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The universal properties of stem cells as pinpointed by a simple discrete model

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Abstract. The ability of a few stem-cells to repopulate a severely damaged bone marrow (BM) guarantees the stability of our physical existence, and facilitates successful BM transplantations. What are the basic properties of stem cells that enable the maintenance of the system's homeostasis? In the present work we attempt to answer this question by investigating a discrete (in time and phase-space) dynamical system. The model we present is shown to retrieve the essential properties of homeostasis, as reflected in BM functioning, namely, (a) to produce a constant amount of mature cells, and (b) to be capable of returning to this production after very large perturbations. The mechanism guaranteeing the fulfillment of these properties is *extrinsic* - negative feedback control in the micro-environment - and does not need additional stochastic assumptions. Nevertheless, the existence of a simple *intrinsic* control mechanism, a clock which determines the switch to differentiation, ascertains that the system does not admit non-trivial extinction states. This result may help clarifying some of the controversy about extrinsic versus intrinsic control over stem cell fate. It should be stressed that all conclusions are valid for any system containing progenitor and maturing cells.

0. Introduction

As was already recognized by the ancients, blood is our "life preserving fluid", whose major three functions are nutrients and oxygen supply to the tissues, self-immunity and defense against pathogens. In order to carry out these tasks human blood contains a variety of cells, each precisely adapted to its specific objective. All the different blood cells develop from a kind of master cell - the hemopoietic (blood forming) stem cell, which inhabits primarily the BM. Hemopoietic cells vary in their degree of "stemness", ranging from the most primitive stem-cells, which can only replicate, through intermediate levels, which can replicate and differentiate, to fully differentiated, mature blood cells, which migrate into the peripheral blood. The transition of stem cells from quiescence into proliferation, or differentiation, is governed by their cell-cycling status, by stimulatory hormones secreted

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by neighboring cells in the micro-environment and by the level of amplification of the stem-cell population [5, 12].

It is commonly believed that one primitive stem cell is sufficient for reconstituting the entire blood and immune systems [4]. This extraordinary regenerative ability of the BM is not surprising, bearing in mind that its vital role must remain unaffected by stem cells depletion, e.g. as a result of chemotherapy, radiation or disease. It should be emphasized that though the supply of blood cells in the periphery is steady, the BM, considered as a physical entity, is not remotely static. Rather, it is dynamic in the sense that it constantly changes in its constitution and arrangement, and these changes occur at varying rates.

Our aim in this paper is to mathematically investigate BM *homeostasis*. More precisely, we wish to define simple properties that enable the BM to rapidly return to a steady supply of blood cells after relatively large perturbations in stem-cell numbers.

Our model contains the following basic types of cells:

- **Stem** cells, denoted by S, which can either proliferate, generating new stem cells, or differentiate, resulting in new mature cells.
- **Differentiated** cells, denoted by D, which are the product of stem cells. After maturation they leave the BM and circulate in blood, leaving the space they occupied earlier empty.
- **Null** cells, which simply represent vacant space in the BM , and can be thought of as its resources. We denote these by N.

The BM is represented geometrically as a connected, locally finite undirected graph. This describes neighborhoods of BM cells. The reader may think of the two or three dimensional discrete lattices, although the results hold for any arbitrary locally finite graph, whose cells do not necessarily have uniform neighborhoods.

The family of models we present is aimed to simulate a situation in which a cell's behavior is determined by a combination of (1) the states of cells in its proximity and (2) its cell cycle:

(1) Stem cell behavior is determined by the number of its stem cell neighbors. This assumption is aimed at simply describing the fact that cytokines, secreted by cells into the micro-environment are capable of activating quiescent stem cells into proliferation and differentiation [5].

(2) Each cell has internal counters, which determine stem cell proliferation, stem cell transition into differentiation, as well as the transit time of a differentiated cell before migrating to the peripheral blood.

1. Description of the model

We begin with a few notations.
Let $G = (V, E)$ be a connected, locally finite undirected graph. Its vertices V and edges E describe the cells and their neighborhood of influence in the BM respectively.

