# Transcription factor network supports phenotypic heterogeneity cancer

David J. Wooten[1], Akshata Udyavar[2], Vito Quaranta[1]

***Short Abstract* — Cancer is recognized as a phenotypically heterogeneous disease, however the underlying causes of this heterogeneity are not well understood. Genomic instability in cancer has generally cited as the mechanism by which tumors acquire a phenotypically diverse profile, however recent results suggest the existence of epigenetically mediated heterogeneity as well. We hypothesized that epigenetic heterogeneity in cancer is supported by transcription factor regulatory networks and adopt the view that stable cell phenotypes can be described as attractors of this system. We test this hypothesis by building a Boolean network model of transcriptional regulation in small cell lung cancer, and conclude that experimentally observed heterogeneity is captured by the model.**

***Keywords* — Cancer heterogeneity, epigenetic landscape, attractors, transcription factor dynamics, small cell lung cancer**

## I. Introduction

UNDERSTANDING the origins and roles of inter- and intra-tumor heterogeneity remains a significant challenge facing cancer researchers. Molecular and genetic subtyping has introduced the promise of personalized therapies, however success has been limited in practice by a lack of well classified subtypes and the emergence of treatment resistant tumors. Both genetic and epigenetic intra-tumor heterogeneity has been implicated in the emergence of resistance in multiple tumor types [1,2], and overcoming this will be critical to the future development of more effective therapies.

A popular framework in theoretical systems biology suggests that a cell's phenotype may be understood as an attractor of the dynamical gene regulatory process [3-5]. This view reflects Waddington's epigenetic landscape in which undifferentiated cells roll "downhill" as they adopt distinct and differentiated identities. In this work, we test the hypothesis that heterogeneous cell states in small cell lung cancer (SCLC) can be explained as attractors of a transcription factor (TF) regulatory network.

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[1]Department of Cancer Biology, Vanderbilt University, 2201 West End Ave, Nashville, TN. E-mail: david.j.wooten@vanderbilt.edu

[2]Department of Chemical and Physical Biology, Vanderbitl University, 2201 West End Ave, Nashville, TN.

## II. Methods and Results

Using 53 SCLC cell lines from the Cancer Cell Line Encyclopedia we applied weighted gene co-expression network analysis (WGCNA) [6] to identify clusters of co-regulated genes. Two clusters were found whose expression patterns were significantly anti-correlated, and determined to be enriched for neuroendocrine/epithelial (NE) and mesenchymal (ML) phenotypes, respectively.

To derive a set of TFs which regulate the expression of these genes we cross-referenced results from ARACNE [7] with TF-DNA binding databases [8]. The resulting TF network was simulated as a Boolean network to identify stable attractors. These theoretically predicted attractors were found to correlate significantly with the observed expression profiles for both the NE and ML phenotypes. Western blots verify the differential expression of key transcription factors, and single cell flow cytometry reveals the heterogeneous presence of both cell types across multiple cell lines.

## III. Conclusion

We have derived a transcription factor regulatory network which is capable of capturing observed phenotypic heterogeneity in SCLC. This work provides a foundation for future studies exploring the controllability of heterogeneity in cancer.

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