



Stem cell regulation: Implications when differentiated cells regulate symmetric stem cell division



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HIGHLIGHTS

- Differentiated cells (DCs) might regulate symmetric stem cell (SC) division.
- This implies that changes in the dynamics of DCs can affect the fitness of SCs.
- Tyrosine kinase inhibitors (TKIs) are used to treat chronic myeloid leukaemia (CML).
- TKIs increase the death rate of DCs, but have most likely no direct effect on SCs.
- TKIs might have an indirect effect on SCs if DCs regulate symmetric SC division.

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ABSTRACT

We use a mathematical model to show that if symmetric stem cell division is regulated by differentiated cells, then changes in the population dynamics of the differentiated cells can lead to changes in the population dynamics of the stem cells. More precisely, the relative fitness of the stem cells can be affected by modifying the death rate of the differentiated cells. This result is interesting because stem cells are less sensitive than differentiated cells to environmental factors, such as medical therapy. Our result implies that stem cells can be manipulated indirectly by medical treatments that target the differentiated cells.

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1. Introduction

Most tissues of the body go through continuous cell turnover due to apoptosis. This cell turnover can also give tissues the ability to self-repair after injury. In general, tissues are maintained by a small group of slowly replicating cells with the capacity to both self-renew and generate differentiated progeny required by a given tissue (Morrison et al., 1997; Reya et al., 2001). Cells that have these two capabilities are called *stem cells*. Differentiated cells perform their function and eventually die – they go through a number of divisions, obtaining various stages of differentiation, until the fully differentiated cells stop dividing (Donohue et al., 1958; Cronkite and Fliedner, 1964; Ogawa, 1993). Although it seems reasonable to propose that all tissues arise from tissue-specific stem cells, rigorous identification and isolation of these

stem cells have only been accomplished in a few instances. For example, *haematopoietic stem cells* have been isolated and shown to be responsible for the generation and regeneration of the blood-forming system and the immune system, called the *haematopoietic system* (Baum et al., 1992; Morrison and Weissman, 1994). The haematopoietic stem cells are located within the bone marrow and segregated among different bones throughout the body. Like several other models (Loeffler and Wichmann, 1980; Agur et al., 2002; Østby et al., 2003; Østby and Winther, 2004; Coiljn and Mackey, 2005; Adimy et al., 2006; Dingli and Michor, 2006; Dingli et al., 2007a,b; Wodarz, 2008; Marciniak-Czochra et al., 2009; Stiehl and Marciniak-Czochra, 2012; Lenaerts et al., 2010; Manesso et al., 2013), the model presented in this paper is inspired by the haematopoietic system. However, it applies to all other tissues that have similar architecture.

An important aspect, related to self-renewal and generation of differentiated cells, is the fate of the two daughter cells when a stem cell divides (Dingli et al., 2007b; Morrison and Kimble, 2006; Yamashita et al., 2003). *Symmetric division* is defined as generation

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of daughter cells destined to acquire the same fate. In this paper, symmetric stem cell division is defined as *symmetric self-renewal* if both daughter cells are stem cells and *symmetric differentiation* if both daughter cells are differentiated. In the former case the number of stem cells increases by one, whereas in the latter case the number of stem cells decreases by one. Stem cells can rely completely on symmetric division. On the other hand, if one daughter cell has stem cell identity and the other daughter cell starts to differentiate, it is called an *asymmetric stem cell division*. This type of division is particularly attractive because the stem cells manage to both self-renew and produce differentiated cells with a single division (Yamashita et al., 2003). However, a disadvantage of asymmetric stem cell division is that it leaves stem cells unable to expand in number. Serial haematopoietic transplantation supports the existence of all three types of divisions (McKenzie et al., 2006).

1.1. Stem cell niche

Since the number of stem cells is much smaller than the number of differentiated cells, the stem cells must be protected and tightly regulated. As discussed by Gentry and Jackson (2013), the *stem cell niche*, which is the restricted region in an organ that supports stem cell behaviour, may be crucial in both aspects (Fuchs et al., 2004; Nikolova et al., 2006; Yin and Li, 2006; Simons and Cleavers, 2011). The niche is composed of both localised signalling cells and an extracellular matrix that control stem cell fate. However, relatively little is known about the exact behaviour of most types of stem cells, and one of the reasons for this is that it is not possible to reconstruct niches scientifically, which makes it difficult to maintain stem cells *in vitro*, because signals from the niche affects stem cell survival, self-renewal, and differentiation.

Germline stem cells are unique stem cells in that they are solely dedicated to reproduction and transmission of genetic information from generation to generation. Through the use of genetic techniques in *Drosophila germline stem cells*, exciting progress has been made in understanding molecular mechanisms underlying interactions between stem cells and stem cell niches (Morrison and Kimble, 2006; Yamashita et al., 2003; Wong et al., 2005). The knowledge gained from studying *Drosophila* germline stem cells has provided an intellectual framework for defining the stem cell niche and molecular regulatory mechanisms for other adult stem cells, such as the haematopoietic stem cells.

The number of cells in a given tissue is approximately constant under normal conditions. It is generally believed that the number of stem cells is approximately constant under normal conditions, and that they differentiate and self-renew at relatively constant rates to replace mature cells and to keep the stem cell number at a certain normal level (Loeffler et al., 1988; Shortman and Naik, 2009). One strategy which stem cells can accomplish these two tasks is asymmetric stem cell division. A classical example of asymmetric division is provided by *Drosophila* germline stem cells. The outcome of a *Drosophila* germline stem cell division depends on the spindle orientation relative to the Hub cells in the stem cell niche, and results from the unequal distribution of intracellular regulators and extracellular (Hub-derived) signals between daughter cells during mitosis (Morrison and Kimble, 2006; Yamashita et al., 2003; Wong et al., 2005). The result is that when a *Drosophila* germline stem cell divides, one daughter remains in the stem cell niche and retains stem cell identity, and one daughter is left outside the stem cell niche and begins to differentiate. Research on *Drosophila* germline stem cells has provided a clear-cut example of how the stem cell niche promotes stem cell maintenance. Similarly, the haematopoietic microenvironment in the bone marrow also plays an important role in the regulation of haematopoietic

stem cell organisation (Lemischka, 1997; Bertolini et al., 1997; Aiuti et al., 1998; Thiemann et al., 1998). Self-renewal depends on local growth conditions, namely, on the direct contact between stem cells and stroma cells (Wineman et al., 1996; Verfaillie, 1998; Koller et al., 1999). However, there are no *in vivo* experiments that reveal exactly how proliferation of haematopoietic stem cells is regulated. Thus, it is not clear whether these cells divide asymmetrically or symmetrically under normal conditions. Serial haematopoietic transplantation indicates that both types of divisions occur under steady state (McKenzie et al., 2006). As discussed later in Section 1.3, theoretical work by Shahriyari and Komarova (2013) and McHale and Lander (2014) illustrate that the symmetric stem cell division can protect against cancer, and this indicates that stem cells divide symmetrically.

Although the number of haematopoietic stem cells remains nearly constant under normal conditions, they can expand rapidly in response to injury to the bone marrow, such as stem cell transplantation (McKenzie et al., 2006). This means that asymmetric stem cell division cannot be the complete story, because it leaves stem cells unable to expand in number. Since the number of stem cells increases with one after symmetric self-renewal, it is likely that the rate of such divisions depends on the number of stem cells, since the haematopoietic stem cells can regenerate after tissue damage. Indeed, *Drosophila* germline stem cells, which normally divide asymmetrically, can be induced to self-renew symmetrically to regenerate an additional stem cell after an experimental manipulation in which one stem cell is removed from the stem cell niche (Morrison and Kimble, 2006; Yamashita et al., 2003; Wong et al., 2005).

1.2. Extracellular regulation

Extracellular signalling molecules regulate the dynamics of cell proliferation and differentiation. However, the precise nature of these processes are in general not known (Layton et al., 1989; Aglietta et al., 1989; Metcalf, 2008; Fried, 2009). An example of extracellular signalling molecules is the *haematopoietic cytokines* that control the production of haematopoietic cells. Each of these cytokines has multiple actions mediated by receptors that can initiate various responses – survival, proliferation, differentiation, maturation, and functional activation. Individual haematopoietic cytokines can either regulate one specific lineage or multiple lineages (Metcalf, 2008). Moreover, for some haematopoietic cell types, such as stem cells or megakaryocyte progenitors, the simultaneous action of multiple cytokines is required for proliferative responses. Unlike other extracellular signalling molecules, like hormones, that have a limited, or single, organ source, the haematopoietic cytokines have many tissue sources, e.g. kidney, liver, lung, muscle and membrane-displayed factors on local stromal cells (Aglietta et al., 1989; Metcalf, 2008). This is one of the reasons why it is difficult to establish the precise source of a haematopoietic cytokine in any particular situation and to predict its ultimate fate. Results from theoretical work regarding the haematopoietic system (Wodarz, 2008) and crypt cells (Potten and Loeffler, 1990) indicate that changes in stem cell number and their cyclic activity are associated with changes in the demand of the mature cell stages. Marciniak-Czochra et al. (2009) designed a six-compartment model to test different hypotheses concerning regulation of self-renewal and differentiation by a feedback signalling factor. Since the precise nature of how extracellular signalling molecules such as cytokines control proliferation and differentiation is still unknown, Marciniak-Czochra et al. assume that the signal intensity is

$$s = \frac{1}{1 + kC_6} \quad (1)$$

where k is a constant and C_6 is the number of mature cells. Marciniak-Czochra et al. compare three different cases:

1. Only proliferation rates are regulated by feedback signals.
2. Only differentiation rates are regulated by feedback.
3. Both proliferation and differentiation rates are regulated by feedback.

They show that the best results are obtained when both proliferation and differentiation rates are regulated by feedback.

Lander et al. (2009) investigate how secreted negative feedback factors may be used to control the output of multistage cell lineages, as exemplified by the actions of GDF11 and activin in a self-renewing neural tissue, the mammalian olfactory epithelium. Similar to Marciniak-Czochra et al. (2009), Lander et al. find that two feedback loops are in general better than one. That is, when feedback loops are added, good control (robustness, stability, low progenitor load, and fast regeneration from a variety of conditions) is found over an increasing fraction of the parameter space. Lander et al. discuss different strategies for how stem cell self-renewal and generation of differentiated progeny can be regulated by negative feedback from differentiated cells. The first scenario is that asymmetric stem cell division is regulated by differentiated cells. In this case, the rate of asymmetric stem cell division increases when the number of differentiated cells is less than under normal conditions, which means that more differentiated cells are produced while the number of stem cells remains constant. On the other hand, it is also possible that symmetric stem cell division is regulated by differentiated cells. In this case, the rate of symmetric self-renewal increases when the number of differentiated cells is less than under normal conditions, which means that the number of stem cells increases and that more differentiated cells are produced than under normal conditions. Since research by Gokoffski et al. (2011) on mice indicates that stem cell populations expand when there are less differentiated cells than under normal conditions, Lander et al. consider the latter case in their model. Similarly, in the model presented in this paper, symmetric stem cell division is regulated by differentiated cells.

Manesso et al. (2013) propose a model where mild perturbations of differentiated cells do not influence the stem cell dynamics – steady state is re-established by increasing the self-renewal rate of the differentiated cells. After a critical threshold level is reached in terms of cell numbers, a second response is activated by increasing the commitment rates from the directly upstream cell types. The second response can influence the stem cell dynamics. The model was able to recapitulate the fundamental steady-state features of haematopoiesis and simulate the re-establishment of steady-state conditions after haemorrhage and bone marrow transplantation in adult mice. However, as discussed in Section 1.3, increasing the self-renewal rate of the differentiated cells can increase the risk of cancer. This might be one of the reasons why several other models, like the ones proposed by Loeffler and Wichmann (1980), Østby and Winther (2004), Wodarz (2008), Gentry and Jackson (2013) and Rodriguez-Brenes et al. (2013), assume that stem cell self-renewal and differentiation are regulated by a negative feedback from more mature cells. In particular, the models proposed by Gentry et al. and Wodarz include both extrinsic and intrinsic chemical signalling and interaction with the niche to control self-renewal, and this novel feature is also investigated in this paper. However, unlike our model, Wodarz' model assumes that when there are only healthy cells in the system, the rate of symmetric stem cell division depends only on the number of stem cells and the rate of asymmetric stem cell division depends only on the number of differentiated cells. Thus, according to Wodarz' model, changes in the population dynamics of the differentiated cells do not influence the dynamics of the stem cell population when there are only

healthy cells in the system. On the contrary, if symmetric stem cell division is regulated by the differentiated cells, then changes in population dynamics of the differentiated cells, such as increased death rate, can influence the dynamics of the stem cell population. In Section 2, we investigate the implications when the rate of symmetric self-renewal depends on both the number of stem cells and the number of differentiated cells.

1.3. Mutations and stem-cell-driven tumours

Genetic changes called *mutations* can occur in any cell that divides (Araten et al., 2005). Even though most mutations are harmless to the body, progressive accumulation of mutations can lead to cancer (Vogelstein and Kinzler, 2004). Indeed, results from theoretical work regarding stem cell self-renewal and differentiation indicate that the tissue architecture, where only a small number of stem cells have the ability to self-renew, has evolved to minimise the risk of malignant transformations (Dingli et al., 2007b; Wodarz and Komarova, 2005; Komarova and Cheng, 2006). That is, if a mutation occurs in a differentiated cell, it is likely to be washed out of the system before it becomes a cancer phenotype, because differentiated cells do not self-renew. On the other hand, mutation in a stem cell can generate a different type of stem cell, denoted *mutant stem cell*. This can lead to an evolutionary process with competition between the mutant stem cells and the normal stem cells (Nowak, 2006a; Dingli et al., 2010). A critical aspect is the fate of the daughter cells when the stem cells divide (Morrison and Kimble, 2006). The model proposed by Dingli et al. (2007b) shows that if the mutant stem cells divide only asymmetrically, their population size remains constant. A high probability of symmetric self-renewal increases the fitness of the stem cells, because this type of division increases the population size. Symmetric differentiation, on the other hand, decreases the population size. Thus, stem cells that differentiate symmetrically with a high probability have decreased fitness.

