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A Diffusion-Reaction Model for Producing a Suitable Environment for Stem Cells in Tissues

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Since 2000, studies have proven the existence of a stem cell niche necessary for stem cell maintenance in various tissue types. Despite a lack of consensus on the microenvironment structures of stem cell niches, strong evidence indicates that stem cells, most cancer cells, and proliferating cells are likely to survive in acidic and hypoxic microenvironments. In this study, we use diffusion-reaction equations for the lactic acid and oxygen concentrations to simulate glycolysis and oxidative phosphorylation reactions in tissues. We find the diffusion-reaction model can produce and sustain the right micro-environment for stem cells. We chose three sets of cell and capillary densities (the two most important model parameters) to respectively represent the heart, liver, and skin tissues. Simulation results are qualitatively consistent with the experimental observations and provide a possible explanation for the high frequency of epithelium-origin cancer and the low frequency of cardiomyocyte-origin cancer.

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I. INTRODUCTION

The stem cell niche was proposed [1] in 1978 and first observed [2] in 2000. It has since been found in various types of tissue [3]. It is a micro-environment which hosts only a limited number of stem cells which can produce progenitor cells, but the progenitor cells cannot further differentiate into mature cells until they leave the stem cell niche [4]. Potential applications for the stem cell niche in regeneration and cancer therapy have attracted considerable attention. However, a decade of effort has yet to produce a consensus in regards to the structure of the stem cell niche [5]. Since the stem cell niche is believed to provide a stable microenvironment for stem cell maintenance, the priority is to determine what environments are suitable for stem cells.

Stem cells are usually observed in environments characterized by low pH value and low pO_2 tension [6]. To differentiate into mature cells, the progenitor cells move to regions characterized by higher pH and higher pO_2 tension [6]. This movement from a lower pO_2 tension to higher pO_2 tension area corresponds to the switch from glycolysis mode to the oxidative phosphorylation (OXPHOS) mode, where glycolysis and OXPHOS are the two main metabolic modes for all animal cells. Evidence has also shown that the non-

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transformed proliferating cells and tumor cells prefer to use glycolysis mode, even given adequate oxygen tension [7, 8], a phenomenon known as aerobic glycolysis or the Warburg effect. Aerobic glycolysis is believed to play an important role in tumor growth and carcinogenesis. The reactions in glycolysis and OXPHOS modes indicate that lactic acid is one of the net products of glycolysis. The main reactions of glycolysis and OXPHOS are listed in Table I [9].

TABLE I: Main reactions of glycolysis and OXPHOS [9].

I. glycolysis *(in cytosol)*
(1) *1 glucose* $\rightarrow$ *2 pyruvate + 2NADH + 2ATP*
(2) *2 pyruvate* $\rightarrow$ *2 lactic acid*
II. OXPHOS *(oxidative phosphorylation, in mitochondria)*
(3) *2 pyruvate* $\rightarrow$ *8NADH + 2FADH*$_2$ *+ 2 GTP*

Here we focus on the roles played by lactic acid and oxygen for the existence of stem cells. We simulate lactic acid and oxygen concentrations in the various tissue types using a diffusion-reaction equation that describes the glycolysis and OXPHOS processes. We find that a suitable microenvironment for stem cells, characterized by low pH values and low pO_2 tension, naturally exists in the tissues. That is, unlike the cases simulated in Ref. [4], the stem cell niches in mammalian tissues may not necessarily have a definite geometric structure. A stem cell niche is any place in the tissue that provides and sustains the right environment through chemical reaction and diffusion.

