Reconstructing the Lineage Hierarchies within the Human Breast Epithelium
In Single Cell Resolution

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Breast cancer is one of the most prevalent forms of cancer in women worldwide. Despite recent advances in understanding the mechanisms driving the initiation and progression of breast cancer, prognosis still remains poor especially due to late diagnosis and subsequent high mortality from metastatic tumor. Breast cancer arises from breast epithelial cells that acquire pro-tumorigenic mutations leading to uncontrolled proliferation and subsequent loss of tissue homeostasis. Over the past decades, we have significantly advanced our knowledge about the genetic mutations driving tumorigenesis and how these mutations alter signaling circuits within a cell. However, it remains unclear how these mutations affect the circuits between different breast epithelial cell populations on a systems-level during the initial steps of tumor formation.

We utilized single cell RNA sequencing to first understand the spectrum of cellular heterogeneity in the mammary epithelial system and to follow tumorigenic events in single cell resolution. We isolated human basal and luminal mammary epithelial cells (MECs) from reduction mammoplasty samples by FACS and subjected them to single cell RNA-seq on the Fluidigm® C1 platform. We sequenced 400 MECs and analyzed these datasets using principal component analysis (PCA), hierarchical clustering methods and t-embedded stochastic neighbor embedding (t-SNE). We identified 5 distinct subpopulations within the basal cell cluster and between 2 and 5 subpopulations within the luminal cell cluster. Our analysis faithfully reflects expected expression patterns of known lineage markers and revealed hundreds of additional specific markers for mammary subpopulations including cell surface markers for prospective isolation and functional studies. Our work provides novel insights into the spectrum of heterogeneity within the mammary epithelial system under normal homeostasis, which will form the basis for ongoing experiments to compare these findings to early neoplastic and cancerous specimens. Understanding the early stages of breast tumorigenesis in high enough resolution will lead to the identification of novel biomarkers for cancer early detection and may form the basis for the development of therapeutic approaches to even prevent breast cancer from progressing into a life threatening condition.

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