Shahriyari and Komarova (2013) and McHale and Lander (2014) illustrate that symmetrically dividing cells might delay double-hit mutant production compared to an equivalent system with asymmetrically dividing stem cells. More precisely, if stem cells only divide asymmetrically, then a mutation acquired in a stem cell will remain in the system indefinitely, and it is only a matter of time before the second mutation occurs. On the contrary, a mutant stem cell generated in a symmetric division has a less certain fate – half of the lineages will differentiate out after the very first division and only $1/K$ of all lineages will expand to size K . Thus, that the uncertainty of the fate of single mutant stem cells can be the reason for the statistically longer time it takes for the symmetrically dividing stem cell model to produce a double-hit mutant.

Rodriguez-Brenes et al. (2011) propose a model that illustrates that a key event in the development of cancer is the escape from feedback loops. In a genetically heterogeneous population, selection favours cells with advantageous traits (Wodarz and Komarova, 2005; Nowak, 2006b). Since cancer is a product of somatic evolution, it is important to investigate how mutants that originally appear in very small numbers are able to invade a cell population that is initially at dynamic equilibrium (Mangel and Bonsall, 2008). Rodriguez-Brenes et al. use computational models that are applied to experimental data, to study the evolutionary dynamics of feedback escape. Their model predicts different patterns of emerging tumour growth that fit previously published experimental data that describe tumour growth dynamics *in vitro* and *in vivo* (Rozenblum et al., 1997; Massagué, 2000, 2001; Derynck et al., 2001; Woodford-Richens et al., 2001; Wu et al., 2008). Of particular interest are non-standard growth patterns, both predicted by the model and found in published experimental

data, which indicates that feedback regulatory mechanisms are still partly at work in growing tumours (Rozenblum et al., 1997; Massagué, 2000, 2001; Derynck et al., 2001; Woodford-Richens et al., 2001; Wu et al., 2008). This gives rise to the notion that tumours not only retain some of the architectural aspects of the underlying healthy tissue, but also some of the regulatory mechanisms.

Stiehl and Marciniak-Czochra (2012) present a model of cancer cell dynamics where it is assumed that the leukemic cell population consists of an ordered sequence of cell statuses similar to the healthy haematopoietic cell lines. Moreover, it is assumed that leukemic stem cells are stimulated by the same cytokines as healthy stem cells. Similar to the models presented in this paper, a negative feedback function regulates self-renewal. However, unlike our model, the feedback function in the model proposed by Stiehl et al. only depends on the fully mature cells, namely,

$$s(t) = \frac{1}{1 + k^c c_m + k^l l_m},$$

where c_m and l_m are the number of fully mature healthy cells and fully mature leukemic cells, respectively, and k^c and k^l are constants. Moreover, the feedback that regulates healthy cells and cancer cells is the same – the difference between leukemic cells and healthy cells is captured by different constants associated with rates of self-renewal, differentiation and cell death. On the contrary, the model investigated in Section 2.1 assumes that the only difference between healthy cells and leukemic cells is the strength at which they regulate self-renewal.

2. Mathematical models

In Sections 2.1 and 2.2, we explore a simple model that only considers two types of cells, namely, stem cells and differentiated cells. This model provides analytic results and captures the basic idea of this paper, which is that changes in the population dynamics of the differentiated cells can lead to changes in the population dynamics of the stem cells when symmetric stem cell division is regulated by differentiated cells. An extension of the model, which includes various stages of differentiation, is presented in Section 2.3. The extended model is explored numerically, since it is too complex to analyse analytically. The numerical analysis shows that the analytic results obtained from the simple model also apply to the extended model.

2.1. Model with two layers of differentiation

The basic model considers two layers of the differentiation hierarchy: Stem cells have the potential for indefinite self-renewal and to give rise to differentiated cells. The differentiated cells are the cells without stem cell characteristics. Let x_s denote the number of stem cells and x_d the number of differentiated cells. As discussed in the introduction, signalling molecules such as cytokines and interaction with the stem cell niche control stem cell behaviour, but the precise nature of this regulation is still unknown (Fuchs et al., 2004; Nikolova et al., 2006; Yin and Li, 2006; Simons and Cleavers, 2011; Layton et al., 1989; Aglietta et al., 1989; Metcalf, 2008; Fried, 2009). We assume that the signalling intensity is approximately

$$\Psi = \exp(-\theta x_s - \gamma x_d), \quad (2)$$

where θ and γ are positive constants. This function captures the fact that the secretion of cytokines is very fast in comparison to cell proliferation and differentiation (Metcalf, 2008). Moreover, the signal intensity reaches its maximum under complete absence of cells, and it decreases exponentially towards zero as the number of

cells increases. In the simple model presented in this subsection, only symmetric self-renewal is regulated by the feedback signals. It is assumed that the stem cells produce immature differentiated cells by asymmetric division and symmetric differentiation at constant rates, g and d_0 , respectively, and die at constant rate, d_1 . The differentiated cells go through a number of divisions, obtaining various stages of differentiation, until the fully mature cells stop dividing. This differentiation process is investigated in more details in Section 2.3. Here we simply assume that the process occurs at constant rate, f , which means that differentiated cells are generated at rate $P = (2d_0 + g)f$. The differentiated cells die at constant rate Q . Hence, the model is given by the following set of ordinary differential equations:

$$\frac{dx_s}{dt} = (r\Psi - d)x_s, \quad (3)$$

$$\frac{dx_d}{dt} = Px_s - Qx_d, \quad (4)$$

where $d = d_0 + d_1$ and r is a positive constant. The system has two equilibrium solutions, namely,

$$(x_s^{0*}, x_d^{0*}) = (0, 0), \quad (5)$$

$$(x_s^*, x_d^*) = \left(\frac{1}{\theta + \frac{P}{Q}\gamma} \ln\left(\frac{r}{d}\right), \frac{P}{Q} \frac{1}{\theta + \frac{P}{Q}\gamma} \ln\left(\frac{r}{d}\right) \right). \quad (6)$$

We only consider the case when $r > d$, which means that (x_s^*, x_d^*) is stable, whereas (x_s^{0*}, x_d^{0*}) is unstable (Appendix B). The former equilibrium solution describes the system under normal conditions. Note that the number of differentiated cells is much larger than the number of stem cells, and that the death rate of the differentiated cells, Q , is much higher than the rate at which the stem cells die and differentiate, d . The pseudo-steady state hypothesis is that the population dynamics of the differentiated cells occurs at a very high rate compared with the stem cell population dynamics. Hence, it is assumed that the differentiated cells are always in equilibrium. Mathematically, we use the approximation $\frac{dx_d}{dt} = \frac{P}{Q}x_s - x_d \approx 0$ to obtain $x_d \approx \frac{P}{Q}x_s$. Thus, the population dynamics of the stem cells is approximately described by the following differential equation:

$$\frac{dx_s}{dt} = \left(\text{rexp} \left(- \left(\theta + \gamma \frac{P}{Q} \right) x_s \right) - d \right) x_s. \quad (7)$$

Starting with any population size (x_s^0, x_d^0) , where $x_s^0 > 0$, the system given in Eqs. (3) and (4), converges towards (x_s^*, x_d^*) (Appendix B). Fig. 1 shows an example where the whole system is regenerated, starting with a single stem cell. For comparison, the figure also shows the regeneration in the absence of feedback from differentiated cells (dashed line). From Fig. 1, we can see that feedback from differentiated cells enables the system to regenerate faster.

Changes in the population dynamics of the differentiated cells lead to changes in the rate of symmetric stem cell division, since the function Ψ is dependent on the variable x_d . The factors that influence the population dynamics of the differentiated cells are included in the model by modifying the death rate to $\hat{Q} \neq Q$. If $\hat{Q} > Q$, then the number of differentiated cells starts decreasing, whereas if $\hat{Q} < Q$, then the number of differentiated cells starts increasing. This triggers changes in the function Ψ as follows: Ψ increases if the number of differentiated cells decreases, and Ψ decreases if the number of differentiated cells increases. The number of stem cells converges towards the following steady

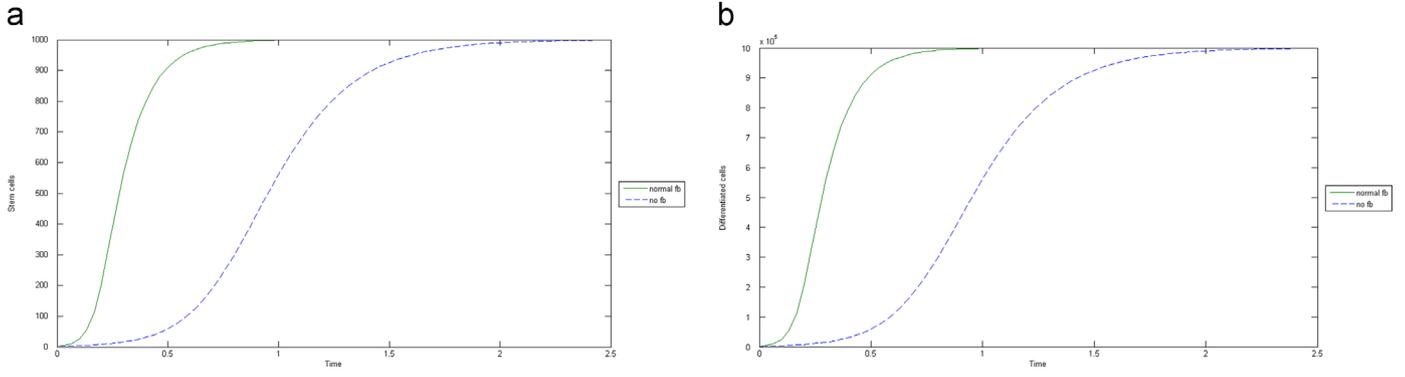


Fig. 1. Regeneration. The whole system is regenerated, starting with a single stem cell. The green line is an example where stem cell self-renewal is regulated by both stem cells and differentiated cells. The blue, dashed line is the regeneration with the same feedback from the stem cells, but no feedback from the differentiated cells. Both examples have the following parameter sizes: $\theta = 10^{-3}$, $d = 0.1353$, $P = 10^6$, $Q = 10^3$. In addition, the example with normal feedback has $\gamma = 10^{-6}$, $r = 1$, whereas the example without feedback from differentiated cells has $\gamma = 0$ and $r = \exp(-10^{-6} \times \gamma_d^*) = \exp(-1)$. (a) and (b) display the stem cells and the differentiated cells, respectively.

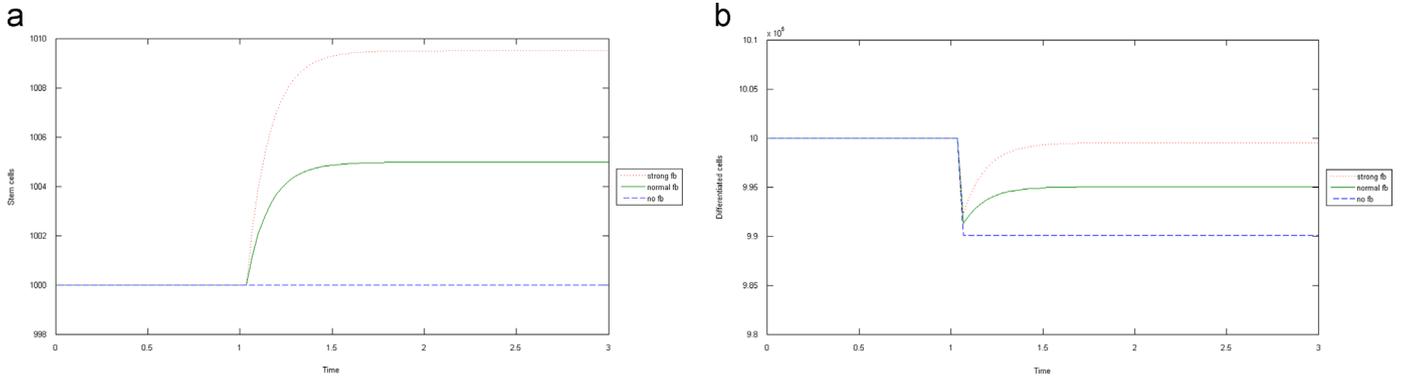


Fig. 2. Increased death rate of the differentiated cells. If the death rate of the differentiated cells increases, then the number of differentiated cells starts decreasing, leading to an increased self-renewal rate, resulting in an increased number of stem cells, and ultimately the number of differentiated cells increases. The red, dotted line shows an example where the feedback from the differentiated cells is much stronger than the feedback from the stem cells. In this case, the number of differentiated cells remains approximately the same as under normal conditions when the death rate of the differentiated cells changes. The blue, dashed line is an example where stem cell self-renewal is not regulated by feedback from differentiated cells, and the number of stem cells remains constant when the death rate of the differentiated cells changes. Consequently, the number of differentiated cells decreases sufficiently. The green line shows an example where the feedback from the stem cells and the differentiated cells have approximately the same strength. All examples have the following parameter sizes: $r = 1$, $d = 0.1353$, $P = 10^6$ and $Q = 10^3$. In addition, the example with strong feedback from the differentiated cells has $\theta = 10^{-4}$ and $\gamma = 1.9 \times 10^{-6}$, whereas the example where the feedback from stem cells and differentiated cells are the same has $\theta = 10^{-3}$ and $\gamma = 10^{-6}$, and finally, the example with no feedback from the differentiated cells has $\theta = 2 \times 10^{-3}$ and $\gamma = 0$. (a) and (b) display the stem cells and the differentiated cells, respectively.