II. THE REACTION-DIFFUSION MODEL

Many reactions are needed to describe the glycolysis and OXPHOS processes listed in Table I. There is evidence that these two processes mutually switch depending strongly on the pH value and oxygen tension, while the change in lactic acid and oxygen concentrations depends on the relative strength of these two processes [7, 8]. Here we focus on the changes of lactic acid and oxygen in the tissues. Through a series of lengthy but appropriate simplifications [see Appendix], we arrive at the following set of partial differential equations:

$$\begin{aligned} \partial u/\partial t &= D_u \nabla^2 u + \rho u^2 v - eu, \\ \partial v/\partial t &= D_v \nabla^2 v - 2\rho u^2 v + e, \end{aligned} \tag{1}$$

where u and v respectively stand for the concentrations of lactic acid and oxygen. The first term on the right hand side of the equations describes the diffusion of the lactic acid and oxygen in the tissues with respective diffusion constants D_u and D_v. The interaction between the lactic acid (u) and oxygen (v), characterized by the non-linear term u^2v, tends to increase the amount of lactic acid while decreasing oxygen. The strength of the

interaction is controlled by the cell density ρ. In addition to this interaction, oxygen is supplied at a rate determined by the capillary density e, and the lactic acid is degraded through the tissues in proportion to its concentration. Aside from the diffusion constants, this simple model only keeps two important parameters: ρ and e. Oxygen diffuses much more quickly than the lactic acid in the tissues. Based on the experimental values reported in references [11] and [12], we set $D_v = 20D_u$ in our analysis and simulations.

The results of the standard linear analysis [13] for the reaction-diffusion equation (1) show that stable patterns of u and v exist in the parameters range $2 < \rho/e < 8$ for $D_u > 0$. One can also easily find that the fixed points (for the cases without diffusion) are given $u_0 = 0.5$ and $v_0 = 2e/\rho$. Thus the distributions of lactic acid (u) and oxygen (v) is solely dependent on the ratio of the cell and capillary density, ρ/e, once D_u is given.

III. MODEL SIMULATION RESULTS

This report aims to show that an environment suitable for stem cells can be stably sustained in tissues through a diffusion-reaction process. For the sake of simplicity the diffusions of lactic acid and oxygen were only considered in a one-dimensional space. We arbitrarily set $D_u = 4.2$ and $e = 0.1$ and found the stable distributions of the lactic acid (u) and oxygen (v) in space. The results for three different values of ρ/e between 2 and 8 are plotted in Fig. 1. The lactic acid and oxygen distributions "oscillate" near their fixed values u_0 and v_0 respectively along the space. From Fig. 1 we see that, for tissues with a higher ρ/e value, the environment will have a smaller oxygen density, and both the lactic acid and oxygen concentrations vary more slowly with respect to position.

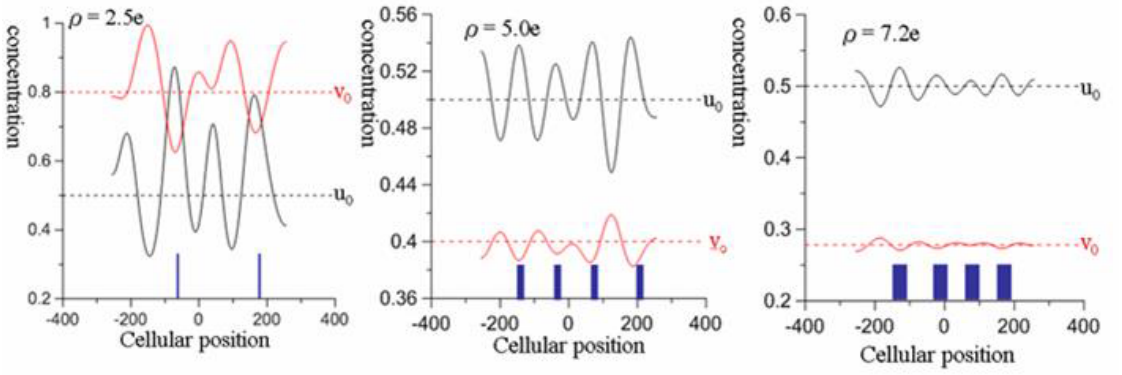

FIG. 1: Concentration of lactic acid (u, black curve) and oxygen (v, red curve) as a function of cell position. $e = 0.1$ and $D = 4.2$. Tissue regions marked in blue are stem cell niches where $u > u_0$, $v < v_0$, and the concentration gradients are small.

