Identification of Combinations of Targets for Claudin-Low Triple Negative Breast Cancer Reversion

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INTRODUCTION

Claudin-Low Triple Negative Breast Cancer (CL TNBC) is an aggressive subtype of TNBC with a poor prognosis[1-4] (Fig 1). Current breast cancer therapies are not effective in treating this cancer, creating the need for a new therapeutic approach[5].

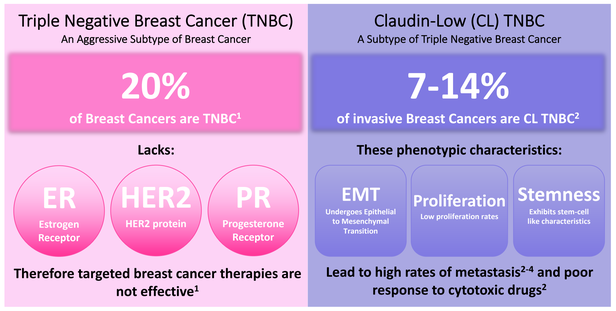
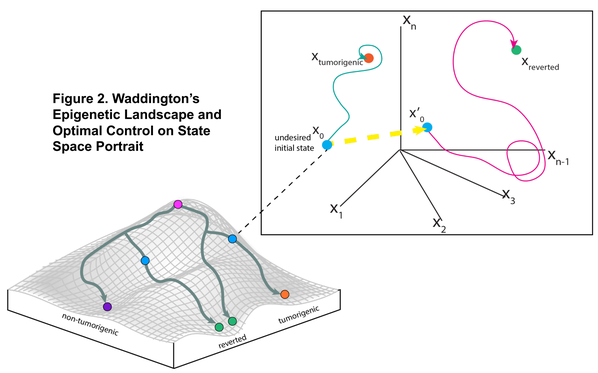


Figure 1. Triple Negative Breast Cancer Characteristics

Tumor reversion is the biological process by which tumor cells lose a significant fraction of their malignant phenotype[6]. Tumor reversion has been observed *in vitro, in vivo, and ex vivo for* over a century. In particular, tumor reversion has been achieved with the CL cell line MDA-MB-231 and MDA-MB-231 xenograft mice models[7-12].

At the cellular level, the development of cancer can be seen as a systems-level dynamical process driven by a tumorigenic intracellular signaling network. Attractors of this network correspond to cell phenotypes[13]. **Cancer attractors** are attractors presenting a malignant phenotype that are pre-existing in the network but not typically accessible[13]. They can be accessed through genetic mutations or changes in the tumor microenvironment. **Tumor reversion can be viewed as an optimal control problem in dynamical systems where the objective is to shift the system away from a cancerous attractor and towards normal-like attractors.**



Structure-based attractor-based control methods for non-linear systems study the controllability of the system based on the structure of the network and aim to restrict the target states to attractors [14,15] (Fig 2). The newly proposed Feedback Vertex Set Control (FC) framework is especially suited for systems with non-linear dynamics[15]. The objective of FC is to **identify combinations of network nodes** that drive the network from an arbitrary initial state to any desired dynamical attractor of the system through an override of their initial states.

OBJECTIVES

- Develop and apply a computational systems biology pipeline for the construction and control of a CL TNBC intracellular signaling network
- Identify and experimentally validate combinations of therapeutic targets to aid in the reversion of Claudin-Low Triple Negative Breast Cancer

METHODS

STEP 1. Reconstruction of Tumorigenic Network Using Multi-Omics Data (Fig 3). RNA-seq data[16], methylation profiles[17], mutational profiles[18], and protein abundance[19] for the CL TNBC cell line MDA-MB-231 and the normal breast cell line MCF10A were used to construct a tumorigenic intracellular signaling network[20-26].