state:

$$x_s^{W*} = \frac{1}{\theta + \frac{P}{Q}\gamma} \ln\left(\frac{r}{d}\right). \tag{8}$$

Note that if $\hat{Q} > Q$, then the number of stem cells increases, whereas if $\hat{Q} < Q$, then the number of stem cells decreases. Thus, for the former case the number of differentiated cells ultimately increases, and for the latter case the number of differentiated cells decreases to the steady state

$$x_d^{W*} = \frac{P}{Q} x_s^{W*}.$$

Fig. 2 illustrates the cell dynamics when the death rate of the differentiated cells is increased. Note that it follows from Eq. (7), that if the pseudo-steady state hypothesis holds, then two different examples of the system given in Eqs. (3) and (4), with $(\theta_0, \gamma_0) \neq (\theta_1, \gamma_1)$, where

$$\theta_0 + \frac{P}{Q}\gamma_0 = \theta_1 + \frac{P}{Q}\gamma_1, \tag{9}$$

and all other parameters are the same, behave approximately identically. Indeed, this is the case in Fig. 2, which shows three different examples of the system given in Eqs. (3) and (4). Because the parameters satisfy the relations described in Eq. (9) when time is less than one, they behave approximately identically in this time interval. When time equals one, the death rate of the differentiated cells changes from Q to \hat{Q} , and the parameters do not satisfy the relations described in Eq. (9) anymore. The blue, dashed line is an example where stem cells are not regulated by feedback from differentiated cells. Hence, when the death rate of the differentiated cells changes to \hat{Q} , the number of stem cell remains constant, x_s^* given in Eq. (6), and the number of differentiated cells decreases to $\frac{P}{Q}x_s^*$. The green line shows an example where stem cells are regulated by feedback from differentiated cells, and $\frac{P}{Q}\gamma_{no}$ has the same order as θ_{no} . When the death rate of the differentiated cells changes to \hat{Q} , the number of stem cells increases to x_d^{W*} , given in Eq. (8), and the number of differentiated cells converges towards $\frac{P}{Q}x_d^{W*}$. The red, dotted line is an example where the stem cells are regulated by strong feedback from the differentiated cells. That is, $\frac{P}{Q}\gamma_{st}$ has a much higher order than θ_{st} . The number of stem cells increases to x_d^{W*} , given in Eq.

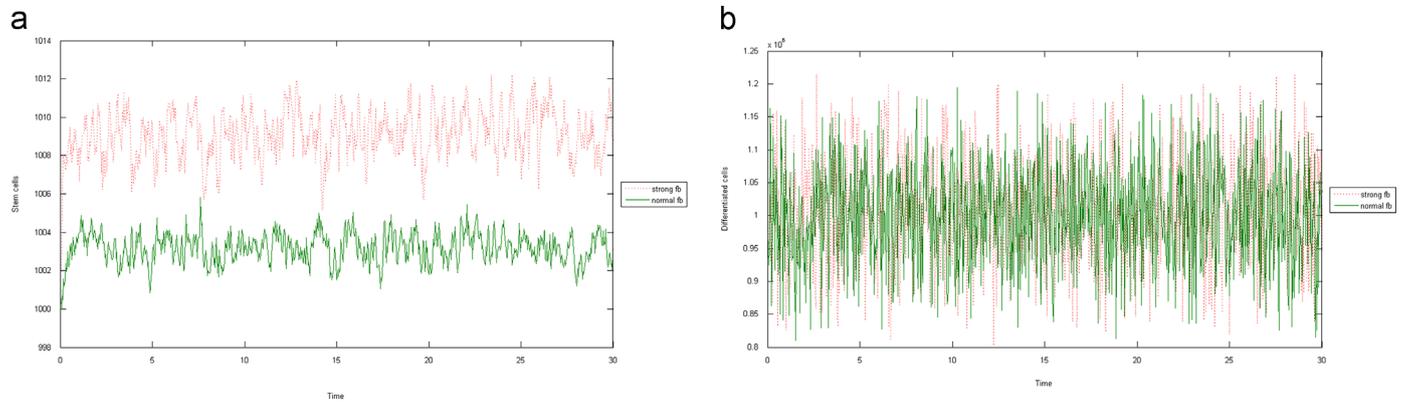


Fig. 3. Parameter sensitivity. In the examples displayed in this figure, all six parameters of the system are continuously varying with up to 20 per cent to test the robustness of the system. More precisely, every time interval $[T, T+1]$ is subdivided into 30 000 time steps. At each time step, every parameter is given a new random value within the interval $[P \times 0.9, P \times 1.1]$, where P is the mean value of the parameter. The green line shows an example where the feedback from the stem cells and the differentiated cells are of the same strength on average. The red, dotted line shows an example where the feedback from the differentiated cells is on average stronger than the feedback from the stem cells. Even though both examples are robust, the former example is less parameter sensitive than the latter. Both examples have the following average parameter sizes: $r = 1$, $d = 0.1353$, $P = 10^6$ and $Q = 10^3$. In addition, the example with strong feedback from the differentiated cells has average parameter sizes $\theta = 10^{-4}$ and $\gamma = 1.9 \times 10^{-6}$, and the example where the feedback from stem cells and differentiated cells are the same has average parameter sizes $\theta = 10^{-3}$ and $\gamma = 10^{-6}$.

(a) and (b) display the stem cells and the differentiated cells, respectively.

(8), and the number of differentiated cells converges towards $\frac{P}{Q}x_d^{W_{st}0*}$ when the death rate of the differentiated cells changes to \bar{Q} . Note that

$$\frac{P}{\bar{Q}}x_s^* < \frac{P}{\bar{Q}}x_d^{W_{no}*} < \frac{P}{\bar{Q}}x_d^{W_{st}*} \approx x_d^*,$$

where x_d^* is as given in Eq. (6). Thus, Fig. 2 illustrates that the worst outcome is obtained in the absence of feedback from differentiated cells. Moreover, it also shows that if the feedback from the differentiated cells is strong, i.e. $\frac{P}{\bar{Q}}\gamma \approx \theta$, then the number of differentiated cells remains approximately the same as under normal conditions when the death rate of the differentiated cells changes. However, the system is less parameter sensitive when $\frac{P}{\bar{Q}}\gamma \approx \theta$ than when $\frac{P}{\bar{Q}}\gamma \gg \theta$, and as discussed by Lander et al. (2009), robustness, which is the ability to maintain performance when perturbations and uncertainties occur, is a key property of living systems (Stelling et al., 2004). How the system responds to perturbations and uncertainties when $\frac{P}{\bar{Q}}\gamma \approx \theta$ and $\frac{P}{\bar{Q}}\gamma \gg \theta$ is illustrated in Fig. 3. The green graph is an example where the former relation holds, and the red, dotted graph is an example where the latter relation holds. Even though both examples are robust, Fig. 3 shows that the variance and the mean number of stem cells increase more when $\frac{P}{\bar{Q}}\gamma \gg \theta$ than when $\frac{P}{\bar{Q}}\gamma \approx \theta$. This is the reason why we choose parameters that satisfy the latter relation in all examples in this paper, and denote it *normal feedback*. However, as illustrated in Fig. 2, a system with strong feedback from the differentiated cells performs much better than a system with normal feedback when the death rate of the differentiated cells decreases. One way to compensate for this is to assume that the differentiated cells also have the ability to self-renew. This is investigated by Manesso et al. (2013). However, as discussed in the introduction, increasing capacity of self-renewal among differentiated cells can lead to increasing probability of cancer (Dingli et al., 2007b; Wodarz and Komarova, 2005; Komarova and Cheng, 2006). In Section 2.3, where various stages of differentiation are included, we investigate another strategy to increase the production of differentiated cells.

2.2. Competition dynamics

When the stem cells divide, a mutation might occur (Araten et al., 2005; Vogelstein and Kinzler, 2004). The stem cells that

harbour a mutation are denoted *mutant stem cells*, whereas the other stem cells are denoted *wild-type stem cells*. When a mutant stem cell divides, both daughter cells also harbour the mutation. The differentiated cells that harbour the mutation are denoted *mutant differentiated cells*, and the other differentiated cells are denoted *wild-type differentiated cells*. Like Rodriguez-Brenes et al. (2011), we want to investigate the case when the mutant cells not only retain the architectural aspects of the wild-type cells, but also the regulatory mechanisms. Similar to Stiehl and Marciniak-Czochra (2012), we assume that the mutant stem cells are stimulated by the same cytokines as the wild-type stem cells, but the two cell types respond to these cytokines with different strength. More precisely, it is assumed that the only difference between the mutant cells and the wild-type cells is that the functions that regulate symmetric self-renewal of the wild-type stem cells and the mutant stem cells, denoted Ψ_x and Ψ_y , respectively, are different. Moreover, we neglect continuous production of mutant stem cells from wild-type stem cells. Let y_s denote the number of mutant stem cells and y_d denote the number of mutant differentiated cells. The basic model is given by the following set of ordinary differential equations:

$$\frac{dx_s}{dt} = (r\Psi_x - d)x_s, \quad (10)$$

$$\frac{dx_d}{dt} = Px_s - Qx_d, \quad (11)$$

$$\frac{dy_s}{dt} = (r\Psi_y - d)y_s, \quad (12)$$

$$\frac{dy_d}{dt} = Py_s - Qy_d, \quad (13)$$

where

$$\Psi_z = \exp\left(-\theta_x^z x_s - \theta_y^z y_s - \gamma_x^z x_d - \gamma_y^z y_d\right),$$

and $\theta_v^z, \gamma_v^z > 0$ for $z, v \in \{x, y\}$. Moreover, it is assumed that

$$\theta_z^x \neq \theta_z^y \quad \text{and} \quad \gamma_z^x \neq \gamma_z^y.$$

This means that wild-type cells can either inhibit growth of mutant stem cells *more* than they inhibit growth of wild type stem cells, or they inhibit growth of mutant stem cells *less* than they inhibit growth of wild type stem cells. Clearly, the fitness of

the wild-type cells is higher in former case than in the latter case. Similarly, the mutant cells have higher fitness if they inhibit growth of the wild-type stem cells more than they inhibit growth of the mutant stem cells. Thus, the terms \mathcal{P}_x and \mathcal{P}_y introduce competition between mutant stem cells and wild-type stem cells.

The system given in Eqs. (10)–(13) has three equilibrium solutions where at least one of the populations gets extinct, namely,

$$(x_s^{0*}, x_d^{0*}, y_s^{0*}, y_d^{0*}) = (0, 0, 0, 0),$$

$$(x_s^{1*}, x_d^{1*}, y_s^{1*}, y_d^{1*}) = \left(\frac{1}{\theta_x^x + \frac{P}{Q}\gamma_x^x} \ln\left(\frac{r}{d}\right), \frac{P}{Q}x_s^{1*}, 0, 0 \right),$$

$$(x_s^{2*}, x_d^{2*}, y_s^{2*}, y_d^{2*}) = \left(0, 0, \frac{1}{\theta_y^y + \frac{P}{Q}\gamma_y^y} \ln\left(\frac{r}{d}\right), \frac{P}{Q}y_s^{2*} \right),$$

and one equilibrium solution with coexistence, $(x_s^{3*}, x_d^{3*}, y_s^{3*}, y_d^{3*})$, where

$$\begin{bmatrix} x_s^{3*} \\ y_s^{3*} \end{bmatrix} = \begin{bmatrix} \theta_x^x + \frac{P}{Q}\gamma_x^x & \theta_y^y + \frac{P}{Q}\gamma_y^y \\ \theta_y^y + \frac{P}{Q}\gamma_y^y & \theta_x^x + \frac{P}{Q}\gamma_x^x \end{bmatrix}^{-1} \begin{bmatrix} \ln\left(\frac{r}{d}\right) \\ \ln\left(\frac{r}{d}\right) \end{bmatrix},$$

$$x_d^{3*} = \frac{P}{Q}x_s^{3*}, \quad y_d^{3*} = \frac{P}{Q}y_s^{3*}. \tag{14}$$

It is assumed that the matrix is non-degenerate. As discussed in Section 2.1, the number of differentiated cells is much larger than the number of stem cells, and we expect the pseudo-steady state hypothesis

$$x_d \simeq \frac{P}{Q}x_s, \quad y_d \simeq \frac{P}{Q}y_s,$$

to hold when the system approaches the given equilibrium solution. Moreover, it is assumed that $r > d$. This means that the equilibrium solution where all types of cells get extinct is unstable. The stability of the remaining equilibrium solutions depends on the following four parameter regimes (Appendix C):

