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TITLE: Elucidating Early Events in HGSC Pathogenesis: A Single-Cell Multiomics Approach to Robustly Trace Cell Lineage, Clonality, and Phenotypes of TP53-Mutated Cells

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14. ABSTRACT : 200 WORDS SUMMARY OF MOST SIGNIFICANT FINDINGS DURING THE RESEARCH PERIOD

The purpose of this project is to characterize lineage relationships within epithelial cells of the fallopian tube using single cell assays leveraging mitochondrial DNA mutations to determine clonality. Better understanding the cell makeup and lineages in the fallopian tube may give insight into the early stages and initiation of tubo-ovarian high-grade serous carcinoma. To accomplish this we are collecting fresh human fallopian tube, ovarian, and endometrial tissues and profiling the cells at the single cell level to simultaneously assess cell states and clonality. To date, we have profiled 6 samples from 2 patients using mitochondrial-single cell ATACseq, with the computational analyses and additional sample collection/processing underway. We have been technically successful in obtaining and processing the samples, acquiring high quality sequencing data. The significance of the results and final conclusions will be better understood with the additional samples and more in-depth computational analyses.

15. SUBJECT TERMS
None listed.

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1. INTRODUCTION:

The most common and lethal subtype of ovarian cancer is high grade serous carcinoma (HGSC). It has been increasingly accepted that the fallopian tube may be the origin of most if not all so-called ovarian HGSC, but little is understood about the epithelial cells in the fallopian tube at the single cell level and which cells may develop into HGSC. This research project uses mitochondrial DNA-based lineage tracing, accessible chromatin profiling, and cell phenotyping using antibody labeling, at the single cell level to assess cell subtypes/states and clonal relationships between epithelial cell types in the fallopian tube. To do this we are collecting human fallopian tube, ovarian, and endometrial tissues to profile and determine cell properties and clonal relationships using scATACseq with mitochondrial DNA mutation tracing and scASAPseq. The purpose is to better understand the cell context in which HGSC arises.

2. KEYWORDS:

Fallopian tube, ATACseq, high-grade serous carcinoma, clonality, mitochondrial mutations

3. ACCOMPLISHMENTS:

What were the major goals of the project?

1. Obtain multiple gynecologic tissues from 4 different patients.
A. submit paperwork for IRB approval exemption (1-3 months): **completed**
B. Submit paperwork for HPRO approval (2-3 month): **completed**
C. Generate viable single cell suspensions from human tissues received for the study, and process for mtscATACseq (3-15 months): We have collected and made viable single cell suspensions for 4 different patients to date, and have processed the samples from 3 patients for mtscATACseq as of 12 months. **(75% completed)**
2. Analysis of mtscATACseq data (6-18 months): Currently in process. Preliminary data shows the identification of 7 different cell subtypes, of which 6 clusters appear to be epithelial in origin and one cluster appears to be an immune cell cluster that expressed EPCAM at the time of processing by flow cytometry to identify the cells of interest. Some clusters appear to be patient-specific while others overlap between all 3 patients. **(~50% completed)**
3. Obtain tissue samples from 4 patients with high-grade serous carcinoma
A. submit paperwork for IRB approval exemption (1-3 months): **completed**
B. Submit paperwork for HPRO approval (2-3 month): **completed**
C. Generate viable single cell suspensions from human tissues received for the study (3-18 months): We have collected and made viable single cell suspensions for samples from 2 patients **(50% completed).**
4. Determine optimal monoclonal antibodies for oligo conjugation and process samples for ASAP-seq
A. Obtain monoclonal antibodies for oligo-conjugation and determine optimal clones for use in ASAP-seq (1-6 months). Optimal antibodies have been selected.
B. Process samples with optimal antibody conjugates for ASAP-seq (6-18 months): **pending repeat processing since the first round failed to demonstrate high quality antibody staining.**
5. Analysis of ASAP-seq data: Antibody staining on 2 samples did not meet Q/A standards. We will be planning a repeat run (**thus analysis still pending**).

What was accomplished under these goals?

1) *major activities*: During this reporting period we have focused on and accomplished the collection of high-quality clinical patient samples and generating viable single cell suspensions from fallopian tube, endometrium, and HGSC tissues. Additionally we have processed a subset of these samples for mtscATACseq and analysis is underway for refining cell subtypes/states and analyzing their clonal relationship to one another. Preliminarily, we identified 7 clusters based on accessible chromatin clustering UMAP, of which 6 appear to be consistent with epithelial cell subtypes and one cluster (Cluster #5) appears to be an immune cell cluster. This is particularly interesting and exciting as we have shown through other mechanisms that there is a predicted immune (T lymphocyte) cell subtype that expresses EPCAM to varying degrees. The cells selected for ATAC-seq analysis in this study were first identified using sorting for EPCAM+ expression in order to enrich for the epithelial cell populations of interest. We have also successfully identified mitochondrial mutations in these samples, and have mapped them to the cell clusters. A more refined analysis of clonality and relationships of cells between the different cell clusters is pending.