For every $v, u \in V$ denote by $\rho(u, v)$ the distance between these vertices in the shortest-path metric induced by G.
Let $N(v) = \{u \in V | \rho(u, v) = 1\}$ denote the neighborhood of a vertex $v \in V$, i.e. the set of vertices joined to v by an edge.
Similarly, the *ball* of radius n centered in $v \in V$ is the set of all vertices such that their distances from v do not exceed n. We write $B(v, n) = \{u \in V | \rho(u, v) \leq n\}$.
If $U \subseteq V$ is a nonempty subset of vertices then for every $v \in V$ let $\rho_U(v) = \min_{u \in U} \rho(u, v)$ be the distance between v and the set U.
A *state* of a vertex is a 2-tuple. The first coordinate denotes the cell's character (either S, D or N) while the second is a non-negative integer τ which denotes its internal counter. Let Ω be the set of states of a vertex.
A map $x : V \to \Omega$ is the state of the entire graph.
The set of all states of G is denoted by Ω^V.
A state $x \in \Omega^V$ at time t is denoted by x^t.
We are ready to define an iterative operator on Ω^V. It depends on three non-negative integers Φ, Ψ, Θ.

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

$$x^t(v) = (S, \tau) \Longrightarrow x^{t+1}(v) = \begin{cases} (D, 0) & \text{if } \forall u \in N(v), x^t(u) = (S, *) \wedge \tau = \Psi \\ (S, \tau) & \text{if } \exists u \in N(v), x^t(u) \neq (S, *) \wedge \tau = \Psi \\ (S, \tau + 1) & \text{otherwise;} \end{cases} \tag{2}$$

$$x^t(v) = (N, \tau) \Longrightarrow x^{t+1}(v) = \begin{cases} (S, 0) & \text{if } v \text{ has a stem neighbor and } \tau = \Theta \\ (N, \tau + 1) & \text{if } v \text{ has a stem neighbor and } \tau < \Theta \\ (N, 0) & \text{otherwise.} \end{cases} \tag{3}$$

Explanation. Rule (1) reflects the time Φ of a cell maturation in the BM before it migrates to the peripheral blood.
Rule (2) states that a stem cell matures, if its internal counter – representing its cycling phase – exceeds a threshold Ψ, and its neighborhood consists of stem cells alone (which corresponds to receiving signals that the environment is saturated with stem cells; see [5] for biological evidence for such negative feedback).
In (3) we state that when a stem cell identifies an empty neighboring site, it proliferates after Θ time steps, such that one of the descendants occupies the vacant site.

Remark. The above can be regarded as a family of cellular automata. It is also easy to see how one can change this definition to get other threshold-like automata. Threshold cellular automata have been employed for the analysis of biological information processing in [1,3] (for general definition and other types of threshold automata see, e.g. [2,7,8,11,13]).

We show next that the above model has strong 'homeostatic' properties.

1.1. Expansion of stem cells

We begin by investigating the property of stem cells to expand throughout the BM. The following lemma shows that any point becomes occupied by a stem cell, given that initially there is at least one stem cell in the BM.

Proposition 1.1. *For any Ψ, Φ, Θ if there exist two vertices $v, u \in V$ such that at some time t, the vertex v is not occupied by a stem cell and u is, then there exists an $s > 0$ such that v will be occupied by a stem cell at time $t + s$.*

Proof. From (2) and (3) we conclude that if u and v are neighbors then u keeps its stem value as long as v is not a stem cell. The vertex v itself turns into stem in no more than $\Phi + \Theta$ time steps, which is the time for v to migrate (in case it was a differentiated cell), turn into a null cell and as it is a neighbor of a stem cell, become a stem cell after Θ time steps.
We proceed by a way of induction on the distance $\rho(u, v)$ to obtain a bound on the time that is needed for v to turn into a stem cell:

