The phenotypic equilibrium of cancer cells: From average-level stability to path-wise convergence

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Abstract

The phenotypic equilibrium, i.e. heterogeneous population of cancer cells tending to a fixed equilibrium of phenotypic proportions, has received much attention in cancer biology very recently. In previous literature, some theoretical models were used to predict the experimental phenomena of the phenotypic equilibrium, which were often explained by the average-level stabilities of the models. Here we present a stochastic multi-phenotype branching model by integrating conventional cellular hierarchy with phenotypic plasticity mechanisms of cancer cells. Based on our model, it is shown that: (i) our model can serve as a framework to unify the previous models for the phenotypic equilibrium, and then harmonizes the different kinds of average-level stabilities proposed in these models; and (ii) path-wise convergence of our model provides a deeper understanding to the phenotypic equilibrium from stochastic point of view. That is, the emergence of the phenotypic equilibrium is rooted in the stochastic nature of (almost) each sample path, the average-level stability is just a direct result of it by averaging stochastic samples.
Figure 1: The phenotypic equilibrium of cancer cells. The figure is generated from the data (SW620 colon cancer cell line) in [7]. In this experiment, two cellular phenotypes were identified: cancer stem cells (CSCs) and non-stem cancer cells (NSCCs). It is shown that no matter where the initial state is (as four different cases shown in the figure), the CSCs proportion will converge to a fixed proportion as time passes. The same is true for NSCCs proportion. This phenomenon is termed the phenotypic equilibrium [6].

1 Introduction

Stability is ubiquitous in biology, ranging from physicochemical homeostasis in cellular microenvironments to ecological constancy and resilience [1, 2, 3]. It is noteworthy that not only can the stability phenomenon arise in normal living systems, but it can also happen in abnormal organisms such as cancer. As a large family of diseases with abnormal cell growth, cancer is generally acknowledged to be the malignant progression along with a series of stability-breaking changes (e.g. genomic instability) within the normal organisms [4]. However, some recent researches reveal the other side of cancer. An interesting phenotypic equilibrium was reported in some cancers [5, 6, 7]. That is, the population composed of different cancer cells will tend to a fixed equilibrium of phenotypic proportions over time regardless of initial states (Fig. 1). These findings provided new insights to the research of cancer heterogeneity.
The experimental works also stimulate theoreticians to put forward reasonable models for interpreting the phenotypic equilibrium \([6, 8, 9, 10, 11, 12, 13, 14]\). In particular, it was reported that the intrinsic interconversion between different cellular phenotypes, also called *phenotypic plasticity* \([15, 16]\), could play a crucial role in stabilizing the mixture of phenotypic proportions in cancer. As a pioneering work, Gupta *et al* proposed a discrete-time Markov chain model to describe the phenotypic transitions in breast cancer cell lines \([6]\). In their model, three phenotypes were identified: stem-like cells (S), basal cells (B) and luminal cells (L). The phenotypic transitions among them can be captured by the transition probability matrix as follows:

\[
P = \begin{pmatrix}
1 - P_{S\rightarrow B} & -P_{S\rightarrow L} & 0 \\
-P_{B\rightarrow S} & 1 - P_{B\rightarrow S} & -P_{B\rightarrow L} \\
0 & -P_{L\rightarrow S} & 1 - P_{L\rightarrow S} - P_{L\rightarrow B}
\end{pmatrix},
\]

where \(P_{i\rightarrow j}\) represents the probability of the transition from phenotype \(i\) to \(j\). According to the limiting theory of discrete-time finite-state Markov chain, there exists unique equilibrium distribution \(\vec{\pi} = (\pi_S, \pi_B, \pi_L)\) such that \(\vec{\pi} = \vec{\pi}P\) provided \(P\) is irreducible and aperiodic \([17]\). The chain will converge to \(\vec{\pi}\) regardless of where it begins. By fitting the Markov chain model to their experimental data, the equilibrium proportions of stem-like, basal and luminal cells were predicted by the equilibrium distribution \(\pi_S, \pi_B, \pi_L\) respectively.

