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CONTRACTING ORGANIZATION: Fred Hutchinson Cancer Research Center

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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 RNAseq to assess the tissue expression of selected markers. To date we have conducted the assays to measure IL-6, IL1b, TNFR2 in blood. Peritoneal fluid samples are at the laboratory and are currently being assay. We have also successfully completed our pilot study of endometriosis tissue DNA/RNA extractions and sequencing and are currently pulling tissues from the biorepository to be send for DNA/RNA extraction. Our work this passed year was delay to lab shut downs due to the COVID-19 pandemic but now that labs are running again we are making progress towards having all of the necessary lab work done and when it is complete we will start data analysis.

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 RNASeq 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. Specimen identification and processing

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

Key outcomes: RNA quality was verified [insert results]. We have now identified the 58 participants with tissue, peritoneal, and blood samples to be processed (Aim 1) and 100 total participants (42 additional from Aim1) to have peritoneal and blood samples processed (Aim 2).

Status: 100% complete

Goal 2. Assays

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

Key outcomes: IL-6, IL-beta, TNFalpha have been assay in the blood samples of all 100 participants. Peritoneal fluid samples are currently in the laboratory being assayed. We have completed the endometriosis tissue DNA/RNA extraction and sequencing pilot (described below). Tissue is now being pulled from the biorepository to send to the lab for DNA/RNA extraction. Germline DNA have been extracted, and are in the queue to be identified, pulled, and shipped to the sequencing core lab for exome sequencing.

Status: 70%

Endometriosis tissue DNA/RNA extractions and sequencing pilot

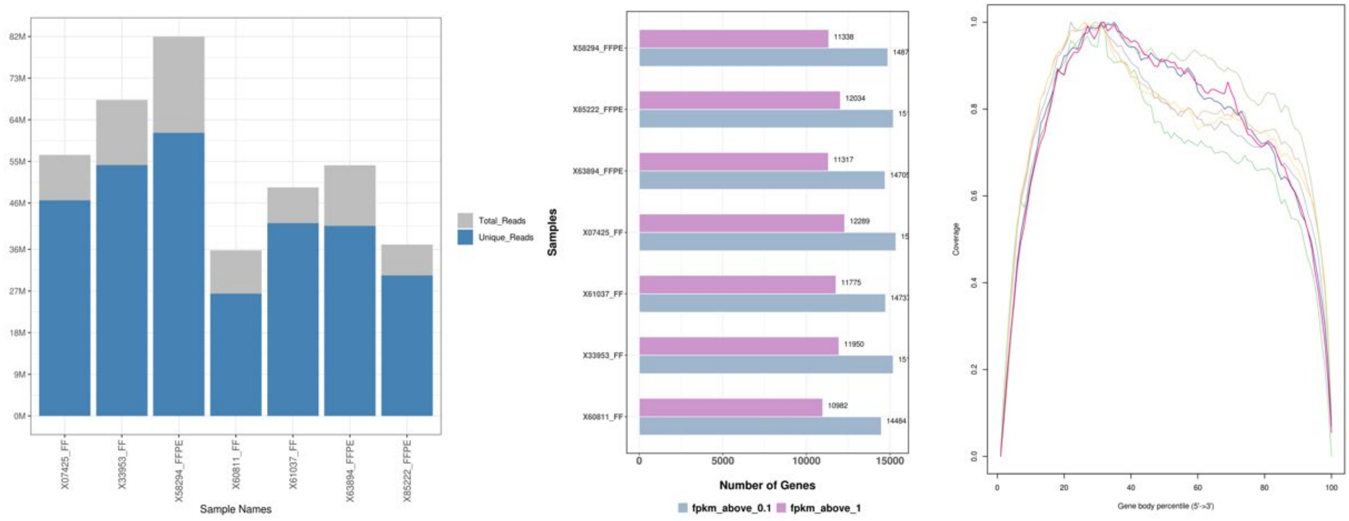
We have successfully extracted RNA from 8 superficial endometriosis tissue samples (4 fresh frozen samples and 4 formalin-fixed paraffin embedded (FFPE) samples) at the Dana-Farber Cancer Institute Molecular Pathology Core Laboratory and successfully generated RNA sequencing data on 7 samples (4 fresh frozen and 3 FFPE samples) working with the Molecular Biology Core Facilities at Dana-Farber Cancer Institute. As shown in **Table 1**, we observed successful RNA extraction with yielded RNA concentrations between 10 and 45 ng/uL for the 3 FFPE samples and between 10 and 512 ng/uL for 4 fresh frozen samples. All 4 fresh frozen samples had percentage of fragments greater than 200 nucleotides (DV200) > 70% and 3 of the 4 FFPE samples had DV200 ≥ 50%.

We performed RNA sequencing on RNA extracted from 4 fresh frozen and 3 FFPE samples. As shown in **Figure 2**, we were able to detect about 15,000 genes regardless of sample type and observed good read counts and 5' to 3' coverage for both fresh frozen and FFPE samples. Preliminary analyses of these data were conducted further examining the number of genes that were mapped and detailed quality control metrics of the generated data, and potential batch effect by basic covariates. We did not observe clear batch effect by basic covariates including sample type (i.e. fresh frozen, FFPE), age, year at surgery, hospital type, or body mass index at surgery, further supporting the feasibility of this assay method.

