Supplementary Information

Cancer stem cells as a proportion of the total tumour mass

The mathematical model described in Johnston *et al.*[23,24], as based on Fig. 1, assumes that the stem (respectively transit) cells either die, differentiate or renew at rates α_1, α_2 and α_3 (respectively β_1, β_2 and β_3), and that the fully-differentiated cells die and are shed into the lumen at a rate γ. The model equations for stem cells (N_0), transit cells (N_1) and fully-differentiated cells (N_2) are given by

$$\frac{\mathrm{d}N_0}{\mathrm{d}t} = \alpha N_0 - \frac{k_0 N_0^2}{1 + m_0 N_0}, \tag{1}$$

$$\frac{\mathrm{d}N_1}{\mathrm{d}t} = \beta N_1 - \frac{k_1 N_1^2}{1 + m_1 N_1} + N_0\left(\alpha_2 + \frac{k_0 N_0}{1 + m_0 N_0}\right), \tag{2}$$

$$\frac{\mathrm{d}N_2}{\mathrm{d}t} = -\gamma N_2 + N_1\left(\beta_2 + \frac{k_1 N_1}{1 + m_1 N_1}\right), \tag{3}$$

where $\alpha = \alpha_3 - \alpha_1 - \alpha_2$ and $\beta = \beta_3 - \beta_1 - \beta_2$. α and β represent the net per-capita growth rates of stem and transit cells, α_2 and β_2 represent the linear parts of the differentiation rates of stem and transit cells, and the terms involving k_0 and k_1 represent, respectively, the nonlinear feedback associated with stem and transit cells. m_0 and m_1 represent feedback saturation parameters, and γ is the per capita rate of fully-differentiated cell population removal.

In the exponential growth phase of the tumour, when the cell populations are very large, $N_0, N_1, N_2 >> 1$, so that we can approximate (1)–(3) by

$$\frac{\mathrm{d}N_0}{\mathrm{d}t} \approx (\alpha - k_0/m_0)N_0, \tag{4}$$

$$\frac{\mathrm{d}N_1}{\mathrm{d}t} \approx (\beta - k_1/m_1)N_1 + (\alpha_2 + k_0/m_0)N_0, \tag{5}$$

$$\frac{\mathrm{d}N_2}{\mathrm{d}t} \approx -\gamma N_2 + (\beta_2 + k_1/m_1)N_1. \tag{6}$$

These are linear ordinary differential equations that can be solved exactly to produce

$$N_0 = n_0 \mathrm{e}^{(\alpha - k_0/m_0)t} \qquad (7)$$

$$N_1 = A\mathrm{e}^{(\alpha - k_0/m_0)t} + (n_1 - A)\mathrm{e}^{(\beta - k_1/m_1)t} \qquad (8)$$

$$N_2 = B\mathrm{e}^{(\alpha - k_0/m_0)t} + C\mathrm{e}^{(\beta - k_1/m_1)t} + (n_2 - B - C)\mathrm{e}^{-\gamma t}, \qquad (9)$$

where n_0, n_1 and n_2 are the initial cell populations of stem, semi-differentiated and fully-differentiated cells, respectively, and constants A, B, C are given by

$$A = \frac{(\alpha_2 + k_0/m_0)n_0}{\alpha - k_0/m_0 - \beta + k_1/m_1}, B = \frac{(\beta_2 + k_1/m_1)A}{\alpha - k_0/m_0 + \gamma} \text{ and } C = \frac{(\beta_2 + k_1/m_1)(n_1 - A)}{\beta - k_1/m_1 + \gamma}. \qquad (10)$$

We note that numerical solution of the full system of equations (1)-(3) gives good agreement with the solutions (7)-(9) of the approximated equations (4)-(6). We now consider the two different cases of whether the cancer stem cell (CSC) originates from tissue stem or transit cells.