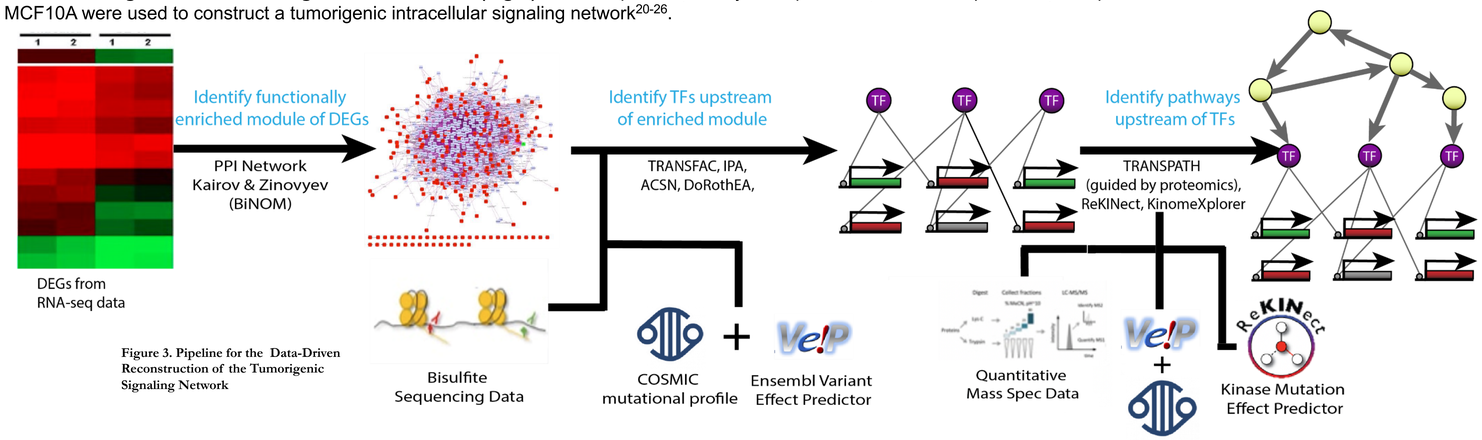


Figure 3. Pipeline for the Data-Driven Reconstruction of the Tumorigenic Signaling Network

STEP 2. Estimating Attractor Landscape with Topological Signal Flow Analysis[27] (SFA). (Fig 4)

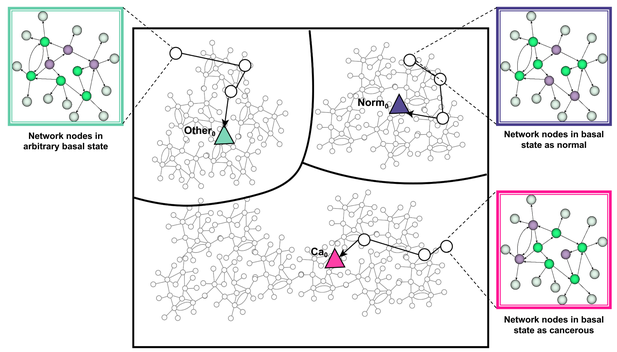


Figure 4. Attractor Landscape. Attractor landscape including the associated attractors for the 2 conditions of interest: Cancerous (Ca) and Normal (Norm).

STEP 3. Estimating Phenotype Landscape with Unsupervised Machine Learning. (Fig 5)

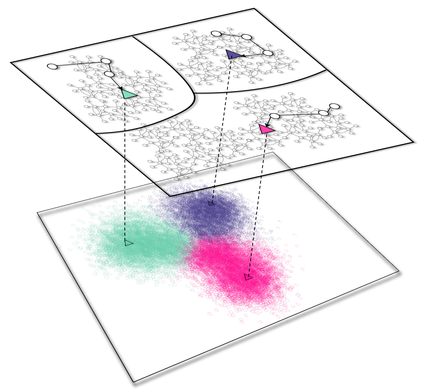


Figure 5. Phenotype Landscape After Unsupervised K-Means Clustering.

