Mathematical Methods and Models in Biomedicine

Series: Lecture Notes on Mathematical Modelling in the Life Sciences

Ledzewicz, U.; Schättler, H.; Friedman, A.; Kashdan, E. (Eds)

2012 ,2012, IX, 419 p. 94 illus., 64 in color.

ISBN 978-1-4614-4177-9

Due: September 30, 2012

Chapter Title: Deciphering Fate Decision in Normal and Cancer Stem Cells: Mathematical Models and Their Experimental Verification

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Online Abstract:

Stem cells (SCs) control tissue development and maintain tissue homeostasis. The SC fate decision – continued replication or commitment to maturation – is decided in a dynamical mechanism according to the changing requirements of the tissue. According to cancer stem cell (CSC) theory, derangements of SC fate decision allow CSCs to stimulate and control tumor progression.

This chapter reviews a series of mathematical models aimed at elucidating fate decision mechanisms in SC and CSC populations. The first, general tissue model was designed to decipher the basic regulation of SC fate decision. The model assumes negative feedback through SC-to-SC interactions, referred to as quorum sensing (QS). Analysis shows that QS is the simplest fate decision model sufficient for maintaining tissue homeostatic properties. Further refinement and analysis of the model confirm that excessive SC proliferation, which can cause a homeostatic tissue to become cancerous, may be triggered by a change in the intensity of intercellular communication. Subsequently, a model describing the behavior of a cancerous tissue was developed. Its simulations suggest the necessity of combinational therapy, targeting both proliferation and differentiation, in order to effectively eliminate CSC population. *In vitro* experiments with CSCs from breast cancer cell-line supported the concept of QS, and also confirmed model prediction that tumor radius grows linearly with time, implying power law tumor growth rate.

A separate model is aimed to identify the molecular mechanism underlying fate decision control in a single SC, by incorporating intracellular signaling pathways that are sensitive to microenvironmental signals. This intracellular model was integrated within the previously studied tissue model. Analysis and simulations of the consequent multi-scale model show that the Dickkopf1 (Dkk1) ligand, secreted by SCs, may serve as a potential modulator of the QS mechanism. The model predicts existence of a threshold level of Dkk1, above which proliferating SCs switch to differentiation. This dose effect of Dkk1 on SC population was corroborated experimentally in breast CSCs.

The presented models suggest that the experimentally supported QS concept is the key to SC fate decision regulation. The generality of the models enables using them both for gaining global insights into cancer therapy, and for distinguishing specific possible therapeutic targets, as the implementation of the molecular scale processes can be done differently for specific cancer types.

Deciphering Fate Decision in Normal and Cancer Stem Cells – Mathematical Models and Their Experimental Verification

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1 Introduction

All tissues in the body are derived from stem cells (SCs). SCs are undifferentiated cells with two essential properties: unlimited replication capacity and the ability to differentiate into one or more specialized cell types. Embryonic SCs are pluripotent, meaning that they can give rise to nearly all cell types. Non-embryonic, adult SCs are found in various tissues and are capable of generating a limited set of tissue-specific cell types. The first discovered and most extensively studied type of adult SC is the hematopoietic SC, found in the bone marrow, which can give rise to all lineages of mature blood cells [12, 84]. Organ-specific SCs have been identified in many other tissues, including the liver, skin, brain and mammary gland (see [19] for review).

Adult SCs are responsible for tissue maintenance and renewal throughout the life of an organism. They replenish cell populations after normal cell death and following more extensive tissue damage caused by disease or injury. This regenerative ability has made SCs a key focus of scientific research, much of which is aimed at developing treatment for a broad variety of diseases [86, 87]. For many years, hematopoietic SCs have been successfully used to treat leukemia and other hematological disorders, through bone marrow transplantation [32]. Recently, clinical trials have been conducted to evaluate SC-based treatment for cardiovascular diseases [20], neurological diseases [43], spinal cord injuries [93] and diabetes [63]. Researchers have also attempted to exploit SCs in tissue engineering, aspiring to replace damaged tissues or cells by transplanting SCs that have been induced in vitro to differentiate into specific phenotypes [37].

SCs do not proliferate or differentiate at a constant rate. Rather, their behavior is highly complex and closely regulated, attuned to the exact needs of the tissue at any given time. For example, under normal conditions SCs might produce only a few differentiated tissue cells (DCs) at a continuous rate, but if a tissue is injured, the SCs may suddenly be required to produce larger quantities of DCs to repair it. It is crucial that SC proliferation and differentiation correspond precisely to the requirements of the tissue. Insufficiently rapid proliferation and differentiation may impair tissue

function, whereas overproliferation may result in uncontrolled growth and increase the occurrence of mutations, which might be cancerous [7]. The need to maintain the delicate balance between proliferation and differentiation implies the existence of a dynamic regulatory mechanism that, at each point in time, determines the *fate* of each SC in the tissue: according to the requirements of the tissue, the SC either proliferates, differentiates, or is quiescent.

The SC fate decision mechanism is a key component of *homeostasis*, or the maintenance of a stable internal environment, which is a fundamental condition for life. The fate decision mechanism is responsible, for example, for ensuring that the blood continuously contains enough red blood cells to carry oxygen to remote corners of the body, while at the same time triggering immune responses to unexpected, immediate threats. An understanding of SC fate decision can shed light on the very essence of homeostasis. Correspondingly, if we examine what happens when the fate decision mechanism malfunctions, we might be able to understand what happens in diseases in which homeostasis is interrupted – such as cancer.

One approach to investigating the role of SC fate decision in cancer relates to the theory of cancer stem cells. This theory suggests that, like healthy tissues, cancers are characterized by a hierarchical structure, in which a small minority of cancer cells (called cancer stem cells, or CSCs) have stem cell-like properties [6, 18, 75]. CSCs can proliferate indefinitely and are responsible for tumor growth, whereas the majority of (differentiated) cancer cells have only a limited ability to proliferate [57]. Even a few CSCs can regenerate a depleted tumor following treatment, and therefore, according to the CSC theory, the only way of effectively curing disease is to eliminate the CSC population [39]. Therapeutic approaches that target CSCs may entail simply killing these cells (elimination therapy) or, alternatively, inhibiting their proliferation (inhibition therapy), or driving them to differentiation (differentiation therapy), which eliminates their unlimited replication capacity [78]. The latter therapy involves interfering with CSC fate decision mechanisms. A deeper understanding of SC and CSC fate decision could be instrumental in the development of such treatments.

Herein we review a series of mathematical models formulated by Agur and colleagues, aimed at elucidating fate decision mechanisms in SC and CSC populations. These models are, then, used to gain insight into cancer therapy.

The first SC model by Agur et al. is aimed to decipher homeostasis in developing systems, using as few assumptions as possible [3]. This model is a cellular automaton, general enough to represent any normally functioning tissue. The model assumes that SC fate decision is determined by negative feedback, depending on local cell-cell interactions between the SCs. Specifically, Agur and colleagues assume that cells are able to ”count” the numbers of cells in their area and make decisions accordingly. This counting ability is known to exist in bacteria and is referred to as quorum sensing (QS). Analysis of this model [3, 45] shows that QS is sufficient for maintaining the homeostatic properties of a tissue. Moreover, this is the simplest model capable of retrieving homeostasis.

This model was followed by an effort to study the derangement of homeostasis, i.e., to learn what causes a normal, homeostatic tissue to become cancerous. To this end, Agur et al.’s original model was refined to incorporate a specific three dimen-

sional structure of the tissue and varying intensities of intracellular signaling (i.e., variation of the distance at which cells can detect the presence of other cells) [5]. Results confirmed that excessive SC proliferation may be triggered by change in the intensity of intercellular communication.

In a subsequent study, the model was adjusted in order to explore the behavior of a cancerous tissue containing CSCs [90]. Exploring the system behavior under various parameter values enabled the authors to identify general therapeutic approaches that are likely to be effective in targeting CSC populations.

A separate model aimed to identify the molecular mechanism underlying fate decision control in a single SC, by incorporating intracellular molecular signaling pathways that are sensitive to microenvironmental signals [4, 44]. This intracellular model was integrated within the previously studied tissue model, to create a multiscale model, which, if verified experimentally, could also serve as a useful tool for distinguishing specific possible therapeutic targets for eliminating CSCs [4]. Mathematical analysis [44] and simulations [4] of this model show that one of the key factors for fate decision regulation is the Dickkopf1 (Dkk1) ligand, which is secreted by SCs into the microenvironment, and may serve as a potential modulator of the negative feedback (QS) mechanism.

The rest of this chapter is organized as follows. Sections 2 and 3 provide background about the SC fate decision mechanism and about the theory of CSCs. Section 4 discusses mathematical modeling of SC fate decision. Section 5 discusses the tissue models, and section 6 discusses the molecular mechanism model. Section 7 discusses the results of the analysis of these models, the implications of considering the concept of feedback regulation through SC-to-SC interactions, and possible future applications for these models in CSC research.

