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TITLE: Disordered Differentiation of Tubal Epithelia: Setting the Stage for Neoplastic Transformation

PRINCIPAL INVESTIGATOR: Kate Lawrenson

CONTRACTING ORGANIZATION: Cedars-Sinai Medical Center, Los Angeles, CA

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4. TITLE AND SUBTITLE

Disordered Differentiation of Tubal Epithelia: Setting the Stage for Neoplastic Transformation

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5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Most high-grade serous 'ovarian' cancers derive from cells lining the fallopian tube. Research by our laboratories and others has shown that in women with inherited mutations in genes such as BRCA1 that confer a high risk of ovarian cancer, fallopian tube cells fail to fulfill the normal cellular life cycle and become 'stuck' in a state that is more prone to carcinogenesis. We believe that this failure of fallopian tube cells to properly differentiate explains, at least in part, why these women are more at risk of ovarian cancer. Similar changes also occur in post-menopausal women, albeit to a lesser extent. The overarching idea underlying this project is that if we can force these cancer-prone cells to complete the normal differentiation process that brings them into a less cancer prone state then we could effectively eliminate a woman's risk of ovarian cancer. Our long-term goal is to develop an oral drug that could be taken around the time of menopause to force the normal differentiation process to occur, and prevent ovarian cancer from occurring. This represents an entirely new paradigm for ovarian cancer prevention and is conceptually linked to the highly effective differentiation therapy for acute promyelocytic leukemia, which induces a complete remission in most patients due to forced differentiation of leukemic cells.

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	9	**19b. TELEPHONE NUMBER** *(include area code)*

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1. Introduction

Most high-grade serous 'ovarian' cancers derive from cells lining the fallopian tube. Research by our laboratories and others has shown that in women with inherited mutations in genes such as *BRCA1* that confer a high risk of ovarian cancer, fallopian tube cells fail to fulfill the normal cellular life cycle and become 'stuck' in a state that is more prone to carcinogenesis. We believe that this failure of fallopian tube cells to properly differentiate explains, at least in part, why these women are more at risk of ovarian cancer. Similar changes also occur in post-menopausal women, albeit to a lesser extent. The overarching idea underlying this project is that if we can force these cancer-prone cells to complete the normal differentiation process that brings them into a less cancer prone state then we could effectively eliminate a woman's risk of ovarian cancer. This project will perform single cell profiling of tubal cells from high-risk and average risk women to identify the key transcription factor networks disrupted in mutation carriers. An orthogonal *in vitro* screen will also prioritize critical factors based on function. Key factors will then be individually validated in organoid models. In the long-term, we intend to leverage these insights into disordered differentiation to identify novel approaches for chemoprevention of tubal cancers.

Keywords

Fallopian tube secretory epithelial cells, Fallopian tube ciliated epithelial cells, high-grade serous carcinoma, transcription factors, PAX8, FOXJ1, organoids, single-cell genomics,

2. Accomplishments

What were the major goals of the project?

Specific Aim 1: To map transcriptional circuitries in BRCA1 mutant tubal epithelia.

Major Task 1: Obtain HRPO approval

Major Task 2: Single cell analysis of human specimens, target validation

Specific Aim 2: To identify inducers of the transitional state in tubal epithelia.

Major Task 2: cDNA overexpression screen and validation

What was accomplished under these goals?

Major Task 1: We successfully obtained HRPO approval for the project prior to the start of the work. (E03070.1a, approved June 28 2022)

Aim 1.
Major Task 2: Single cell analysis of human specimens, target validation

1) Major activities: We have used 10x single cell RNA-sequencing to profile fimbria brushings from 8 additional patients and are currently performing integrated analyses of those data. We aim to add profiles for another 6 mutation carriers and 8 controls to complete the targeted sample size for this aim.
2) Specific objectives: To identify features of BRCA mutation in the fallopian tube fimbria; To identify key transcription factor networks disrupted in BRCA-mutant fallopian tube epithelial cells; To validate key TFs *in vitro*
3) Significant results or key outcomes: Analyses of these data are ongoing but preliminary results are consistent with our hypothesis that epithelial differentiation is perturbed in carriers of high-risk mutations (Figure 1).
4) Other achievements: none to report

