Defining the Mammary Epithelial Changes Induced by UTI in wildtype and *Brca1*-KO Mouse Models

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INTRODUCTION

The mammary gland is a glandular organ that functions in production of milk for breast-feeding. Contrasting with other organs, the mammary gland completes development during adulthood. Local infection, such as mastitis, has been implicated in altering MEC proportions in the mammary gland during inflammation in bovine models by inducing apoptosis to alveolar cells. There is little known about the effects of distal infection on MEC plasticity in the mammary gland. Previous work has shown changes in the extracellular matrix (ECM) and immune cells in the mammary gland in murine models induced with Urinary Tract Infection (UTI), a clinically relevant infection. Considering the stromal changes influenced during UTI, this research uncovers how the epithelial environment of the mammary gland is altered in wildtype mice induced with UTI and *Brca1*-KO mice induced with UTI by analyzing single-cell RNA sequencing data with Seurat Package in R.

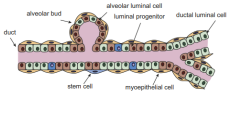
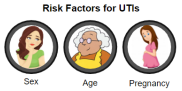
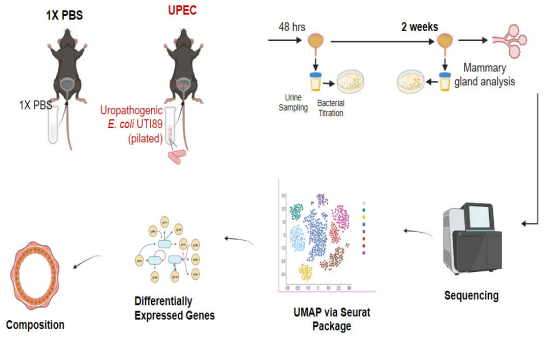


Fig 1: The epithelial environment of the mammary gland. (Fu et al. 2020)



- 1 in 2 women develop a UTI in their lifetime
- 2 in 5 are asymptomatic

METHODS AND MATERIALS



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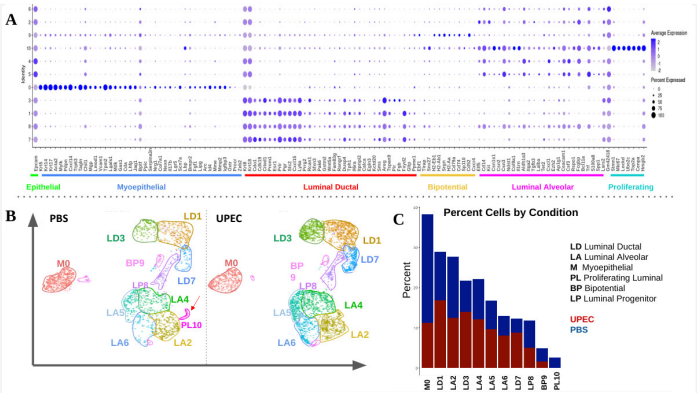

Fig. 2: Clustering of wildtype datasets with UPEC infection versus PBS treated mice. **A)** Dotpot showing expression of epithelial markers in each cluster. Intensity of color represents average expression and size of dot represents percent of cells expressing the specific marker. **B)** UMAP clustering of data split by condition. Each dot represents a single cell. **C)** The percent of cells in each cluster by condition.

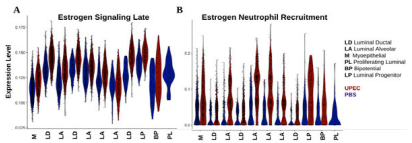

Fig. 3: Violin plots of signature scoring. Vertical axis shows expression level whereas horizontal axis is by MEC clusters. **A)** The signature scoring for estrogen signaling from Hallmark gene list. **B)** The signature scoring for neutrophil recruitment.

Table 1: Upregulated genes in unique *Brca1*-KO hormone sensing epithelial cell cluster LD7.

Gene*	avg_log2FC	Implication	Source
Krt6a	3.95	Tumor forming bipotential MEC	Bu et al. (2011)
Areg	3.64	Estrogen Signal in Erα BC	Peterson et al. (2015)
F3 (TF)	3.64	Cancer cell surface marker	Hu Z. (2020)
Ngf	3.56	Breast Cancer agiogenesis	Romon et al. (2010)

P-value = 0

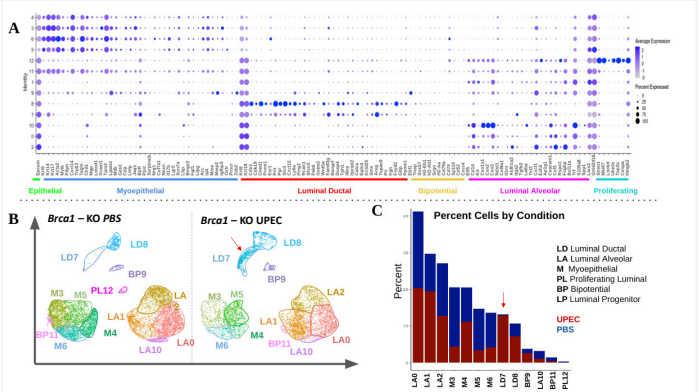

Fig. 4: Clustering of *Brca1*-KO datasets with UPEC infection versus PBS treated mice. **A)** Dotpot showing expression of epithelial markers across clusters. Intensity of color represents average expression and size of dot represents percent of cells expressing the specific marker. **B)** UMAP clustering of data split by condition. Each dot represents a single cell. **C)** The percent of cells in each cluster by condition.

CONCLUSIONS

- A higher proportion of luminal epithelial cells was seen in the UPEC infected wildtype mice.
- A unique population of proliferating luminal cells was not present in the UTI condition of the wildtype mice.
- Neutrophil recruitment signatures elevated in UPEC infected wildtype mice suggest a potential effect of infection on mammary gland environment.
- There was an enrichment of bipotential populations in *Brca1*-KO UTI model.
- Luminal ductal population (LD7) shows higher expression of genes implicated in breast cancer.

FUTURE STUDY

- Investigate causes of elevated estrogen signaling and neutrophil pathways in wildtype UPEC infected model and potential role in breast cancer development.
- Use trajectory analysis to determine possible differentiation pathways of epithelial clusters of interest.
- New studies could assess how repeat infection, chronic infection, or other UTI-inducing microbes affect the mammary gland environment.

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