Table 1. Quality control metrics of the extracted RNA samples from 8 SPE tissue samples

		RiboGreen Quant			BioAnalyzer RNA QC		
Sample Type	**ID**	**RNA Con.c** [ng/uL]	**RNA Volume** [uL]	**RNA Yield** [ng]	**RNA Con.c** [pg/uL]	**RIN#**	**DV200**
Fresh Frozen	**X60811**	14.24	25	356.00	1997	N/A	>70%
Fresh Frozen	**X33953**	512.10	25	12802.50	24109	5.3	/
Fresh Frozen	**X61037**	25.28	25	632.00	2767	7.3	/
Fresh Frozen	**X07425**	9.97	25	249.25	657	8.2	/
FFPE scroll	**X17281**	0.75	25	18.75	59	1.2	<30%
FFPE scroll	**X63894**	44.93	25	1123.25	2575	2.2	30-50%
FFPE scroll	**X85222**	10.31	25	257.75	392	2.4	50-70%
FFPE scroll	**X58294**	19.00	25	475.00	1431	2.1	50-70%

Figure 2. Quality control metrics on RNAseq data from 4 fresh frozen and 3 FFPE ectopic endometriosis samples.



Furthermore, we were able to successfully generate exome sequencing data using DNA extracted from 4 fresh frozen and 2 FFPE samples. More than 20,000 variants were identified in both fresh frozen and FFPE samples, demonstrating that the exome sequencing quality does not differ between these different sample type (**Table 2**). Identified variants included missense variants, frameshift variants, structural interaction variants. Of the 8 cancer driver mutations that were hypothesized in this application, there were up to 3 somatic mutations detected in these superficial endometriosis tissue samples (**Table 3**). We are planning to sequence germline DNA samples which will allow us to detect copy number variants and identify somatic mutations with more precision.

Table 2. Summary of variants detected

	Number of variants				
Sample	**Raw**	**PASS filters**	**On target unfiltered (multiallelic split)**	**PASS on target**	**PASS panel**
20201215_FFPE_X58294_KT8112_S187	21,348	5,132	21,741	5,132	2
20201215_FFPE_X85222_KT8112_S186	18,673	4,924	18,976	4,924	1
20201215_FF_X07425_KT8112_S189	24,940	5,623	25,640	5,623	1
20201215_FF_X33953_KT8112_S191	29,137	4,893	30,153	4,893	2
20201215_FF_X60811_KT8112_S188	21,753	4,949	22,290	4,949	0
20201215_FF_X61037_KT8112_S190	26,080	4,844	26,940	4,844	3

Table 3. Number of somatic mutations detected

Sample	# of somatic variants (out of the 8*)	Type of somatic mutation
FFPE_X58294	2	ARID1A, PTEN
FFPE_X85222	1	PPP2R1A
FF_X07425	1	PTEN
FF_X33953	2	KLLN, ERBB2
FF_X60811	0	
FF_X61037	3	ARID1A, PTEN, KRAS

*ARID1A, BRAF, CTNNB1, ERBB2, KRAS, PIK3CA, PPP2R1A, PTEN

Goal 3. Data analysis and manuscript publication

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

Key outcomes: Data cleaning has begun on the blood biomarker assays that have been completed, including calculating CVs and identifying outliers. No other work can be initiated until the other assays are complete.

Status: 5%

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.

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.

None.

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 but now that laboratories are open we are working diligently to complete the remaining lab work and begin data analysis.

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.
Grant P30 CA015704 has expired and the 3 new grants below have been funded.

BCRF-20-085 10/1/2020 – 9/30/2021 1.8 CM
Breast Cancer Research Foundation (Kensler)
Estrogen related dietary patterns and body composition: impacts on hormone levels, breast density and premenopausal breast cancer risk
This study will examine whether a diet associated with estrogen levels is associated with estrogen levels is associated with risk of breast cancer. We will assess breast density in younger women, under the age of 40, and examine the influence of estrogen levels measured in blood on breast density. In addition, we will examine how breast density and body fat distribution are related.
Role: Co-Investigator

CASCADIA DATA ALLIANCE Collaboration Award 11/1/2020 – 10/31/2021 1.2 CM
(Huntsman, Harris, Morgan, Bashashati)
Pathology AI for a Federated Quality Assurance Program: Ovarian Cancer Pilot
Our vision is to establish an international network for AI-based pathology quality assurance across different sites, which can operate without explicitly sharing any patient data. In this project, as a proof of concept, we propose to develop and deploy an ML-based ovarian cancer histopathology classifier, using privacy-preserving data sharing mechanisms.
Role: PD/PI

RFP #75N92019R0030 (Anderson) 2/1/2021 – 1/31/2026 0.48 CM
NHLBI
Women's Health Initiative (WHI) - Clinical Coordinating Center
The primary objectives of the Women's Health Initiative (WHI) Clinical Coordinating Center (CCC) for the Extension Study (ES-IV) are: To maximize the knowledge gained from the WHI resource to impact public health, particularly of older women, by conducting high quality studies examining the epidemiology, biologic mechanisms, and health outcomes among postmenopausal women; To maintain and expand the WHI resource to address these public health concerns through additional active and passive follow-up (2020-2025) and collection of high quality outcomes data; To provide an efficient service infrastructure to support the governance of the WHI and facilitate the use of this resource by the broader research community; To engage and facilitate access to researchers not previously associated with WHI, particularly early career and under-represented minority investigators.
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