
Mathematical Modeling of Stem Cells Related to Cancer

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Abstract

In this chapter we present an overview of different mathematical and numerical approaches to describe stem cell proliferation and differentiation and the development of small cancer stem cell populations that are origins of neoplasm disease. The purpose of this chapter is not to scare the reader with complex mathematical and numerical analysis. Instead we aim to summarize the wide range of possibilities that mathematics and computer sciences can offer to experimentalists and theoretical biologists.

We briefly introduce recently developed models that address different aspects of stem cell dynamics and cancer development. First we focus on models of periodic hematological diseases having origin in abnormal behavior of hematopoietic stem cells in bone marrow. We also present models, less organ specific, describing the differentiation and possible mutation of stem cells which can give rise to cancer. We introduce a simple

model which simulates the crucial interplay of proliferating and quiescent stem cells, and we discuss its potential application to treatment design. One of the presented models illustrates in detail the role of nuclear factor κ B-complex in proliferation of adult neural stem cells. Finally the reader will become acquainted with a computational model approach able to simulate stem cell dependent tissue development, homeostasis, and recovery from external perturbations.

Introduction

Recent research confirms that many neoplastic diseases like breast cancer [1], [2], prostate cancer [3], liver cancer [4], or leukemia [5], can occur because of mutations in normal stem and/or early progenitor cells. Moreover, it has been shown that various genes regulating the self-renewal in normal cells are also found in cancer cells [6]. It is known that most cancers are not clonal, but consist of heterogeneous sub-populations with distinct characteristics within a single neoplasm. These sub-populations are similar to the hierarchical tree of stem cell lineages. These results manifest the so-called stem cell cancer hypothesis, claiming that some cancers have stem cell origin. Moreover, it is known that the self-renewal ability in cancer stem cells (CSCs) is poorly controlled, leading to abnormal differentiation and faster proliferation in cancer tissue [7]--[9]. Hence, it appears likely that CSCs are often responsible for recurrences of the disease after treatment.

It is well known that key to tumor control is early detection of neoplastic changes in healthy tissue. It is important to understand the mechanism of carcinogenesis and the complexity of its progress and development. Modern life sciences, such as biology, molecular biology, medicine or oncology, are based primarily on experiments and clinical observations. Scientists try to understand the dynamics and particular function of selected signaling pathways, proteins and drugs focusing mainly only on statistical results of experiments. Although necessary, this knowledge does not reveal the general dynamics and the complexity of the problem even in case of single cell. It is crucial for future research and application in medicine to understand the 'engine' that drives the whole biochemical machinery.

Mathematical modeling and computational approaches have become more accepted by experimentalists and clinicians in recent years as contributing to new understandings of complicated cell mechanisms and tissue physiology. Indeed, even a single cell or small tissue samples are complex dynamical systems that adapt to environmental challenges in space and time - which renders them suitable to modeling. Mathematical models and numerical simulations can explain and uncover some still unknown aspects of cell behavior and tissue function. Models based on key biological mechanisms can give interesting insights and formulate predictions that cannot be derived from specific experiments or statistical data alone. Therefore, novel research approaches should incorporate interdisciplinary dialogs between biology, mathematical modeling and computer simulations to validate experimental data and non-intuitive scenarios such as the stem cell hypothesis (Figure 1).

Mathematical models can be classified into macroscopic and microscopic models depending on their level of description. Macroscopic models usually describe the evolution of sub-populations and interactions (competition and/or cooperation) between them rather than between individual entities.

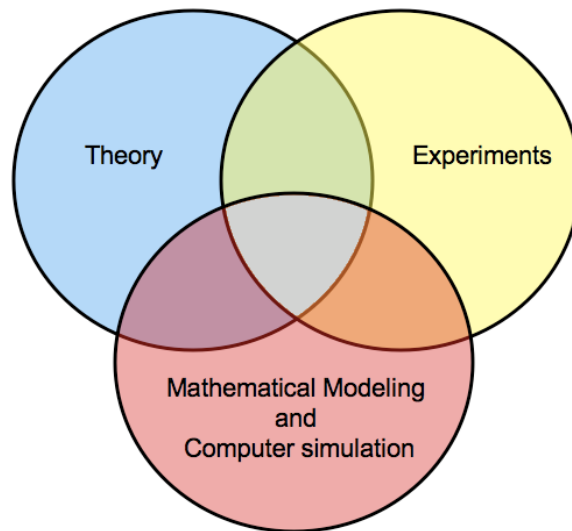


Figure 1. The schematic representation of modern biology, which should base on experiments run in laboratories, theory and should be also supported by mathematical models and computer simulations.

Microscopic models describe cellular and sub-cellular interactions between cells, and are based on processes like (a) chemical signaling between cells, and between cell and surrounding environment through the emission of activating or/and inhibiting cytokine signals; (b) protein and cyclin synthesis; (c) molecular interactions between proteins and protein complexes which can take place in the cell cytoplasm, in the nucleus or at the membrane.

From a mathematical and numerical point of view we furthermore distinguish between continuous and discrete models with respect to time. Continuous models describe the rate of change of for example cell density or protein concentrations over time. On the other hand in discrete models individual entities such as cells in populations or molecules in chemical concentrations are described at fixed separated time points. According to this distinction, in the following we first present some continuous modeling approaches that discuss stem cell dynamics in terms of cell populations or protein concentrations. Later on we discuss discrete models and develop an agent-based model of cell dynamics.

Continuous Mathematical Models

Continuous Models of Periodic Hematological Diseases

All blood cells arise from a common origin in the bone marrow, the hematopoietic stem cells. These stem cells can differentiate into one of three major cell lines: leukocytes, platelets, and erythrocytes. The exact details of how the numbers of circulating cells of each type are regulated remain somewhat obscure, though the broad outlines are clear. Interestingly, mathematical modeling of periodic hematological diseases has allowed us great insight into these regulatory processes.

In periodic chronic myelogenous leukemia (PCML) the leukocyte count varies periodically, typically between values of 30 to 200×10^9 cells/L. This is far above the normal value of 6×10^9 cells/L. The variation occurs with a period in the range of 40 to 80 days, which is very long in comparison with the maturation and lifespan of stem cells and leukocytes. In addition, oscillations may also occur in platelets and occasionally in reticulocytes. In these cases the platelet and reticulocyte periods are the same as the leukocyte periods [29] [30]. It has been argued that this, in addition to the occurrence of the Philadelphia chromosome in all differentiated lineages, is indicative of the stem cell origins of PCML [30].

Cyclical neutropenia (CN) is characterized by oscillations that are most prominent in neutrophils. Neutrophil numbers fall from normal or above normal levels to almost zero, and rise again, with a period of about 19-21 days in humans [31]--[33]. The disease also occurs in grey collies, with a shorter period of 11-16 days [34]. Interestingly, the platelet numbers typically oscillate as well, with the same period as neutrophils, but with a mean around the normal platelet level. Reticulocyte levels may also oscillate, again with the same period as neutrophils and platelets.

In both PCML and CN, the hypothesis that oscillations originate in stem cells is related to the fact that oscillations occur in different lines. However, in many earlier mathematical models, only one cell line, or one line coupled to the stem cells, is represented. In this context it is not possible to examine the effects of a destabilization in one line or in the stem cell compartment on whole system. For example, while Pujo-Menjouet et al., ([35]) explored how long period oscillations (as seen in PCML) could arise within the context of a G_0 stem cell model, the stem cell model alone could not predict whether leukocytes and platelets would oscillate at the levels observed in PCML. Similarly, Bernard et al., ([36]) were able to duplicate various features of cyclical neutropenia with an integrated mathematical model of the HSC and peripheral neutrophil control. However, since their model did not include platelet and erythrocyte regulation it is unknown if their simulated neutropenic conditions would be consistent with observed platelet and erythrocyte data in CN.

