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Mathematical Modeling of Tumor Growth and Metabolism

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DEDICATION

To Mom, Dad (1941-2013), Hang, and Dinh
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The objective of this work is to use mathematical modeling to understand the mechanisms that regulate tumor growth and metabolism. A hallmark of cancer is cell-intrinsic metabolic reprogramming that enhances anabolic support of cell proliferation and leads to metabolic heterogeneity in tumors at larger scales. Here we report a self-organizing pattern of metabolism in xenograft colon tumors, where Pyruvate Dehydrogenase Kinase (PDK1), a negative regulator of oxidative phosphorylation, is highly active in clusters of cells arranged in a spotted array. To explore the basis for this pattern, we developed reaction-diffusion models describing the interactions between two different metabolic cell types, nutrients, and growth factors. Our first model is based on Gierer-Meinhardt’s activator-depleted substrate model and characterizes the relationship between cells, glucose, and lactate. In the other mathematical models reported here, we incorporated Wnt, a signal known to upregulate PDK1 and Warburg metabolism, so that Wnt promotes a metabolic switch to glycolysis. Diffusive instability analysis, or classic Turing analysis, is used to determine parameter sets in which patterns are expected to form and is found to be in good agreement with numerical simulations. Partial inhibition of Wnt alters the pattern in tumors and in the model, which also predicts that inhibition of Wnt alters the expression of proteins that increase the range of Wnt ligand diffusion. This prediction is validated in xenograft tumors and is consistent with expression data in primary human colon cancer. The model also
predicts that inhibitors that target glycolysis or Wnt signaling are not so effective as single therapies for cancer as they are in combination for synergistic reduction of tumor growth. We present another mathematical model similar to the Wnt signaling model, but which includes other factors known to be involved in cancer metabolism. This larger mathematical model and numerical results demonstrate good agreement with the Wnt signaling model, and we therefore conclude that the smaller model contains all essential elements necessary to capture the effects we observe, to make predictions for the mechanisms that cause the altered metabolic pattern in Wnt-inhibited tumors, and to investigate treatment programs in silico. It is hoped that these mathematical models will be useful in informing researchers and collaborators about cancer and metabolism.
Introduction

Understanding how cancer cells metabolize nutrients for energy is an important step toward developing new treatment programs and eradicating this disease. One hallmark of cancer is reprogrammed metabolism [4], and it has been observed for decades that many cancers exhibit an altered cellular energy metabolism compared to normal, noncancerous cells [28]. In normal differentiated tissues in the human body and in the presence of oxygen, glucose is completely oxidized to CO$_2$ through the TCA cycle and oxidative phosphorylation. In the absence of oxygen, such as in muscle cells during exercise, glucose metabolism is limited to glycolysis and is fermented into lactate. In cancer cells or proliferating cells, even in the presence of oxygen, the cells continue to limit their metabolism to glycolysis and fermentation to lactate [28]. This type of metabolism, called aerobic glycolysis or Warburg metabolism, is counterintuitive, as glycolysis results in the waste product lactic acid, which is harmful to most cells. Moreover, glycolysis is much less efficient than oxidative phosphorylation in producing adenosine triphosphate (ATP), a unit of energy transfer. The prevailing hypothesis for this altered metabolism is that cancer cells have to support rapid cell division which requires biomass production, and that this pathway is sluggish enough to allow byproducts to be siphoned off [26], [17]. Lactate is also pro-angiogenic, thus increasing nutrient supply to the tissue [6].

However, it has been observed that not all cancer cells exhibit a completely glycolytic phenotype: Spatio-temporal microenvironmental factors are known to play a role in cellular
metabolism. At least two examples of a magnificent symbiotic relationship between cells un-
dergoing glycolysis and cells undergoing oxidative phosphorylation have been hypothesized. These are described in more detail in the next chapter. We aim to model the interplay between these different cell types, nutrients, and various growth and signaling factors to gain new insight into cancer metabolism.

The remainder of this thesis is organized as follows. The first chapter describes some basic background in the biology of reprogrammed metabolism in cancer cells. In the second chapter we investigate evidence that provides another profound example of metabolic symbiosis in cancer, in the form of a striking spotted pattern in immunohistochemical (IHC) stains of xenograft tumors of colon cancer cell line SW-480. We discuss a mathematical model that was built on the Gierer-Meinhardt activator-depleted substrate model which describes the relationship between populations of glycolytic and oxidative cell types, glucose, and lactate. There is good qualitative agreement between the model and IHC data, with some limitations that are addressed in the next chapter. In Chapter 3 another mathematical model is described in which the relationship between the Wnt signaling pathway and a Wnt inhibitor is the mechanism that induces the spotted pattern. This model was built on the Gierer-Meinhardt activator-inhibitor model, where Wnt activates a Wnt inhibitor (e.g., SFRP5). The numerical results are qualitatively similar to experimental data. We also perform diffusive instability analysis, or classic Turing analysis, for the Wnt signaling model. This analysis is useful for determining parameter sets in which patterns are expected to form, and was found to be in good agreement with numerical simulations. Predictions were made with this model for how Wnt and Wnt inhibitor activity is altered in tumors in which Wnt signaling was interfered with, which were subsequently validated by further experiments. Simulations of various therapy regimes are also presented in this chapter, and it is predicted that a combination of drugs that target Wnt signaling and metabolism may be highly effective. In Chapter 4, another mathematical model that characterizes the pattern of IHC stains is presented. This model is similar to the one presented in Chapter 3, but includes
other factors that are known to be involved in cancer metabolism. The mathematical model
and numerical results are presented and demonstrate good agreement with the Wnt signaling
model from Chapter 3, and we therefore conclude that the Wnt signaling model contains all
essential elements needed to describe the metabolic symbiosis we observe.
Chapter 1

Cancer and Metabolism

A hallmark of cancer is reprogrammed metabolism [4], and a better understanding of the processes and mechanisms that cause this altered metabolism can be useful in developing new treatment programs. This chapter discusses some of the biology in cancer and metabolism as background for the sections that follow.

1.1 Warburg Metabolism

Normal, noncancerous cells typically undergo the process of oxidative phosphorylation (OX-PHOS) to metabolize nutrients in environments with normal oxygen concentrations, but it has been observed for decades that many cancers exhibit an altered cellular energy metabolism. The Warburg Effect, also referred to as aerobic glycolysis and discovered by Nobel Prize winner Otto Warburg in 1956 [28], describes one form of this alteration—the use of glycolysis rather than oxidative phosphorylation as the main form of energy metabolism, even under normal oxygen levels. This is counterintuitive, as glycolysis results in the waste
product lactic acid, which is harmful to most cells. Moreover, glycolysis is much less efficient than oxidative phosphorylation in producing adenosine triphosphate (ATP), a unit of energy transfer in cells: Glycolysis results in two molecules of ATP for each molecule of glucose, whereas oxidative phosphorylation yields 36 molecules [26]. The mechanism for the shift to Warburg metabolism is widely studied and not yet fully understood. Recent research suggests that cancer cells adjust their metabolism to meet various demands, so although aerobic glycolysis is less efficient in terms of energy production, this pathway may in fact result in a growth advantage, because metabolic byproducts can crosstalk with other signaling pathways to promote cell growth and proliferation and support biomass production [26], [17]. A byproduct of glycolysis, lactic acid, dissociates into lactate and H+ ions, so that there is an accumulation of lactate in the tumor. It has been found that lactate is pro-angiogenic [6], another major advantage to tumor cells because new blood vessels increase the supply of nutrients and growth factors to the tumor. Furthermore, it has been proposed that tumor cells have evolved to survive in this highly acidic environment, where normal cells cannot [2].

1.2 Metabolic Symbiosis

Although the Warburg Effect has been observed for many years, not all cancer cells exhibit a completely glycolytic phenotype at all times. Oxidative cells (cells whose primary metabolic program is OXPHOS) are often found close to blood vessels, while glycolytic cells (cells whose primary metabolic program is glycolysis) are found away from blood vessels, and cells can switch from one metabolic program to another depending on temporal and other microenvironmental conditions. In 2008, a symbiotic relationship between oxidative and glycolytic cell was proposed by Sonveaux et al. ([23]; see Figure 1.1): Glycolytic cells uptake glucose, producing lactic acid which dissociates into lactate and H+ ions, so that oxygenated
cells can preferentially uptake the lactate, using it for metabolic fuel, thereby allowing more glucose to diffuse toward glycolytic cells. Thus, these two metabolic cell types work together in a mutually beneficial symbiotic relationship.

Moreover, it has been observed that cancer-associated fibroblasts (CAFs) undergo aerobic glycolysis, and the resulting lactate is used directly by tumor cells for oxidative phosphorylation [19]. These examples of metabolic symbiosis have the potential to be highly clinically relevant. If the symbiotic relationship between metabolic cell types can be targeted, this may lead to better treatment options.

1.3 Metabolism and Wnt Signaling

The canonical Wnt signaling pathway is a critical signaling pathway that controls cell proliferation and differentiation during development and healing. This widely studied pathway is known to cause cancer when mutations arise that upregulate Wnt signaling [11]. In the Wnt pathway, $\beta$-catenin accumulates in the cytoplasm in the cell, translocating to the nucleus, and coactivating transcription factors in the TCF/LEF (T-cell factor/lymphoid enhancing...
factor) family. A recent study by Pate et al. found that there is a clear connection between Wnt signaling and Warburg metabolism: Wnt signaling directs glycolysis and angiogenesis in colon cancer [18]. Moreover, the study identified pyruvate dehydrogenase kinase 1 (PDK1), a direct Wnt target gene, as a driver of Warburg metabolism and vessel development.

In that study, Wnt signaling in colon cancer xenograft tumors was interfered with by the over-expression of a dominant negative form of lymphoid enhancer factor 1 or T-cell factor (dnLEF or dnTCF respectively), both of which lack the β-catenin binding domain and whose resulting tumors undergo limited Wnt signaling. It was found that the dnLEF/dnTCF tumors had reduced lactate levels and higher ATP production compared to so-called mock tumors (tumors in which Wnt was not interfered with), implying a shift towards OXPHOS and away from glycolysis. The study also used fluorescence lifetime imaging microscopy (FLIM), which measures relative levels of glycolysis and oxidative phosphorylation, to evaluate metabolism in living cells. Results from FLIM analysis showed a decrease in glycolysis in dnLEF and dnTCF tumors. These are all clear indications that Wnt plays a significant role in promoting glycolysis and angiogenesis in colon cancer metabolism.
Chapter 2

Glucose-Lactate Model

2.1 Background

In this chapter, we investigate a possible simple mechanism behind a cellular switch in cancer metabolism, discussed in further detail below, and a mathematical model that describes it. We present the results from experiments performed on colon cancer xenograft tumors that show evidence of a remarkable symbiotic relationship between two different metabolic cell types. We propose a mathematical model patterned after an existing model to characterize this relationship. It is hoped that this will help inform biology to predict behaviors, to test mechanisms, and to confirm them with experiments.

2.1.1 Patterning in Cancer Metabolism

Cells from human colon cancer cell line SW480 were subcutaneously injected into immunocompromised mice and the resulting xenograft tumors were excised, sliced, and stained for
phosphorylated pyruvate dehydrogenase (pPDH) by immunohistochemistry (IHC). Three different types of xenograft tumors were grown. “Mock” tumors refers to ordinary xenograft tumors in which Wnt signaling has not been not interfered with and can be thought of as control experiments. Tumors were also grown in which a dominant negative form of lymphoid enhancer factor 1 (“dnLEF” or “dnLEF-1”) was overexpressed. These tumors lack the necessary $\beta$-catenin binding domain for Wnt signaling to occur, thus reducing the level of Wnt activity, and hence glycolysis [18], in the tumor. Note that it is not possible to produce a tumor in which Wnt signaling is completely disrupted. Finally, dnLEF xenograft tumors were also grown in which pyruvate dehydrogenase kinase 1 (PDK1) was overexpressed (“dnLEF + PDK1”). Because PDK1 drives glycolysis, these were grown to test whether glycolytic activity could be restored in the dominant negative tumors. Images of phospho-PDH stains are shown in Figure 2.1, where the lower left quadrant corresponds to expression of dnLEF, the lower right corresponds to the expression of both dnLEF and PDK1, and both upper quadrants are controls. The two figures that follow Figure 2.1 are zoomed images.

The IHC stains revealed a striking spotted pattern, appearing as areas of localized clusters of cells, or “spots,” indicating regions with a higher expression of pPDH compared to neighboring areas. Because pPDH is a marker for PDK1 activity, which suppresses OXPHOS in favor of glycolysis, the spots suggest a spatial pattern of metabolism in the xenograft tumors. The darker-stained areas in the IHC panels indicate regions undergoing relatively higher levels of glycolysis, and lighter-stained areas are regions undergoing relatively higher levels of oxidative phosphorylation. We propose that this spotted pattern is evidence of a symbiosis between these metabolic cell types: Cells localize into small regions that undergo glycolysis, producing lactate for neighboring oxidative cells, thereby allowing more efficient metabolism of glucose throughout the tumor. However, this spatial symbiosis is different from the one proposed by Sonveaux et al. [23], which predicts a gradient in glycolytic to oxidative metabolism, or Pavlides et al. [19], which predicts a relationship between cancer-associated fibroblasts and stroma. The dominant negative tumors also present in a spotted
Figure 2.1: Spotted patterns in immunohistochemical phosphorylated PDH stains colon cancer xenograft tumors. Mock and dnLEF1 tumors are evidence of a spatial symbiotic relationship between glycolytic cells (brown spots), which produce lactate that can be uptaken by surrounding oxidative cells. PDK1 rescues glycolysis in tumors, so that the dnLEF1 + PDK1 tumor does not show a spotted pattern.

pattern, but it is distinctly different: The spots are significantly larger, fewer, and farther apart when compared to mock. The background is also noticeably lighter, indicating that overall more OXPHOS is occurring. The spotted pattern was eradicated in the tumors in which both dnLEF and PDK1 were overexpressed, and showed a high level of pPDH activity, indicating that PDK1 rescues the dnLEF-induced suppression of glycolysis.
Figure 2.2: Magnified image of Figure 2.1. Dark blue stains are nuclei.
Figure 2.3: Magnified image of Figure 2.1. Dark blue stains are nuclei.
2.1.2 Existing Mathematical Model

We hope to understand this spotted pattern by developing a mathematical model to characterize the different metabolic cell types and their interactions with nutrients and growth factors. An existing mathematical model, developed by Mendoza-Juez et al. [16], was motivated by the Sonveaux symbiosis hypothesis [23] discussed in Chapter 1, and adequately describes the relationship between \textit{in vitro} oxidative and glycolytic cells, allowing them to switch from one metabolism type to another. The model includes an equation for the population \( p_g \) of cells undergo glycolysis; an equation for the population \( p_o \) of cells undergoing oxidative phosphorylation; an equation for glucose \( G \); and an equation for lactate \( L \). In the Mendoza-Juez paper, as well as in this work, cells are referred to as either glycolytic or oxidative, but this does not mean a binary switch between the two metabolism programs; we use this terminology to refer to the predominant program that the cells are undergoing.

