**Introduction**

**Triple Negative Breast Cancer (TNBC)** is an aggressive, heterogenous subtype of breast cancer (BC) accounting for 20% of BC cases1. Unlike other subtypes of BC which are characterized by the presence of estrogen receptors, progesterone receptors, or HER2 amplification, TNBC is characterized by the lack of all three of these markers. As a result, it is difficult to treat TNBC with current targeted therapies. Claudin-low (CL) TNBC is a subtype of TNBC making up 7-14% of invasive breast cancers diagnoses2. It is characterized by low expression of tight junction proteins Claudin 3, 4, and 7 and the cell adhesion molecule E-cadherin2,3,4. CL tumors are prone to epithelial to mesenchymal transition (EMT) as well as exhibiting stem-cell like characteristics, two hallmarks of cancer marking high metastasis rates2,5. The low proliferation rate of the subtype sets it apart from most tumors, making it difficult to treat with cytotoxic drugs2. CL TNBC has a poor prognosis and a clear need for new treatment options.

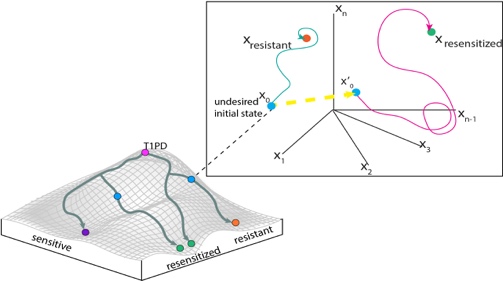
One potential therapeutic strategy is **tumor reversion**, the biological process by which tumor cells lose a significant fraction of their malignant phenotype7. Tumor reversion has been observed for over a century (SOURCE). It has also been achieved both *in vitro*, *in vivo*, and *ex vivo*. In particular, tumor reversion has been achieved in vitro with the CL cell line MDA-MB-231, and in vivo in a mice xenografted with MDA-MB-231 cells8-13.

At the cellular level, the development of cancer can be seen as a systems-level dynamical process driven by an intracellular signaling network. Attractors of this network correspond to cell phenotypes14. **Cancer attractors** are attractors presenting a malignant phenotype that are pre-existing in the network but not typically accessible and therefore not occupied by cells14. They can be accessed through genetic mutations or changes in the tumor microenvironment. Cancer 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 control methods** study the controllability of systems based solely on the structure of the network15-18. Attractor-based control methods focus on the controllability of the system by restricting the target states to attractors. Recently, structure-based attractor-based methods for non-linear systems have been proposed17,18(Fig 1). The newly proposed Feedback Vertex Set Control (FC) framework is especially suited for systems with non-linear dynamics8. 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 by perturbing the initial state of identified nodes.

**Objectives:** Develop and apply a computational systems biology pipeline for the construction and control of an intracellular signaling network of reversion of Claudin-Low Triple Negative Breast Cancer. The ultimate goal is to identify and experimentally validate combinations of therapeutic targets to aid in the reversion of CL TNBC.

**Figure 1. Waddington’s Epigenetic Landscape and Optimal Control on State Space Portrait**



DATA

To calculate differential gene expression, we have RNA-seq data for the CL TNBC cell line MDA-MB-231 and the normal breast cell line MCF10A19. We use bisulfite data and mass spectrometry data for MDA-MB-231 to consider the epigenetics and protein abundance of the cell-line, respectively20,22. Single Nucleotide Variation and Copy Number Variation profiles for MDA-MB-231 were taken from the Catalog of Somatic Mutations in Cancer (COSMIC)21.

METHODS

**STEP 1. Reconstruction of Tumorigenic Network.** Multi-Omics Profilng data was used to identify functionally related differentially expressed genes (FunDEGs), the transcriptions factors (TFs) transcribing them, and the upstream molecules regulating FunDEGs and TFs.( .(Binom, rekinect, vep, transpath, transfac, acsn, ipa))

**STEP 2. Estimating Attractor Landscape with Topological Signal Flow Analysis (SFA).** We apply SFA19 to the network using 100,000 random initial conditions to estimate the attractor landscape based on the network topology (Fig 3).