- (I) $\theta_y^y + \frac{P}{Q}\gamma_y^y > \theta_x^x + \frac{P}{Q}\gamma_x^x$ and $\theta_x^x + \frac{P}{Q}\gamma_x^x > \theta_y^y + \frac{P}{Q}\gamma_y^y$. For these parameter relations both the wild-type cells and the mutant cells inhibit growth of mutant stem cells more than growth of wild-type stem cells. The only stable equilibrium solution is extinction of the mutant cells and survival of the wild-type cells, $(x_s^{1*}, x_d^{1*}, y_s^{1*}, y_d^{1*})$. Moreover, starting with any population size $(x_s^0, x_d^0, y_s^0, y_d^0)$, where $x_s^0, y_s^0 > 0$, the system converges towards $(x_s^{1*}, x_d^{1*}, y_s^{1*}, y_d^{1*})$.
- (II) $\theta_y^y + \frac{P}{Q}\gamma_y^y < \theta_x^x + \frac{P}{Q}\gamma_x^x$ and $\theta_x^x + \frac{P}{Q}\gamma_x^x < \theta_y^y + \frac{P}{Q}\gamma_y^y$. For these parameter relations both the wild-type cells and the mutant cells inhibit growth of wild-type stem cells more than growth of mutant stem cells. The only stable equilibrium solution is extinction of the wild-type cells and survival of the mutant cells, $(x_s^{2*}, x_d^{2*}, y_s^{2*}, y_d^{2*})$. Furthermore, starting with any population size $(x_s^0, x_d^0, y_s^0, y_d^0)$, where $x_s^0, y_s^0 > 0$, the system converges towards $(x_s^{2*}, x_d^{2*}, y_s^{2*}, y_d^{2*})$.
- (III) $\theta_y^y + \frac{P}{Q}\gamma_y^y > \theta_x^x + \frac{P}{Q}\gamma_x^x$ and $\theta_x^x + \frac{P}{Q}\gamma_x^x < \theta_y^y + \frac{P}{Q}\gamma_y^y$. For these parameter relations the wild-type cells inhibit reproduction of wild-type stem cells more than reproduction of mutant stem cells, and likewise, the mutant cells inhibit reproduction of mutant stem cells more than reproduction of wild-type stem cells. In this case the only stable equilibrium solution is coexistence, $(x_s^{3*}, x_d^{3*}, y_s^{3*}, y_d^{3*})$. Starting with any population size $(x_s^0, x_d^0, y_s^0, y_d^0)$, where $x_s^0, y_s^0 > 0$, the system converges towards $(x_s^{3*}, x_d^{3*}, y_s^{3*}, y_d^{3*})$.
- (IV) $\theta_y^y + \frac{P}{Q}\gamma_y^y < \theta_x^x + \frac{P}{Q}\gamma_x^x$ and $\theta_x^x + \frac{P}{Q}\gamma_x^x > \theta_y^y + \frac{P}{Q}\gamma_y^y$. When the mutant cells inhibit reproduction of wild-type stem cells more than

reproduction of mutant stem cells, and likewise, the wild-type cells inhibit reproduction of mutant cells more than reproduction of wild-type stem cells more than both the equilibrium solutions where only one type of cells survives, $(x_s^{1*}, x_d^{1*}, y_s^{1*}, y_d^{1*})$ and $(x_s^{2*}, x_d^{2*}, y_s^{2*}, y_d^{2*})$, are stable. Starting with any population size $(x_s^0, x_d^0, y_s^0, y_d^0)$ where $y_s^0, x_s^0 > 0$, then if $y_s^0 < x_s^0 Y$, the system converges towards $(x_s^{1*}, x_d^{1*}, y_s^{1*}, y_d^{1*})$, whereas if $y_s^0 > x_s^0 Y$, the system converges towards $(x_s^{2*}, x_d^{2*}, y_s^{2*}, y_d^{2*})$, and if $y_s^0 = x_s^0 Y$, the system converges towards the equilibrium solution $(x_s^{3*}, x_d^{3*}, y_s^{3*}, y_d^{3*})$, where

$$Y = \frac{\theta_x^y + \frac{P}{Q}\gamma_x^y - \left(\theta_x^x + \frac{P}{Q}\gamma_x^x\right)}{\theta_y^x + \frac{P}{Q}\gamma_y^x - \left(\theta_y^y + \frac{P}{Q}\gamma_y^y\right)}.$$

2.2.1. Changes in the competition dynamics

In this subsection we show how changes in the population dynamics of the differentiated cells can lead to changes in the population dynamics of the stem cells. We include changes in the population dynamics of the differentiated cells by modifying the death rate of these cells. Clearly, changes in this death rate can effect the dynamics of the whole system, since the stability of all the equilibrium solutions depends on the inequality

$$\theta_z^v + \frac{P}{Q}\gamma_z^v < \theta_z^w + \frac{P}{Q}\gamma_z^w,$$

for $z, v, w \in \{x, y\}$, $v \neq w$. The stability of the system is changed when the death rate is modified from Q to \hat{Q} , such that the inequality is changed to

$$\theta_k^i + \frac{P}{Q}\gamma_k^i > \theta_z^j + \frac{P}{Q}\gamma_k^j,$$

for at least one triple $i, j, k \in \{z, v, w\}$. There are three different cases:

- (I) $\theta_z^v < \theta_z^w$ and $\gamma_z^v < \gamma_z^w$. This inequality cannot be changed for any $\hat{Q} > 0$.
- (II) $\theta_z^v < \theta_z^w$ and $\gamma_z^v > \gamma_z^w$. This inequality is changed for any $\hat{Q} < \frac{\gamma_z^v - \gamma_z^w}{\theta_z^w - \theta_z^v} P$.
- (III) $\theta_z^v > \theta_z^w$ and $\gamma_z^v < \gamma_z^w$. This inequality is changed for any $\hat{Q} > \frac{\gamma_z^w - \gamma_z^v}{\theta_z^v - \theta_z^w} P$.

These mathematical results can be summarised as follows:

- The equilibrium solution where the mutant cells survive and the wild-type cells get extinct is stable when the mutant cells inhibit growth of wild-type stem cells more than growth of mutant stem cells. If the death rate of the differentiated cells is changed such that the mutant cells inhibit the mutant stem cells more than the wild-type stem cells, then this equilibrium solution becomes unstable.
- The equilibrium solution where the wild-type cells survive and the mutant cells get extinct is stable when the wild-type cells inhibit growth of mutant stem cells more than growth of wild-type stem cells. If the death rate of the differentiated cells is changed such that the wild-type cells inhibit the wild-type stem cells more than the mutant stem cells, then this equilibrium solution becomes unstable.
- The equilibrium solution with coexistence is stable when the mutant cells inhibit growth of mutant stem cells, and likewise, the wild-type cells inhibit growth of wild-type stem cells more than growth of mutant stem cells. This equilibrium solution becomes

unstable if either the death rate of the differentiated cells is changed such that the mutant cells inhibit growth of wild-type stem cells more than growth of mutant stem cells and/or if the death rate of the differentiated cells is changed such that the wild-type cells inhibit growth of mutant stem cells more than growth of wild-type stem cells.

2.2.2. Numerical simulations

We have performed numerical simulations for different parameter regimes to illustrate how changes in the population dynamics of the differentiated cells can affect the competition dynamics of the stem cells. The goal of this paper is to point out that the relative fitness of stem cells can be affected by changes in the population of differentiated cells. Thus, the parameters are not scaled with respect to a specific tissue. Moreover, since the feedback mechanism within the stem cell area cannot be measured directly, it is not possible to give a precise estimate for all parameters. Just like the examples in Wodarz' (2008) paper, the time is given in an arbitrary unit.

Note that if

$$\theta_x^x = \theta_y^x, \gamma_x^x = \gamma_y^x \quad \text{and} \quad \theta_y^y = \theta_x^y, \gamma_y^y = \gamma_x^y,$$

then exactly one of the equilibrium solutions, where one type of cell gets extinct, is stable. An example of this is shown in Fig. 4. Initially we have that

$$\theta_z^y + \frac{P}{Q}\gamma_z^y < \theta_z^x + \frac{P}{Q}\gamma_z^x,$$

for $z \in \{x, y\}$. Thus, starting with only one mutant stem cell, the system converges towards the equilibrium solution where the mutant cells invade and the wild-type cells get extinct:

$$(x_s^{2*}, x_d^{2*}, y_s^{2*}, y_d^{2*}) = \left(0, 0, \frac{1}{\theta_y^y + \frac{P}{Q}\gamma_y^y} \ln\left(\frac{r}{d}\right), \frac{P}{Q}y_0^{2*} \right).$$

At time 350 the death rate of the differentiated cells is increased to \hat{Q} , such that the inequalities

$$\theta_z^y + \frac{P}{\hat{Q}}\gamma_z^y > \theta_z^x + \frac{P}{\hat{Q}}\gamma_z^x,$$

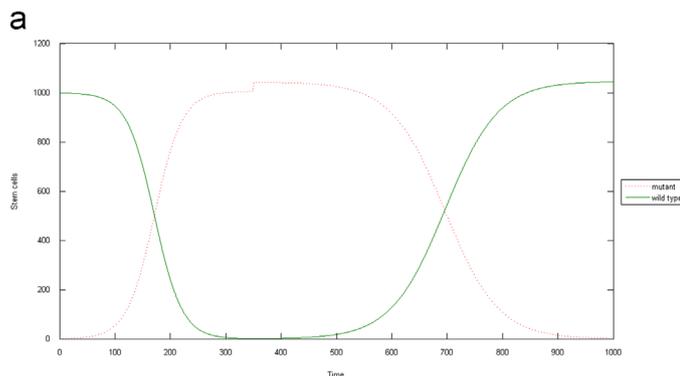


Fig. 4. One stable equilibrium solution. Initially, both the wild-type cells and the mutant cells inhibit growth of wild-type stem cells more than growth of mutant stem cells. Thus, if one mutant stem cell is generated at time zero, the system converges towards the only stable equilibrium solution, which is extinction of the wild-type cells and survival of the mutant cells. At time 350 the death rate of the differentiated cells is modified such that both the wild-type cells and the mutant cells inhibit growth of mutant stem cells more than growth of wild-type stem cells. Hence, extinction of the mutant cells and survival of the wild-type cells become the only stable equilibrium solution, and the system converges towards this solution.

The parameter sizes are: $\theta_x^x = \theta_y^x = 0.0012$, $\gamma_x^x = \gamma_y^x = 1.15 \times 10^{-6}$, $\theta_x^y = \theta_y^y = 1.18 \times \theta_x^x$, $\gamma_x^y = \gamma_y^y = 0.8 \times \gamma_x^x$, $r = 1$, $d = \exp\left(-\left(\theta_x^x + \frac{P}{Q}\gamma_x^x\right)10^{10}\right)$, $P = 10^6$ and $Q = 10^3$.

(a) and (b) display the stem cells and the differentiated cells, respectively.

hold, and the system converges towards the equilibrium solution where the mutant cells get extinct and the wild-type cells survive:

$$(x_s^{1*}, x_d^{1*}, y_s^{1*}, y_d^{1*}) = \left(\frac{1}{\theta_x^x + \frac{P}{Q}\gamma_x^x} \ln\left(\frac{r}{d}\right), \frac{P}{Q}x_0^{1*}, 0, 0 \right).$$

Fig. 5 shows an example where initially the inequalities

$$\theta_z^y + \frac{P}{Q}\gamma_z^y < \theta_z^x + \frac{P}{Q}\gamma_z^x$$

hold for $z \in \{x, y\}$. Thus, only $(x_s^{2*}, x_d^{2*}, y_s^{2*}, y_d^{2*})$ is stable, and the system converges towards this equilibrium solution. By changing the death rate of the differentiated cells to \hat{Q} , we obtain that

$$\theta_y^y + \frac{P}{\hat{Q}}\gamma_y^y < \theta_y^x + \frac{P}{\hat{Q}}\gamma_y^x \quad \text{and} \quad \theta_x^x + \frac{P}{\hat{Q}}\gamma_x^x > \theta_x^y + \frac{P}{\hat{Q}}\gamma_x^y.$$

This means that both $(x_s^{2*}, x_d^{2*}, y_s^{2*}, y_d^{2*})$ and $(x_s^{1*}, x_d^{1*}, y_s^{1*}, y_d^{1*})$ become stable. Thus, which of the equilibrium solutions the system converges towards, depends on the time that the death rate is modified.

2.3. Multi-compartment model

In this subsection, we present an extension of the simple model proposed in Section 2.1, which includes various stages of the differentiation process. As discussed in the introduction, the differentiated cells are produced by the stem cells through asymmetric division and symmetric differentiation, and they go through a number of divisions, obtaining various stages of differentiation, until the fully mature cells stop dividing (Donohue et al., 1958; Cronkite and Fliedner, 1964; Ogawa, 1993). However, as discussed by Dingli et al. (2007a), there is no unambiguous determination of the number of stages connecting stem cells and fully differentiated cells, let alone how fast cells go through different stages of maturation (Donohue et al., 1958; Cronkite and Fliedner, 1964). Similar to Dingli et al., we model differentiation as a multi-step process where cell replication and differentiation are coupled with cells moving through successive stages – compartments – of maturation in a series of steps from the stem cells all the way down to the fully differentiated cells. More precisely, when differentiated cells are produced by stem cells through asymmetric division and symmetric differentiation, they move to compartment 1. Furthermore, it is assumed that when a cell in compartment i

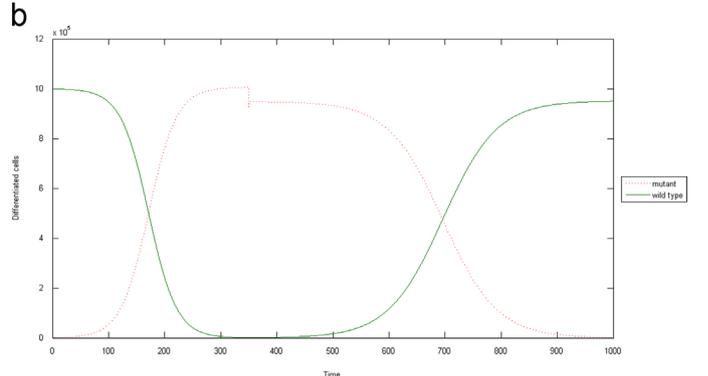


Fig. 4. One stable equilibrium solution. Initially, both the wild-type cells and the mutant cells inhibit growth of wild-type stem cells more than growth of mutant stem cells. Thus, if one mutant stem cell is generated at time zero, the system converges towards the only stable equilibrium solution, which is extinction of the wild-type cells and survival of the mutant cells. At time 350 the death rate of the differentiated cells is modified such that both the wild-type cells and the mutant cells inhibit growth of mutant stem cells more than growth of wild-type stem cells. Hence, extinction of the mutant cells and survival of the wild-type cells become the only stable equilibrium solution, and the system converges towards this solution.