The assumptions in the Mendoza-Juez model are that the \( p_g \) and \( p_o \) cells can proliferate, and switch between metabolic programs based on lactate and glucose levels: If lactate is high, cells are more likely to switch to OXPHOS, and if lactate is low, the cells are more likely to be glycolytic. Cells can only switch from OXPHOS to glycolysis if the glucose level is sufficiently high. Glucose can be uptaken by both cell types, and lactate is produced by glycolytic cells and is uptaken by oxidative cells.

This model was validated against five different human cancer cell lines \textit{in vitro}. However, it only describes the relationship between oxidative and glycolytic cells temporally, not spatially, and furthermore is not used to describe that relationship \textit{in vivo}. Modifying the model by the addition of a spatial component in the form of a diffusion term failed to generate any spatial patterns, both numerically and through diffusive instability analysis, for several parameter sets.
In 2014, another mathematical model by McGillen et al. [15] was built off of the Mendoza-Juez model to include a spatial component that could help further capture the metabolic symbiosis, which intrinsically requires a spatial feature. McGillen’s model is a direct extension, with some simplifications, of the Mendoza-Juez model. The model describes a one-dimensional ray in polar coordinates assuming spherical symmetry. It characterizes symbiosis for a substantial portion of parameter space, but does not confer a spotted pattern such as the one observed in IHC stains. A reaction-diffusion model by Phipps et al. [20] was developed to describe concentrations of oxygen, lactate and glucose surrounding a single microvessel, a model that captures the symbiotic relationship but is on the microscale and does not yield a spotted pattern. We therefore developed a new mathematical model, one that captures not only a symbiotic relationship between the two different metabolic cell types, but also the striking spotted pattern we observe.

2.2 Mathematical Model

2.2.1 Augmenting the Symbiosis Hypothesis

Since the existing models do not generate spatial patterns under several parameter sets, there may be some detail in the symbiosis hypothesis that is missing (see Figure 2.4). There is evidence that lactate stimulates production of hypoxia-inducible factor 1α (HIF1α) [12], a transcription factor that induces transcription of glucose transporters and glycolytic enzymes. Accumulation of glycolytic metabolites in turn promotes activation of HIF1α [14]. In addition, HIF1 directly enhances glycolysis [14]. Moreover, lactate influx through monocarboxylate transporter 1 (MCT1) stimulates nuclear factor κ-light-chain-enhancer of activated B cells (NFκB) [27], which are proteins that control the transcription of DNA, in endothelial cells; incorrect regulation of NFκB is linked to cancer. Finally, activation of
NFκB causes an increase in the rate of aerobic glycolysis and upregulates glucose transporter 3 (GLUT3) [8], which facilitates glucose transport across plasma membranes. These facts all suggest that there is a feed-forward loop in glycolysis, and that lactate production, which is dependent on glucose, is nonlinear.

If we simplify the left side of Figure 2.4, we obtain Figure 2.5, so that glucose uptake upregulates lactate, lactate upregulates itself, and lactate upregulates glucose uptake.

Figure 2.5: Lactate production increases with more lactate, but depletes glucose.

### 2.2.2 Activator-Depleted Substrate Model

This makes the Gierer-Meinhardt activator-depleted substrate model (Equations 2.1a-2.1b; [3]) a natural choice for modeling lactate and glucose (see Figure 2.6). The reaction-diffusion
equations describe the spatio-temporal relationship between $a$, the activator, and $s$, the substrate. This model is known to yield patterns in certain parameter regimes. The first term on the right hand side of each equation is for diffusion. The activator level depends not only on itself but also on the substrate. As the activator level increases, the substrate is depleted (reflected in the nonlinear production term $ca^2s$ in either equation). This reduces the production of the activator, which allows the substrate to increase, and the cycle begins again. There are decay terms $\mu a$ and $\nu s$. The term $c_0$ is a source term for $s$. The patterning results from the depletion of the highly diffusible substrate, causing long-range inhibition of the activator.

Figure 2.6: Gierer-Meinhardt activator-depleted substrate model. The activator $a$ is autocatalytic and upregulates nonlinear uptake of the substrate $s$. The substrate helps produce the activator.

\[
\begin{align*}
\frac{\partial a}{\partial t} &= D_a \nabla^2 a + ca^2 s - \mu a \\
\frac{\partial s}{\partial t} &= D_s \nabla^2 s + c_0 - ca^2 s - \nu s
\end{align*}
\]

2.2.3 Equations

We incorporated a modified version of the Gierer-Meinhardt activator-substrate equations into our model (modified to allow for different coefficients in the nonlinear terms), where lactate is the activator and glucose is the substrate. The glucose was modeled so that there is a constant glucose source and so that it is consumed during the production of lactate
through glycolytic cells. The lactate was modeled to be autocatalytic, so that its production is dependent on the current level of lactate. Both glucose and lactate can diffuse throughout the domain. The two cell types, $P_o$ and $P_g$, can diffuse and “switch” metabolism programs depending on the level of lactate and glucose in the domain. Similar to the Mendoza-Juez model, if lactate is high then the cells are more likely to switch to OXPHOS, and if lactate is low then the cells are more likely to switch to glycolysis. The switch to glycolysis can happen only if sufficient glucose is present. Each term in the equations is described below.

Equations 2.2a and 2.2b describe the population of oxidative and glycolytic cells $P_o$ and $P_g$ respectively. These equations, as well as the transition functions for $\chi_L$, $\chi_*^L$, are similar to those in the Mendoza-Juez model described above, but diffusion, or random motion, is now included in the equations ($D_o\Delta P_o$ and $D_g\Delta P_g$ terms). We allow lactate and glucose to diffuse as well, with the variables $D_o$, $D_g$, $D_L$, and $D_G$ defined as diffusion coefficients. The parameters $\kappa_L$ and $\kappa_o$ are lactate and glucose consumption rates by oxidative cells. The terms $\kappa_{NL}$ and $\kappa_{NG}$ are the rates for the nonlinear production of lactate and uptake of glucose through the glycolytic cells, respectively. Lactate and glucose decay with rates given by $\mu$ and $\nu$ respectively. The term $R$ represents a bulk source of glucose. We denote oxidative cell proliferation, glycolytic cell proliferation, switch time from oxidative to glycolytic phenotype, and switch time from glycolytic to oxidative phenotype by $\tau_o$, $\tau_g$, $\tau_{og}$, and $\tau_{go}$, respectively. The transition function $\chi_G$ is used so that cells can switch to glycolysis only if sufficient glucose is present.

\[
\begin{align*}
\frac{\partial P_o}{\partial t} &= D_o\Delta P_o + \frac{1}{\tau_o} \left( 1 - \frac{P_o + P_g}{P^*} \right) P_o + \frac{1}{\tau_{go}} \chi_L(L) P_g - \frac{1}{\tau_{og}} \chi_*^L(L) \chi_G(G) P_o \quad (2.2a) \\
\frac{\partial P_g}{\partial t} &= D_g\Delta P_g + \frac{1}{\tau_g} \left( 1 - \frac{P_o + P_g}{P^*} \right) P_g - \frac{1}{\tau_{go}} \chi_L(L) P_o + \frac{1}{\tau_{og}} \chi_*^L(L) \chi_G(G) P_o \quad (2.2b) \\
\frac{\partial L}{\partial t} &= D_L\Delta L - \kappa_L L P_o + \kappa_{NL} L P_g - \mu L \quad (2.2c)
\end{align*}
\]
\[
\frac{\partial G}{\partial t} = D_G \Delta G - \kappa_o G P_o + R - \kappa_{NG} G L^2 P_g - \nu G
\] (2.2d)

The functions \( \chi_L(L) \) and \( \chi^*_L(L) \) are transition functions (to transition from the glycolytic phenotype to the oxidative phenotype and back); we use the same functions as in the Mendoza-Juez model.

\[
\chi_L(L) = \frac{1}{2} [1 + \tanh (\gamma_L (L - L^*_1))]
\] (2.3a)

\[
\chi^*_L(L) = \frac{1}{2} [1 - \tanh (\gamma_L (L - L^*_2))]
\] (2.3b)

\( L^*_1 \) and \( L^*_2 \) indicate where the level of lactate at which the switch occurs, and \( \gamma_L \) determines the sharpness of the curve (for very high values of \( \gamma_L \), the curve is nearly a step function). The \( \chi_G(G) \) term is a function that only allows the switch from oxidative to glycolytic metabolism to occur if there is sufficient glucose, defined by the parameter \( G_{\text{min}} \), in the system. We use a function similar to the \( \chi_L(L) \) function:

\[
\chi_G(G) = \frac{1}{2} [1 + \tanh (\gamma_G (G - G_{\text{min}}))]
\] (2.4)

After nondimensionalizing (see Appendix), the equations become

\[
\frac{\partial P_o}{\partial t} = D_o \Delta P_o + \frac{1}{\tau_o} (1 - P_o - P_g) P_o + \frac{1}{\tau_{go}} \chi_L(L) P_g - \frac{1}{\tau_{og}} \chi^*_L(L) \chi_G(G) P_o
\] (2.5a)

\[
\frac{\partial P_g}{\partial t} = D_g \Delta P_g + \frac{1}{\tau_g} (1 - P_o - P_g) P_g - \frac{1}{\tau_{go}} \chi_L(L) P_g + \frac{1}{\tau_{og}} \chi^*_L(L) \chi_G(G) P_o
\] (2.5b)

\[
\frac{\partial L}{\partial t} = D_L \Delta L - \kappa_L L P_o + \kappa_{NL} G L^2 P_g - \mu L
\] (2.5c)
\[
\frac{\partial G}{\partial t} = \Delta G - \kappa_o P_o + 1 - GL^2 P_g - G
\]  
\text{(2.5d)}

The above system has a reduced parameter set and is used subsequently to obtain the numerical results that follow in the next section.

### 2.2.4 Diffusive Instability Analysis

The lactate and glucose equations are a modified version of Gierer and Meinhardt’s activator-depleted substrate model, allowing for different rates for the nonlinear terms, and include contributions of uptake and proliferation by \(P_o\) and \(P_g\) cells. The lactate is modeled as the activator and the glucose as the substrate, since glucose is necessary for \(P_g\) cells to create lactate. If we make the simplifying assumption that \(P_g = 1\) and \(P_o = 0\), then the lactate and glucose equations are reduced to the activator-depleted substrate model. In order to determine the parameters necessary for diffusive instability in the system, we reduced the stability analysis to this simpler model, which indicates parameter values in the glucose and lactate equations for which induced patterns in the \(P_o\) and \(P_g\) cell populations are possible.

Nondimensionalizing the activator-substrate equations, where \(a\) is the activator and \(s\) is the substrate, gives

\[
\begin{align*}
\frac{\partial a}{\partial t} &= D_a \frac{\partial^2 a}{\partial x^2} + ca^2 s - \mu a \\
\frac{\partial s}{\partial t} &= \frac{\partial^2 s}{\partial x^2} + 1 - a^2 s - s
\end{align*}
\]  
\text{(2.6a)}

We performed classic diffusive instability analysis for this model (see, for example, [1]). In order for patterns to be expected to emerge, we first found the steady states without diffusion,
which must be asymptotically stable. We then linearized about the steady state after including diffusion and sought an unstable solution, meaning that an initial perturbation will grow over time. In a two-equation system, the conditions for patterning reduce to the following expressions, i.e., all of the following must hold, where each $a_{ij}$ is the partial derivative of equation $i$ with respect to $j$, where $i = 1, 2$ and $j = 1, 2$ (1 corresponds to the activator and 2 corresponds to the substrate), evaluated at the steady states $\bar{a}$ and $\bar{s}$, after setting the time derivative and space derivative to 0:

1. $a_{11} + a_{22} < 0$

2. $a_{11}a_{22} - a_{12}a_{21} > 0$

3. $a_{11} + D_a a_{22} > 2\sqrt{D_a(a_{11}a_{22} - a_{12}a_{21})} > 0$

Computing the partial derivatives and evaluating at the steady states $\bar{a}$ and $\bar{s}$, we have

$a_{11} = 2c\bar{a}\bar{s} - \mu$,  $a_{12} = c\bar{a}^2$,  $a_{21} = -2\bar{a}\bar{s}$,  and  $a_{22} = -\bar{a}^2 - 1$.

The first steady state does not yield any patterns. The second steady state must satisfy

\[
\begin{align*}
a_{11} + a_{22} &= \frac{-c^2 - 2\mu^3 + c\sqrt{c^2 - 4\mu^2}}{2\mu^2} \\
&= \mu - \frac{c^2 + c\sqrt{c^2 - 4\mu^2}}{2\mu^2} \\
&< 0 \\
a_{11}a_{22} - a_{12}a_{21} &= \frac{c^2 - 4\mu^2 + c\sqrt{c^2 - 4\mu^2}}{2\mu} \\
&> 0 \\
a_{11} + D_a a_{22} &= \mu - D_a \frac{c^2 + c\sqrt{c^2 - 4\mu^2}}{2\mu^2}
\end{align*}
\]
\[
> \sqrt{\frac{2D_a}{\mu}} \sqrt{c^2 - 4\mu^2 + c \sqrt{c^2 - 4\mu^2}}
\]

There are three parameters that can be varied: \(D_a, c,\) and \(\mu\). The above conditions dictate the parameter spaces (see shaded areas in Figure 2.7) in which patterns are predicted to form in Gierer-Meinhardt’s model. The shaded area decreases as the diffusion coefficient of the activator increases, so that as the diffusion of \(a\) gets closer to the diffusion of \(s\) (normalized to 1 here), the parameter space in which patterns can form is decreased. This analysis helps determine which parameter values can yield patterns in the full four-equation model. For the base case parameters listed in the next section, the third steady state is unstable in time, so their Turing spaces are not included here.
Figure 2.7: Diffusive instability plots varying $c$ (nonlinear production of activator $a$) and $\mu$ (decay of $a$) (refer to Equations 2.6a-2.6b). Shaded region indicates values of $c$ and $\mu$ for which patterns are expected to form during early times. (A) Diffusion coefficient of activator $D_a = 0.001$. (B) $D_a = 0.01$. (C) $D_a = 0.05$. (D) $D_a = 0.1$.