**STEP 3. Estimating Phenotype Landscape with Unsupervised Machine Learning.** We apply K-Means algorithm to cluster the simulated attractors (Fig 4).

**STEP 4. Applying FC Control and In-Silico Screenings.** We apply FC control8 to the network to identify control sets (FCs). We perform *in-silico* screenings using SFA and applied K-Nearest Neighbors (KNN) classifier to identify combinations of perturbations of nodes in each FC set that can shift attractors from the Tumorigenic to the Normal basin of attraction (Fig 5).

**Discussion**

We have successfully constructed a pipeline for the reconstruction of the CL TNBC signaling network. Based on preliminary data, we were able to capture known TNBC dysregulated genes, as well as genes related to EMT and Stemness.

Our *in silico* perturbation screenings did generate different Tumor Reversion Sets….

Future work includes prioritizing 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.

**Results**

**Claudin-Low TNBC Network.** The constructed network has 230 nodes and 583 edges (Fig 6). 90 of the nodes are hallmarks of cancer, 27 are breast disease ontology associated, and 4 are claudin-low markers. 142 of the network nodes are in the ACSN. (SOURCES)

**Attractor Landscape Estimation and Unsupervised Attractor Classification.** We initialized the network with basal expression levels for MCF10A and MDA-MB-231, and ran SFA to estimate the corresponding attractors. We generated 100,000 random initial states of the system and simulated their corresponding attractors to estimate the attractor landscape of the network. All attractors were classified with K-Means with 10,000 initializations and optimal k of 6. The MCF10A and MDA-MB-231 attractors appeared in different clusters (Fig 7).

**FC Control Analysis.** We identified 6 FC sets in network. Each FC set contained 28 source nodes and 14 FVS nodes. We identified a subset of FVS nodes that were present in all 6 FC set. This FVS subset consists of 11 nodes: MAP2K6, MAP2K3, MAPK1, AURKA, CTNNB1, FOXM1, JUN, RELA, STAT3, TCF3, and AKT1. We applied all (311=177,147) perturbations on the FVS nodes parting from the cancerous state. INCLUDE KNN RESULTS

sources

1. Boyle (1 from grant)
2. Dias, PloS one 2017
3. Prat, Breast Cancer Research, BioMed Central, 2010
4. Holliday, Breast Cancer Research, BioMed Central, 2011
5. Salvador, Currentpathobiology reports, 2016
6. Chalakur-Ramireddy, *Biosci*, 2018
7. Telerman, *Nat. Rev. Cancer,* 2009
8. Chu*,* *PLoS One*, 2013
9. Chu*,* *Oncogene*, 2012
10. Yan, *J. Biol. Chem,* 2010
11. Dydensborg*,* *Oncogene*, 2009
12. Kong*,* *Mol. Syst. Biol,* 2014
13. Matossian*, Oncoscience*, 2018
14. Huang, Ernberg, and Kauffman, Seminars in Cell & Developmental Biology, 2009
15. Ching-Tai Lin. Structural controllability. 1974;19(3).
16. Liu Y-Y, Slotine J-J, Barabási A-L Nature. 2011;473(7346).
17. Mochizuki A, Fiedler B, Kurosawa G, Saito D. J Theor Biol. 2013;335
18. 8.Zañudo JGT, Yang G, Albert R. 2016. doi:10.1073/pnas.1617387114
19. <https://bmcgenomics.biomedcentral.com/articles/10.1186/s12864-018-4533-0>
20. <https://genomebiology.biomedcentral.com/articles/10.1186/gb-2013-14-10-r110>
21. <https://cancer.sanger.ac.uk/cell_lines/sample/overview?id=905960>
22. <https://www.sciencedirect.com/science/article/pii/S2211124715003411?via=ihub>
23. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6315782/pdf/cancers-10-00525.pdf>
24. <https://breast-cancer-research.biomedcentral.com/articles/10.1186/s13058-019-1125-0>
25. <https://www.researchgate.net/publication/232610667_Control_of_Breast_Cancer_Growth_and_Initiation_by_the_Stem_Cell-Associated_Transcription_Factor_TCF3>
26. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6518732/pdf/13046_2019_Article_1206.pdf>
27. <https://www.researchgate.net/publication/270907408_A_novel_role_for_the_SUMO_E3_ligase_PIAS1_in_cancer_metastasis>