$$s \leq \Phi + \rho(u, v)\Theta. \tag{4}$$

□

The arguments above provide that the distance $\rho_{U(t)}(v)$ between a vertex v, which is not occupied by a stem cell at time t, to the subset $U(t) \subseteq V$ of vertices which entertain a stem cell at time t is a non-increasing function. Furthermore, there exists $s \leq \Phi + \rho_{U(t)}(v)\Theta$ such that $\rho_{U(t+s)}(v) = 0$.
What happens then? We assert that if $\Psi > 0$ and $r \geq t + s$ then $\rho_{U(r)}(v) \leq 2$, This means that from the time $t + s$ on there always is a stem cell not farther than two edges from v. We record this discussion in the following proposition. Note that its formulation excludes the pathological initial state of synchronized stem cells (discussed in subsection 1.3) by demanding a change of the state of v at a certain time t_0.

Proposition 1.2. *Let $\Psi > 0$. Suppose that a vertex v becomes a stem cell at time t_0, then for every $t \geq t_0$ there is a vertex $u \in B(v, 2)$ which is occupied by a stem cell.*

Proof. A necessary condition for the production of a stem cell in v at time t_0 is that $\exists v' \in N(v), x^{t_0-1}(v') = (S, \tau)$ for some $\tau \geq \Theta$. Note that the internal counter of v differs from the internal counter of v' (and they do not equalize at least until the counter of v is Ψ). Now, the vertex v stays stem until both conditions in (2) hold. Therefore, if v differentiates at time $t_1 > t_0$, either it still has a stem neighbor at time t_1 or all of its neighbors differentiate simultaneously with v. The second scenario can happen only if there is at least one vertex $v'' \in B(v, 2)$ that turned to stem at time $t_1 - 1$. In any case, as long as the vertex v is not a stem cell, there is a stem cell in $B(v, 2)$. Apply now proposition 1.1 to ensure that until the next time the vertex v is occupied by a stem cell (and then the arguments can be repeated inductively), the distance from v to the closest stem cell will not exceed 2. □

A direct conclusion from 1.2 is an estimation for the density of stem cells in a bounded vicinity. Although it can be formulated for every connected, locally compact graph, we state the following corollary for graphs with bounded degree (as will be explained herein after), since in this case sizes of balls can be estimated uniformly.
We need two more notations:
If the graph G has the property that there exists d such that $|N(v)| \leq d, \forall v \in V$, we say that G has *bounded degree*, and write $deg(G) \leq d$ (It is indeed reasonable to assume that the BM is described as a graph of bounded degree).
The *density* of stem cells in a given finite subset of vertices $U \subset V$ at time t is the proportion at time t of the number of stem cells S in U and the total number of vertices in U. It is denoted by $\delta^t(U)$. We have:

Corollary 1.3. *Let G be a graph of bounded degree and let $\Psi > 0$. Suppose that at some time t_0 a vertex v is not occupied by a stem cell and a vertex u is, then for every ball $B = B(v, 2) \subset G$,* $\liminf_{t\to\infty} \delta^t(B) \geq \frac{1}{deg(G)^2+1}$.

Proof. By 1.1 and 1.2, any ball of radius 2 admits a stem cell from a certain moment on. The size of such a ball contains no more than $deg(G)^2 + 1$ vertices. □

In essence propositions 1.1, 1.2 and 1.3 show that not only is it true that one stem cell is sufficient to bring back the system to a normal state, that is a state where each vertex is used at some time for cell proliferation, it is also true that the BM has a 'built-in' mechanism guaranteeing that stem cells do not become too scattered.

1.2. Production of mature cells

We have seen that stem cells do fill the graph nicely. In this subsection we show that the system generates enough mature blood cells.

