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Modeling of Interactions between Cancer Stem Cells and their Microenvironment: Predicting Clinical Response

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Abstract

Mathematical models of cancer stem cells are useful in translational cancer research for facilitating the understanding of tumor growth dynamics and for predicting treatment response and resistance to combined targeted therapies. In this chapter, we describe appealing aspects of different methods used in mathematical oncology and discuss compelling questions in oncology that can be addressed with these modeling techniques. We describe a simplified version of a model of the breast cancer stem cell niche, illustrate the visualization of the model, and apply stochastic simulation to generate full distributions and average trajectories of cell type populations over time. We further discuss the advent of single-cell data in studying cancer stem cell heterogeneity and how these data can be integrated with modeling to advance understanding of the dynamics of invasive and proliferative populations during cancer progression and response to therapy.

Keywords

Breast cancer; Cancer stem cell; Mathematical model; Optimal therapy design

1. Introduction

Mathematical modeling of cancer stem cells has proven useful in several important areas of translational cancer research. Those include, for example: understanding evolutionary dynamics of clonal populations and prediction of therapeutic resistance [1–3]; understanding tumor growth dynamics [4]; inferring the evolutionary dynamics that occur during cancer initiation and progression [5]; understanding the dynamics of stem cell state transitions and estimation of dedifferentiation rates [6, 7]; understanding the complex regulatory pathways that modulate stem cell behavior; and predicting clinical responses to combination therapies targeting the cancer stem cell niche [7].

Based on predictions from modeling, clinical oncologists are able to optimize dosing, frequency, and duration of therapies (e.g., dose dense treatments in adjuvant breast cancer therapies), which increase efficacy and minimize side effects, leading to improved outcomes [8–10]. Statistical modeling has also proven valuable in selecting prognostic and predictive markers in clinical trials in translational oncology [11]. There are many opportunities where modeling can expand its contribution to translational oncology. As single-cell transcriptomics and epigenetic data become more readily available and methods of simulation become more sophisticated, multiscale modeling will permit the integration of data that will inform models and improve predictions, which will ultimately lead to more effective therapies. In this chapter, we will review the methods that are currently being used

in mathematical oncology, and suggest areas where modeling could further be applied in cancer stem cell systems biology research.

2. Compelling Research Questions in (Cancer) Stem Cell Research That Can Be Addressed with Mathematical Modeling

2.1 Single-Cell Gene Expression and Epigenetic Data: How to Extract Information to Best Inform Models?

Single-cell sequencing and transcriptomics on a genome-wide level has advanced greatly in recent years. Statistical methods have been developed to analyze single-cell data in order to characterize tumor heterogeneity [12, 13], demonstrate clonal evolution [14], and infer phylogenetic relationships and ordering of mutations [15].

Genetic and epigenetic patterns that emerge during the processes of stem cell quiescence, activation, and differentiation can be captured using single-cell analysis. Intra-tumoral heterogeneity creates a challenge for the study of the interconnecting molecular events that guide these processes. Single-cell gene expression analysis has been used to explore cell heterogeneity in breast cancer and unravel gene expression variation in both cell line and patient- derived xenograft samples [16,17]. By examining expression levels of 96 genes from pathways involved in cell self-renewal, adhesion, and differentiation, three different patterns of expression in these genes were observed in single cells obtained from cell lines and from patient-derived xenograft samples. These patterns correspond to three distinct cell populations: epithelial Breast cancer stem cells (BCSCs), mesenchymal BCSCs, and non-stem cancer cells. Applying these methods to populations of circulating tumor cells will allow for the characterization of cell types within a patient at diagnosis and in response to treatment.

Whole transcriptome RNA-sequencing is used to transcriptional events that are continuously changing within a cell over time. Changes that are observed using this technology include alternative gene spliced transcripts, post-transcriptional modifications, gene fusion, mutations, and alterations in gene expression. Additionally, whole genome bisulfite sequencing is used to generate genome-wide analysis of DNA methylation. As these technologies become available within single-cell studies, sophisticated methods will need to be developed to analyze these data and distinguish relevant patterns from inherent noise that is anticipated within a single cell over time. Signaling pathways can be reconstructed from genome, transcriptome, and proteome data [18, 19]. While statistical inference has been successful in studying these components individually, combining information from each level is essential for understanding the system as a whole [20]. As our understanding of cellular networks improves, these results can be integrated with dynamic modeling approaches to estimate rates of stem cell state transitions and to identify regulatory nodes. As samples from circulating tumor cells from patients exposed to therapeutic combinations become available, these methods could be used to sort cell populations and track responses of each population to therapy.