Cyclical thrombocytopenia (CT) is a rare hematological disorder described mostly in adults and characterized by periodic platelet count fluctuations of unknown etiology. The incidence of the statistically significant periodic platelet data is equally distributed between men and women. Sometimes this disease is associated with bleeding symptoms which have no apparent cause other than thrombocytopenia. Although, in general, human platelet levels remain relatively stable for years (150×10^9 - 450×10^9 platelets/L with an average of 290×10^9 platelets/L), many factors can influence an individual's platelet count (e.g. exercise, racial origin, some diseases, pregnancy). In CT the platelet counts oscillate from very low (1×10^9 platelets/L) to normal or very high levels (2000×10^9 platelets/L). This hematological disorder was reviewed by Go ([37]), Swinburne and Mackey ([38]), Cohen and Cooney ([39]), and has been the subject of mathematical modeling (Santillan et al. ([40]), Von Schulthess and Gessner ([41])).

In previous work leukocyte ([42], [43], [36]), erythrocyte ([44]--[46]) and platelet ([47], [40]) dynamics have been modelled separately, with the goal of building quantitative understanding of cellular production within the context of periodic hematological disorders. Colijn and Mackey [48] linked these models together, connecting models for the three distinct

cell lines to a mathematical model of the stem cell population ([49]--[51], [35]). The model has four distinct compartments representing hematopoietic stem cells and circulating leukocytes, platelets and erythrocytes. The stem cells are pluripotential and self-renewing, and can differentiate into the leukocyte, erythrocyte or platelet lines. Alternatively, stem cells may re-enter the proliferative phase of the stem cell compartment. The stem cell and leukocyte compartments are modelled using the stem cell model connected to a neutrophil population as in [36]. The platelet and erythrocyte compartments are simplified approximations of earlier modelling efforts. The full model is described by a system of five highly nonlinear differential delay equations, cf. [48], [52] for full details of the model development as well as its usage in understanding PCML and CN.

In [48] Colijn and Mackey analyzed data taken from published studies of periodic chronic myelogenous leukemia. These data were previously analyzed for significant periodicity using Lomb periodogram ([53]) techniques by Fortin and Mackey ([30]). Each primary study presented time series of patient leukocyte counts, and some also provided platelet and reticulocyte data. Based on estimates of parameters for a typical normal human, they systematically explored the changes in some of these parameters necessary to account for the quantitative data on leukocyte, platelet and reticulocyte cycling in these PCML patients.

Their results indicate that the oscillatory nature of PCML is probably generated through a bifurcation in the dynamics of the coupled hematopoietic stem cell compartment and the regulation of differentiated leukocytes. Based on the simulations, the critical model parameter changes required to simulate the periodic chronic myelogenous leukemia patient data are the amplification in the leukocyte line, the differentiation rate from the stem cell compartment into the leukocyte line, and the rate of apoptosis in the stem cell compartment. There was a suggestion that changes in the numbers of proliferating stem cells may be important in generating PCML.

In [52] Colijn and Mackey used the model from [48] to understand the dynamics of CN in nine grey collies and 27 CN patients and the effects of treatment with granulocyte colony stimulating factor (G-CSF). Their results lend credibility to the hypothesis that the origins of oscillation in cyclical neutropenia are a destabilization in the stem cell compartment, induced by changes in the neutrophil line; the oscillations are then transmitted to the other lines. A biological interpretation of their model simulations is that CN is due to a decreased amplification (increased apoptosis) within the proliferating neutrophil precursor compartment, and a decrease in the maximal rate of re-entry into the proliferative phase of the stem cell compartment.

An analysis of data from the grey collies as well as human patients under treatment with G-CSF implies that G-CSF leads to an increased amplification (lower rate of apoptosis) in the proliferating neutrophil precursors, and there was on average a higher rate of differentiation into the neutrophil line than without the treatment. As in the untreated subjects all of these changes are consistent with laboratory and clinical findings.

Recently Apostu and Mackey ([54]) used the model presented in [48] to elucidate the nature of cyclical thrombocytopenia. They concluded that the platelet fluctuations in amegakaryocytic CT are caused by a cyclic inhibition of megakaryocytopoiesis, accentuated by an increased platelet maturation time and a reduced release of platelets per megakaryocyte. Their results suggest that the onset of oscillations in autoimmune CT can be explained by an

accelerated peripheral destruction of platelets, exacerbated by an increased maturation of megakaryocytes and a slow relative growth rate of megakaryocytes.

A Continuous Model of Stem Cell and Cancer Stem Cell Proliferation, Differentiation and Maturation

The hypotheses and implications of mathematical models describing population dynamics of CSCs and their differentiation have recently been discussed [10]. In this paper a predictive model concerning self-renewing brain CSCs has validated principles according to which cancers can occur as a result of mutations in normal stem cells, early progenitor cells and even mature cells.

The model, a large system of ordinary differential equations, contains many nonlinear terms. We refrain from discussing the mathematics in detail, and refer to the original publication [10]. However, in the following we describe the model assumptions, present the main results and their biological interpretation. Ganguly and Puri distinguish seven main types of cells - stem cells (SC); early progenitor cells (EP); late progenitor cells (LP); mature cells (MC); abnormal stem cells (SC_A); abnormal early progenitor cells (EP_A) and abnormal progeny (tumor) cells (AP). Each cell type is considered as a separate model compartment, with cell population growth being modeled by considering individual rate expressions for each given cell type. Figure 2a shows a schematic representation of the model. SCs can self-renew with probability P_{SC} (both daughter cells retain stem cell features) or differentiate and transfer to the EP compartment. Stem cell DNA can mutate during the replication with probability M_{SC} such that the daughter cell which inherits the mutated gene is transferred into the SC_A population. EP cells, as well as EP_A cells, undergo only a limited number (k) of self-renewal steps. Thus, cells with identical self-renewal capacity are grouped into k sub-compartments. However, cells belonging to the k^{th} compartment can not self-renew any more. If EP_1 ($EP_{A,i}$) cells undergo cell division they self-renew into a subgroup EP_{i+1} ($EP_{A,i+1}$) with given probability P_{EP} ($P_{EP,A}$), which is assumed to be equal for all sub-populations, respectively. Dividing cells that do not supply the EP_{i+1} ($EP_{A,i+1}$) compartment differentiate into LP (AP, in case of abnormal cells) cells. Moreover, at each division EP_i cells are subject to mutations defined by mutation probability M_{EP} , which is assumed to be identical for each EP_i compartment. Naturally, the AP compartment is supplied by EP_A cells. Finally, cells that reach the MC or AP compartment die due to apoptosis.

Without mutations SC, EP and LP populations converge to a steady state, and for specific apoptosis rates the MC population remains constant. A sudden damage to the mature tissue (caused for example by acute radiation or surgery) activates tissue healing signals resulting in increased SC and EP cell proliferation rates. If mutations are enabled, SC and EP cells can produce SC_A or EP_A cells, respectively, that eventually form the AP compartment. Stochastic numerical simulations of this model show that an oncogenic event in SC leads to faster enrichment in AP cells, compared to the case of mutations in EP cells. Hence, the increase of the growth rate of EP_A cells leads to faster proliferation and increased cancer risk. It has been also concluded that AP cell growth rate increases as the mean time between two consecutive insults decreases.

