\chapter{Introduction}

\section{Claudin-Low Triple Negative Breast Cancer}

Triple Negative Breast Cancer (TNBC) is an aggressive, heterogeneous subtype of breast cancer (BC) accounting for $20\%$ of BC cases. 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. This, coupled with the disease’s aggressive nature, makes it difficult to treat TNBC with current targeted therapies. While over $80\%$ of clinical trials studying TNBC therapeutics are exploring combinations of targets, there is currently no approved targeted treatment for TNBC.

Prior to 2007, breast cancer was classified into four intrinsic subtypes : Luminal A, Luminal B, HER2-enriched, and basal-like. As gene expression technologies evolved, a new subtype accounting for $7-14\%$ of invasive breast cancer was classified and termed Claudin-Low (CL) [SOURCE]. This subtype is characterized by low to absent expression of tight junction proteins Claudin 3, 4, and 7, as well as the cell adhesion molecule E-Cadherin. These molecular deficiencies are associated with the epithelial-to-mesenchymal transition (EMT) and the cancer stem cell (CSC) phenotype. EMT is the process by which epithelial cells gain migratory and invasive properties like that of mesenchymal stem cells [29462906]. EMT also allows for the formation of CSCs, which are associated with therapeutic resistance and metastasis [20813035]. Furthermore, CL tumors progress through the cell cycle slower than other BC subtypes, making them resistant to traditional cytotoxic chemotherapies that depend of rapid cell division for selectivity. Due to its lack of response to targeted and cytotoxic therapies, this aggressive disease has a poor prognosis and low survival rates. New therapeutic strategies are required for the treatment of CL TNBC.

\textbf{Include details about increased immune response?}

\section{Tumor Reversion}

Conventional cancer therapies are designed to induce apoptosis in cancerous cells. These therapies work by targeting rapidly dividing cells or malignant cells with characteristic cell-surface receptors. The limitations to this approach lie in that it is difficult to selectively choose which cells to kill, and ultimately normal cells are damaged in the process. Furthermore, not all tumors are rapidly dividing or present cell-surface receptors that can be targeted, rendering conventional therapies ineffective. An alternative therapeutic approach for cancer therapy is tumor reversion. Tumor reversion is the biological process by which tumor cells lose a significant fraction of their malignant phenotype (source). It is unlike conventional therapies in that instead of inducing apoptosis of cancerous cells, tumor reversion is aimed at reverting them to normal-like cells. In doing so, this approach reduces the deleterious impact on normal cells.

Tumor reversion has been observed for over a century. It was first observed by Boveri and Rous in 1907 when ovarian teratoma cells spontaneously differentiated into a normal somatic cell lineage [SOURCE]. Since then, it has been achieved \textit{in vitro}, \textit{in vivo}, and \textit{ex vivo} [SOURCE]. In particular, tumor reversion has been achieved \textit{in vitro} with the Claudin-Low Triple Negative Breast Cancer cell line MDA-MB-231, and \textit{ex vivo} in mice xenografted with MDA-MB-231 cells (sources).

Tumor reversion is not merely the reversal of the development of a malignant cell. Instead, it is its own biological process involving cell reprogramming through epigenetic and genetic tools that supersede the tumorigenic characteristics of a cell [SOURCE]. Molecular components of a cell such as transcription factors can be targeted to induce the reversion of tumorigenic cells by altering the cell's transcriptonal profile. Reversion targets differ from tumor suppressor targets because only reversion targets can trigger the shift from a malignant to a normal-like phenotype, rather than solely suppressing the malignant phenotype (Telerman, 2009).

\section{Cancer as a Developmental Disease}

So often is cancer reduced to its genetic component, that the larger picture of tumor cells in the context of development is lost. Normal cells, such as blood cells and epithelial cells, differentiate into distinct phenotypes despite having identical genomes. Genetic mutations do not indicate how these cells differentiate. The development of cancer can be viewed the same way. It has long been suggested that cancer cells are simply a cell type resulting from an error in the complex cell developmental processes that guide the differentiation of normal cells (source). Thus, the development of cancer can be seen as a systems-level dynamical process driven by an intracellular signaling network where signaling pathways have been dysregulated.

\section{Intracellular Signaling Networks}

An intracellular signaling network can accurately represent large-scale signaling processes that occur within cells (\textbf{Figure~\ref{fig:network}}). Represented as a graph, the nodes of an intracellular signaling network represent molecular components of a cell and edges are the regulatory interactions in between them. Unlike mathematical models, which require knowledge of system dynamics such as kinetic parameters or logic formalisms, signaling networks can be constructed when this information is unknown or difficult to determine. The intracellular signaling network consists of three layers. The first layer contains functionally related differentially expressed genes. These genes may not be highly differentially expressed, but they form a functional core of genes that are biologically relevant to development of the cell type. The next layer consists of transcription factors regulating those genes, and the last layer contains master regulators upstream of transcription factors.

\begin{figure} [hb!]