2.2 Modeling Cell-Cell Interactions Between Cancer Stem Cells and Their Microenvironment

Because tumors consist of many cell types that interact with each other, as well as with the numerous cell types that are present in the tumor microenvironment, models that account for these interactions are required. Evolutionary game theory has been useful in modeling these interactions [1]. Models based on evolutionary game theory have been employed to examine mechanisms of growth control under conditions of competing resources [21], and have predicted the evolution of cooperation among tumor cells [22].

The breast cancer stem cell microenvironment consists of a number of diverse cell types including more differentiated tumor cells, stromal cells, endothelial cells, and immune cells. These cells interact with each other through a number of signaling mechanisms involving cytokines, growth factors, and other signaling molecules, such as miRNAs [23–29].

Under normal conditions, the stem cell niche regulates how stem cells participate in tissue generation, maintenance, and repair, preventing stem cell depletion and overpopulation. The interaction between these normal, tissue-specific stem cells and their niche is required for balanced tissue maintenance, and aberrant function of the niche may contribute to malignant transformation.

The cancer stem cell niche plays an important role in the regulation of tumor growth, and metastasis as well as in modulating therapeutic response. Here, we will describe the cellular elements of the breast cancer stem cell niche.

Breast cancer stem cells, exist in either a proliferative, epithelial state characterized by expression of ALDH as well as epithelial markers such as E-cadherin, or in a quiescent, invasive, mesenchymal state, characterized by expression of CD44 as well as additional mesenchymal markers such as vimentin, N-cadherin, Twist, and Slug and Snail [30]. When a BCSC is in the proliferative state, it can undergo symmetric self-renewal, or asymmetric self-renewal, giving rise to one identical copy of itself and one **bipotent progenitor cell** [31, 32]. Alternatively, it can undergo symmetric differentiation generating two bipotent progenitors (see Fig. 1). Mathematical modeling has shown that slight disruption in the balance between symmetric self-renewal, asymmetric self-renewal, and symmetric differentiation can lead to Gompertzian growth kinetics in tumors [7]. The bipotent progenitors give rise to either **luminal cells** or **basal cells**. These differentiated cancer cells comprise the bulk of the tumor, and currently most cancer treatment modalities are focused on this population. Other cells that are present in the stem cell microenvironment include **mesenchymal stem cells** that give rise to and maintain the stroma, **endothelial cells** that reside in the tumor vasculature and various elements of the immune system. In fact, recent studies have indicated that myeloid-derived suppressor cells (MDSCs) are able to directly stimulate BCSC self-renewal through the activation of the Notch pathway [33].

All of these cell types, as well as the microenvironmental signaling pathways that guide their interaction, need to be considered in a multiscale model of the breast cancer stem cell niche. These models may be helpful in predicting patient responses to combinatorial therapies

targeting angiogenesis, for promoting activation of cancer stem cells that are quiescent, and the prevention of invasion.

2.3 Relevance of Spatial Factors?

Spatial organization is a key factor for growth and tissue renewal during development and regeneration of healthy tissues [34]. It was first observed in the germ stem cell niche of *Drosophila mela-nogaster* that during cell division, the mitotic spindle is aligned with support cells of the niche so that the daughter cell that remains within the niche retains stem cell identity, whereas the daughter cell that is displaced outside the niche (away from self-renewal signals) initiates differentiation [35]. These oriented divisions have also been observed in mammalian epithelia. For example, the position of a stem cell within a hair follicle predicts whether it is likely to remain committed, generate precursors, or progress to a different fate [34]. Another example is that of stratified epithelial cells. Alignment of the stem cell niche along rigid basal lamina leads to regular morphologies, whereas alignment along a freely moving basal lamina leads to distorted epithelial morphologies [36].

The dynamics of the stem cell niche have been well described in the hematopoietic system.

