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TITLE: Inflammation and the Malignant Transformation of Endometriosis

PRINCIPAL INVESTIGATOR: Holly Harris, ScD

CONTRACTING ORGANIZATION: Fred Hutchinson Cancer Research Center
Seattle, WA 98109

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6. AUTHOR(S)

Holly Harris, ScD

E-Mail: hharris@fredhutch.org

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

Fred Hutchinson Cancer Research Center
1100 Fairview Ave N, Seattle, WA 98109

Michigan State University
17 Michigan Street NE, Grand Rapids, MI 49503

Brigham and Women's Hospital, Inc.
221 Longwood Ave, Boston, MA 02115

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

We are conducting a study to examine the association between inflammatory markers in peritoneal fluid and driver mutations and immunohistochemical (IHC) markers of cell proliferation and invasiveness in endometriosis tissue. We will also evaluate whether inflammation-related epidemiologic factors and systemic inflammation (e.g., CRP, IL-6 plasma levels) are associated with inflammatory markers in peritoneal fluid. Data and specimens for this study have been previously collected from A2A cohort, a longitudinal cohort of women oversampled for those surgically diagnosed with endometriosis. This project consists of selecting appropriate individuals with peritoneal fluid, endometriosis tissue, blood, and epidemiologic data, accessing samples for biospecimen assays, and conducting analyses. Targeted sequencing is being used to identify our genes of interest, ELISA is being used to measure inflammatory biomarkers in peritoneal fluid and blood, and IHC to assess the tissue expression of selected markers. To date we have conducted the necessary pilot studies to ensure a robust workflow. Currently our work has been paused because of the COVID-19 pandemic and there are no results to present at this time.

15. SUBJECT TERMS

endometriosis, ovarian cancer, cancer driver mutations, peritoneal fluid, inflammation, epidemiology, risk factors

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Introduction

We are conducting a study to examine the association between inflammatory markers in peritoneal fluid and driver mutations and immunohistochemical (IHC) markers of cell proliferation and invasiveness in endometriosis tissue. We will also evaluate whether inflammation-related epidemiologic factors and systemic inflammation (e.g., CRP, IL-6 plasma levels) are associated with inflammatory markers in peritoneal fluid. Data and specimens for this study have been previously collected from A2A cohort, a longitudinal cohort of women oversampled for those surgically diagnosed with endometriosis. This project consists of selecting appropriate individuals with peritoneal fluid, endometriosis tissue, blood, and epidemiologic data, accessing samples for biospecimen assays, and conducting analyses. Targeted sequencing is being used to identify our genes of interest, ELISA is being used to measure inflammatory biomarkers in peritoneal fluid and blood, and IHC to assess the tissue expression of selected markers.

Keywords

endometriosis, ovarian cancer, cancer driver mutations, peritoneal fluid, inflammation, epidemiology, risk factors

Accomplishments

What were the major goals of this project? What was accomplished under these goals?

Goal 1. Administrative tasks

Specific objectives: Execute subcontract and material transfer agreements, submit Fred Hutch IRB application, and submit DOD HRPO application.

Key outcomes: Drs. Harris, Terry, and Missmer led staff in preparation and revision of documents necessary to obtain these approvals.

Status: Complete.

Goal 2. Specimen identification and processing

Specific objectives: Identify and locate suitable existing samples for study inclusion, create master surgical and participant characteristics database.

Key outcomes: We have identified 214 cases eligible for this study (peritoneal fluid, endometriosis tissue, blood collection, and questionnaire data). As part of this process we conducted a pilot study to ensure that the inflammatory markers we wanted to examine could be adequately assayed in peritoneal fluid. This pilot was successful, with CVs ranging of 1% for hsCRP and IL-6, 3% for IL-10, 4% for TNFR2, and 8% for IL1-beta. We also piloted test DNA and RNA extraction from the endometriosis tissue samples and the extraction was successful. The final step in this process is to verify the RNA quality, however this work was halted by the COVID-19 pandemic. After receiving these results we will identify the 58 participants with tissue, peritoneal, and blood samples to be processed

Status: 25% complete

Goal 3. Assays

Specific objectives: DNA sequencing and gene expression profiling, RNA seq, and peritoneal and blood inflammatory assays. Biomarker data merged with covariate data.

