AWARD NUMBER: W81XWH-22-1-0391

TITLE: Deciphering the Role of Progesterone in Ovarian Cancer Risk for BRCA Mutation Carriers

PRINCIPAL INVESTIGATOR: Facundo Fernandez

CONTRACTING ORGANIZATION: Georgia Institute of Technology, Atlanta, GA

REPORT DATE: June 2023

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
June 2023	Annual	15May2022-14May2023

4. TITLE AND SUBTITLE

Deciphering the Role of Progesterone in Ovarian Cancer Risk for BRCA Mutation Carriers

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-22-1-0391

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Jaeyeon Kim/Facundo Fernandez

E-Mail: jaeyeonk@iu.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

Indiana University School of Medicine, Indianapolis, IN 46202
Georgia Institute of Technology, Atlanta, GA 30332

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

Prevention and effective treatment of cancer require a deep understanding of the pathogenesis of a malignancy. As ovarian cancer is mostly diagnosed in advanced stages, however, the mechanisms underlying its etiology, inception, and progression remain poorly understood. Our proposed research aims to define progesterone signaling as a vital early intrinsic factor determining ovarian cancer development, particularly for high-risk women carrying a pathogenic BRCA1 or 2 mutation. High-grade serous ovarian cancer, also known as high-grade serous carcinoma (HGSC), is the most common and deadliest ovarian cancer type. One approach to overcome the present knowledge gap in ovarian cancer pathogenesis would be to use an animal model faithfully reproducing the clinical disease with molecular similarity to the human cancer. To this end, we developed mouse models that recapitulate the clinical metastases of human HGSC with histological, molecular, and genomic similarities. Applying one of these mouse models of HGSC, we have demonstrated that the ovarian hormone progesterone is a pivotal intrinsic factor inducing the development of HGSC equipped with metastatic capability. Crucially, mifepristone, an FDA-approved inhibitor of progesterone signaling, markedly extended survival of these mice by blocking the development and metastases of HGSC. Accordingly, these preclinical findings point to a compelling concept that progesterone signaling is a potential prophylactic target for ovarian cancer prevention, particularly for high-risk women, such as BRCA1/2-mutation carriers. In the proposed research, we will examine this progesterone hypothesis by interrogating the vital early role and mechanism of progesterone signaling in the development and metastasis of HGSC using a Brca1 model; and by examining genomic and metabolomic abnormalities influencing steroid hormone synthesis and response in BRCA1/2-mutation carriers. These studies will bring new insights into the role and mechanism of hormones in the development and metastasis of ovarian cancer. Additionally, they will offer sound rationale and useful guidance for future human trials targeting progesterone signaling with antiprogestins, such as mifepristone, for ovarian cancer prevention. Ultimately, these studies will pave the way for reliable risk prediction and effective nonsurgical prevention of this deadly malignancy in BRCA1/2-mutation carriers.

15. SUBJECT TERMS

None listed.

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
Unclassified	Unclassified	Unclassified	Unclassified	10	**19b. TELEPHONE NUMBER** *(include area code)*

TABLE OF CONTENTS

1. INTRODUCTION: The central hypothesis of this project is that ovarian progesterone (P4) is a crucial intrinsic factor determining ovarian cancer risk among *BRCA1/2*-mutation carriers. The specific hypotheses are: (1) P4 is a vital *early* factor inducing HGSC development and metastasis; and (2) genetic alterations impacting P4 levels determine individual ovarian cancer risks in *BRCA1/2* carriers. To test these hypotheses, we will examine the early role of ovarian P4, and the blocking effect of P4 signaling by an antiprogestin, in the development and metastasis of HGSC using a Brca1 mouse model; and comprehensively evaluate the genomic and metabolomic factors that influence steroid hormone synthesis and metabolism in *BRCA1/2*-mutation carriers.

