system from mounting effective anti-tumor responses. Moreover, the hypoxic tumor microenvironment plays an important role in immune escape by favoring immune suppression and tumor resistance. Tumor hypoxia is thought to promote the immunosuppressive phenotypes of both tumor cells as well as infiltrating immune cells. However, the mechanisms by which hypoxia promotes immunosuppression in ovarian cancer remains to be elucidated and may have important therapeutic implications in the treatment of metastatic ovarian cancer. We hypothesize that hypoxia through HIF-1 signaling in regulatory T cells promotes angiogenic and immunosuppressive phenotypes, each contributing to metastatic ovarian cancer tumor growth. Here we will determine the functional role of HIF-1 in Treg cells by utilizing a genetic approach to dissect the functions of HIF in the context of ovarian cancer metastatic tumor growth, angiogenesis, and immunosuppression.

KEYWORDS: Hypoxia, tumor microenvironment, ovarian cancer, regulatory T cell, HIF-1, angiogenesis, therapy, metastasis, immune suppression.

ACCOMPLISHMENTS:

The major goals of this project are to determine the functional role of hypoxic HIF signaling in regulatory T cells and the impact on ovarian cancer metastasis. In aim 1 we propose to determine the role of HIF-1 deletion in Treg cells in ovarian tumor metastasis. In the second aim, we will determine the role of HIF-1 deletion in regulating proangiogenic activities of Treg cells. In the third aim, we will test the role of HIF-1 in mediating the suppressive function of Treg cells. This project investigates the role of hypoxia inducible factors in driving the metastatic phenotype of ovarian cancer and proposes to block these factors and associated pathways as therapeutic strategies for the treatment of ovarian cancer.

The major goals of the project during this funding period are as stated in the approved SOW are as follows:

TASK 1. To determine the role of HIF-1 deletion in Treg cells on metastatic ovarian cancer growth (years 1 and 2).

Task 1a. Generate FOXP3-Cre and FOXP3-HIF-1 mice with existing FOXP3-Cre and HIF-1 floxed homozygous. Two rounds of breeding are required and we need a total of 50 female mice to be generated FOXP3-Cre and FOXP3-HIF-1 (n = 10 each, July 31 2015- July 31 2016).

The goal in the first and second reporting period was to generate mice in which we could investigate the functional role of HIF-1 signaling (inactivation) in regulatory T cells (Tregs) and its impact on ovarian cancer metastasis. To test the functional importance of HIF-1 in Treg cells on metastatic ovarian cancer growth, we have utilized a genetic approach in which conditional deletion of HIF-1 in Treg cells will be achieved using Cre-loxP mediated recombination with a Treg specific promoter. The conditional allele for HIF-1

contains loxP sites that flank exon 2 which encodes the bHLH DNA binding domain resulting in an out-of-frame deletion of exon 2 and inactivation of HIF-1 upon Cre-mediated recombination (Ryan et al., 1998). HIF-1 floxed mice on the C57BL/6 background were a gift from Dr. Randall Johnson and have been part of our breeding colony for many years (Rankin et al., 2012). FOXP3-YFP/Cre mice express a knocked in yellow fluorescent protein/Cre-recombinase fusion protein from the *Foxp3* locus without disruption endogenous *Foxp3* expression. These mice were recently purchased from the Jackson Laboratory on a C57BL/6 background and have been previously used to study the functional role of specific factors in

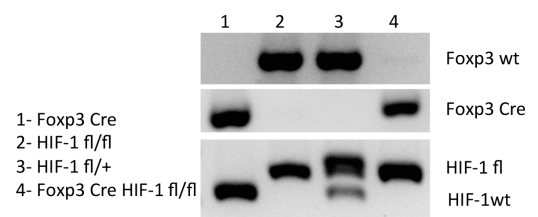


Figure 1. Generation of FOXP3-Cre HIF-1 deficient mice. Genomic PCR of the FOXP3-Cre, HIF-1 floxed alleles in control and FOXP3-HIF-1 deficient mice.

Treg cells (Rubtsov et al., 2008). Mice homozygous for the HIF-1 conditional allele (floxed/floxed) were crossed to FOXP3-Cre mice to generate FOXP3-HIF-1 heterozygous male and female mice. These mice were bred to generate control (FOXP3-Cre) and FOXP3-HIF-1 floxed/floxed deficient female mice that have FOXP3-Cre present on both X alleles (Fig.1). We conclude that FOXP3-HIF-1 deficient mice are viable and the number of Tregs within the spleen and mesenteric lymph node of FOXP3-Cre control and FOXP3-HIF-1 deficient mice were not significantly different under homeostatic and tumor bearing conditions (Fig. 2).

