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Characterization of stem cells using mathematical models of multistage cell lineages

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ABSTRACT

Stem cells dynamics is an important field of research with promising clinical impacts. Due to the revolutionary new technologies of biological data collection, an enormous amount of information on specific factors and genes responsible for cell differentiation is available. However, the mechanisms controlling stem cell self-renewal, maintenance and differentiation are still poorly understood and there exists no general characterization of stem cells based on observable cell properties. We address these problems with the help of mathematical models. Stem cells are described as the cell type that is most responsive to certain environmental signals. This results in a dynamic characterization of stemness that depends on environmental conditions and is not necessarily linked to a unique cell population.

1. Introduction

Complex multicellular organisms mostly consist of a large number of cell types, which are well characterized with respect to morphology, function, histological appearance, gene expression and biochemical composition. These cells are called differentiated, i.e., specialized. During embryogenesis, as well as in adult tissues, differentiated cells emerge from undifferentiated or less differentiated cells in the course of a complex, well regulated process, called differentiation. Such nonspecialized cells having the ability to give rise to cells with different biological properties are called stem cells.

Embryonic stem cells exist during early embryogenesis and all cell types found in a neonate are derived from them. In comparison, adult stem cells (tissue stem cells), which exist throughout the whole life, are able to give rise to a limited set of different cell types. Adult stem cells can be found in different tissues, e.g., in skin, bone marrow, intestine, liver, muscle or brain. They are responsible for tissue homeostasis and tissue repair after injuries. In fast proliferating tissues, such as blood or intestinal epithelium, a considerable number of cells has to be generated each day, e.g., 10^{11}–10^{12} blood cells per day in the case of adult humans [1,2]. There is a hope that adult stem cells can be used to repair or replace impaired organs [3]. Importantly, blood stem cell (hematopoietic stem cell) transplantation has been used for decades to treat leukemias and other diseases [4]. It seems possible that changes in properties of adult stem cells may be linked to impairment of tissue maintenance and repair during aging [5,6].

Understanding of the mechanisms governing stem cells differentiation and maintenance is of central interest in developmental biology and regenerative medicine, increasingly interested in the molecular characterization of stem cells. Recently the concept of stem cells has been extended to include cancer stem cells, i.e., malignant cells that maintain and

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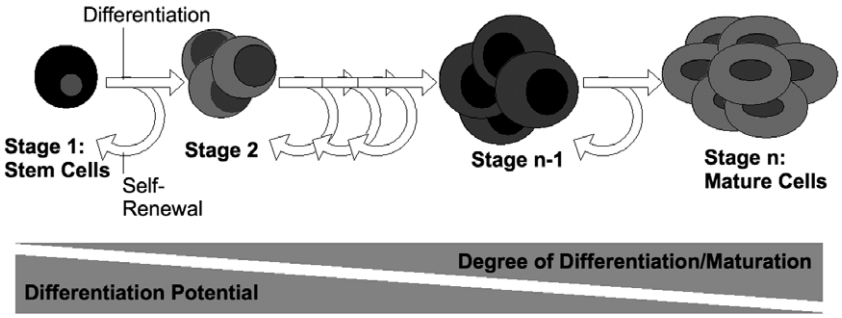

Fig. 1. Hierarchy of cells of different maturation stages.

give rise to tumor cell populations [7,8]. In spite of intensive research no morphological or biochemical characterization of stem cells is available. Adult stem cells are characterized by the following properties (compare [9,7,3,10]):

1. Stem cells are able to maintain the size of their population by producing offspring with stem cell properties (so called self-renewal).
2. Stem cells are able to give rise to cells with different biological properties (multipotency).
3. Stem cells are functionally nonspecialized.

Stem cell populations are morphologically and biochemically heterogeneous [11]. In some tissues stem cells cannot be identified and it is only possible to decide whether a subset of cells contains stem cells [12]. The question arises whether stemness can be defined on the level of a single cell and which properties are suitable to characterize stem cells.

In this paper we approach the above questions using mathematical models describing dynamics of cell differentiation and self-renewal regulated by extracellular signaling feedback. The point of departure is a multi-compartment model of a discrete collection of cell subpopulations, which we have recently proposed to investigate dynamics of blood reconstitution after chemotherapy and bone marrow transplantation [13]. Models we developed were calibrated based on clinical data obtained from patients with multiple myeloma after high-dose chemotherapy and stem cells transplantation.

In the present paper we study a general system of hierarchical proliferation system regulated by a signaling feedback. One established method of modeling such hierarchical cell systems is to use multi-compartment models, a discrete collection of ordinary differential equations, each of which describes a well-defined differentiation stage. Such framework is based on the traditional understanding that in each lineage of blood cell precursors, there exists a discrete chain of maturation stages, which are sequentially traversed. In most cases, the number of cells increases with each step of maturation and many orders of magnitude separate the size of the stem cell population from the number of mature cells [14]. In general, the differentiation potential, i.e., the ability of a cell to give rise to cells of different types, decreases with ongoing maturation (see Fig. 1). Stem cells divide much slower than more differentiated cell types, which is often interpreted as protecting against mutations and cytotoxic agents. The model involves a special biologically justified mechanism of cell regulation, including varying in time asymmetry of cell divisions and proliferation rates controlled by a negative feedback from the mature blood cells. Mathematical analysis of the model equations leads to a generalization of the concept of self-renewal potential, which can be helpful to define the stem cell compartment. This concept helps to explain why stem cell behavior arises as a property of a whole population and not as a property of single cells.

2. Mathematical model

In this paper we present a generalization of our model proposed in [13]. The model is based on the traditional assumption that in each lineage of blood cell precursors, there exists a discrete chain of maturation stages, which are sequentially traversed. Cell behavior is characterized by the proliferative activity, the probability to differentiate and the probability to die. We assume that the cell properties change during maturation process.

In this section we restrict our model to one type of mature cells. We assume that the regulation of differentiation of different lineages is independent on each other. The evidence for that are, for example, hypoxia, where red blood cell counts raise, while white blood cell counts remain constant, immune response related to the change of white blood cell counts or blood loss, where the loss of white cells is neglectable with respect to the loss of red cells. As a further simplification we will assume that the considered cell system is regulated by one single cytokine. One example for such system is the regulation of red blood cell production by erythropoietin (EPO), [15,16]. Another example is the formation of granulocytes, which is mainly regulated by G-CSF, [16]. This reasoning results in the following assumptions.

Assumptions 2.1. (A1) One type of mature cells is taken into account.
(A2) To become mature, the cells have to pass a discrete number of subsequent maturation steps (maturation stages); none of these steps can be skipped. Cells pass these steps in one direction from the primitive to the more mature stages. Transition in the opposite direction (dedifferentiation) is neglected.