2 Fate Decision in Stem Cells: Managing the Replication-Differentiation Balance

Tissues containing SCs are organized as cellular hierarchies, in which SCs make up a small fraction of the cell population [34]. SCs can divide either symmetrically or asymmetrically. In symmetric division, two similar SCs are produced, i.e., the SC proliferates. Asymmetric division, in contrast, yields one SC and one daughter cell that is more differentiated, termed a progenitor cell (PC). The PC transiently amplifies, meaning that it replicates for a limited time. The PC produces either additional PCs that are at an even more advanced stage of differentiation or terminally differentiated cells (DCs), which cannot replicate (Figure 1). DCs fulfill the tissue's functionality (e.g., blood cells, skin cells).

As noted above, the SC proliferation and differentiation rates must conform to the tissue's development and changing needs. The SCs must constantly supply the required quantities of DCs under various constraints, for example, in growing tissues or following disease or injury. At the same time, the size of the SC population must be restricted in order to prevent uncontrolled growth and crowding out of the DC population, and to decrease the risk of cancerous mutations [7].

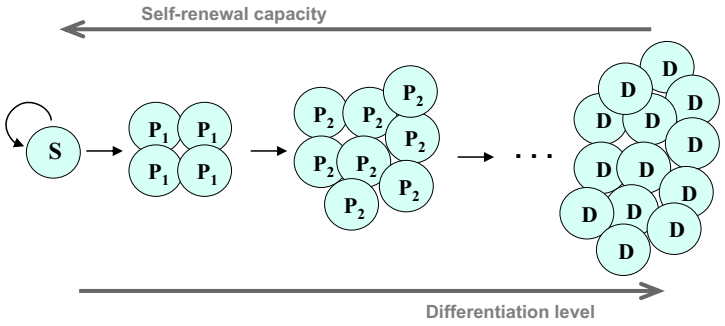

Fig. 1. Schematic description of the cell hierarchy in a tissue. A stem (S) cell can replicate indefinitely, while producing early progenitor cells (P_1), which in turn produce a larger population of more differentiated progenitors (P_2). The differentiation process is naturally continuous, and can go on through several lineages of PCs, eventually resulting in fully differentiated (D) cells.

Control over an SC's fate is exerted through the cell's microenvironment. The SC receives signals from its environment and, according to these signals, "decides" whether to replicate, differentiate, die (apoptosis) or remain quiescent. The signals regulating SC decisions might come from any number of sources: they may be determined by biochemical and mechanical characteristics of the environment, such as cytokine concentrations, cell-to-cell signals, extracellular matrix properties, and possibly somatic properties of the SC itself [65, 70, 89]. Some theories suggest that an external, physical tissue structure, transmits the various signals that regulate SC fate [14]. Other theories propose that SCs are capable of sending signals to one another without relying on additional structures. The QS theory, which forms the basis of the work by Agur et al., stems from the latter approach.

The SC fate decision mechanism controls the cell-production rate, and this control is key to tissue homeostasis. Derangement of this mechanism might lead to the development of cancer. The theory of CSCs, elaborated in the following section, creates an opportunity to further explore this notion.

3 Cancer Stem Cell Theory

The CSC theory asserts that some elements of the normal cellular hierarchy exist also in cancer. The theory states that in cancerous tissue, as in normal tissue, a small percentage of cells possess the ability of unlimited self-renewal [6, 18, 75]. These cells, called CSCs, drive the growth and spread of the disease, whereas their more differentiated progeny are destined to die, as they have limited or no ability to undergo further mitotic divisions [57]. It was originally postulated that CSCs arose from normal SCs that escaped the bounds of self-renewal [29, 52]. However, it is also possible that these cells are the result of mutations that caused a progenitor cell to re-acquire the ability of self-renewal [18].

In the 1990s, studies in patients with chronic myelogenous leukemia (CML) and acute myelogenous leukemia (AML) provided compelling evidence for the existence of CSCs [11, 29, 88]. Since then, cells with SC characteristics have been identified in solid cancer diseases, such as brain cancer and breast cancer. Putative SC populations have also been observed in cancer types such as colon, pancreas, prostate and melanoma (see review by Lobo et al. [57]). However, there is still controversy about the generality of the CSC theory [1, 42].

CSCs seem to be relatively resistant to conventional therapy. In several *in vitro* experiments, putative SCs in different cancer types, for example multiple myeloma and breast cancer, did not respond to conventional chemotherapeutic agents [56, 62]. Radioresistance was also shown for ex-vivo Glioma stem cells [9]. This may be because CSCs have a slow proliferation rate, in comparison to differentiated transiently amplifying tumor cells, while chemotherapy and radiotherapy generally targets rapidly proliferating cells [92]. Moreover, owing to their limitless replication capacity, CSCs that have survived treatment are capable of replenishing a depleted tumor. This may explain the high occurrence of cancer relapse after seemingly successful therapy with strong clinical response [66]. According to this hypothesis, effective tumor eradication must include agents that target CSCs [23]. Recently, outcomes of clinical trials in both myeloma [40] and breast cancer [21] patients have supported this theory by showing correlation between CSC quantities and patient survival after treatment.

Agents that efficaciously attack CSCs and cause their death (elimination therapy) are scarce, owing to these cells' resistance to drugs. Alternative therapy modalities that target CSCs include inhibiting CSC proliferation (inhibition therapy), or driving them to differentiate into transiently amplifying tumor cells (differentiation therapy), which leads to their terminal differentiation and eventual death, and facilitates their elimination through conventional therapy [78].

CSC theory suggests that cancerous tissues might have some kind of homeostatic regulation analogous to that in normal tissues. Thus, an understanding of fate decision mechanisms can shed light on CSC population sizes and dynamics, just as it can for SCs in normal tissue. Some of the main signaling pathways that participate in the regulation of SC fate decision in developmental processes have been found to be mutated in cancer [57, 83]. Researchers have begun to seek ways of targeting

CSCs by blocking or modifying these pathways, with the aim of allowing specific CSC therapy without affecting normal SCs [77].

4 Mathematical Modeling of Stem Cell Fate Decision

Understanding the mechanisms regulating SC fate decision is fundamental to understanding homeostasis – a basic condition for life. Specifically, deciphering fate decision in CSCs may be key to controlling and eliminating tumor growth. Although more and more biological data have become available regarding multiple factors in the microenvironment that affect SC fate decision [57], it is still not fully understood what controls an SC's decision to replicate or to differentiate into self-amplifying progenitors.

Over the last few years, mathematical models based on biological data have been proposed to describe SC fate decision processes at the cellular and intracellular levels. Some models have described the kinetics of molecular dynamical mechanisms, such as signaling pathways (e.g., [2, 44]). Systems biology approaches have been employed to investigate intracellular signaling pathways and transcription factor networks that play a role in determining SC fate (for a review see [70]).

In order to understand the dynamics of normal and cancerous tissues, which might enable researchers to identify drug targets for controlling tumor cell populations, it is not sufficient to investigate intracellular molecular processes. Rather, it is necessary to examine the tissue as a whole. Several mathematical models have been proposed to describe the role of SCs and CSCs in tissue balance. Many of these models used continuous ordinary differential equations (ODE) systems to describe the dynamics of different cell sub-populations (e.g., SCs and DCs) [22–24,30,51,60,61,67,71,73,80,85,96]. Others are discrete cellular automata models, where the behavior of individual cells is followed [3–5, 8, 28, 59, 64, 91]. Most of these studies did not focus on the regulation of fate decision and did not examine the validity of the methods used to model this decision. SC control was either considered stochastic, with fixed probabilities of differentiation and replication (e.g., [85]) or described by generic feedback from a homogeneous environment, with no specified underlying mechanisms [22–24, 30, 61, 67, 73, 80]. [71], [51] and [96] introduce regulation by specific environmental signals (e.g., NF-κB, GDF11 or EGFR), but they did not consider cell-to-cell interactions. Many of the models apply to specific systems and cannot be generalized [8, 59, 60, 64, 91].

In what follows we describe a series of models by Agur and colleagues, which focus both on tissue-level cell population dynamics and on intracellular molecular signaling in order to describe SC and CSC behavior. The models rely on a minimum of assumptions, all of which concern the SC fate decision mechanism. This minimalism enables the models to provide generalizable conclusions and concrete therapeutic recommendations that are not restricted to specific tissue or disease types.

5 General Description of Stem Cell Dynamics in Tissue: A Discrete Model

5.1 A general cellular automaton tissue model

The first model by Agur et al. was a general model describing tissues with hierarchical (SC-based) structures [3]. This model formed the basis for all SC models that followed, and its aim was to describe the simplest possible system capturing the essential properties of developing tissues which is capable of retrieving homeostasis in living systems.