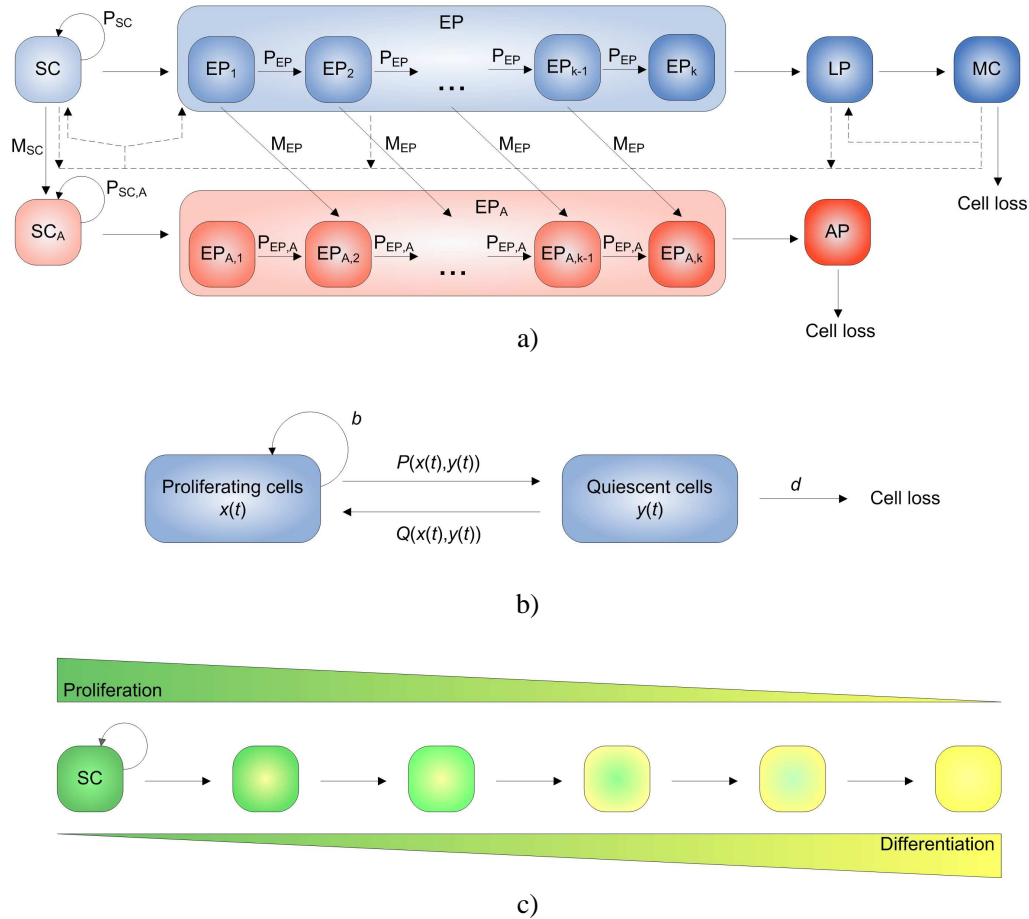


Figure 2. a) Multi compartment block diagram of a mathematical model developed by Ganguly and Puri, [10]. Here: SC represents the population of stem cells; EP early progenitor cells; LP late progenitor cells; MC mature cells; SC_A abnormal stem cells; EP_A abnormal early progenitor cells and AP abnormal progeny (tumor) cells. The EP cells as well as EP_A cells are split into k sub-compartments that contain identical cells regarding the type, which differ only with respect to the number of times they have undergone self-renewal. For more details see text. The solid lines indicate the direct transition from one compartment to another one, while dashed lines stand for feedback interaction loops. b) Two compartment block diagram of the mathematical model developed by Solyanik et al., [9]. Here $x(t)$ is the fraction of proliferating cells given at time t ; $y(t)$ is the fraction of quiescent cells given at time t ; b is the cell division rate of the proliferating cells and d is the cell death rate of quiescence cells. $P(x(t), y(t))$ and $Q(x(t), y(t))$ describe the intensity of cell transition from proliferating to quiescent compartment and vice versa, respectively. c) Stem cells and differentiated cells defined by their proliferation capacity and differentiation level. Non-differentiated stem cells have unlimited replicative potential and self-renewing ability. Stem cell progeny will differentiate with each proliferation and lose replication capacity.

Interaction between Quiescent and Proliferating Stem Cells Described by a Continuous Mathematical Model

Another interesting model postulated by [11], later discussed by [12], investigates the behavior of proliferating ($x(t)$) and quiescent cancer cell ($y(t)$) populations, based on

experimental data. Proliferating cells can divide with constant rate β or lose their division ability and therefore transit to the resting phase. Resting cells can either return to the proliferating state or die with constant rate δ (Figure 2b). The interactions between these two cancer cell sub-populations are described by the following system of coupled ordinary differential equations

$$\begin{aligned} \frac{d}{dt} x(t) &= \overbrace{\beta x(t)}^{\text{cell proliferation}} - \overbrace{P(x(t), y(t))x(t)}^{\text{transition from proliferating to quiescent compartment}} + \overbrace{Q(x(t), y(t))y(t)}^{\text{transition from quiescent to proliferating compartment}}, \\ \frac{d}{dt} y(t) &= \overbrace{P(x(t), y(t))x(t)}^{\text{transition from proliferating to quiescent compartment}} - \overbrace{Q(x(t), y(t))y(t)}^{\text{transition from quiescent to proliferating compartment}} - \overbrace{\delta y(t)}^{\text{cell death}}, \end{aligned} \quad (1)$$

where $P(x(t), y(t))$ and $Q(x(t), y(t))$ describe the intensity of cell transition from proliferating to quiescent state per day and vice versa. It is additionally assumed that the transition from the quiescent to the proliferating state depends on the number of proliferating cells only. Moreover, the transition of quiescent cells into the proliferating compartment $Q(x(t), y(t))$ increases with increasing $x(t)$ at low levels, but decreases when the number of proliferating cells becomes very large. It has been experimentally observed that cells can only proliferate in the sufficient presence of biological and physical factors. Hence, $P(x(t), y(t))$ depends on the number of all cells, and the authors proposed the following function

$$P(x(t), y(t)) = \gamma[x(t) + \alpha y(t)],$$

where α and γ describe the proliferating and quiescent cells' nutrient consumption, respectively.

With negligible transition from quiescent to proliferating compartment due to the large duration of growth delay i.e. $Q(x(t), y(t)) = 0$ [11] the system goes to a stable equilibrium at $(r/(a+r), a/(a+r))$. If $Q(x(t), y(t)) \neq 0$ a mathematical analysis of (1) becomes more difficult, and numerical simulations have to be used to reproduce the experimental data [12]. The experiments were done by Wallen et al., [13], [14], who cultivated the three unfed mouse mammary tumor cell lines 66, 67 and 68H for two weeks. In both, simulations and experiments it has been shown that more than 98% of unfed cells were alive after two weeks (initially the population grew exponentially before reaching a plateau), and only a small fraction of cells was still proliferating (for details see [13], [14]). We understand that a certain ratio of cells dies over time. However, the overall cell population remains constant as long as there is a sufficient fraction of proliferating cells.

The advantage of this kind of modeling approach is the estimation of treatment success prior to clinical application, as most cancer treatment protocols mainly eradicate proliferating cells. It seems to be possible to fit experimental data for a potential two weeks trial to estimate the long-term behavior of proliferating and quiescent populations such as cancer stem cells.

A Continuous Mathematical Model for Nuclear Factor κ B Complex Determinating the Proliferation of Adult Neural Stem Cells

The mathematical models described above simulate macroscopic properties of tissues or cell cultures, and neglect details of molecular mechanisms governing cell proliferation and cancer development. Since molecular biology can only investigate subsets of mechanisms that determine the cell behavior, mathematicians should develop multi-scale models that link different levels of complexity, for example, overexpression of proteins at the cellular level with the proliferation of cells at tissue level. The next model we would like to discuss in more detail focuses on the role of sub-cellular processing on single stem cell dynamics.

In a recent study it has been shown that the nuclear factor κ B (NF- κ B) plays an essential role in proliferation of neural stem cells (NSCs) [15]. In fact, most substances or conditions positively modulate proliferation of NSCs via the NF- κ B pathway. Following these findings, Piotrowska et al., [16] have proposed a simple continuous mathematical model (containing two ordinary differential equations) for NF- κ B dependent proliferation of NSCs.