(A3) Cell behavior is regulated by feedback signaling. One type of signal is taken into account.
(A4) Behavior of cells at maturation step i at time t only depends on the signal at time t and the quantity of cells in maturation step i. Especially this means that behavior of cells in stage i and influx to stage i from stage $i-1$ are independent.
(A5) Concentration of signaling molecules depends on the density of mature cells. The lower is the density of mature cells the higher is the concentration of signaling molecules.

Denote by $c_i(t)$ the population density of cell type i at time t. The density of the stem cell population at time t is denoted as $c_1(t)$ and the concentration of signaling molecules as $s(t)$. Time evolution of cell system is described by the following system of ordinary differential equations,

$$\begin{aligned}
\frac{dc_1(t)}{dt} &= f_1(s(t), c_1(t)),\\
\frac{dc_2(t)}{dt} &= f_2(s(t), c_2(t)) + g_1(s(t), c_1(t)),\\
&\vdots\\
\frac{dc_n(t)}{dt} &= f_n(s(t), c_n(t)) + g_{n-1}(s(t), c_{n-1}(t)).
\end{aligned} \tag{1}$$

$g_i(s(t), c_i(t))$ denotes the flux of cells from the maturation stage i to the maturation stage $i+1$ due to differentiation, which is nonnegative (based on Assumption (A2)). The term $f_i(s(t), c_i(t))$ denotes the change of $c_i(t)$ that is caused by processes at the ith stage of maturation. If gain of cells caused by proliferation and self-renewal is stronger than the loss caused by differentiation or death, then $f_i(s(t), c_i(t))$ is positive. Otherwise $f_i(s(t), c_i(t))$ is negative. Since mature cells are postmitotic, i.e., they cannot proliferate, the term $f_n(s(t), c_n(t))$ accounts only for cell death and is, therefore, negative. To close the system, we assume that e.g., $s(t) = s(c_n(t))$, i.e., the regulatory signal depends on the concentration of mature cells. This assumption is based on the idea that the feedback signal fulfills a quasi-steady state assumption, i.e. the dynamics of the signaling molecules takes place on a faster time scale than the process of cell proliferation and differentiation. Changes of hematopoietic cytokines have been reported to occur promptly after injuries: serum cytokine levels are observed to change within the first 90 min, [17]. On the contrary, the standard estimate for mammalian cell cycle transit of 20–24 h, [18], is quite long. From a more general point of view, this assumption holds for the systems, in which feedback hormones are stored in readily releasable vesicles. Then, hormone release and kinetics are much faster than all cellular responses, which require signal transduction and gene expression. Furthermore, the control function given by $s(t) = s(c_n(t))$ corresponds to the assumption that signal level depends only on the mature cells. Such regulation holds in erythropoiesis, where signal levels depend on oxygen transport, which is only performed by mature red cells, [15]. For granulopoiesis the assumption is a good approximation of reality, since the number of signal receptors on mature cells is much higher than that on immature cells, [19].

The functions f and g are characterized by the following assumptions.

Assumptions 2.2. (A6) $f_i(s, 0) = 0$ for all s.
(A7) $g_i(s, c_i) \geq 0$ and $g_i(s, 0) = 0$ for all s.
(A8) If $c_i > 0$ then $f_i(s, c_i)$ is strictly monotone increasing in s.
(A9) For each $i < n$, there exists a signal concentration $\tilde{s}_i > 0$ such that $f_i(\tilde{s}_i, c_i) = 0$ for all $c_i > 0$.
(A10) $\frac{\partial}{\partial c_i} f_i(s, c_i)|_{c_i=0} > 0$ if $s > \tilde{s}_i$ and $\frac{\partial}{\partial c_i} f_i(s, c_i)|_{c_i=0} < 0$ if $s < \tilde{s}_i$.
(A11) In healthy tissues cell densities are in equilibrium.

Assumptions (A6) and (A7) reflect the fact that empty population of the stage i cannot proliferate, die nor differentiate to the stage $i+1$ and that cell flux is unidirectional. Assumption (A8) is motivated by the hypothesis that signaling molecules bind to receptors at the cell surface with a probability increasing with the number of signaling molecules. The average number of receptors that are occupied at a given time point and the cellular response increase with the increase of the concentration of signaling molecules. Assumption (A9) reflects the hypothesis that the concentration of signaling molecules triggers transition between an expanding, i.e., self-renewal dominated, or a declining, differentiation dominated, state. A great number of systems where changes in concentration of a signal molecule or in activity of a type of receptors lead to changes in functional cell properties (e.g., conservation of current state versus differentiation) has been described (e.g. [20–22]).

Remark 2.1. Assumption (A8) implies that $\tilde{s}_i$ is unique, i.e., $f_i(s, c_i) > 0,\ \forall c_i > 0$ if $s > \tilde{s}_i$ and $f_i(s, c_i) < 0,\ \forall c_i > 0$ if $s < \tilde{s}_i$. Assumptions (A8) and (A9) can be replaced by the assumption that there exists a unique $\tilde{s}_i > 0$ such that $f_i(\tilde{s}_i, c_i) = 0$ for all $c_i > 0$.

Assumption (A10) states that, for low cell concentrations, in the expanding state the cell gain per unit of time increases with the increasing cell concentration. This assumption is biologically justified, since the more cells in stage i exist, the more cells proliferate and therefore the number of offspring per unit of time also increases.

3. Stationary system

To explore possible differences between the stem cells and more differentiated cells, we analyze system (1) under steady state conditions. Population density and a concentration of signaling molecules in equilibrium are denoted by $\bar{c}_i$ and $\bar{s}$, respectively.

Assumptions (A6)–(A7) guarantee the following properties of the model solutions.

Proposition 3.1. *Solutions of system (1) with f and g fulfilling Assumptions (A6)–(A7) remain nonnegative for nonnegative initial conditions. Moreover, the trivial solution is a steady state of the model.*

Important stem cell systems in healthy organisms are known to be organized hierarchically in the following sense [23,14].

Definition 3.2 (*Hierarchically Organized Stationary State*)**.** A stationary state is called hierarchical if for all i with $\bar{c}_i > 0$, it holds $g_j(\bar{s}, \bar{c}_j) > 0$ for all $j \geq i$.

The above notion means that all existing immature cell populations contribute to maintain the next downstream population, and thus maintain indirectly the pool of mature cells. We propose to define stem cell population as a population that gives rise to a hierarchically organized system of cells maintaining the mature cell population.