### 2.2.5 Wavenumber Analysis

We first selected a set of parameters that are predicted to yield patterns in the activator substrate model; then we expanded this to a set of values for the four-equation model and again performed diffusive instability analysis to determine whether patterns are possible. In this analysis, we assumed a solution to each equation of the form $\alpha\cos(qx)e^{\sigma t}$. The reason we used this form is because of its relationship to its partial derivatives (the first partial derivative with respect to time $t$ and the second partial derivative with respect to the space variable $x$); that is, equations of this form appear to be good candidates for solutions to the
model. Moreover, by the Fourier Theorem, we know that the perturbation can be written as a Fourier cosine series; here we isolate one component for the linear analysis. We desired wavenumbers for which the eigenvalue $\sigma$, the growth rate of the perturbation in time, has positive real part. “Wavenumber” is defined as the integer $n$ in $q = 2\pi n/d$ where $d$ is the size of the spatial domain. The wavenumber translates to the number of peaks in a sinusoidal wave in a one-dimensional spatial domain of length $d$. Further details of this analysis are given in Chapter 3. Figure 2.8 shows wavenumber versus maximum eigenvalue for the base case parameters $d = 8$, $\kappa_{NL} = 3$, $\mu = 1$, $\tau_o = \tau_g = \tau_{og} = \tau_{go} = 1$, $D_L = D_o = D_g = 0.01$. The graph shows that spatial perturbation of time-stable steady states is linearly unstable, with mode 6 the fastest growing mode. The growth rates of modes 4 through 8 are very close. Any of these may emerge under nonlinear interactions.

![Figure 2.8](image)

Figure 2.8: Patterns are predicted for wavenumbers whose growth rate is positive (analysis was done using base case parameters listed in the text). In this example, the maximum growth rate is at wavenumber 6, but the growth rate of modes 4 through 8 are similar; a pattern with any of these modes may emerge under nonlinear interactions.
2.3 Numerical Results

We used a finite difference method with explicit time step and periodic boundary conditions. The numerical results presented here are for parameters $\kappa_{NL} = 3$, $\mu = 1$, $\tau_o = \tau_g = \tau_{og} = \tau_{go} = 1$, $D_L = D_o = D_g = 0.01$ (see Table 2.1). A random initial perturbation was seeded in the lactate and glucose equations, and initial oxidative and glycolytic cell concentrations were constant at 0 and 1 respectively. See Figures 2.9 through 2.12 for the time evolution of the cells and nutrients. The parameters here give a pattern persisting in both space and time and are in good agreement with the wavenumber analysis from the previous section with dominant wavenumber 7. The mean glycolytic cell concentration at the end time is 0.8, consistent with Warburg metabolism. The concentration of oxidative cells is high where lactate is high, which is consistent with the idea that these cells rely on greater levels of lactate.

For this simulation and the one-dimensional simulations that follow, we also performed wavenumber analysis and computed the Fourier transform (using MATLAB’s FFT function and plotting the modulus of the FFT divided by the length of the vector versus nonnegative integers) of the final pattern in each simulation, to determine whether the wavenumber in the analysis is similar to the wavenumber given by the simulation. In the previous section, we noted that diffusive instability predicted that the growth rate (eigenvalue) for the base case parameters was highest at wavenumber 6, but that the rates for wavenumbers 4 through 8 were similar. The base case simulation has a wavenumber of 7, demonstrating good agreement with our analysis.

Next we modeled the effects of overexpressing dnLEF, which suppresses glycolysis. There are a few ways to suppress glycolysis: require a higher level of glucose for the switch from oxidative to glycolytic metabolism (increase $G_{\text{min}}$), or suppress the switch from oxidative phosphorylation to glycolysis. We again performed diffusive instability analysis, now with
these effects: increasing $G_{\min}$ to 1, or setting $L^*_2 = 0$. Peak growth rates have shifted to the left; they have also increased, meaning that perturbations are more unstable (see Figure 2.13). The time evolution of these effects are presented in the next section (Figures 2.14 through 2.22). We see that increasing $G_{\min}$ results in a lower peak wavenumber and lower average $P_g$ cells, but the effect is transient. Suppressing the transition to glycolysis results in a persisting pattern with lower peak wavenumber and average $P_g$ cells is reduced to 0.74. Our wavenumber analysis also shows good agreement with the simulation results: The analysis predicts that wavenumber will decrease to 5, and which is the number of peaks we see in our numerical solution.

We next examined the overexpression of both dnLEF1 and PDK1, whose overall effects are to enhance glycolysis. To enhance glycolysis, we considered two mechanisms: Require a lower level of glucose for the switch from oxidative to glycolytic metabolism (decrease $G_{\min}$), or suppress the switch from glycolysis to oxidative phosphorylation. The results from diffusive instability analysis, now with the effects of decreasing $G_{\min}$ to 0.1, or setting $L^*_1 = 8$, are shown in Figure 2.23. In this case, perturbations are less unstable. The time evolution (Figures 2.24 through 2.31) shows that if $G_{\min}$ is decreased to 0.1, then we observe a subtle, persisting pattern with average $P_g$ concentration 0.95. If we require more lactate to switch to OXPHOS, the result is 100% $P_g$ cells with no spatial pattern. The wavenumber analysis again agrees with our numerical results with a shift to a wavenumber of 5.

Finally, we extended the model to two spatial dimensions and performed the same numerical simulations (Figures 2.15 through 2.36). We observed the same effects as those in the one-dimensional model, with small hotspots (darkest red spots in Figure 2.32) of glycolytic cells in the mock simulations. The dominant negative LEF simulations yield fewer, larger spots as desired. The PDK1 rescue simulations show an abundance of glycolytic cells, with a subtle pattern if we decrease $G_{\min}$ and eradication of the pattern if we suppress the switch from glycolysis to OXPHOS.
Table 2.1: **Glucose-lactate model base case parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_o$</td>
<td>Diffusion coefficient of oxidative cells</td>
<td>0.01</td>
</tr>
<tr>
<td>$D_g$</td>
<td>Diffusion coefficient of glycolytic cells</td>
<td>0.01</td>
</tr>
<tr>
<td>$\tau_{go}$</td>
<td>Switch time from glycolytic to oxidative phenotype</td>
<td>1</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>Sensitivity switch parameter</td>
<td>100</td>
</tr>
<tr>
<td>$L_1^*$</td>
<td>Switch threshold for $\chi_L$</td>
<td>3</td>
</tr>
<tr>
<td>$G_{min}$</td>
<td>Switch threshold for $\chi_G$</td>
<td>0.3</td>
</tr>
<tr>
<td>$P_o$</td>
<td>Initial $P_o$ concentration</td>
<td>0</td>
</tr>
<tr>
<td>$P_g$</td>
<td>Initial $P_g$ concentration</td>
<td>1</td>
</tr>
<tr>
<td>$D_L$</td>
<td>Diffusion coefficient of lactate</td>
<td>0.01</td>
</tr>
<tr>
<td>$\kappa_L$</td>
<td>Lactate consumption by oxidative cells</td>
<td>1</td>
</tr>
<tr>
<td>$\kappa_o$</td>
<td>Glucose consumption by oxidative cells</td>
<td>1</td>
</tr>
<tr>
<td>$\kappa_{NL}$</td>
<td>Production rate of lactate</td>
<td>3</td>
</tr>
<tr>
<td>$\mu$</td>
<td>Decay rate of lactate</td>
<td>1</td>
</tr>
<tr>
<td>$L$</td>
<td>Initial $L$ level</td>
<td>0.99</td>
</tr>
<tr>
<td>$G$</td>
<td>Initial $G$ level</td>
<td>0.99</td>
</tr>
</tbody>
</table>
Figure 2.9: Time evolution of $P_o$ and $P_g$ cells with base case parameters

Figure 2.10: Time evolution of $L$ and $G$ with base case parameters
Figure 2.11: $P_o$, $P_g$, $L$, and $G$ at end of simulation with base case parameters.

Figure 2.12: Fourier transform at end of simulation with base case parameters. The wavenumber with highest positive FFT value corresponds to the number of peaks in the previous figure.
Figure 2.13: Wavenumber analysis for $G_{\text{min}} = 1$ or $L_2^* = 0$. Circles indicate peaks of growth rate.

Figure 2.14: Time evolution of $P_o$ and $P_g$ cells with $G_{\text{min}} = 1$.
Figure 2.16: Time evolution of $L$ and $G$ with $G_{\text{min}} = 1$

Figure 2.17: $P_o$, $P_g$, $L$, and $G$ cells at end of simulation with $G_{\text{min}} = 1$
Figure 2.18: Fourier transform at end of simulation with $G_{\text{min}} = 1$. The wavenumber with highest positive FFT value corresponds to the number of peaks in the previous figure.

Figure 2.19: Time evolution of $P_o$ and $P_g$ cells with $L_z^2 = 0$
Figure 2.20: Time evolution of $L$ and $G$ cells with $L_2^* = 0$

Figure 2.21: $P_o$, $P_g$, $L$, and $G$ at end of simulation with $L_2^* = 0$
Figure 2.22: Fourier transform at end of simulation with $L_2^* = 0$. The wavenumber with highest positive FFT value corresponds to the number of peaks in the previous figure.

Figure 2.23: Wavenumber analysis for $G_{\text{min}} = 0.1$ or $L_1^* = 8$. Circles indicate peaks of growth rate.
Figure 2.24: Time evolution of $P_o$ and $P_g$ cells with $G_{\text{min}} = 0.1$

Figure 2.25: Time evolution of $L$ and $G$ cells with $G_{\text{min}} = 0.1$
Figure 2.26: $P_o, P_g, L$, and $G$ at end of simulation with $G_{\text{min}} = 0.1$

Figure 2.27: Fourier transform at end of simulation with $G_{\text{min}} = 0.1$. The wavenumber with highest positive FFT value corresponds to the number of peaks in the previous figure.
Figure 2.28: Time evolution of $P_o$ and $P_g$ cells with $L_1^* = 8$

Figure 2.29: Time evolution of $L$ and $G$ cells with $L_1^* = 8$
Figure 2.30: $P_o, P_g, L, \text{ and } G$ at end of simulation with $L_1^*=8$

Figure 2.31: Fourier transform at end of simulation with $L_1^*=8$. The wavenumber with highest positive FFT value corresponds to the number of peaks in the previous figure.
Figure 2.15: Initial conditions for 2D simulations

Figure 2.32: 2D simulation with base case parameters
Figure 2.33: 2D simulation with $G_{\text{min}} = 1$

Figure 2.34: 2D simulation with $L_2^2 = 0$
Figure 2.35: 2D simulation with $G_{\text{min}} = 0.1$

Figure 2.36: 2D simulation with $L_1^* = 8$
2.4 Discussion

Though it has long been observed that many cancer cells exhibit the Warburg Effect, whereby cells undergo glycolysis rather than oxidative phosphorylation for energy metabolism under normal oxygen levels [28], the reason for this is not fully understood. Despite the prevalence of the Warburg Effect, it is known that cancer cells are not completely glycolytic: Cells can switch between different metabolic regimes depending on environmental influences in space and time [23]. One hypothesis, developed by Sonveaux et al. in 2008, is that there is a symbiosis between the different metabolic programs: Glycolytic cells secrete lactic acid, which dissociates into H+ ions and lactate; the lactate is then uptaken by oxidative cells, allowing more glucose to be consumed by the glycolytic cells [23]. In 2014 Pate et al. showed that dnLEF-1 tumors exhibit suppressed glycolysis, that PDK1 enhances glycolysis, and that PDK1 rescues the suppression of induced glycolysis in dnLEF-1 tumors [18]. Immunohistochemical stains of colon cancer xenografts show a pronounced spotted pattern in the control experiments and in experiments in which dnLEF-1 was overexpressed, while no pattern exists in the images with overexpression of both dnLEF-1 and PDK1. These findings are evidence of a symbiotic relationship, different from the Sonveaux hypothesis, which predicts a gradient of metabolism. Here, rather than a gradient, the two cell types self-organize into a regular spotted pattern, where the spots are assumed to be localized areas that undergo relatively more glycolysis and the surrounding areas are assumed to undergo relatively more OXPHOS.

In this chapter we presented a mathematical model, a modification of an existing model, that describes both Sonveaux’s hypothesis and the heterogeneous spotted patterns. We added a modification of the Gierer-Meinhardt activator-depleted substrate model to account for the nonlinear production of lactate and nonlinear dependence of glucose uptake on lactate. We selected parameters and performed diffusive instability analysis to show that patterns are
predicted to form, for mock, dnLEF-1, and dnLEF-1 + PDK1 simulations. For the latter two simulations, we found that our model predicts larger, fewer spots with more than one configuration of the parameter set: We can either modify $G_{\text{min}}$, or modify the switch value between glycolysis and oxidative phosphorylation. Either of these methods are possibilities. The effects from changing $G_{\text{min}}$ are transient, but it is not known whether the spotted pattern in the xenograft tumors are stable over time. The numerical results presented in this paper are in good qualitative agreement with the Sonveaux hypothesis as well as the IHC data, and demonstrate that the mechanisms for the switch in metabolic phenotypes can be understood through our model, in all three tumor types.

Despite the agreement between the math model and our data, there are limitations that indicate that a different model may be more effective in characterizing the metabolic symbiosis. The base case parameters in two dimensions gives a labyrinthine pattern rather than a spotted pattern. However, this could perhaps be explained by poor sensitivity of IHC staining in being able to capture relatively lower levels of glycolysis, and instead is capturing only the highest levels ("hot spots") in the $P_g$ cells in Figure 2.32. Additionally, diffusive instability requires that the diffusion coefficient of glucose is at least an order of magnitude higher than that of lactate for patterns to form. These diffusion coefficients have not been measured in xenograft tumors such as the ones investigated here, but in media in which they have been measured, their values are reported to be on the same order of magnitude. We considered techniques such as keeping the diffusion coefficients similar and instead modifying uptake or decay rates, but this did not result in patterns. Furthermore, this model lacks any connection to the Wnt signaling pathway, which is known to promote glycolytic metabolism. The following chapter illustrates a different mathematical model that takes these limitations into account.

Nevertheless, this model can be used to characterize the relationship between glycolytic and oxidative cell types and glucose and lactate. It could be extended to include terms such
as oxygen and the effects of vasculature and pH. Different cell types can be included, such as cancer stem cells, committed progenitor cells, and terminally differentiated cells. It is hoped that predictions from this model can be validated by biological experiments, which will further inform the modeling.