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\includegraphics[width = .5\textwidth]{images/network.png}

\caption{Toy Example of Intracellular Signaling Network}

\label{fig:network}

\end{figure}

Advancements in high throughput measurement technologies have enabled experiments to produce various genomic, transcriptomic, proteomic, and epigenomic profiles to describe a system. The combination of these "omics" can be used to infer the underlying signaling processes of a cell. Taking a data-driven approach to constructing an intracellular signaling network reduces bias and allows for a genome-wide representation of the cell. The resulting intracellular signaling network can be used to identify regulators that control the transcriptome of the cell, and therefore may be targets for reprogramming the cell.

The state of the intracellular signaling network evolves over time according to the regulatory relationships between each node until it reaches a steady state, known as an \textit{attractor}. Each network state corresponds to a gene expression pattern of the cell, and each attractor corresponds to a steady state gene expression profile associated with a cell fate (Kauffman). The state space of the intracellular signaling network is a high-dimensional space consisting of all possible states of the network. Attractor states are points in the state space characterized as “low energy states” at the bottom of a “potential well”. This potential well is the basin of attraction, and consists of unstable states that are close to the attractor in the state space and eventually converge to the attractor. An intracellular signaling network of N nodes has an N-dimensional state space, which cannot be visualized by the human eye. However, Waddington’s Epigenetic Landscape can be helpful for visualizing the difference in potential of network states.

\section{Waddington’s Epigenetic Landscape}

Waddington’s Epigenetic Landscape was first developed in the 1950s as a metaphor for cell differentiation. It is a concept from developmental biology in which a differentiating cell is likened to a ball rolling down a hill. Just as a ball rolls down the landscape until it comes to a stop in a valley, a stem-cell starts at the top of the landscape with potential to follow any accessible developmental path until it reaches a valley as fully differentiated cell. In this sense, the valley's of Waddington's epigenetic landscape each represent a cell type.

Waddington's Epigenetic Landscape has since been formally explained as a generalized potential landscape for the state space of an intracellular signaling network, derived from its dynamics. The height of a state in Waddington’s epigenetic landscape corresponds to how much “potential” the state has, or how unstable it is (\textbf{Figure~\ref{fig:landscape}}). Hence, the valleys of Waddington's landscape hold low potential states - the attractors of the intracellular signaling network - and hills correspond to unstable, transient states separating attractors. It is important to note that the term “epigenetic” in Waddington’s epigenetic landscape does not refer to the molecular biology term used to describe chromatin modifications. Instead, it is more closely related to the notion of an “epigenetic state” from physics – a state that arises from genetic interactions (Kauffman, 2009). In this framework, a switch between two cell phenotypes corresponds to the transition of a cell from one valley to another.

\textbf{Say that multiple attractors can represent the same phenotype?}

\begin{figure}

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\includegraphics[width = \textwidth]{images/waddington\_landscape.png}

\caption{Waddington's Epigenetic Landscape as a Potential Landscape of the N-Dimensional State Space of an Intracellular Signaling Network.}

The trajectory from the top to the bottom of the landscape represents cell development. Network states at the bottom of the landscape have low potential and are the attractors of the intracellular signaling network, corresponding to cell phenotypes.

\label{fig:landscape}

\end{figure}

\subsection{Cancer Attractors}

Since attractors of intracellular signaling networks correspond to cell types, and cancer is an abnormal cell type, then there must be attractors of the intracellular signaling network representing cancer cells. Kauffman described the cancer attractor in 1971 as a preexisting attractor of the intracellular signaling network that cannot be accessed by normal cells. This concept stems from the idea that intracellular signaling networks of normal cells can have many attractors that are not associated with normal physiological cell types but rather a byproduct of its complex dynamics. Many of these attractors may correspond to non-viable gene expression profiles, but some may be associated with a viable, proliferative phenotype, representing cancer. Normal developmental trajectories steer cells away from these attractors, but when those processes are dysregulated, as they are in the development of cancer, cells can fall into the basin of these cancer attractors.

The concept of the cancer attractor allows for the consideration of genetic mutations in the context of development. In a signaling network without genetic mutations, cancer attractors are not accessible. However, genetic mutations alter the structure of the intracellular signaling network by removing a node or edge in the case of loss-of-function mutations, and strengthening or adding additional edges in the case of gain-of-function mutations. These changes in the intracellular signaling network structure alter the topography of the epigenetic landscape by lowering barriers to reach cancer attractors, or in the case of a bifurcation event, creating a de novo cancer attractor from a previously unstable state. These mutations increase the probability that developing cells falls into a cancer attractor. Other factors, such as changes in the tumor micro environment, can have a similar effect on the topography of the landscape and increase the probability of the development of cancer.

