Angiogenesis: A Model of Cell Differentiation

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1 Abstract

Angiogenesis is the formation of blood vessels, and is of great importance in the growth of tumours. Attempts have been made to desgin experiments in petri-dishes that mimic the conditions of tumour growth. The first of the experiments is the 'matrigel' assay. Matrigel provides a matrix for the endothelial cells to grow on, and contains all the nutrients that the cells need. It is found that in the matrigel assay blood vessels didn't form, although some transient strucutres formed at early times in the experiment. The second experiment is the 'biocure' assay. In this experiment the petri dish is filled with both endothelial and fibroblast cells. The fibroblasts form a strucutal supporting network for the endothelial cells. Tubules resembling blood-vessels formed after about ten days in the biocure asssay.

The process of cell differentiation is thought to be important in the growth of blood vessels. Cells can sense that they are part of a blood vessel, and change their shape to form tubules. Also it is likely that they change their chemical messaging properties, and their abilities to bind to other endothelial cells.

A model is developed that describes cell differentiation, and separates cells into different classes. For simplicity the spatial distribution of cells in different classes is ignored. Using simple population dynamics, a set of coupled non-linear ODEs is developed to describe the dynamics of the system. The system is found to have two different long-time states, one corresponding to the formation of blood vessels and one where vessels did not form. The ratio of the cell proliferation rate to the cell maturity rate (the time it takes to realise that it is part of a blood vessel) is critical in determining which is the final state of the system.

2 Cell Classification

The endothelial cells at the start of each experiment were the same. However, by the end of the two experiments the cells had not only formed different structures, but the cells had different properties. The process by which cells change their properties is called differentiation. Cells with similar properties can be grouped together into classes. The aim of any model will be to describe mathematically how cells change from one class to another. First we have to define the different classes.

1. *Young and Single (class a)*: These are new cells that have just been formed, and can differentiate into other cell types at a later times. These cells have the ability to reproduce themselves, and to join other cells to form structures.

2. *Young and Structured (class b)*: Cells can move and link together to form desirable structures (i.e. blood vessels).

3. *Young and Clumped (class c)*: Sometimes large clumps of cells can form where each cell is joined to many neighbours. The cells can sense that this is an undesirable structure.

4. *Mature Structure (class d)*: When cells are joined together in a desirable structure for a long enough time the cells differentiate again. The structure they form becomes very stable, and is hard to break-up (i.e. blood vessels).

5. *Dead (class e)*: Cells can die either of old age, or because they have received a message telling them to die.

6. *Inactive (class f)*: If the density of cells in the petri dish becomes too high then the cells become inactive. These cells are alive, but are incapable of reproducing or forming structures.

Cells in different classes can undergo certain processes. These processes often involve the cells differentiating into another cell class. This allows a dynamical model of the different cell class populations to be developed. The processes modelled are:

1. *Proliferation*: Young single cells can sub-divide increasing the total cell population (with rate g).

2. *Structure Formation*: Cells release chemical messages that diffuse throughout the matrix. Other cells sense these chemical gradients, and move up them to join with other cells (with rate k_1).

3. *Structure Maturing*: When cells come into contact with each other receptors on opposing cell membranes link, sending messages to the nuclei of the cells. This causes the cells to differentiate (with rate k_3), and change their chemical properties. One effect of this differentiation is to make the structures stable, so it is hard for other cells to attach themselves to the structure (blood vessel).

4. *Clumping*: Before cells in structures have time to mature, it is possible for other cells to join onto the structure (with rate k_2) forming undesirable clumps of cells instead of desirable blood vessels.

5. *Clump Separation*: When cells form clumps together, receptors from many cell membranes are joined together. This can be sensed by the cell, which realises that this is an undesirable state to be in. Further chemical messages are realised telling the clump to separate (with rate k_4).

6. *Inactivity*: When the density of the cells becomes high the concentration of clumps of cell becomes high. Therefore the concentration of the chemical messager telling the clumps to separate will get very high. High concentrations of this chemical could act as a toxin that causes the cells to differentiate and become inactive (with rate k_6 if the clump population is above a threshold value).