The model is a simple, discrete dynamical system that can represent any tissue containing SCs. As the replication-differentiation balance in SCs is essential for maintenance of tissue homeostasis, the model assumes that replication and differentiation decisions are regulated by feedback regarding the condition of the tissue as a whole. Specifically, an SC's fate is assumed to be determined by feedback it receives from neighboring cell populations (referred to as *quorum sensing*, QS). The SC 'reads' and responds to signals from other SCs in its local microenvironment. Thus, QS is the fate decision mechanism controlling the SC replication-differentiation balance. The QS mechanism exists among Gram-negative bacteria, e.g., *Vibrio harveyi* and *Vibrio cholera* [10, 54]. In these bacteria, gene expression is regulated through the monitoring of population density, using diffusible molecules for communication.

To be able to take cell-cell feedback interactions into account, without assuming spatial homogeneity of the environmental signals, Agur et al. [3] used a *cellular automata* (CA) model, in which the behavior of each individual cell is tracked. In CA models, cells are discrete sites on a lattice. Time is also discretized, and at every time step, the state of each cell is defined by fixed rules. The rules can be deterministic or include stochasticity and probability distributions, but they must be determined by local conditions at the site of the specific cell.

The basic conceptual model includes the minimum of details necessary to represent a normally functioning tissue, as can be seen in the scheme in Figure 2. Tissue cells are represented by three types of automata cells: stem (S), differentiated (D) and null (N) cells, the latter representing vacant space in the tissue. An SC can either replicate, generating new SCs, or differentiate and become a DC. A DC is assumed to live in the system for a certain maturation time, and then die or migrate from the tissue, leaving an unoccupied space (N cell). This N cell may eventually become occupied by a new SC, created via a proliferation process (i.e., when a neighboring SC replicates). A DC in the model represents an entire cell line of progenitors and differentiated cells before they die or migrate from the tissue, generalized in the model through the DC life span. An SC's 'decision' to differentiate or proliferate depends on the number of SCs and N cells in its neighborhood, respectively. This dependency represents the effects of a variety of secreted cytokines in the cell's microenvironment, enabling the cell to sense which types of cells are in its proximity.

Mathematically, this system is represented by dynamics on a connected undirected graph $G = (V, E)$, where V and E are sets of vertices and edges, respectively. Each vertex is a cell, and the edges connect each vertex with its closest neighbors.

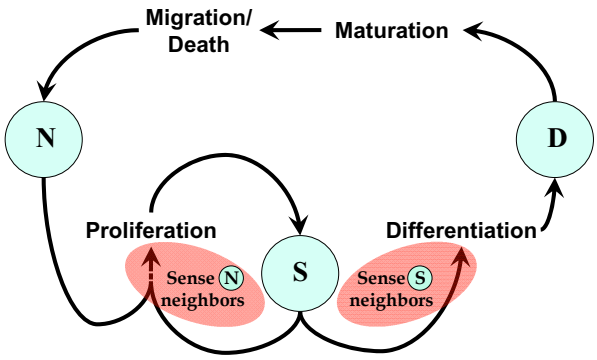

Fig. 2. Schematic description of the general tissue model. Three cell types – stem (S), differentiated (D) and null (N) cells – are represented. The pink areas show QS regulation on the SC fate decision.

The distance between each two vertices joined by an edge is defined as 1. Each vertex is equipped with an internal counter τ, measuring the cell's progress towards replication or differentiation, if it is an SC, or progress of maturation in the case of DCs. Note that the connected graph formulation compels no restrictions of the geometrical structure or dimensionality of the cellular automaton.

The state x of a vertex v at any time t (denoted $x^t(v)$) is a two-component variable, the first dimension denoting the cell's 'type' (either S, D or N), while the second is a non-negative integer that denotes its internal counter status. Agur et al. assumed that at each time step, the cell state can be changed due to differentiation (from S to D), proliferation (from N to S) or cell death (from D to N). These changes happen according to the following rules, depending on three non-negative integer parameters, namely Φ, Ψ and Θ:

A DC increases its life-time counter at each time step from τ to $\tau+1$, until when $\tau=\Phi$ it dies, and its state becomes $(N,0)$. Φ represents DC maturation time.

An SC increases its internal counter in the same way, until $\tau=\Psi$, where Ψ represents the duration of SC differentiation time. Then, if all of the SC's closest neighbors are SCs, the cell differentiates (its state becoming $(D,0)$). However, if an SC has a non-stem neighbor when $\tau=\Psi$, it does not differentiate but remains in the same state. This stipulation corresponds to the QS hypothesis of an SC receiving negative feedback signals from the other SCs in its microenvironment.

An N cell does not change its state, unless it has a stem neighbor, which provides the N cell with the potential to become occupied by the SC's daughter cell following the SC's replication. If the N cell has a stem neighbor, it increases its internal counter over time, until $\tau = \Theta$, where Θ represents the cell cycle time-period for SC proliferation. Then is the N cell is replaced with a new SC (i.e., its state becomes $(S,0)$).

These rules are described by an iterative operator, which defines what happens to a single vertex during the transition between time t and time $t+1$. This operator is applied simultaneously at each time step on all vertices in V, to define the state of the system at any time t. The operator definition is as follows:

$$x^t(v) = (D,\tau) \longrightarrow x^{t+1}(v) = \begin{cases} (N,0) & \text{if } \tau = \Phi, \\ (D,\tau+1) & \text{otherwise;} \end{cases} \tag{1}$$

$$x^t(v) = (S,\tau) \longrightarrow x^{t+1}(v) = \begin{cases} (D,0) & \text{if } \tau = \Psi \text{ and each } v\text{'s neighbor} \\ & \quad \text{is a stem cell,} \\ (S,\tau) & \text{if } \tau = \Psi \text{ and } v \text{ has a non-stem} \\ & \quad \text{neighbor} \\ (S,\tau+1) & \text{otherwise;} \end{cases} \tag{2}$$

$$x^t(v) = (N,\tau) \longrightarrow x^{t+1}(v) = \begin{cases} (N,0) & \text{if } v \text{ has no stem neighbor,} \\ (S,0) & \text{if } v \text{ has a stem neighbor} \\ & \quad \text{and } \tau = \Theta, \\ (N,\tau+1) & \text{otherwise;} \end{cases} \tag{3}$$

where a vertex is defined as a neighbor of v if the distance between the two vertices in the shortest-path metric induced by G is equal to 1.

5.2 Tissue homeostasis

In order to prove that this simple description of fate-decision regulation is sufficient to reproduce tissue homeostasis, Agur and colleagues conducted a mathematical analysis of the model [3, 45]. This resulted in a set of propositions, analytically proven, that together show that the model retains the basic properties essential for maintaining tissue homeostasis, reaching stable SC and DC populations. These theorems are non-quantitative and are robust for any potential refinements involving more elaborate rules. In other words, the model represents a family of cellular automata, and it can be modified to describe more specifically the cell-population control of specific cell types in different tissues. For example, imposing limitations on the kinetic parameters Φ, Ψ and Θ or imposing a certain geometrical structure will not affect the system's homeostatic properties, since the theorems that follow directly from the basic model assumptions will stay valid.

It was proven that, after some limited initial number of time steps, the tissue model sustains a minimal density of SCs at any time point. A constant supply of

mature cells is also assured, owing to the existence of a lower bound for the rate of production of DCs. (The proofs are detailed in [3].) The authors also analyzed the dynamics leading to a state in which the system dies out, i.e., when all vertices are in the state of N. They proved that the system never dies out, regardless of the initial SC population size, except under specific extreme conditions. This feature of the model reflects the tissue's ability to recover after SC depletion.

As will be shown later, the homeostatic balance reproduced by the model depends primarily on the minimal fraction of SCs in the particular SC's immediate neighborhood that leads to initiating its differentiation. For simplicity, in the first, general model this parameter (referred to as the QS parameter) was set to 1. The second condition guaranteeing homeostasis is a strictly positive time-delay between a cell's "birth" and its differentiation (Ψ). Since the latter condition exists for all biological cells, it will not be discussed any further. The other parameters of the model determine factors such as speed of cell production but do not influence the ability of tissue cell populations to reach homeostasis. This demonstrates the importance of the negative feedback, depicted in the model by rule 2, in which an SC does not differentiate unless its immediate microenvironment is saturated with SCs. This regulatory feedback has a crucial role in the homeostatic characteristics described above.