2. KEYWORDS: Ovarian cancer, high-grade serous carcinoma, high-grade serous ovarian cancer, HGSC, BRCA1, BRCA2.

3. ACCOMPLISHMENTS:

Specific Aim 1: Evaluate whether P4 is a vital early factor determining HGSC development and metastasis	**Timeline (Months)**	**Site 1 (IUSM)**	**Site 2 (Georgia Tech)**	**Current Status**
Major Task 1 Hypothesis: early P4 exposure is vital to development of HGSC and acquiring its metastatic potential.				
Subtask 1: Effect of early, short-term P4 exposure on HGSC development and metastasis. • Submission of institution approved animal protocols and related material for DoD's ACURO approval. • Receive ACURO approval before initiating animal experiments. • 20 mice per group x 8 groups = 160 mice total.	1-24	x		Necessary animal protocol has been approved by IUSM IACUC and DoD ACURO Mouse breeding and generation is in progress to obtain experimental mice
Subtask 2: P4- and mifepristone-specific early molecular alterations in HGSC development • Submission of institution approved animal protocols and related material for DoD's ACURO approval. • Receive ACURO approval before initiating animal experiments. • 5 mice per group x 12 groups = 60 mice total.	1-24	x		Necessary animal protocol has been approved by IUSM IACUC and DoD ACURO Mouse breeding and generation is in progress to obtain experimental mice
Milestone(s) Achieved: Determination of a critical role of P4 in HGSC initiation; characterization of early molecular changes in P4-induced HGSC development.	24			
Major Task 2 Hypothesis: Blocking P4 signaling early for a short period effectively inhibits HGSC development and metastases.				
Subtask 1: Preventive effect of early, short-term blocking of P4 signaling with the antiprogestin mifepristone.	12-36	x		

• Submission of institution approved animal protocols and related material for DoD's ACURO approval. • Receive ACURO approval before initiating animal experiments. • 20 mice per group x 6 groups = 120 mice total.				
Subtask 2: Inhibitory effect of mifepristone on transformation of premalignant fallopian tube cells • Submission of institution approved animal protocols and related material for DoD's ACURO approval. • Receive ACURO approval before initiating animal experiments. • 5 mice per group x 4 groups x 3 repeats = 60 mice total.	12-30	x		
Milestone(s) Achieved: Determination of the preventive effect of short-term antiprogestin intervention on ovarian cancer.	36			
Specific Aim 2: Define genetic factors affecting steroid hormone levels in *BRCA1/2*-mutation carriers	**Timeline (Months)**	**Site 1 (IUSM)**	**Site 2 (Georgia Tech)**	
Major Task 1 hypothesis: *BRCA*-mutation carriers may harbor additional genomic abnormalities affecting steroidogenesis.				
Subtask 1: Examine genomic alterations affecting steroidogenesis among BRCA-mutation carriers. • Whole-exome sequencing (WES) analysis using genomic DNA samples from 70 unaffected and 70 affected BRCA carriers as well as 50 sporadic HGSC cases. • IRB protocol not required: de-identified, tissue-bank human samples were determined as Not Human Subjects Research by IRB. Subtask 2: Association between hormone levels and ovarian cancer development.	6-48	x		Early stages: in collaboration with IUSCCC Komen Tissue Bank (KTB), DNA sequencing data of BRCA carriers is being analyzed.
Milestone(s) Achieved: Identification of specific genomic alterations associated with hormone synthesis and response; Publication of 1-2 peer-reviewed papers.	48			
Major Task 2 hypothesis: Different levels of progesterone (P4) elicit distinct metabolomic changes.				