Even though the Markov chain model fitted their experimental results very well, Zappperi and La Porta \([8]\) questioned the validity of the phenotypic transitions and gave an alternative explanation to the phenotypic equilibrium, which was based on the conventional cancer stem cell model with imperfect cancer stem cell (CSC) biomarkers. Moreover, Liu *et al* showed that the negative feedback mechanisms of non-linear growth kinetics of cancer cells can also control the balance between different cellular phenotypes \([18]\). Their works suggested that the phenotypic plasticity may not be the only explanation to the phenotypic equilibrium. To further reveal the mechanisms giving rise to the phenotypic equilibrium, it is more convincing to study the models integrating the phenotypic conversions with the conventional cellular processes of cancer. Motivated by this, a series of works discussed the phenotypic equilibrium by establishing the population dynamics coordinating with both hierarchical cancer stem cell paradigm and phenotypic plasticity \([9, 10, 11, 12, 13, 14]\). In these works, the phenotypic equilibria were intimately related to the stable steady-state behavior of the corresponding ordinary differential equations (ODEs) models. In other words, if one can model the dynamics of the phenotypic proportions as the following system of ODEs

\[
\frac{d\vec{x}}{dt} = \vec{F}(\vec{x}),
\]

the unique stable fixed point \(\vec{x}^*\) (if exists) corresponds to the equilibrium proportions.
The aforementioned works have showed that the phenotypic equilibrium can be explained by different types of stabilities in different models. Thus a natural question is whether there exists a unified framework to harmonize the equilibrium distribution of the Markov chain model and the stable steady-state behavior of the ODEs model. In this study, we try to discuss this issue by establishing a multi-phenotype branching process [19]. On one hand, our model integrates the phenotypic plasticity with the cellular processes (such as cell divisions) that have extensively been studied in biology. On the other hand, the model is stochastic and closer to the reality with finite population size [20][21]. Based on this model, it is shown that the ODEs model can be derived by taking average of our model. More specifically, the ODEs model is just the proportion equation of the multi-phenotypic branching model. Besides, the Markov chain model is also closely related our model. That is, the proportion equation can reduce to the Kolmogorov forward equation of continuous-time Markov chain provided that the growth rates of the whole population due to different phenotypes are the same. This result also implies the scope of application of the Markov chain model with phenotypic transitions. More importantly, by showing the almost sure convergence of our model, whether the stationarity of the Markov chain model or the stability of the ODEs model can be unified as the average-level stability of our model. Note that the almost sure convergence indicates the path-wise stability of stochastic samples, providing a more profound explanation to the phenotypic equilibrium. In other words, the phenotypic equilibrium is actually rooted in the stochastic nature of (almost) each path sample, the average-level stability is just a direct result of it by averaging all the stochastic samples. Furthermore, we can also show that, not only can the model with phenotypic plasticity give rise to the path-wise convergence, but the conventional cancer stem cell model without phenotypic plasticity can also lead to the convergence only if the net growth rate of cancer stem cells is larger than that of more committed cancer cells. This echoes the works by [8][13] that the phenotypic plasticity is not the only explanation to the phenotypic equilibrium.

The paper is organized as follows. The model is presented in Section 2. In Section 3, we discuss the relations of our model with the ODEs and Markov chain models. The path-wise convergence of our model is investigated in Section 4. Conclusions are in Section 5.

2 Model

In this section we describe the assumptions of our model. Consider a population composed of different cancer cell phenotypes. For pure theoretical investigations, the number of the phenotypes can be any \( n \) in general [13][22]. However, to better illustrate our theoretical results on the basis of more concrete biological background, enlightened by [6],
Table 1: Model description

| Reaction rate | Cellular mechanism    | Cellular reaction          |
|---------------|-----------------------|----------------------------|
| $\alpha_1$    | Symmetric division    | $S \xrightarrow{\alpha_1} S+S$ |
| $\alpha_2$    | Asymmetric division   | $S \xrightarrow{\alpha_2} S+B$ |
| $\alpha_3$    | Asymmetric division   | $S \xrightarrow{\alpha_3} S+L$ |
| $\beta_1$     | Symmetric division    | $B \xrightarrow{\beta_1} B+B$ |
| $\beta_2$     | De-differentiation    | $B \xrightarrow{\beta_2} S$ |
| $\beta_3$     | Phenotypic switching  | $B \xrightarrow{\beta_3} L$ |
| $\beta_4$     | Cell death            | $B \xrightarrow{\beta_4} \emptyset$ |
| $\gamma_1$    | Symmetric division    | $L \xrightarrow{\gamma_1} L+L$ |
| $\gamma_2$    | De-differentiation    | $L \xrightarrow{\gamma_2} S$ |
| $\gamma_3$    | Phenotypic switching  | $L \xrightarrow{\gamma_3} B$ |
| $\gamma_4$    | Cell death            | $L \xrightarrow{\gamma_4} \emptyset$ |

we here focus on the specific model consisting of three phenotypes: stem-like cells (S), basal cells (B) and luminal cells (L).