Moreover, further analysis of the model shows that under certain assumptions, the model guarantees stability in the proportion of SCs in the population [45]. Minimalistic and biologically plausible limitations on the cells' kinetic parameters, and some constraints on the symmetry of the initial SC subset, enable derivation of an expression for the fraction of SCs (and of DCs) in the population, averaged over a period of $\Psi+\Theta+\Phi+3$ time steps. During this time period, which is the minimal time for an automaton cell to go through all states (proliferation, differentiation and death), the SC population size fluctuates. However, for a special case of tube-like tissues, the size of the SC population is bounded from above and from below. When cylindrical symmetry is imposed on the graph, by constructing it as $h+1$ similar-sized layers, the numbers of all SCs and DCs at each time step do not differ from the average value by more than γ%, where

$$\gamma = \frac{400(\Psi+\Theta+\Phi+3)}{h+1} < \frac{1600(\Phi+1)}{h} \tag{4}$$

(proof in [45]). Importantly, given such a cylindrical structure, it is possible to calculate how many initial SCs are needed in the system in order to generate a stable cell population. This is of interest for tissue engineering, where tube-like tissues are constructed using SCs implemented in an artificial scaffold [81].

What can go wrong in tissue homeostasis? To examine the effect of deranged intercellular communication in the microenvironment, Agur and colleagues modified the model slightly [5]. They allowed the QS parameter to be less than unity, now denoting it K_i, representing the intensity of a signal that reaches an SC from another SC located at a distance of i on the connected graph. Rule 2 of the CA iterative operator was generalized, such that an SC differentiates only if the overall signal intensity it is exposed to (from all SCs in its proximity) is above a certain threshold.

The model was modified to have a cubic geometrical structure in order to simplify quantification of this demand (see figure 1 in [5]).

Numerical simulations of this model were performed under various values of K_i and with $\sim 10^4$ possible triplets of values for cell kinetic parameters Φ, Ψ, Θ, and different randomly chosen initial states. Most of the simulations resulted in one of two states: (i) system death, i.e., when all SCs differentiate and eventually die, or (ii) uncontrolled proliferation, i.e., when most of the SCs keep proliferating and do not differentiate throughout the duration of the simulation. In the latter case, when the modeled tissue becomes saturated with cells, the system achieves a quasi-steady-state, where a small stable fraction of the cell population is DCs, and a much greater part of the CA is occupied by SCs. Statistical segmentation of all simulation results showed that the magnitude of intercellular communication, represented by the QS parameter, dominantly affects the probability of uncontrolled proliferation and the probability of system death. The conclusion is that tissue homeostatic balance is highly dependent on signal intensity, which implies that QS is a crucial mechanism in fate decision.

Analysis and simulations, examining the effect of relations between the kinetic parameters, show that shortening DC lifespan can increase the proliferation of SCs. Analysis also shows that proliferation may become unlimited when the initial SC population is large. A possible implication for SC therapy would be a necessity to limit the initial number of implanted SCs. Regarding cancer, these results are consistent with the CSC theory rationalization that conventional therapy fails because it mainly eliminates non-CSC tumor cells (as represented in the simulation of shortening DC lifespan). Moreover, these results imply that such therapy may intensify CSC proliferation. Implications of the conceptual QS model for a cancerous tissue will be discussed in detail in the following subsection.

5.3 Model of cancerous tissue

The existence of the QS mechanism implies that the trigger for cancer may lie in the SC's ability to sense its microenvironment. The results of the model analysis described above suggest that excessive cell proliferation may result from changes in the kinetic parameters of the SCs changing their inherent ability to receive signals, or from changes in the microenvironment, affecting the magnitude of the signals transduced to SCs. Hence, cancer initiation may be stimulated by factors that cause microenvironmental changes (e.g., inflammation,) rather than by increased mutagenesis, as suggested elsewhere [58]. On the other hand, a natural outcome of excessive proliferation is an increase in the expected number of random mutations, including irreversible oncogenic mutations. If this explanation for carcinogenesis is valid, it means that in the first stage of cancer development, namely, during extensive proliferation of normal SCs, carcinogenesis can be reversed by inducing environmental changes that modify cell signaling intensity.

This also means that the SCs' microenvironment is where we should look for keys to possibly control, prevent or reverse the direction of tumor growth. If we

adopt the theory that CSCs are largely responsible for tumor growth, then controlling the dynamics of cancer progression might become possible through imposing changes in the environment of these SC-like cells. Drugs affecting local signals in the interactions between CSCs can be used for manipulating their differentiation and proliferation rates. Yet any attempt to eliminate CSCs must take into consideration the feedback of the CSC population on itself. For example, elimination of DCs may accelerate the CSC replication rate, owing to the negative feedback that CSCs receive from the population. Hence, cancer therapy based on targeting only DCs (or progenitor tumor cells) may be counterproductive, as it may stimulate CSC proliferation.

To analyze the dynamics of cancer cell populations containing CSCs, Vainstein et al. [90] adapted the SC model by Agur and colleagues, under the CSC theory assumption that hierarchical dynamics in cancer resemble those of normal tissues. Several changes were made in an attempt to increase the model's realism. In Vainstein et al.'s model, a CSC can be in a non-cycling (quiescent) state, or in a cycling state, in which a proliferation process takes place. Furthermore, whereas the original model described proliferation as a 'decision' of an empty space to become occupied by an SC, in this model proliferation is initiated by the proliferating cell (i.e., the internal counter for proliferation belongs to the dividing cell and not to the vacant space). Finally, the model is probabilistic, where QS control is achieved by setting the probability of differentiation and of entering proliferation cycle as a function of numbers of stem and vacant neighbor cells, respectively.

The model is implemented in a honeycomb-shaped CA grid, where each automata cell has six neighbors. The probability p_d of a non-cycling CSC A to differentiate is:

$$p_d = p_{\max} - \frac{a^m (p_{\max} - p_{\min})}{a^m + \Big(den(A) \Big)^m} \tag{5}$$

where $den(A) = N_1 + \dfrac{N_2}{2k}$ is the density of SCs in the proximity of A, N_i being the number of CSCs at a distance i from A, and k is the damping coefficient reflecting a reduction in signal intensity as the distance from the neighbor grows. $1/a$ represents the sensitivity to this microenvironmental signal, and m, p_{max} and p_{min} are parameters for steepness and maximal and minimal borders of the function, respectively.

The probability p_c of a non-cycling CSC A to enter the proliferation cell-cycle is:

$$p_c = 1 - (1 - p_0)^n \tag{6}$$

where n is the number of vacant automata cells in the proximity of A, calculated in the same way as $den(A)$, and p_0 is a parameter representing the proliferation probability when one neighboring vacant cell is available. When a CSC enters the cell cycle, an adjacent empty cell is randomly chosen, and after a certain proliferation time Θ this site becomes occupied by a new CSC. As in the previous models [3, 5], DCs possess an internal counter as well, to force their death after an estimated lifespan Φ.

The model was simulated under many different combinations of model parameter values, in biologically plausible ranges based on published information (see [90] for details). These model parameters include parameters determining a CSC's level

of sensitivity to microenvironmental signaling (a, k) and other parameters that influence proliferation and differentiation rates (p_{max}, p_0, Φ), as well as initial size and distribution of the cell population subsets (i.e., CSCs and DCs) in the CA.

Numerical simulations of the model, under almost all conditions tested, reproduced the dynamics of tumor growth in three phases: initial slow growth in the cell population size, accelerated growth, and decelerated growth until a state of saturation (due to the space limitations of the CA model). This saturation constitutes a "quasi-steady state" of cell population size with small fluctuations, which demonstrates the homeostatic tissue balance induced by the QS control mechanism, similar to the quasi-stability observed in simulations of the previous model [5]. This is also similar to the QS-controlled SC-DC balance that was observed in the analysis of the first general model [3] described in subsection 5.1. Multiple simulations of the CSC model showed that in the quasi-steady state, cell densities and spatial distributions of the cells were robust to stochastic effects, as well as to changes in the initial conditions and CA size.

The model can be used to examine possible methods of controlling tumor progression, by trying to pinpoint critical parameters that can be targeted in order to eliminate the CSC population. For this purpose, we can look at the simulation results (summarized in table 1) to observe what happens to the various cell populations when each model parameter is manipulated in various ways. Stimulating differentiation by increasing p_{max} or decreasing a or k (see eq. 5) reduced the density of non-cycling CSCs but did not affect cycling-CSC and DC cell populations. Shortening DC lifespan Φ, which is expected to indirectly also cause acceleration of CSC differentiation (see eq. 6), resulted in a decrease in the size of the total tumor cell population; however, the cycling-CSC density increased. On the other hand, decreasing the proliferation rate (p_0) caused a reduction in cycling-CSC density, but the non-cycling CSC population was not affected. The effect of changing each of the parameters was found to be independent of other changes.

These results indicate that there is no single parameter that can be manipulated in order to decrease densities of all cell types. Rather, only therapy that both inhibits proliferation and promotes differentiation can be effective. Simulation results of this combinational therapy showed that it can indeed successfully eradicate tumor cells of all cell types.