Firstly, when the CSC originates from the tissue stem cells, $e^{(\alpha - k_0/m_0)t}$ will be much greater than $e^{(\beta - k_1/m_1)t}$ and so the limiting ratio as $t \to \infty$ is given by $N_0 : N_1 : N_2 = n_0 : A : B$, which can be expressed as

$$N_0 : N_1 : N_2 = 1 : \frac{\alpha_2 + k_0/m_0}{\alpha - k_0/m_0 - \beta + k_1/m_1} : \frac{(\alpha_2 + k_0/m_0)(\beta_2 + k_1/m_1)}{(\alpha - k_0/m_0 - \beta + k_1/m_1)(\alpha - k_0/m_0 + \gamma)}. \qquad (11)$$

In particular, this yields the key ratio

$$\frac{N_0}{N_0 + N_1 + N_2} = \frac{1}{1+P}, \quad \text{where } P = \frac{\alpha_2 + k_0/m_0}{\alpha - k_0/m_0 - \beta + k_1/m_1}\left(1 + \frac{\beta_2 + k_1/m_1}{\alpha - k_0/m_0 + \gamma}\right). \qquad (12)$$

Secondly, when a transit cell is the CSC (or driving cell of the tumour), the stem cell population is in a steady state given by $N_0^* = \alpha/(k_0 - m_0\alpha)$. In this case the transit and fully-differentiated cell populations grow without bound while the last term in (5), representing stem cell differentiation, remains constant. The ratio of the populations in the limit as $t \to \infty$ is given by

$$N_0 : N_1 : N_2 = 0 : 1 : \frac{\beta_2 + k_1/m_1}{\beta - k_1/m_1 + \gamma}, \qquad (13)$$

and, in particular, the key ratio is given by

$$\frac{N_1}{N_1+N_2}=\frac{1}{1+P}, \quad \text{where } P=\frac{\beta_2+k_1/m_1}{\beta-k_1/m_1+\gamma}. \tag{14}$$

We note that qualitatively these results are unchanged if linear feedback is used instead of saturating feedback. For the stem cells, linear feedback corresponds to the case where $m_0 = 0$, and saturating feedback occurs when $m_0 \neq 0$.

CSCs from transit cells compared to fully-differentiated cells

Alternatively, we could consider the ratio of the transit cell population to the fully-differentiated cells during unbounded growth. Since we are only interested in the ratio N_1 / N_2 with the N_1 population driving the cancer, we assume that the stem cell population is constant to ease computation. We choose the constant $D=\alpha_2 N_0^* + k_0 (N_0^*)^2 / (1+m_0\, N_0^*)$ to represent the stem cell differentiation rate. Using equations (2) and (3), we get

$$\frac{\mathrm{d}}{\mathrm{d}t}\left(\frac{N_1}{N_2}\right)=(\beta+\gamma)\frac{N_1}{N_2}+\frac{D}{N_2}-\beta_2\left(\frac{N_1}{N_2}\right)^2-\frac{k_1 N_1{}^2}{N_2(1+m_1 N_1)}\left(1+\frac{N_1}{N_2}\right). \tag{15}$$

When N_1 and N_2 are large, corresponding to the exponential growth phase, this equation can be written approximately as

$$\frac{\mathrm{d}}{\mathrm{d}t}\left(\frac{N_1}{N_2}\right)=\left(\beta-\frac{k_1}{m_1}+\gamma\right)\frac{N_1}{N_2}-\left(\beta_2+\frac{k_1}{m_1}\right)\left(\frac{N_1}{N_2}\right)^2. \tag{16}$$

This can be solved directly to give

$$\frac{N_1}{N_2}=\frac{\beta-k_1/m_1+\gamma}{\beta_2+k_1/m_1+Ce^{-(\beta-k_1/m_1+\gamma)t}}, \tag{17}$$

where C is a constant. Therefore, as long as $\beta + \gamma > k_1/m_1$ (which is true in the exponential growth phase in the saturating feedback model), as $t \to \infty$ the limiting ratio is

$$\frac{N_1}{N_2} = \frac{\beta - k_1/m_1 + \gamma}{\beta_2 + k_1/m_1}. \tag{18}$$

Hence, the general ratio can be expressed as

$$\frac{N_1}{N_2} = \frac{\text{Net transit cell growth rate} + \text{Differentiated cell removal rate}}{\text{Maximum transit cell differentiation rate}}. \tag{19}$$