**Materials and methods**

*Numerical simulations*

Numerical simulations were performed in MATLAB, using an explicit forward time, centered space finite difference method. Periodic boundary conditions were used for all fields. The initial condition for \( G \) and \( L \) was a uniform random perturbation with an average magnitude of 0.05 about 0.99 throughout the domain. Initial \( P_g \) and \( P_o \) were constant at 1 and 0 respectively.
Chapter 3

Wnt Signaling Model

3.1 Background

There are some limitations to the model in the previous chapter. For example, we see a labyrinthine pattern instead of a spotted pattern in the two-dimensional mock simulations, but it is possible that the IHC stains are not very sensitive to phosphorylated PDH, so that the stains detect only the highest levels of glycolysis (darkest red spots in $P_g$ cells in Figure 2.32), which reveals itself as a spotted pattern. Another limitation is that the previous model requires that glucose diffuses much farther range than lactate. In reality, the diffusion coefficients of glucose and lactate are difficult to measure. To our knowledge, these values have not been measured in xenograft tumors such as the ones we study, and in what has been measured, it has been found that glucose and lactate diffuse on approximately the same order of magnitude. On the other hand, there are many complications involved in computing these numbers (measurement errors, numerical approximations, different microenvironments), so we cannot be certain about their true values. We therefore developed a new mathematical
model, described in the following sections, to characterize the spatial pattern we observe, with the addition of the Wnt signaling pathway, which has recently been implicated in glycolysis in colon cancer [18], and an inhibitor of Wnt.

3.1.1 Cancer Metabolism, Symbiosis, and the Wnt Signaling Pathway

Reprogrammed metabolism is a hallmark of cancer [4], and recently the Wnt signaling pathway has been implicated in driving this change in colon cancer [18]. Understanding the mechanism behind this is crucial for developing more effective treatments of this disease. Many mathematical models have been developed to understand the relationship between different metabolic phenotypes, nutrients, and growth factors [16], [15], [20], but none of these models include the effects of the Wnt pathway on metabolism. Here, we present data and a mathematical model for a symbiotic relationship between metabolic phenotypes in colon cancer cells, a relationship that forms an intricate spatial arrangement that is influenced by Wnt signaling.

The Wnt signaling pathway is a critical pathway that controls cell proliferation and differentiation during development and healing. If key signaling components are genetically inactivated (e.g., Adenomatous Polyposis Coli, APC), the pathway becomes chronically activated and this can lead to cancer. It was demonstrated that Wnt signaling directs glycolysis and angiogenesis in colon cancer cells through direct upregulation of Pyruvate Dehydrogenase Kinase 1 (PDK1) expression [18]. PDK1 is a mitochondrial kinase that inhibits the Pyruvate Dehydrogenase Complex (PDC) via phosphorylation of Pyruvate Dehydrogenase (PDH), the first component enzyme of PDC that converts pyruvate to acetyl CoA for mitochondrial respiration. Through phosphorylation of PDH, PDK1 suppresses
OXPHOS modes of metabolism to favor glycolytic modes of metabolism that produce lactate [18].

In the course of our study of Wnt signaling and cancer metabolism in human colon cancer cell lines, we observed heterogeneous patterns of metabolism in xenograft tumors. Heterogeneity was observed via immunohistochemical stains of PDK1 activity and Wnt signaling, appearing as a pattern of discrete clusters of cells, or “spots,” indicating groups of cells with different levels of glycolysis relative to OXPHOS, and differences in Wnt activity. We will refer to these groups of cells as “glycolytic ($P_g$)” and “OXPHOS ($P_o$)” to indicate that they differ in the balance between these two modes of metabolism. As the spotted array appeared throughout the tumor at regular intervals, we developed a mathematical model to study the spatial pattern and probe a potential link to Wnt signaling. Here we propose that the spotted pattern of metabolism is heavily influenced by Wnt signaling and one or more Wnt inhibitors.

A non-spatial mathematical model has previously been developed to describe interactions between oxidative cells, glycolytic cells, lactate, and glucose [16], and other models have been developed that include a spatial component [15], [20]. However, these models do not describe a spotted pattern, and moreover they do not include any connection to Wnt. To achieve a better understanding of the relationship between different metabolic states and Wnt signaling, we present a mathematical model consisting of reaction-diffusion equations for Wnt activity, a diffusible Wnt inhibitor, nutrients, and populations of different metabolic cell types (oxidative cells and glycolytic cells). We show that the model is in good qualitative agreement with experimental data. We also investigate the parameter space and study the effects of interference with Wnt signaling, the results of which are validated with experiments. Finally, we consider possible therapeutic regimes in silico and conclude that they can be effective in completely eradicating the tumor if they adequately reduce Wnt activity. Although many mathematical models have been developed to understand tumor metabolism, to our
knowledge, this is the first to describe the intricate spatial relationship between metabolic types together with their relationship to Wnt signaling.

3.2 A Spotted Pattern of PDK Activity and LEF1 Expression in Xenograft Tumors

Xenograft tumors of colon cancer cell line SW480 (containing homozygous loss of function mutations in APC and activated Wnt signaling) were produced by subcutaneous injection of cells in immunocompromised mice. To investigate metabolic changes within the tumor, 10µm slices of formalin-fixed, paraffin-embedded tumor were probed with antisera specific for phosphorylated-PDH (pPDH) as an indicator of PDK1 activity and Lymphoid Enhancer Factor-1 (LEF1), a Wnt signaling transcription factor and Wnt target gene [5]. Both stains revealed a general, high level of pPDH and LEF-1, but also heterogeneity in the form of a striking spotted pattern within the tumor (Figure 3.1A-B, where “mock” refers to tumors from parental SW480 cells). The pPDH pattern appeared as discrete localized clusters of cells with increased levels of pPDH, and these clusters, or spots, were detected throughout the tumors at regular intervals. Since pPDH staining is an indicator of PDK activity, the darker stained cell clusters indicate increased rates of glycolysis relative to neighboring cells in the more lightly stained areas. The lighter staining neighboring cells are utilizing greater levels of OXPHOS since PDH is less inhibited (less phosphorylated). It is known that cells undergoing OXPHOS can preferentially use lactate, a secreted byproduct of glycolysis, rather than glucose as metabolic fuel [23]. Thus, this pattern points to a metabolic spatial relationship between these two cell populations, and following others’ findings [23], [19], indicates a potential symbiosis between them: Glycolytic cells uptake glucose, localize into distinct regions and produce metabolic fuel for surrounding oxidative cells, thereby allowing more glucose to be uptaken by the glycolytic cells.
In addition to the spotted metabolic pattern, an overlying gradient in pPDH staining level was observed: The spots were denser toward the outer edge of the tumor, and decrease in frequency near the center of the tumor, suggesting that overall more glycolysis occurs at the outer regions of the tumor where there is more vasculature [18]. This is in opposition to the hypothesis [23] that oxidative cells are located closer to blood vessels, and glycolytic cells are located in more hypoxic areas. In our xenograft images, we did not observe this same spatial relationship between metabolism and vasculature.

A spotted pattern was also evident in LEF1 immunohistochemical stains (Figure 3.1B), indicating that the heterogeneous pattern may be influenced by heterogeneity of Wnt signaling. Automated image analysis was used to quantify the spatial parameters of each of the spotted patterns. Figure 3.1C shows the quantification of each spot area and distances to each nearest neighbor relative to mock average pPDH spot size and distance. Interestingly, the analysis revealed a significant overlap in the spatial arrangement of pPDH and LEF1 spots, suggesting that the heterogeneity of metabolism and Wnt signaling might be linked.

Xenograft tumors from colon cancer cell lines are different from primary human colon cancers, which develop in immunocompetent patients and contain a variety of cell types and greater stromal involvement. In Figure 3.1D we show pPDH and LEF1 stains in primary human colon tumors, demonstrating significant spatial heterogeneity in the stains. While a regular spotted array is not apparent, the heterogeneous pattern of high/low glycolysis and high/low LEF1 in the epithelial colon cancer cells indicates that although xenograft tumors are artificial and have a radically different microenvironment, understanding the mechanism of this spatial patterning can provide insight into the metabolic heterogeneity in human tumors.
SW480 (Mock) Xenograft Tumor

A. Phosphorylated PDH

B. LEF1

C. Mock tumor spot size and distance

D. Primary Human Colorectal Carcinoma

Phosphorylated PDH

LEF1
3.3 Mathematical Model

The spotted pattern in the xenograft IHC stains led us naturally to build our mathematical model with reaction-diffusion equations such as those first described by Alan Turing in 1952 [24], who demonstrated that an initial perturbation in the level of chemical concentrations can grow in the presence of diffusion, thus allowing these chemicals, or morphogens, to self-regulate into a spatial pattern. It was shown that this can happen if an activator, which diffuses short-range, is inhibited by a long-range inhibitor. There are several “local activation, long-range inhibition” Turing models that produce patterns in specific parameter spaces. The most relevant to the xenograft stains are Gierer-Meinhardt’s activator-inhibitor and activator-depleted substrate models.

Because the relationship between oxidative and glycolytic cells can be modulated by glucose and lactate levels, we first considered a model based on Gierer-Meinhardt’s activator-depleted substrate model, detailed in Chapter 2, in which lactate acts as the activator and glucose as the substrate. The equations for the oxidative and glycolytic cells are similar to those in [16], where lactate levels modulate a switch between metabolic programs. The results from this model somewhat agree qualitatively with the xenograft data, if the diffusion coefficient of glucose, the inhibiting substrate, is significantly higher than that of lactate. Diffusive
instability, which can lead to patterning in this system, requires that this is true. However, values for the diffusion coefficients of glucose and lactate are reported to be very similar, though these values have not been measured in xenograft tumors. If the diffusion coefficients for glucose and lactate are set equal in the mathematical model, patterns will not arise. Even if the diffusion coefficient of lactate is set to one order of magnitude less than that of glucose, no distinct spotted pattern emerges in the mock simulations to mimic the pattern observed in the IHC stains for the Turing spaces investigated here. Instead, there is a general labyrinthine pattern, with irregular localized areas of elevated lactate and glycolytic activity. It is possible that the IHC stains only capture the highest levels of pPDH activity, revealing only “hot spots” of elevated pPDH, which could emerge as a spotted pattern (see Figure 2.32).

Since the glucose-lactate model does not fully explain the pattern, we focused on the evidence that Wnt influences metabolism and the observation that the spotted pattern of expression of the Wnt target gene LEF1 was also evident [18]. We therefore developed a model (Equations 3.1a through 3.1f) for simulating the spatial and temporal dynamics of different metabolic phenotypes, nutrients, and growth and inhibition factors through a system of reaction-diffusion equations connected to Wnt signaling. We included populations of oxidative and glycolytic cells, a diffusible substrate that accounts for concentrations of nutrients such as glucose and growth factors, Wnt activity, and Wnt inhibitor activity. The Wnt and Wnt inhibitor equations are based on the Gierer-Meinhardt activator-inhibitor model [3], where Wnt is the activator which produces a factor that inhibits Wnt activity. See Figure 3.2A-B for an illustration of the interactions between the most essential terms.
Figure 3.2: A mathematical model for Wnt signaling regulation of metabolism. This model describes the change over time of oxidative and glycolytic cell populations, Wnt signaling activity, Wnt inhibitor activity, and nutrients (see Equations 3.1a through 3.1f). (A) The cells can diffuse, proliferate, “switch” metabolism programs depending on Wnt signaling activity and nutrient levels, and die from lack of nutrient. (B) Wnt and Wnt inhibitor activity equations are based on the Gierer-Meinhardt activator-inhibitor model. The Wnt signal diffuses short range relative to the longer-range diffusion of the Wnt inhibitor. Wnt also auto-upregulates its activity in glycolytic cells at a rate proportional to nutrient level, is inhibited by a Wnt inhibitor, is constitutively upregulated in both cell types, and decays (downregulation term). The Wnt inhibitor diffuses long range, is nonlinearly upregulated by Wnt, and decays. (C) Three-dimensional numerical simulations that model the spatial distribution and level of glycolytic and oxidative cells, Wnt, and Wnt inhibitor reveal an emergent self-organizing pattern of metabolic heterogeneity (spots). The simulations shown depict the heterogeneity in a 3D and 2D representation. The 3D representation includes a portion of the “tumor” removed to visualize the interior of the domain. The 2D representation is a horizontal slice of the respective 3D simulation in the center of the domain. (D) Summary of parameter effects on the spotted pattern.

Biological assumptions for the model are as follows: We included terms for random motion (diffusion) in space. Each cell type (oxidative and glycolytic, or $P_o$ and $P_g$ respectively) can proliferate, die, and switch to the other cell type depending on various factors. We included an equation to account for dead cells, which consists of $P_o$ and $P_g$ cells that have died from lack of nutrient, and which can diffuse and decay. Wnt ($W$) and Wnt inhibitor ($W_I$) activity are nonlinear with respect to Wnt, meaning that their rates are proportional to Wnt activity. Nonlinear Wnt activity is dependent on $P_g$ levels while nonlinear Wnt inhibitor activity equations are based on the Gierer-Meinhardt activator-inhibitor model.
activity is proportional to both $P_g$ and $P_o$ levels. We also included a term for constitutive Wnt signaling through both cell types and decay terms for $W$ and $W_I$. The general nutrient term $N$ can diffuse, decay, and taken up by the different cell populations. We include a bulk source and Dirichlet boundary condition for the nutrient, both of which are dependent on the average level of glycolytic cells in the domain, a simplified way to incorporate increased angiogenesis driven by glycolysis [18]. In this way we also take into account the effects of considerably less vasculature in tumors in which Wnt signaling was blocked by dominant interfering forms of the Wnt transcription factors Lymphoid Enhancer Factor (dnLEF) or T Cell Factor (dnTCF) [18]. We assume sufficient oxygen is present throughout the domain for OXPHOS to operate, even at a minimal level.