5.4 Model prediction of power law tumor growth rate and supporting experimental results

Examining the simulated macroscopic dynamics of tumor growth reveals an interesting result regarding tumor growth rate. Model dynamics in the intermediate stage of accelerated growth support previous results, suggesting power law tumor growth [26, 31, 36], as opposed to the widely-accepted assumption that the tumor growth rate is exponential or Gompertzian (e.g., [49]). In the simulation results for the two-dimensional CA, the total number of cells is well approximated by a parabola, i.e., it is proportional to the square of time [90]. Similar model simulations of a one-dimensional automaton show that growth of the total number of cells is

Table 1. Summary of effects of varying model parameters on all population sizes and on the total tumor size. Up arrow means increasing effect of the parameter on the specified cell density, down arrow means decreasing effect, and '−' means no effect. A change of no single parameter reduced both cycling and non-cycling CSC densities [90].

	Cycling CSC density	Non-cycling CSC density	DC density	Total tumor cell population
Increasing differentiation rate	–	↓	–	↓
Shortening DC lifespan	↑	–	↓	↓
Decreasing proliferation rate	↓	–	↓	↓

linear [48]. Therefore, the model suggests that a tumor radius should grow linearly with time. This is corroborated by experimental findings in breast cancer [36] and malignant glioma [82].

To test this, *in vitro* experiments [48] have been conducted in a breast cancer MCF-7 cell line. Small colonies of these cells were seeded in a thin channel or a Petri dish, and their growth was monitored for several days. One-dimensional growth of cells in channels showed that the progression rate of the cell-colony front line was linear (Figure 3). The two-dimensional area growth of cell colonies showed good fit with the model's prediction of quadratic growth (Figure 4). Measurements of 3D tumor growth, done in a mouse xenograft model of human breast cancer cells, also support this hypothesis of linear growth of tumor radius (data not shown). Analysis of these results and of the possible implications of power law tumor growth rate on clinical therapy is to be published in [48].

5.5 Experimental results supporting the quorum sensing concept

In vitro experiments [5] with CSCs or "stem-like cells" from the breast cancer MCF-7 cell line were conducted in order to test the theory of the QS control mechanism underlying the model. CSCs, or "stem-like cells" positive for the CD44 marker, were isolated from the breast cancer cell line and plated at different proportions with remaining cell populations. The proportion of CSCs was evaluated several times, until the culture was confluent, and cell populations' proportions reached equilibrium.

The experimental results revealed that, eventually, CSCs reached a constant proportion in the population, regardless of their initial plating density. These results imply the existence of some additional factor, beyond CSCs' intrinsic replication rate, that determines the proportion of CSCs in the population. The experimental setup dictates that this factor could only have come from the CSCs themselves (for example, through intercellular communication among CSCs), proving the existence of QS [5].

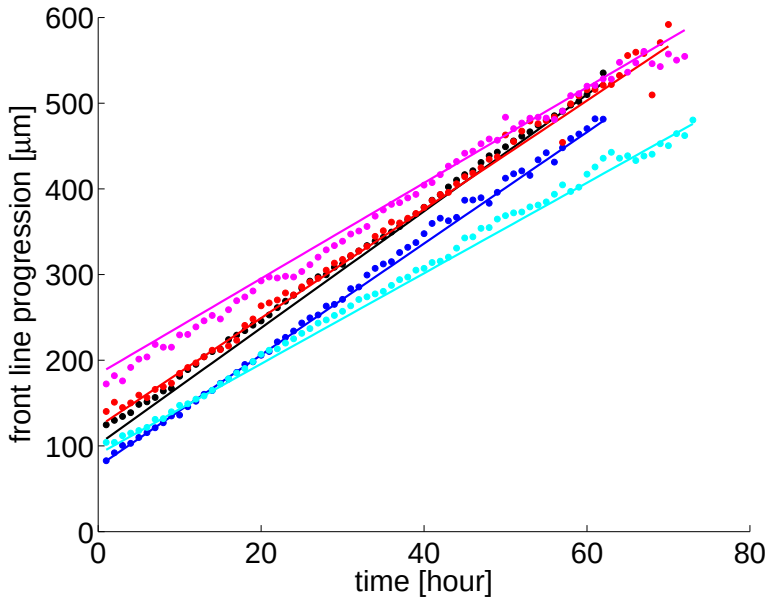

Fig. 3. Experiments prospectively confirming model's predictions in one dimension. One-dimensional front line progression of five MCF-7 cell colonies (each denoted by a different color) shows a linear growth pattern. The colony growth in a thin channel was experimentally measured in 1-hr intervals (dots). Comparing the slopes of the linear fit (lines) of all the different independent replicates shows that the growth rate is similar in the different replicates. (In collaboration with Bjoern Boysen, Andreas Lankenau, Claus Duschel, Fraunhofer Institute for Biomedical Engineering; IBMT)

6 A Molecular Model and its Implementation in the Large-Scale Tissue Model

6.1 Stem cell intracellular molecular model

The models described in section 5 show that the balance between SCs and DCs in a tissue (normal or cancerous) is controlled by QS, and specifically by SCs' sensitivity to microenvironmental signals. If one wishes to control the balance of different cell populations in a tissue, it is necessary to understand the molecular mechanisms that enable SCs to monitor their environment and, thus, to modulate tissue homeostasis. Understanding this molecular mechanism could enable prediction of the consequences of specific environmental changes, and this knowledge may be used to find ways to externally influence SC fate.

Several intracellular signaling pathways are known to be important for SC fate decision. These include the Wnt canonical pathway and the Notch and Shh pathways. These pathways take part in the fate decision process in embryonic SCs and are also suspected of being active in CSCs. Mutations in these pathways have been found in different cancer types [57, 83, 94].

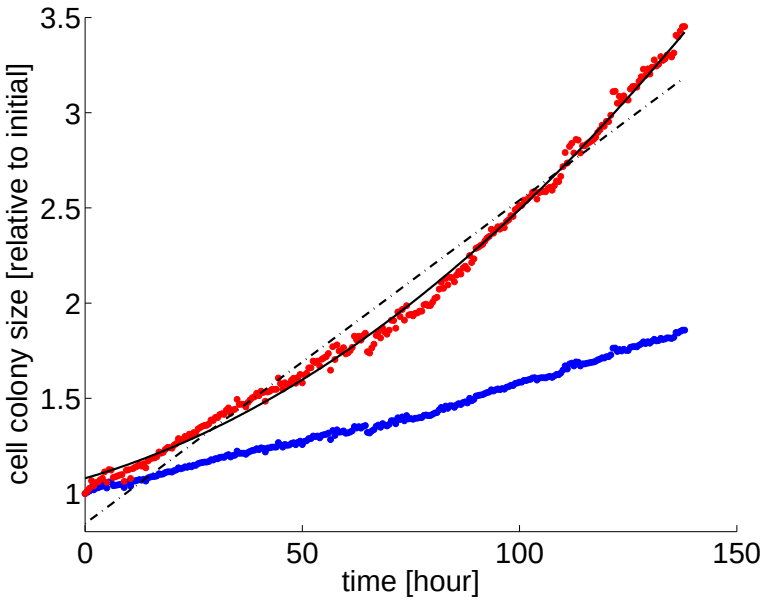

Fig. 4. Experiments prospectively confirming model's predictions in two dimensions. Two-dimensional growth of an MCF-7 cell colony shows a quadratic growth pattern. The colony area growth in a Petri dish was experimentally measured in 0.5-h intervals (red dots). Quadratic fit (solid line) is presented, in comparison to linear fit (dashed-dotted line). Growth of the cell colony radius (blue dots), calculated from area measurements assuming a circle structure, demonstrates the linearity of radial growth rate. (In collaboration with Bjoern Boysen, Andreas Lankenau, Claus Duschel, Fraunhofer Institute for Biomedical Engineering; IBMT)

Agur et al. [4] and Kirnasovsky et al. [44] formulated a new model, describing the pathways in a single breast cancer stem cell (BCSC). They implemented this intracellular network within the tissue model. Since the model's objective was to evaluate the fate decision process in BCSCs, the Wnt and Notch pathways were selected to be modeled in this work, because of their central role in the mammary tissue homeostasis, and in transformation to breast cancer [15, 25, 35, 74].

The Wnt canonical pathway is activated by binding of the Wnt ligands to Frizzled/LRP membrane receptors, causing accumulation of β-catenin [72, 76]. High β-catenin levels in the nucleus induce transcription of target genes, which leads to cell proliferation [13]. β-catenin is also involved in regulation of the adhesion molecule E-cadherin, which mediates SC contacts with neighboring cells [17]. The bound E-cadherins affect the efficiency of gene transcription induced by β-catenin [38]. The Notch pathway also plays an important role in SC self-renewal [35, 95]. The binding of the membrane-bound Notch receptors to neighbor cell transmembrane ligands, Delta, Serrate, Lag-2 (DSL), activates transcription of genes such as Hes, which suppress differentiation. A kinetic model of the intracellular steps of the Wnt

pathway, down to the level of β-catenin regulation, was first introduced by Lee and Heinrich [53], and further extended and analyzed by others (see review by [46]). The Notch pathway was also mathematically modeled [2]. However, to our knowledge, [4, 44] is the first model that specifically merges these pathways together. The approach used in this model is supported by recent information about crosstalk between the pathways [83].