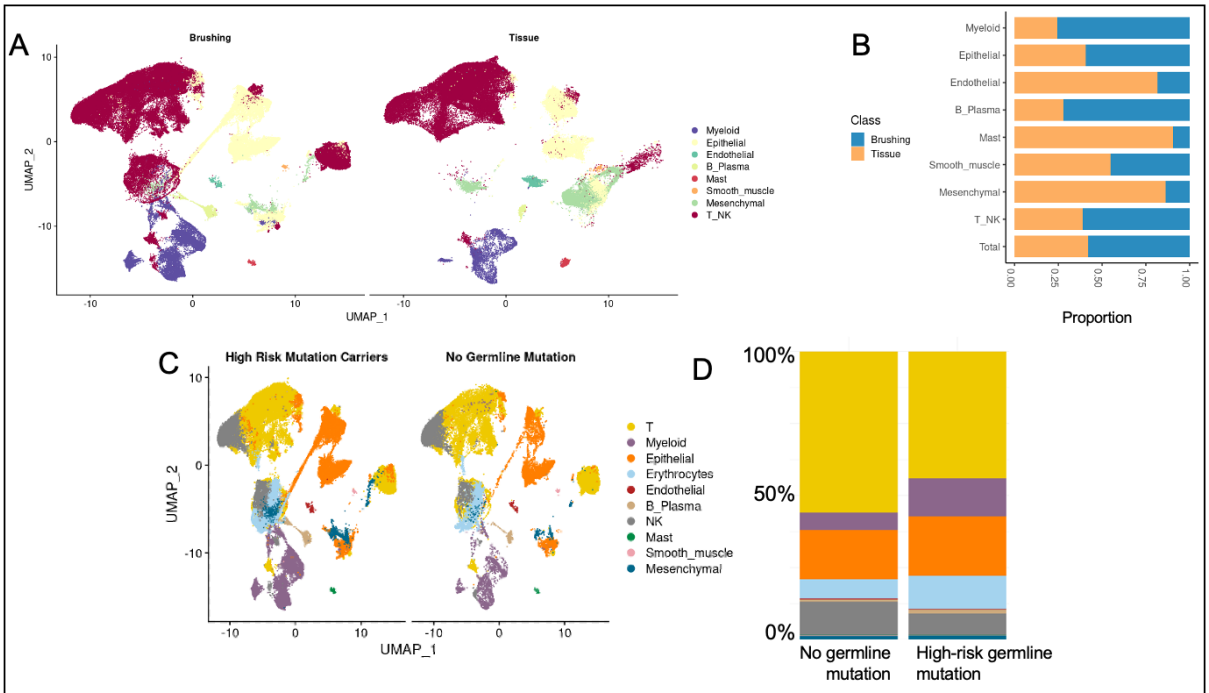

Figure 1. Single cell analysis of human fallopian tube tissues and brushings in carriers and non-carriers of high-risk mutations. (A) Fimbria brushings from average and high-risk women are broadly similar to tissue profiles with notable key alterations. (B) Mesenchymal, endothelial and mast cells are depleted in brushings which preferentially enriched epithelial cells and intra-epithelial and blood immune cells. Total column shows the expected distribution under the null. (C, D) Brushings of mutation carriers have an expanded epithelial fraction compared to non-carriers. Immune populations are also altered.

Aim 2.
Major Task 2: cDNA overexpression screen and validation

1) Major activities: activities have focused on reagent preparation – we have been preparing the DNA and virus for the screen, expanding organoid cultures, and optimizing viral delivery to prepare for performing the screen and subsequent validation in year 2.
2) Specific objectives: To perform a transcription factor screen to identify modifiers of epithelial differentiation; To validate key TFs *in vitro*
3) Significant results or key outcomes: Optimization of viral delivery continues. Protocols and antibodies for imaging have been optimized.
4) Other achievements: At the time of submission all our organoids were from non-carriers. We have optimized our protocols for culture of cells harvested by exfoliative cytology and have been able to grow cells from *BRCA2* mutation carriers. We will incorporate those unique models into this project as appropriate.

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 the goals?

