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).

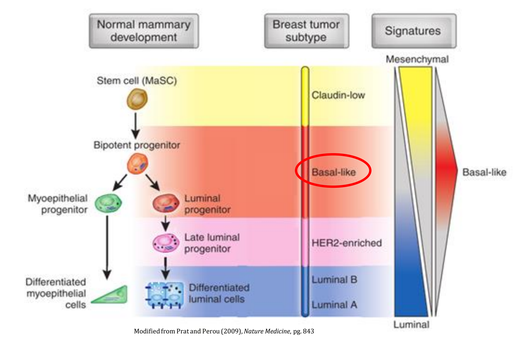
Aim 2: Identify chemotherapeutic and chromatin remodeling agents that initiate or block tumor cell plasticity.

Background

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

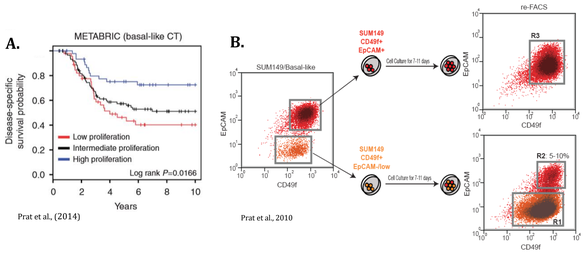
- TNBC is *5th leading cause of cancer deaths* in women in the USA and ~70% TNBCs are basal-like breast cancers (BLBCs).
- Lack of therapeutic targets, aggressive tumor formation, and variation in tumor sensitivity to chemotherapy = poor prognosis.

Mammary Gland Epithelial Hierarchy and Molecular Subtype



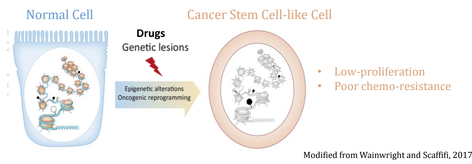
Modified from Prat and Perou (2009), *Nature Medicine*, pg. 843

Cellular heterogeneity associated with resistance to chemo



- TNBC/BLBCs with basal-like signatures (high proliferation) show **greater** chemo-sensitivity 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)

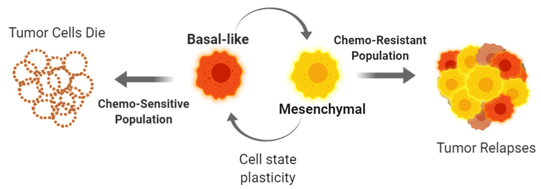
Chromatin remodeling involved in cell state switching/plasticity



Modified from Wainwright and Scaffidi, 2017

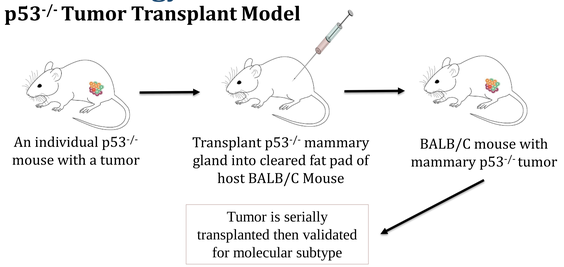
Hypothesis

Plasticity between subpopulations of heterogeneous BLBCs leads to changes in tumor sensitivity to chemo- and targeted therapies.



Methodology

p53$^{-/-}$ Tumor Transplant Model



Single Cell RNA-Sequencing (scRNA-seq)

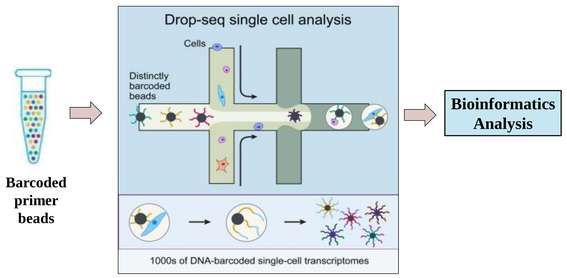


Image adapted from Macosko et al., (2015)

Aim 1 Results: Flow Cytometry

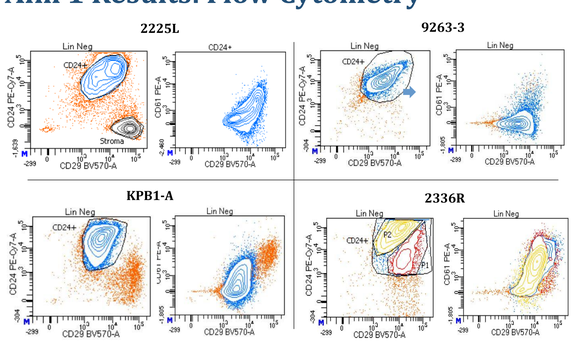


Figure 1. Flow cytometry profiles of 4 p53-null basal-like mouse tumors show some heterogeneity. GEM BLBC tumors (2225L, 9263-3R, KPB1-A, and 2336R) stained with CD24/29/61 antibody panel.