STEP 4. Applying FC Control[15] and In-Silico Screenings. (Fig 6)

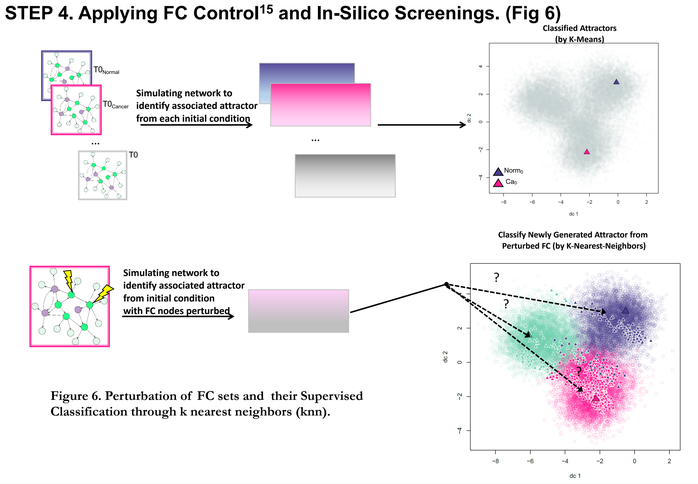


Figure 6. Perturbation of FC sets and their Supervised Classification through k nearest neighbors (knn).

RESULTS

Claudin-Low TNBC Network. The constructed network has 230 nodes and 583 edges (Fig 7). 90 of the nodes are hallmarks of cancer, 27 are breast disease ontology associated, and 5 are claudin-low markers[28,29]. 142 of the network nodes are in the ACSN.

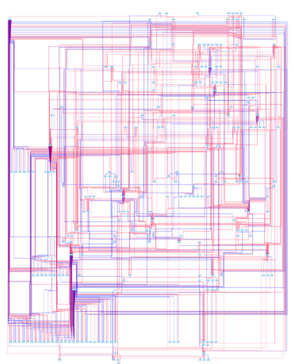


Figure 7. **Tumorigenic Network.** 230 nodes and 583 edges. Edges in red are activating edges, while blue edges are inhibitory.

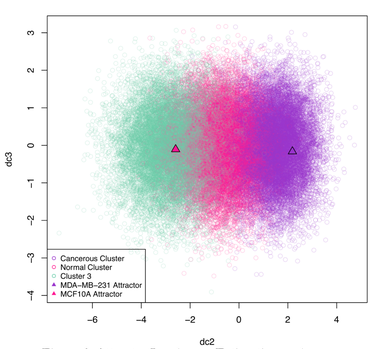


Figure 8. Attractor Landscape Estimation and Unsupervised Attractor Classification. Part of the phenotype landscape is shown above. The MCF10A and MDA-MB-231 attractor appear in different clusters.

FC Control Analysis. We identified 6 different FC sets in network. Each FC set contained 28 source nodes and 14 Feedback Vertex Set (FVS) nodes. We randomly chose 100,000 perturbations of the 14 FVS nodes and simulated their resulting attractors. Using the phenotype landscape (Fig 8) and knn to classify the perturbations, we found 54 perturbations that shifted the attractor of the network from the cancerous cluster to the normal cluster. One such perturbation is FC_1:

$$FC_1 = \left\{ \begin{array}{l} \textbf{Downregulation:} \\ \text{FOXM1} \\ \text{GSK3B} \\ \text{STAT3} \\ \text{TCF3} \\ \\ \textbf{Upregulation:} \\ \text{PIAS1} \\ \text{MAPK1} \end{array} \right\}$$

These genes have been related to EMT, stemness, and migration in TNBC, and STAT3 is a drug target in on going clinical trials[30-34].

DISCUSSION

We have successfully constructed a pipeline for the reconstruction of a CL TNBC signaling network that captures known TNBC dysregulated genes as well as genes related to EMT and Stemness, two CL TNBC characteristics.

Our *in silico* perturbation screenings generated different tumor reversion FC sets whose override have the potential to shift the resulting attractor away from the cancerous attractor and towards the normal attractor. We will improve our classification of these results by refining our clustering method for estimating the phenotype landscape or revisiting the network reconstruction to ensure the normal and cancerous clusters are not, or only slightly overlapping.

Future work includes simulating more FVS perturbations, including source nodes in our perturbations, and screening perturbations of the other FC sets.
We will also prioritize tumor reversion sets to select a few for experimental validation. We plan to extend our analysis with dynamical modeling to compare results and potentially obtain additional therapeutic targets.

ACKNOWLEDGEMENTS:

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