Task 1b. Evaluate ID8 metastatic tumor growth in 6-8 week old FOXP3-HIF-1 mice generated above (July 31 2016-July 31 2017).

We have obtained a highly metastatic derivative of the ID8 syngeneic ovarian cancer cell line, ID8-ascites that was generated by Dr. Katherine Fuh at Washington University. Intraperitoneal injection of ID8 cells results in the development of ascites and solid tumor lesions within the omentum and peritoneum within 30 days post injection (Fig. 3). We have compared the metastatic tumor growth of ID8-ascites cells in FOXP3-Control and FOXP3-HIF-1 deficient mice. Our studies suggest that HIF signaling in FOXP3 Treg cells does not significantly impact ID8 ascites tumor metastasis as ascites volume and total tumor volume was comparable between FOXP3-Cre control and FOXP3-HIF1 deficient mice (Fig. 3). Furthermore, inactivation of HIF-1 in FOXP3-Cre expressing regulatory T cells is not sufficient to modulate CD4+ or CD8+ T cell infiltration or activation within the ID8-ascites tumor model (Fig. 4). These findings suggest that inactivation of HIF-1 in regulatory T cells does not affect the protumorigenic or immunosuppressive properties of regulatory T cells in the ID8 ovarian cancer model.

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

This grant is a career development grant where I am an active member and participant of the Ovarian Cancer Academy. During this funding period (July 31, 2016- July 31, 2017) I have attended the DOD Ovarian Cancer Academy (DOD OCA) meeting in Seattle (September, 2016) in which I had the opportunity to network and meet with the Deans of the Academy, Drs. Nita Maihle and Doug Levine, as well as all of the other early career investigators within the Ovarian Cancer Academy. Additionally, I attend and participate in monthly DOD OCA webinars where I have had the opportunity to present my work and receive feedback, learn about others work to identify collaborations, and receive career development lectures. Finally, I have also had the opportunity to attend the Marsha Rivkin Ovarian Cancer meeting in Seattle (September 2016). Additional professional development activities include organizing and hosting an Ovarian Cancer Focus Group meeting at Stanford University where Ovarian cancer researchers (Oliver Dorigo, Jonathan Berek, Mickey Hu, Nelson Teng, and Wendy Fantl) present their work in an informal setting to establish collaborations and receive constructive feedback for their work. For my training activities, I meet with my mentor, Dr. Jonathan Berek, monthly to discuss the progress and growth of my ovarian cancer research and identify opportunities for growth. As a result of these meetings, I have applied and received extramural funding from the Marsha Rivkin Center for Ovarian Cancer Research and the Mary Kay Foundation to support my ovarian cancer research.

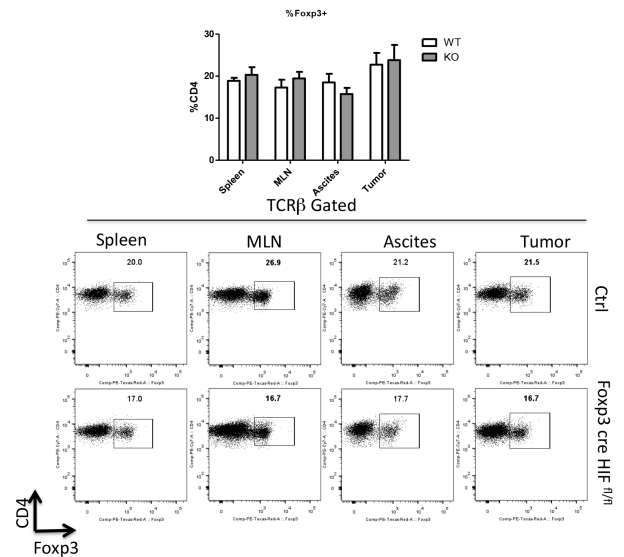


Figure 2. HIF-1 inactivation in FOXP3 regulatory T cells (Tregs) does not affect the frequency of Tregs in the spleen, mesenteric lymph node, ascites, or tumor of ID8 tumor bearing mice. FOXP3-Control (WT) or FOXP3-HIF-1 deficient mice were injected i.p. with 10⁶ ID8-Ascites cells. Mice were monitored daily. 30 days after injection mice developed symptoms of ovarian cancer. Ascites volume was measured using a syringe. Macroscopic tumors evident in the omenta of mice were weighed. The frequency of TCRβ, CD4, and FOXP3 positive Tregs within each tissue was determined by FACS analysis.

How were the results disseminated to communities of interest?

