Cells, cancer, and rare events: homeostatic metastability in stochastic non-linear dynamics models of skin cell proliferation

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A recently proposed single progenitor cell model for skin cell proliferation [Clayton et al., Nature 446, 185 (2007)] is extended to incorporate homeostasis as a fixed point of the dynamics. Unlimited cell proliferation in such a model can be viewed as a paradigm for the onset of cancer. A novel way in which this can arise is if the homeostatic fixed point becomes metastable, so that the cell populations can escape from the homeostatic basin of attraction by a large but rare stochastic fluctuation. Such an event can be viewed as the final step in a multi-stage model of carcinogenesis. This offers a possible explanation for the peculiar epidemiology of lung cancer in ex-smokers.

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The epidermis is the outermost part of the skin barrier. It comprises 10–20 layers of skin cells, which are predominantly keratinocytes \[1\]. Keratinocytes proliferate in the basal layer, move up through the middle layers, and are finally shed from the outermost layer at a desquamation rate of the order \(10^3 \text{cells hr}^{-1} \text{mm}^{-2}\) \[2\]. This process means that cells have to proliferate continuously in the basal layer to replenish the supra-basal layers. Faulty cell proliferation in the basal layer has important health-care consequences, for example basal cell carcinoma is one of the most prevalent forms of cancer \[3\].

Motivated by elegant \textit{in vivo} experiments on labelled keratinocyte clones, Clayton \textit{et al.} \[4, 5, 6\] recently proposed a novel single progenitor cell (SPC) model for basal layer proliferation. In the present paper I examine an extension of this SPC model to include autoregulation, and homeostasis as a dynamical fixed point. Interestingly, something resembling the onset of cancer (\textit{i.e.} carcinogenesis) arises naturally in the new model if the homeostatic fixed point loses stability in the direction of unlimited growth of the cell populations. One intriguing possibility is ‘homeostatic metastability’, in which a large but rare stochastic fluctuation results in the cell populations escaping from the homeostatic basin of attraction. Such a rare escape event can be viewed as the final step in a multi-stage model of carcinogenesis \[4, 8\].

In the SPC model, there are two cell types, or ‘compartments’: progenitor cells A, and post-mitotic cells B. These proliferate according to

\[
\begin{align*}
A & \to A + A \text{ rate } \lambda_1, \quad A \to A + B \text{ rate } \lambda_2, \\
A & \to B + B \text{ rate } \lambda_3, \quad B \to \emptyset \text{ rate } \Gamma.
\end{align*}
\]

The first three processes represent possible progenitor cell division pathways. The last process represents post-mitotic cells leaving the basal layer. Obviously, the progenitor cell division pathways must be finely balanced otherwise the cell populations either grow or vanish exponentially \[8\]. Making the assumption therefore that \(\lambda_1 = \lambda_3\), Clayton \textit{et al.} \[4, 5\] write \(\lambda_1 = \lambda_3 = \lambda r\) and \(\lambda_2 = \lambda (1 - 2r)\). For mouse skin, they find \(\lambda \approx 0.16 \text{day}^{-1}\) for the overall progenitor cell division rate, and \(2r \approx 0.16\) for the branching ratio into the symmetric division pathways (in later work \(2r \approx 0.4\) \[3\] but the actual value is not too important). A steady state condition for the cell populations is

\[
\lambda \rho = \Gamma (1 - \rho)
\]

where \(\rho = n_A/n \approx 0.22\) is the fraction of progenitor cells, \(n = n_A + n_B\) is the total cell density, and \(n_A\) and \(n_B\) are the individual cell densities. From this one determines \(\Gamma \approx 0.045 \text{day}^{-1}\), compatible with the above-mentioned desquamation rate.

A phase space portrait and a representative stochastic trajectory for the SPC model are shown in Fig. 1(a). The trajectory was generated using a standard kinetic Monte-Carlo algorithm \[10\], starting from a patch of area \(A\) comprising initially \(N = An = 200\) cells, and assuming the cell types remain well-mixed. The actual value of \(A\) is irrelevant since the processes in Eq. (1) are all first order. In Fig. 1 the ordinate is \(n/n_0\) where \(n_0\) is the initial cell density (number).

While providing an eminently satisfactory explanation for the keratinocyte labelling data, the original SPC model includes a fine-tuning assumption, \(\lambda_1 = \lambda_3\), making it structurally unstable in the language of dynamical systems theory \[11\]. As a consequence the model lacks homeostasis in the sense that it possesses a \textit{line} of stable fixed points, shown in Fig. 1(a). Also it is not generally robust against perturbations, for example it cannot accommodate the introduction of a small population of stem cells \[12\], without making some additional fine-tuning assumptions. One obvious solution to this is to suppose that the cell division rates depend on the cell population densities \(n_A\) and \(n_B\), representing the fact that cellular fates are governed by integration of autocrine and paracrine signalling factors \[12, 13, 14\]; indeed this idea was already suggested by Jones and Simon as an avenue for further investigation \[15\]. In such an autoregulating SPC (ASPC) model, the fine-tuning would arise as a consequence of the cell population dy-
namics driving the system to a homeostatic fixed point. An ASPC model would be structurally stable from the point of view of dynamical systems theory, and also able to withstand perturbations, such as the presence of a small number of stem cells.