7. *Inactivity Spreads*: Once an inactive region has been formed it acts as a sink, so that any other cells that accidently join it will become inactive themselves. This process can effect all cells (with rates k_7, k_8, k_9, k_{10}).

8. *Mature Structure Stability*: The receptors on the cell membranes of mature structures (blood vessels) will be in a form such that it is hard for additional cells to bind to the structure (thus making the structure stable). However, occasionally a cell will be able to bind in an undesirable place. The cell in the mature structure (blood vessel) will sense this, and will protect the structure by sending out a messages to kill the intruding cell and itself (with rate k_5). There is strong experimental evidence that a process similar to this takes place. Cells that have been cultivated such that they are almost immortal cannot form blood vessels, suggesting that cell death is important in the process of blood vessel formation.

The rate at which cells proliferate and structures mature, are assumed to be much slower than the rate at which single cells move to form structures. The rate at which mature cells kill themselves to protect the blood vessels is also very slow, as it is hard for single cells to attach to a mature structure.

3 Mathematical Model

A mathematical model of the above processes can be formed by using simple population dynamics. This gives a set of coupled non-linear ODEs which we can solve, to find the long-term behaviour of the system.

$$\begin{aligned}
\dot{a} &= ga - 2k_1a^2 - k_2ab + 3k_4c - k_9af - k_5ad \\
\dot{b} &= k_1a^2 - k_3b - k_2ab - k_{10}bf \\
\dot{c} &= k_2ab - k_4c - k_6H(c - c_{th}) - k_7cf \\
\dot{d} &= k_3b - k_8df - k_5ad \\
\dot{e} &= k_5ad \\
\dot{f} &= k_6H(c - c_{th}) + k_9af + k_{10}bf + k_7cf + k_8df
\end{aligned} \tag{1}$$

Note that the population of dead cells (e), decouples from the rest of the system, so can be disregarded in the analysis of the system.