I have reached out to the greater Stanford community to make them aware of my project activities and involvement with the DoD Ovarian Cancer Academy. I was interviewed by the Stanford Medicine Scope Blog, an online publication for the Stanford Community and donors, where I described the need for ovarian cancer research, the goals of the DoD Ovarian Cancer Academy, as well as my professional and research goals within this program. I have also presented an invited oral presentation on my work on Hypoxia and Ovarian cancer supported by this grant at 1) the Keystone Symposia in Whistler, British Columbia, Canada in March 2017 and 2) the Tumor Microenvironment Workshop in Miami, FL in May 2017.

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

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

The goal of the research in the next reporting period is to:

TASK 2. Determine the role of Treg HIF-1 on tumor angiogenesis (years 3 and 4.5).

Task 2a. The role of Treg HIF-1 in regulating angiogenesis in ID8 tumors. (Months 24-30)

To test the functional role of Treg HIF-1 in regulating ovarian cancer angiogenesis in vivo, ID8 tumor sections and ascites will be analyzed from FOXP3-Cre control mice and FOXP3-HIF-1 mice described in Aim 1. VEGA protein levels will be measured in the ascites using a mouse VEGFA ELISA kit from RandD. I have previous experience measuring VEGF in tissue and serum from mice using this kit (Rankin et al., 2012). Tumor sections will be stained and quantified for CD31, an endothelial cell marker. The number of CD31 positive vessels per field will be counted.

Task 2b. The role of Treg HIF-1 in regulating angiogenesis in vivo. (Months 30-36)

To directly assess the role of Treg HIF-1 in regulating angiogenesis in vivo, the number of CD31+ endothelial cells in subcutaneous matrigel plugs that contain conditioned media from normoxic or hypoxic (2% oxygen) CD4+CD25+ T cells isolated from FOXP3-Cre control or FOXP3-HIF-1 deficient mice will be determined after 72 hours of incubation.

IMPACT:

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

Nothing to Report.

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. Nothing to Report.

What was the impact on other disciplines?

Nothing to Report.

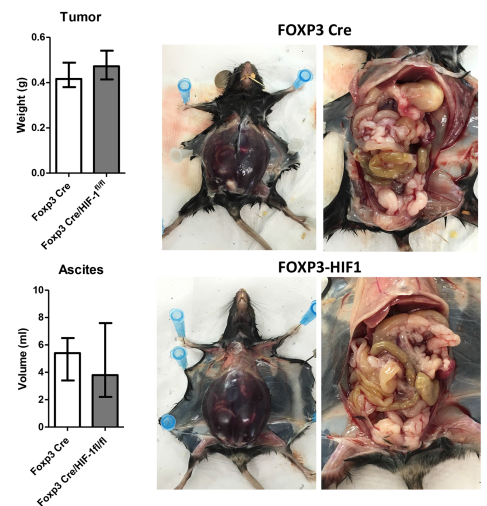


Figure 3. Metastatic tumor burden in the ID8-ascites model of ovarian cancer. FOXP3-Cre control and FOXP3-HIF1 deficient female mice were injected i.p. with 1×10^6 ID8-Ascites cells. Mice were monitored daily. At 30 days of injection mice developed symptoms of ovarian cancer. Ascites volume was measured using a syringe. Macroscopic tumors evident in the omentum of mice were weighed.

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.

What was the impact on technology transfer?

Nothing to Report.

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

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:

Nothing to Report.