The nondimensionalized equations for the rate of change in the population of oxidative ($P_o$) cells and glycolytic ($P_g$) cells respectively are shown in Equations 3.1a-3.1b and are illustrated in Figure 3.2A. Equations 3.1d-3.1e and Figure 3.2B show the rate of change in the concentration of Wnt and Wnt inhibitor ($W$ and $W_I$) activity respectively. Equations 3.1c and 3.1f are the respective equations for dead cells and nutrient.
\[
\frac{\partial P_o}{\partial t} = D_o \nabla^2 P_o + \frac{1}{\tau_o} N (1 - P_o - P_g - P_d) P_o + \frac{1}{\tau_o} \chi(W) P_g \\
- \frac{1}{\tau_{og}} \chi_W(W) \chi_N(N) P_o - \mu_o \chi_N(N) P_o \\
\text{random motion} \quad \text{proliferation} \quad \text{switch to OXPHOS} \\
\text{switch from OXPHOS} \quad \text{death} \\
(3.1a)
\]

\[
\frac{\partial P_g}{\partial t} = D_g \nabla^2 P_g + \frac{1}{\tau_g} \alpha_W + W N (1 - P_o - P_g - P_d) P_g - \frac{1}{\tau_g} \chi(W) P_g \\
+ \frac{1}{\tau_{og}} \chi_W(W) \chi_N(N) P_o - \mu_g \chi_N(N) P_g \\
\text{random motion} \quad \text{proliferation} \quad \text{switch from glycolysis} \\
\text{switch to glycolysis} \quad \text{death} \\
(3.1b)
\]

\[
\frac{\partial P_d}{\partial t} = D_d \nabla^2 P_d + \mu_o \chi_N(N) P_o + \mu_g \chi_N(N) P_g - \mu_d P_d \\
\text{random motion} \quad \text{dead } P_o \text{ cells} \quad \text{dead } P_g \text{ cells} \quad \text{decay} \\
(3.1c)
\]

\[
\frac{\partial W}{\partial t} = D_W \nabla^2 W + \frac{1}{a + bW_I} \kappa_W NW^2 P_g + S_W(P_o + P_g) - \mu_W W \\
\text{random motion} \quad \text{inhibition upregulation} \quad \text{upregulation} \quad \text{downregulation} \\
(3.1d)
\]

\[
\frac{\partial W_I}{\partial t} = D_W I \nabla^2 W_I + \kappa_W NW^2 (P_o + P_g) - \mu_W I W_I \\
\text{random motion} \quad \text{upregulation} \quad \text{downregulation} \\
(3.1e)
\]

\[
\frac{\partial N}{\partial t} = D_N \nabla^2 N - \nu_{NG} N P_g - \nu_{NG} N P_o - \mu_N N + N_s \\
\text{random motion} \quad \text{uptake by } P_g \text{ cells} \quad \text{uptake by } P_o \text{ cells} \quad \text{decay} \quad \text{bulk source} \\
(3.1f)
\]

The first term on the right side of the equality in the \( P_o \) and \( P_g \) equations (Equations 3.1a and 3.1b) refers to diffusion, or random motion of the cells. The next terms are standard logistic proliferation terms, with proliferation dependent on nutrient level \( N \), cell type, and
the current total population of cells in the domain. The model sets the proliferation of
glycolytic cells to be dependent on Wnt activity according to Michaelis-Menten dynamics,
given by the term \( \frac{W}{\tau_o + W} \), which saturates at high levels of \( W \). The parameters \( \frac{1}{\tau_o} \) and \( \frac{1}{\tau_g} \) are
proliferation rates and \( \frac{1}{\tau_{go}} \) and \( \frac{1}{\tau_{og}} \) are switching rates. The last terms in these equations are
cell death terms; death is modeled such that it occurs if the nutrient supply \( N \) drops below
some threshold \( N_d \). The death rates are given by \( \mu_o \) and \( \mu_g \).

The glycolytic and oxidative cells can “switch” metabolism programs depending on \( W \), Wnt
activity, which is reflected in the third and fourth terms of each equation, where \( \chi_W \) and \( \chi^*_W \)
are switch functions. Essentially, these functions are used so that if Wnt activity is below
a parameter \( W^* \), then the cells are more likely to switch to a more dominant OXPHOS
program from a glycolytic program, and if Wnt activity is above \( W^* \), then the cells are more
likely to switch to glycolysis from OXPHOS. We assume that oxidative cells can switch to
glycolysis only if sufficient nutrient is present, given by parameter \( N_g^* \). The cells will die if
nutrient is below the parameter \( N_d \). We can adjust the steepness \( \gamma \) of the functions so that
they are more step-like and hence more sensitive to \( W \) and \( N \). We use a moderate value of
1 for \( \gamma_W \), the steepness of the Wnt switch. We use a large value of 100 for \( \gamma_N \), the steepness
of the switch to glycolysis; we could alternatively have used piecewise functions for \( \chi_N \) and
\( \chi^*_N \). The switch functions \( \chi_W \), \( \chi^*_W \), \( \chi_N \) and \( \chi^*_N \) are given by modified hyperbolic tangent
functions

\[
\chi_W(W) = \frac{1}{2} [1 - \tanh(\gamma_W(W - W^*))] \quad (3.2a)
\]
\[
\chi^*_W(W) = \frac{1}{2} [1 + \tanh(\gamma_W(W - W^*))] \quad (3.2b)
\]
\[
\chi_N(N) = \frac{1}{2} [1 - \tanh(\gamma_N(N - N_d))] \quad (3.2c)
\]
\[
\chi^*_N(N) = \frac{1}{2} [1 + \tanh(\gamma_N(N - N_g^*))] \quad (3.2d)
\]
The dynamics of dead cells are described by another reaction-diffusion equation (Equation 3.1c). This is the population of cells that have died from lack of nutrient, which is given by the second and third terms in this equation. These cells can also diffuse and decay.

Equations 3.1d and 3.1e are illustrated in Figure 3.2B and describe $W$ (Wnt) and $W_I$ (Wnt inhibitor, e.g., SFRP5) activity. $D_W$ and $D_{W_I}$ are constant diffusion coefficients. It has been shown in epidermal cells that Wnt target genes produce Wnt signals as well as long-range secreted Wnt inhibitors [10], so the inhibitor is assumed to diffuse much longer range than Wnt, i.e., $D_W$ must be significantly smaller than $D_{W_I}$. Wnt signaling activity is assumed to be nonlinear with respect to Wnt and is inhibited by the Wnt inhibitor through the term $\frac{1}{a+bW_I}$. We assume the Wnt inhibitor is being produced by Wnt activity through both cell types. The terms $\mu_W$ and $\mu_{W_I}$ are decay rates. The term $S_W(P_o + P_g)$ in the Wnt equation refers to constitutive Wnt signaling through the cells.

The equation for nutrient (Equation 3.1f) describes the diffusion and uptake, decay, and source of nutrient. The nutrient term has Dirichlet (fixed) boundary conditions and diffuses in from the boundary of the spatial domain, so that the boundary can be considered as regions where vasculature is high. The second and third terms refer to uptake of nutrient by the two different cell types. The term $\mu_N N$ is a natural decay term. The last term, $N_s$, refers to the nutrient source, which is a small source term applied to the entire domain. This source term is based linearly on the glycolytic activity of the cells and is given by

$$N_s = N_s \left( \int P_g \right) = \left[ (1 - \alpha_N) \int P_g / S_x S_y + \alpha_N \right]$$

where $\alpha_N$ is a parameter less than 1, $\int P_g$ is the integral of $P_g$ cells, and $S_x$ and $S_y$ are the lengths of the sides of the spatial domain. This function was chosen so that $N_s(0) = \alpha_N$ and $N_s(S_x S_y) = 1$ ($S_x S_y$ is the maximum that $\int P_g$ can reach). We chose to have the nutrient source $N_s$ depend on $P_g$ cells because glycolysis induces angiogenesis, allowing more nutrients and growth factors to be delivered to the tumor [18]. We use a linear function in the model as the simplest form for the dependence between

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$N$ and $P_g$ and because the vessel density for mock and dominant negative tumors can be fit by a line; see Figure 3.3.

Figure 3.3: Vessel density versus phospho-PDH expression relative to mock. We quantified the vasculature of the tumors by staining for CD31, an endothelial cell marker (panels C, D, E). We graphed the vessel density per field and the level of pPDH expression on the $x$-axis, both values normalized to Mock tumors (panel A). Mock tumors demonstrated significantly higher pPDH levels and vasculature than dnLEF or dnTCF tumors. The nutrient scaling factor is used for the nutrient bulk source and boundary conditions in the mathematical model (panel B).
This mathematical model is a simplified version of a larger model (presented in Chapter 4) that includes similar equations for $P_o$, $P_g$, $W$, $W_I$, and $N$, as well as additional equations for PDK activity, HIF concentration (a hypoxia-inducible transcription factor that activates transcription of PDK1), and lactate concentration. The cellular metabolism switch was modulated by PDK activity rather than Wnt. The results from that model are qualitatively similar to the results in this section, so we conclude that the most important elements are retained in the Wnt signaling model.

### 3.4 Diffusive Instability Analysis

In this section, we present the details for classic diffusive instability analysis which is performed on the Wnt signaling model in this chapter in a similar way to that described in Chapter 2 and in [1]. Due to the complexity of the system of equations, the entire analysis is performed numerically rather than in closed-form. Because of boundary conditions, we perform the analysis only on the equations for $P_o$, $P_g$, $W$, and $W_I$ and assume that $N$ is constant at 1. The analysis presented here is for one spatial dimension.

In classic Turing analysis, we desire steady states that are stable in time and unstable in space. This is first done by considering the model as a set of ordinary differential equations by excluding diffusion. The steady states of the ODEs are computed and their stability is determined by linearization of the equations about their steady states. The steady states are asymptotically stable if the real part of every eigenvalue of the Jacobian matrix of partial derivatives, evaluated at the steady state, is negative. Diffusion is then included and the Jacobian matrix and its eigenvalues are recomputed. If any of the real parts of these eigenvalues is positive, then we have diffusive instability, meaning that a perturbation from the initial condition may grow over time.
More specifically, assuming a system of reaction-diffusion equations

\[ u_t = \Delta u + f(u), \]

we omit the diffusion term and assume a stationary uniform state (i.e., \( f(u_0) = 0 \), where \( u_0 \) is a steady state). Linearizing and setting \( w = u - u_0 \), we get

\[ w_t = Jw, \]

where \( J \) is the Jacobian matrix of partial derivatives of \( f \) evaluated at \( u_0 \). If the eigenvalues of \( J \) have real part all negative, then the steady state \( u_0 \) is stable and we continue with the analysis by including diffusion and linearizing again. We obtain

\[
\begin{bmatrix}
D_1 & 0 \\
D_2 & \ddots \\
0 & \cdots & D_n
\end{bmatrix}
\begin{bmatrix}
\partial_{xx}w
\end{bmatrix}
\]

where \( D_i \) is the diffusion coefficient of the \( i^{th} \) variable. Assuming, as we did in Chapter 2, a solution of the form \( \alpha \cos(qx)e^{\sigma t} \), we can rewrite our operator as the matrix-differential linear operator

\[
\tilde{J} = \begin{bmatrix}
a_{11} - q^2 D_1 & a_{12} & \ldots & a_{1n} \\
a_{21} & a_{22} - q^2 D_2 & \ddots \\
\vdots & & \ddots & \\
a_{n1} & \ldots & a_{nn} - q^2 D_n
\end{bmatrix}
\]

where \( a_{ij} \) is the partial derivative of the \( i^{th} \) equation with respect to the \( j^{th} \) variable, evaluated at the given steady state, and \( D_i \) is the diffusion coefficient of the \( i^{th} \) variable. We compute the eigenvalues of \( \tilde{J} \) and require at least one of them to have positive real part. If this occurs, then we assume that this parameter set will yield patterns, at least for short times. The greater the magnitude of the real part of the positive eigenvalue, the more likely a perturbation will grow, hence the more likely a pattern is to form.
We first narrowed our parameter range in the Wnt and Wnt inhibitor equations by investigating the two-equation activator-inhibitor system, i.e., classic diffusive instability analysis was done on this smaller system to narrow down the parameter space that is expected to give patterns. After finding and fixing suitable parameters for these equations, we performed diffusive stability analysis on the larger system by numerically solving the equations without diffusion for 100 time units, fixing all other values and varying two parameters at a time. If a steady state was not found (i.e., the difference between the minimum and maximum of the ODE solution at the last ten time steps was greater than some tolerance), then that set of parameters was assumed to give us oscillations or to be otherwise unstable and so the linear stability analysis could not be performed with those parameters.

We loop over integers $k$ from 1 to 12, where $k$ is the wavenumber in $q = 2\pi k/d$, and $d$ is the length of the one-dimensional spatial domain. If any of these new eigenvalues has a positive real part, then that set of parameters is expected to result in a pattern during early times.

In Figures 3.4 through 3.13, areas that are white are associated with parameters where no patterns are expected to form. Areas that are neither white nor blue show the eigenvalue and wavenumber associated with the pattern that is predicted to emerge. Any area that is blue is a region in which the solution of the ordinary differential equation (ODE) versions of the equations is oscillatory rather than steady, hence the equations could not be linearized about a steady state and so the analysis could not be performed. That is not to say, however, that patterns could not form in the PDE system with those parameter values.

We plot the maximum positive eigenvalue, which quantifies the instability of an initial perturbation, and associated wavenumber $k$, which predicts the dominant number of spikes or peaks in a one-dimensional spatial domain, and is thus an indicator of number of spots. If a set of parameters has at least one positive eigenvalue, it is expected that a perturbation grows in time, hence a pattern should form in early times. The larger the eigenvalue, the higher the instability and hence more likelihood for a pattern to emerge and remain over
the long term. Therefore, a nonzero eigenvalue that is very small may yield a pattern only in very early times in the simulation. Moreover, a nonzero eigenvalue and wavenumber may mean a pattern exists in the system, but it may translate to a pattern only in the Wnt and inhibitor, and not necessarily in the cells. We have found that we see good agreement between the parameter space predicted to form patterns through this analysis and in our numerical results.
Figure 3.4: Maximum eigenvalues for diffusive instability analysis varying $\kappa_W$ and $\kappa_{W_I}$. Patterns are expected to form at early times in colored regions, except blue regions indicating oscillating ODE solutions where the analysis cannot be performed; white means that the maximum eigenvalue is negative or 0. Higher eigenvalues indicate more instability.

Figure 3.5: Wavenumbers for diffusive instability analysis varying $\kappa_W$ and $\kappa_{W_I}$. Patterns are expected to form at early times in colored regions, except blue regions indicating oscillating ODE solutions where the analysis cannot be performed; white means that the associated maximum eigenvalue is negative or 0. Wavenumber is an indication of number of spots.
Figure 3.6: Maximum eigenvalues for diffusive instability analysis varying $\mu_W$ and $\mu_{W_I}$. Patterns are expected to form at early times in colored regions, except blue regions indicating oscillating ODE solutions where the analysis cannot be performed; white means that the maximum eigenvalue is negative or 0. Higher eigenvalues indicate more instability.

Figure 3.7: Wavenumbers for diffusive instability analysis varying $\mu_W$ and $\mu_{W_I}$. Patterns are expected to form at early times in colored regions, except blue regions indicating oscillating ODE solutions where the analysis cannot be performed; white means that the associated maximum eigenvalue is negative or 0. Wavenumber is an indication of number of spots.
Figure 3.8: Maximum eigenvalues for diffusive instability analysis varying $a$ and $b$. Patterns are expected to form at early times in colored regions, except blue regions indicating oscillating ODE solutions where the analysis cannot be performed; white means that the maximum eigenvalue is negative or 0. Higher eigenvalue indicates more instability.