Mathematical models designed to explore the mechanisms by which stem cells communicate with the niche, as well as the fact that cancer arises as a result of failure of this communication, have shown that coupled lineages allow for more controlled regulation of total blood cell numbers than uncoupled lineages and respond better to random perturbation to maintain homeostatic equilibrium [37].

In a model of the breast cancer stem cell niche, it would be ideal to also consider spatial effects. Spatial stochastic models have been used to study cancer initiation and progression [38] as well as mutational heterogeneity [39]. Spatial models have the potential to be helpful for the optimization of therapies targeting the stem cell niche.

2.4 Do Hypoxic Microenvironments Promote Late Recurrence?

The vasculature of tumors is very important in determining how nutrients and drugs are delivered to tumor cells. Recent evidence from mouse xenograft studies demonstrates that hypoxia, mediated by hypoxia-inducible factor 1 α , drives the stem/progenitor cell enrichment, and activates the Akt/ β -catenin cancer stem cell regulatory pathway [40]. Hypoxia stimulates ALDH⁺ epithelial BCSCs, located in the interior hypoxic zones of breast tumors, while the invasive mesenchymal cells are located on the leading edge of the tumor. Models that take into consideration the fractal geometric properties of tumor vascular networks, as well as the spatial gradients in resources and metabolic states, have been used to predict metabolic rates of tumors and derive universal growth curves to predict growth dynamics in response to targeted treatments [41]. Extensions of these growth equations including necrotic, quiescent, and proliferative states have been used to understand growth trajectories across tumor types. This type of modeling may be ideally suited to answer questions related to the growth of stem cell compartments in response to hypoxia, and for the selection of combined, targeted treatments for the eradication of both quiescent and proliferative BCSCs. Another potential option would be to use recent updates to stochastic simulation methods that include spatial effects. Introducing the spatial aspects of the stem

cell niche into simulation is required to answer questions related to hypoxic regulation of BCSC behavior.

2.5 Integration of Immunotherapy with molecularly Targeted and Cytotoxic Therapies

The advent of immunotherapy has led to a dramatic shift in the treatment and survival of several tumors, such as melanoma, renal cell carcinoma, lung cancer, and Hodgkin lymphoma [42–49]. Approximately one-quarter of patients with triple negative breast cancer respond to immunotherapy [50]. Immunotherapy is particularly successful in aggressive malignancies, where the percentage of tumor-initiating cells is high. For example, in melanoma the majority of tumor cells have capacity for self-renewal [51]. These tumors were the first where immunotherapy was shown to be successful. Immunotherapy, informed by mathematical modeling, may have a greater chance of leading to durable remissions [52].

Successful immunotherapy should target stem-like cells as well as bulk tumor cells. Mathematical modeling can be helpful in predicting the variable response to immunotherapy based on different proportions of cell types comprising a tumor. These models are especially relevant in the adjuvant setting, where tumor growth and invasion are driven by a small number of cells on a longer time scale, and where considerably more time and resources are required to directly observe survival outcomes in relation to therapy. If immunotherapy is successful in activating the immune system to target the stem cell compartment, it should eventually lead to eradication of the tumor. However, the required duration of therapy required to observe an appreciable change in bulk tumor size is unknown. Stochastic models can be used to predict extinction times of the cell populations comprising the tumor, allowing the estimation of the treatment duration required to eradicate cancer cells [53]. Models should also take into account the potential costs of immunotherapy, including autoimmune side effects. These models would allow selection of the optimal treatment dosing and duration that would have the best chance of tumor eradication while minimizing the risk of side effects.

Another area in immunotherapy where mathematical modeling may prove useful is in determining optimal combinations of therapies. A branching process model has been used to predict success of combination therapy under assumptions of mutations conferring resistance [54]. In models combining cytotoxic chemotherapy, vaccine therapy, CTLA4 and PD-1 inhibitors, and drugs targeting the BRAF and MEK pathways and other molecular pathways [55, 56], it will be important to model dosing and effectiveness in order to address the need to minimize potentially debilitating side effects, including autoimmune processes as well as the development of secondary malignancies.