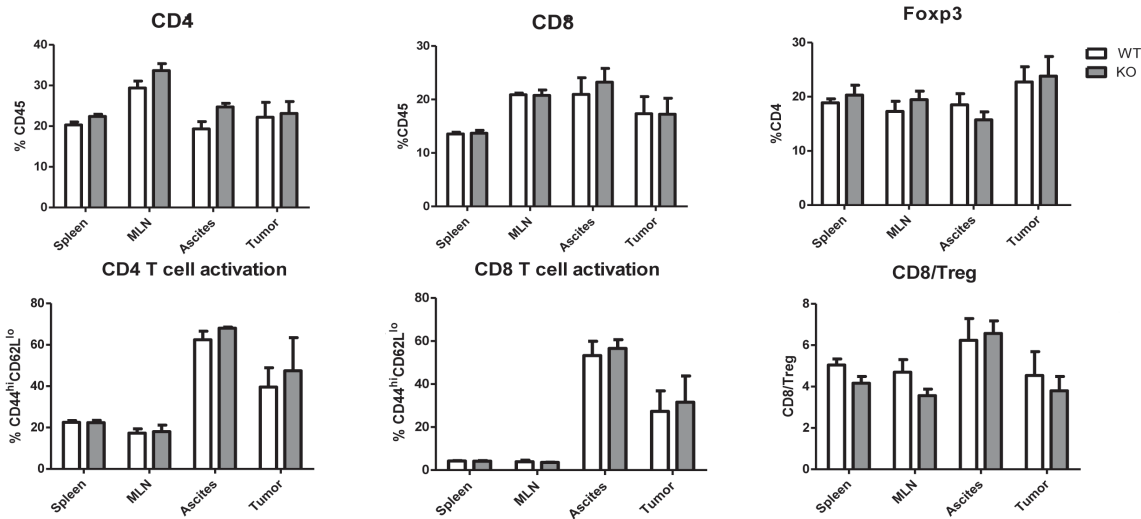


Figure 4. Conditional inactivation of HIF-1 in FOXP3-Cre expressing regulatory T cells does not impact CD4 and CD8 T cell infiltration or activation in the ID8 ovarian cancer tumor model. Shown are the percentage of CD4+, CD8+ cells within the CD45+ population of the spleen, mesenteric lymph node, ascites, and tumor, the percentage of Foxp3+ CD4+ T cells, percentage of CD44^{hi} CD62L^{lo} CD4 and CD8 T cells, and the CD8/Treg ratios as determined by FACS analysis.

CHANGES/PROBLEMS:

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.

Nothing to report.

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

Nothing to report.

PRODUCTS:

Publications, conference papers, and presentations

Zhao H, Nolley R, Chan AM, **Rankin EB**, Peehl DM. Cabozantinib inhibits tumor growth and metastasis of a patient-derived xenograft model of papillary renal cell carcinoma with MET mutation. **Cancer Biol Ther.** 2016 Aug 11:1-9; published; acknowledgement of federal support (yes).

Rankin EB and Giaccia AJ. The Receptor Tyrosine Kinase AXL in Cancer Progression. **Cancers (Basel)**, 2016 Nov 9;8(11); published; acknowledgement of federal support (yes).

Rankin EB, Nam JM, and Giaccia AJ. Hypoxia: Signaling in the metastatic cascade. **Trends in Cancer.** 2016 Jun 2 (6): 295–304; published; acknowledgement of federal support (yes).

Rankin EB and Giaccia AJ. Hypoxic control of metastasis. **Science.** 2016 Apr 8;352(6282):175-80; published; acknowledgement of federal support (yes).

Other Products:

We have generated FOXP3-HIF-1 mice in which HIF-1 is conditionally inactivated in regulatory T cells (Tregs). These mice can be useful for a variety of applications investigating the impact of HIF-1 signaling in Treg function.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name:	Erinn Rankin
Project Role:	Primary Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	3
Contribution to Project:	Dr. Rankin has designed and assisted Ms. Foreman in all proposed experimental design and execution.
Funding Support:	DOD, Marsha Rivkin, Mary Kay

Name:	Jonathan Berek
Project Role:	Mentor
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1.2
Contribution to Project:	Dr. Berek mentors Dr. Rankin by ensuring that Dr. Rankin's research and career development is progression.
Funding Support:	N/A

Name:	Katie Foreman
Project Role:	Research Assistant
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	7.44
Contribution to Project:	Ms. Foreman has performed all proposed experiments with Dr. Rankin.
Funding Support:	N/A

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

My research assistant Michaela Soriano left for medical school in June 2016. Katie Foreman, research assistant joined the project at this time (June 2016).

What other organizations were involved as partners?

Nothing to Report.

SPECIAL REPORTING REQUIREMENTS

Nothing to Report.

APPENDICES

Erinn B. Rankin cv

ERINN B. RANKIN, Ph.D.
Assistant Professor
Department of Radiation Oncology
Division of Radiation Biology
Stanford University School of Medicine
CCSR-South, Room 1245A
269 Campus Drive
Stanford, CA 94305-5152
(650) 497-8742
erankin@stanford.edu
Education

- 2007 University of Pennsylvania, Philadelphia, PA Ph.D.
Cell Growth and Cancer (Dr. Volker Haase)
- The role of hypoxia inducible factors family members in the development of VHL disease. January 1, 2007. Dissertation available from ProQuest. Paper AAI3260971.
- 2000 University of Illinois Urbana-Champaign, IL B.S.
Microbiology

Professional Appointments

- 2014–present Assistant Professor
Department of Radiation Oncology, Department of Obstetrics and Gynecology, Stanford University, Stanford, CA
- 2012–2014 Research Associate
Department of Radiation Oncology, Stanford University, Stanford, CA
- 2010–2011 Visiting Research Scholar (Dr. Ernestina Schipani)
Endocrine Unit, Massachusetts General Hospital, Boston, MA
- 2007–2012 Postdoctoral Scholar (Dr. Amato J. Giaccia)
Department of Radiation Oncology, Stanford University, Stanford, CA