Before discussing the results of Fig. 1, we first present experimental data for cell and capillary density. Table II lists the cell sizes and capillary density collected from Refs. [14–18] for epithelium (skin), hepatocyte (liver), and cardiomyocyte (heart) cells. We estimate

the relative values of ρ/e among skin, liver, and heart cells as follows:

$$\frac{\rho/e\ (\text{skin})}{\rho/e\ (\text{heart})} \approx 6^2 \times 90 \times (2400)^{\frac{3}{2}}/5^2 \times 65 \times (100)^{\frac{3}{2}} = 235, \tag{2}$$

$$\frac{\rho/e\ (\text{liver})}{\rho/e\ (\text{heart})} \approx 6^2 \times 90 \times (2400)^{\frac{3}{2}}/15^3 \times (700)^{\frac{3}{2}} = 6. \tag{3}$$

TABLE II: Cell size and capillary density of skin, liver, and heart [14–18].

Cell types	**Cell size**	**Capillary density**
Cardiomyocyte (heart)	*Diameter* 10–15 um *Length* 80–100 [14] um	2400 [16]/mm^2
Hepatocyte (liver)	*Diameter* 30 [15] um	700 [17]/mm^2
Epithelium (skin, columnar)	*Diameter*10 um *Length* 65 [14] um	100 [18]/mm^2

We see that the order of ρ/e values for these three tissues is

$$\rho/e\ (\text{heart}) < \rho/e\ (\text{liver}) < \rho/e\ (\text{skin})\ . \tag{4}$$

The values of ρ/e used in our simulations for producing stable patterns shown in Figs. 1(a), 1(b), 1(c) are in ascending order of size, as in Eq. (4). Thus, although their values cannot be directly compared in size with the experimental data, we assume that the concentration patterns of the lactic acid and oxygen in the heart, liver, and skin tissues can be qualitatively represented by Figs. 1(a), 1(b), and 1(c), respectively. Comparing these three figures, we see the average oxygen concentration is reduced as ρ/e increases in the tissues. This is qualitatively consistent with the experimental fact that, as shown in Table III, the oxygen threshold is the largest for the heart tissues and smallest for the skin tissues [8].

TABLE III: Oxygen threshold for different tissues [8].

Tissue type	**Normoxic pO_2 level (%)**
Heart	17.7
Liver	10.0
Skin	5.02

As previously noted, stem cells prefer an environment characterized by low pH and low pO_2 tension [6]. In our reaction-diffusion model for lactic acid and oxygen concentrations,

we expected that tissue regions with $u > u_0$ and $v < v_0$ would be suitable for stem and progenitor cells, and the regions with $u < u_0$ and $v > v_0$ suitable for differentiated cells. In addition, it is widely accepted that the gradient of growth factors plays an important role in inducing differentiation [9]. That is, our model has a large gradient of lactic acid and oxygen concentrations, which will easily induce stem cells to differentiate into mature cells. For our simulation results shown in Fig. 1, the concentration gradients (both for u and v) change more abruptly in Fig. 1(a) than in Figs. 1(b) and 1(c). Thus we expect that for the heart tissues (represented by Fig. 1(a)), only the small regions near the local maxima of the lactic acid are suitable for stem cells, while for the skin tissues (represented by Fig. 1(c)), stem cells can exist in most regions in the tissues. Notice that, given an excess of stem cells in tissues not differentiating into mature cells, tissues may easily develop cancers. From our simulation results for the heart, liver, and skin tissues (Fig. 1), the relatively small ρ/e value of the heart tissue allows for only a small number of stem cells, thus reducing the incidence of cancer. On the other hand, skin cancers are more likely to occur, because, as represented in Fig. 1(c), the environment in skin tissues is more favorable to stem cells. These results are consistent with clinical observations of the high frequency of epithelium-origin cancer and the near absence of cardiomyocyte-origin cancer.