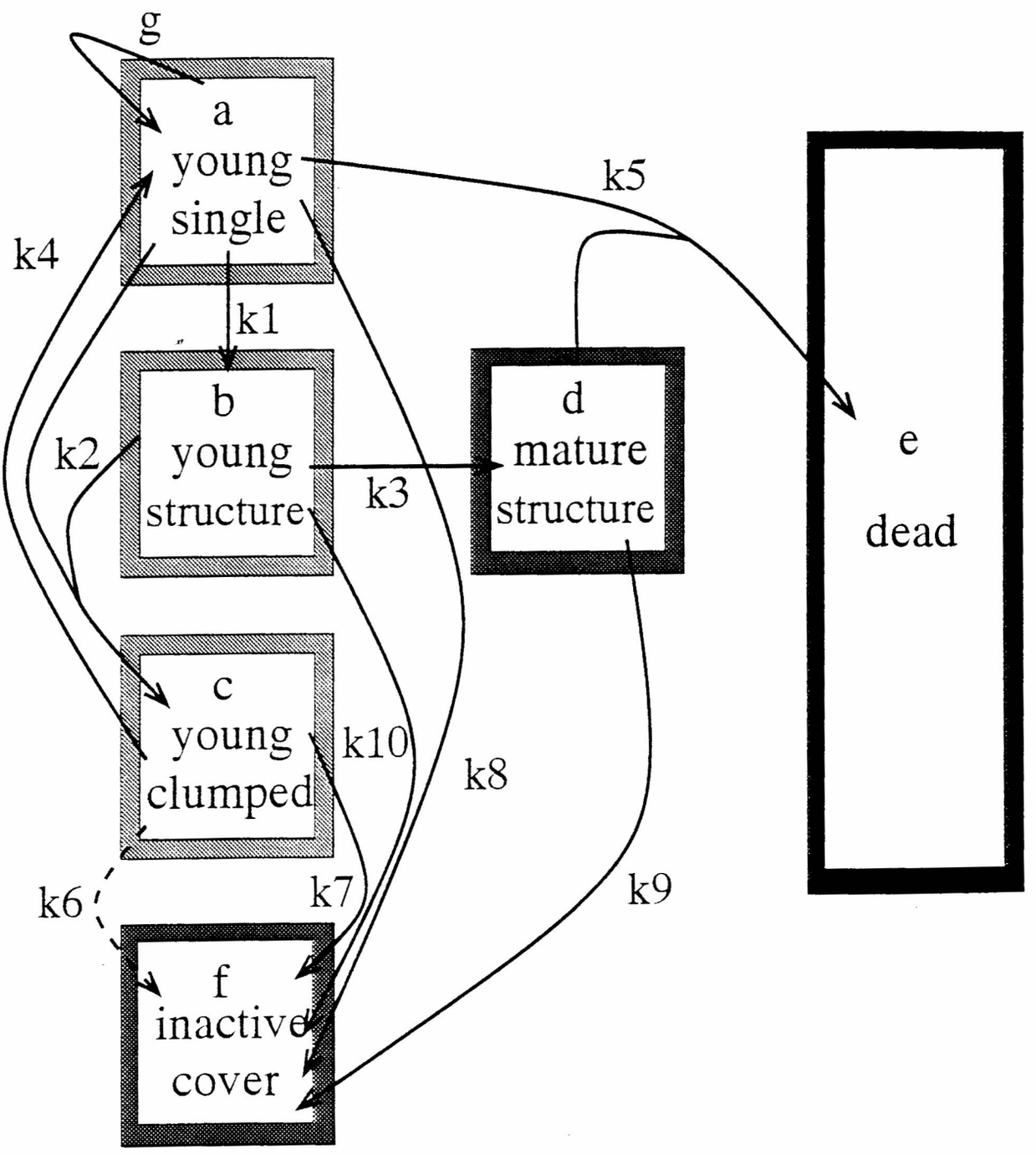

Figure 1: The processes and cells classes of the model.

3.1 Steady-State Analysis

The system has three obvious steady-states. Later when we take advantage of the separation of time-scales, we will show that these are the only steady-states in that limit.

3.1.1 All Dead

This is a state where all the cells have killed each other during the formation of structures. Mathematically it is given by:

$$a, b, c, d, f = 0 \tag{2}$$

We now want to determine the stability of this state. First we calculate the Jacobian matrix, and its eigenvalues (λ).

$$J = \begin{pmatrix} g & 0 & 3k_4 & 0 & 0 \\ 0 & -k_3 & 0 & 0 & 0 \\ 0 & 0 & -k_4 & 0 & 0 \\ 0 & k_3 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \end{pmatrix} \tag{3}$$

$$\lambda = g, -k_3, -k_4, 0, 0 \tag{4}$$

So for any positive proliferation rate ($g > 0$), this state is linearly unstable. This agrees with the experimental observation that this is never seen as a final state, providing the cells are fed and oxygenated.

3.1.2 All Inactive

This is a state when all the live cells are inactive (class f). This state is not a fixed point, but a 1-dimensional line in state-space. Mathematically it is given by:

$$a, b, c, d = 0 \bigcap f = f^* \tag{5}$$

Again, we want to calculate the stability of this state by calculating the eigenvalues of the Jacobian matrix.

$$J = \begin{pmatrix} g - k_9 f^* & 0 & 3k_4 & 0 & 0 \\ 0 & -k_3 - k_{10} f^* & 0 & 0 & 0 \\ 0 & 0 & -k_4 - k_7 f^* & 0 & 0 \\ 0 & k_3 & 0 & -k_8 f^* & 0 \\ k_9 f^* & k_{10} f^* & k_7 f^* & k_8 f^* & 0 \end{pmatrix} \tag{6}$$

$$\lambda = g - k_9 f^*, -k_3 - k_{10} f^*, -k_4 - k_7 f^*, -k_8 f^*, 0 \tag{7}$$

The eigenvector corresponding to the eigenvalue 0 is parallel to the f-axis in state-space. Therefore, any instability caused by this eigenvalue being equal to 0, will not move the system away from the manifold that describes the steady-state. Secondly we

should note that $\dot{f} \geq 0 \ \forall \ a, b, c, d, f \geq 0$ (i.e. for all physiologically and mathematical possible states). Therefore this state is a stable steady state iff:

$$f^* > \frac{g}{k_9} \tag{8}$$

This is the final state of the experimental set-up with the matrigel assay, where no blood vessels formed, and the petri dish was covered in a uniform layer of inactive cells.