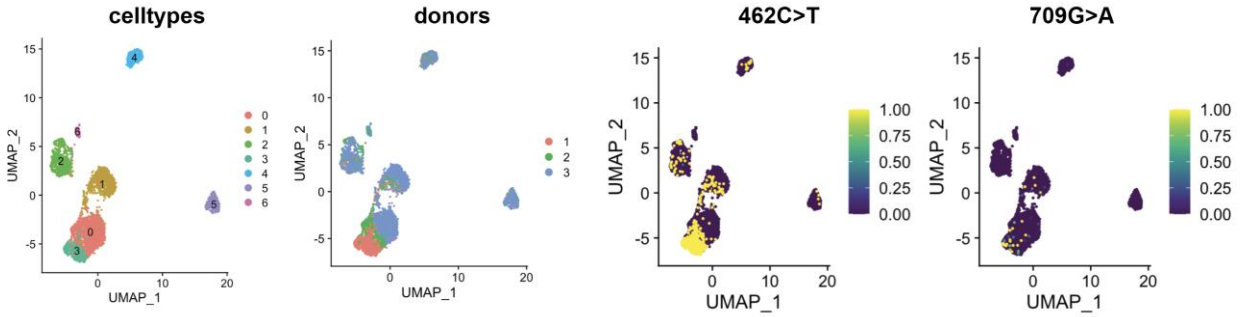
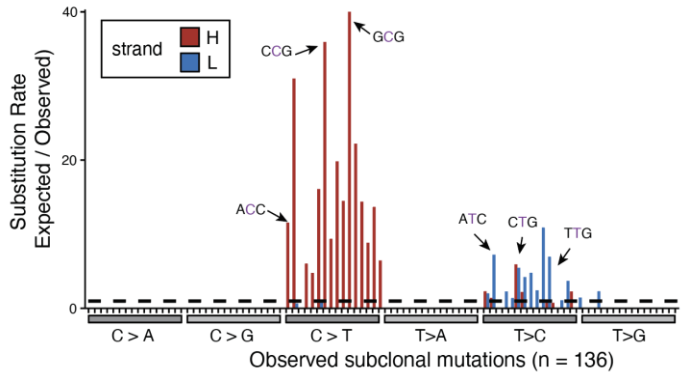


FIGURE 1 (above). This data corresponds to the major goals 1 and 2 of this project, representing a subset of the collected samples that have undergone mtscATAC-seq with preliminary data analysis. On the far left is a UMAP showing clustering of the cells analyzed amongst the 3 patients. Seven distinct clusters were identified, all of which appears to be epithelial cell subsets except for cluster 5 which based on accessible chromatin profiles, is most consistent with an immune cell subset. Most clusters were represented across all 3 patients however there is variation in the proportion of patients represented in each cluster. For example, patient 1 (second from left UMAP) is heavily represented in cluster 3 (far left UMAP plot). Two example mitochondrial mutations identified in this cohort of patients are mapped to the cell clusters (two right-side UMAPs).

Figure 2 (below). 136 distinct mitochondrial mutations were identified across the 3 patients.



2) *specific objectives*: specific objectives include sample collection, producing viable high-quality single cell suspensions from samples, processing samples for single cell sequencing, and analyzing the data. Our sample collection and single cell suspension generation is going well and on track for the projected timeline. We have processed a subset of the samples for single cell sequencing and the analysis is underway.

3) *significant results or key outcomes*: To date the most significant result or key outcome is the ability to obtain and process clinical samples. Data analysis is underway currently so no significant data-related results to date.

Thus far we have been meeting our stated goals per the projected timeline.

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?

We plan to continue the sample collection to obtain the target sample/patient numbers, and complete the processing of the remaining samples. We need to repeat the ASAP-seq attempt as the first round failed. Data analysis will also need to be completed in the next reporting period.

4. **IMPACT:**
What was the impact on the development of the principal discipline(s) of the 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 on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:

Caleb Lareau has taken a faculty position at MSKCC, and he will continue to collaborate with us on this project.

Our first attempt at ASAP-seq had a failure of antibody staining, so we will need to repeat this entire process in a second attempt.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to report.

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

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

  Journal publications.

  Nothing to report.

  Books or other non-periodical, one-time publications.

  Nothing to report.

  Other publications, conference papers and presentations.

  Nothing to report.

- **Website(s) or other Internet site(s)**

  Nothing to report.

- **Technologies or techniques**

  Nothing to report.

- **Inventions, patent applications, and/or licenses**

  Nothing to report.

- **Other Products**

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*Brooke Howitt*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	*0000-0002-0309-6680*
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Howitt has coordinated and supervised all aspects of this project.*
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?

Nothing to report for PI/key personnel

What other organizations were involved as partners?

Nothing to report currently; however Caleb Lareau has taken a faculty position at MSKCC so he will now be a partner from an outside organization.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: Not applicable.

QUAD CHARTS: Not applicable.

9. APPENDICES: None.