The model of a single BCSC [4,44] was built on the basis of the above biological information. It comprises descriptions of the Wnt, Notch and E-cadherin pathways, including feedback loops and crosstalk between the pathways. This intracellular network was implemented [4] within a tissue model, where SCs and non-SCs are interconnected through signals in the microenvironment. The CA tissue model is similar to that described in the previous section (subsection 5.3), except that the SC decision to differentiate and its decision to enter the proliferation cycle are not simply a function of numbers of neighbor cells. Rather, these decisions are dictated deterministically by accumulation of proliferation factors (PF) and differentiation factors (DF) above certain thresholds (C_P and C_M, respectively). These factors are quantitatively estimated for each SC, taking into account the specific inter-cellular signal intensities, as illustrated in the scheme shown in Figure 5 [4].

In [4] and [44], the intracellular processes in a BCSC are modeled according to the following assumptions: activated LEF/TCF transcription factors (denoted in the equations as L) encourage proliferation by increasing PF levels (denoted as P). The activation of LEF/TCF is positively controlled by the Wnt signal intensity (denoted as S) and negatively controlled by the E-cadherins, which are bound to E-cadherins in neighboring cells. (The levels of total and bound E-cadherins are denoted as E and E_b, respectively.) E-cadherin synthesis is negatively regulated by Wnt signal intensity. The Wnt pathway is assumed to be activated by the Wnt ligand (W) in the close environment of the cell, while Dkk1 proteins (D) form a negative feedback loop on the pathway, since their secretion is enhanced as a function of the signal intensity, and they in turn inhibit the Wnt signal [16]. The Notch pathway is activated by Notch receptors (N) binding to DSL proteins in neighboring cells, which are assumed to be expressed by every cell in the model at a constant level. An activated Notch receptor stimulates a sequence of molecular events that increases Hes protein (H) synthesis, which inhibits cell differentiation by reducing DF (M). The Notch pathway is also regulated by a positive feedback loop, as LEF/TCF inhibits the degradation of the Notch receptor [41].

On the basis of these assumptions, a hybrid model was constructed, where cells in the tissue were represented as automata cells, while the intracellular realm of each SC in the tissue model was described by an ODE model of the protein-protein interactions in the Wnt and Notch signaling pathways. The ODE system is as follows:

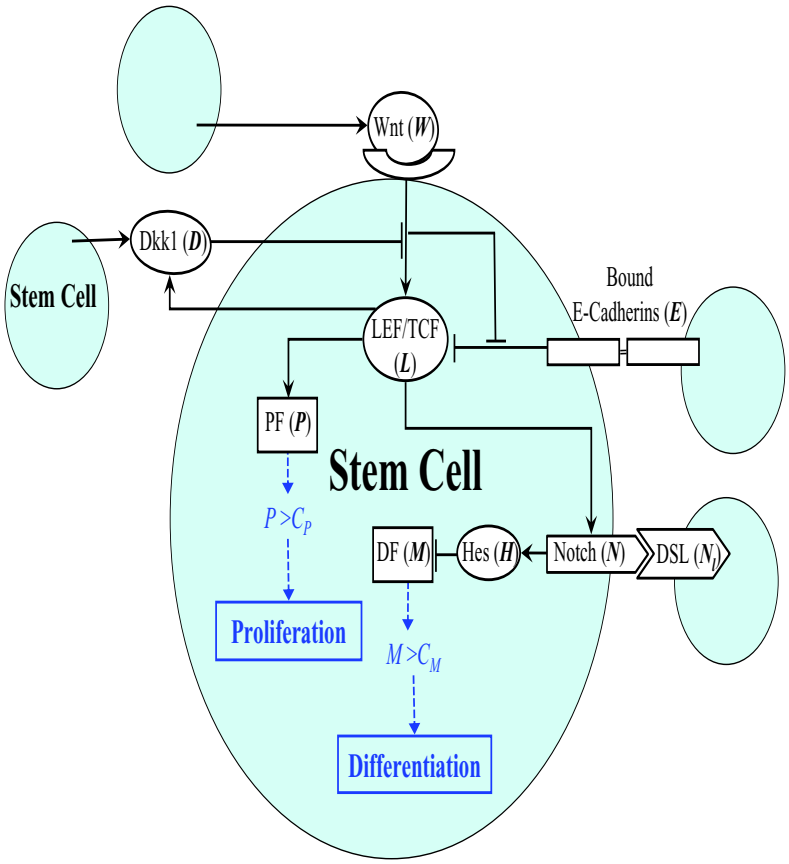

Fig. 5. Schematic representation of the mathematical model of an SC fate decision, regulated by signals in the cell's microenvironment. In this model, the SC's decision to proliferate and to differentiate are caused by the accumulation of proliferation and differentiation factors (PF and DF), respectively, above certain thresholds (C_P and C_M, respectively). The regulation of these factors by Wnt, Notch and E-cadherin signaling pathways is represented, including feedback loops and crosstalk between the pathways. The role of the proteins Wnt, Dickkopf1 (Dkk1), LEF/TCF and Hes, and of the cell-surface receptors Notch, Delta, Serrate, Lag-2 (DSL) and E-cadherin in these pathways is demonstrated, using pointed arrows (→) to represent activation and blunt arrows (⊣) to represent inhibition. Neighboring cells (blue circles) increase the levels of Wnt, E-cadherins and Notch-DSL bindings. A stem neighbor may also increase Dkk1 levels. The threshold-dependent effects of PF and DF, respectively, on the SC fate decision are shown in blue (dashed arrows). Notation for the level of every factor/protein, as used in the equations, is written in parentheses [4].

$$S(t) = f_F^{\uparrow}\left(W_t(t)\right) \cdot f_S^{\downarrow}\left(D_t(t)\right) \tag{7}$$

$$\dot{D} = f_D^{\uparrow}(L) - \mu_D \cdot D \tag{8}$$

$$\dot{L} = S \cdot f_L^{\downarrow}(E_b) - \mu_L \cdot L \tag{9}$$

$$\dot{E} = f_E^{\downarrow}(S) - \mu_E \cdot E \tag{10}$$

$$E_{b,i}(t) = \max\left(E_{b,i}(0), \max_{\tau \le t}(k_b \cdot E(t) \cdot E_i(t))\right) \tag{11}$$

$$\dot{P} = f_P^{\uparrow}(L) - \mu_P \cdot P \tag{12}$$

$$\dot{N} = p_N - f_N^{\downarrow}(L) \cdot N \tag{13}$$

$$\dot{H} = f_H^{\uparrow}(N_r) - \mu_H \cdot H \tag{14}$$

$$\dot{M} = f_M^{\downarrow}(H) - \mu_M \cdot M, \tag{15}$$

where the dependence of each protein on another protein is described using a sigmoid-shaped Hill function of the form: $f(x) = \dfrac{u \cdot a^m + v \cdot x^m}{a^m + x^m}$. Different functions (with different parameters u, v, a, m for each, which determine the exact shape and limits of the sigmoid)) are denoted by subscripts (f_F, f_S, etc.). Increasing and decreasing functions are denoted by $f^{\uparrow}$ and $f^{\downarrow}$, respectively.

In eq. 7, W_t is the total expression level of Wnt proteins in the environment of the considered cell, calculated as $W_t = \dfrac{1}{2} \cdot \left(W + \dfrac{W_n}{6}\right)$, where W is Wnt produced by the particular cell, and W_n is the sum of Wnt produced by all of the cells in the adjacent environment of that cell (maximum of 6 neighbors, due to the CA grid structure). D_t, representing the total expression level of Dkk1 in the environment of the cell, is calculated in a similar way, while D is the Dkk1 produced by the cell itself.

The parameters μ are the constant degradation rates for each different protein, respectively denoted by subscript (μ_D, μ_L, etc.).

The number of bound E-cadherins in the cell, E_b (eq. 9), is the sum of the number of E-cadherins bound to any adjacent cell: $E_b(t) = \sum_{i=1}^{6} E_{b,i}(t)$, where $E_{b,i}(t)$ is the number of E-cadherins bound to the neighboring cell in the i-th direction. $E_{b,i}(t)$ is dependent (eq. 11) on the level of E-cadherins in the considered cell E, in the considered neighboring cell E_i, and on the E-cadherin binding coefficient k_b.

p_N (eq. 13) is the Notch receptor synthesis rate.