Status: On hold. Due to the COVID-19 pandemic we are waiting for the labs to re-open so that we can initiate these assays.

Goal 4. Data analysis and manuscript publication

Specific objectives: This task includes data cleaning, analyses, and preparation and submission of manuscripts.

Status: Not yet initiated.

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

Nothing to report.

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 these goals?

In the next reporting period we plan to complete the remaining study activities as soon as COVID-19 restrictions are lifted.

Impact

What was the impact on the development of the principal disciplines of this 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 of society beyond science and technology?

Nothing to report.

Changes/Problems

Changes in approach and reasons for change.

In discussion with pathologists regarding the sufficiency of endometriosis tissue lesion size (which are substantially smaller than most malignant tumors) we determined would need to pivot from IHC to using RNA seq to assess Ki-67, E-cadherin, α- and ß-catenin status. This approach has been successful used in other studies. This is a change in assessment method but does not impact the objectives and scope of the project.

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

The COVID-19 pandemic and subsequent laboratory shut-downs caused delays to our study.

Changes that had a significant impact on expenditures.

Nothing to report.

Significant changes in use or care of human subjects.

Nothing to report.

Significant changes in use or care of vertebrate animals.

n/a

Significant changes in biohazards and/or select agents.

Nothing to report.

Products

Publications, conference papers, and presentations

Nothing to report.

Websites or other internet sites

Nothing to report.

Technologies or techniques

Nothing to report.

Inventions, patent applications, and/or licenses

Nothing to report.

Other products

Nothing to report.

Participants & Other Collaborating Organizations

What individuals have worked on this project?

Name	Degree	Project Role	Researcher Identifier	Person Months	Contribution to Project	Funding Support
Harris, Holly	ScD	Principal Investigator	0000-0002-2572-6727	1	PI responsible for all facets of the study	N/A

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

Yes, Dr. Harris' other support has changed as follows.
Grants R03 HD081064 and K22 CA193860 have expired and the 3 new grants below have been funded.

BCRF-18-085 10/1/2018 – 9/30/2020 1.2 CM
Breast Cancer Research Foundation (Kensler) $248,283
Adolescent and early adulthood adherence to an anti-inflammatory diet and effects on breast cancer intermediate endpoint/risk factors
Our goal is to identify actionable ways that we can reduce breast cancer risk through healthy dietary changes.
Role: Co-Investigator

R01 NR017951-01A1(Harris) 2/11/2019 – 12/31/2022 2.4 CM
NIH $2,051,225
An AHEI Dietary Intervention to Reduce Pain in Women with Endometriosis
Endometriosis affects approximately 10% of reproductive age women and incurs significant health care costs and morbidity. This randomized controlled trial will investigate a 12-week dietary intervention based on the Alternative Healthy Eating Index-2010, examining its effects on pain, quality of life, and inflammatory biomarkers among women with laparoscopically-confirmed endometriosis. The study results will help us identify evidence-based, modifiable, dietary factors that decrease pain and improve quality of life among women with endometriosis.
Role: PD/PI

W81XWH910307 (Kaaks) 9/1/2019 – 8/31/2021 0.6 CM
Department of Defense (DOD) $400,000
PREDICT: the Prospective Early Detection Consortium for Ovarian Cancer
The overarching goal of this project is to establish "PREDICT" – the Prospective Early Detection Consortium for Ovarian Cancer – for the application of state-of-the-art "omics" technologies for biomarker discovery and validation. This international consortium is comprised of large-scale prospective cohort studies and biobanks with blood samples collected prior to diagnostic surgery. Within PREDICT we will calibrate previous CA125 measurements across all studies, validate a set of candidate tumor associated autoantibodies, and leverage existing data to determine diagnostic microRNA signatures for ovarian cancer to be prospectively validated in future studies.
Role: Co-Investigator

What other organizations were involved as partners?

Organization Name: Brigham and Women's Hospital, Inc.
Location of Organization: Boston, MA 02115
Partner's Contribution: Collaboration

Organization Name: Michigan State University
Location of Organization: Grand Rapids, MI
Partner's Contribution: Collaboration

Special Reporting Requirements

n/a

Appendices

n/a