Figure 3.9: Wavenumbers for diffusive instability analysis varying $a$ and $b$. Patterns are expected to form at early times in colored regions, except blue regions indicating oscillating ODE solutions where the analysis cannot be performed; white means that the associated maximum eigenvalue is negative or 0. Wavenumber is an indication of number of spots.
Figure 3.10: Maximum eigenvalues for diffusive instability analysis varying $D_W$ and $S_W$. Patterns are expected to form at early times in colored regions, except blue regions indicating oscillating ODE solutions where the analysis cannot be performed; white means that the maximum eigenvalue is negative or 0. Higher eigenvalues indicate more instability.

Figure 3.11: Wavenumbers for diffusive instability analysis varying $D_W$ and $S_W$. Patterns are expected to form at early times in colored regions, except blue regions indicating oscillating ODE solutions where the analysis cannot be performed; white means that the associated maximum eigenvalue is negative or 0. Wavenumber is an indication of number of spots.
Figure 3.12: Maximum eigenvalues for diffusive instability analysis varying $\gamma$ and $W^*$. Patterns are expected to form at early times in colored regions, except blue regions indicating oscillating ODE solutions where the analysis cannot be performed; white means that the maximum eigenvalue is negative or 0. Higher eigenvalues indicate more instability.

Figure 3.13: Wavenumbers for diffusive instability analysis varying $\gamma$ and $W^*$. Patterns are expected to form at early times in colored regions, except blue regions indicating oscillating ODE solutions where the analysis cannot be performed; white means that the associated maximum eigenvalue is negative or 0. Wavenumber is an indication of number of spots.
3.5 Reaction-Diffusion Modeling Mimics the Self-Organizing Patterns of PDK Activity and Glycolysis in Xenograft Tumors

In the numerical results presented here, we used no-flux boundary conditions for all terms except $N$, which has Dirichlet boundary conditions ($N$ at the boundary is equal to the value $\beta_N N_s$, where $\beta_N$ is a parameter and $N_s$ is described above). The initial conditions were set for a random distribution of $P_g$ cells located near the boundary, and small random values of $W$ and $W_I$ in the same areas where initial $P_g$ cells are located. A constant high level of nutrient throughout the domain was provided, and the initial condition contained no $P_o$ cells. All parameter values used are given in Table 3.2 and were chosen to be in qualitative agreement with experimental data.

Figure 3.2C-D shows the numerical two- and three-dimensional results for the fraction of glycolytic and oxidative cells and concentration of Wnt and Wnt inhibitor, where each two-dimensional plot is a horizontal slice through the center of the three-dimensional spatial domain. Consistent with our xenograft data, a distinct spotted pattern in the population of glycolytic cells is produced by the model over time. Over the entire domain, there is a high level of glycolytic-dominant cells (light red background) with localized areas of very high levels of glycolytic cells (dark red spots). Similar to the xenograft tumors, the spots are denser toward the boundary of the tumor space, where there is a higher density of vasculature, a spatial pattern that agrees with the overall pattern of pPDH staining of the mock tumors in Figure 3.1A. The oxidative cells are close to 0 in the same spots where glycolysis is high, and their levels are relatively higher in regions surrounding these spots. Wnt and Wnt inhibitor activity show a similar spotted pattern, with high levels distributed in a spotted array throughout the domain, surrounded by lower levels in the neighboring
regions. Like the pattern of glycolysis, the frequency of spots is higher near the boundary relative to the interior. We conclude that this model and its assumptions are sufficient to reproduce, in a qualitative manner, similar results to our experimental data.

3.6 Parameter effects on metabolic phenotype

Because the parameters required for our model (rates of production, decay, upregulation, proliferation, etc.) are largely unknown, we performed a parameter study (Figure 3.2D) to explore this space. We included diffusive instability analysis to determine the ranges of values for which patterns are predicted to occur (see diffusive instability section in this chapter). Restricting the model to the parameter space that results in patterning, we modified the parameters one by one and tested for phenotype changes in metabolism and patterning. Increasing the diffusion coefficient or decreasing the decay of Wnt produced the effect of spreading out Wnt activity, so that the spots of glycolysis (i.e., cell clusters) increased in size. Decreasing the decay of the Wnt inhibitor caused the inhibitor to stay within the system for more time, and resulted in fewer spots. Modifying the proliferation parameters only changed the number of time steps it took to reach a steady state but otherwise had no effect on the self-organization of a spotted array. In contrast, modifying the switch time parameters changes the proportion of \( P_o \) and \( P_g \) cells, a modification which greatly affected the patterning. Increasing or decreasing the Wnt switch changed the background of \( P_g \) cells and size of the spots (cell clusters) without affecting the number of spots. Small reductions to \( S_W \), which can be thought of as reducing overall Wnt signaling, reduced the background of glycolytic cells without much effect on size or number of spots. Sufficiently reducing \( S_W \) resulted in all terms (\( P_o, P_g, W, \) and \( W_I \)) decreasing to 0. When \( \kappa_W \) (nonlinear Wnt activity) was decreased or \( b \) (a constant of inhibition) was increased, a greater number of glycolytic cells was observed. The change occurred because of the nonlinear effect; making
these parameter changes only affected the nonlinear Wnt upregulation term, and results in less Wnt inhibitor. Less inhibitor resulted in more Wnt activity, which in turn increased the number and density of glycolytic cells. When $\mu_W$ (Wnt decay rate) was increased, a drop in Wnt activity and a corresponding decrease in the size of glycolytic spots was observed. Figure 3.1D provides a summary of the parameter effects on the pattern.

3.7 Interfering with Wnt Signaling Alters Colon Cancer Metabolism In Vivo

We propose that the system of metabolism in xenograft tumors and the spotted pattern that reflects its heterogeneity is influenced by Wnt signaling, so that a change in Wnt activity should result in a change in patterning. Therefore, to test whether Wnt is an influential signal, we interfered with the pathway by blocking downstream activity through the overexpression of dominant negative LEF-1 (dnLEF), or dominant negative TCF-1 (dnTCF). Both dominant negative versions are naturally occurring transcription factor isoforms that lack the $\beta$-catenin binding domain and therefore interfere with the activation/expression of Wnt target genes. Expression of moderate, physiological levels of dnLEF or dnTCF expression partially, but not completely, disrupts Wnt target gene expression in the xenograft tumors. Partial disruption is necessary because complete inhibition of Wnt activity would block the formation of tumors altogether.

Parental SW480 colon cancer cells were lentivirally transduced and selected for dnLEF or dnTCF expression. These cells were subcutaneously injected in immunocompromised mice for tumor formation. Experiments showed that, as a result of overexpressing dnLEF, PDK1 activity was reduced, Warburg metabolism was diminished as measured by ATP and lactate levels, and tumor mass was reduced by approximately 4-5-fold [18]. Immunohistochemical
staining of the levels of phospho-PDH in these tumors (Figure 3.14A) revealed a lighter background and lower pPDH level overall. Interestingly, pPDH positivity remained easily visible as a spotted pattern, but with striking changes. The pPDH spots were comprised of a greater number of cells – with greater distances between each spots (compared to parental or “mock” transduced cells; Figure 3.14D).

We also utilized immunohistochemical staining for the Wnt-mediating factor β-catenin in the dnLEF tumors (Figure 3.14B) (dnLEF-expressing tumors cannot be stained for LEF1). These stains likewise revealed a heterogeneous pattern, with clusters of cells (spots) having higher levels of β-catenin in the nucleus than neighboring cells, although because of the very high levels of β-catenin in SW480 cells, there is an overall strong intensity of the IHC stain. Automated image analysis identified contours of cell clusters that were not larger, but similar in size to pPDH cell clusters (spots) in the Mock/parental tumors.
SW480 dnLEF1 Xenograft Tumor

A. Phosphorylated PDH

B. β-catenin

C. [Images of time series data for different cell types]

D. [Graph showing relationship between spot area and distance to nearest neighbor]

 mocks pPDH spots
 mocks pPDH average
 dLEF pPDH spots
 dLEF pPDH sim average
 dLEF β-catenin spots
 dLEF/ΔTGF sim average
3.8 Modeling Interference of Wnt Signaling

To understand the phenotypic alterations in the metabolic pattern, we used the model to identify changes in parameters that could recapitulate the experimental observation. The simplest change in the model that mimics interference of Wnt signaling is to reduce $S_W$, which lowers intrinsic Wnt activity throughout the domain – a manipulation that represents the effect of the Wnt-interfering, dominant negative LEF/TCF expression in every cell. When $S_W$ is reduced slightly (from 7.5 to 7; see Figure 3.15), the background of $P_g$ cells lightens, but the number of spots and sizes of spots remain the same. As this parameter is reduced further, lowering the background of Wnt signaling and glycolytic metabolism even more, the frequency and sizes of spots remain the same. When it is reduced to a critical level (in this case, 5.5), all terms decrease to 0. Thus, the modeling results obtained from simply modifying overall Wnt signaling ($S_W$) are inconsistent with the experimental data.
Figure 3.15: Effects of varying the rate $S_w$ of constitutive Wnt signaling. Oxidative and glycolytic populations at end of simulation are shown.

Clearly, the effects of dnLEF/dnTCF expression are more complex than simply decreasing Wnt throughput in the nucleus. The changes in the size and density of the pPDH-positive cell clusters suggested that dnLEF/dnTCF might be triggering a cell-extrinsic response that connects collections of cells in the microenvironment. Specifically, the increase in the cell cluster size suggested the existence of an extracellular soluble factor that manipulates and crosstalks with the stability of Wnt. Therefore, following our parameter exploration above, we considered additional modifications of parameters to develop a better qualitative agreement with the metabolic pattern in the dnLEF/dnTCF tumors and to take into account a diffusible signal that influences Wnt signaling: decrease $S_w$ as described above (so there is less overall Wnt signaling activity in the entire domain); increase the diffusion coefficient $D_w$ of the Wnt signal (this spreads the Wnt signal, which causes the development of larger spots); and increase the diffusion coefficient $D_{wI}$ of its inhibitor (which spreads the Wnt inhibitor farther to yield fewer spots). Changing these parameters resulted in a lighter background and the development of larger, fewer spots similar to that observed in the dnLEF/dnTCF-expressing tumors. Alternatively, to achieve the same trend in patterning as
in the experiments, instead of increasing $D_W$ and $D_{W_I}$, we obtained similar results when we decreased $\mu_W$ and $\mu_{W_I}$. Additionally, since nutrient $N$ in the model is tied to the proportion of $P_g$ cells, a natural decrease in $N$ in both the interior and boundary was observed. This observation is consistent with our experimental data, as we observed significantly fewer blood vessels in the dnLEF and dnTCF tumors compared to mock [18]. Figure 3.14C presents the simulation results from these parameter changes. Overall, the simulated tumors self-organize with larger spots of glycolysis (pPDH-positivity) and Wnt activity, with a lighter background overall, and fewer spots. Image analysis of the xenograft tumor staining gives a scatter plot (Figure 3.14D) of the area of each spot versus distance to nearest neighbor (centroid to centroid), relative to mock average spot area or distance. In addition, the average spot size versus distance of the simulation tumors, relative to mock simulation averages, is shown on the plot. Overall, mock spots of pPDH are smaller and closer together compared to those in the dnLEF or dnTCF tumors and the simulation results correlate very well. The exception is the heterogeneous $\beta$-catenin staining, which was detected by the image analysis as an array of spots with an average size and distance similar to the Mock-parental xenograft tumors, an effect which needs to be investigated further.

3.9 In Vivo Validation of Model Predictions

Only a few studies have directly examined the diffusion range of Wnt ligands, a range which is extremely limited, in part because the ligands are post-translationally modified by palmitoylation and are highly lipophilic for membranes and extracellular matrix proteins [29]. Regulators that modify the range of ligand diffusion are even less well characterized (Figure 3.16A). Secreted Wnt inhibitors are better studied and include the Dickkopf (DKK) family of secreted inhibitors of the Wnt co-receptor LRP5/6, and the SFRP protein family, secreted inhibitors that bind directly to Wnt ligands to prevent receptor binding (Figure
Given that our mathematical model predicts the diffusion coefficients of Wnt ligands and their inhibitors to have increased in the dnLEF and dnTCF xenograft tumors, we identified candidate regulators and tested the prediction that one or more were elevated in their expression. We used Q-RTPCR analysis of mRNA purified from both colon cancer cells cultured in 2D conditions and from snap-frozen 3D xenograft tumors. Using RNA-seq data as a guide for identifying candidate inhibitors expressed in SW480 cells, we designed primers for both diffusion regulators and inhibitors that were detectably expressed in this cell line. Expression analysis of these candidate regulators revealed that the Wnt diffusers SPOCK2 and GPC4 are overexpressed in our dnLEF and dnTCF tumors while the Wnt inhibitor SFRP5 is modestly elevated in the dnLEF and dnTCF tumors, but not in the 2D cultures (Figure 3.16B, D). Interestingly, clinical data from The Cancer Genome Atlas (TCGA) (Figure 3.16E) shows that SPOCK2 mRNA and SFRP5 mRNA are downregulated in colon cancer samples relative to normal colon tissue, results that are consistent with our model predictions because expression of diffusion regulators and inhibitors likely decreases as high Wnt signaling and metabolic heterogeneity develop in primary colon cancer.
### A. Fly Gene | Human Gene | Reference | Function
--- | --- | --- | ---
Swim | LCN7/TINAGL1 | Mulligan et al. PNAS 2012 | Binds to Wg in a lipid-dependent manner to increase range. At high concentrations, can inhibit Wls activity.
CDC42 | Stanganello, et al. Nature Communications 2015 | CDC42/N-Wasp regulates formation of Wnt-positive filopodia
Dally/Dip | GPC1-6 | Lin, et al. Nature 1999 | Co-receptor with Fzd2 to modulate range of Wg
Cow | Testican-2/SPOCK2 | Chang, et al. Plos One 2014 | HSPG that binds to Wg, increasing Wg mobility. Can act as an inhibitor in high Wg concentration.

### B. Expression of Wnt Ligand Diffusing Proteins

**in vitro**

- Fold expression over Mock
- CDC42, LCN7, SPOCK2, GPC1, GPC2, GPC4

**in xenograft tumors**

- Fold expression over Mock
- CDC42, LCN7, SPOCK2, GPC1, GPC2, GPC4

* p < 0.05. N = 3, SEM shown.

### C. Human Gene | Reference | Function
--- | --- | ---
DKK4 | Baehs, et al. Cancer Letters 2009 | Prevents Wnt binding to LRP co-receptor
SFRP5 | Suzuki, et al. Nature Genetics 2004 | Secreted; Binds to Wnt ligands extracellularly, preventing binding to Fzd receptors

### D. Expression of Wnt Signaling Inhibitors

**in vitro**

- Fold expression over Mock
- DKK4, SFRP5

**in xenograft tumors**

- Fold expression over Mock
- DKK4, SFRP5

* p < 0.05. N = 3, SEM shown.
E.