Subtask 1: Assess metabolomic alterations associated with P4 levels and steroidogenesis in a Brca1 mouse model and among *BRCA* carriers by mass spectrometry analysis. • Serum samples from the mouse studies from Specific Aim 1 Major Task 1 and 2: 340 samples total. • Serum samples from Specific Aim 2 Major Task 1: 190 samples total. Subtask 2: Development of in-depth steroidomics analysis to quantify metabolites in the steroid biosynthesis pathway.	1-48		x	Early stages: Analytic pipeline for in-depth steroidomics is under development
Milestone(s) Achieved: Determination of specific metabolites linked to different levels of P4; Mapping the abundance of all metabolites in the P4 biosynthesis pathway.	48			

Abbreviations

ACURO: Animal Care and Use Review Office
BRCA1: Breast Cancer 1 gene.
BRCA1/2: Breast Cancer 1 or 2 gene.
BRCA carriers: women carrying a pathogenic BRCA1 or 2 mutation.
HGSC: high-grade serous carcinoma, or high-grade serous ovarian cancer.
IUSM: Indiana University School of Medicine
IUSCCC: Indiana University Simon Comprehensive Cancer Center
P4: progesterone.

What was accomplished under the goals?

In the first year of the project, we had made expected progress in line with the goals laid out in the SOW. We had obtained the IUSM IACUC and DoD ACURO approval for mouse experiments. Mouse breeding had been progressing accordingly without any major issues or obstacles. Also, DNA sequencing analysis had been initiated using whole-genome sequencing (WGS) data from BRCA1 or 2 carriers and women without these mutations. Additionally, we have begun to develop an analytical liquid chromatography-mass spectrometry (LC-MS/MS) pipeline for in-depth steroidomics of animal serum using mass spectrometry.

Specific Aim 1: Evaluate whether P4 is a vital early factor determining HGSC development and metastasis.

To examine the hypothesis that early P4 exposure is critical to HGSC development and its metastatic potential, we have been in the process of generating the experimental female mice: Brca1$^{+/-}$-DKO mice (*Brca1* $^{flox/+}$ *Dicer1* $^{flox/flox}$ *Pten* $^{flox/flox}$ *Amhr2* $^{cre/+}$). To generate these experimental mice, we have also been generated breeding pairs with proper genotypes: male *Brca1* $^{flox/+}$ *Dicer1* $^{flox/flox}$ *Pten* $^{flox/flox}$ *Amhr2* $^{cre/+}$ mice; female *Brca1* $^{+/+}$ *Dicer1* $^{flox/flox}$ *Pten* $^{flox/flox}$ *Amhr2* $^{+/+}$ mice. With a steady flow of active breeding pairs being established, we expect to produce a total of 400 experimental mice throughout the project period.

Specific Aim 2: Define genetic factors affecting steroid hormone levels in *BRCA1/2*-mutation carriers.

BRCA1/2 carriers have high genetic risks of developing ovarian and breast cancer. Despite the high risks, not all BRCA1/2 carriers develop these cancers. Incomplete penetrance suggests that additional modifying factors (genetic, environmental, or both) are necessary for oncogenesis in BRCA1/2 carriers. Our previous research suggests that ovarian progesterone (P4) may be a critical determinant of cancer development in BRCA1/2 carriers. Thus, we hypothesize that BRCA1/2 carriers may harbor additional genomic abnormalities affecting steroidogenesis.

One gene of interest is CYP21A2, which encodes 21α-hydroxylase. This enzyme converts (i) P4 to 11-deoxycorticosterone and (ii) 17α-hydroxyprogesterone (17α-OHP4) to 11-deoxycortisol. Thus, defects in this enzyme leads to an accumulation of P4 and 17α-OHP4, which could enhance P4 signaling. Interestingly, mutations in the CYP21A2 gene are common in Ashkenazi Jews (1 in 3), who also have high incidence of BRCA1/2 mutations (1 in 40 women; 1:400 in the general population). Thus, we hypothesize that BRCA1/2 carriers might be more likely to harbor CYP21A2 mutations than women without BRCA1/2 mutations.