The parameter sizes are: $\theta_x^x = \theta_y^x = 0.0012$, $\gamma_x^x = \gamma_y^x = 1.15 \times 10^{-6}$, $\theta_x^y = \theta_y^y = 1.18 \times \theta_x^x$, $\gamma_x^y = \gamma_y^y = 0.8 \times \gamma_x^x$, $r = 1$, $d = \exp\left(-\left(\theta_x^x + \frac{P}{Q}\gamma_x^x\right)10^{10}\right)$, $P = 10^6$ and $Q = 10^3$.

(a) and (b) display the stem cells and the differentiated cells, respectively.

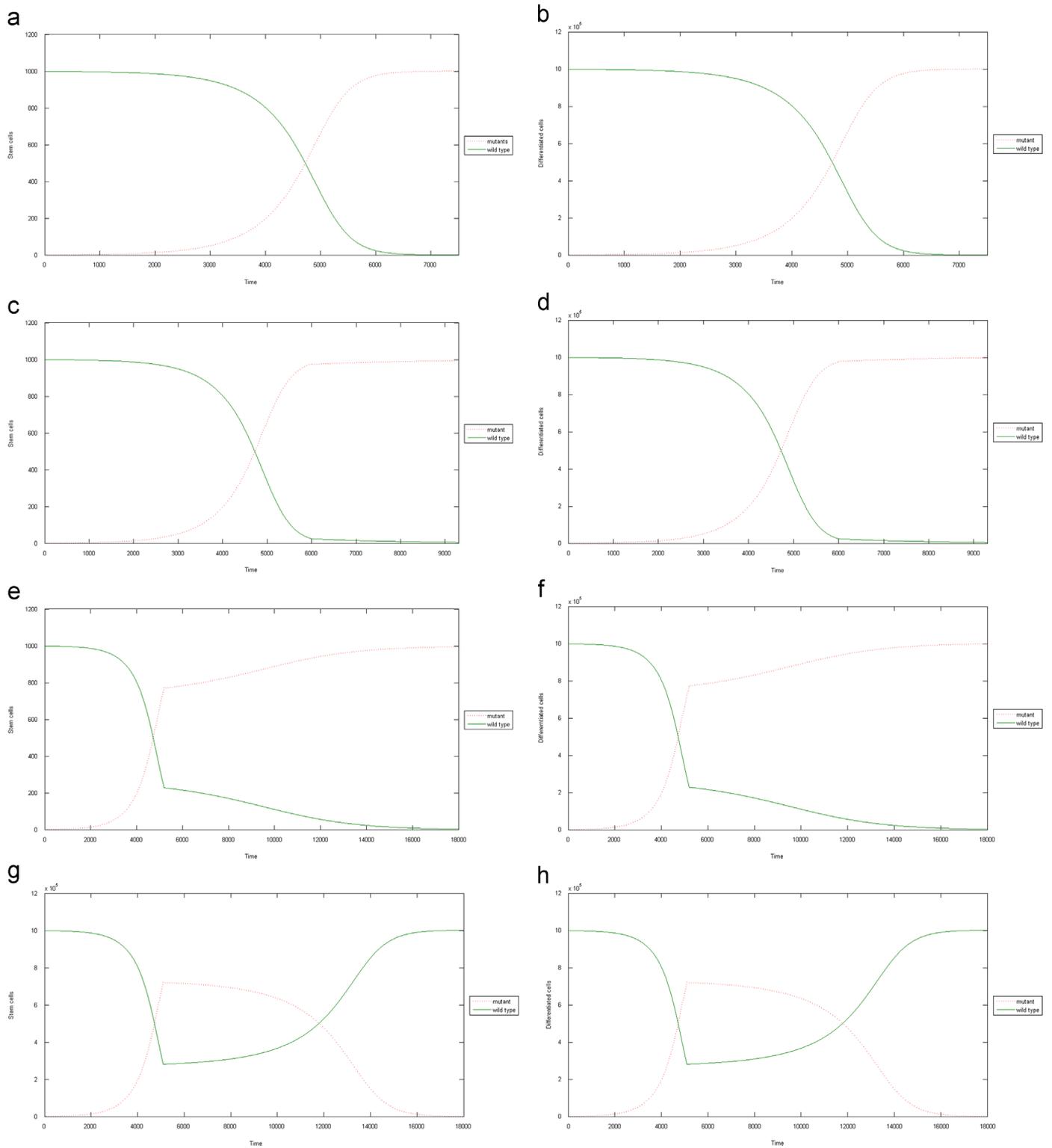


Fig. 5. Two stable equilibrium solutions. Initially, both the wild-type cells and the mutant cells inhibit growth of wild-type stem cells more than growth of mutant stem cells. Thus, if one mutant stem cell is generated at time zero, the system converges towards the only stable equilibrium solution, which is extinction of the wild-type cells and survival of the mutant cells. By modifying the death rate of the differentiated cells, the equilibrium solution, where the wild-type cells survive and the mutant cells get extinct, also becomes stable. Which of the equilibrium solutions the system converges towards, depends on the time that the death rate is modified. The parameter sizes are: $\theta_x^x = \theta_y^x = 0.0012$, $\gamma_x^x = \gamma_y^x = 1.15 \times 10^{-6}$, $\theta_x^y = \theta_y^y = 0.8 \times \theta_x^x$, $\gamma_x^y = 1.2083 \times \gamma_x^x$, $\gamma_y^y = 1.2077 \times \gamma_x^x$, $r = 1$, $d = \exp\left(-\left(\theta_x^x + \frac{P}{Q}\gamma_x^x\right)10^{10}\right)$, $P = 10^6$ and $Q = 10^3$. (a) and (b) display the stem cells and the differentiated cells, respectively, when the death rate is not modified. (c) and (d) display the stem cells and the differentiated cells, respectively, when the death rate is modified at time 6000. (e) and (f) display the stem cells and the differentiated cells, respectively, when the death rate is modified at time 5200. (g) and (h) display the stem cells and the differentiated cells, respectively, when the death rate is modified at time 5100.

divide, both daughter cells are placed in compartment $i + 1$, for $1 \leq i < N$ where N is the total number of compartments of differentiated cells. When the cells reach compartment N , they stop dividing and eventually die. Let x_0 denote the number of stem cells and x_i denote the number of differentiated cells in compartment i . It is assumed that when the cells in all compartments are approximately in normal conditions, then the cells in compartments 1 to $N - 1$ divide and die at the approximately same, constant rates, c and s , respectively, where $c > s$. For simplicity, it is assumed that the death rate of the cells in compartment N is $q = c + s$, and that $p = 2c = 2d_0 + g$, where d_0 and g are the rates at which the stem cells differentiate symmetrically and divide asymmetrically, respectively. Hence, if the number of stem cells is in equilibrium, x_0^* , then the number of differentiated cells in compartment i is expected to converge towards

$$x_i^* = \left(\frac{p}{q}\right)^i x_0^* \quad (15)$$

(approximately). The approximation of the signalling intensity given in (2) considers the average feedback from all differentiated cells. Here, an approximation of the signalling intensity that includes different stages of differentiation is presented:

$$\Psi = \exp(-\theta x_0 - \sum \gamma_{x_i} x_i).$$

It is assumed that for any pair $1 \leq i, j \leq N$, $\gamma_{x_i} \left(\frac{2p}{p+q}\right)^i$ and $\gamma_{x_j} \left(\frac{2p}{p+q}\right)^j$ have the same order and that $\sum_{i=1}^N \gamma_{x_i} \left(\frac{2p}{p+q}\right)^i$ has the same order as θ , because our numerical results indicate that the systems with these parameter relations are most robust.

A second feedback mechanism is considered in this subsection, namely, that cells in compartment i inhibit cell division in compartment $i - 1$ for $1 < i \leq N$, and that cells in compartment one inhibit asymmetric stem cell division. As discussed in the introduction and in Section 2.1, molecules such as cytokines regulate cell behaviour, and the secretion of cytokines is very fast compared with cell activity such as differentiation. However, the precise nature of this regulation is still unknown (Layton et al., 1989; Aglietta et al., 1989; Metcalf, 2008; Fried, 2009). We assume that the signalling intensity from compartment j is approximately

$$\Gamma_{x_j} = \exp(-\nu_j x_j),$$

for $1 \leq j \leq N$. Since the rates of differentiated cell division and asymmetric stem cell division are approximately constant under

normal conditions, ν_j must be sufficiently large, such that

$$\exp(-\nu_j x_j^*) < \epsilon, \quad (16)$$

for some small number $\epsilon \approx 0$, where x_j^* is given in Eq. (15). The extended model is given by the following set of ordinary differential equations:

$$\frac{dx_0}{dt} = (r\Psi - d)x_0, \quad (17)$$

$$\frac{dx_i}{dt} = (p + 2W\Gamma_{x_i})x_{i-1} - (q + W\Gamma_{x_{i+1}})x_i, \quad (18)$$

$$\frac{dx_N}{dt} = (p + 2W\Gamma_{x_N})x_{N-1} - qx_N, \quad (19)$$

for $1 \leq i < N$, and where W is a positive constant. The system has two equilibrium solutions, namely,

$$(x_0^{0*}, x_1^{0*}, \dots, x_N^{0*}) = (0, 0, \dots, 0),$$

$$(x_0^{1*}, x_1^{1*}, \dots, x_N^{1*}) \approx (x_0^*, x_1^*, \dots, x_N^*),$$

where x_i^* is given in (15) for $1 < i \leq N$ and

$$x_0^* = \frac{1}{\theta + \sum_{j=1}^N \gamma_{x_j} \left(\frac{p}{q}\right)^j \ln\left(\frac{r}{d}\right)}.$$

For $r > d$ the former equilibrium solution is unstable and the latter is stable. Moreover, the numerical analysis shows that starting with any population size $(x_0^0, x_1^0, \dots, x_N^0)$ where $x_0^0 > 0$, the system converges towards the stable equilibrium solution.

The work by Komarova (2013) indicates that a well-regulated N -compartment model must have at least $N + 1$ control loops, and that all the $N + 1$ different cell populations must control at least one process. Moreover, the differentiation decision for stem cells must be controlled by another population, and the control of stem cell divisions must be negative. The multi-compartment model presented in this subsection satisfy all these conditions. Fig. 6 illustrates that this model performs better than a model that contains less control loops. That is, the figure shows an example where the whole system is regenerated, starting with a single stem cell. For comparison, the figure also shows the regeneration in the absence of feedback between the compartments. From Fig. 6, we can see that feedback between the compartments enables the system to regenerate faster.

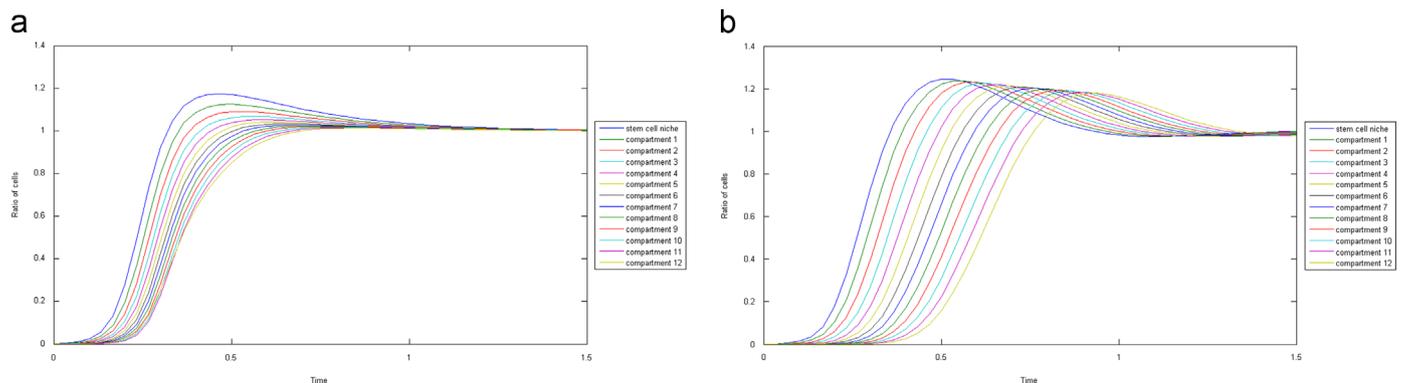


Fig. 6. Regeneration of the multi-compartmental model. The whole system is regenerated, starting with a single stem cell. The stem cells reach their normal population size first, and the differentiated cells in compartment i reach their normal population size before the differentiated cells in compartment $i + 1$. The figure displays the following ratios:

$$\frac{\text{number of cells}}{\text{normal population size}}$$

for the stem cell niche and all compartments of differentiated cells. The regeneration-time depends on the strength of the feedback and on the number of feedback-loops. The parameter sizes are: $\theta = 0.0012$, $\gamma_{x_i} = 10^{-4} \times \left(\frac{q}{p}\right)^i r = 1$, $W = 2$, $d = 0.0907$, $p = 2$, $q = 1.1$, $\nu_{x_i} = \frac{\ln(10^3)}{x_i^*}$. (a) displays regeneration with two feedback-loops. (b) displays regeneration with one feedback-loop.