3. Mathematical Modeling and Simulation Tools in Translational Oncology

In silico experiments can be used in concert with cell line experiments, animal xenograft model studies, and patient-oriented translational studies to complement and improve cancer stem cell research. Signaling networks in the cancer stem cell microenvironment are complex and much work is needed to understand the regulatory dynamics of this system. While gene knock-out experiments allow the delineation of the importance of each individual molecular component of the cancer stem cell microenvironment, the combination

of mathematical modeling with laboratory research allows studying of the emergent properties and provides a framework for elucidating the integrative dynamics of this complex system [57–60].

Given the levels of complexity of the cancer stem cell niche, selection of the most appropriate mathematical model remains challenging. We will describe a variety of mathematical modeling approaches and situations where specific methods can address this challenge by providing important biological insights.

3.1 Defining the Model

The breast cancer stem cell niche is a complex system comprised of cancer stem cells, and the surrounding cells and molecular signals that govern the behavior of the stem cells. Multiple overlapping feedback loops regulate whether a cancer stem cell undergoes self renewal, quiescence, differentiation, or apoptosis. The niche also regulates the rare event of partially differentiated breast epithelial cancer cells undergoing dedifferentiation into a stem-like state.

The scope of a model is defined by the *reactant species* involved, and by the *reactions* or *events* that take place. Examples of species involved in the breast cancer stem cell niche include cancer stem cells (quiescent and invasive versus proliferative), progenitor cells, differentiated luminal and basal cells, endothelial cells, mesenchymal cells, immune cells as well as the elements of signaling pathways, which regulate the transitions and interactions between these cell types [26, 61]. Those signaling pathway elements include cytokines (e.g., IL-6, IL-8, TGF- β , BMPs), receptors (e.g., HER2 and CXCR1), and intracellular signals, including protein kinases (e.g., Akt), transcription factor proteins (e.g., Lin28, I κ B, Stat3), microRNA precursors (e.g., let-7), and microRNAs (e.g., mir-93) [23–29]. The reactions of a model describe the important events that change the abundance of reactant species. Examples of reactions in the breast cancer stem cell niche include stem cell self renewal, quiescence, differentiation, and apoptosis. In general, a model should be kept as simple as possible, adding sufficient complexity to address the biological principles involved.

Figure 2 shows a simplified model of the state transitions that occur between the proliferative epithelial (MET) state of breast cancer stem cells (BCSCs) and their invasive quiescent mesenchymal (EMT) state (for illustration, a small number of species and reactions have been included here). The species include cell types (EMT and MET states of the BCSCs) and the factors (cytokines and intracellular signaling molecules) that regulate transitions between these two states. Reaction types include receptor binding and dissociation, as well as the state transitions. A more biologically complete model also includes the regulatory feedback loops that exist within this system, such as IL-6 activation of the Akt/Stat/ NF κ B pathway leading to increased transcription of IL-6, and the interaction of Lin-28/Let-7 and HER2 leading to activation of β -catenin driving self-renewal of epithelial BCSCs. The inclusion of such regulatory feedback loops thus enables the model to more closely simulate responses to environmentally stressful conditions.

3.2. Deterministic Versus Stochastic Models

Deterministic models can provide insight into many important aspects of microenvironmental signaling, including the understanding of dynamic control (as revealed by time-course studies), the impact of cellular cross-talk and identification of control points, and an indication of possible target points for treatment, as well as the exploration of dose-response relationships [62, 63]. There are several software packages in Matlab® and Mathematica® that enable investigators to explore nonlinear dynamics of complex systems based on a series of reaction rate equations. Examples of these include the Systems Biology Toolbox [40] in Matlab®, and ReactionKinetics [64] in Mathematica®.