**CDC42**

- **LCN7**

- **SPOCK2**

- **GPC1**

**Legend:**
- Red: Tumor
- Blue: Normal

**COAD:** Colon Adenocarcinoma
**COREAD:** Colorectal Adenocarcinoma
**READ:** Rectal Adenocarcinoma
Figure 3.16: Model predictions revealed in xenograft tumors and human colorectal cancer. The model predicted that lowering Wnt signaling results in an increase in the expression of factors that increase the range of diffusion of Wnt and Wnt inhibitors. (A) Known regulators of Wnt ligand diffusion. (B) Quantitative PCR of diffusion regulators in SW480 mock, dnLEF, and dnTCF transduced cells and xenograft tumors show human SPOCK2 mRNA and GPC4 mRNA notably upregulated in xenograft tumors but not 2D culture in vitro. (C) Wnt signaling antagonists that are expressed in SW480 colon cancer cells. (D) Quantitative PCR of Wnt antagonists reveals human SFRP5 mRNA to be upregulated in the dominant negative (dnLEF and dnTCF) tumors (but not in 2D culture in vitro). In vitro data represent an average of three sample sets (+/− SEM), xenograft tumor data represents the average of five independent tumor sets (+/− SEM); *P < 0.05. (E) Clinical data from The Cancer Genome Atlas (TCGA) show a downregulation in the expression of SPOCK2 mRNA (0.463, 0.458 fold compared to normal tissue) and SFRP5 mRNA (0.0676, 0.0479 fold compared to normal tissue) in colon cancer samples compared to normal tissue. Colon (C; 459 patients) and colorectal (CR; 626 patients). Expression levels generated by RSEM (RNA-Seq by Expectation Maximization) analysis of TCGA data using Firebrowse [13]. These results align with model predictions in that expression of diffusion regulators and inhibitors likely decreases as high Wnt signaling and metabolic heterogeneity develop in primary colon cancer.

3.10 Predicted Sensitivities of Glycolytic and Oxidative Cell Phenotypes to Targeted Therapy

To identify the most sensitive subpopulation of cells in the tumors, we compared the effectiveness of a hypothetical therapy program (see Figure 3.17) that targeted one metabolic population versus another by independently varying the death rates of $P_o$ and $P_g$ cells as proxy for targeted killing (using new death terms $\mu_{P_o}P_o$ and $\mu_{P_g}P_g$). The simulation applied the targeted therapy to a fully developed tumor at steady state for different lengths of time (nondimensional time units), followed by removal of the therapy and a recovery time for tumor development. These simulations revealed that regardless of the targeted population, modest rates of cell death suppressed tumor development transiently, followed by full recovery of the system, a pattern more evident and more robust when cell killing was directed towards
the $P_o$ population. In contrast, targeting the $P_g$ population led to a complete loss of the simulated tumor at lower time intervals or death rates. Thus, the simulation predicts that $P_g$ cells are the most sensitive population and that targeting these cells will more effectively lead to a crash of the system and loss of the tumor.

Figure 3.17: Simulations suggest that the glycolytic cell population is a more sensitive drug target than the oxidative cell population. We targeted either $P_o$ (left panels) or $P_g$ (right panels) cells selectively, starting from a metabolically patterned state, for 2.5, 5, or 7.5 time units, with a death rate between 0.25 and 1. After therapy is stopped, the cells are allowed to evolve according to the original model (Figure 3.2). The total cell population relative to initial cell population is shown.
3.11 Modeling Therapeutic Treatments for Cancer

There are no known therapies that directly target glycolytic cells, so we considered drugs that target them indirectly. Since selective targeting frequently resulted in full recovery of the simulated tumor, we also considered dual targeting of two features. For example, by simulating the effects of known small molecule inhibitors that inhibit Wnt signaling (e.g., porcupine inhibitors such as IWP2/C59 that block secretion of Wnt ligands, or Tankyrase inhibitors such as XAV939 that reduce β-catenin levels), coupled with agents that directly inhibit PDK (e.g., dichloroacetic acid, DCA), we asked whether combination therapy is more effective than single agent therapies. See Figure 3.18A. To simulate less Wnt secretion, the diffusion coefficient of Wnt was decreased. To model the inhibition of β-catenin by XAV939, which is similar to dominant negative tumors, we changed the parameters similarly: The general Wnt signaling term $S_W$ was decreased, and $D_W$ and $D_{WI}$ were increased linearly with respect to the decrease in $S_W$ (linear with respect to the change from mock to dnLEF parameters). Since inhibition of PDK releases inhibition of pyruvate dehydrogenase complex (PDC) and increases OXPHOS capacity, we modeled the effects of DCA by increasing the rate $\frac{1}{\tau_{go}}$ at which the switch from glycolysis to OXPHOS occurs.

In the mock DCA+IWP2 combination simulations, we varied $D_W$ between its original value of $4 \times 10^{-3}$ (normalized to 1 in Figure 3.18B) and $10^{-6}$ and $\frac{1}{\tau_{go}}$ between 1 and 24. The figure shows the response from an initial patterned state after applying this treatment for 50 time units. We depict the response as the ratio between the integrals of treated cells versus untreated cells. As we decrease $D_W$ from its original parameter value of 0.004, and increase $\frac{1}{\tau_{go}}$ from its original value of 1, the oxidative cells increase linearly with $\frac{1}{\tau_{go}}$ up to some point, after which there is a gradual decrease, followed by a sharp decrease. There is a corresponding gradual drop in the $P_g$ cells, followed by a sharp drop to 0, so that the total of all cells ($P_o$ and $P_g$ combined) is either close to 0 or close to 1. This suggests that this type
of treatment is an “all-or-nothing” response, so that either the entire population of cells is eradicated, or all cancer cells return, though perhaps with different fractions and different spatial patterns for oxidative or glycolytic phenotypes. When the oxidative cells reach a certain threshold, there is insufficient room for glycolytic cells to survive, so that nutrient levels and Wnt and Wnt inhibitor activity are substantially decreased, which in turn forces all cells to die.

Pure IWP2/C59 treatment (only reducing Wnt diffusion), without combining it with DCA therapy, does not appear to be effective in eradicating the cells. The Wnt secretion is reduced and the spotted pattern is more difficult to resolve, but the cells appear to reach a steady state, with total cells ($P_o + P_g$ cells) equal to 100%. Pure DCA treatment merely changes the balance between glycolysis and OXPHOS but does nothing to eradicate any of the cells. When we combine DCA and XAV939 treatment, a result similar to DCA+IWP2 treatment is obtained (Figure 3.18C). Fixing XAV939 concentration, as DCA concentration increases, the population of oxidative cells increases, until there is an insufficient level of $P_g$ cells, and hence Wnt and nutrient, to sustain the tumor, resulting in a crash in the system.

In general, these treatment simulations indicate that a combination of two therapies will be effective, as long as one or the other has adequately been applied. For example, a value of $\frac{1}{\tau_{go}} = 12$ is effective in eradicating the cells as long as $D_W$ is $10^{-4}$ or less. In other words, if Wnt secretion or $\beta$-catenin expression has not been sufficiently suppressed, then PDK inhibition must be adequately increased, and vice versa.
A. Combined DCA & IWP2 Treatment

- **Lrp Fzd**
- **Wnt**
- **ß-Catenin**
- **mTORC2**
- **PDK1**
- **Glycolysis**

<table>
<thead>
<tr>
<th>Function</th>
<th>IWP2/C59/LGK974</th>
<th>XAV939</th>
<th>DCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porcupine inhibitor. Prevents secretion/production of Wnt ligands, decreasing extracellular signals.</td>
<td>Tankyrase inhibitor. Prevents dissolution of ß-catenin destruction complex, increasing degradation of ß-catenin.</td>
<td>Pan-PDK inhibitor, prevents shutting down mitochondrial respiration, encourages OXPHOS</td>
<td>Decreased diffusion coefficient of W (D_w)</td>
</tr>
<tr>
<td>Model</td>
<td>Clinical</td>
<td>Preclinical</td>
<td>Clinical</td>
</tr>
</tbody>
</table>

B. Combined DCA & IWP2 Treatment

C. Combined DCA & XAV939 Treatment
Figure 3.18: Therapies targeting metabolism and Wnt signaling synergize for tumor death in mathematical simulations. (A) Modeled therapies, their targets and the model parameters influenced by therapy. (B) Starting with a metabolically patterned state, treatment of tumors with dichloroacetic acid (DCA) and IWP2 combined leads to an effective crash in the system, as shown by the complete loss of cells (B.1e and B.2e). The panels on the left show the oxidative ($P_o$, top) and glycolytic ($P_g$, bottom) cell arrangements (metabolic patterning) and the three graphs on the right show the fractions of $P_o$ or $P_g$ cells relative to their initial values, after applying the therapy for 50 time units. The panels all use a single Wnt diffusion value ($D_W/D_{W\text{\ mim}=0.25}$, corresponding to red curve on the graphs). Each individual simulation panel corresponds to $1/\tau_{go}$ values 1, 4, 8, 14, and 15 respectively (denoted on the graphs by labels (1a) through (2e)). The effects of therapy on the total cell population, relative to the initial cell population, for the same $D_W$ and $1/\tau_{go}$ values, are shown in the third graph. (C) Treatment with DCA and XAV939 triggers a similar, sudden decrease in cell populations. XAV939 treatment is modeled by decreasing $S_W$ and increasing $D_W$ and $D_{Wj}$ linearly with respect to the decrease in $S_W$; legend values are listed relative to mock $S_W$. The panels on the left correspond to the purple curve in the graphs and show the effect on patterning for $1/\tau_{go}$ values 1, 4, 12, 17, and 18 respectively (denoted by labels (3a) through (4e)).

3.12 Discussion

Though the role of Wnt signaling in cancer growth and development has been studied for many years, only recently has the contribution of Wnt signaling to the regulation of cancer cell metabolism been described. In this paper we generated colon cancer xenograft tumors and examined changes in cellular metabolism by immunohistochemical staining for phospho-PDH. We showed that the tumors exhibited a pronounced spotted pattern, where the spots are presumed to be areas in which the predominant metabolic pathway is glycolysis and the areas surrounding the spots are regions in which cells are primarily undergoing oxidative phosphorylation. We proposed a mathematical model for this symbiotic spatio-temporal relationship between these cell types, including equations for populations of these two metabolic phenotypes (an oxidative population and a glycolytic population); Wnt; a Wnt inhibitor (e.g., SFRP5); and a generic nutrient term. The Wnt and Wnt inhibitor equations are driven by Gierer-Meinhardt’s activator-inhibitor model, which describes the
feedback relationship between Wnt and its inhibitor, from which a spotted pattern emerges. Our equations also enable us to describe the cellular switching of metabolism regimes through Wnt levels, and results in a spotted pattern in the cell types. The results from our model demonstrate good qualitative agreement with our xenograft stains.

Metabolism is a complex process, involving many different pathways, nutritional stresses, and a changing microenvironment, and as such requires coping mechanisms such as the symbiosis reported here. Although xenograft tumors are an artificial construct, heterogeneity in metabolism does not occur only in these tumors. In the normal colon, pPDH stains show a gradient, with high PDK activity in the base of the crypt, and less PDK activity closer to the top. In orthotopic tumors, in which cancer cells were implanted in the mouse intestine, we also observed a spotted pattern in pPDH stains (not shown). In human patient tumors, there is no regular pattern but there is definite heterogeneity in the epithelial portion with abundant stroma. We were able to eradicate the spotted pattern in the xenografts by overexpressing PDK1 in each of the tumors, evidence that metabolism is dynamic and responsive to various factors.

Our model shows that interference in Wnt signaling is not only the result of a decrease in overall Wnt activity. Making the simple parameter change of decreasing overall Wnt signaling throughput does lead to less glycolytic activity surrounding localized spots of high glycolytic activity (lower background of $P_g$ cells), which was validated in experiments in which Wnt transcription was blocked by the overexpression of dominant negative LEF-1, but in addition to less glycolytic activity in the background, the dnLEF stains also revealed larger, fewer regions of PDK activity (i.e., larger, fewer spots). In the model, the effect of larger and fewer spots is achieved by increasing the diffusion coefficients for Wnt and its inhibitor; these changes result in spreading out of Wnt and Wnt inhibitor activity. This model prediction led us to investigate the expression of Wnt diffusers and inhibitors. We found that the diffusers SPOCK2 and GPC4 were overexpressed in our xenografts and not
in vitro. The expression of SFRP5, which acts as a Wnt inhibitor by preventing binding Fzd receptors, also showed higher expression in our dominant negative xenografts compared to mock.

We also modeled the effects of various possible therapy agents that affect glycolysis. We considered agents such as IWP2 or C59 that block the secretion or production of Wnt ligands; XAV939, which inhibits β-catenin; and DCA, which inhibits PDK, thereby increasing OXPHOS. We modeled these agents respectively by reducing the diffusion coefficient of Wnt; decreasing the rate of constitutive Wnt signaling and concurrently increasing the diffusion of Wnt and its inhibitor; or by increasing the rate at which the cells can switch to oxidative phosphorylation. The model predicts that these therapeutic regimes are less effective if applied individually, and that the novel combination of DCA treatment together with either a Wnt ligand inhibitor such as IWP2 or β-catenin inhibitor like XAV939 can be highly effective in eradicating the tumor. The tumor type and whether to target Wnt secretion or β-catenin will determine which drug combination to use.

The mathematical model presented in this section is an abstract idealization and simplification of tumor proliferation and metabolism, and real tumors are much more complex than presented here. Although it is clear that Wnt has a strong influence on the pattern, since the spots change drastically when Wnt is interrupted, it is possible that Wnt and a Wnt inhibitor are not the only factors contributing to the pattern. We consider a larger model with more details in Chapter 4, but that model is again a representation, and numerical results are qualitatively similar to those presented here. In this paper, we had to make several assumptions for our model parameters, since these values are largely unknown. Because we investigated only xenograft tumors in the work presented here, we do not know how the results would differ with tumors in human subjects. The mock LEF1 spots are similar in shape and distribution to mock pPDH spots, but the β-catenin spots in the dnLEF tumors are not similar to the pPDH dnLEF spots, an observation which should to be studied in
more depth. We have not yet been able to test the treatment regimes discussed here, but the modeling provides promising results and testable hypotheses.

Future work includes experimentally validating the treatment predictions discussed here. An extension to the mathematical model could be to include an oxygen term, and to include explicitly the effects of angiogenesis, rather than having the general nutrient depend linearly on the population of glycolytic cells.