NF- κ B protein is a transcription factor, crucially involved in many biological and physical processes such as regulation apoptosis and survival genes, inflammation, cancer, innate immunity [17], [18], as well as memory formation and learning [19]. Furthermore, NF- κ B is directly responsible for cell growth and proliferation [20]. The most frequent form of NF- κ B is a heterodimer composed of two subunits: p50 and p65. Activation of NF- κ B is mainly controlled at the posttranscriptional level by complex formation with the inhibitory protein I κ B in the cytoplasm. After binding of the stimulating agent (e.g., tumour necrosis factor (TNF) or erythropoietin (EPO)) to a receptor in the cell membrane, the signal is transduced via intermediate kinases to the IKK $\alpha/\beta/\gamma$ complex (Figure 3a). This leads subsequently to phosphorylation of I κ B and its proteasome degradation. This degradation triggers the translocation of NF- κ B into the nucleus followed by binding to regulatory DNA-sequences and initiation of a transcription process [21].

The changes of active NF- κ B concentration in a cell ($x(t)$) can be described by a nonlinear ordinary differential equation, such as

$$\frac{d}{dt} x(t) = \underbrace{\alpha \frac{1 - x(t)}{J_1 + 1 - x(t)}}_{\text{activation of NF-}\kappa\text{B}} - \underbrace{\beta \frac{\delta(t)x(t)}{J_2 + x(t)}}_{\text{deactivation of NF-}\kappa\text{B due to I}\kappa\text{B binding}} - \underbrace{kx(t)}_{\text{proteasomal degradation of NF-}\kappa\text{B}}, \quad (2)$$

where $1 - x(t)$ and $\delta(t)$ denote the scaled concentrations of inactive NF- κ B and I κ B at time t , respectively, and J_1 , J_2 , k , α and β are nonnegative constants [16]. The first term in Eq. (2) represents the activation of NF- κ B via phosphorylation of I κ B by the IKK complex due to the stimulus represented by α ; while the second term models the deactivation of active NF- κ B by NF- κ B driven autoregulatory expression of I κ B. Both terms are governed by so-called Michaelis-Menten kinetics, with J_1 and J_2 being the Michaelis-Menten constants [22]. The last term in Eq. (2) corresponds to the degradation of active NF- κ B.

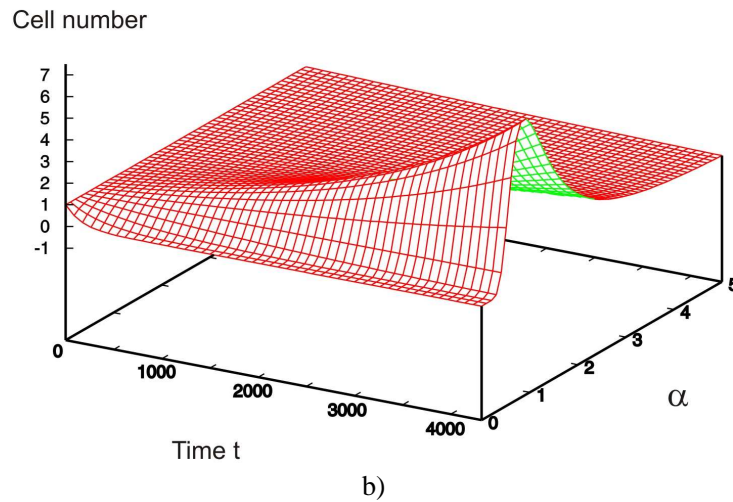
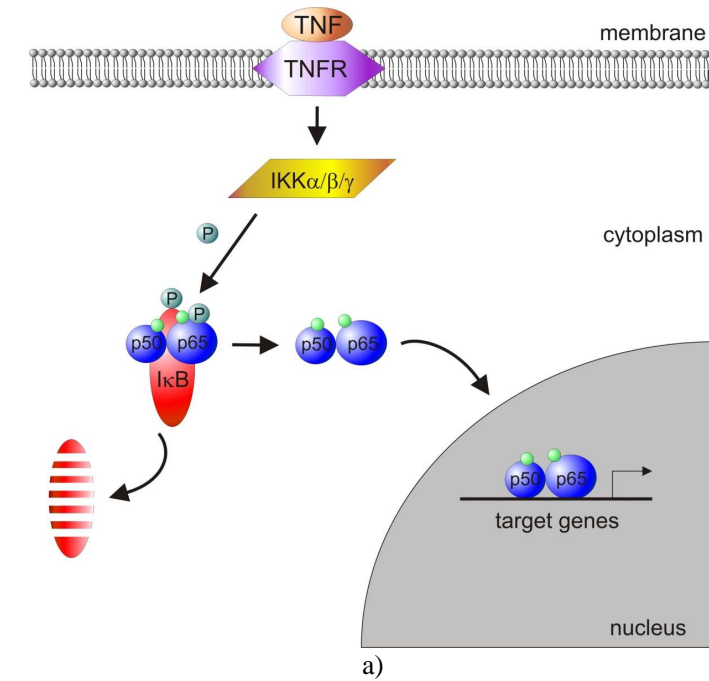


Figure 3. a) Schematic representation of signal transduction leading to NF- κ B driven proliferation. After binding of an NF- κ B activator (i.e. TNF α or EPO) to its receptor (TNFR), the signal is transduced to the IKK $\alpha/\beta/\gamma$ complex. This complex phosphorylates inhibitory protein I κ B, which is then ubiquitinated and proteosomally degraded. The degradation triggers the translocation of NF- κ B into the nucleus of the cell, followed by initiation of transcription by binding to regulatory DNA sequences. It leads to transcription of specific target genes and finally to proliferation of NSCs. Figure modified after Piotrowska et al. [16]. b) Graph of $y(t)$ -solutions to Eqs. (2)–(5) proposed in [16] for different values of parameter $\alpha \in [0,5]$, which represents the relative cell number. Here α stands for relative concentration of the NF- κ B activator such as TNF α or EPO. Simulation has been performed for the initial data $x(0) = 0.1$, $y(0) = 1$. All other model parameter values are as in Table 1 in [16]. Figure is reproduced from [16] by permission.

Experiments have shown a negative correlation between active NF- κ B and I κ B [23]. Therefore, the concentration of I κ B (denoted by $\delta(t)$) is assumed to be a decreasing function of active NF- κ B (denoted by $x(t)$), which can be modelled by a Hill function:

$$\delta(t) = \delta_0 \frac{\theta}{\theta + x(t)}, \quad (3)$$

where δ_0 and θ are positive constants.

Piotrowska et al. were able to relate the cellular concentration of active NF- κ B with NSC proliferation speed. Since *in vitro* cultivated cells are not affected by factors that limit proliferation, the change of cell number $y(t)$ can be described by

$$\frac{d}{dt} y(t) = \overbrace{\gamma f[x(t)]}^{\text{proliferation}} y(t), \quad (4)$$

dependence
on NF- κ B

with positive constant γ . Both, low and pathologically high NF- κ B concentrations (hyperactivation) lead to cell death in the NSC population and only for intermediate values of active NF- κ B cells are stimulated to proliferate [19] (compare with Fig. 3 in [16]). Based on these observations a reproduction function such as

$$f(x(t)) = b^2 - (x(t) - d)^2, \quad (5)$$

can be assumed, where b and d are positive constants to mark the physical level of NF- κ B needed for cells to proliferate, with $d > b$ and $d + b < 1$. The local and global existence, non-negativity and boundness of solutions for bounded initial data as well as the analytical analysis of steady state existence and stability can be found in [16]. The solutions of the system (2-5) strongly depend on the constants d and b , and parameter α (the stimulus) as well. For fixed parameter values (see Table 1. in [16]) and $\alpha \neq 0.5244$ or $\alpha \neq 3.408$ there is only one biologically relevant steady state of the system (2-5). For $\alpha \in (0.5244, 3.408)$, the population of *in vitro* cultivated NSCs will either grow infinitely, or the population will die out, otherwise.