In Table 1 we list all the cellular processes included in our model. We can see that the model integrates the phenotypic plasticity mechanisms with the well-known cellular reactions in conventional cancer stem cell scenario [23]. For example, S cell can divide symmetrically into two identical S cells, and can differentiate into more committed cells (B cell or L cell) by asymmetric divisions as well. Meanwhile, for B cell (or L cell), besides symmetric division and cell death, it can also convert into the other two phenotypes by cell plasticity. In particular, we term the conversion from more committed cells (B cell and L cell) to stem-like cell the de-differentiation [24].

Based on the stochastic theory of cellular reaction systems (Chapter 11 in [25]), each cellular process in Table 1 happens with independent exponential reaction time. For example, the reaction “$S \xrightarrow{\alpha_1} S+S$” means that for a stem-like cell, it will live an exponential time with expectation $1/\alpha_1$, and then divides into two identical stem-like cells. Similar explanation can be made to other reactions. If we let $X_1(t), X_2(t)$ and $X_3(t)$ be the cell numbers of S, B and L phenotypes respectively, then $\dot{X}(t) = (X_1(t), X_2(t), X_3(t))^T$ can be modeled as a continuous-time multi-phenotype branching process [19]. If we define $\Pr(\vec{x}; t)$ be the probability of $\vec{X}(t) = \vec{x} = (x_1, x_2, x_3)^T$, according to the theory of Chemical Master Equation (CME), the rate of change of $\Pr(\vec{x}; t)$ is equal to the rate of transition from all the other possible states to $\vec{x}$ minus the rate of transition from $\vec{x}$ to all the other
possible states, i.e.
\[
\frac{d\Pr(\bar{x}; t)}{dt} = \sum_{\bar{x} \neq \bar{x}^*} T_{\bar{x} \to \bar{x}^*} \Pr(\bar{x}^*; t) - \sum_{\bar{x}^* \neq \bar{x}} T_{\bar{x}^* \to \bar{x}} \Pr(\bar{x}; t),
\]
(2)

where \( T_{\bar{x} \to \bar{x}^*} \) is the transition rate from \( \bar{x}^* \) to \( \bar{x} \) and \( T_{\bar{x}^* \to \bar{x}} \) is the transition rate from \( \bar{x} \) to \( \bar{x}^* \) (see [A] for more details).

In next section we will show that the ODEs model [11, 12] and Markov chain model [6] can be derived from our model. For convenience we term our multi-phenotype branching model the MPB model.

3 The relations of the MPB model, ODEs model and Markov chain model

3.1 Proportion equation of MPB model

To relate our MPB model to the ODEs model, we consider the mean dynamics of the MPB model by averaging all the stochastic samples of it.

Let \( \langle \bar{X}(t) \rangle \) be the expectation of \( \bar{X}(t) \), that is, for each component we define
\[
\langle X_i(t) \rangle := \sum_{\bar{x}} x_i \Pr(\bar{x}; t) \quad (1 \leq i \leq 3).
\]

We multiply \( x_i \) on the both sides of Eq. (2), and then calculate the summation over all \( \bar{x} \)
\[
\sum_{\bar{x}} x_i \frac{d\Pr(\bar{x}; t)}{dt} = \sum_{\bar{x}} x_i \left( \sum_{\bar{x}^* \neq \bar{x}} T_{\bar{x}^* \to \bar{x}} \Pr(\bar{x}^*; t) - \sum_{\bar{x}^* \neq \bar{x}} T_{\bar{x}^* \to \bar{x}} \Pr(\bar{x}; t) \right),
\]
then it is not difficult to obtain that
\[
\frac{d\langle \bar{X}(t) \rangle}{dt} = G\langle \bar{X}(t) \rangle,
\]
(3)

where
\[
G = [g_{ij}] = \begin{pmatrix}
\alpha_1 & \beta_2 & \gamma_2 \\
\alpha_2 & \beta_1 - \beta_2 - \beta_3 - \beta_4 & \gamma_3 \\
\alpha_3 & \beta_3 & \gamma_1 - \gamma_2 - \gamma_3 - \gamma_4
\end{pmatrix}.
\]

(4)

Since Eq. (3) describes the dynamics of the absolute numbers of different cellular phenotypes, we term it the number equation. However, we are more concerned about the
dynamics of the relative numbers (i.e. proportions) of different cellular phenotypes. So we let
\[ \vec{p}(t) = \frac{\langle \vec{X}(t) \rangle}{\langle X_1(t) + X_2(t) + X_3(t) \rangle} \]
be the proportion vector of cellular phenotypes. By converting \( \langle \vec{X}(t) \rangle \) to \( \vec{p}(t) \) in Eq. (3) we have the equation governing the phenotypic proportions as follows
\[ \frac{d\vec{p}(t)}{dt} = G\vec{p}(t) - \vec{p}(t)e^T G\vec{p}(t), \]
where \( e = (1, 1, 1)^T \). We term Eq. (5) the proportion equation. It is noteworthy that Eq. (5) just corresponds to the ODEs model investigated in [11]. The proportion equation thus connects the MPB model and the ODEs model, implying that ODEs model can be seen as the average-level counterpart of the stochastic dynamics of the MPB model.