Other Professional Positions

- 2000-2002 Research Specialist (Dr. EunRan Suh)
University of Pennsylvania, Philadelphia, PA

Honors and Awards

- 2016 Mary Kay Foundation Research Award
- 2016 Rivkin Center for Ovarian Cancer Research Pape Family Pilot Award
- 2016 Department of Defense Ovarian Cancer Academy Award
- 2014-2016 Gabilan Faculty Award, Stanford University
- 2012 J. Martin Brown Award for Outstanding Achievements in the Radiation Sciences

2011	Keystone Symposia Travel Award
2007-2012	NCI Postdoctoral Trainee
2007	Saul Winegrad Award for Outstanding Dissertation (University of Pennsylvania)
2005-2007	American Heart Association Pre-Doctoral Trainee

Memberships

2015-Present	American Association for Cancer Research
--------------	--

Publications (Peer Reviewed)

1. Sinha S, Thomas D, Chan S, Gao Y, Brunen D, Torabi D, Reinisch A, Hernandez D, Chan A, **Rankin EB**, Bernards R, Majeti R, Dill DL. Systematic discovery of mutation-specific synthetic lethals by mining pan-cancer human primary tumor data. **Nat Commun.** 2017 May 31;8:15580
2. Kariolis MS, Miao YR, Diep A, Nash SE, Olcina MM, Jiang D, Jones DS 2nd, Kapur S, Math II, Koong AC, **Rankin EB**, Cochran JR, Giaccia AJ. Inhibition of the GAS6/AXL pathway augments the efficacy of chemotherapies. **J Clin Invest.** 2017 Jan 3;127(1):183-198. doi: 10.1172/JCI85610. (PMCID: PMC5199716)
3. Aguilera TA, Rafat M, Castellini L, Shehade H, Kariolis MS, Hui AB, Stehr H, von Eyben R, Jiang D, Ellies LG, Koong AC, Diehn M, **Rankin EB**, Graves EE, Giacca AJ. Reprogramming the immunological microenvironment through radiation and targeting Axl. **Nat Commun.** 2016 Dec 23;7:13898. doi: 10.1038/ncomms13898. (PMCID: PMC5196438)
4. Divine LM, Nguyen MR, Meller E, Desai RA, Arif B, **Rankin EB**, Bligard KH, Meyerson C, Hagemann IS, Massad M, Thaker PH, Hagemann AR, McCourt CK, Powell MA, Mutch DG, Fuh KC. AXL modulates extracellular matrix protein expression and is essential for invasion and metastasis in endometrial cancer. **Oncotarget.** 2016 Nov 22;7(47):77291-77305. doi: 10.18632/oncotarget.12637. (PMID: 27764792)
5. Zhao H, Nolley R, Chan AM, **Rankin EB**, Peehl DM. Cabozantinib inhibits tumor growth and metastasis of a patient-derived xenograft model of papillary renal cell carcinoma with MET mutation. **Cancer Biol Ther.** 2016 Aug 11:0. doi: 10.1080/15384047.2016.1219816. [Epub ahead of print] (PMID: 27715452)
6. Mirzamohammadi F, Papaioannou G, Inloes JB, **Rankin EB**, Xie H, Schipani E, Orkin SH, Kobayashi T. Polycomb repressive complex 2 regulates skeletal growth by suppressing Wnt and TGF- β signaling. **Nat Commun.** 2016 Jun 22;7:12047. doi: 10.1038/ncomms12047. (PMCID: PMC4917962)
7. Zhou L, Liu XD, Sun M, Zhang X, German P, Bai S, Ding Z, Tannir N, Wood CG, Matin SF, Karam JA, Tamboli P, Sircar K, Rao P, **Rankin EB**, Laird DA, Hoang AG, Walker CL, Giaccia AJ, Jonasch E. Targeting MET and AXL overcomes resistance to sunitinib therapy in renal cell carcinoma. **Oncogene.** 2016 May;35(21):2687-97. doi: 10.1038/onc.2015.343. Epub 2015 Sep 14. (PMCID: PMC4791213)
8. Wu C, **Rankin EB**, Castellini L, Fernandez-Alcudia J, LaGory EL, Andersen R, Rhodes SD, Wilson TL, Mohammad KS, Castillo AB, Guise TA, Schipani E, Giaccia AJ. Oxygen-sensing PHDs regulate bone homeostasis through the modulation of osteoprotegerin. **Genes Dev.** 2015 Apr 15;29(8):817-31. doi: 10.1101/gad.255000.114. Epub 2015 Apr 6. (PMCID: PMC4403258)
9. Finger EC, Castellini L, **Rankin EB**, Vilalta M, Krieg AJ, Jiang D, Banh A, Zundel W, Powell MB, Giaccia AJ. Hypoxic induction of AKAP12 variant 2 shifts PKA-mediated protein phosphorylation to enhance migration and metastasis of melanoma cells. **Proc Natl Acad Sci USA.** 2015 Apr 7;112(14):4441-6. doi: 10.1073/pnas.1418164112. Epub 2015 Mar 19. (PMCID: PMC4394282)
10. **Rankin E**, Fuh KC, Castellini L, Viswanathan K, Finger EC, Diep A, LaGory E, Kariolis M, Chan A, Lindgren D, Axelson H, Miao Y, Krieg A, Giaccia AJ. Direct regulation of GAS6/AXL signaling by HIF promotes renal metastasis through SRC and MET. **Proc Natl Acad Sci USA.** 2014 Sep