To test this hypothesis, we have first sought to examine CYP21A2 mutations in unaffected BRCA1/2 carriers and affected BRCA1/2 carriers with breast cancer as well as women negative for these BRCA1/2 mutations, in collaboration with the Komen Tissue Bank (KTB) at IUSCCC. We are currently analyzing WGS data from 51 BRCA1/2 carriers and 410 women negative for BRCA1/2 mutations. This hypothesis would be supported if the proportion of BRCA1/2 carriers with CYP21A2 mutations is significantly higher than that of BRCA1/2-negative women with CYP21A2 mutations. We will report the results in the year 2 progress report.

To examine both metabolism products and reactants in the progesterone signaling pathway, we have surveyed the cancer literature and compiled a list of target analytes that we will quantify using chemical standards as well as high resolution accurate mass tandem mass spectrometry. We are currently in the process of sourcing these standards from a number of chemical vendors and have started to optimize the conditions for ionization of these analytes using atmospheric pressure chemical ionization in an Orbitrap system. The next steps will be to construct external calibration curves for each analyte alone and in combination, followed by investigation of pilot animal serum samples in their native form as well as spiked with the target analytes. Recovery calculations will be performed and the method optimized as needed.

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

This project has allowed our lab and me to initiate collaborations with other investigators within the institution, nationally, and internationally. We have recruited a new graduate student to the project and she is being trained in metabolomics and lipidomics methodologies.

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

Specific Aim 1: Evaluate whether P4 is a vital early factor determining HGSC development and metastasis.
Major Task 1 Hypothesis: early P4 exposure is vital to HGSC development and its metastatic potential.

Subtask 1: Effect of early, short-term P4 exposure on HGSC development and metastasis.

To test whether early P4 exposure is crucial to HGSC development and metastasis, we will treat Brca1$^{+/-}$-DKO mice with P4 (or a placebo) for 3 wk (6 mg total) at a different time point after ovariectomy: (i) upon ovariectomy at 5-6 wk of age, (ii) 1 week after ovariectomy, (iii) 3 wk after ovariectomy, or (iv) 3 months (m) after ovariectomy (20 mice in each group). Tumor development and metastasis will be examined using histopathological and molecular analyses.

Subtask 2: P4- and mifepristone-specific early molecular alterations in HGSC development.

To capture the P4-specific early molecular alterations leading to HGSC development, Brca1$^{+/-}$-DKO mice will be treated with P4 or mifepristone for variable lengths (1 wk to 3 m). In this experiment, Brca1$^{+/-}$-DKO mice, upon ovariectomy at 5-6 weeks of age, will be treated with P4 or a placebo: (i) 1 wk (2 mg total), (ii) 3 wk (6 mg), or (iii) 3 m (25 mg). In parallel, Brca1$^{+/-}$-DKO mice, without ovariectomy, will be treated at 5-6 weeks of age with mifepristone or a placebo: (i) 1 wk (0.7 mg), (ii) 3 wk (2.1 mg), or (iii) 3 m (9 mg). (12 groups; 5 mice/group.) Mice in each group will be sacrificed at 1 wk, 3 wk, or 3 m after treatment. Premalignant or tumor tissues in the fallopian tube will be used for RNA-Seq analysis.

Major Task 2 Hypothesis: Blocking P4 signaling early for a short period effectively inhibits HGSC development and metastases

Subtask 1: Preventive effect of early, short-term blocking of P4 signaling with the antiprogestin mifepristone

To assess whether blocking P4 signaling early for a short period is sufficient for HGSC prevention, we will treat Brca1$^{+/-}$-DKO mice with the antiprogestin mifepristone for variable lengths at a premalignant stage. In this experiment, Brca1$^{+/-}$-DKO mice will be treated with mifepristone (or a placebo), starting at 5-6 wk of age, for 1 wk (0.7 mg), 3 wk (2.1 mg), or 3 m (9 mg) (20 mice in each group). Mouse survival as well as tumor development will be examined. This experiment will likely begin toward the end of year 2.

Subtask 2: Inhibitory effect of mifepristone on transformation of premalignant fallopian tube cells

We will establish primary premalignant fallopian tube cultures using Brca1$^{+/-}$-DKO mice.