3.2 Markov chain as a special case of the proportion equation

Note that the Markov chain model Eq. (1) is discrete-time and the MPB model is continuous-time, to compare the two models in the same time scale, we turn our attention from discrete-time Markov chain to continuous-time Markov chain. Consider the standard model of continuous-time Markov chain. That is, let \( P_i(t) \) be the probability of the Markov chain being in state \( i \) at time \( t \), its dynamics can be captured by the Kolmogorov forward equation:
\[ \frac{d\vec{P}(t)}{dt} = Q^T \vec{P}(t), \]
where \( Q \)-matrix \([ q_{ij} ]\) satisfying
\[ q_{ij} \geq 0 \ \forall i \neq j, \]
\[ q_{ii} = -\sum_{j:j \neq i} q_{ij}. \]

In the remaining part of this section we will show the relation of the Kolmogorov forward equation Eq. (6) and the proportion equation Eq. (5). If we let the sum of each column of \( G \) in Eq. (4) is the same, i.e.
\[ \alpha_1 + \alpha_2 + \alpha_3 = \beta_1 - \beta_4 = \gamma_1 - \gamma_4 = \kappa, \]
then Eq. (5) becomes
\[
\frac{d\bar{p}(t)}{dt} = (G - \kappa I)\bar{p}(t),
\]  
where $I$ is identity matrix. If we denote $G^* = (G - \kappa I)^T$, it can be shown that $G^*$ satisfies the conditions (7) and (8) for the $Q$-matrix (see [3]). In other words, the Kolmogorov forward equation Eq. (6) is a special linear case of the proportion equation Eq. (5). This indicates that the proportion dynamics of the MPB model can equivalently be captured by the distribution dynamics of the Markov chain model provided that the sum of each column of $G$ is the same.

It should be pointed out that the column sum of $G$ is of great biological significance. For example, note that in first column $\alpha_1$, $\alpha_2$ and $\alpha_3$ are the symmetric and asymmetric division rates of stem-like cells, so the sum of the first column $\alpha_1 + \alpha_2 + \alpha_3$ represents the growth rate of the population due to stem-like cells. That is, its column sum characterizes the contribution of stem-like cells to the whole population growth. Moreover, the sum of the second column is $\beta_1 - \beta_4$, corresponding to the net growth rate (symmetric division rate minus death rate). Note that the phenotypic transitions do not change the size of the whole population, i.e. $\beta_2$ and $\beta_3$ do not contribute to the growth of the whole population, $\beta_1 - \beta_4$ can also be seen as the contribution of basal cells to the whole population growth. Similar explanation can be made to luminal cells. Therefore, the condition of equal column sums of $G$ implies the scope of application of the Markov chain model, that is, the growth rates due to different cellular phenotypes should be the same. Otherwise, one should be more cautious about the validity of the Markov chain model. Similar result can be extended to more general cell population dynamics with any finite $n$ phenotypes, see another recent work [22] of ours.

4 Path-wise convergence of the MPB model

In last section we can see that the MPB model provides a unified framework for the ODEs model and Markov chain model. In this section, we will show path-wise convergence of the MPB model, which provides a much stronger concept of stability by which both the stable steady-state behavior of the ODEs model and the equilibrium distribution of the Markov chain model will serve as average-level stabilities of the MPB model.

Much attention has long been paid to the limit theorems of multi-type branching processes by mathematicians [26, 27, 28, 29]. Here we are not going to discuss the rigorous mathematical theory in general (which is the focus of our another work [22]). Instead we are more interested in the specific results with biological significance. Let
\[
\tilde{p}(t) = \frac{\tilde{X}(t)}{X_1(t) + X_2(t) + X_3(t)},
\]
we are concerned about the conditions under which \( \vec{p}(t) \) converges to a constant vector \( \vec{\mu} \). Note that \( \vec{p}(t) \) is stochastic, the “convergence” means \textit{almost sure convergence}. That is, if the convergence of \( \vec{p}(t) \) holds, almost all the stochastic paths will tend to a fixed equilibrium. We term it the \textit{path-wise convergence}. Before presenting our main results, we have two remarks:

1. The path-wise convergence of our concern is conditioned on \textit{essential non-extinction} \[23, 22\], see \textit{C} for more details on the definition of it. Here we only need to know that, to ensure the essential non-extinction of the process, it is sufficient to have the non-extinction of stem-like cells in our main theorems. Note that our model assumes no death of stem-like cells (see Table 1), the model will never become essentially extinct as long as the initial number of S cells is positive.