\subsection{Tumor Reversion on Waddington's Epigenetic Landscape}

Transient non-genetic perturbations to the network state can move it from one site in the landscape to another, ultimately changing which basin of attraction it falls into (\textbf{Figure~\ref{fig:landscape control}}). Alternatively, constitutively controlling target nodes can alter the dynamics of the intracellular network, and therefore the topography of the epigenetic landscape, making it possible for a cell to move from the cancerous to normal attractor. The landscape is robust to small perturbations (SOURCE), but concerted perturbations of multiple network nodes may be sufficient to alter the network state. Thus, tumor reversion can be viewed as an optimal control problem in dynamical systems theory to identify combinations of targets that can trigger the shift out of the basin of the cancer attractor and towards that normal attractor.

\begin{figure} [H]

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\includegraphics[width = \textwidth]{images/waddington\_control.png}

\caption{Altering the initial condition of a cell can send it down a new developmental trajectory.}

\label{fig:landscape control}

\end{figure}

\section{Control Of Intracellular Signaling Networks}

Structure based control methods study the controllability of systems based solely on the structure of the network (SOURCES). Attractor based control methods aim to restrict the target states of a network to attractors. Structure-based attractor-based control methods aim to do both. Many structure based control methods are designed for linear systems or require dynamics to be linearized when applied to nonlinear systems. These approaches are not appropriate for applications to intracellular signaling networks, which are governed by nonlinear dynamics. Recently, a structure-based attractor-based control method for systems with non-linear dynamics, Feedback Vertex Set Control (FC), has been proposed. FC is a an extension of mathematical control theory applied to dynamical systems focused on the controllability of the system by restricting the target states to attractors.

The Feedback Vertex Set (FVS) of a network is a subset of nodes whose removal leaves the network without directed cycles. Mochizuki et al. mathematically proved that, for a network governed by non-linear dynamics, controlling the dynamics of the FVS is sufficient to drive the dynamics of the whole system to converge to any dynamical attractor. Overriding the state variables of the FVS nodes into the trajectory specified by a given dynamical attractor ensures that the network will approach that desired dynamical attractor.

Za\~{n}udo further expanded this framework to include source nodes (nodes with no incoming edges) as necessary control targets. Mochizuki and colleagues assume that source nodes converge to a unique trajectory and do not need independent control, but Za\~{n}udo asserts that source nodes can denote external stimuli a cell responds to, which could result in different attractors for source node states. The combination of the FVS and source nodes constitutes the FC set. Therefore, the identification of overrides of FC nodes that steer the network away from the cancer attractor and towards the normal associated attractor is equivalent to the identification of perturbations that should be applied to cancerous cells to trigger a shift towards a normal-like phenotype.

\textbf{Refer to experimental validation of FC? Talk about mFVS vs FVS?}

\section{Estimation of Network Dynamics}

In order to determine the effect of perturbations on the long-term behavior of a signaling network, an estimation of the system dynamics is necessary. Though signaling networks do not describe the temporal behavior of a system, dynamical systems based approaches for network analysis have recently been developed. One such approach is the estimation of network dynamics based on network structure. Lee and Cho developed an algorithm to estimate signal flow between network nodes to predict network attractors from user provided initial conditions based on the network topology. In this signal propagation algorithm, the activity of a node $i$ at time $t+1$ is:

\begin{equation\*}

a\_i(t+1) = \bigg(\prod\_ja\_j(t)^{W\_{ij}}\bigg)^\alpha a\_b(i)^{1-\alpha}

\end{equation\*}

Where $a\_j(t)$ is the activity of node j regulating node i at the previous time step, $W\_{ij}$ is the weight of the interaction between the two nodes, $a\_b(i)$ is the basal activity of node I, and $\alpha$ is a hyper parameter between 0 and 1. The algorithm assumes that input stimulation does not change according to time. Taking the logarithm and using matrix notation produces:

\begin{equation\*}

x(t+1) = \alpha W x(t) + (1-\alpha)b

\end{equation\*}

Where x is $log(a) \in P^N$, b is $log(a\_b) \in P^N$, and $W\in P^{N\times N}$ is a weight matrix. This equation can be solved iteratively until it convergences at some tolerance level, or the steady state solution of the equation can be solved with:

\begin{equation\*}

x\_s = (1-\alpha)(I-\alpha W)^{-1}b

\end{equation\*}

The link weight matrix, W, is normalized so that all links become a decay link. This ensures that the vale of signal flow between two molecules will be smaller than the activity of the source of the signal. W is defined as:

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\begin{equation\*}

W = D\_{in}^{-1/2}AD\_{out}^{-1/2} \\

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(D\_{in})\_{ii} = \sum\_j\abs{A\_{ij}}

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\begin{equation\*}

(D\_{out})\_{jj} = \sum\_j\abs{A\_{ij}}

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Where $A$ is the adjacency matrix of the network, and $D\_{in}$ and $D\_{out}$ are diagonal matrices consisting of the in-degree and out-degree for each node, respectively.

To compare the effects of several perturbations, the difference between log steady state attractor values can be calculated as

\begin{equation\*}

x^{fold} = x^{c\_1} – x^{c\_2}

\end{equation\*}

Where $x^{c\_i}$ denotes the log steady state activity change for each condition, $x^{fold}$ is essentially a log fold change. The sign (positive or negative) of $x^{fold}$ denotes if the activity of each node is up- or down-regulated in the compared conditions, rather than predicting the accurate amount of change.

\textbf{What else should I say about SFA?}