Specific Aim 2: Define genetic factors affecting steroid hormone levels in *BRCA1/2*-mutation carriers.
Major Task 1 hypothesis: *BRCA*-mutation carriers may harbor additional genomic abnormalities affecting steroidogenesis.

Subtask 1: Examine genomic alterations affecting steroidogenesis among BRCA-mutation carriers.

In addition to examining the correlation between BRCA1/2 mutations and CYP21A2 mutations, we will also determine whether CYP21A2 mutations are correlated with increased risk of breast cancer in BRCA1/2 carriers, using the KTB WGS data. Besides CYP21A2 mutations, we will also examine other gene variants, including progesterone receptor variants, which are linked to steroidogenesis, to evaluate the potential contributions to cancer risks in BRCA1/2 carriers. We will also expand this analysis to ovarian cancer risk in BRCA1/2 carriers.

Subtask 2: Association between hormone levels and ovarian cancer development.

To examine whether serum P4 levels correlate with ovarian cancer development among BRCA1/2 carriers, we will begin to obtain serum samples, via collaboration, from unaffected carriers (*BRCA*-mutation carriers without cancer) and affected carriers (*BRCA*-mutation carriers diagnosed with ovarian or breast cancer).

Major Task 2 hypothesis: Different levels of progesterone (P4) elicit distinct metabolomic changes.

Subtask 1: Assess metabolomic alterations associated with P4 levels and steroidogenesis in a Brca1 mouse model and among *BRCA* carriers by mass spectrometry analysis.

Serum samples will be collected from Brca1$^{+/-}$-DKO mice with or without P4 or mifepristone. Serum samples obtained from Aim 2 Main Task 1 (Subtask 2) will also be used for metabolomics analysis, which will be performed once all samples are collected.

Subtask 2: Development of in-depth steroidomics analysis to quantify metabolites in the steroid biosynthesis pathway.

Quantitation of a number of metabolites in the progesterone signaling pathway will be achieved using LC-MS/MS approaches. These methods will be carefully validated to ensure the accuracy and precision of the results.

4. IMPACT: ***Nothing to Report.***

5. CHANGES/PROBLEMS: ***Nothing to Report.***

6. PRODUCTS: ***Nothing to Report.***

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Jaeyeon Kim
Project Role: Principal Investigator
Contribution to Project: Dr. Kim has designed and supervised the project.

Name: Soojin Park
Project Role: Postdoctoral researcher
Contribution to Project: Dr. Park has performed mouse experiments as well as mouse management.

Name: Thu-Huyen Pham
Project Role: Postdoctoral researcher
Contribution to Project: Dr. Pham has performed mouse experiments as well as mouse management.

Name: Andro Botros
Project Role: Research Assistant
Contribution to Project: Mr. Botros has performed mouse genotyping as well as mouse management.

Name: Nicole Harris
Project Role: Research Assistant
Contribution to Project: Ms. Harris has performed mouse genotyping.

Name: Emily Massa
Project Role: Research Assistant
Contribution to Project: Ms. Massa has performed mouse genotyping.

Name: Elisabeth Schwiebert
Project Role: Graduate student (Georgia Institute of Technology)
Contribution to Project: Elisabeth is developing the LC-MS/MS steroidomics method.

Name: Dr. Facundo Fernández
Project Role: Partnering PI (Georgia Institute of Technology)

Contribution to Project: Dr. Fernández has designed and supervised the steroidomics analysis of the project.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period? Nothing to Report.

What other organizations were involved as partners?

Organization Name: Georgia Institute of Technology.
Location of Organization: Atlanta, Georgia.
Partner's contribution to the project: Collaboration.

8. SPECIAL REPORTING REQUIREMENT

COLLABORATIVE AWARDS: Dr. Facundo Fernández, the Partnering PI at Georgia Institute of Technology, will submit his Annual Report separately.

9. APPENDICES: None.