- 16;111(37):13373-8. doi: 10.1073/pnas.1404848111. Epub 2014 Sep 3. (PMCID: PMC4169907)
11. Mangiavini L, Merceron C, Araldi E, Khatri R, Gerard-O'Riley R, Wilson TL, **Rankin EB**, Giaccia AJ, Schipani E. Loss of VHL in mesenchymal progenitors of the limb bud alters multiple steps of endochondral bone development. **Dev Biol.** 2014 Sep 1;393(1):124-36. doi: 10.1016/j.ydbio.2014.06.013. Epub 2014 Jun 24. (PMCID: PMC4335807)
 12. Taniguchi CM, Miao YR, Diep AN, Wu C, **Rankin EB**, Atwood TF, Xing L, Giaccia AJ. PHD inhibition mitigates and protects against radiation-induced gastrointestinal toxicity via HIF2. **Sci Transl Med.** 2014 May 14;6(236):236ra64. doi: 10.1126/scitranslmed.3008523. (PMCID: PMC4136475)
 13. Finger E C, Cheng C-F, Williams T R, **Rankin EB**, Bedogni B, Tachiki L, Spong S, Giaccia A J, Powell M B. CTGF is a therapeutic target for metastatic melanoma. **Oncogene.** 2014 Feb 27;33(9):1093-100. doi: 10.1038/onc.2013.47. Epub 2013 Feb 25. (PMCID: PMC3965577) **Participated in study design, interpreted the data, and revised all components of the manuscript text.*
 14. **Rankin EB**, Wu C, Khatri R, Wilson TLS, Araldi E, Rankin AL, Yuan J, Kuo CJ, Schipani E, Giaccia AJ. The HIF signaling pathway in osteoblasts directly modulates erythropoiesis through the production of EPO. **Cell.** 2012 Mar 30;149(1):63-74. doi: 10.1016/j.cell.2012.01.051. (PMCID: PMC3408231)
 15. **Rankin EB**, Fuh KC, Taylor TE, Krieg AJ, Musser M, Yuan J, Wei K, Kuo CJ, Longacre TA, Giaccia AJ. AXL is an essential factor and therapeutic target for metastatic ovarian cancer. **Cancer Res.** 2010 Oct 1;70(19):7570-9. doi: 10.1158/0008-5472.CAN-10-1267. Epub 2010 Sep 21. (PMID: 20858715).
 16. Krieg AJ, **Rankin EB**, Chan D, Razorenova O, Fernandez S, Giaccia AJ. Regulation of the histone demethylase JMJD1A by hypoxia-inducible factor 1 alpha enhances hypoxic gene expression and tumor growth. **Mol Cell Biol.** 2010 Jan;30(1):344-53. doi: 10.1128/MCB.00444-09. (PMCID: PMC2798291) **Designed studies, performed experiments, analyzed data, performed statistical analysis, and authored text for Figure 2.*
 17. **Rankin EB**, Rha J, SelakMA, Unger TL, Keith B, Liu Q and Haase VH. HIF-2 regulates hepatic lipid metabolism. **Mol Cell Biol.** 2009 Aug;29(16):4527-38. doi: 10.1128/MCB.00200-09. Epub 2009 Jun 15. (PMCID: PMC2725738)
 18. **Rankin EB**, Rha J, Unger TL, Wu CH, Shutt HP, Johnson RS, Simon MC, Keith B, Haase VH. Hypoxia-inducible factor-2 regulates vascular tumorigenesis in mice. **Oncogene.** 2008 Sep11;27(40):5354-8. doi: 10.1038/onc.2008.160 Epub 2008 May 19. (PMCID: PMC2575082)
 19. Peyssonnaud C, Zinkernagel AS, Schuepbach RA, **Rankin E**, Vaulont S, Haase VH, Nizet V, Johnson RS. Regulation of iron homeostasis by the hypoxia-inducible transcription factors (HIFs). **J Clin Invest.** 2007 Jul;117(7):1926-32. (PMCID: PMC1884690)
 20. **Rankin EB**, Biju MP, Liu Q, Rha J, Johnson RS, Simon CM, Keith B, Haase VH. Hypoxia inducible factor (HIF)-2 regulates hepatic EPO expression in vivo. **J Clin Invest.** 2007 Apr;117(4):1068-77. (PMCID: PMC1838939)
 21. **Rankin EB**, Tomaszewski JE, Haase VH. Renal cyst development in mice with conditional inactivation of the von Hippel-Lindau tumor suppressor. **Cancer Res.** 2006 Mar;66(5):2576-2583. (PMCID: PMC3514875)
 22. **Rankin EB**, Higgins DF, Walisser JA, Johnson RS, Bradfield CA, Haase VH. Inactivation of the arylhydrocarbon receptor nuclear translocator (Arnt) suppresses von Hippel-Lindau disease associated vascular tumors in mice. **Mol Cell Biol.** 2005 Apr;25(8):3163-72. (PMCID: PMC1069599)
 23. **Rankin EB**, Xu W, Silberg DG, Suh E. Putative intestine-specific enhancers located in 5' sequence of the CDX1 gene regulate CDX1 expression in the intestine. **Am J Physiol Gastrointest Liver Physiol.** 2004 May;286(5):G872-80. doi: 10.1152/ajpgi.00326.2003. Epub 2004 Jan 8. (PMID: 14715525)
 24. **Rankin EB**, Yu D, Jiang J, Shen H, Pearce EJ, Goldschmidt MH, Levy DE, Golovkina TV, Hunter CA, Thomas-Tikhonenko A. An essential role of Th1 responses and interferon gamma in infection-mediated suppression of neoplastic growth. **Cancer Biol Ther.** 2003 Nov-Dec;2(6):687-93. (PMID: 14688478)
 25. Suh ER, Ha CS, **Rankin EB**, Toyota M, Traber PG. DNA methylation down regulates CDX1 gene expression in colorectal cancer cell lines. **J Biol Chem.** 2002 Sep 27;277(39):35795-800. doi: 10.1074/jbc.M205567200. (PMID: 1212493)

