Disrupted epithelial differentiation and mitochondrial remodeling as early events in BRCA-associated carcinogenesis

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Introduction

- Hereditary breast and ovarian cancers share common molecular roots, with germline *BRCA1* and *BRCA2* mutations conferring profound lifetime risk for both malignancies. Although high-grade serous ovarian carcinoma (HGSC) is now thought to arise primarily from the secretory epithelium of the fallopian tube, the earliest events that predispose BRCA mutation carriers to malignant transformation across tissues, including both the breast and the fallopian tube, remain incompletely defined.
- Understanding these early alterations is essential, as they may reveal shared pathways of cancer susceptibility that transcend individual organ sites and provide opportunities for risk prediction, interception, and early detection in BRCA carriers.

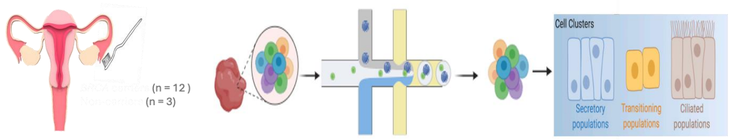
Materials and Methods

Samples were obtained during risk-reducing salpingo-oophorectomy (RRSO) using exfoliative cytology with a brush technique.

The study included:

- 3 samples from individuals at average risk (non-carriers)
- 11 samples from high-risk individuals (*BRCA^{mut}* carriers). (*BRCA1* mutation carriers, n=4; *BRCA2* mutation carriers, n=8).

scRNA-seq was performed using the 10x 3' gene expression platform. Raw data were processed with Cell Ranger, and quality control and doublet removal were performed. Total, 200,000 high-quality cells were retained for subsequent analysis.



The scRNA-seq data were then subjected to comprehensive analysis utilizing state-of-the-art computational tools, including Scanpy, for initial data preprocessing, followed by further analysis using scVI. These powerful tools facilitated the detailed exploration of the single-cell transcriptomes, enabling the identification of distinct cellular populations, elucidation of differential gene expression patterns, and the discovery of potential molecular drivers associated with ovarian cancer development

Results

Single-Cell transcriptomics unveils cellular and molecular features associated with germline *BRCA1/2* mutations

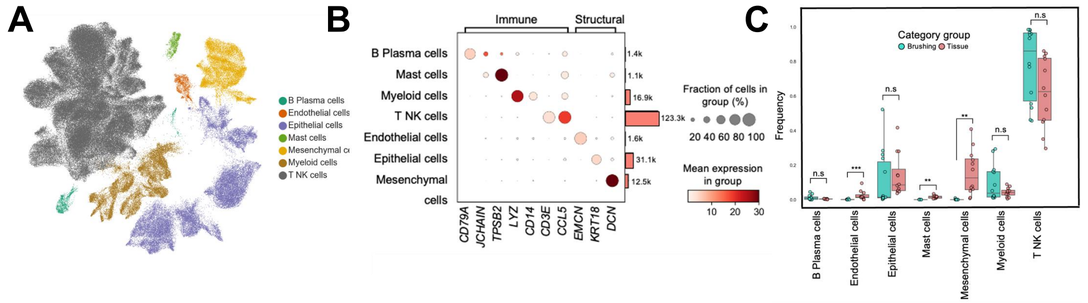


Figure 1: Cellular landscapes of dissociated fallopian tissues and fimbria brushings. scRNA-seq of 14 fimbria brushing are compared to 12 publicly available dissociated fallopian tubes, allowing the identification of A) 8 major cell types by using B) canonical markers. C) The frequency across the two categories show an expected significant depletion of endothelial and mast cells in the brushing samples but a correct representation of the epithelial cells.