3.1.3 Blood Vessels

This steady-state describes a system where all the cells are in stable mature structures (class d). This state is not a fixed point, but a 1-dimensional line in state-space. Mathematically it is given by:

$$a, b, c, f = 0 \ \bigcap \ d = d^* \tag{9}$$

Again, we want to calculate the stability of this state by calculating the eigenvalues of the Jacobian matrix.

$$J = \begin{pmatrix} g - k_5 d^* & 0 & 3k_4 & 0 & 0 \\ 0 & -k_3 & 0 & 0 & 0 \\ 0 & 0 & -k_4 & 0 & 0 \\ -k_5 d^* & k_3 & 0 & 0 & -k_8 d^* \\ 0 & 0 & 0 & 0 & k_8 d^* \end{pmatrix} \tag{10}$$

$$\Rightarrow \lambda = g - k_5 d^*, -k_3, -k_4, 0, (k_8 d^*) \tag{11}$$

For the same reason as in section 3.1.2, the 0 eigenvalue does not effect the stability of the manifold describing the steady-state. The eigenvalue $k_8 d^*$ seems to make the manifold unstable. However, this is not the case unless $c(t) > c_{th}$, since $f = 0$ until this threshold value has been reached. Therefore the steady-state is stable iff:

$$d^* > \frac{g}{k_5} \quad \text{providing that,} \quad c < c_{th} \ \forall \ t \tag{12}$$

This steady state corresponds to blood vessels forming in the petri-dish. This is the final state of the experimental set-up with the fibroblast assay, where blood vessels formed in the petri-dish.

3.2 Multiple-Scales Analysis

As mentioned in section 2, different processes occur on different time-scales. The time-scales involved in the movement and ordering of cells into structures, are much quicker than the time-scales involved with cell proliferation, cell maturing, and cell death. Note that this separation of time-scales implies that $\epsilon_i \ll K_j$, then:

$$\epsilon_g = \frac{g}{k_1}, \quad \epsilon_3 = \frac{k_3}{k_1}, \quad \epsilon_5 = \frac{k_5}{k_1}, \quad K_2 = \frac{k_4}{k_1}, \quad K_4 = \frac{k_2}{k_1}, \tag{13}$$

We will also assume that we are working with a system where $c < c_{th}\ \forall t$. Define T as the total number of young cells that are capable of forming blood vessels:

$$T \equiv a + 2b + 3c, \tag{14}$$

There has now been a separation of time-scales for different processes with $\dot{a}$, $\dot{b}$, $\dot{c} \sim O(1)$, while $\dot{T}, \dot{d}, \sim O(\epsilon)$. This allow us to solve the steady state equations for a, b, c, as functions of T, and d, then:

$$\begin{aligned}
\dot{T} &= (\epsilon_g - \frac{2\epsilon_3}{K_2} - \epsilon_5 d) A(T) \\
\dot{d} &= (\frac{\epsilon_3}{K_2} - \epsilon_5 d) A(T) \\
A(T) &= \frac{-(2K_4 + K_2K_4) + ((2K_4 + K_2K_4)^2 + 12K_4K_2^2T)^{\frac{1}{2}}}{6K_2} \\
&\text{note,}\quad A(T) > 0 \quad \forall T > 0
\end{aligned} \tag{15}$$

We can now look for other possible steady-states. For $\dot{T} = 0$, and $\dot{d}$, then one of the following conditions must be met:

$$a(T) = 0 \Rightarrow T = 0 \Rightarrow a, b, c = 0, \quad or, \quad \epsilon_g - \frac{2\epsilon_3}{k_2} - \epsilon_5 d = 0 \bigcap \frac{\epsilon_3}{k_2} - \epsilon_5 d = 0 \tag{16}$$