2. Since the phenotypic equilibrium characterizes the heterogeneity of cancer, we particularly care about when \( \vec{\mu} \) is positive, \textit{i.e.} all the components of \( \vec{\mu} \) are positive. In other words, we intend to find the conditions under which the equilibrium proportions of all the S, B, and L phenotypes are positive.

We present our main results in the following two theorems (see \textit{C} for the proofs):

**Theorem 1 (Case with phenotypic plasticity)** Assume \( G \) in Eq. (4) is positive, \textit{i.e.}, all the elements of \( G \) are positive, then \( \vec{p}(t) \) will tend to a fixed positive vector \( \vec{\mu} \) almost surely as \( t \to \infty \) provided that the initial number of S cells is positive.

**Theorem 2 (Case without phenotypic plasticity)** Assume that

1. all the phenotypic transition rates are zero, \textit{i.e.} \( \beta_2, \beta_3 \) and \( \gamma_2, \gamma_3 \) are zero;
2. \( \alpha_i > 0, \ i = 1, 2, 3; \)
3. \( \alpha_1 > \beta_4 \) and \( \alpha_1 > \gamma_4 \),

then \( \vec{p}(t) \) will tend to a fixed positive vector \( \vec{\mu} \) almost surely as \( t \to \infty \) provided that the initial number of S cells is positive.

The above two theorems are applicable to different cases. Theorem 1 assumes the positivity of \( G \), implying that the phenotypic plasticity mechanisms are accounted for in this case. In contrast, all the phenotypic plasticity mechanisms are not included in Theorem 2 since their reaction rates are zero. Even though their assumptions are basically different, both theorems can lead to the path-wise convergence. Fig. 2 and Fig. 3 illustrate Theorems 1 and 2 respectively by using stochastic simulations. In both cases, even though all the stochastic paths fluctuate at the beginning of the process, the proportions of S, B, and L cells eventually converge to their equilibrium proportions as time passes. Since the path-wise convergence indicates the stability of (almost) each stochastic sample, the convergence of the mean dynamics is just a direct result of it by taking average of all the stochastic samples (see lower panels of Fig 2 and 3). Note that both the Kolmogorov forward equation of the Markov chain and the ODEs model can be seen as the mean
Figure 2: Illustration of Theorem 1 by stochastic simulations. Upper panel shows the stochastic path-wise dynamics of the phenotypic proportions of S (blue), B (black) and L (red). The initial cell numbers of S, B and L cells are assumed to be 20, 0 and 0 respectively, that is, the initial proportions of S, B and L cells are 100%, 0% and 0%. Since Theorem 1 requires all the elements of $G$ are positive, we set $\alpha_1 = 0.7$, $\alpha_2 = 0.8$, $\alpha_3 = 0.6$; $\beta_1 = 1$, $\beta_2 = 0.2$, $\beta_3 = 0.25$, $\beta_4 = 0.1$; $\gamma_1 = 1$, $\gamma_2 = 0.15$, $\gamma_3 = 0.23$, $\gamma_4 = 0.12$. 30 stochastic samples for each phenotype were produced. It is shown that even though the stochastic paths fluctuate at the beginning of the process, the proportions of S, B and L phenotypes eventually path-wisely tend to their equilibrium proportions respectively. Lower panel shows the mean dynamics of the phenotypic proportions by averaging all the 30 samples shown in upper panel.
Figure 3: Illustration of Theorem 2 by stochastic simulations. Upper panel shows the stochastic path-wise dynamics of the phenotypic proportions. The initial states are the same as those in Fig. 2. According to the conditions of Theorem 2, we set $\alpha_1 = 0.9$, $\alpha_2 = 0.8$, $\alpha_3 = 0.6$; $\beta_1 = 0.6$, $\beta_2 = 0$, $\beta_3 = 0$, $\beta_4 = 0.2$; $\gamma_1 = 0.7$, $\gamma_2 = 0$, $\gamma_3 = 0$, $\gamma_4 = 0.3$. Similarly, 30 samples for each phenotype were produced. It is also shown that each stochastic path can converge to its equilibrium. Lower panel shows the mean dynamics.
dynamics of the phenotypic proportions, their stabilities just correspond to the average-level stabilities of the MPB model, which can be derived from the path-wise convergence. Moreover, in D we can see that, starting from different initial states, the process will still tend to the fixed equilibrium. In this way, the path-wise convergence provides a deeper understanding to the phenotypic equilibrium from the stochastic point of view.