Proposition 1.4. *Suppose that a vertex $v \in V$ is occupied by a stem cell at time t. Then either v or one of its neighbors will be occupied by a differentiated cell within* $\max(\Psi + 1, \Theta + 2)$ *iterations.*

Proof. Assume that v has no differentiated neighbors (otherwise we are done), then $N(v)$ will consist only of stem cells by at most $\Theta + 1$ time steps, unless one of its neighbors differentiates in the meantime (and again we are done). The time that v has to wait for its differentiation in this situation is no longer than $\Psi - \Theta$, if this term is nonnegative. □

Note that in this model one cannot guarantee that a particular stem cell will differentiate. The lemma above does guarantee that in the close vicinity of any stem cell some cell differentiates during a fixed bounded time interval.
An immediate consequence of the above is a lower bound on the supply of mature cells to the peripheral blood:

Corollary 1.5. *Let $\Psi > 0$. Suppose that at some time t_0 a vertex v is not occupied by a stem cell and a vertex u is, then every ball of radius* 3 *eventually supplies at least one mature cell every* $\max(\Psi + 1, \Theta + 2) + \Phi$ *iterations.*

Proof. By 1.3, in this situation every ball of radius 2 admits a stem cell from a certain moment on. Proposition 1.4 says that either this cell or one of its neighbors (and so we argue about balls of radius 3) differentiate within $\max(\Psi + 1, \Theta + 2)$ iterations and migrate from the BM as mature cells after Φ additional iterations. □

1.3. Steady states and dying out states

We consider the (unique) state satisfying $\forall v \in V, x(v) = N$ as the *death state* of the system. A state x^t for which there exists a $k \in Z^+$ such that x^{t+k} is the death state, will be called a *dying out state*. Clearly, any state consisting of no stem cells is a dying out state. The other extreme case, a state consisting only of stem cells the counters of which are identical (we regard such states as *synchronized*), is a dying out state. We claim that if $\Psi > 0$ then there are no other dying out states.

Proposition 1.6. *Let $\Psi > 0$, then the only dying out states are either those consisting of no stem cells or only of synchronized stem cells.*

Proof. Let $x^t \in \Omega$ be a state, which is not one of the dying out states as in the hypothesis. If there exists $v \in V$ which is not a stem cell at time t, then since there exists a stem cell at time t, v turns to a stem cell (by 1.1) and so by 1.2 there is always a stem cell in $B(v, 2)$. The system does not die out.
Assume, therefore, that V admits only stem cells at time t. By the hypothesis, the counters are not synchronized. This condition ensures that they do not differentiate together and again the system does not die out. □

Next, we are after states $x \in \Omega$ for which for all $k \in Z^+$, $x^{t+k} = x^t$. These are the *steady states* of the system. The fact that each differentiated cell matures and leaves the BM eventually, combined with Proposition 1.1 implies

Proposition 1.7. *For every Φ, Ψ, Θ the model does not have steady states other than the death state.*

Finally, we would like to demonstrate the significance of the condition $\Psi > 0$. The following example shows that 1.2, 1.3, 1.5 and 1.6 do not hold when $\Psi = 0$.

Example. Let $G = (V, E)$ be the graph whose vertices V correspond to the integers such that every two consecutive integers are connected by an edge in E. Let $\Psi = 0$ (Φ and Θ are arbitrary), and start iterating beginning with the initial state $x^0 \in \Omega$ as follows:

$$x^0(v) = \begin{cases} (S, 0) & \text{if } v \equiv 0 (mod 3) \\ (N, 0) & \text{otherwise} \end{cases}$$

Clearly, at time $t = \Theta + 1$ we have $x^{\Theta+1}(v) = (S, 0)$ for every v.
The synchronization of stem cell counters yields a death state at time $\Theta + 2$.

2. Discussion

In this work we attempted to characterize the universal properties of stem cells which account for a constant production of mature cells and for the ability to recover from severe perturbations. Our model determines the common sense ground rules governing stem cell behavior, while allowing factors which influence the "decision" of individual stem cells to proliferate, differentiate or remain quiescent to be modulated.