To develop such an ASPC model, I start by introducing a control parameter $q$ to describe a possible bias in the symmetric cell division fates, thus

$$
\begin{align*}
\lambda_1 &= \lambda r (1 - q), \\
\lambda_2 &= \lambda (1 - 2r), \\
\lambda_3 &= \lambda r (1 + q).
\end{align*}
$$

The parameters $\lambda$, $r$ and $q$ replace $\lambda_1$, $\lambda_2$ and $\lambda_3$ without loss of generality. The steady state conditions are given by $q = 0$ and Eq. (2). From this point onwards, I deliberately adopt a ‘physics-oriented’ approach in which the model is kept as simple as possible to expose the general mechanisms at work. In particular it is not claimed that the biology is accurately represented. The basic idea is to introduce a minimal assumption about $q$ and $\lambda$ on the cell population densities, to represent the integrated effect of the intercellular signals. I start by making $q = q(\rho)$. Then the steady state condition selects the value of $\rho$ for which $q(\rho) = 0$. I additionally suppose that $\lambda = \lambda(n)$ is a decreasing function of the total number density ($\lambda' < 0$) representing the fact that the progenitor cell proliferation rate should be reduced if the overall cell density increases [10]. With these assumptions, fixed points of the dynamics are determined by $q(\rho) = 0$ and $\lambda(n) = \Gamma(1 - \rho)/\rho$. It is a straightforward exercise to show, in the language of dynamical systems theory, that a fixed point is a stable node if $q' > 0$, and a saddle if $q' < 0$.

A concrete model of this type has

$$
\lambda = \lambda_0 \left( \frac{n_0}{n} \right)^\alpha, \\
q = \tanh \left[ \frac{\beta \rho_0 (1 - \rho_0) (\rho - \rho_0)}{\rho (1 - \rho)} \right],
$$

where $\lambda_0 = \Gamma(1 - \rho_0)/\rho_0$. This model has a stable node at a target population density $n = n_0$ and progenitor cell fraction $\rho = \rho_0$. I emphasise that these functions have been arbitrarily chosen for illustrative purposes, though with care to make sure that they have the appropriate limiting behaviours. In Eq. (4) $\alpha$ and $\beta$ are ‘stiffness’ coefficients. They are related to derivatives of the functions at the fixed point by $\alpha = -n \lambda'/\lambda$ and $\beta = q'$. For results presented below I use $\alpha = 2$ and $\beta = 10$ which allows for stochastic fluctuations of moderate amplitude, balanced between the two cell types. I have checked that the results are qualitatively insensitive to this choice.

Fig. (b) shows the phase space portrait and a representative stochastic trajectory for this ASPC model with a fixed point chosen to lie at $\rho_0 = 0.22$ and a target population size $N_0 = A n_0 = 200$. Again $A$ does not need to be explicitly specified though in this case it could be interpreted as representing the influence of diffusible intercellular signalling factors. In contrast to Fig. (a), there is an isolated stable fixed point, whose basin of attraction extends to cover the whole plane. The stochastic trajectory is strongly localised to the vicinity of the fixed point. It is clear that this behaviour should be generic to a wide class of ASPC models since the existence of an isolated stable fixed point is a structurally stable feature of the dynamics. The fixed point in a model of this type represents homeostasis in several ways. Firstly, if the cell populations are perturbed, they will tend to return to the original fixed point. Secondly, fluctuations in the cell populations will be limited to the vicinity of the fixed point. Thirdly, the model itself can be perturbed, for example by the inclusion of stem cells, without leading to a qualitative change in behaviour.

A key requirement of such models is that they retain compatibility with the keratinocyte labelling data of Clayton et al. [4 5]. An (admittedly mean-field) argument that this is true can be made as follows. Imagine labelling a small fraction of keratinocytes. If the label is passive, proliferation of labelled cells will be determined by fixed parameter values, corresponding to the homeostatic fixed point. In particular the symmetric division pathways of labelled progenitor cells will be automatically balanced.