IV. CONCLUSION

In summary, we used a diffusion-reaction equation to simulate the glycolysis and OXPHOS processes in various tissues. We found that a microenvironment characterized by low pH, low pO_2 tension, and small concentration gradients is conducive to the existence of stem cells in the tissues. Our diffusion-reaction equation used only two important parameters to characterize tissue type: the cellular density ρ and the capillary density e. Tissues with a small ρ/e value (e.g., heart tissue) were found to have a small niche with only a few stem cells, and stem cells outside the niche will differentiate into mature cells. On the other hand, tissues with large ρ/e values (e.g., skin tissue) have a large region suitable for stem cells and thus may contain a large number of stem cells that do not differentiate into mature cells. Since an excess of stem cells which do not differentiate into mature cells can lead to the development of cancer in the tissue, our model provides an explanation for the near total lack of cardiomyocyte-origin cancer and the high frequency of epithelium-origin cancer.

APPENDIX A

Here we describe briefly some key steps in obtaining Eq. (1). Further details can be found in Ref. [10].

In the glycolysis mode, glucose (denoted by a) produces pyruvate (P) and NADH

(N):

$$a \rightarrow 2P + 2N. \tag{A1}$$

and the interaction between the pyruvate (P) and NADH (N) in turn yields lactic acid (u):

$$P + N \rightarrow u. \tag{A2}$$

When the OXPHOS mode dominates, the pyruvate (P) will change into NADH (N) plus other chemicals:

$$P \rightarrow N + \ldots \tag{A3}$$

All reactions proceed with the presence of the oxygen (v) which is supplied by the source (d) and reacts with NADH (N) to produce water and NAD^+:

$$2N + v + 2H^+ \rightarrow 2\mathrm{H_2O}{+}2\mathrm{NAD}^+ \tag{A4}$$

Given these reactions, we can write down the production rates of u, v, P, and N as

$$\partial u/\partial t = kNP - eu, \tag{A5}$$

$$\partial v/\partial t = -cN_2v + d, \tag{A6}$$

$$\partial P/\partial t = a - bP - kNP, \tag{A7}$$

$$\partial N/\partial t = a + bP - kNP - cN_2v, \tag{A8}$$

where in (A5) we have added the term $-eu$ to reflect the fact that the lactic acid decreases through capillaries with a rate proportional to its total amount.

We now focus on the variations of u and v. The interaction terms NP and N_2v in (A5) and (A6) can be rewritten in terms of $\partial P/\partial t$ and $\partial N/\partial t$ with the help of (A7) and (A8). According to the strengths of the glycolysis and oxphos modes, which dictate the relative strength of the interaction constants, these two interactions NP and N_2v can be approximated by an effective term u_2v. That is (A5) and (A6) can be written as the coupled differential equations:

$$\partial u/\partial t = c_1\, u_2v - eu, \tag{A9}$$

$$\partial v/\partial t = -c_2\, u_2v + d, \tag{A10}$$

where the term u_2v shows that the lactic acid is more influentially than the oxygen in affecting the change of their quantities.

Finally, arbitrary constants $c1$, $c2$, and d relative to the strengths e are chosen for the investigation in this article. Together with the diffusion terms for u and v, we have arrived at Eq. (1).

Eq. (1) is a very much simplified, and maybe even over-simplified, equation for describing the glycolysis and oxphos processes. We also lack experimental justification in choosing interaction constants in our model. Nevertheless, we have seen the reaction-diffusion equations have been successfully in describing the formation mechanism for some biological systems. In this research we attempt to explore how a reaction-diffusion model can be implemented in studying cancer formation.

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