The main properties of our model are achieved from the negative feedback demand in (2), namely that a stem cell does not differentiate unless its immediate micro-environment is saturated with stem cells. The second demand in (2) is also significant: If we assume the existence of an internal clock determining the differentiation onset to $\Psi > 0$ (We understand this condition as guaranteeing desynchronization of neighboring stem cells), then we obtain the results that stem cells are eventually dense (1.2, 1.3) and that, let alone sporadic cases, the system never dies out (1.6). The other constituents of the system, such as the geometry of the graph, and the parameters of maturation, Φ, and of proliferation, Θ, seem to play a secondary role only. They do determine factors such as speed of cell manufacturing and lengths of periods, but not the basic behavior of the system.

The above conclusion is very significant. It states that even though our representation of the developing system is very simple, the properties that emerge are general, and hold for more complex descriptions. That is to say that the above principles will not be upset if BM's description as a conglomerate of cells, each subjected to cell-to-cell signaling in the micro-environment and to its internal mitotic clock, will be replaced by more elaborate rules. We would like to mention two examples of refining the model, still obtaining the same results.
1) The internal counter of the cells can be updated as a function of the number of stem cells in the micro-environment. This refinement may represent the stimulation of stem cell-cycle by cytokines secreted by other stem cells in the micro-environment .
2) An empty cell resets its counter to 0 if any of its empty neighbors became a stem cell. This condition, representing a strong negative feedback control of stem cells on their neighbors' cell cycle time, somehow limits a stem cell's proliferating potential. Note that this demand gives a more subtle control on level of cell-to-cell signaling.
One can easily see that the non-quantitative theorems are not affected by these two refinements.

Other options of bringing the model closer to reality are obviously possible. Nevertheless, the basic rules of this model are sufficient, so that one does not need to assume the existence of more elaborate mechanisms in order to explain the fundamental rules governing stem cell behaviour in general and BM homeostasis in particular. For example, unlike previous authors [9], we believe that stem cells' behavior in a functioning BM does not need to be stochastic (one may take the existence of a stochastic generator in each cell as an additional complexity).

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References

1. Agur, Z.: Fixed points of majority rule cellular automata with application to plasticity and precision of the immune system, Complex System **5**, 351–357 (1991)
2. Agur, Z., Fraenkel, A.S., Klein, S.T.: The number of fixed points of the majority rule, Discrete Math., **70**, 295–302 (1988)
3. Agur, Z., Kerszberg, M.: The emergence of phenotypic novelties through progressive genetic change, The American Naturalist., **129**(6), 862–875 (1987)
4. Barnes, D.W.H., Ford, C.E., Gray, S.M., Louit, J.F.: Spontaneous and induced changes in cell populations in heavily irradiated mice, Progr. Nuclear Energy Ser VI., **2**, 1–10 (1959)
5. De Haan, G., Dontje, B., Nijhof, W.: Concepts of hemopoietic cell amplification. Synergy, redundancy, and pleiotropy of cytokines affecting the regulation of erythropoiesis, Leukemia Lymphoma., **22**, 385–394 (1996)
6. Durrett, R., Steif, J.E.: Some rigorous results for the Greenberg-Hastings model, J. Theoret. Probab., **4**(4), 669–690 (1991)
7. Ginosar, Y., Holzman, R.: The majority action on infinite graphs: strings and puppets, Discrete Math., **215**, 59–71 (2000)
8. Goles, E., Martinez, S.: *Cellular Automata, Dynamical Systems and Neural Networks*, Kluwer, Dordrecht (1994)
9. Gordon, M.Y., Amos, T.A.S.: Stochastic Effects in Hemopoiesis, Stem Cells., **12**, 175–179 (1994)
10. Greenberg, J.M., Hastings, S.: Spatial patterns for discrete models of diffusion in excitable media, SIAM J. Appl. Math., **34**(3), 515–523 (1978)
11. Moran, G.: On the period-two-property of the majority operator in infinite graphs, Trans. Amer. Math. Soc., **347**(5), 1649–1667 (1995)
12. Veiby, O.P., Mikhail, A.A., Snodgrass, H.R.: Growth factors and hematopoietic stem cells, Hem. Oncol, Clinics North Amer., **11**, 1173–1184 (1997)
13. Wolfram, S.: *Theory and Applications of Cellular Automata*, World Scientific, Singapore (1986)