An example of a reaction rate equation, applied to our simplified model of stem cell state transitions, describes the rate of change in E over time, the concentration of epithelial BCSCs and the rate of change in M over time, the concentration of mesenchymal BCSCs:

$$\frac{dE}{dt} = -(k_1y_1 + k_2y_2)E + (k_3y_3 + k_4y_4 + k_5y_5) \quad (1)$$

$$\frac{dM}{dt} = -(k_3y_3 + k_4y_4 + k_5y_5)M + (k_1y_1 + k_2y_2)E \quad (2)$$

where y_1 through y_5 are the concentrations of the microenvironmental factors (IL-6 • gp130, TGF- β • TGF- β R2, mir-93, BMP, and HER) that interact with the two cellular species, and k_1 through k_5 are rate constants describing the impact of the interaction of sets of species. In this simple model, we note that the rates of change over time for E and M are related as follows:

$$\frac{dE}{dt} = -\frac{dM}{dt} \quad (3)$$

If symmetric self-renewal, a process that results in an increase in the number of BCSCs was to be added into this mathematical model, as well as apoptosis, which decreases the number of BCSCs the system of equations would be:

$$\frac{dE}{dt} = -(k_1y_1 + k_2y_2)E + (\beta - \delta + k_3y_3 + k_4y_4 + k_5y_5)M \quad (4)$$

$$\frac{dM}{dt} = -(k_3y_3 + k_4y_4 + k_5y_5)M + (k_1y_1 + k_2y_2)E \quad (5)$$

where β and δ are the rates of symmetric self-renewal and apoptosis, respectively. In this case, the rate of change in epithelial and mesenchymal BCSCs would be equal only when $\beta = \delta$.

3.3 Visualizing the Model

Petri nets are diagrams that are used in systems biology to describe transitions and interactions that occur in complex systems [65]. In these graphs, boxes represent the occurrence of transitions, ovals represent species, and directed arcs delineate which reactant species enter the reaction (i.e., arrow flows from species to reaction) and products that are produced during the reaction (i.e., arrow flows from the reaction to the species). Figure 3 shows the petri net generated by our simplified model, which describes the state transitions between the quiescent and proliferative BCSC states. While Petri nets are based on strong mathematical foundation, they are also helpful for use as a visual communication aid to understand system behavior. The Petri net graphs in Fig. 3 were made using the GraphViz package in the Julia language.

3.4 Stochastic Simulation

In certain situations, stochastic models provide additional information when approaching scientific questions in mathematical oncology. Rare events, such as mutation and extinction, can be accounted for with stochastic models, as can random fluctuations in species counts that may greatly impact the population dynamics of the system. Using probabilistic models, one is able to calculate how frequently a population would become extinct under a given condition or treatment, as well as the required duration of therapy that would be needed to eradicate a stem cell population [58].

As the system gains increasing layers of complexity, more sophisticated models are required and these models become difficult to solve analytically. In this scenario, stochastic simulation techniques are helpful in studying niche dynamics where there can be large numbers of species and reactions and multiple overlapping feedback loops. In the stochastic reaction kinetics framework, a propensity must be specified for each reaction, as well as the net change in count of each species. For our simplified example of the stem cell state transitions, the species counts for epithelial BCSCs, mesenchymal BCSC, IL-6 • gp130, and HER2 • EGFR would be x_1 to x_4 , respectively, allowing the calculation of the propensity of each reaction as well as the stoichiometric change in each species for each reaction. Table 1 shows the propensity and stoichiometric change for two example reactions.

Stochastic simulation algorithms proceed by updating the state vector, which consists of particle counts for each of the reactant species, after each reaction (or set of reactions) is allowed to fire. In the stochastic simulation algorithm [66] the counts are updated after each reaction fire. As a result, this algorithm is the most accurate, but also the slowest. Approximate algorithms, such as the τ -leaping algorithm, leap over a set of reactions, in which the mean number of times a given reaction fires during the interval is given by the product of its propensity and the length of the leap interval [44]. While these methods increase computational speed, they can compromise accuracy in situations where the propensity is abruptly changing. An update to the τ -leaping algorithm, the step-anticipation

τ -leaping algorithm, allows the user to anticipate the change in propensity during the leap and leads to improved accuracy without compromising speed [67]. Outputs from stochastic simulation include full distributions of cell counts, as well as trajectories of cell counts over time. Figure 3 shows the full distributions (panel A) and mean trajectories (panel B) for epithelial BCSCs while varying the rate of symmetric self-renewal of epithelial BCSCs. Full distributions may be advantageous over the mean trajectory when one is interested in the frequency with which a population of cancer stem cells falls below a threshold of detectability or when investigating how frequently that population is eradicated in response to therapy.