Definition 3.3 (*Stem Cell Population*)**.** If $\bar{c}_i > 0, \ldots, \bar{c}_n > 0, g_i(\bar{s}, \bar{c}_i) > 0, \ldots, g_{n-1}(\bar{s}, \bar{c}_{n-1}) > 0$ and $g_{i-1}(\bar{s}, \bar{c}_{i-1}) = 0$, then $\bar{c}_i$ is called a stem cell population of the steady state $(\bar{c}_1, \ldots, \bar{c}_n)$.

The definition implies the following.

Proposition 3.4. (i) *If $\bar{c}_1$ is a stem cell population of the steady state $(\bar{c}_1, \ldots, \bar{c}_n)$, then $\tilde{s}_1 < \tilde{s}_i$ for all $i > 1$, where $\tilde{s}_j$ are defined in Assumption (A9).*

(ii) *For each $j \in \{1, \ldots, n\}$, if there exists $i > j$ such that $\tilde{s}_i \leq \tilde{s}_j$, then $\bar{c}_j$ cannot be a stem cell population.*

Proof. (i) For $\bar{c}_1 > 0$, the steady state condition and Assumption (A9) imply $\bar{s} = \tilde{s}_1$. Since $\bar{c}_1$ is a stem cell population, we obtain $g_i(\bar{s}, \bar{c}_i) > 0$ for all $i > 1$. If there exists $j > 1$ with $\tilde{s}_j < \tilde{s}_1$, Assumption (A9) implies $f_i(\bar{s}, \bar{c}_i) > 0$ and therefore, $\frac{dc_i(t)}{dt} = f_i(\bar{s}, \bar{c}_i) + g_{i-1}(\bar{s}, \bar{c}_{i-1}) > 0$.

(ii) If $\bar{c}_j$ is a stem cell population, then $g_{j-1}(\bar{s}, \bar{c}_{j-1}) = 0, g_j(\bar{s}, \bar{c}_j) > 0, \ldots, g_{n-1}(\bar{s}, \bar{c}_{n-1}) > 0$. The steady state condition and Assumption (A9) lead to $\bar{s} = \tilde{s}_j$. Since $\tilde{s}_i \leq \tilde{s}_j$, then $\frac{dc_i(t)}{dt} = f_i(\bar{s}, \bar{c}_i) + g_{i-1}(\bar{s}, \bar{c}_{i-1}) > 0$. □

The next question to address is what happens if the least differentiated cell compartment that can be non-void in a steady state equals zero.

Theorem 3.5. *Define $i := \max\{j | \tilde{s}_k \geq \tilde{s}_j, \text{for all } k = 1, \ldots, n\}$. If $\bar{c}_i = 0$ in a hierarchical steady state, which implies that the steady state is of the form $(0, \ldots, 0, \bar{c}_l, \ldots, \bar{c}_n)$ with $\bar{c}_l > 0, \ldots, \bar{c}_n > 0$, where $l > i$, then this steady state is unstable.*

Proof. Consider a linearization around the steady state $(0, \ldots, 0, \bar{c}_l, \ldots, \bar{c}_n)$ with $l > i$. Then, $\bar{s} = \tilde{s}_l$ and the Jacobian matrix A is of the following form

$$A := \begin{pmatrix} M_1 & 0 \\ C & M_2 \end{pmatrix}, \tag{2}$$

where M_1 is a submatrix of A, consisting of rows $1, \ldots, l-1$ and columns $1, \ldots, l-1$ and M_2 is a submatrix of A, consisting of rows $l, \ldots, n$ and columns $l, \ldots, n$ and

$$C := \begin{pmatrix} 0 & \cdots & 0 & \alpha \\ 0 & \cdots & 0 & 0 \\ \vdots & \vdots & \vdots & \vdots \\ 0 & \cdots & 0 & 0 \end{pmatrix},$$

where $\alpha = \frac{\partial g_{l-1}}{\partial c_{l-1}}$. The matrix M_1 is lower triangular. The characteristic polynomial takes the form

$$\chi_{A(\lambda)} := \prod_{i=1}^{l-1} \left(\lambda - \frac{\partial}{\partial c_i} f_i(\tilde{s}_l, 0) \right) \cdot \det[(I \cdot \lambda - M_2)].$$

Since $l > i$ and, due to Assumption (A10), $\frac{\partial}{\partial c_i} f_i(\tilde{s}_l, 0) > 0$ and we conclude that the considered steady state is unstable. □

This leads to the following result.

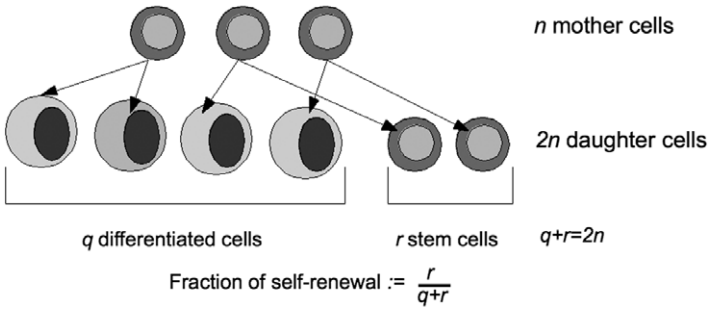

Fig. 2. Definition of the *fraction of self-renewal*.

Corollary 3.6 (*Characterization of a Stem Cell Population*)**.** *Stem cell population of a hierarchically organized steady state is given by* $\bar{c}_i > 0$ *with*

$$i := \max\{j | \tilde{s}_k \geq \tilde{s}_j, \text{for all } k = 1, \ldots, n\}.$$

The conclusion is that the stem cell population needs less signal molecules to maintain its size than any other cell population in the system considered.

The given calculations are also valid for a more complex signal behavior of the form $\frac{ds}{dt} = h(c_1, \ldots, c_n, s)$, if the hierarchical steady states fulfill $h(\bar{c}_1, \ldots, \bar{c}_n, \bar{s}) = 0$.

3.1. Biological interpretation of $\tilde{s}_i$

Time evolution of cell densities in case of all cells beside stem cells depends on influx from differentiation of the cells belonging to the previous differentiation stage. Therefore, to compare the capacity of different cell populations, we will analyze the system without the influx from the compartment $i-1$ to the compartment i. In such case, time evolution of cells of the type i is given by

$$\frac{dc_i(t)}{dt} = f_i(s(t), c_i(t)).$$

A signal concentration $\tilde{s}_i$ is, following Assumption (A9), the concentration of signal molecules satisfying

$$\frac{dc_i(t)}{dt} = f_i(\tilde{s}_i, c_i(t)) = 0,$$

i.e., the concentration at which cell population i is stimulated sufficiently to maintain its size without influx from other populations.