We will now consider competition dynamics in the multi-compartment model. Like in Section 2.2, it is assumed that the wild-type cells and the mutant cells have the same differentiation hierarchy. However, the mutant cells and the wild-type cells inhibit symmetric stem cell self-renewal at different strength. Moreover, in this subsection it is also assumed that the mutant differentiated cells have a lower death rate than the wild-type cells. Let y_0 denote the number of mutant stem cells and y_i denote the number of differentiated cells in compartment i . The competition dynamics is given by the following set of ordinary differential equations:

$$\begin{aligned} \frac{dx_0}{dt} &= (r\psi_x - d)x_0, \\ \frac{dx_i}{dt} &= (p + 2W\Gamma_{x_i+y_i})x_{i-1} - (q_x + W\Gamma_{x_{i+1}+y_{i+1}})x_i, \\ \frac{dx_N}{dt} &= (p + 2W\Gamma_{x_N+y_N})x_{N-1} - q_x x_N, \\ \frac{dy_0}{dt} &= (r\psi_y - d)y_0, \\ \frac{dy_i}{dt} &= (p + 2W\Gamma_{y_i+y_i})y_{i-1} - (q_y + W\Gamma_{y_{i+1}+x_{i+1}})y_i, \\ \frac{dy_N}{dt} &= (p + 2W\Gamma_{y_N+y_N})y_{N-1} - q_y y_N, \end{aligned}$$

for $1 \leq i < N$, where $q_y < q_x$,

$$\Gamma_{x_k+y_k} = \exp(-\nu_k(x_k+y_k))$$

where ν_k satisfies the inequality given in (16) for $1 \leq k \leq N$, and

$$\psi_z = \exp\left(-\theta_x^z x_0 - \theta_y^z y_0 - \sum_{j=1}^N (\gamma_{x_j}^z x_j + \gamma_{y_j}^z y_j)\right)$$

for $z \in (x, y)$. Note that if the parameters $\gamma_{y_j}^z$ are of the same order as $\gamma_{x_j}^z$, respectively, then the total number of stem cells decreases if the mutant population starts to grow. Since we are interested in investigating the case where the number of mutant differentiated cells increases beyond the normal level, when the total number of stem cells remains approximately constant, it is assumed that $\gamma_{y_j}^z \left(\frac{q_y}{q_x}\right)^j$ is of the same order as $\gamma_{x_j}^z$.

The system has three equilibrium solutions where at least one type of cells gets extinct, namely,

$$(x_0^{0*}, \dots, x_N^{0*}, y_0^{0*}, \dots, y_N^{0*}) = (0, \dots, 0), \tag{20}$$

$$(x_0^{1*}, \dots, x_N^{1*}, y_0^{1*}, \dots, y_N^{1*}) = (x_0^*, \dots, x_N^*, 0, \dots, 0), \tag{21}$$

$$(x_0^{2*}, \dots, x_N^{2*}, y_0^{2*}, \dots, y_N^{2*}) = (0, \dots, 0, y_0^*, \dots, y_N^*), \tag{22}$$

where

$$z_0^* \approx \frac{1}{\theta_z + \sum_{j=1}^N \gamma_{z_j}^z \left(\frac{p}{q_z}\right)^j} \ln\left(\frac{r}{d}\right),$$

and

$$z_j^* \approx \left(\frac{p}{q_z}\right)^j z_0^*,$$

for $z \in \{x, y\}$ and $1 \leq j \leq N$. The system has also one equilibrium solution with coexistence, $(x_0^{3*}, \dots, x_N^{3*}, y_0^{3*}, \dots, y_N^{3*})$, where

$$\begin{aligned} \begin{bmatrix} x_0^{3*} \\ y_0^{3*} \end{bmatrix} &\approx \begin{bmatrix} \theta_x^x + \sum_{j=1}^N \gamma_{x_j}^x \left(\frac{p}{q_x}\right)^j & \theta_x^y + \sum_{j=1}^N \gamma_{y_j}^x \left(\frac{p}{q_y}\right)^j \\ \theta_y^x + \sum_{j=1}^N \gamma_{x_j}^y \left(\frac{p}{q_x}\right)^j & \theta_y^y + \sum_{j=1}^N \gamma_{y_j}^y \left(\frac{p}{q_y}\right)^j \end{bmatrix}^{-1} \begin{bmatrix} \ln\left(\frac{r}{d}\right) \\ \ln\left(\frac{r}{d}\right) \end{bmatrix}, \\ z_j^{3*} &\approx \left(\frac{p}{q_z}\right)^j z_0^*. \end{aligned}$$

The equilibrium solution where all cells get extinct is unstable for $r > d$. The numerical analysis shows that the analytic results

obtained in Section 2.2 also apply to the extended model. That is, the equilibrium solution with survival of the wild-type cells and extinction of the mutant cells given in (21) is stable if the wild-type cells inhibit reproduction of mutant cells more than reproduction of wild-type cells, i.e.

$$\theta_x^x + \sum_{j=1}^N \gamma_{x_j}^x \left(\frac{p}{q_x}\right)^j < \theta_y^x + \sum_{j=1}^N \gamma_{y_j}^x \left(\frac{p}{q_y}\right)^j.$$

On the other hand, if the wild-type cells inhibit reproduction of wild-type cells more than reproduction of mutant cells, then the equilibrium solution is unstable. Likewise, the equilibrium solution with survival of the mutant cells and extinction of the wild-type cells given in (22) is stable if the mutant cells inhibit reproduction of wild-type cells more than reproduction of mutant cells, i.e.

$$\theta_x^y + \sum_{j=1}^N \gamma_{x_j}^y \left(\frac{p}{q_x}\right)^j > \theta_y^y + \sum_{j=1}^N \gamma_{y_j}^y \left(\frac{p}{q_y}\right)^j.$$

On contrary, if the mutant cells inhibit reproduction of mutant cells more than reproduction of wild-type cells, then the equilibrium solution is unstable. If both the equilibrium solutions given in (21) and (22) are unstable, then the equilibrium solution with coexistence is stable. Moreover, if there is only one stable equilibrium solution and both types of stem cells are present, then the system converges towards this solution. On the other hand, if there are two stable equilibrium solutions and both types of stem cells are present, then the system converges towards one of the equilibrium solutions.

Fig. 7 shows an example where a mutant stem cell is generated when the wild-type cells are in normal condition. Since both the wild-type cells and the mutant cells inhibit growth of wild-type cells more than mutant cells, the only stable equilibrium is extinction of the wild-type cells and invasion of the mutant cells, and the system converges towards this solution. The death rate of the mutant differentiated cells is lower than the death rate of the wild-type cells. Hence, the number of differentiated cells increases beyond the normal level. Moreover, since the mutant differentiated cells have weak feedback to the stem cells, the number of stem cells remains approximately constant. When the death rate of the differentiated cells is reduced, the equilibrium solution where the wild-type cells survive and the mutant cells get extinct also becomes stable. Which of the two stable solutions the system converges, depends on the time that the death rate is reduced.

3. Discussion

In this paper we use a mathematical model to investigate implications when the rate of symmetric self-renewal is regulated by both differentiated cells and stem cells, and show that changes in the population dynamics of the differentiated cells can lead to changes in the population dynamics of the stem cells. This result implies that a medical treatment that targets differentiated cells can change the competition dynamics of the stem cells, even if the treatment has no direct effect on the stem cells.

Research suggests that a subset of cancer cells within some tumours, the so-called *cancer stem cells*, may drive the growth and metastasis of these tumours (Reya et al., 2001; Clarke and Fuller, 2006). Understanding the pathways that regulate proliferation, self-renewal, survival and differentiation of malignant and normal stem cells may shed light on mechanisms that lead to cancer and suggest better modes of treatment (Rodriguez-Brenes et al., 2011). For most types of cancer, the target cell of transforming mutation is unknown. However, there is considerable evidence that certain types of leukaemia, such as *chronic myeloid leukaemia* (CML), arise

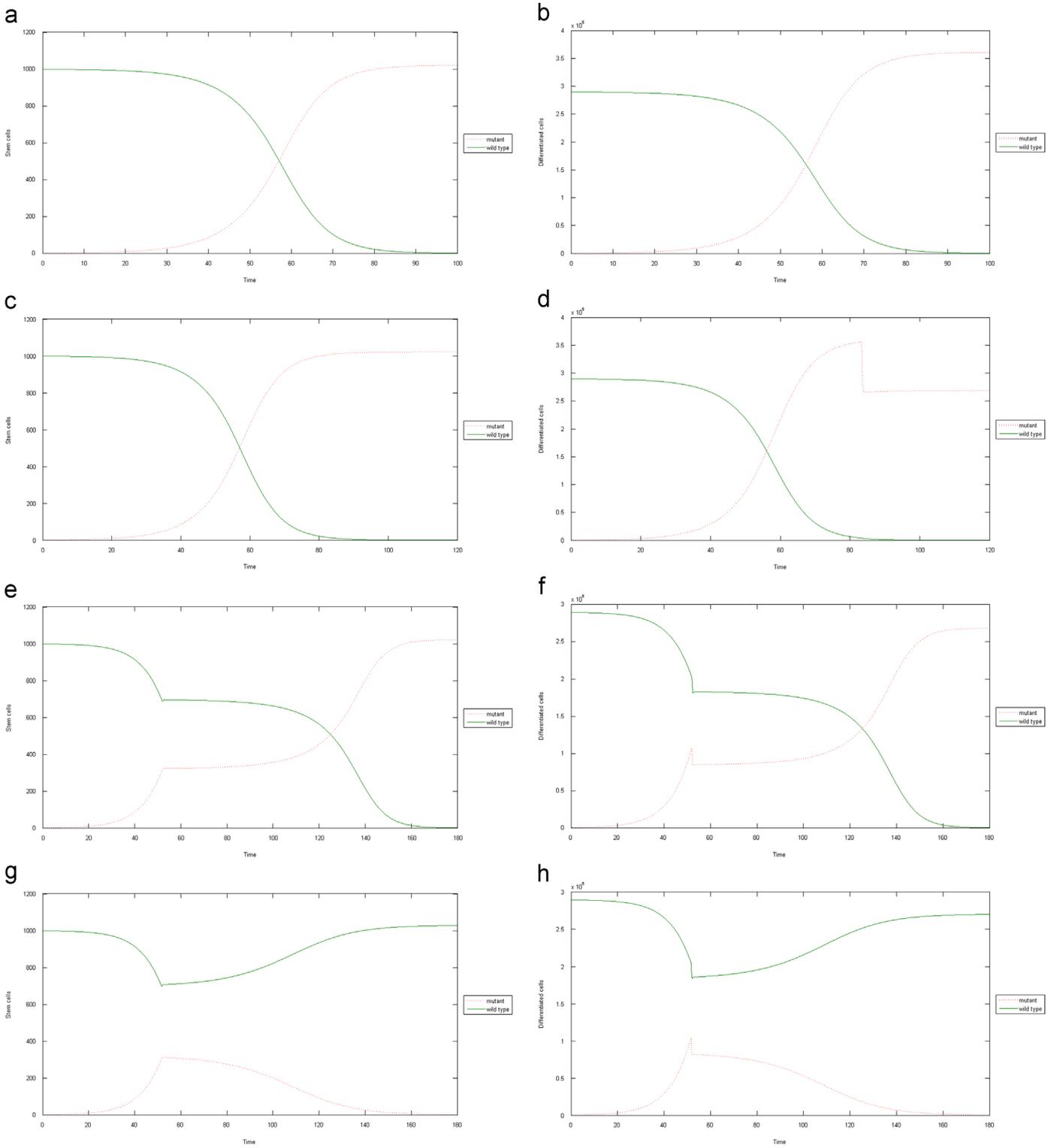


Fig. 7. Two stable equilibrium solutions in the multi-compartmental model. Initially, the mutant differentiated cells have a low death rate and weak feedback to the stem cells. Moreover, both the wild-type cells and the mutant cells inhibit growth of wild-type stem cells more than growth of mutant stem cells. Consequently, if one mutant stem cell is generated, the total number of differentiated cells increases and mutants invade the system, whereas the wild-type cells get extinct. If the death rate of the differentiated cells is decreased enough, the equilibrium solution, where the wild-type cells survive and the mutant cells get extinct, also becomes stable. Which of the equilibrium solutions the system converges to, depends on the time that the death rate is modified.

The parameters sizes are: $\theta_x^x = 0.0012$, $\theta_y^x = 0.0024$, $\theta_x^y = 0.0024$, $\theta_y^y = 0.0023$, $r = 1$, $d = 0.0907$, $p = 2$, $q_x = 1.1$, $q_y = 1.08$, $\hat{Q} = 1.11$, $\gamma_{x_i}^x = 10^{-4} \times (\frac{q_x}{p})^i$, $\gamma_{y_i}^x = 10^{-7} \times 8.3 \times (\frac{q_y}{p})^i$, $\gamma_{x_i}^y = 10^{-7} \times 8.3 \times (\frac{q_x}{p})^i$, $\gamma_{y_i}^y = 10^{-7} \times 8.3 \times (\frac{q_y}{p})^i$, $W = 1$, $\nu_{x_i+y_i} = \frac{\ln(10)}{x_i^2}$.

(a) and (b) display the stem cells and differentiated cells, respectively, when the death rate of the differentiated cells is not modified.

(c) and (d) display the stem cells and the sum of all differentiated cells, respectively, when the death rate of the differentiated cells is modified at time 83.

(e) and (f) display the stem cells and the sum of all differentiated cells, respectively, when the death rate of the differentiated cells is modified at time 52.