As the end of this section, we need to explain the biological meanings of the assumptions in Theorem 2. The assumptions (1) and (2) together indicate the cellular hierarchy proposed by conventional cancer stem cell theory [23]. That is, cancer stem cells (S cells) are capable of self-renewal and differentiation into other more committed cancer cells (B and L cells) but not vice versa. In this way, cancer stem cells are at the apex of this cellular hierarchy. Furthermore, note that $\alpha_1, \beta_1 - \beta_4$ and $\gamma_1 - \gamma_4$ are all the diagonal elements of $G$ in Eq. (11), corresponding to the net growth rates of S, B and L cells respectively. The assumption (3) thus indicates that S phenotype is the dominant type in the population (see C). Therefore, Theorem 2 shows that the phenotypic equilibrium can still hold in the paradigm of conventional cancer stem cell theory only provided that S phenotype is dominant.

5 Conclusions

In this study, we have presented a multi-phenotypic branching model of cancer cells. On one hand, this model can serve as a underlying model from which the ODEs model and the Markov chain model can be deduced. On the other hand, the almost sure convergence of the model refreshes our understanding of the phenotypic equilibrium, from average-level stability to path-wise stability. Furthermore, our results have indicated that, even though the phenotypic plasticity facilitates the phenotypic equilibrium, it is not indispensable in some cases. It has been shown that the conventional cancer stem cell model can also stabilize the mixture of the phenotypic proportions, providing an alternative explanation to the phenotypic equilibrium.

Moreover, it should be noted that even though this work is focused on the issue of cancer, our methods can conveniently be used to more generalized cell population dynamics [22]. To further reveal the biological mechanisms of the phenotypic equilibrium, more detailed dynamic models of cancer cells are needed. For instance, the hypothesis of cooperation among cancer cells has been put forward [30]. In particular, self-sufficiency of certain growth signals of cancer cells supports the concept of mutualism and could be an important mechanism supporting the phenotypic equilibrium. Therefore, the models of capturing the interactions among cancer cells, e.g. evolutionary game models [31], could be a promising research direction in future. Furthermore, the genetic and epigenetic state networks [32, 33] of cancer will enable us to explore the molecular mecha-
mechanisms of the phenotypic equilibrium, which is poorly understood. The network methods have been used to investigate the processes of cellular pluripotent reprogramming \cite{34} and epithelial-mesenchymal transitions (EMT) \cite{35}. Note that EMT could play a key role in regulating the phenotypic heterogeneity in cancer \cite{36}, further studies on it should be another important tasks in future plans.

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**A Complete form of Eq. (2)**

Here we show more details about the master equation Eq. (2)

$$\frac{d\text{Pr}(\vec{x}; t)}{dt} = \sum_{\vec{x}^* \neq \vec{x}} T_{\vec{x}^* \rightarrow \vec{x}} \text{Pr}(\vec{x}^*; t) - \sum_{\vec{x}^* \neq \vec{x}} T_{\vec{x} \rightarrow \vec{x}^*} \text{Pr}(\vec{x}; t).$$

To obtain the complete form of Eq. (2), we need to confirm all possible $T_{\vec{x}^* \rightarrow \vec{x}}$ and $T_{\vec{x} \rightarrow \vec{x}^*}$ correspondingly. Based on the cellular processes listed in Table 1, we can calculate $T_{\vec{x}^* \rightarrow \vec{x}}$ and $T_{\vec{x} \rightarrow \vec{x}^*}$ correspondingly. For example, let $\vec{x}^* = (x_1 - 1, x_2, x_3)^T$, the event "$(x_1 - 1, x_2, x_3)^T \rightarrow (x_1, x_2, x_3)^T$", will happen if any one of "$S \overset{\alpha_1}{\rightarrow} S + S$" happens. Since the number of S cells is $x_1 - 1$ in current population, the transition rate of $(x_1 - 1, x_2, x_3)^T \rightarrow (x_1, x_2, x_3)^T$ should be $(x_1 - 1) \times \alpha_1$. On the other hand, the reaction "$S \overset{\alpha_1}{\rightarrow} S + S$" can also lead to the transition from $(x_1, x_2, x_3)^T \rightarrow (x_1 + 1, x_2, x_3)^T$ with rate $x_1 \times \alpha_1$. Along this way we can determine all the transition rates similarly. Therefore,

$$\sum_{\vec{x}^* \neq \vec{x}} T_{\vec{x}^* \rightarrow \vec{x}} \text{Pr}(\vec{x}^*; t) =$$

$$(x_1 - 1)\alpha_1 \text{Pr}(x_1 - 1, x_2, x_3; t) + x_1\alpha_2 \text{Pr}(x_1, x_2 - 1, x_3; t)$$