Definition 3.7 (*Signal Intensity of Self-Maintenance*)**.** Signal intensity of self-maintenance is the intensity of signal (cytokine level) s_i that is needed to maintain the size of population i without cell influx from downstream compartments.

Corollary 3.6 characterizes a stem cell population as a cell population with the lowest signal concentration for self-maintenance. In this sense stem cells are more sensitive to environmental signals and more successful in competition for signal molecules.

4. Analysis of a special version of the model

To obtain more insight into possible characterization of stem cells, in this section we focus on a special case of model (1), which has been proposed in Ref. [13]. In this case, f_i and g_i are given by explicit functions. The choice of the functions f_i and g_i enables us to relate the results from the last paragraph to specific quantitative cell properties. The model is based on the assumption that the differentiation process is strictly related to cell division, i.e., differentiation takes place only during cell division and therefore, the rate of cell differentiation is proportional to the rate of cell proliferation.

To describe self-renewal quantitatively we introduce the *fraction of self-renewal* that describes, which fraction of the progeny cells is identical to the mother cells (see Fig. 2). This parameter can be interpreted as the probability that a daughter cell has the same properties as the mother cell.

Remark 4.1. Note that the fraction of self-renewal is defined as an average over the whole population. It is, therefore, not related in a simple way to the occurrence of asymmetric cell divisions. For example, the fraction of self-renewal is equal to $\frac{1}{2}$, if all cells divide asymmetrically but also if 50% of the dividing cells produces two differentiated progeny cells and the other 50% give rise to two undifferentiated cells. In the latter case all divisions are symmetric (as illustrated in Fig. 2).

Denote the proliferation rate of the subpopulation of type i at time t by $p_i(t)$, the fraction of self-renewal by $a_i(t)$ and the death rate by $d_i(t)$. Then, the flux to mitosis at time t is given by $p_i(t)c_i(t)$. The fraction $a_i(t)$ of daughter cells stays undifferentiated. Therefore, the influx to cell population i after cell division is given by $2a_i(t)p_i(t)c_i(t)$ and the flux to the next cell compartment is given by $2(1-a_i(t))p_i(t)c_i(t)$. The flux to death at time t is given by $d_i(t)c_i(t)$. We obtain

$$
\begin{aligned}
f_i(t) &= (2a_i(t)-1)p_i(t)c_i(t) - d_i(t)c_i(t), \quad \text{for } i < n,\\
g_i(t) &= 2(1-a_{i-1}(t))p_{i-1}(t)c_{i-1}(t), \quad \text{for } 1 < i < n,\\
f_n(t) &= -d_n(t)c_n(t).
\end{aligned}
$$

As proposed in Ref. [13], we assume that the feedback signal depends on the concentration of mature cells and is given by

$$
s \equiv s(c_n(t)) := \frac{1}{1+kc_n(t)}
$$

with a positive constant k. This dependence can be justified using a quasi-steady state approximation of the plausible dynamics of the cytokine molecules [13]. The expression reflects the heuristic assumption that signal intensity achieves its maximum under absence of mature cells and decreases asymptotically to zero if the level of mature cells increases. To evaluate the expression in absence of mature cells is – of course – a mathematical idealization since vital function of any organism would collapse if there exist no mature cells of a given type.

Considering different plausible regulatory feedback mechanisms leads to different types of nonlinearities in the model equations. In particular, in Ref. [13] three different regulatory modes were proposed:

(M1) constant p_i and $a_i(s) = \frac{a_{i,\max}}{1+kc_n}$,
(M2) $p_i(s) = \frac{p_i}{1+kc_n}$ and constant a_i, and
(M3) $p_i(s) = \frac{p_i}{1+kc_n}$ and $a_i(s) = \frac{a_{i,\max}}{1+kc_n}$.

It was demonstrated that regulation of the output of hematopoiesis is more efficient and can be achieved in the clinically relevant time scale only if the regulated parameters are the proportions of cells (stem cells or committed cells), which do not differentiate following division (the fractions of daughter cells which stay in the same compartment, parameters a_i) [13]. Regulation of the rates of proliferation (parameters p_i) is not sufficient for that purpose.

As shown numerically in Ref. [13], such feedback control of cell self-renewal fractions is an efficient mechanism regulating cell differentiation and the vicinity of the positive equilibrium can be achieved in a clinically relevant time scale. Therefore, we assume the proliferation rates to be constant in time and self-renewal fractions to be controlled by the signal feedback, $a_i(t) \equiv a_i(s(c_n(t))) = a_{i,\max} \cdot s(c_n(t))$, where $a_{i,\max}$ is the maximal fraction of self-renewal. Furthermore, death rates are assumed to be constant in time. This results in the following set of equations,

$$
\begin{aligned}
\frac{\mathrm{d}c_1}{\mathrm{d}t} &= (2a_{1,\max}s-1)p_1c_1 - d_1c_1,\\
&\dots\\
\frac{\mathrm{d}c_i}{\mathrm{d}t} &= (2a_{i,\max}s-1)p_ic_i + 2(1-a_{i-1,\max}s)p_{i-1}c_{i-1} - d_ic_i,\\
&\dots\\
\frac{\mathrm{d}c_n}{\mathrm{d}t} &= 2(1-a_{n-1,\max}s)p_{n-1}c_{n-1} - d_nc_n,\\
s &= \frac{1}{(1+kc_n)}.
\end{aligned}
\tag{3}
$$

The following, biologically relevant, assumptions are made for the model parameters and initial data:

$$
\begin{aligned}
&t \in [0,\infty),\\
&c_i(0) \geq 0, \quad \text{for } i = 1\dots n,\\
&d_i \geq 0, \quad \text{for } i = 1\dots n-1,\\
&d_n > 0,\\
&p_i > 0, \quad \text{for } i = 1\dots n-1,\\
&a_{i,\max} \in [0,1) \quad \text{for } i = 1\dots n-1,\\
&k > 0.
\end{aligned}
$$

Analysis of the model equations in case of regulatory mode (M2) and (M3) leads to similar results.