N_r (eq. 14) is the level of Notch receptor ready to be activated, which is dependent on the number of Notch receptors in the cell (N) and also on the level of DSL in the microenvironment, in the following way: $N_r = \min(N, N_l)$, $N_l = \dfrac{1}{6} \cdot \sum_{i=1}^{6} N_{l,i}$, where $N_{l,i}$ is the DSL level of the neighboring cell being in i-th direction from the considered cell, and N_l is the total level of DSL directed to the cell.

6.2 Hybrid cellular automata (HCA) multi-scale model

The new tissue model [4], formed by implementation of this ODE model into the CA model described above, is considered a hybrid cellular automata (HCA) model since it contains both continuous protein activities and discrete cellular developmental and spatial states. This multi-scale model can be used to study consequences of specific intracellular changes on the structure of the tissue.

In order to analyze the molecular proliferation-differentiation regulation mechanism, the ODE system describing the signaling pathways was slightly simplified, and stability analysis was conducted, concluding that the system has a unique equilibrium point (i.e., stable values for all variables, in particular P and M), which is locally asymptotically stable [44]. Simulations showed that the system converges to an equilibrium point under a wide range of biologically relevant values of parameters and initial conditions.

The authors studied how the steady-state values for P and M, which reflect the SC's tendency to proliferate or differentiate, are dependent on the microenvironmental conditions [44]. This was performed by examining the system's response to changes in the various external signals, e.g., Dkk1 level, Wnt level, and the level of DSL receptors on the neighboring cells. Results of this analysis showed that the steady-state values for P and M depend on the level of local cell density. Under high local density, the high E-cadherin signal is dominant and causes differentiation. Under lower cell density, Wnt and Dkk1 signal intensities are dominant, and SCs proliferate at a rate that is dependent on the Dkk1 signal intensity. As will be explained later, the Dkk1 signal reflects the feedback regulation of the SC proportion in the population. Low cell density is generally characterized by a high proliferation rate; however, under extremely low cell density, low Notch signal leads to SC differentiation.

In addition, numerical simulations of the HCA model dynamics have been carried out. The CA honeycomb grid was initially seeded with randomly placed cells. To provide a stable model, parameter values for normal SCs were chosen in a range that promises tissue survival to confluence. These parameters were estimated to fit real characteristics of mammary SCs, based on relevant literature (for details see [4]). Then, for every time step, intracellular dynamics for all the cells were simulated by calculation of per-cell expression levels of all modeled proteins. Accordingly, cell fate was determined for each of the automata cells. This way, the effects of changes in specific protein concentrations, e.g., Dkk1, on the tissue dynamics, could be explored.

Simulations were also used to examine possible effects of defects in signaling pathways on SC proliferation-differentiation balance. Parameters were changed such that Notch receptor synthesis, Wnt ligand expression, or E-cadherin concentration required for LEF/TCF activity inhibition would increase by $5-20\%$. The model was re-simulated, and results were compared to the control simulation result with "normal" parameter values.

6.3 Dkk1 as a key regulating factor for fate decision regulation

Mathematical analysis of the model [44] showed that Dkk1 is a key, biologically plausible factor in fate decision regulation. The protein Dkk1 is secreted by SCs into the microenvironment and hence may serve as a potential QS modulator, as it can indicate the number of SCs in the close neighborhood. The model predicts that above a specific level, Dkk1 reduces proliferation, thus increasing differentiation.

The numerical simulation results suggest that the Dkk1 effect is biphasic. Below a critical concentration Dkk1 will not affect, and may even somewhat increase, the proportion of SCs in the population. Above this threshold, increasing Dkk1 concentration leads to a significant decrease in the numbers of both proliferating and quiescent SCs, as a result of differentiation.

Simulating dose effects of Dkk1 with changed model parameters, representing increasingly activated pathways due to mutations, did not change the qualitative dependence of SC proportion on Dkk1. However, the critical Dkk1 concentration under which proliferating SCs switch to differentiation depends on the pathway activity, as affected by the specific mutation. This implies that application of exogenous Dkk1 can be used to control the number of SCs that transition from proliferation to differentiation, and thus to maintain tissue homeostasis, even in situations of derangement of the intracellular mechanism controlling SC fate decision.

6.4 Experimental validation in breast cancer stem cells

To test model predictions, *in vitro* experiments on breast cancer MCF-7 cell line, and on primary cells from breast cancer patients, were conducted [4]. Agur and colleagues treated these cell colonies with graded doses of Dkk1, and measured each dose's effect on the proportion of breast cancer cells characterized by the SC phenotype $CD44^{+}CD24^{-/low}$ [6, 33] and on mammosphere formation [33]. For both the cell line and primary breast cancer cells, the results validated the model prediction that high Dkk1 levels would reduce the number of CSCs. This is demonstrated in Figure 6, where mammosphere formation under high Dkk1 levels is shown to decrease in a dose-dependant manner. At low levels of Dkk1, there was no increase in CSC numbers or mammosphere formation in primary breast cancer cells, but there was a significant increase in the number of CSCs observed for MCF-7 cells. Overall, the results were variable and highly dependent on the particular experimental protocol, i.e., duration of treatment with Dkk1 before and during the mammosphere-forming assay (see figure 4 in [4]), or before the flow cytometry of $CD44^{+}CD24^{-/low}$ cells. The latter result lends support to the prediction that the CSC proliferation rate under low Dkk1 levels may vary in different tissues, depending on parameters such as pathway activity levels and DCs' mortality.

In addition, the effect of Notch pathway activation and inhibition was examined. For this purpose, Agur and colleagues investigated the response of MCF-7 cells to a recombinant human Notch-receptor ligand DLL4, and exposure to DAPT, an inhibitor that blocks Notch receptor activity, as well as to knocking out Notch4 expression by siRNA. The experimental results confirm the role of Notch activation in increasing proliferation rate in BCSCs, as predicted by the model.

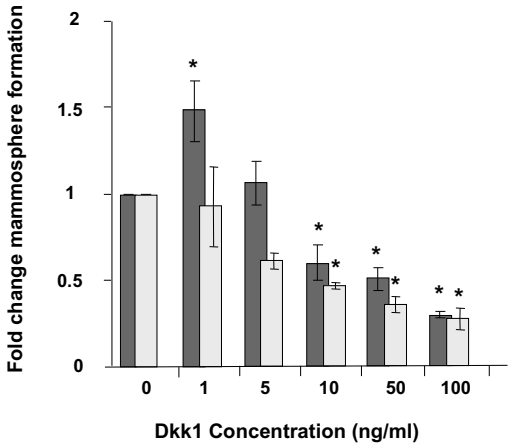

Fig. 6. Effect of Dkk1 treatment on mammosphere formation. Dkk1 effect was measured by MCF-7 cells (dark grey bars) and by primary human invasive breast cancer cells (bright grey bars). MCF-7 cells were pre-incubated with graded concentrations of Dkk1 in serum-free medium for 24 h, and then plated for mammosphere-forming assays for 7 days. Primary breast cancer cells were plated in the presence of Dkk1 and cultured for 7 days. Data for each concentration of Dkk1 are expressed as the fold change in mammosphere formation compared to untreated controls (0 ng/mL). Asterisks mark statistically significant differences [4].

7 Conclusion and Discussion

7.1 Establishment of the quorum sensing theory in healthy stem cells and in cancer

The series of mathematical models reviewed in this chapter was aimed at revealing what determines homeostasis in developing tissues. The fundamental question of homeostasis of tissue composition can, actually, be narrowed down to the question of how SC fate is decided, between continued replication and commitment to maturation. The understanding of this important control function might also illuminate the possible derangements of SC fate decision in cancer, thus leading to improved ways of controlling cancer progression.

The first SC model formulated by Agur and colleagues aimed to decipher the basic regulation of SC fate decision that yields homeostasis in developing tissues. Using a simple mathematical model, Agur et al. [3] showed that an extrinsic control – QS, or negative feedback on SC replication – is sufficient for maintaining homeostasis in a developing tissue, given the existence of an intrinsic control – a cell-cycle clock.

The developed CA model was general, describing only basic universal properties of SCs. No specific assumptions were made about tissue spatial structure, growth rate, duration of cell-cycle, or DC population characteristics, such as their lifespan. This underlines the generality of the model's conclusion, regarding the significance of the feedback of cell densities in the SC's environment on its fate decision. In other words, the SC's ability to 'count' its stem neighbors lies at the core of homeostasis. The QS concept in the context of oncogenesis was established experimentally in BCSCs as described above [5].