Figure 3b visualizes the change of cell number over time ($y(t)$ -solution to Eqs. (2-5)) for α parameter values in the interval $[0,5]$. Recall that α is the stimulus for the activation of NF- κ B via phosphorylation of I κ B by the IKK complex. For a very small stimulating signal there is a little activation of NF- κ B, insufficient for cells to proliferate (compare to Figure 5 and Figure 9 in [16]). As α increases the concentration of active NF- κ B increases, and after reaching a threshold level the cells will start to proliferate. However, increasing α above another critical threshold results in hyperactivation of NF- κ B and subsequent cell apoptosis. The model was compared with *in vitro* experimental data of NSCs after exposure to 0 (control) and 10 ng/ml TNF [16]. In the model as well as in experiments presented in [16],

adult NSCs respond to TNF with significantly increased proliferation compared to untreated cells, see Figure 10 in [16].

The discussed model differs from previous models describing the NF- κ B activation in fibroblasts [23]--[25]. For simplicity, only interactions between IKK complex and inactive NF- κ B, I κ B and active NF- κ B, and proteasomal degradation of active NF- κ B are considered. Focussing only on these crucial interactions reduces the system to two nonlinear ordinary differential equations, which enables mathematical analysis of the dynamics. For more complex systems (20 equations with more than 30 independent model parameters, [23], [24]) such an analysis is impossible and numerical simulations alone have to be used to study system properties.

So far we have discussed NF- κ B as a modulator of NSC proliferation. However, the dependence of proliferating cancer stem cells on NF- κ B has been observed recently, too [26] - [28]. With parameter adaptation, the discussed model can be adapted to study cancer cell and cancer stem cell dynamics. Such a simple model could give useful insights into cancer development, as it can provide a potent tool for predicting results of proliferation assays and NSCs expansion.

Discrete Mathematical Models

Systems of coupled differential equations simulate behavior of cells or concentrations of molecules. In discrete models the fate of individual cell or molecule is followed. In fact, many individual events are crucial to determinate the cells' phenotype and behavior. Moreover, the cell phenotype can change depending on the input it receives from the microenvironment according to certain probability distributions. Cellular automaton and agent-based models can treat discrete features of cells and the resulting phenotype variability. Thus, in discrete mathematical and computer models, time is discretized, and at each time step every cell (or agent, for general agent-based models) follows certain defined rules that determine the state of the cell and consequently the whole system at this time point. The rules can, and often do so, include stochasticity and probability distributions giving the system more realistic non-deterministic behavior, which allows to study the impact of varying environmental factors. Dynamics of single cells are combined to populations and a complex system behavior emerges. By virtue of this approach properties of individual agents and their mutation give rise to multiple distinct subpopulations, which in cancer modeling can be interpreted as different cell types such as stem cells or differentiated cells, and more or less aggressive tumor clones.

In a recent interdisciplinary study using a hybrid continuous-discrete model it has been shown that the tumor microenvironment can orchestrate tumor phenotype development and selection [55]. Another automaton model has been designed to predict proliferating and quiescent cell populations and the tumor volume doubling time which is clinically important [56]. It is known that actively proliferating cells are more susceptible to treatment such as radiation and chemicals than quiescent cells, resting in G_0 phase. These cells stay arrested until DNA damage is completely repaired or the cell is sent into apoptosis. Stem cells are surrounded and protected by adjacent cells, and are thought to be quiescent under normal

conditions. Resting at the time of an environmental insult, stem cells have a higher chance of surviving and subsequently re-populating the tissue.

A Discrete Cellular Automaton Model of Stem Cells and Tissue Homeostasis

A discrete mathematical model of stem cells and their role in tissue homeostasis has been developed by Agur and co-workers [57]. The model considers: bone marrow stem cells S , differentiated cells D , and null cells N , i.e. empty lattice space. Each cell's behavior is determined by their cell cycle, their internal state and by number and type of cells in the neighborhood. The model is defined as a connected, locally finite undirected graph $G = (V, E)$ where the vertices V describe the cells and the edges E describe their neighborhood. Operators on the set of all states on the graph define the dynamic behavior of the systems.

The rules of the system are defined such that differentiated cells mature until time Φ before they leave the domain (i.e., the bone marrow). Stem cells mature into differentiated cell if their age, i.e. internal counter, exceeds Ψ and its neighbors consist of stem cells alone. Finally, stem cells proliferate into an empty site after time Θ . A detailed explanation of all rules and the proofs can be found in [57].

The model characterizes some universal properties of stem cells to produce mature cells and recover from severe perturbations. The direct stem cell environment modulates the decision to remain quiescent or to proliferate. Eventually the system results in a dense stem cell population and the system never dies out. Stem cell maturation Ψ drives the system dynamics, and stem cell proliferation Θ and differentiated cell maturation Φ play a secondary role only. The disadvantage of such a cellular automaton approach is the necessary simplicity - and due to lack of simulations there is a need to prove the dynamics of the proposed rules.

An Agent Based Model of Stem Cell and Non-Stem Cell Tissue Dynamics

A more complex discrete modeling technique is agent-based modeling. Agents are autonomous entities whose behavior is based on a certain set of rules and in response to the local environment [55], [56]. The agents make non-deterministic decisions independently from large systems or aims of complex populations. However, as many agents not only respond to the environment but also modify it, changes in single cell state and behavior can lead to system catastrophes.

We now construct a small-scale agent-based stem cell and non-stem cell model to simulate single cell dynamics and cell-cell interactions as they occur in the early stages of tissue or tumor development [58]. We define rules from general biological observations to describe individual cell behavior and simulate dynamic patterns of the emerging population system. We distinguish between a stem cell and a non-stem cell phenotype, and allow for variations of some of the implemented rules to discuss aberrations that lead to cancer development.

Key cellular behavior events are cell division and cell death. For both events, a chain of internal and external signals must occur. Tissue homeostasis is a complex dynamic process and cells obey external signals that define cell cycle progression. The environment determines the fate of each cell, for example by diffusion of signals or cell-cell signaling. Cells either proceed through the cell cycle to undergo mitosis, or rest in the so-called G_0 phase. Resting cells may re-enter the cell cycle and proceed into mitosis if nearby cells died and need to be replaced. In a stable tissue the change in cell number over time should be negligible. Whenever a cell dies another one will proliferate, and when a cell proliferates another one is sent into apoptosis. This phenomenon is called Moran Process [59]. To avoid overcrowding we believe there is a contact inhibition between cells i.e. cells are less likely to divide if they are surrounded by other cells, and more likely to undergo mitosis in a microenvironment without spatial constraints. The potential to undergo mitosis is different for stem cells and differentiated cells. Stem cells are undifferentiated cells with unlimited replicative potential and self-renewal capacity. They can divide symmetrically to either form two stem cells or two differentiated cells, or asymmetrically to maintain one stem cell and send the offspring into differentiation. With each cell division non-stem cells become more differentiated and fulfill specific functions. However, these cells lose their replicative potential and die within a short time frame (Figure 2c). We define the proliferative potential $p_s = \infty$ for stem cells and $p_d = 12 \pm 3$ for differentiated cells. As the non-stem cells divide their proliferative potential decreases, and the daughter cells inherit the potential. Progeny of stem cells with differentiation fate get assigned a random p_d within the above stated interval.

In our simulation, we increment time at discrete 1-hour time steps. Cell age is increased and the cells progress in the cell cycle subject to certain environmental conditions. At the end of mitosis (M phase) proliferation capacity is checked. If the proliferation potential is exhausted, i.e. $p_d = 0$, the cell is sent into apoptosis. The flowchart of the simulation process and the decisions made by every cell at each time point is shown in Figure 4.