Lemma 4.1 (*Global Solutions*). *Solutions of model (3) exist for all positive times.*

Proof. Since $u_i(t) \geq 0$ for $u_i(0) \geq 0$, it holds

$$\frac{\mathrm{d}}{\mathrm{d}t}\sum_{i=1}^{n} u_i(t) = \sum_{i=1}^{n-1} p_i(t)u_i(t) - \sum_{i=1}^{n} d_i u_i(t) \leq p_{\max}\sum_{i=1}^{n} u_i(t),$$

where $p_{\max} = \max\{p_i \mid i = 1, \ldots, n-1\}$, which is a finite positive constant. Gronwall's Lemma implies that $\sum_{i=1}^{n} u_i(t)$ is bounded for each finite time point. Similarly, boundedness by the exponential function holds also for each u_i, since they are nonnegative for nonnegative initial conditions. □

Proposition 4.2 (*Positive Steady States*). *System (3) has a unique positive steady state $\bar{c}_1, \ldots, \bar{c}_n$, if and only if the following conditions are satisfied.*

(1) $(2a_{1,\max} - 1)p_1 > d_1$
(2) $2a_{1,\max}p_1(d_i + p_i) - 2a_{i,\max}p_i(d_1 + p_1) > 0$, *for* $i = 2, \ldots, n-1$.

The steady state is given by

$$\bar{c}_l = \bar{c}_n \prod_{l+1}^{n} \Theta_i, \quad \text{for } l = 1, \ldots, n, \tag{4}$$

where

$$\bar{c}_n = \frac{1}{k}\left(\frac{2a_{1,\max}p_1}{d_1 + p_1} - 1\right),$$
$$\Theta_i := \frac{d_i + p_i - 2a_{i,\max}p_i\bar{s}}{2(1 - a_{i-1,\max}\bar{s})p_{i-1}} > 0, \quad \text{for } i = 2, \ldots, n-1,$$
$$\Theta_n := \frac{d_n}{2(1 - a_{n-1,\max}\bar{s})p_{n-1}} > 0, \quad \text{and}$$
$$\bar{s} := \frac{d_1 + p_1}{2a_{1,\max}p_1}.$$

Proof. The proof follows by a direct calculation. □

In some cell systems, such as the granulopoietic system, the death of immature cells can be neglected. This leads to the following special case.

Corollary 4.3 (*Positive Steady States: Special Case*). *If $d_1 = \cdots = d_{n-1} = 0$, $d_n > 0$, there exists a unique positive steady state if and only if the following conditions are satisfied:*

(1) $a_{1,\max} > \frac{1}{2}$,
(2) $a_{1,\max} > a_{i,\max}$, *for* $i = 2, \ldots, n-1$.

It holds

$$\bar{c}_l = \bar{c}_n \prod_{l+1}^{n} \Theta_i, \quad \text{for } l = 1, \ldots, n, \tag{5}$$

where

$$\bar{c}_n = \frac{1}{k}(2a_{1,\max} - 1),$$
$$\Theta_i := \frac{(a_{1,\max} - a_{i,\max})p_i}{(2a_{1,\max} - a_{i-1,\max})p_{i-1}}, \quad \text{for } i = 2, \ldots, n-1, \quad \text{and}$$
$$\Theta_n := \frac{a_{1,\max}d_n}{(2a_{1,\max} - a_{n-1,\max})p_{n-1}}.$$

In the following, we characterize the so called semitrivial steady states, i.e., nonnegative steady states with at least one variable equal to zero.

Lemma 4.4. *All semitrivial steady states are of the form $\bar{c}_1 = \cdots = \bar{c}_k = 0$ and $\bar{c}_l \neq 0$ for $l = k+1, \ldots, n$, with $1 \leq k \leq n$.*

Proof. Assume $\bar{c}_i = 0$ in a steady state. Therefore,

$$0 = \frac{dc_i}{dt} = (2a_{i,\max}\bar{s} - 1)p_i\bar{c}_i + 2(1 - a_{i-1,\max}\bar{s})p_{i-1}\bar{c}_{i-1} - d_i\bar{c}_i = 2(1 - a_{i-1,\max}\bar{s})p_{i-1}\bar{c}_{i-1}.$$

Nonnegativity of $\bar{c}_n \geq 0$ provides $0 \leq \bar{s} = \frac{1}{1+k\bar{c}_n} \leq 1$. Since $a_{i,\max} \in [0, 1)$ and $p_i > 0$ by assumption, we obtain $2(1 - a_{i-1,\max}\bar{s})p_{i-1} > 0$. Therefore $0 = 2(1 - a_{i-1,\max}\bar{s})p_{i-1}\bar{c}_{i-1}$ implies $\bar{c}_{i-1} = 0$. In summary, $\bar{c}_i = 0 \Rightarrow \bar{c}_{i-1} = 0$ and by induction $\bar{c}_1 = \cdots = \bar{c}_i = 0$. □

Proposition 4.5 (*Semitrivial Steady States*). *Assume that* $(2a_{1,\max} - 1)p_1 > d_1$, *and let*

$$s_i := \frac{a_{1,\max}p_1}{d_1 + p_1} - \frac{a_{i,\max}p_i}{d_i + p_i},$$
$$\mathbb{J} := \{i \mid s_i \leq 0\},$$
$$\mathbb{S} := \{s_i \mid s_i \leq 0\}, \quad \textit{and}$$
$$k := \max\{i \in \mathbb{J} \mid s_i = \min \mathbb{S}\}.$$

If $\bar{c}_k > 0$, *then* $\bar{c}_l = 0$ *for* $l < k$ *and the steady state values* $\bar{c}_k, \ldots, \bar{c}_n$ *are positive and unique.*

Proof. The assertion of the proposition follows from calculations taking into account the sign of $2(1 - a_{i,\max}s) > 0$. After renumbering of equations steady state population concentrations can be calculated by the formula given in Proposition 4.2. □

Corollary 4.6 (*Instability*).

(i) *If there exists a positive steady state, each semitrivial steady state is unstable. The trivial steady state is also unstable.*
(ii) *If there exists a steady state with k positive components, each steady state with less than k positive components is unstable.*

Proof. The corollary results from Theorem 3.5. □

4.1. Biological interpretation of the mathematical constraints

Assumption (1) in Proposition 4.2, i.e., $(2a_{1,\max} - 1)p_1 > d_1$, is equivalent to the existence of signal with intensity $s \in (0, 1)$, such that $dc_1/dt > 0$. In other words, for existence of a positive steady state, it is necessary that there exist signal levels such that death rate of stem cells is smaller than the reproduction rate. In the case, in which stem cell death is neglected, this assumption is equivalent to the assumption that the maximal fraction of self-renewal of a stem cell population is larger than $\frac{1}{2}$, i.e., in the stem cell compartment self-renewal is more probable than differentiation, if the signal concentration is high enough.