Aim 1 – goals for the next 12 months

- *Profile an additional 14 patients*
- *Integrated analysis of transcription factor networks in high-risk and average risk women*
- *Validation of top candidate TFs*
- *Write, submit and revise manuscript describing tissue-based profiling and validation*

Aim 2 – goals for the next 12 months

- *Perform TF overexpression screen, following on from final stages of optimization of virus delivery*
- *Validation of top candidate TFs*
- *Write, submit and revise manuscript describing screen and validations [predicted to extend into post-award period]*

4. Impact

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

Little is known about the mechanisms that regulate fallopian tube epithelial differentiation. The mucosal lining of fallopian tubes comprises a columnar epithelium of predominantly ciliated and secretory epithelial cells. The ratio of ciliated to secretory cells may be modulated by changes in steroid hormones, age, and certain germline genetic risk factors including highly penetrant mutations in *BRCA1* or *BRCA2*. Our single cell profiling of fallopian tubes and a recent mouse model support a model in which a unipotent epithelial progenitor differentiates first into secretory cells and then into ciliated cells. However, the profiles of high-risk fallopian tubes are poorly characterized, largely because standard-of-care protocols require tissues to be fully embedded in paraffin. Our data set of fimbria brushings is the first of its kind for this subset of patients, and is showing perturbed differentiation pathways in tubal epithelium that we expect contribute to cancer risk. This opens up a new niche for chemoprevention research in high-risk mutation carriers. It is also possible that this approach could be used in the future to determine the impact of inherited genetic variants of unknown significant.

- **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**
    - *HPRO approval took around 2 months longer to complete than expected. However we are on track to make up for the lost time cause by this small delay.*
- **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 agents**
    - *Nothing to Report*

6. Products

Nothing to Report

7. Participants & Other Collaborating Organizations

Name:	*Kate Lawrenson*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	*0000-0002-6469-2515*
Nearest person month worked:	1
Contribution to Project:	*Dr. Lawrenson has directed the research, reviewed and interpreted the results and had oversight of all implementation, analysis and troubleshooting. She also obtained and maintained regulatory permissions required for the research*
Funding Support:	

Name:	*Marcela Haro*
Project Role:	*Co-I*
Researcher Identifier (e.g. ORCID ID):	*0000-0002-1696-8441*
Nearest person month worked:	3
Contribution to Project:	*Dr. Haro performed the single cell sequencing, analysis and interpretation plus the organoid culture and optimizations for Aim 2.*
Funding Support:	

Name:	*Forough Abbasi*
Project Role:	*Research Associate*
Researcher Identifier (e.g. ORCID ID):	*0000-0002-1696-8441*
Nearest person month worked:	2
Contribution to Project:	*Ms Abbasi provided technical support for human tissue processing and organoid culture.*
Funding Support:	

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

Dr. Lawrenson is PI on the following new awards:

Title: *iPSC Modeling of Ovarian Cancers in BRCA1&2 Mutation Carriers*
Effort: 5%
Supporting Agency: Ovarian Cancer Research Alliance
Performance Period: 1/1/23 – 12/31/25
Funding Amount:
Major Goals: Our goal is that the development and characterization of human avatars of fallopian tube epithelium (FTE) differentiated from induced pluripotent stem cell (iPSC) models of women carrying germline BRCA1 or BRCA2 mutations can identify novel molecular mechanisms and biomarkers associated with early-stage pathogenesis of high grade serous ovarian cancers (HGSOCs).
Specific Aims:

1. Establish human iPSC models of FT developing from a germline background of BRCA1&2 mutation
2. Create human avatars of HGSOC developing from a genetic background of BRCA1&2 & TP53 mutations using iPSC from women with BRCA 1&2 mutations engineered to express functional TP53 mutations
3. Evaluate candidate proteins associated with BRCA 1/2 mutations as early stage predictive and diagnostic biomarkers of HGSOC

Title: *A Multifaceted Approach to Biomarker Discovery for BRCA-Associated Fallopian Carcinoma*
Effort: 5%
Supporting Agency: DOD OCRP
Grants Officer: grants@ocrahope.org, 2212-268-1002
Performance Period: 9/30/23 – 9/29/26
Funding Amount:
Major Goals: We propose that by combining cutting edge single cell genomics technologies that can deconvolute fallopian tube heterogeneity, induced pluripotent stem cell (iPSC) methods to accurately model molecular changes in fallopian tube pathology during the earliest stages of BRCA-associated neoplastic development, and state-of-the art methylation based screening methods we can identify novel candidate biomarkers of early stage HGSC.
Specific Aims:
Specific Aim 1. To map cellular and molecular hallmarks of high-risk fallopian tube epithelium
Specific Aim 2. To identify candidate biomarkers in a step-wise iPSC model of BRCA1-associated HGSC
Specific Aim 3. To establish a methylation-based biomarker screening panel for early-stage HGSC

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

Nothing to Report

7. Special Reporting Requirements

Not applicable.

8. Appendices

None.