$$+ x_1\alpha_3 \text{Pr}(x_1, x_2, x_3 - 1; t) + (x_2 - 1)\beta_1 \text{Pr}(x_1, x_2 - 1, x_3; t)$$

$$+ (x_2 + 1)\beta_2 \text{Pr}(x_1 - 1, x_2 + 1, x_3; t) + (x_2 + 1)\beta_3 \text{Pr}(x_1, x_2 + 1, x_3 - 1; t)$$

$$+ (x_2 + 1)\beta_4 \text{Pr}(x_1, x_2 + 1, x_3; t) + (x_3 - 1)\gamma_1 \text{Pr}(x_1, x_2, x_3 - 1; t)$$

$$+ (x_3 + 1)\gamma_2 \text{Pr}(x_1 - 1, x_2, x_3 + 1; t) + (x_3 + 1)\gamma_3 \text{Pr}(x_1, x_2 - 1, x_3 + 1; t)$$

$$+ (x_3 + 1)\gamma_4 \text{Pr}(x_1, x_2, x_3 + 1; t),$$
and
\[ \sum_{\vec{x}^* \neq \vec{x}} T_{\vec{x} \rightarrow \vec{x}^*} \Pr(\vec{x}; t) = x_1 \sum_{i=1}^{3} \alpha_i \Pr(\vec{x}; t) + x_2 \sum_{i=1}^{4} \beta_i \Pr(\vec{x}; t) + x_3 \sum_{i=1}^{4} \gamma_i \Pr(\vec{x}; t). \]

**B  Proportion equation and Kolmogorov forward equation**

Here we show how the proportion equation of the MPB model relates to the Kolmogorov forward equation of continuous-time Markov chain. Consider the proportion equation Eq. (5)
\[ \frac{d\vec{p}(t)}{dt} = G\vec{p}(t) - \vec{p}(t)e^T G\vec{p}(t). \] (10)

If the column sums of $G$ are the same and equal to $\kappa$, i.e.
\[ \alpha_1 + \alpha_2 + \alpha_3 = \beta_1 - \beta_4 = \gamma_1 - \gamma_4 = \kappa, \]
then
\[ \frac{d\vec{p}(t)}{dt} = G\vec{p}(t) - \kappa\vec{p}(t) = (G^\ast)^T \vec{p}(t). \]

where $G^\ast = (G - \kappa I)^T$ and $I$ is identity matrix. For any $i \neq j$, it is easy to see that $g^\ast_{ij} = g_{ji}$. Note that all the off-diagonal elements of $G$ are non-negative, so $g^\ast_{ij} \geq 0$, satisfying the condition Eq. (7). Meanwhile, $g^\ast_{ii} = g_{ii} - \kappa$. Note that $\kappa = \sum_j g_{ji}$,
\[ g^\ast_{ii} = g_{ii} - \kappa = -\sum_{j \neq i} g_{ji} = -\sum_{j \neq i} g^\ast_{ij}, \]
satisfying the condition Eq. (8). Hence $G^\ast$ corresponds to the $Q$-matrix of a continuous-time Markov chain, whose Kolmogorov forward equation is just the proportion equation of the MPB model provided that the growth rates due to different phenotypes are the same.

**C  Proofs of Theorems 1 and 2**

The proofs of Theorems 1 and 2 are both on the basis of the Theorem 5 in another work [22] of ours. Since the Theorem 5 holds for the general multi-type branching processes with $n$ phenotypes, it naturally holds for our MPB model with three phenotypes:

\[ \text{As far as we know, the Theorem 5 in [22] requires minimal constraint to the path-wise convergence of our concern. However, it should be noted that technically our main results can also be proved based on the Theorem 3.1 in [28].} \]
Lemma 1 (Theorem 5 in [22]) Assume that the Perron-Frobenius eigenvalue $\lambda_1$ of $G$ in Eq. (4) is simple and positive. Conditioned on essential non-extinction, $\vec{p}(t)$ will tend to $\vec{\mu}$ almost surely as $t \to \infty$. $\vec{\mu}$ is the normalized right eigenvector of $\lambda_1$, which is non-negative.