3.5 3D Simulation and Agent-Based Modeling

Agent-based modeling is a microscale approach that combines elements of game theory, complex systems, emergence, and evolutionary programming to simulate the actions and interactions of individual cells and collective groups of cells to assess their effects on the system. They are particularly useful in accounting for details of smaller levels of systems and the prediction of the appearance of complex phenomena that occur at a higher level. Open source simulation packages have recently become available that allow simulation of the behavior of millions of cells in three-dimensional tissues. These methods have been applied to patient-calibrated models of ductal carcinoma in situ to predict clinical progression [68]. While these methods currently do not distinguish stem cell states, it would be a useful extension of this approach to predict bulk tumor response when cancer stem cells are therapeutically targeted.

3.6 .How to Integrate Models with Data

Mathematical modeling combined with experimental techniques in single-cell expression and epigenetic analysis represent a powerful combination to understand the dynamics of the cancer stem cell niche. An iterative approach is employed, where experimental data are used to validate models and further inform mathematical modeling parameters, and modeling predictions are used to guide experiments and suggest new ones [69]. In situations where known molecular mechanisms represented in the model are sufficient to account for physiologic or cell biological phenomena, the model can be used to explore the emergent system properties. When there are additional phenomena not explained by molecular mechanisms, the model could suggest new experiments to identify additional molecular mechanisms to explain these phenomena.

We anticipate that single-cell genomic and transcriptomic profiling will advance our understanding of intra-tumoral heterogeneity of cancer stem cells, the role of circulating CSC populations during cancer development and tumor progression and in the response to treatment. As our understanding of cellular interactions within the tumor and its tissue microenvironment advances, we will be able to design novel therapies that will more effectively target the tumor microenvironment. The ability to track the evolution of the cancer stem cell compartments in circulating tumor cells in response to therapy will be particularly helpful in the adjuvant setting where eradication of cancer stem cells is most critical.

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Fig. 1.

Types of stem cell division. A stem cell or stem-like cell can undergo symmetric self-renewal, giving rise to two identical copies of themselves, or asymmetric self-renewal, giving rise to one identical copy of itself and one partially differentiated progenitor cell. It can also undergo symmetric differentiation, in which it gives rise to two partially differentiated daughter cells.

Species: epithelial BCSC, mesenchymal BCSC, IL-6 and its receptor (gp130), TGF- β and its receptor (TGF- β R2), mir-93, BMP, HER2 and its receptor (EGFR).

Reactions:

Receptor binding and dissociation

Stem cell state transitions

Fig. 2.

Schematic of microenvironmental signals governing BCSC state transitions. In this simplified model of the BCSC niche, we identify the species involved, including cell types (the proliferative epithelial BCSCs and the quiescent mesenchymal BCSC populations) and cytokines and intracellular signals that regulate transition between these two states. The reactions included in our model directly or indirectly play a role in regulating the BCSC state transitions

Fig. 3.

Petri net generated by the simplified model of factors regulating transitions between proliferative and quiescent BCSC states. The Petri net demonstrates the interconnectivity of the model, defining its reactant species (ovals) and the transitions and events (boxes) that relate them to each other

Fig. 4. Sample output from stochastic simulation of stem cell state transitions. The first panel shows the full distribution of epithelial BCSC cell counts over 1000 simulations for a fixed period of time. For slower birth rates, BCSC cell populations reach smaller final counts. In the second panel, the average trajectories of epithelial-like BCSC populations are shown. When the birth rate is faster, BCSC cell counts initially diminish in response to therapy but later increase over time

Table 1

Propensity and stoichiometric change for two example reactions

Reaction	Propensity	Stoichiometric matrix
$\text{MET} + \text{IL-6} \bullet \text{gp130} \rightarrow \text{EMT} + \text{IL-6} \bullet \text{gp130}$	$C_1 \cdot x_1 \cdot x_2$	$V_{11} = -1, V_{12} = 0, V_{13} = +1$
$\text{EMT} + \text{HER2} \bullet \text{EGFR} \rightarrow \text{MET} + \text{HER2} \bullet \text{EGFR}$	$C_2 \cdot x_3 \cdot x_4$	$V_{21} = +1, V_{23} = -1, V_{24} = 0$

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