FVS nodes:

* Mapk
  + <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6144355/pdf/ol-16-04-4984.pdf>
* AKT1 (
  + <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6144355/pdf/ol-16-04-4984.pdf>

Clinical study with drug targeting it: <https://www.futuremedicine.com/doi/full/10.2217/pgs-2017-0117>

* FOXM1:
  + Targeting of CBP/β-catenin/FOXM1 with ICG-001 eliminated CSCs
* GSK3B
  + Inhibition of it decreases migration, EMT and Stemness
  + Inhibitiymal cells
  + <https://digitalcommons.library.tmc.edu/utgsbs_dissertations/806/>
  + <https://breast-cancer-research.biomedcentral.com/articles/10.1186/s13058-019-1125-0>
* STAT3
  + Stemness, proliferation, migration, EMT
  + Several inhibitors of STAT3 have been tested and shown to inhibit tumor growth/migration and stem cell properties
  + <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6518732/pdf/13046_2019_Article_1206.pdf>
* TCF3
  + <https://www.researchgate.net/publication/232610667_Control_of_Breast_Cancer_Growth_and_Initiation_by_the_Stem_Cell-Associated_Transcription_Factor_TCF3>
  + Stem cells, cell growth
* PIAS
  + Prevents EMT, stemness, metastisis
  + <https://www.researchgate.net/publication/270907408_A_novel_role_for_the_SUMO_E3_ligase_PIAS1_in_cancer_metastasis>
* JUN

1. Boyle (1 from grant)
2. Dias et al., PloS One, 2017, 28045912
3. Prat et al., Breast Cancer, 2010, 20813035
4. Holliday et al., Breast Cancer Research, 2011, 21884641
5. Chalakur-Ramireddy et al., *Biosci*, 2018, 29298879
6. Telerman et al., *Nat. Rev. Cancer,* 2009, 19180095
7. Chu et al.*,* *PLoS One*, 2013, 23577196
8. Chu et al.*,* *Oncogene*, 2012, 21892208
9. Yan et al., *J. Biol. Chem,* 2010, 20189993
10. Dydensborg et al.*,* *Oncogene*, 2009, 19483726
11. Kong et al.*,* *Mol. Syst. Biol,* 2014, 21878914
12. Matossian*, Oncoscience*, 2018, 29854878
13. Huang, Ernberg, and Kauffman, Seminars in Cell & Developmental Biology, 2009
14. Mochizuki et al., J Theor Biol., 2013
15. Zañudo et al., pnas, 2016
16. <https://bmcgenomics.biomedcentral.com/articles/10.1186/s12864-018-4533-0>
17. <https://genomebiology.biomedcentral.com/articles/10.1186/gb-2013-14-10-r110>
18. <https://cancer.sanger.ac.uk/cell_lines/sample/overview?id=905960>
19. <https://www.sciencedirect.com/science/article/pii/S2211124715003411?via=ihub>
20. Kairov, Ulykbek et al. “Network analysis of gene lists for finding reproducible prognostic breast cancer gene signatures.” *Bioinformation* vol. 8,16 (2012): 773-6. doi:10.6026/97320630008773
21. Wingender, E et al. “TRANSFAC: an integrated system for gene expression regulation.” *Nucleic acids research* vol. 28,1 (2000): 316-9. doi:10.1093/nar/28.1.316
22. Krämer, Andreas et al. “Causal analysis approaches in Ingenuity Pathway Analysis.” *Bioinformatics (Oxford, England)* vol. 30,4 (2014): 523-30. doi:10.1093/bioinformatics/btt703
23. Krull, Mathias et al. “TRANSPATH: an integrated database on signal transduction and a tool for array analysis.” *Nucleic acids research* vol. 31,1 (2003): 97-100. doi:10.1093/nar/gkg089
24. Kuperstein I, Bonnet E, Nguyen HA, Cohen D, Viara E, Grieco L, Fourquet S, Calzone L, Russo C, Kondratova M, Dutreix M, Barillot E, Zinovyev A. Atlas of Cancer Signalling Network: a systems biology resource for integrative analysis of cancer data with Google Maps. Oncogenesis. 2015 Jul 20;4:e160. doi: 10.1038/oncsis.2015.19. Pubmed ID: 26192618.
25. Creixell *et al.* [Kinome-wide Decoding of Network Attacking Mutations Rewiring Cancer](http://dx.doi.org/10.1016/j.cell.2015.08.056) 2015, Cell 163, 1-16. DOI:10.1016/j.cell.2015.08.056
26. McLaren W, Gil L, Hunt SE, Riat HS, Ritchie GR, Thormann A, Flicek P, Cunningham F.  
    **The Ensembl Variant Effect Predictor.**  
    Genome Biology Jun 6;17(1):122. (**2016**)  
    [doi:10.1186/s13059-016-0974-4](https://genomebiology.biomedcentral.com/articles/10.1186/s13059-016-0974-4)
27. Lee D, Cho K-H. Sci Rep. 2018;8(1):5262
28. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5207440/pdf/pone.0168669.pdf>
29. <https://cancerres.aacrjournals.org/content/77/9/2213>
30. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6315782/pdf/cancers-10-00525.pdf>
31. <https://breast-cancer-research.biomedcentral.com/articles/10.1186/s13058-019-1125-0>
32. <https://www.researchgate.net/publication/232610667_Control_of_Breast_Cancer_Growth_and_Initiation_by_the_Stem_Cell-Associated_Transcription_Factor_TCF3>
33. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6518732/pdf/13046_2019_Article_1206.pdf>
34. <https://www.researchgate.net/publication/270907408_A_novel_role_for_the_SUMO_E3_ligase_PIAS1_in_cancer_metastasis>