Assumption (2) in Proposition 4.2, i.e., $2a_{1,\max}p_1(d_i+p_i) - 2a_{i,\max}p_i(d_1+p_1) > 0$, for $i = 2, \ldots, n-1$, is equivalent to the assumption that the signal concentration for self-maintenance of the stem cell compartment is smaller than that of all other mitotic cell types. This means that stem cells need less signal than other cells to maintain the size of their compartment without influx from other compartments. In the special case where death of immature cells is neglected, Assumption (2) is equivalent to the assumption that the maximal fraction of self-renewal of the stem cell population has to be larger than the maximal fraction of self-renewal of all other mitotic cell types. In this case stem cells are defined by their higher potential to self-renew. This characterization is well known in biology, e.g., [24,25]. The characterization of the stem cell population as the population with the smallest signal concentration for self-maintenance is more general. If death rates of immature cells are greater than zero or if the dependence of self-renewal on signal concentration is different for different cell types, the fraction of self-renewal is no longer sufficient to characterize the stem cell population.

The results of this section are summarized in the following "Theorem".

Theorem 4.7. (*Stem Cell Theorem*) *In the given model a stem cell population can be characterized by the following properties:*

(1) *For some cytokine levels the death rate is smaller than the reproduction rate.*
(2) *The signal intensity (cytokine level) needed for maintenance of population size is smaller than that of all other cell populations.*

Corollary 4.8. *Let* p_i, $a_{i,\max}$, d_i *satisfy conditions* (1) *and* (2) *of Theorem 4.7, then it holds,*

(1) *There exists a unique steady state with* $\bar{c}_1 = \cdots = \bar{c}_{i-1} = 0$ *and* $\bar{c}_i > 0, \ldots, \bar{c}_n > 0$.
(2) *All other steady states are unstable.*
(3) *There exists no nonnegative steady state with* $\bar{c}_k > 0$ *for* $k < i$.
(4) *To ensure* $\bar{c}_i > 0$ *without influx from other compartments, a signal of intensity* $s_i := \frac{d_i+p_i}{2a_{i,\max}p_i}$ *is needed.*

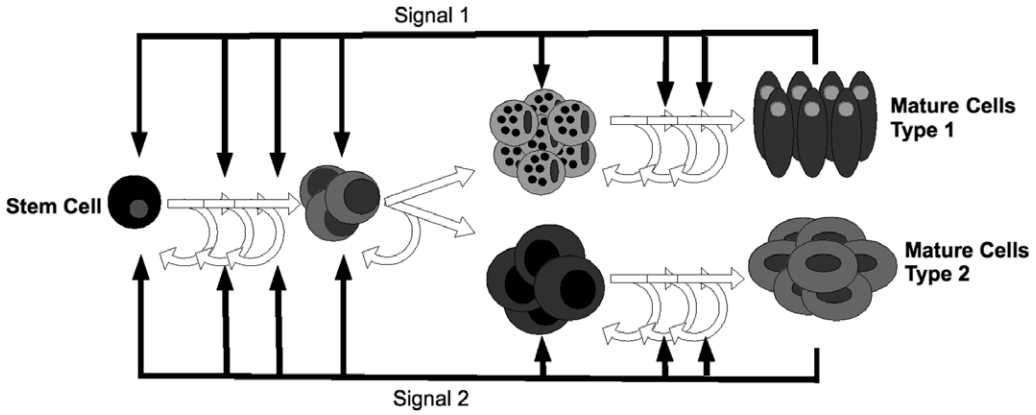

Fig. 3. Stem cell population giving rise to two types of mature cells and simple feedback regulation: For each type of mature cells there exists one signal that regulates the differentiation of immature cells to the mature cells of the corresponding type. Stages common for both lineages depend on both signals, lineage specific stages depend on one signal.

5. A system with more than one type of mature cell

In this section we consider a system of stem cells that are able to give rise to more than one type of mature cells. Interactions between different cell lineages are very complex (compare [26,27,16,28,29]) and little is known about the regulation of lineage selection. Recently, it has been demonstrated that signal molecules play a key role in the regulation of lineage selection [26]. To investigate this problem we propose a simple version of a two-lineage system. The following assumptions are made.

Assumptions 5.1. (B1) To become mature, cells of each lineage have to pass a number of serial maturation steps; none of these steps can be omitted. Cells pass these steps only in one direction, i.e., from primitive to more mature stages. Early developmental stages are the same for cells of both lineages (Fig. 3).

(B2) Cell behavior is regulated via feedback signaling. For each lineage, cell dynamics is controlled by one type of signaling molecules. Stages that are specific for one lineage depend on one signal, stages that are common to both lineages depend on two signals. This assumption is motivated by the fact that in some cell systems, such as the hematopoietic system, dynamics of important lineages is regulated by one or two types of signaling molecules (see, e.g., [30–32]).

(B3) The concentration of the first type of signaling molecules depends on the density of mature cells of one of the lineages, while the concentration of the second type of signaling molecules depends on the density of mature cells of the other lineage. The lower is the density of mature cells, the higher is the concentration of the corresponding signaling molecules.

Time evolution of the cell system fulfilling the above assumptions can be described by the following system of ordinary differential equations.

Common stages of both lineages

$$\begin{aligned}
\frac{dc_1(t)}{dt} &= f_1(s^{(1)}(t), s^{(2)}(t), c_1(t)),\\
\frac{dc_2(t)}{dt} &= f_2(s^{(1)}(t), s^{(2)}(t), c_2(t)) + g_1(s^{(1)}(t), s^{(2)}(t), c_1(t)),\\
&\vdots\\
\frac{dc_{m-1}(t)}{dt} &= f_{m-1}(s^{(1)}(t), s^{(2)}(t), c_{m-1}(t)) + g_{m-2}(s^{(1)}(t), s^{(2)}(t), c_{m-2}(t)).
\end{aligned}$$

Lineage specific stages

$$\begin{aligned}
\frac{dc_m^{(2)}(t)}{dt} &= f_m^{(2)}(s^{(2)}(t), c_m^{(2)}(t)) + g_{m-1}^{(2)}(s^{(1)}(t), s^{(2)}(t), c_{m-1}(t)),\\
&\vdots\\
\frac{dc_{n_2}^{(2)}(t)}{dt} &= f_{n_2}(s^{(2)}(t), c_{n_2}^{(2)}(t)) + g_{n_2-1}^{(2)}(s^{(2)}(t), c_{n_2-1}^{(2)}(t)),
\end{aligned}$$

where $s^{(2)}(t) \equiv s(c_{n_2}^{(2)}(t))$.

$$\frac{dc_m^{(1)}(t)}{dt} = f_m^{(1)}(s^{(1)}(t), c_m^{(1)}(t)) + g_{m-1}^{(1)}(s^{(1)}(t), s^{(2)}(t), c_{m-1}(t)),$$
$$\vdots$$
$$\frac{dc_{n_1}^{(1)}(t)}{dt} = f_{n_1}(s^{(1)}(t), c_{n_1}^{(1)}(t)) + g_{n_1-1}^{(1)}(s^{(1)}(t), c_{n_1-1}^{(1)}(t)),$$

where $s^{(1)}(t) \equiv s(c_{n_1}^{(1)}(t))$. Stages of lineage 1 are marked with superscript $^{(1)}$, while specific stages of lineage 2 are marked with superscript $^{(2)}$. Common stages have no superscript. The meaning of the functions $f_i, f_i^{(1)}$ and $f_i^{(2)}$ is analogous to that of function f_i from above, the meaning of the functions $g_i, g_i^{(1)}$ and $g_i^{(2)}$ is analogous to that of g_i from above.