Now we present some simulations that show the cellular behavior based on the implemented rules. First we initialize our system with a single non-stem cell with proliferation capacity $p_d = 10$ in the center of the domain. If the cell has passed through the cell cycle it will divide because no neighbors are inhibiting it. As a result of this division, the original cell and its offspring have both now a proliferation capacity of $p_d = 9$. Both cells will cycle and reach mitosis, resulting in four cells all with $p_d = 8$. The emerging population will initially grow exponentially without contact inhibition. As the number of cells increases the available space for each cell decreases. If there is no space available, then the cell ready to proliferate will be sent into G_0 phase and stay there until an adjacent grid point becomes vacant. In Figure 5 proliferation capacity is color-coded, with maximum proliferation potential being red and no cell division left - black. As the population grows a proliferation capacity gradient from the center to the outer rim is formed. Limited proliferation (Figure 5a) results in population number oscillations already at very small cell numbers. Cells in the core of the population are contact inhibited and rest. Cells at the outer rim proliferate until all their proliferation capacity is exhausted and subsequently die. Previously resting cells will re-enter the cell cycle and start to proliferate again and occupy the freed space. This ‘die off – re-population’ process continues until all cells have exceeded their proliferation potential and the cluster vanishes. However, if we initialize a single stem cell with unlimited replicative potential, all direct

offspring of this stem cell will start to populate the domain with maximum proliferation capacity $p_d = 10$ (Figure 5b).

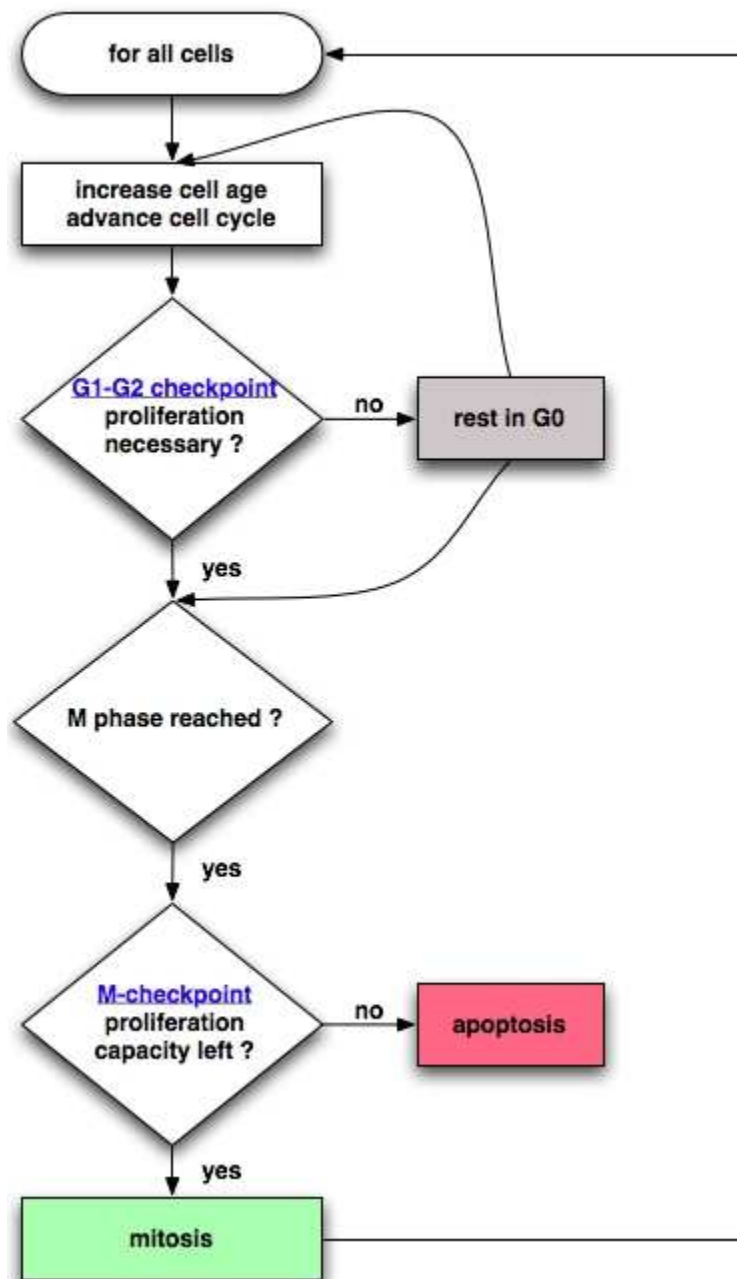


Figure 4. Cell life cycle scheme. At each time step the cell age increases. The cell will rest in G_0 if the microenvironment is saturated. If there is space for the cell to divide it will proceed into mitosis (M phase). If the proliferation capacity is exhausted the cell will undergo apoptosis; otherwise it will produce a daughter cell.

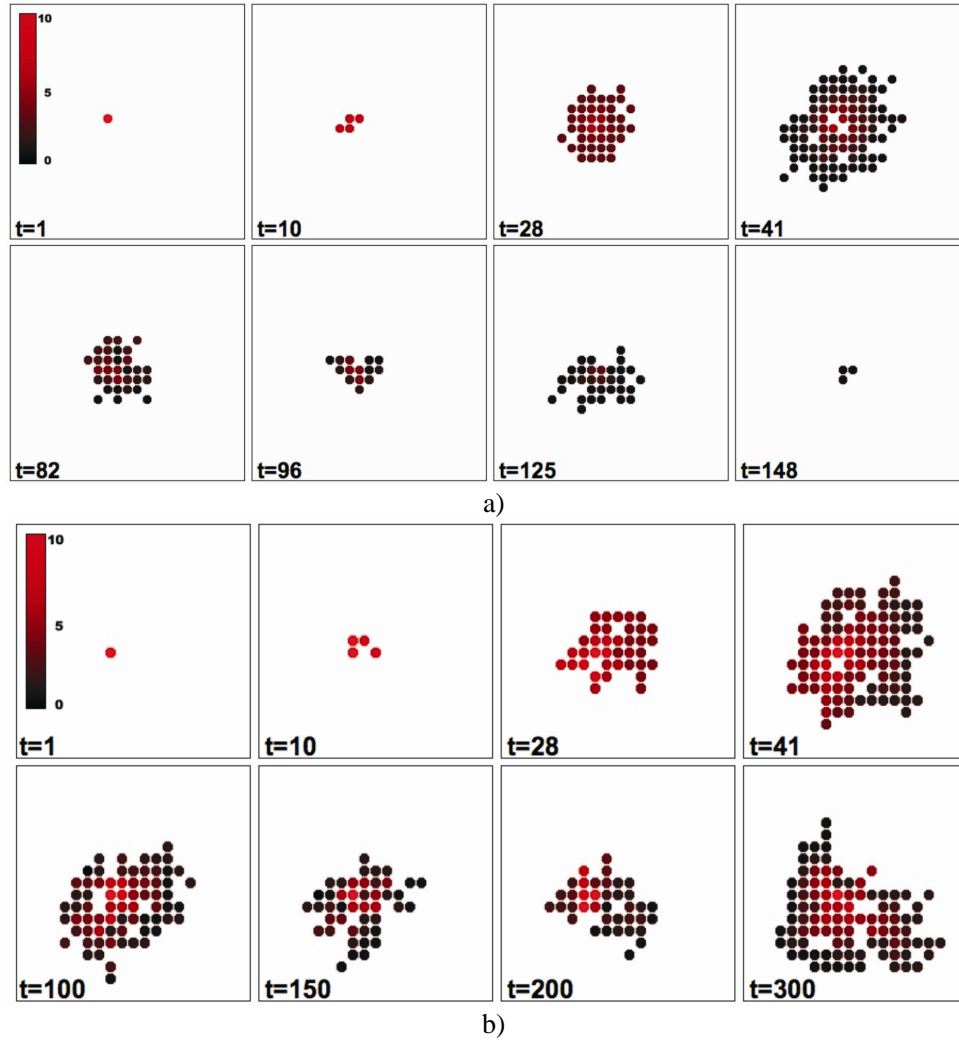
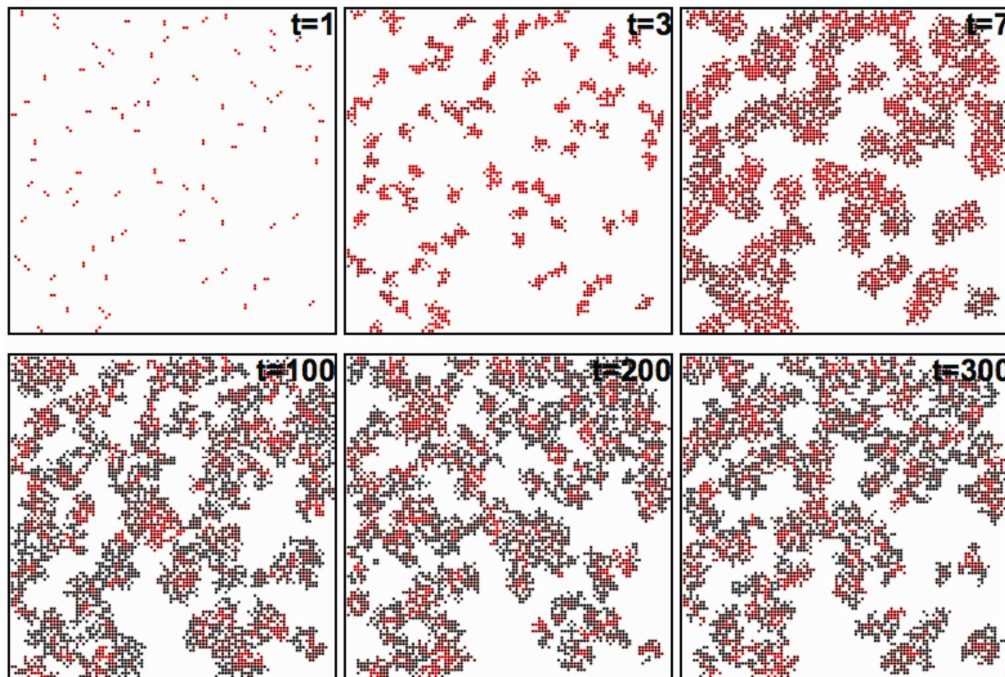