The first condition gives us the three steady-states we have already found, while the second condition imposes two conditions on d, so is impossible to obtain. Therefore, we have found the only 3 possible steady-states already. A second question that should be asked is whether there are oscillatory solutions. If we divide the equation for $\dot{T}$ by the equation for $\dot{d}$, we get an equation we can integrate to give $T(d)$. Therefore the evolution of the system can be described by a single first order ODE of one variable, so the solution cannot be oscillatory.

$$T = T_0 + d + \left(\frac{3\epsilon_3 - \epsilon_g K_2}{\epsilon_5 K_2}\right) \ln\left(1 - \frac{\epsilon_5 K_2 d}{\epsilon_3}\right) \tag{17}$$

We have imposed the boundary conditions that $T(0) = T_0$, and that $d(0) = 0$ (i.e. that at $t = 0$ no blood vessels have formed).

3.3 Long-term Behaviour

The important question we want to answer is which of the 2 stable steady-states the system ends up in for a given set of parameters. If the final state is $d = d^*$, then $T(\infty)$ must go to 0. If the final state is $f = f^*$, then in this approximation T will increase at large times (at high T, $c > c_{th}$, the current approximation breaks down). If the final state is $T = 0$, we can calculate d^* explicitly using equation 17.

$$0 = T_0 + d + \left(\frac{3\epsilon_3 - \epsilon_g K_2}{\epsilon_5 K_2}\right) \ln\left(1 - \frac{\epsilon_5 K_2 d}{\epsilon_3}\right) \tag{18}$$

This is a transcendental equation. Since $T_0 > 0$, we can immediately see that for a solution d^* to exist given that T is always finite, then:

$$\frac{\epsilon_g}{\epsilon_3} < \frac{3}{K_2} \tag{19}$$

So the ratio of the proliferation rate to the maturity rate is crucial in determining whether or not this fixed point exists. Secondly, if a solution exists, then for all $0 < T_0 < \epsilon_3/\epsilon_5$, then $d^* > d_c$ if:

$$d_c \geq -\left(\frac{3\epsilon_3 - \epsilon_g K_2}{\epsilon_5 K_2}\right) \ln\left(1 - \frac{\epsilon_5 K_2 d_c}{\epsilon_3}\right) \tag{20}$$

If a solution exist, then for it to be the long-time solution it must be linearly stable. Evaluating the eigenvalues of the Jacobian matrix of the reduced system, gives us the stability condition that:

$$d^* > \frac{\epsilon_g K_2 - 2\epsilon_3}{\epsilon_5 K_2} \tag{21}$$

This gives us d_c, so the fixed point is stable iff:

$$\frac{\epsilon_g K_2 - 2\epsilon_3}{\epsilon_5 K_2} \geq -\left(\frac{3\epsilon_3 - \epsilon_g K_2}{\epsilon_5 K_2}\right) \ln\left(1 - \frac{\epsilon_5 K_2 \frac{\epsilon_g K_2 - 2\epsilon_3}{\epsilon_5 K_2}}{\epsilon_3}\right) \tag{22}$$

This equation is simplified by defining the quantity y, and using the fact that $\epsilon_3 > 0$, then:

$$1 \geq y(1 - ln(y)), \qquad \text{where,} \quad y \equiv 3 - \frac{\epsilon_g K_2}{\epsilon_3} \tag{23}$$

Note, that for d^* to exist then $0 < y < 3$. On this range the largest value that y can take is 1, so the inequality is never broken. Therefore if d^* exists, it is linearly stable.

4 Conclusions

A model was constructed to describe cell differentiation. The model had 2 possible long-time steady-states: one where the petri-dish would be covered by inactive cells (the matrigel assay); and, one where blood vessels would be formed (the biocure assay). The ratio of the proliferation rate to the maturity rate of the cells was found to be critical in determining which was the final state. If the proliferation rate is too rapid then the petri-dish will be covered with inactive cells. If the proliferation rate is less than a critical value, blood-vessels can be formed. Matrigel is rich in nutrients, and provides a substrate for the cells. These two factors will increases the proliferation rate, suggesting that it would be hard for blood vessels to form. In the biocure assay the proliferation rate is less, so blood vessels are more likely to form.