(g) and (h) display the stem cells and the sum of all differentiated cells, respectively, when the death rate of the differentiated cells is modified at time 51.5.

from mutation in haematopoietic stem cells (Reya et al., 2001; Wang and Dick, 2005; Hope et al., 2004).

Treatment of CML with the *tyrosine kinase inhibitors* (TKIs) imatinib and nilotinib represents a successful application of molecularly targeted anti-cancer therapy (Druker et al., 1996, 2001; Kantarjian et al., 2002). TKIs reduce the fitness of leukemic differentiated cells. However, the effect of TKIs on leukemic stem cells remains incompletely understood. Several mathematical models of CML and treatment with TKIs have been proposed (Dingli and Michor, 2006; Wodarz, 2008; Michor et al., 2005; Rodriguez-Brenes et al., 2011; Roeder et al., 2006). These models are discussed and compared by Michor (2008). Discontinuation of TKIs results in a relapse of the disease in many patients within a few months (Cortes et al., 2004). Explanations have been put forward for this phenomenon. For example, the drug might have no effect on the CML stem cells (Dingli and Michor, 2006; Michor et al., 2005), or the CML stem cells can be susceptible to drug therapy when they are in an active state, but are not susceptible when they are in quiescent state (Roeder et al., 2006). In contrast to these arguments a small study involving 12 patients has shown that in some individuals the disease has remained undetected for two years after discontinuation of TKIs, raising the possibility that TKIs have eradicated the disease in these patients (Rousselot et al., 2006). Moreover, all studies indicate that the effect of TKIs increases when treatment starts early in disease progression (Rousselot et al., 2006; Gorre et al., 2001; Houchhause et al., 2002; Roche-Lestienne et al., 2002). These results can be explained by the mechanisms described in our model: Suppose that the treatment with TKIs has no direct effect on the leukemic stem cells. However, since the treatment changes the population dynamics of the differentiated cells, and the differentiated cells regulate the proliferation of the stem cells, treatment indirectly effects the stem cells and can lead to changes in the competition dynamics of the stem cells. More precisely, let us revisit the examples illustrated in Figs. 5 and 7. In both figures the wild-type cells represent the healthy cells, the mutant cells represent the leukemic cells, and treatment is represented by modifying the death rate of the differentiated cells. Subfigures (a) and (b) show the disease progression without any treatment – the number of leukemic cells expands and the healthy cells get extinct. In (c) and (d), treatment starts too late to have any significant effect on the disease progression. In (e) and (f), treatment starts early enough to slow down the disease progression and the healthy cells survive a bit longer. However, ultimately, the leukemic cells invade the population and the healthy cells get extinct. Finally, in (g) and (h), treatment starts early enough to reverse the competition dynamics – the healthy cells survive and the leukemic cells get extinct. Fig. 7 shows an example of the extended model, which captures the fact that the number of differentiated leukemic cells increases beyond the normal level, whereas the number of stem cells remains approximately constant (Wang and Dick, 2005; Hope et al., 2004). However, the competition dynamics in both examples is determined by the feedback functions that regulate self-renewal, and this is best captured by the example of the simple model illustrated in Fig. 5.

Lenaerts et al. (2010) illustrate that the results from studies of TKIs treatment (Cortes et al., 2004; Rousselot et al., 2006; Gorre et al., 2001; Houchhause et al., 2002; Roche-Lestienne et al., 2002) can also be explained by the stochastic nature of the haematopoietic stem cells. A deterministic model does not capture neither neutral drift nor that a disadvantageous phenotype can outcompete an advantageous phenotype in a finite population. Since stem cell populations in general are small, their population dynamics are highly sensitive to stochastic fluctuations. Under steady state, the number of stem cells is approximately constant, and Lenaerts et al. show that the stem cell population dynamics can be captured by the Moran process, which describes the probabilistic dynamics in a finite population of constant size N . The Moran process

predicts that if there are i mutant stem cells and $N-i$ wild-type stem cells in the population, while the mutants have relative fitness r and the wild-types relative fitness 1, then the probability that the mutant cells eventually invade the whole population is

$$p_i = \frac{i}{N} \quad (23)$$

if $r = 1$, and

$$p_i = \frac{1-r^{-i}}{1-r^{-N}}$$

if $r \neq 1$. The mutants are advantageous if $r > 1$, disadvantageous if $r < 1$, and neutral if $r = 1$. Moreover, the probability that the mutant population eventually gets extinct is $1-x_i$. Hence, the Moran process predicts that coexistence is only temporary – ultimately the population consists of only one type of cells. Lenaerts et al. assume that the competition between the healthy stem cells and the CML stem cells is captured by a neutral Moran process and that TKIs treatment has no effect on stem cells. If CML is discovered early, then the number of CML stem cells, i , is in general much smaller than the total number of stem cells, N . It follows from Eq. (23) that the probability that the CML stem cells get extinct is $1-\frac{i}{N} \approx 1$. Hence, there is a very good chance of full recovery, even though the TKIs treatment has no effect on the stem cells. On the other hand, if CML is discovered relatively late, then the number of CML stem cells is typically very high, such that $1-\frac{i}{N} \approx 0$. This means that full recovery is very unlikely.

Lenaerts et al. (2010) illustrate the importance of stochastic fluctuations in stem cell populations, and the response dynamics predicted by the model closely matches data from clinical trials. Since stem cell regulation is an extremely complex process, a model that treats self-renewal and differentiation as purely random events fits general data better than a deterministic model with a single regulation mechanism. Thus, the model proposed by Lenaerts et al. gives a general picture of how stem cells behave under steady state. However, Lander et al. (2009) show that linear models, e.g. the one proposed by Lenaerts et al., are very parameter sensitive. Since parameter sensitivities tend to be undesirable in well-regulated biological systems, stochastic behaviour cannot be the complete story. A deterministic model of stem cell dynamics with only one regulation mechanism can be designed to describe more specific data. For instance, research results by Gokoffski et al. (2011) indicate that the number of stem cells increases when the number of differentiated cells decreases. This can be explained by a model of stem cell self-renewal and differentiation, where symmetric stem cell division is regulated by differentiated cells, like the model proposed by Rodriguez-Brenes et al. (2013) and the models presented in this paper.

The model proposed by Lenaerts et al. (2010) and the model presented in this paper have different explanations for successful TKIs treatment. However, this does not mean that one of the conclusions must be false. It is possible that the CML stem cells are advantageous before TKIs treatment and, because the differentiated cells regulate symmetric stem cell division, the CML stem cells are disadvantageous during the TKIs treatment. In this case, the average behaviour of the CML stem cells can be approximately neutral, as assumed by Lenaerts et al.

The main purpose of the simple model proposed in Section 2.1 is to investigate implications when symmetric stem cell division is regulated by differentiated cells. Similar results can be obtained by replacing the signal intensity function given in (2) with another function that reaches its maximum under complete absence of cells and decreases towards zero as the number of cells decreases. For instance

$$S = 1/(\theta x_s + \gamma x_d + 1),$$

which is similar to the function proposed by Marciniaak-Czochra et al. (2009) given in (1). Yet, the function in (2) is used in our model because it makes the stability analysis simple. It would also be interesting to investigate implications when differentiated cells also regulate symmetric differentiation. However, in this paper, the model is kept simple to obtain analytic results. In Sections 2.2 and 2.3 the competition dynamics is investigated. It is possible that the mutant cells have other properties than the ones investigated in this paper. For instance, the mutant differentiated cells in compartment i could have a much weaker feedback to compartment $i - 1$ than the wild-type cell in compartment i . This could radically change the dynamics of the cells. However, investigation of these types of mutation is beyond the scope of this paper. Finally, the timescale in all examples are the same. This illustrates that regeneration of the system in general occurs much faster than the invasion of a mutation. Moreover, Figs. 4, 5 and 7 indicate that the timescale of the competition dynamics depends on the ratios

$$\frac{\theta_v^w + \frac{P}{Q} \gamma_v^w}{\theta_v^z + \frac{P}{Q} \gamma_v^z}$$

where $v, w, z \in \{x, y\}$, $w \neq z$. That is, the closer these ratios are to one, the slower the competition dynamics occurs.

4. Conclusion

In this paper, we use a mathematical models where symmetric stem cell division is regulated by negative feedback from the differentiated cells, to show that changes in the population dynamics of the differentiated cells can lead to changes in the population dynamics of the stem cells. This result is interesting because it can explain how medical treatments that have no direct effect on the stem cells can change the competition dynamics of these cells. For example, the model can reproduce some of the results from studies of TKIs treatment of CML patients (Cortes et al., 2004; Rousselot et al., 2006; Gorre et al., 2001; Houchhause et al., 2002; Roche-Lestienne et al., 2002):

- The effect of TKIs increases when treatment starts early in disease progression.
- In some cases the treatment slows down the disease progression without erasing the CML stem cells, which drive the disease.
- In other cases the treatment reverses the disease progression and seems to erase the CML stem cells.

The results from these studies seem contradictory if a classical deterministic model of stem cells and differentiation is used, where stem cell activity is not regulated by the differentiated cells (Dingli and Michor, 2006; Michor et al., 2005). Our model shows that the results from the different studies can be explained by negative feedback from differentiated cells that regulate symmetric stem cell division: TKIs treatment reduces the fitness of the CML differentiated cells, but has little or no direct effect on the CML stem cells. However, since the differentiated cells regulate the proliferation of the stem cells, the treatment indirectly affects the stem cells and can lead to changes in the competition dynamics of the stem cells, which in some cases results in the extinction of the CML stem cells.

Appendix A

Proposition 1. Consider the systems of differential equations given in (3) and (4) and (10)–(13). If Q is sufficiently large, then the pseudo-

state hypothesis (Appendix C)

$$\frac{dz_d}{dt} \frac{1}{Q} = \left(\frac{P}{Q} z_s - z_d \right) \approx 0 \tag{A.1}$$

holds for $z \in \{x, y\}$ and $t \geq \frac{1}{r}$, when it is given that the parameters r and d are of significantly lower order than the parameters P and Q , $r > d$ and $P > Q$, and initial values are non-negative.

Proof. By re-scaling the systems of given in (3) and (4) and (10)–(13) with respect to the constant r , we obtain

$$\frac{dv_s}{dT} = (\Psi_v - d_r)v_s, \tag{A.2}$$

$$\frac{dv_d}{dT} = P_r v_s - Q_r v_d, \tag{A.3}$$

where $d_r = \frac{d}{r}$, $P_r = \frac{P}{r}$, $Q_r = \frac{Q}{r}$, and $T = r \times t$. For the system with only one type of cells $v = x$, whereas $v \in \{x, y\}$ for the system with both wild-type cells and mutant cells. Note that if $v_s = 0$, then $\frac{dv_s}{dT} = 0$, and if $v_d = 0$ and $v_s \geq 0$, then $\frac{dv_d}{dT} \geq 0$. Consequently, a solution of the system with non-negative initial values will never obtain negative values.

We now use the perturbation methods presented by Fowler (1997) to analyse the system. Given that $0 < d_r < 1$, $Q_r \gg 1$ and $P_r > Q_r$, the pseudo-state hypothesis states that if Q_r is sufficiently large, then

$$\frac{dv_d}{dT} \frac{1}{Q_r} = \left(\frac{P_r}{Q_r} v_s - v_d \right) \approx 0 \tag{A.4}$$

when $T \geq O(1)$. However, the approximation given in (A.4) does not generally hold for the initial values, i.e. when T is close to zero. This means that the neglect of $\frac{dv_d}{dT} \frac{1}{Q_r}$ is wrong in a region that contains $T = 0$. We will now show that if Q_r is sufficiently large, then this is only a thin region, termed the *boundary layer*. We bring back the term $\frac{dv_d}{dT}$ in the boundary layer by rescaling the time as

$$T = \frac{1}{Q_w} \tau,$$

where Q_w is $O(Q_r)$. To obtain variables that are $O(1)$, we rescale as follows:

$$v_s = v_s(0) V_s, \\ v_d = v_d(0) V_d,$$

where $v_s(0)$ and $v_d(0)$ are the initial values of v_s and v_d , respectively. By substituting this into the system of differential equations given in (A.2) and (A.3), we obtain

$$\frac{dV_s}{d\tau} = \frac{1}{Q_w} (\Psi_v - d_r) V_s, \\ \frac{dV_d}{d\tau} = \frac{P_r v_s(0)}{Q_w v_d(0)} V_s - \frac{Q_r}{Q_w} V_d.$$

Note that V_s is $O(1)$ when τ is close to zero. Thus, since $0 < d_r, \Psi_v < 1$ and $P_r \gg 1$, the variable V_s is approximately constant, i.e. $\frac{dV_s}{d\tau} \approx 0$ when τ is $O(1)$. On the other hand, $\frac{Q_r}{Q_w}$ is $O(1)$, while $\frac{P_r}{Q_w} \geq O(1)$. Thus, we obtain the approximate solution

$$V_d(\tau) = \frac{P_r v_s(0)}{Q_r v_d(0)} V_s(\tau) + \left(1 - \frac{P_r v_s(0)}{Q_r v_d(0)} \right) \exp\left(-\frac{Q_r}{Q_w} \tau \right).$$

By substituting the original variables, we obtain

$$v_d(T) = \frac{P_r}{Q_r} v_s(T) + \left(v_d(0) - \frac{P_r}{Q_r} v_s(0) \right) \exp\left(-\frac{Q_r T}{Q_w} \right).$$

Hence, outside the boundary layer, i.e. when $T \geq O(1)$, we obtain the approximation

$$v_d(T) = \frac{P_r}{Q_r} v_s(T),$$

which satisfies the pseudo-steady state hypothesis given in (A.4). Thus, we have proved that for sufficient large Q , any solution with non-negative initial values of either the system given in (3) and (4) or the system given in (10)–(13) satisfies the pseudo-state hypothesis given in (A.1) for $t \geq \frac{1}{r}$. \square

Appendix B

Proposition 2. *The system of differential equations given in (3) and (4) has one stable equilibrium solution:*

$$(x_s^*, x_d^*) = \left(\frac{1}{\theta + \frac{P}{Q}\gamma} \ln\left(\frac{r}{d}\right), \frac{P}{Q} \frac{1}{\theta + \frac{P}{Q}\gamma} \ln\left(\frac{r}{d}\right) \right),$$

and one unstable equilibrium solution:

$$(x_s^{0*}, x_d^{0*}) = (0, 0),$$

for $r > d$. The following domain

$$\mathcal{V} = \{(x_s, x_d) \in \mathbb{R}^2 \mid x_s > 0, x_d \geq 0\}$$

is in the basin of attraction of (x_s^*, x_d^*) .