Definition 5.2 (*Stem Cell Population of a Hierarchical System*)**.** Population i is called a stem cell population of the steady state $(\bar{c}_1, \ldots, \bar{c}_{m-1}, \bar{c}_m^{(1)}, \ldots, \bar{c}_{n_1}^{(1)}, \bar{c}_m^{(2)}, \ldots, \bar{c}_{n_2}^{(2)})$ for $i < m$, if $g_{i-1}(\bar{s}^{(1)}, \bar{s}^{(2)}, \bar{c}_{i-1}) = 0$ and

$$\begin{array}{ll}
\bar{c}_i > 0, & g_i(\bar{s}^{(1)}, \bar{s}^{(2)}, \bar{c}_i) > 0 \\
\cdots & \cdots \\
\bar{c}_{m-1} > 0, & g_{m-1}(\bar{s}^{(1)}, \bar{s}^{(2)}, \bar{c}_{m-1}) > 0 \\
\bar{c}_m^{(1)} > 0, & g_m^{(1)}(\bar{s}^{(1)}, \bar{c}_i) > 0 \\
\cdots & \cdots \\
\bar{c}_{n_1}^{(1)} > 0, & \\
\bar{c}_m^{(2)} > 0, & g_m^{(2)}(\bar{s}^{(2)}, \bar{c}_i) > 0 \\
\cdots & \cdots \\
\bar{c}_{n_2}^{(2)} > 0. &
\end{array}$$

In other words the stem cell population is the population that gives rise to all existing cell lineages and maintains them in equilibrium.

It is an important question how a multi-lineage cell system behaves, where there exists more than one type of signaling molecules. A number of different studies was performed to get insights into this issue [30–32]. Most of them aimed to determine which signaling molecules are important for regulation of a specific cell lineage. Such investigations were performed most frequently for the hematopoietic cell system, since hematopoietic cells are easily accessible and countable. The studies indicate that receptor or signal knockouts lead to modified densities of different cell types in equilibrium. Since gene knockouts possibly cause deleterious effects during embryogenesis, only genetic modifications which result in viable organisms (so called viable mutants) can be investigated. It is a disadvantage of this approach that the performed manipulations could result in unresolved pathological effects. Therefore it is a matter of debate in how far results can be extrapolated to healthy organisms. An important, but poorly exploited byproduct of these studies is the fact that even in the absence of unique signals or signal receptors steady states exist, [32], showed that fluctuations of blood cell counts in absence of G-CSF effects are comparable to those found in steady state hematopoiesis in healthy mice. This means that a stem cell population maintains different steady states depending on the signaling conditions. An outstanding example for this stem cell property is the knockout of one specific type of signal molecule (G-CSF) in mice which leads to a reduction of granulocytes count by 80%. Substitution of the knocked-out signal leads, after some days, to normalized marrow and blood cell counts [32]. This study implies that, according to the availability of signals, stem cells maintain different steady states. Transitions between them seem to be reversible.

Although there exist little data on signal dependence of steady states in other cell systems, we make the following assumptions.

Assumptions 5.3. (B5) The stem cell population of a multi-lineage system is also the stem cell population of the corresponding single-lineage systems. It means that if c_i is the stem cell population of the multi-lineage system, then the system consisting of the equations for $c_i, \ldots, c_{m-1}, c_m^{(1)}(t), \ldots, c_{n_1}^{(1)}$ with $s^{(1)}(t) \equiv s(c_{n_1}^{(1)}(t))$ and $s^{(2)}(t) \equiv 0$ also has a hierarchical steady state with stem cell population c_i. The same holds for the system obtained by exchanging $^{(1)}$ and $^{(2)}$.

(B6)
$$\begin{array}{ll}
f_i(s_1, s_2, 0) = 0 & \text{for } i = 1, \ldots, m-1 \text{ and all } s_1, s_2; \\
f_i^{(1)}(s, 0) = 0 & \text{for } i = m, \ldots, n_1 \text{ and all } s; \\
f_i^{(2)}(s, 0) = 0 & \text{for } i = m, \ldots, n_2 \text{ and all } s.
\end{array}$$

(B7)
$$\begin{array}{ll}
g_i(s_1, s_2, 0) = 0 & \text{for } i = 1, \ldots, m-2 \text{ and all } s_1, s_2; \\
g_i^{(1)}(s, 0) = 0 & \text{for } i = m-1, \ldots, n_1 - 1 \text{ and all } s; \\
g_i^{(2)}(s, 0) = 0 & \text{for } i = m-1, \ldots, n_2 - 1 \text{ and all } s.
\end{array}$$

(B8) If $c_i > 0$ then

$$f_i^{(1)}(s, c_i) \text{ is strictly monotone increasing in } s,$$
$$f_i^{(2)}(s, c_i) \text{ is strictly monotone increasing in } s,$$
$$f_i(s_1, s_2, c_i) \text{ is strictly monotone increasing in } s_1 \text{ and } s_2.$$

(B9) For each $i > m - 1$ there exists

$$\text{a signal concentration } \tilde{s}_i^{(1)} > 0, \text{ such that } f_i^{(1)}(\tilde{s}_i, c_i) = 0, \ \forall c_i > 0,$$
$$\text{a signal concentration } \tilde{s}_i^{(2)} > 0, \text{ such that } f_i^{(2)}(\tilde{s}_i, c_i) = 0, \ \forall c_i > 0.$$

(B10) In healthy tissues cell counts are in equilibrium.

The above assumptions are the extension of Assumptions (A6)–(A9) and are based on similar biological motivation.

