Intra-tumoral heterogeneity and plasticity in basal-like breast tumors

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Project Aims

Aim 1: Determine functional and genomic characteristics of the distinct cellular subpopulations within basal-like breast cancers (BLBCs).

Aim 2: Identify chemotherapeutic and chromatin remodeling agents that initiate or block tumor cell plasticity.

Background

Basal-Like Breast Cancer is associated with Triple Negative Breast Cancer (TNBC)

- TNBC is the 5th leading cause of cancer deaths in women in the USA and ~70% TNBCs are basal-like breast cancers (BLBCs).
- Lack of therapeutic targets, aggressive tumor formation, and variation in tumor sensitivity to chemotherapy = poor prognosis.

Mammmary Gland Epithelial Hierarchy and Molecular Subtype

Cellular heterogeneity associated with resistance to chemo

- TNBC/BLBCs with basal-like signatures (high proliferation) show greater chemoresistance compared to tumors with mesenchymal signatures (low proliferation, A)
- Mesenchymal populations give rise to more basal-like population, potential indication of stem-like capabilities and plasticity in human BLBC tumor line (B)

Chromatin remodeling involved in cell state switching/plasticity

Methodology

p53+ Tumor Transplant Model

- An individual p53+/− mouse with a tumor
- Transplant p53+/− mammary gland into dorsal fat pad of host BALB/C Mouse
- Tumor is serially transplanted 3x for subtyping

Single Cell RNA-sequencing (scRNA-seq)

- Drop-seq single cell analysis
- Bioinformatic Analysis

Aim 1 Results

Aim 1: Flow Cytometry

- GEM BLBC tumors (223SL, 9263-3, KRP1-A, and 223SL) stained with CD44/29/61 antibody panel.

Figure 1. Flow cytometry profiles of 4 p53+ mouse basal-like BLBCs show some heterogeneity. GEM BLBC tumors (223SL, 9263-3, KRP1-A, and 223SL) stained with CD44/29/61 antibody panel.

Figure 4. p53+ mouse tumor-derived cell lines show variation in responses to carboplatin, paclitaxel, and I-BET151. A) High throughput dose response curves for 4 BLBC lines and 1 CL (T111) using CellTiter-Glo Luminescent Cell Viability Assay in 384-well format. B) Gene set enrichment analysis (GSEA) for hallmark gene sets for untreated and I-BET151-treated (10 μM, 13.5h) 223SL BLBC cell line using gene signatures derived from DNA microarray (n=2).

Conclusions

1) 9263-3 and several other basal-like tumors are highly heterogeneous with multiple subpopulations = strong candidates for Aim 1 experimental work.
2) These tumors have distinct basal-like, mesenchymal, and proliferative clusters.
3) Variation in tumor cell line sensitivity to carboplatin and paclitaxel.
4) I-BET151 treatment alters EMT gene expression in basal-like tumor cell line in vitro.

Future Directions

- Continue genomic analysis of subpopulations using FACS and single cell RNA-seq to look for enrichment of differential genes.
- Treat tumors with chemotherapeutics (carboplatin/paclitaxel) and I-BET151 to test effect on plasticity of cellular subpopulations in using scRNA-seq and bulk RNA-seq.
- Determine sensitivity of distinct cellular subpopulations to chemotherapeutics.
- Assess functional properties of subpopulations by tumor initiating cell assays, i.e. mammosphere and limited dilution assays.
- Repeat experiments using Patient Derived Xenograft (PDX) samples.

References


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