Figure 5. Cell cluster formation from non-stem cells and stem cells. As cells divide they lose proliferation capacity (red→black). Initially the populations grow exponentially as each cell experiences sufficient space. As the number of cells increases, cells in the core of the population cluster get contact inhibited and sent to rest. When the outer cells die off, cells in the interior will re-enter the cell cycle and start proliferating again until their proliferation capacity is exhausted, too. a) The cell number in populations arising from single cells with limited replicative potential $pd = 12$ (red, $t = 1$) oscillate over time with cells at the outer rim dying and cells in the interior re-enter proliferation. After $t = 148$ days no cells with proliferation capacity is left, and at day $t = 150$ days all cells are dead. b) Populations emerging from a stem cell have initial similar behavior than those arising from non-stem cells. However, more cells are produced, as the core of the population is direct offspring of the stem cell with maximum proliferation capacity. As cells die off the stem cell will generate more potent offspring re-populating the cell cluster.

This results in a larger number of cells in the population. Initially the population growth is similar to the population described above (Figure 5a). As the majority of cells die off, however, the stem cell will again produce potent progeny that repopulate the domain as

before. Hence a population that arises from a stem cell will persist despite intermediate oscillations in cell number.

Tissues and organs are complex, heterogeneous structures with many cells that fulfill specific functions. Different stem cell ratios have been estimated or identified for different tissues. The fraction of stem cells in the breast, for example, has been reported to be between 0.2% and 5% [60]. Tissue homeostasis, a ‘stable and constant’ number of cells in the population, is assured by the interplay of stem cells and non-stem cells. Differentiated cells die off due to normal tissue turnover or when their proliferation capacity is exhausted. Those cells have to be replaced by potent cells to maintain tissue integrity. In a theoretical domain of constant size, say 100×100 grid points, each cell can occupy one grid point. Furthermore, at any time at most one cell can reside on a single grid point. With a small number of stem cells arbitrarily distributed in the domain, the size of each of the resulting cell clusters is not sufficient to occupy all the space in the domain and single cluster dynamics result in large variations in overall cell number. As the stem cell ratio increases multiple clusters arise that eventually compete for space. If progeny of stem cells in one cluster die, cells from adjacent clusters can replace the vacant space. If these cells later die, the original cells can populate this space again and the number of cells in the tissue is maintained. Figure 6a shows a sample simulation of 100 initial stem cells forming individual clusters of cells, each of which follows the growth dynamics as presented above. Despite of oscillations in cell numbers over time the overall tissue structure is preserved. Figure 6b shows plots of cell numbers obtained from simulations with 0.2% stem cell ratio, 1% stem cell ratio, and 5% stem cell ratio for five different simulations each. With increasing number of stem cells the variation of cell numbers in the tissue reduces as discussed.



a)

Figure 6. (Continued).

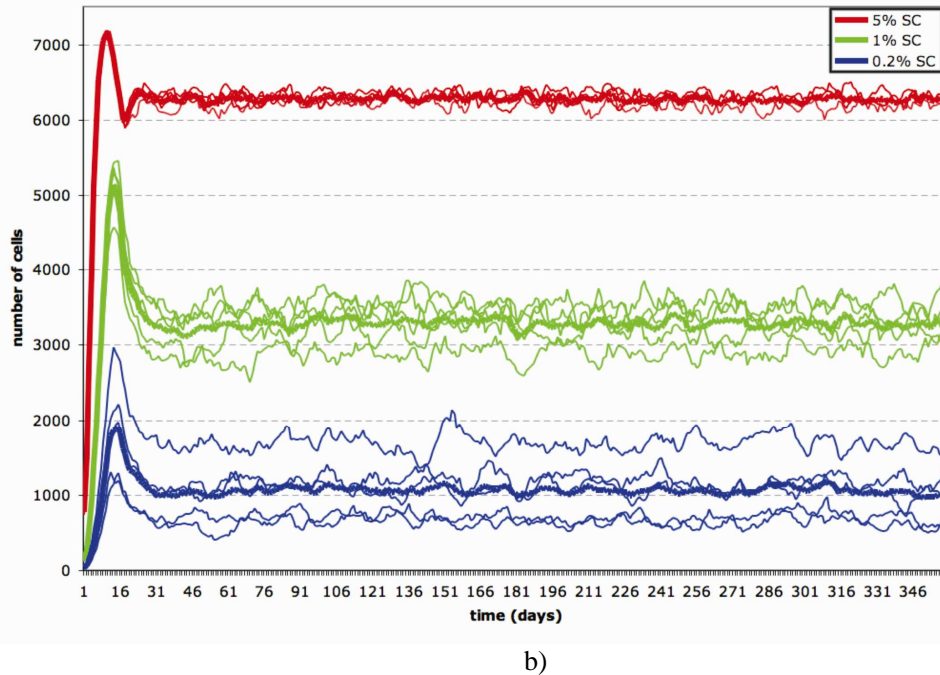
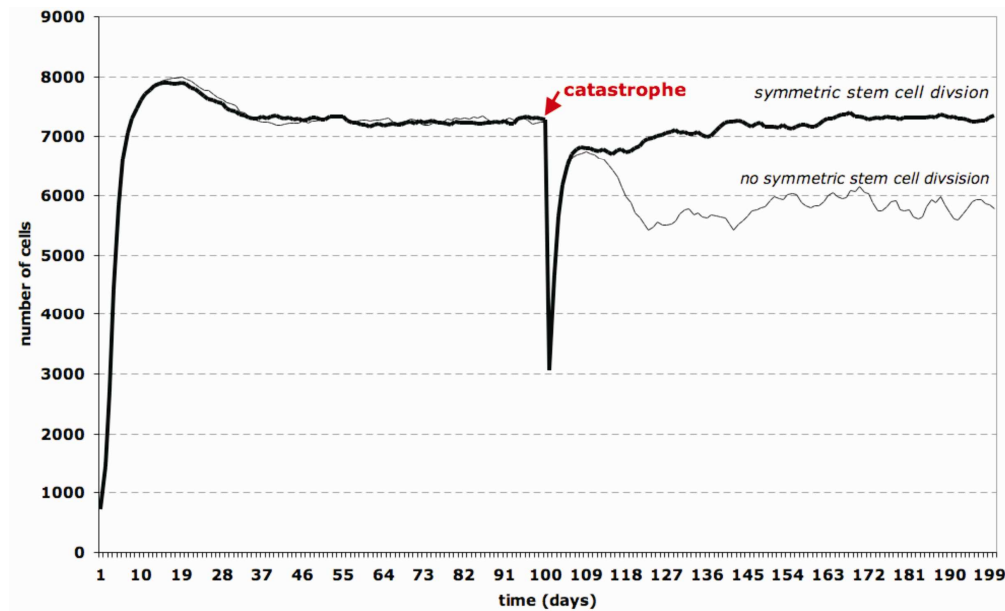
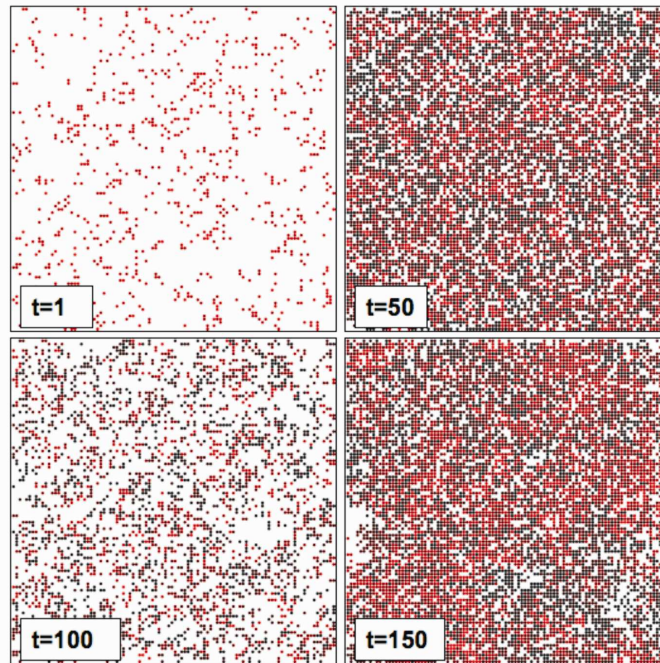