Remark 5.1. Assumption (B9) together with Assumption (B8) imply that $\tilde{s}_i^{(1)}$ and $\tilde{s}_i^{(2)}$ fulfilling

$$\begin{aligned}
&f_i^{(1)}(s, c_i) > 0 \quad \forall c_i > 0 \text{ and } s > \tilde{s}_i^{(1)},\\
&f_i^{(2)}(s, c_i) > 0 \quad \forall c_i > 0 \text{ and } s > \tilde{s}_i^{(2)},\\
&f_i^{(1)}(s, c_i) < 0 \quad \forall c_i > 0 \text{ and } s < \tilde{s}_i^{(1)},\\
&f_i^{(2)}(s, c_i) < 0 \quad \forall c_i > 0 \text{ and } s < \tilde{s}_i^{(2)}
\end{aligned}$$

are unique. Furthermore, for each $i < m-1$, there exist signal concentrations $\tilde{s}_i^{(1)} > 0$ and $\tilde{s}_i^{(2)} > 0$ such that $f_i(\tilde{s}_i^{(1)}, 0, c_i) = 0$ and $f_i(0, \tilde{s}_i^{(2)}, c_i) = 0$.

With the help of the above assumptions it is possible to characterize a stem cell population by its ability to maintain appropriate single-lineage subsystems. In the remainder of this section we will analyze the multi-lineage system. From the previous considerations a corollary follows.

Corollary 5.4. *If a stem cell population c_i of a multi-lineage system satisfying Assumptions* (B1)–(B10) *exists, it is characterized by the following properties: $\tilde{s}_j^{(1)} > \tilde{s}_i^{(1)}$ and $\tilde{s}_j^{(2)} > \tilde{s}_i^{(2)}$ for all $j > i$.*

Proof. This corollary is a consequence of (B5), (B8) and (B9). □

Additional assumptions are necessary to obtain the instability result.

Proposition 5.5. *Let c_i, $i < m$, be a stem cell population of a multi-lineage system satisfying the following conditions,*

(i) *For all (s_1, s_2) such that $f_j(s_1, s_2, c_j) = 0$ for a $c_j > 0$ and $j > i$, it holds $f_i(s_1, s_2, c_i) > 0$ for all $c_i > 0$.*
(ii) *If $f_i(s_1, s_2, c_i) > 0$ for all $c_i > 0$, then also $\frac{\partial f_i(s_1, s_2, c_i)}{\partial c_i}|_{c_i=0} > 0$.*

Then, each steady state of the form $(0, \ldots, 0, \bar{c}_{i+1}, \ldots, \bar{c}_{m-1}, \bar{c}_m^{(2)}, \ldots, \bar{c}_{n_2}^{(2)}, \bar{c}_m^{(1)}, \ldots, \bar{c}_{n_1}^{(1)})$ is linearly unstable.

Proof. The proof is similar to that of Theorem 3.5. $\frac{\partial f_i(s_1, s_2, 0)}{\partial s_l} = 0$ and $\frac{\partial g_i(s_1, s_2, 0)}{\partial s_l} = 0$ for $l \in \{1, 2\}$ and Jacobian matrix has the form given in Eq. (2). Moreover, $\frac{\partial f_i(s_1, s_2, c_i)}{\partial c_i}|_{c_i=0}$ is an eigenvalue. Conditions (i) and (ii) ensure that it is positive. □

Remark 5.2. If conditions (i) and (ii), and Assumptions (B8) and (B9) hold only locally, i.e., for some intervals of signaling intensity, then there may exist multiple steady states of the system. Consequently, a stem cell population may not be unique and may change depending on the signaling. This conclusion is in line with the experimental findings showing that cancer cells may disrupt environmental signaling [33].

Remark 5.3. The results of this section apply without major modifications to cell systems with more than two lineages.

5.1. Biological interpretation

As in the case of single-lineage systems, the stem cell population is more sensitive to signal molecules than the other cell populations. If c_i is the stem cell population, then in a steady state $f_i(\bar{s}^{(1)}, \bar{s}^{(2)}, \bar{c}_i) = 0$, while $f_j(\bar{s}^{(1)}, \bar{s}^{(2)}, \bar{c}_j)$, $f_j^{(1)}(\bar{s}^{(1)}, \bar{c}_j)$ and $f_j^{(2)}(\bar{s}^{(2)}, \bar{c}_j)$ have to be negative. It means that the steady state signal concentrations are sufficient to prevent the stem cell population from declining, while it is not the case in all other populations. In this sense, the stem cell population is more sensitive to the steady state signals than any other population. The same interpretation holds for condition (i) of Proposition 5.5.

6. Discussion

The question of what distinguishes stem cells from non-stem cells is one of the most important questions of developmental and cancer biology and regenerative medicine. In the present paper, this question has been addressed with the help of mathematical models. Mathematical modeling is a powerful approach that has contributed a lot to the understanding of complex biological systems such as the hematopoietic system, [34].

The answer depends on the stem cell concept used. Biologically accepted hypothesis that characterizes stem cells as a cell population with the highest ability to self-renew is related to cell properties and neglects the influence of microenvironment and feedback signaling. The models proposed in this paper characterize the stem cells population as the population that is more sensitive to environmental signals than all other cell populations. This characterization is more general than the classification of stemness by the self-renewal potential. Furthermore, it concerns the behavior of whole cell populations and not single cells. The proposed concept seems to be compatible with well known heterogeneity of stem cell populations [35,36,11], and includes the possibility that heterogeneity may be necessary for maintenance of the population size. This interpretation characterizes stem cells with the help of their behavior in the presence of signal molecules. External signals play an important role in the interactions of cells with their environment [37–39]. Therefore, our characterization of stem cells takes into account interactions of cells with the environment. This is in line with biological findings that stress the importance of microenvironment for stem cell function (so called stem cell niche) [40,23,41,42,38,43].

In recent biological papers it has been demonstrated that cancer cells disrupt signaling between stem cells and their environment [33]. In this respect the importance of signaling for the developed stem cell characterization is in accordance with experimental findings. Stem cell behavior can be interpreted as successful competition for environmental signals. Competition for signal molecules is a widespread mechanism in nature and can be found, for example, during neurogenesis [44] or folliculogenesis [45]. Consequently, malignant transformation of stem or progenitor cells can be understood as enhanced sensitivity to the signal molecules or reduced need of them. From an evolutionary point of view this behavior seems acceptable, since exhaustion of the stem cell pool starts later than exhaustion of all other cell populations.

The classification developed in this paper may be helpful to identify stem cells and stem cell populations [46–48]. The proposed concept reveals stem cells as a population that is most sensitive to the signal concentrations in the current steady state. Since cells respond differently to different conditions, the stemness property is not necessarily linked to a unique cell population. It seems that according to the signal level, different cell populations may exhibit the properties of stem cells. One cell population may be more sensitive to one signal configuration and other cell population to another. Such understanding can be helpful for investigation and treatment of cancer. It might be possible to determine environmental conditions, in which so called cancer stem cells do not longer behave as stem cells. Such conditions might help to find means to eradicate the cancer stem cell population.

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