Proof. We prove the proposition for the case when Q is sufficiently large so that Proposition 1 holds. Then the system given in (3) and (4) is reduced to the following differential equation:

$$\frac{dx_s}{dt} = (r\Psi - d)x_s,$$

where

$$\Psi = \exp\left(-\left(\theta + \gamma \frac{P}{Q}\right)x_s\right).$$

The Jacobian of the system is

$$\mathbf{J}(x_s) = (r\Psi - d) - x_s \left(\theta + \gamma \frac{P}{Q}\right) r\Psi.$$

We have that

$$\mathbf{J}(x_s^{0*}) = (r - d) > 0$$

for $r > d$. Hence, the equilibrium solution (x_s^{0*}, x_d^{0*}) is unstable. Since

$$\mathbf{J}(x_s^*) = -x_s^* \left(\theta + \gamma \frac{P}{Q}\right) r\Psi < 0,$$

the equilibrium solution (x_s^*, x_d^*) is stable.

Note that $\frac{dx_s}{dt} > 0$ for $0 < x_s < x_s^*$. Hence, if the initial number of stem cells is less than x_s^* , then the solution converges towards the stable equilibrium solution. Likewise, $\frac{dx_s}{dt} < 0$ for $x_s > x_s^*$. Hence, if the initial number of stem cells is greater than x_s^* , then the solution converges towards the stable equilibrium solution. Consequently, \mathcal{V} is in the basin of attraction of (x_s^*, x_d^*) . \square

Appendix C

Proposition 3. *The system given in Eqs. (10)–(13) has three equilibrium solutions where at least one of the populations gets extinct, namely,*

$$(x_s^{0*}, x_d^{0*}, y_s^{0*}, y_d^{0*}) = (0, 0, 0, 0),$$

$$(x_s^{1*}, x_d^{1*}, y_s^{1*}, y_d^{1*}) = \left(\frac{1}{\theta_x + \frac{P}{Q}\gamma_x} \ln\left(\frac{r}{d}\right), \frac{P}{Q} \frac{1}{\theta_x + \frac{P}{Q}\gamma_x} \ln\left(\frac{r}{d}\right), 0, 0 \right),$$

$$(x_s^{2*}, x_d^{2*}, y_s^{2*}, y_d^{2*}) = \left(0, 0, \frac{d}{\theta_y + \frac{P}{Q}\gamma_y} \ln\left(\frac{1}{d}\right), \frac{P}{Q} \frac{d}{\theta_y + \frac{P}{Q}\gamma_y} \ln\left(\frac{1}{d}\right) \right),$$

and one equilibrium solution with coexistence, $(x_s^{3*}, x_d^{3*}, y_s^{3*}, y_d^{3*})$, given in Eq. (14). For $r > d$, $(x_s^{0*}, x_d^{0*}, y_s^{0*}, y_d^{0*})$ is unstable. Moreover, given that Q is sufficiently large such that Proposition 1 holds, the behaviour of system depends on the following four parameter relations:

- (a) For $\theta_y + \frac{P}{Q}\gamma_y > \theta_x + \frac{P}{Q}\gamma_x$ and $\theta_x + \frac{P}{Q}\gamma_x > \theta_x + \frac{P}{Q}\gamma_x$, the only stable equilibrium solution is $(x_s^{1*}, x_d^{1*}, y_s^{1*}, y_d^{1*})$. Moreover, the domain

$$\mathcal{U} = \{(x_s, x_d, y_s, y_d) \in \mathbb{R}^4 \mid z_s > 0, z_d \geq 0, z \in \{x, y\}\}, \tag{C.1}$$

is in the basin of attraction of $(x_s^{1*}, x_d^{1*}, y_s^{1*}, y_d^{1*})$.

- (b) For $\theta_y + \frac{P}{Q}\gamma_y < \theta_x + \frac{P}{Q}\gamma_x$ and $\theta_x + \frac{P}{Q}\gamma_x < \theta_x + \frac{P}{Q}\gamma_x$, the only stable equilibrium solution is $(x_s^{2*}, x_d^{2*}, y_s^{2*}, y_d^{2*})$, and \mathcal{U} is in the basin of attraction of $(x_s^{2*}, x_d^{2*}, y_s^{2*}, y_d^{2*})$.

- (c) For $\theta_y + \frac{P}{Q}\gamma_y > \theta_x + \frac{P}{Q}\gamma_x$ and $\theta_x + \frac{P}{Q}\gamma_x < \theta_x + \frac{P}{Q}\gamma_x$, the only stable equilibrium solution is $(x_s^{3*}, x_d^{3*}, y_s^{3*}, y_d^{3*})$. Moreover, \mathcal{U} is in the basin of attraction of this equilibrium solution.

- (d) For $\theta_y + \frac{P}{Q}\gamma_y < \theta_x + \frac{P}{Q}\gamma_x$ and $\theta_x + \frac{P}{Q}\gamma_x > \theta_x + \frac{P}{Q}\gamma_x$, both $(x_s^{1*}, x_d^{1*}, y_s^{1*}, y_d^{1*})$ and $(x_s^{2*}, x_d^{2*}, y_s^{2*}, y_d^{2*})$ are stable and $(x_s^{3*}, x_d^{3*}, y_s^{3*}, y_d^{3*})$ is unstable. Moreover, the domain

$$\mathcal{D}^1 = \left\{ (x_s, x_d, y_s, y_d) \in \mathcal{U} \mid y_s < x_s \frac{\theta_x - \theta_y}{\theta_x - \theta_y} \right\}$$

is in the basin of attraction of $(x_s^{1*}, x_d^{1*}, y_s^{1*}, y_d^{1*})$, and

$$\mathcal{D}^2 = \left\{ (x_s, x_d, y_s, y_d) \in \mathcal{U} \mid y_s > x_s \frac{\theta_x - \theta_y}{\theta_x - \theta_y} \right\}$$

is in the basin of attraction of $(x_s^{2*}, x_d^{2*}, y_s^{2*}, y_d^{2*})$. The basin of attraction of the equilibrium solution $(x_s^{3*}, x_d^{3*}, y_s^{3*}, y_d^{3*})$ is the line

$$\mathcal{L}^3 = \left\{ (x_s, x_d, y_s, y_d) \in \mathcal{U} \mid y_s = x_d \frac{\theta_x - \theta_y}{\theta_x - \theta_y} \right\},$$

where

$$\theta_w^v = \theta_w^v + \frac{P}{Q_v} \gamma_w^v,$$

for $v, w \in \{x, y\}$.

Proof. Given that Q is sufficiently large, such that Proposition 1 holds, the system given in (10)–(13) is reduced to the following two differential equations:

$$\frac{dx_s}{dT} = (\Psi_x - D)x_s, \tag{C.2}$$

$$\frac{dy_s}{dT} = (\Psi_y - D)y_s, \tag{C.3}$$

where $D = \frac{d}{T}$, $T = r \times t$, and

$$\Psi_v = \exp\left(-\theta_x^v x_s - \theta_y^v y_s\right).$$

for $v \in \{x, y\}$. We have that $\frac{dv_s}{dT} = 0$ for $v_s = 0$ and $\Psi_v = D$. Note that $0 < D < 1$. Thus, there are three equilibrium solutions where at least one of the variables is zero, namely,

$$(x_s^{0*}, y_s^{0*}) = (0, 0),$$

$$(x_s^{1*}, y_s^{1*}) = \left(\frac{1}{\theta_x} \ln\left(\frac{1}{D}\right), 0 \right),$$

$$(x_s^{2*}, y_s^{2*}) = \left(0, \frac{1}{\theta_y} \ln\left(\frac{1}{D}\right) \right).$$

The equilibrium solution with coexistence, (x_s^{3*}, y_s^{3*}) , must satisfy

$$\Omega \begin{bmatrix} x_s^{3*} \\ y_s^{3*} \end{bmatrix} = \ln\left(\frac{1}{D}\right) \begin{bmatrix} 1 \\ 1 \end{bmatrix},$$

where

$$\Omega = \begin{bmatrix} \Theta_x^x & \Theta_y^x \\ \Theta_x^y & \Theta_y^y \end{bmatrix}.$$

If $\det\Omega = 0$, we have a solution with coexistence if and only if $\Theta_x^x = \Theta_x^y$ and $\Theta_y^x = \Theta_y^y$, which means that there is no difference between the wild-type cells and mutant cells. Thus, we will only consider the case when $\det\Omega \neq 0$, and obtain the following solution with coexistence:

$$\begin{bmatrix} x_s^{3*} \\ y_s^{3*} \end{bmatrix} = \frac{\ln\left(\frac{1}{D}\right)}{\Theta_x^x \Theta_y^y - \Theta_x^y \Theta_y^x} \begin{bmatrix} \Theta_y^y & -\Theta_y^x \\ -\Theta_x^y & \Theta_x^x \end{bmatrix} \begin{bmatrix} 1 \\ 1 \end{bmatrix}.$$

Note that $x_s^{3*}, y_s^{3*} > 0$ if and only if both $\Theta_y^y > \Theta_y^x$ and $\Theta_x^x > \Theta_x^y$ or both $\Theta_y^y < \Theta_y^x$ and $\Theta_x^x < \Theta_x^y$. The Jacobian of the system is

$$J(x_s, y_s) = \begin{bmatrix} (\Psi_x - D) - \Theta_x^x \Psi_x x_s & -\Theta_y^x \Psi_x x_s \\ -\Theta_x^y \Psi_y y_s & (\Psi_y - D) - \Theta_y^y \Psi_y y_s \end{bmatrix}.$$

We have that

$$J(x_s^{0*}, y_s^{0*}) = \begin{bmatrix} (1-D) & 0 \\ 0 & (1-D) \end{bmatrix}.$$

Since $D < 1$, both eigenvalues are positive. Hence, (x_s^{0*}, y_s^{0*}) is unstable. Moreover,

$$J(x_s^{1*}, y_s^{1*}) = \begin{bmatrix} -\Theta_x^x \Psi_x x_s^{1*} & -\Theta_y^x \Psi_x x_s^{1*} \\ 0 & (\Psi_y - \Psi_x) \end{bmatrix}.$$

Thus, if $\Psi_y > \Psi_x$, i.e. $\Theta_y^y < \Theta_y^x$, then one eigenvalue is positive and (x_s^{1*}, y_s^{1*}) is unstable, whereas if $\Psi_y < \Psi_x$, i.e. $\Theta_y^y > \Theta_y^x$, then both eigenvalues are negative and (x_s^{1*}, y_s^{1*}) is stable. Likewise,

$$J(x_s^{2*}, y_s^{2*}) = \begin{bmatrix} (\Psi_x - \Psi_y) & 0 \\ -\Theta_x^y \Psi_y y_s^{2*} & -\Theta_y^y \Psi_y y_s^{2*} \end{bmatrix}.$$

Hence, if $\Psi_y < \Psi_x$, i.e. $\Theta_y^y > \Theta_y^x$, then one eigenvalue is positive and (x_s^{2*}, y_s^{2*}) is unstable, whereas if $\Psi_y > \Psi_x$, i.e. $\Theta_y^y < \Theta_y^x$, then both eigenvalues are negative and (x_s^{2*}, y_s^{2*}) is stable. Finally,

$$J(x_s^{3*}, y_s^{3*}) = \begin{bmatrix} -\Theta_x^x \Psi_x x_s^{3*} & -\Theta_y^x \Psi_x x_s^{3*} \\ -\Theta_x^y \Psi_y y_s^{3*} & -\Theta_y^y \Psi_y y_s^{3*} \end{bmatrix}.$$

The characteristic equation is

$$(\Theta_x^x \Psi_x x_s^{3*} + \lambda)(\Theta_y^y \Psi_y y_s^{3*} + \lambda) - \Theta_x^y \Theta_y^x \Psi_x \Psi_y x_s^{3*} y_s^{3*} = 0.$$

Hence, (x_s^{3*}, y_s^{3*}) is stable if $\Theta_x^x \Theta_y^y > \Theta_x^y \Theta_y^x$. Thus, the equilibrium solution with coexistence is both stable and positive if $\Theta_x^x > \Theta_x^y$ and $\Theta_y^y > \Theta_y^x$.

By analysing the nullclines of the system given in (C.1) and (C.2), namely,

$$\begin{aligned} \Theta_x^x x_0 + \Theta_y^y y_0 &= -\ln D, \\ \Theta_x^y x_0 + \Theta_y^x y_0 &= -\ln D \end{aligned}$$

we can predict the global behaviour. Since these nullclines are the same as the nullclines of the two-species Lotka–Volterra competition model (Smith, 1978), the global stability analysis is identical. Hence, the basins of attraction of the equilibrium solutions are as described in (a)–(d) (Smith, 1978). \square

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