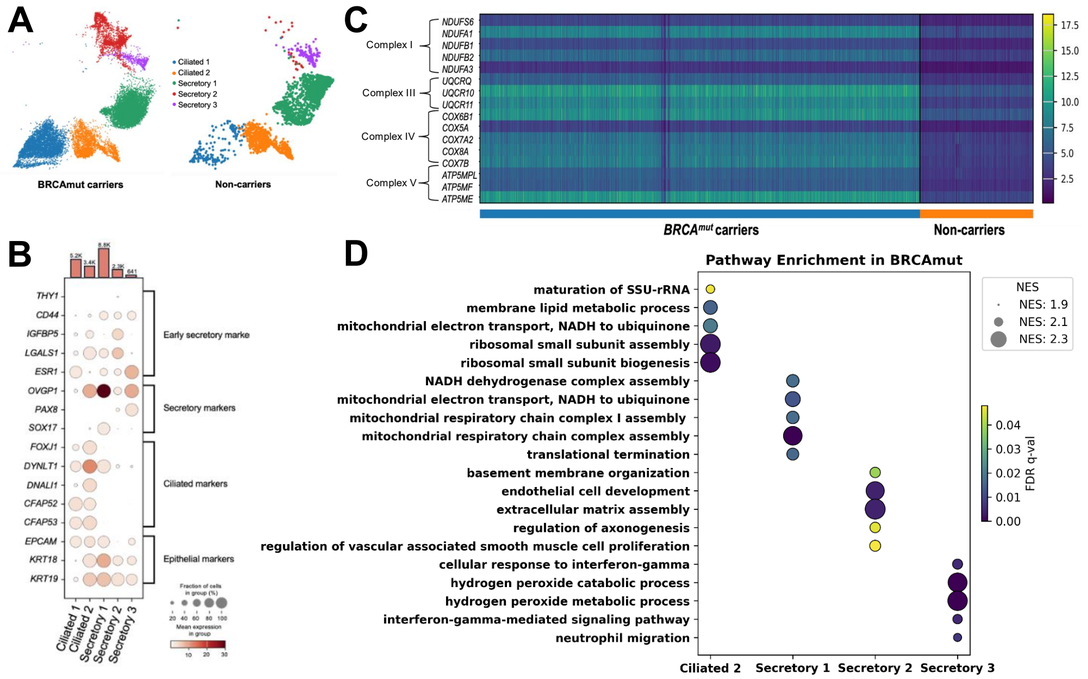


Figure 2: Increased oxphos metabolism in epithelial secretory cells of *BRCA^{mut}* carriers. A) 3 secretory cell clusters and 2 ciliated cell clusters from the epithelial subset of the fimbria brushing samples can be identified by using canonical markers. B) The secretory 1 presents an expression of *SOX17*, a master transcription factor of ovarian cancer. The differential expression analysis of this specific cluster C) shows the upregulation of multiple genes involved in the OXPHOS pathway in the *BRCA^{mut}* carriers. The pathway upregulated in the carriers (D) confirms this increase of metabolism

Conclusion and Significance

- **Effective Sampling Technique:** Exfoliative brushing sampling proved effective in capturing key cellular populations within Fallopian tubes, providing a rich dataset for analysis.
- **Distinct Cellular Composition:** scRNA-seq revealed significant differences in cellular composition between *BRCA^{mut}* carriers and non-carriers, highlighting the complexity of the tumor microenvironment.
- **Increase mitochondrial activity in secretory cells:** A subset of secretory cells in *BRCA^{mut}* carriers present an upregulation of genes involved in the OXPHOS pathway, known to be an increase in cancer that could reflect an early event leading to the development of precancerous lesions.
- **Advancing Personalized Therapies and Future Confirmatory Studies:** The insights gleaned from this study underscore the potential for single-cell transcriptomics to inform personalized therapeutic strategies for ovarian cancer patients. However, it's important to note that these findings should be validated and further explored, particularly through the integration of spatial transcriptomics data. This additional layer of analysis will be crucial in confirming the robustness of our results and enhancing our understanding of the spatial organization of cell types within the tumor microenvironment, ultimately paving the way for more targeted and effective treatments with the potential to improve patient outcomes.
- **Future Directions:** As we continue to explore the intricate biology of ovarian cancer, this work serves as a foundation for further research and the development of targeted treatments, offering hope for a brighter future in the battle against this deadly disease.

Acknowledgments

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