A more realistic model [90], implemented as a probabilistic CA, shed new light on the role of QS-regulated fate decision in CSC-based tumor progression. Simulations showed that the model yielded a quasi-steady state of the proportion of CSCs in the tumor cell population, which is comparable to the homeostatic resilient state of a normal tissue as described by the general model. Examination of how various changes in model parameters affect cell population size resulted in significant conclusions: First, accelerated death of DCs weakened the negative feedback that these cells posed on CSC proliferation, which, rather counter-intuitively, increased the number of cycling CSCs. This observation is in line with the CSC hypothesis, that in order to eliminate the tumor, CSCs must be targeted instead or in addition to the transient amplifying tumor cells [78]. Second, simulation results suggested that neither inhibition of proliferation alone nor stimulation of differentiation alone were sufficient to reduce both cycling and non-cycling CSC populations. Moreover, the model enabled analysis of the tumor growth dynamics, and the results implied that the tumor radius grows linearly with time.

Attempting to decipher the mechanism that enables QS, Agur et al. [4] introduced a new hybrid CA model, which described processes at the molecular level in addition to dynamics at the tissue level. The model included a detailed description of the intracellular system of signaling pathways, triggered by microenvironmental signals received from neighboring cells, that were found to balance SC replication and differentiation in developing tissues, and in particular in the mammary tissue and in breast cancer. Analyzing this model enabled the authors to explore the means by which tissue balance can be controlled. In the case of cancer, that would mean controlling tumor growth. Analysis of this model [44] pinpointed the Dkk1 protein as a key factor in breast cancer SC fate decision regulation, as it increases the probability of SC differentiation, in addition to reducing the probability of its proliferation. Numerical simulations of the model [4], corroborated by experiments, suggested the existence of a critical Dkk1 concentration, below which SC replication remains largely unaffected. Above this threshold, SC replication is significantly suppressed.

Overall, these models present a new concept, in which QS is viewed as the basic regulatory mechanism driving SC and CSC fate decision. This mechanism is the foundation for the maintenance of healthy tissue homeostasis [3, 45], and its disruption is at the source of cancer initiation [5]. Deciphering the explicit molecular mechanism that enables SCs to monitor their environment and, thus, to modulate tissue homeostasis, could pave the way to controlling fate decision. In the case of CSCs, this could lead to identifying new therapeutic agents to be used for control-

ling tumor progression, as demonstrated by a model of the network of intracellular signaling pathways controlling fate decision in breast cancer SCs [4, 44].

Recently, there is growing interest in the theory of the *stem cell niche*, suggesting that SCs reside in a supporting physiological microenvironment of a defined structure within the tissue [55]. The existence of such a niche for CSCs has been proposed, and experimental evidence for this structure has been found at least in colon cancer, where SCs seem to be localized in a narrow ring near the base of the crypts, and in breast cancer (for reviews see [14, 50, 79]). The normal or cancer SC niche is usually described as a physiological microenvironment, consisting of specialized cells that provide the necessary conditions for SCs to remain undifferentiated and proliferate. These supporting niche-cells are thought to participate in the regulation of SC fate decision and control their range of function [14]. However, the QS model presented here shows that the creation of an external, well-defined niche structure is not necessary for controlling the replication-differentiation balance. The niche could be formed spontaneously, with required conditions for SC proliferation and differentiation being supplied by the SC population itself. This proposition is supported by mathematical analysis [44] of the model for intra- and intercellular feedback mechanisms.

7.2 Implications of model analysis for cancer therapy

Theoretical analysis and simulations of these mathematical models have already yielded some conclusions that may help open new directions for cancer therapy. First, the intensity of SC-to-SC signaling was found to be a critical factor in the maintenance of tissue balance. Insufficient signal intensity, either due to environmental factors, or due to insufficient signal receipt, as a result of mutations inherent in the SCs themselves, was shown to lead to excessive SC proliferation until they completely deplete the DC population from the tissue [5]. This implies that cancer initiation may be stimulated by changes in the microenvironment, affecting the magnitude of the signals transduced to SCs, and that this process can be reversible under environmental changes that modify the signal intensity. If cancer initiation is caused by increased mutagenesis [58], no epigenetic process can prevent it.

Exploring the system behavior under various possible manipulations, changing factors that influence proliferation and differentiation rates, suggested that only combinational therapy that targets both CSC proliferation and differentiation can be effective [90]. This is in line with clinical experience, since drugs targeting CSCs were found to be clinically more effective when combined with each other, or with conventional therapy that mainly targets DCs [27, 68].

The implementation of a molecular model of processes on the intracellular scale pointed to Dkk1 as a key factor in SC fate decision regulation [4]. Dkk1 can be used for differentiation therapy, and is expected to be more effective than other agents stimulating SC differentiation, since it also reduces proliferation. According to the model, Dkk1 therapy challenges the QS-regulated fate decision, which is a general cellular homeostasis mechanism; hence, it should be more robust than other methods. However, the generality of the model does not allow parameter estimation that would

be accurate enough to estimate the optimal Dkk1 dosage. Optimizing the dosage of Dkk1 administration is crucial to effectiveness of therapy, since both simulations and experimental results showed that too low administration of Dkk1 may stimulate CSC proliferation. This may not only be ineffective in eliminating the tumor, but lead to the opposite result.

The simulation results also provide new insights into the tumor growth rate. Simulation results imply that tumor radius grows linearly with time, i.e., the growth of tumor volume is cubic in time [90]. This finding is corroborated by experimental results [48]. Yet only a few previous studies have related to the possibility of a power-law tumor growth rate [26, 31, 36], whereas it is widely accepted to model tumor growth as exponential or Gompertzian (e.g., [49]). This could have therapeutic implications, for example with regard to the design of schedules for radiotherapy, which are usually optimized assuming exponential tumor growth during the intervals between irradiation [69].

7.3 A robust tool for exploring and manipulating stem cells behavior

The generality of the basic CA model makes it relevant for the research of adult SCs of any kind. Refinement of the basic model by implementation of various explicit limitations, describing specific tissue-dependent characteristics, could enable researchers to model SC behavior in any tissue of interest, including solid and non-solid tumors. The model can be adjusted to describe, for example, the bone marrow with the migration of mature cells to the peripheral blood, or colon cancer with the specific spatial structure of the crypt.

The CA form of the model allows for consideration of the influence of neighboring cells on fate decision in the dynamical process of tumor growth. This is not possible in continuous CSC dynamical models, which describe the macroscopic behavior of CSCs, and which rely on assumptions about tumor growth rate or spatial homogeneity of environmental signals (cf. [23, 30]). Agur et al. [3] were the first to use a CA formulation to create a general model of SC behavior; other CA models, in contrast, were built to model SC spatial behavior in the specific tissue structure of the colon [59, 64, 91] or breast [8]. Enderling et al. [28] also used a CA model to describe tumor growth dynamics, but they did not try to simulate homeostatic properties in the tissue and had no constraints on the tissue's resilience, considering no feedback of the SC population on SC differentiation.

The multi-scale model [4], which includes modeling of intracellular-level dynamics in conjunction with the dynamics on the tissue level, is used for distinguishing possible therapeutic targets for eliminating CSCs. Notwithstanding, the model still captures the principal mechanism of SC fate decision regulation, i.e., the QS mechanism. Analysis of the model could point out the most effective therapeutic agents, those that attack the main control of CSCs' self-maintenance. The intracellular part was modeled in view of the biological data for BCSCs; however, a different approach could be adopted in order to gain insights for other specific cancer types and therapies.

Currently, the intracellular SC model is being expanded to combine more of the main relevant signaling pathways, including detailed molecular models for these pathways. For example, a detailed model of the Wnt pathway has been built, and its parameters were fitted and validated using experimental data [47]. Implementation of this detailed molecular model in the multi-scale tissue model will have the advantage of parameter availability. Thus, the resultant multi-scale model will be able to make quantitative predictions of the effects of different therapeutic agents. Such a model could be useful for the research of all cancer types, unlike the multi-scale model of van Leeuwen et al. [91], which is specific for colorectal cancer SCs.

Beyond the interest for cancer, manipulating SC fate decision can help in controlling *in vitro* developed tissues, engineered for the purpose of transplantation or designed for experimental research. A model describing the regulation of fate decision in a tissue *in vitro* could contribute to optimization of tissue engineering. For example, analysis of the basic tissue model presented here [45] suggested the possibility of evaluating the minimal number of SCs necessary for replenishing an empty scaffold. Furthermore, the ability to control SC proliferation and differentiation *in vitro* might help to increase the availability of adult SCs for transplantations. In addition, the model can be used for exploring other diseases caused by SC malfunctions.

In conclusion, the presented mathematical models suggest that QS is the key to SC fate decision regulation, and they also begin to decipher the molecular mechanisms underlying it. These efforts bring us closer to the goal of controlling fate decision in real tumors, using mathematical models as tools for quantitative predictions of the efficacy of concrete therapeutic agents for specific cancer types.

Acknowledgements. We thank Yuri Kogan and Karin Halevi-Tobias for helpful discussions and support, Karen Marron for helpful advice and careful editing of the manuscript, and the Chai Foundation for supporting the study.

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