Review Articles

1. Rankin EB and Giaccia AJ. The Receptor Tyrosine Kinase AXL in Cancer Progression. **Cancers (Basel)**. 2016 Nov 9;8(11).
2. Rankin EB, Nam JM, and Giaccia AJ. Hypoxia: Signaling in the metastatic cascade. **Trends in Cancer**. 2016 Jun 2 (6): 295–304.
3. Rankin EB and Giaccia AJ. Hypoxic control of metastasis. **Science**. 2016 Apr 8;352(6282):175-80.
4. Rankin EB, Narla A, Park JK, Lin S, Sakamoto KM. Biology of the bone marrow microenvironment and myelodysplastic syndromes. **Mol Genet Metab**. 2015 Sep-Oct; 116(1-2):24-8. doi: 10.1016/j.ymgme.2015.07.004. PMID: 26210353
5. Wu C, Giaccia AJ, and Rankin EB. Osteoblasts: A novel source of erythropoietin. **Curr Osteoporosis Rep**. 2014 Dec;12(4):428-32. doi: 10.1007/s11914-014-0236-x. PMID: 25204993.
6. Schipani E, Wu C, Rankin EB, and Giaccia AJ. Regulation of bone marrow angiogenesis by osteoblasts during bone development and homeostasis. **Front Endocrinol** 2013 Jul 10;4:85
7. Wu C, Rankin EB, and Giaccia AJ. Blood and bones: Osteoblastic HIF signaling regulates erythropoiesis. **Cell Cycle** 2012 Jun 15;11(12)2221-2.
8. Rankin EB, Giaccia AJ, Schipani E. A central role for hypoxia signaling in cartilage, bone, and hematopoiesis. **Curr Osteoporosis Rep**. 2011 Jun;9(2):46-52.
9. Rankin EB, Giaccia AJ, Hammond EM. Bringing H2AX into the angiogenesis family. **Cancer Cell**. 2009 Jun 2;15(6):459-61.
10. Rankin EB and Giaccia AJ. The role of hypoxia-inducible factors in tumorigenesis. **Cell Death Differ**. 2008 Apr;15(4):678-85.

Book Chapters

Rankin, EB, Erler, J and Giaccia, AJ. The cellular microenvironment and metastasis. In: Abeloff's Clinical Oncology, 5th Edition. Niederhuber, J.E., Armitage, J.O., Doroshow, J.H., Kastan, M.B. and Tepper, J. (eds), Elsevier 2014:40-51.