I now turn to the implications for cancer modelling. As outlined in the introduction, the ASPC models might offer some interesting insights into carcinogenesis, which can be considered as unlimited cell proliferation caused either by a loss of stability of the homeostatic fixed point, or by a large but rare stochastic fluctuation causing the system to exit the homeostatic basin of attraction. Whilst the idea that cancer arises from an instability in the underlying cell population dynamics is rather old [13 17], the second possibility is very intriguing and has apparently not been hitherto considered. My studies of models comprising $A$, $A^*$ and $B$ cells, with a process $A \rightarrow A^*$ representing a somatic mutation (c.f. Klein et al. [3]), shows that the phenomenon of ‘homeostatic metastability’ can easily be observed. However the resulting three-dimensional phase space portraits are tricky to represent and analyse. To illustrate the mechanism of homeostatic metastability therefore, I return to the original ASPC model, but introduce a re-entrant bias control function $q(\rho)$. Such a model is a prototypical example of a system which is near a saddle-node bifurcation.

An example of a re-entrant $q(\rho)$ is given by inserting an extra factor $(\rho_1 - \rho)/(\rho_1 - \rho_0)$ in the argument to the tanh function in Eq. (4). This model has a stable node at $\rho = \rho_0$ and a saddle at $\rho = \rho_1$. The saddle-node bifurcation is approached as $\Delta \rho = \rho_1 - \rho_0$ vanishes. The phase space portrait and a representative stochastic trajectory for this ASPC model are shown in Fig. (c) for $\Delta \rho = 0.04$ (other parameters as for the original ASPC model). The homeostatic basin of attraction is now confined to the lower left region. The saddle lies on the homeostatic basin boundary. The simulations show that the system inevitably escapes from the homeostatic basin, typically in the vicinity of the saddle. After this the cell popula-
To characterise the escape event, I generate a large set of trajectories and compute an escape rate $u$ from the probability of remaining in the homeostatic basin [18]. Fig. 2 shows that $u$ decreases approximately exponentially with $N_0\Delta \rho^2$ [19]. The sensitive dependence on the target population size $N_0$ is reminiscent of system size effects found for other non-equilibrium phase transitions [20]. The sensitivity to the distance $\Delta \rho$ from the saddle-node bifurcation may reflect a quadratic dependence of the height of an ‘action’ barrier [21].

The concept of homeostatic metastability fits neatly into multi-stage models which are widely used to interpret cancer epidemiology [7, 8]. In a multi-stage model, cell lineages slowly transit through one or more precancerous stages before entering a final cancerous stage. The idea presented here is that homeostatic escape could be the final slow step after one or more somatic mutations have taken place, bringing, for instance, the system close to a saddle-node bifurcation. Note that, extrapolating from Fig. 2, the homeostatic escape rate can easily be comparable to multi-stage transition rates which are of the order $10^{-6}–10^{-4}$ yr$^{-1}$ [22]. In this context it is intriguing to note that the initial step in the development of skin cancer often appears to involve a mutation in the ras signalling pathway [23], which is important for controlling cell proliferation.

The mechanism of epithelial renewal in the lungs has much in common with that of the skin, although the turnover time may be somewhat longer [24]. Lung cancer might therefore be expected to have many features in common with skin cancer. A long-standing puzzle in multi-stage models of lung cancer has been the apparent indifference of the rate of the final step to the presence of a carcinogen (tobacco smoke) [25, 26, 27]. This epidemiology has been interpreted as indicating that a non-
mutagenic mechanism might be at work. One suggestion is that the final step is epigenetic in nature [26]. Another suggestion is that the final step somehow involves signalling [27]. Homeostatic metastability can be seen as a specific example of the latter signalling mechanism. As Frank challenges though [8], any such interpretation of the epidemiology should be supported by other evidence. Experiments that directly test the mechanism of homeostatic escape are therefore very desirable! A central feature of the present model, around which experiments may perhaps be designed, is that the cells themselves do not undergo any change if the system escapes from homeostasis; only the micro-environment changes.

Some general points can be made about directions for future research. Firstly, many of the arguments presented here are mean-field in nature. This key point was well appreciated by Klein et al. [6], who showed that many mean-field results still hold in a two-dimensional version of the original SPC model. The extension of the present ASPC models to fully-fledged two dimensional models is clearly a crucial next step. Another direction in which progress could be made is to improve the representation of the biology, for example moving to multi-scale [28], or agent-based models [29], which capture the detailed nature of individual cell fates.

A generic conclusion of the present study is that it may not be valid to examine just the deterministic consequences of somatic mutations, since rare stochastic events may occur at comparable rates. This makes the task of examining the behaviour of more biologically detailed models rather formidable. Brute force methods (i.e., lots of very long simulations) have been used in the present paper since the underlying stochastic processes are rather simple. This may not be possible for more complex models. Instead, it may be necessary to bring to bear more sophisticated techniques such as transition path sampling [30], or forward-flux sampling [31].

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