For proving Theorems 1 and 2, we need to first explain the concept of essential non-extinction. We are not going to discuss the general mathematical definition of it (see Sec. 4.2 in [22]). In our MPB model, essential non-extinction specifically means non-extinction of the phenotype corresponding to the Perron-Frobenius eigenvalue $\lambda_1$. For Theorem 2, the assumption (3) implies that the Perron-Frobenius eigenvalue of $G$ is $\alpha_1$ (we will show this later). In other words, essential non-extinction here just means non-extinction of S phenotype. For Theorem 1, since $G$ is positive, it is possible for any two phenotypes to inter-convert to each other. In this case, non-extinction of one particular phenotype is equivalent to non-extinction of any phenotype. This implies that, no matter which phenotype corresponds to $\lambda_1$, to guarantee essential non-extinction, it is sufficient to assume non-extinction of any one phenotype. Therefore, if we can ensure non-extinction of S phenotype, essential non-extinction will hold in both theorems. Note that S cell can never die in our model, we only need to assume that the initial number of S phenotype is positive in both theorems.

We now prove the two theorem. On one hand, we need to show that $\lambda_1$ of $G$ is simple and positive in both theorems. On the other hand, since Lemma 1 only concludes the non-negativity of $\vec{\mu}$, we need to further show the positivity of $\vec{\mu}$. For Theorem 1, since we assume that $G$ is positive, according to the well-known Perron-Frobenius theory for positive square matrix [17], it is easy to show that $\lambda_1$ is simple and positive, and its normalized right eigenvector $\vec{\mu}$ is positive as well. In this way, by Lemma 1 we have $\vec{p}(t) \to \vec{\mu}$ almost surely as $t \to \infty$, and $\vec{\mu}$ is positive.

For Theorem 2, According to its conditions, $G$ reduces to

$$G = \begin{pmatrix} \alpha_1 & 0 & 0 \\ \alpha_2 & \beta_1 - \beta_4 & 0 \\ \alpha_3 & 0 & \gamma_1 - \gamma_4 \end{pmatrix}.$$  \hspace{1cm} (11)

Since it is a lower triangular square matrix, it is easy to get its eigenvalues: $\lambda_1 = \alpha_1$, $\lambda_2 = \beta_1 - \beta_4$, and $\lambda_3 = \gamma_1 - \gamma_4$. By assumptions (2) and (3), it is easy to obtain that $\lambda_1 = \alpha_1$ is the Perron-Frobenious eigenvalue, which is positive and simple. Meanwhile, the normalized right eigenvector of $\lambda_1$ is

$$\vec{\mu} = \frac{1}{Z} ([\alpha_1 - (\beta_1 - \beta_4)][\alpha_1 - (\gamma_1 - \gamma_4)], \alpha_2[\alpha_1 - (\gamma_1 - \gamma_4)], \alpha_3)^T,$$

\footnote{The Perron-Frobenius eigenvalue is the largest real eigenvalue of $G$ (see chapter 1 in [17]).}
where $Z$ is the normalized constant. To prove $\bar{\mu} > 0$, we only need to check that each component of $\bar{\mu}$ is positive:

1. $\alpha_1 > \beta_1 - \beta_4$ and $\alpha_1 > \gamma_1 - \gamma_4$ $\implies$ $[\alpha_1 - (\beta_1 - \beta_4)][\alpha_1 - (\gamma_1 - \gamma_4)] > 0$.

2. $\alpha_2 > 0$ and $\alpha_1 > \gamma_1 - \gamma_4$ $\implies$ $\alpha_2[\alpha_1 - (\gamma_1 - \gamma_4)] > 0$.

3. $\alpha_3 > 0$ $\implies$ $\alpha_3 > 0$.

This completes the proof.

### D Stochastic simulations for other initial states

Fig. D.4 shows the result in Theorem [1] with the initial states other than those in Fig. 2. We can see that even though starting from different initial states, Fig. D.4 predicts the same equilibrium proportions as Fig. 2. Similar result is shown in Fig. D.5 for Theorem 2.

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Figure 4: The initial cell numbers of S, B and L cells are assumed to be 20, 50 and 60 respectively. The parameters are the same as those in Fig. 2. That is, $\alpha_1 = 0.7$, $\alpha_2 = 0.8$, $\alpha_3 = 0.6$; $\beta_1 = 1$, $\beta_2 = 0.2$, $\beta_3 = 0.25$, $\beta_4 = 0.1$; $\gamma_1 = 1$, $\gamma_2 = 0.15$, $\gamma_3 = 0.23$, $\gamma_4 = 0.12$. 
Figure 5: The initial cell numbers of S, B and L cells are also assumed to be 20, 50 and 60 respectively. The parameters are the same as those in Fig. 3. That is, $\alpha_1 = 0.9$, $\alpha_2 = 0.8$, $\alpha_3 = 0.6$; $\beta_1 = 0.6$, $\beta_2 = 0$, $\beta_3 = 0$, $\beta_4 = 0.2$; $\gamma_1 = 0.7$, $\gamma_2 = 0$, $\gamma_3 = 0$, $\gamma_4 = 0.3$. 
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