Grant Support

1. OCRP (Pilot Award) (Rankin, P.I.) 09/30/17-09/29/19
"Preclinical Testing of FLASH Radiotherapy and Immune Checkpoint Blockade Combination Therapy in Ovarian Cancer"
2. Mary Kay Foundation (Rankin, P.I.) 07/01/16-06/30/18
"Hypoxic signaling in metastasis: Molecular mechanisms and targeted therapy"
3. Marsha Rivkin Center for Ovarian Cancer Pilot Award (Rankin, P.I.) 04/01/16-03/31/17
"Targeting the hypoxic secretome in omental metastasis"
4. NCI RO1 (Giaccia, P.I.; Rankin, co-investigator) 07/01/15-06/30/20
"Preclinical testing of a novel therapy targeting AXL in advanced kidney cancer"
5. DoD Ovarian Cancer Academy Award (Rankin, P.I.) 07/01/15-06/30/20
"The role of hypoxia in the tumor microenvironment: Implications for ovarian cancer therapy"
6. MD Anderson/KCRP (Pilot Award) (Giaccia, P.I.; Rankin, Co-Investigator) 04/01/13-03/31/14
"Mechanisms of tumor resistance to targeted RTK therapy in ccRCC"

Patents

Inhibition of AXL signaling in anti-metastatic therapy.
US Patent PCT/US2011/022125

Modified AXL peptides and their use in inhibition of AXL signaling in anti-metastatic therapy.
US Patent PCT/US2013/074786

Invited Oral Presentations (National)

- 2017 Hypoxic signaling in Tumor-Mesothelial Niche Promotes Collagen Remodeling and Ovarian Cancer Metastasis. 15th International Tumor Microenvironment Workshop, Miami, FL
- 2016 Hypoxic signaling in ovarian cancer metastasis: Molecular mechanisms and targeted therapy. Third annual meeting of the international ovarian cancer consortium, Oklahoma City, OK
- 2011 Hypoxia inducible factor signaling in osteoblasts and the regulation of hematopoiesis. MGH Bone Research Workshop, Boston, MA
- 2010 The role of hypoxia signaling in the osteoblastic niche and the regulation of hematopoiesis, AACR, Washington DC
- 2005 ARNT is required for the development of VHL disease associated renal cysts in mice. ASN, Philadelphia, PA
- 2004 The role of hypoxia inducible factors in VHL disease associated tumorigenesis. ASN, St. Louis, MO

Invited Oral Presentations (International)

- 2017 Hypoxic signaling in the tumor-mesothelial niche. Keystone Symposia, Whistler, Canada
- 2016 Hypoxic signaling in tumor metastasis: molecular mechanisms and targeted therapy. The 3rd GI-CoRE Medical Science and Engineering Symposium, Hokkaido, Japan
- 2015 Hypoxic signaling in metastasis: Molecular mechanisms and targeted therapy. The Tumor Microenvironment Workshop, Vancouver, Canada
- 2008 HIF-2 regulates VHL associated vascular tumorigenesis and hepatic lipid metabolism in vivo. Keystone Symposia, Vancouver, Canada
- 2006 Hypoxic regulation of hepatic erythropoietin. International Conference on EPO, Lubeck, Germany

Poster Presentations

- 2015 The receptor tyrosine kinase, AXL, is a therapeutic target driving the mesenchymal phenotype in ovarian cancer. AACR: Ovarian Cancer Meeting, Orlando, FL
- 2015 Direct regulation of GAS6/AXL signaling by HIF promotes renal metastasis through SRC and MET. Hypoxia Keystone Symposia, Dublin, Ireland
- 2014 Osteoblastic PHD signaling modulates the HSC niche. AACR Radiation Oncology Think Tank, Fort Myers, FL

- 2012 The HIF signaling pathway in osteoblasts directly modulates erythropoiesis through the production of EPO. Keystone Symposia, Banff, Canada
- 2011 Osteoblasts regulate erythropoiesis through HIF. Keystone Symposia, Big Sky, MO
- 2010 AXL is an essential factor and therapeutic target for metastatic ovarian cancer. Keystone Symposia, Keystone, CO

Teaching

- 2017 Guest lecturer CBIO 242: Hypoxia and Angiogenesis (Stanford University)
- 2016 Guest lecturer CBIO 242: Hypoxia and Angiogenesis (Stanford University)
- 2016 Guest Instructor CBIO 280: Cancer Biology Journal Club (Stanford University)
- 2015 Instructor CBIO 280: Cancer Biology Journal Club (Stanford University)
- 2006 Teaching Assistant BIOM 555: Gene Expression (University of Pennsylvania)