Chu, I. M. et al. Expression of GATA3 in MDA-MB-231 Triple-negative Breast Cancer Cells Induces a Growth In-hibitory Response to TGFß. PLoS One8, e61125 (2013).

29.Chu, I. M. et al. GATA3 inhibits lysyl oxidase-mediated metastases of human basal triple-negative breast cancer cells. Oncogene31, 2017–2027 (2012).

30.Yan, W., Cao, Q. J., Arenas, R. B., Bentley, B. & Shao, R. GATA3 inhibits breast cancer metastasis through the re-versal of epithelial-mesenchymal transition. J. Biol. Chem.285, 14042–51 (2010).

31.Dydensborg, A. B. et al. GATA3 inhibits breast cancer growth and pulmonary breast cancer metastasis. Oncogene28, 2634–2642 (2009).

32.Kong, S. L., Li, G., Loh, S. L., Sung, W.-K. & Liu, E. T. Cellular reprogramming by the conjoint action of ER, FOXA1, and GATA3 to a ligand-inducible growth state. Mol. Syst. Biol.7, 526–526 (2014).

33.Matossian, M. D. et al. Panobinostat suppresses the mesenchymal phenotype in a novel claudin-low triple negativepatient-derived breast cancer model. Oncoscience5, 99–108 (2018)

1. Boyle, Annals of Oncology, 2012, 23012306
2. Dias et al., PloS One, 2017, 28045912
3. Prat et al., Breast Cancer Res, 2010, 20813035
4. Holliday et al., Breast Cancer Res, 2011, 21884641
5. Chalakur-Ramireddy et al., *Biosci*, 2018, 29298879
6. Telerman et al., *Nat. Rev. Cancer,* 2009, 19180095
7. Chu et al.*,* *PLoS One*, 2013, 23577196
8. Chu et al.*,* *Oncogene*, 2012, 21892208
9. Yan et al., *J. Biol. Chem,* 2010, 20189993
10. Dydensborg et al.*,* *Oncogene*, 2009, 19483726
11. Kong et al.*,* *Mol. Syst. Biol,* 2014, 21878914
12. Matossian*, Oncoscience*, 2018, 29854878
13. Huang et al., Semin. Cell Dev. Biol, 2009
14. Mochizuki et al., J Theor Biol., 2013