Aim 1 Results: Tumor scRNA-seq

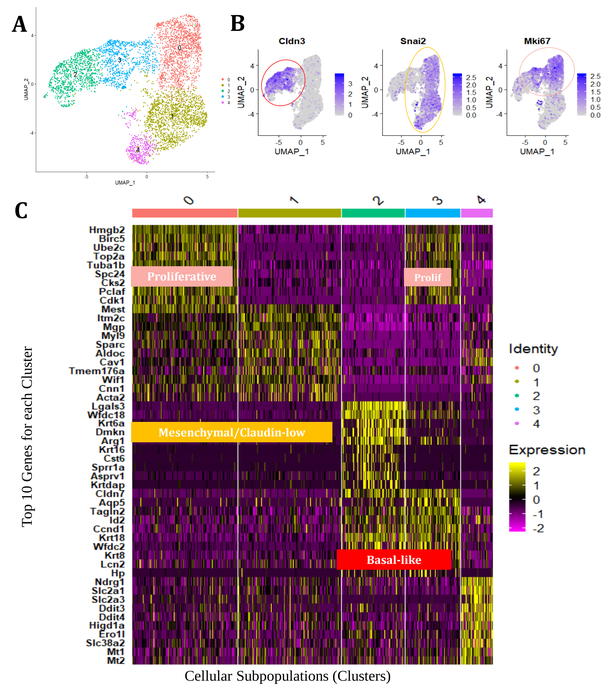


Figure 2. Single cell RNA-sequencing of p53-null, mouse BLBC (9263-3R) cells growing *in vivo* and positive for tumor cell-specific marker Neomycin Resistance Gene (Neo+) shows intra-tumoral heterogeneity. A) UMAP showing 5 distinct clusters (n= 5316 cells) of 9263-3R Neo+ tumor cells. **B)** Sample gene expression profiles with representative markers showing basal-like cells (Cldn3), mesenchymal/Claudin-low cells (Snai2), and proliferative cells (Mki67) in the p53-null GEM tumor. **C)** Heatmap of top 10 differential genes expressed in each subset of tumor cells that express Neo+.

Aim 2 Results: Treatments *in vitro*

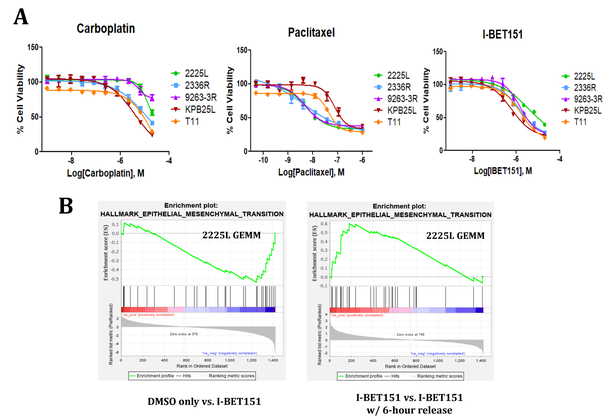


Figure 4. p53-null mouse tumor-derived cell lines show variation in responses to carboplatin, paclitaxel, and I-BET151. A) High-throughput dose-response curves for 4 BLBC lines and 1 CL (T11) using CellTiter-Glo Luminescent Cell Viability Assay in 384-well format. **B)** Gene Set Enrichment Analysis (GSEA) for Hallmark gene sets for untreated and I-BET151-treated (IC_{50} = 13.09μM) 2225L BLBC cell line using gene signatures derived from DNA microarray (n=2) .

Conclusions

1) 9263-3R 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

1. Lim et al. (2010). Transcriptome analyses of mouse and human mammary cell subpopulations reveal multiple conserved genes and pathways. *Breast Cancer Research*, *12*(2).
2. Prat et al. (2013). Characterization of cell lines derived from breast cancers and normal mammary tissues for the study of the intrinsic molecular subtypes. *Breast Cancer Research and Treatment*, *142*(2), 237–255.
3. Prat, A., & Perou, C. M. (2009). Mammary development meets cancer genomics. *Nature Medicine*, *15*(8), 842–844.

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