Figure 6. Simulation of tissue homeostasis in a domain of 100×100 grid points holding at the most 100,000 cells. a) An initial arbitrary distribution of 1% stem cells, i.e. 1000 cells, results in cell cluster formation within the first 7 weeks. Despite oscillations in cell numbers the tissue structure is overall preserved. b) Plot of cell numbers obtained from simulations with 0.2% stem cell ratio (blue), 1% stem cell ratio (green) and 5% stem cell ratio (red). Shown are five different simulations for each stem cell ratio and an average over those runs (thick line). With increasing number of stem cells the variation of cell numbers in the tissue reduces.

So far we have only discussed asymmetric stem cell division. For functional tissue it is crucial to maintain homeostasis especially after sudden loss of cells and stem cells e.g. during exposure to acute radiation or other cellular catastrophes. Symmetric stem cell division is needed to preserve a constant stem cell compartment and the tissue integrity. We assume that stem cells fate is determined by diffusion of signals in the extracellular matrix [61]. Figure 7 shows the development of a tissue from initially about 400 stem cells. Very quickly about 7.200 cells get produced and the domain gets populated to an arbitrarily set density of 75%. We simulate at time $t = 100$ steps a cellular catastrophe by randomly killing 50% of all cells, both stem and non-stem cells. The surviving stem cells start immediately to divide symmetrically until the pre-catastrophe stem cell compartment is re-established. The newly produced stem cells move away arbitrarily and start repopulation of the tissue by asymmetric division. Without symmetric stem cell division, the reduced number of stem cells cannot produce the cell numbers that have been in the domain previous to the perturbation resulting in a less dense tissue (Figure 7b).



a)



b)

Figure 7. Impact of symmetric stem cell division on recovery to homeostasis after catastrophe. Stem cell ratio is 5%. At time $t = 100$ a catastrophe destroys randomly 50% of the cells. a) If stem cells cannot divide symmetrically (thin plot) pre-catastrophe cell number cannot be established. Only symmetric stem cell division (thick plot) and random cell migration enables quick recovery after a disaster. b) Sample simulation for development of a tissue from 5% stem cells with cell migration enabled and recovery from a disaster at time $t = 100$.

Conclusion

Mathematical models applied to biology and medicine describe and simulate possible not yet known scenarios. Complex biological dynamics, for example cell behavior, tissue recovery or cancer development, are reduced to key mechanisms which enable the analysis of how the system changes if any of these mechanisms are interrupted. In this chapter we have tried to discuss a small selection of representative modeling approaches that can have significant implications for stem cell study. Some describe stem cell differentiation and maturation [10], interactions between proliferative and quiescent stem cells [11], [12], or cell proliferation controlled by signaling pathways [15], while others focus on tissue homeostasis for healthy tissue [57], [58]. Most, if not all of the mathematical models of stem cell dynamics have subsequent implications to cancer development and treatment. For example, from the last presented agent based model [58] and simulations it seems obvious that each tumor reaching the clinical significance must contain cancer stem cells to drive the development up to a malignant size. Without stem cells, the tumor cell cluster would remain small and be doomed to die out. *In vivo* tumors are known to be heterogeneous in terms of clonogenicity. Using a model in which human breast cancer cells were grown in immunocompromized mice, it was shown that only a minority of the cancer cells had the ability to form new tumors [62]. The disability to form new tumors by the majority of the cancer cells can be explained by a model with limited proliferation capacity such as seen in normal cell populations in Figure 5a, which are likely to die after a certain number of proliferations [58]. Cancer stem cells have been successfully identified in acute myelogenous leukemia [63], [64], breast cancer [62], and brain tumors [26], [65], [66], and recently pancreas cancer [67].

Current cancer treatment is aimed to eradicate as many cancer cells as possible. A treatment protocol that eliminates 99% of the tumor cells may be considered successful. However, if only parts of the residual 1% of cancer cells have stem-cell properties, i.e. unlimited proliferation capacity, then the tumor will recur. More and more research focuses on this small clonogenic sub-population, as tumor treatment may provide better tumor control if the cancer stem cells are eradicated [68]. Recently, it has been shown that breast cancer initiating cells are more radiosensitive compared to the bulk of cancer cells [69]. Moreover, it also has been experimentally proven that glioblastoma stem cells are radioresistant and may therefore contribute to treatment failures [70].

A major advantage of mathematical models and computer simulations is their ability to make predictions of biological systems and their behavior that would be difficult to conduct experimentally. As an obvious example, it is challenging to maintain an unfed cell line longer than approximately 2 weeks and conducting counts. Moreover, mathematical models give the advantage to perform ‘virtual’ experiments instead of complicated lab experiments, which can be costly and time consuming. Furthermore, models are reproducible and not subject to biological variation, possibly induced by constantly changing external conditions. Mathematical/numerical experiments can give many answers since large numbers of variables and their dependence on time, space, and each other can be measured. Using mathematical models and computer simulations that incorporate new molecular findings may provide a platform to improve understanding of spatio-temporal tumor and cancer stem cell dynamics.

Models can be developed to investigate the effect of treatment sensitizing or cell-cycle synchronizing drugs on treatment outcome, which may subsequently lead to improved tumor control.

In summary, mathematical models have a huge potential in biomedical research, which has not yet reached acceptance in many laboratories. We hope that this chapter gives a flavor of how models can help developing new hypotheses or testing existing theories. To use models as a predictive tool, future research needs to incorporate a dialog between biologists, clinicians and mathematicians. We believe that such an interdisciplinary effort will eventually lead to insights that can be translated into the clinic to the ultimate benefit of patients.

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