**Materials and Methods**

*Numerical simulations*

Numerical simulations were performed in MATLAB, using a forward difference method for each time derivative. $P_o$, $P_g$, $W$, and $W_I$ equations were solved implicitly in centered diffusion terms. The nutrient equation was solved implicitly in uptake, decay, and centered diffusion terms. No-flux boundary conditions were used for all fields except for the nutrient boundary which used a Dirichlet boundary condition. The initial condition for the $P_g$ cell population was a random distribution near the boundary of the domain. $W$ and $W_I$ initial conditions were a random distribution in the same locations where initial $P_g$ cells were located. Initial $N$ was 1 everywhere in the domain; results did not change qualitatively if $N$ was solved as a quasi-steady state equation. Initial conditions for $P_o$ and $P_d$ terms were 0.

*Xenograft tumors*

SW480 stable transductants for xenograft injection were prepared through lentiviral infection with pCDH vector from System Biosciences: alone (Mock), dnLEF-1 or dnTCF-1Emut, with or without PDK1, followed by selection with 500 µg/ml G418. Transduced cells were collected for confirmation of expression, and Wnt signaling activity was measured by a Super-
TOPFlash luciferase reporter (Kira Pate). 2.5 × 10^6 cells were injected subcutaneously into immunodeficient NOG mice. Tumors were removed, fixed in paraformaldehyde overnight, and paraffin embedded four weeks after injection.

**Immunohistochemistry**

For phosphorylated PDH and β-catenin staining, deparaffinized 3.5 µm sections of formalin-fixed-paraffin-embedded (FFPE) mouse xenograft tumor and human colorectal carcinoma tissues followed by pressure cooker antigen retrieval in citrate buffer, sections were blocked in 3% H2O2, goat or horse serum plus MOM block reagent (if mouse primary antibody was used on mouse tissue), avidin, and biotin blocking reagents (Vector Labs). Sections were incubated in primary antibodies: anti-PDHpSer293 (Calbiochem AP1062, 1:200), anti-β-catenin (BD 610153 mouse anti-βCatenin 1:100), anti-LEF1 (REPLACE), anti-PDK1 (REPLACE) followed by biotinylated secondary antibodies and visualization using a peroxidase-conjugated avidin-based Vectastain protocol. Slides were then counterstained with hematoxylin and mounted using Permount mounting medium (Fisher). Images were captured using an Olympus FSX100 system and processed in Adobe Photoshop.

**Quantitative PCR**

RNA was extracted from xenograft tumors and cells using TRIzol (Invitrogen) following manufacturer’s instructions. cDNA was synthesized with 1 µg of total RNA with the High Capacity cDNA Reverse Transcription Kit (Invitrogen), per manufacturer’s instructions. qPCR was performed in triplicate for each experimental condition using Maxima SYBR green qPCR Master Mix (Invitrogen), according to the manufacturer’s instructions. To normalize mRNA levels, GAPDH probes were used.
Primer pairs as follows:

GAPDH — Forward: TCGACAGTCAGCCGCATCTTTCTT,
    Reverse: GCGCCCAATACGACCAAATCC.

TINAGL1 — Forward: ACCAGGTCACTCCTGTCTACC,
    Reverse: GATGCCTCCCTTTGTATAGGAAG.

CDC42 — Forward: CCATCGGAATATGTACCGAC,
    Reverse: CTCAGCGGTCGTAATCTGTC.

SPOCK2 — Forward: CCCGGCAATTTCATGGAGG,
    Reverse: GCGGTTCCAGTGCTTGATC.

GPC1 — Forward: GGCTGGTGCTGCTATGT,
    Reverse: CAGGTTTCCTCCCATCTCGC.

GPC2 — Forward: CACCTGCTGTTCCAGTGAGA,
    Reverse: AGAGAGTGCTGGGCTACTGA.

GPC4 — Forward: GTGGGAAATGTGAACCTGGAA,
    Reverse: CGAGGGACATCTCCGAAGG.

DKK4 — Forward: GGGACACTCTGTGTGAACGA,
    Reverse: TGGTTTTCCTGGACTGGGTG.

SFRP5 — Forward: CTGTACGCGTCATCCTAGCC,
    Reverse: CGGACCAGAAGGGGTCTAT.
Image processing of spots

Image processing (overlay of spot contours, computation of spot size and distance to nearest neighbor) was done using built-in tools in MATLAB’s Image Processing Toolbox. Parameters for built-in tools were chosen manually to give the best fit for pattern contours.

3.13 Tables

Table 3.1: Variables in Wnt signaling model

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_o$</td>
<td>Fraction of cell population undergoing oxidative phosphorylation</td>
</tr>
<tr>
<td>$P_g$</td>
<td>Fraction of cell population undergoing glycolysis</td>
</tr>
<tr>
<td>$P_d$</td>
<td>Fraction of cell population that has died from lack of nutrient</td>
</tr>
<tr>
<td>$W$</td>
<td>Wnt activity</td>
</tr>
<tr>
<td>$W_I$</td>
<td>Wnt inhibitor activity</td>
</tr>
<tr>
<td>$N$</td>
<td>Nutrient</td>
</tr>
<tr>
<td>Parameter</td>
<td>Description</td>
</tr>
<tr>
<td>-----------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>$D_o$</td>
<td>Diffusion coefficient of oxidative cells</td>
</tr>
<tr>
<td>$D_g$</td>
<td>Diffusion coefficient of glycolytic cells</td>
</tr>
<tr>
<td>$D_W$</td>
<td>Diffusion coefficient of Wnt</td>
</tr>
<tr>
<td>$D_{W_I}$</td>
<td>Diffusion coefficient of Wnt inhibitor</td>
</tr>
<tr>
<td>$D_N$</td>
<td>Diffusion coefficient of nutrient</td>
</tr>
<tr>
<td>$\tau_o$</td>
<td>Oxidative cell proliferation time</td>
</tr>
<tr>
<td>$\tau_g$</td>
<td>Glycolytic cell proliferation time</td>
</tr>
<tr>
<td>$\tau_{og}$</td>
<td>Switch time from OXPHOS to glycolysis</td>
</tr>
<tr>
<td>$\tau_{go}$</td>
<td>Switch time from glycolysis to OXPHOS</td>
</tr>
<tr>
<td>$\alpha_W$</td>
<td>Constant for Michaelis-Menten dynamics</td>
</tr>
<tr>
<td>$\kappa_W$</td>
<td>Rate of nonlinear Wnt production</td>
</tr>
<tr>
<td>$\kappa_{W_I}$</td>
<td>Rate of Wnt inhibitor production</td>
</tr>
<tr>
<td>$\mu_o$</td>
<td>Decay rate of $P_o$ cells</td>
</tr>
<tr>
<td>$\mu_g$</td>
<td>Decay rate of $P_g$ cells</td>
</tr>
<tr>
<td>$\mu_d$</td>
<td>Decay rate of $P_d$ cells</td>
</tr>
<tr>
<td>$\mu_W$</td>
<td>Decay rate of Wnt</td>
</tr>
<tr>
<td>$\mu_{W_I}$</td>
<td>Decay rate of Wnt inhibitor</td>
</tr>
<tr>
<td>$\mu_N$</td>
<td>Decay rate of nutrient</td>
</tr>
</tbody>
</table>

Model parameters for mock and dnLEF/dnTCF simulations in Wnt signaling model. Red text indicates changes from mock to dnLEF/dnTCF.
Table 3.3: Wnt signaling model parameters (continued)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Mock value</th>
<th>dnLEF value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S_W$</td>
<td>Rate of Wnt production through cells</td>
<td>7.5</td>
<td>6.5</td>
<td>Parameter estimation</td>
</tr>
<tr>
<td>$a$</td>
<td>Constant of inhibition</td>
<td>$10^{-8}$</td>
<td>$10^{-8}$</td>
<td>Parameter estimation</td>
</tr>
<tr>
<td>$b$</td>
<td>Constant of inhibition by $W_I$</td>
<td>1</td>
<td>1</td>
<td>Parameter estimation</td>
</tr>
<tr>
<td>$\gamma_W$</td>
<td>Sensitivity level of Wnt switch functions</td>
<td>1</td>
<td>1</td>
<td>Parameter estimation</td>
</tr>
<tr>
<td>$\gamma_N$</td>
<td>Sensitivity level of nutrient switch function</td>
<td>100</td>
<td>100</td>
<td>Assumed to be high</td>
</tr>
<tr>
<td>$\nu_{NG}$</td>
<td>Uptake of nutrient by $P_g$ cells</td>
<td>10</td>
<td>10</td>
<td>Parameter estimation</td>
</tr>
<tr>
<td>$\nu_{NO}$</td>
<td>Uptake of nutrient by $P_o$ cells</td>
<td>10</td>
<td>10</td>
<td>Parameter estimation</td>
</tr>
<tr>
<td>$N_s$</td>
<td>Parameter for nutrient source</td>
<td>2</td>
<td>2</td>
<td>Parameter estimation</td>
</tr>
<tr>
<td>$W^*$</td>
<td>Wnt level at which 50% of cells switch metabolism</td>
<td>5</td>
<td>5</td>
<td>Parameter estimation</td>
</tr>
<tr>
<td>$N^*$</td>
<td>Nutrient level below which cells will die</td>
<td>0.07</td>
<td>0.07</td>
<td>Parameter estimation</td>
</tr>
<tr>
<td>$N_g^*$</td>
<td>Nutrient level below which $P_o$ cells cannot switch to glycolysis</td>
<td>0.1</td>
<td>0.1</td>
<td>Parameter estimation</td>
</tr>
<tr>
<td>$\alpha_N$</td>
<td>Value of scaling function when $\int P_g = 0$</td>
<td>0.025</td>
<td>0.025</td>
<td>Parameter estimation</td>
</tr>
<tr>
<td>$\beta_N$</td>
<td>Nutrient boundary condition parameter</td>
<td>1</td>
<td>1</td>
<td>Parameter estimation</td>
</tr>
<tr>
<td>$S_x$</td>
<td>Horizontal length of spatial domain</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>$S_y$</td>
<td>Vertical length of spatial domain</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

Model parameters for mock and dnLEF/dnTCF simulations in Wnt signaling model. Red text indicates changes from mock to dnLEF/dnTCF.
Chapter 4

Wnt-PDK-Lactate-HIF Model

4.1 Background

The model in Chapter 3 was developed to have minimal complexity. Because other signaling pathways and growth or inhibition factors are known to be involved in cancer growth and metabolism, we considered an extension to the Wnt signaling model to include these additional effects.

4.2 Mathematical Model

4.2.1 Augmenting the Wnt Signaling Model

We added more detail to the Wnt signaling model by including equations for PDK, lactate, and HIF. The equations for $P_o$, $P_g$, $P_d$, $W$, $W_I$, and $N$ (Equations 4.1a through 4.1f) are
identical to those in the Wnt signaling model in the previous chapter, except that the metabolic switch between OXPHOS and glycolysis is regulated by PDK activity rather than Wnt levels. If PDK is high, then the cells are more likely to switch to glycolysis, and if PDK is low, the cells are more likely to switch to OXPHOS. This larger model was built with the assumptions that Wnt and HIF promote PDK activity [18], [9], [21], PDK activity promotes lactate production through upregulation of glycolysis [18], and lactate increases HIF production [12].

The equation for $P$, PDK activity, is given by equation (4.1g). The first term on the right of the equality is random motion; the second term represents nonlinear upregulation by Wnt, since Wnt signaling upregulates PDK [18]; the third term represents upregulation by HIF1α [9], [21]; the fourth term stands for upregulation through the cells, with sufficient nutrient; and the last term is a decay term.

Equation (4.1h) is the equation for lactate, which is assumed to diffuse long-range; is upregulated nonlinearly by PDK through the $P_g$ cells, since PDK drives glycolysis [18]; and can decay and be uptaken by $P_o$ cells.

The dynamics for HIF are given by Equation (4.1i). HIF is assumed to diffuse, to be upregulated nonlinearly by lactate, and to decay. The nonlinear upregulation by lactate comes from the assumption that lactate stabilizes HIF [12]. The last two terms in the HIF equation are production terms. There is a small rate ($d_H$) at which HIF is produced everywhere; and there is a large rate ($d_{Hni}$) at which HIF is produced when nutrient is low, which represents the stabilization of HIF in hypoxic environments.
4.2.2 Equations

\[
\frac{\partial P_o}{\partial t} = D_o \Delta P_o + \frac{1}{\tau_o} N (1 - P_o - P_g - P_d) P_o - \mu_o \chi_N(N) P_o + \frac{1}{\tau_{go}} \chi_P(P) P_g - \frac{1}{\tau_{og}} \chi_P^*(P) \chi_N^*(N) P_o
\]

random motion proliferation death

\[
+ \frac{1}{\tau_{go}} \chi_P(P) P_g
\]

switch to OXPHOS

\[
- \frac{1}{\tau_{og}} \chi_P^*(P) \chi_N^*(N) P_o
\]

switch from OXPHOS

\[
\frac{\partial P_g}{\partial t} = D_g \Delta P_g + \frac{1}{\tau_g} W N (1 - P_o - P_g - P_d) P_g - \mu_g \chi_N(N) P_g
\]

random motion proliferation death

\[
- \frac{1}{\tau_{go}} \chi_P(P) P_g + \frac{1}{\tau_{og}} \chi_P^*(P) \chi_N^*(N) P_o
\]

switch from glycolysis switch to glycolysis

\[
\frac{\partial P_d}{\partial t} = D_d \Delta P_d + \mu_o \chi_N(N) P_o + \mu_g \chi_N(N) P_g - \mu_d P_d
\]

random motion dead \( P_o \) cells dead \( P_g \) cells decay

\[
\frac{\partial W}{\partial t} = D_W \Delta W + \frac{1}{a + b W} \kappa_W N W^2 P_g + S_W(P_o + P_g)
\]

random motion inhibition upregulation upregulation

\[
- \mu_W W
\]

downregulation

\[
\frac{\partial W_I}{\partial t} = D_{W_I} \Delta W_I + \kappa_{W_I} N W^2 (P_o + P_g) - \mu_{W_I} W_I
\]

random motion upregulation downregulation

\[
\frac{\partial N}{\partial t} = D_N \Delta N - \nu_{NG} N P_g - \nu_{NO} N P_o - \mu_N N + N_s
\]

random motion uptake by \( P_g \) cells uptake by \( P_o \) cells decay bulk source

(4.1a)

(4.1b)

(4.1c)

(4.1d)

(4.1e)

(4.1f)
\[
\frac{\partial P}{\partial t} = D_P \Delta P + \nu_{PW} \frac{W^2}{\alpha_{PW} + W} N + \nu_{PH} \frac{H}{\alpha_{PH} + H} N \tag{4.1g}
\]
\[
\quad + \nu_P N (P_o + P_g) - \mu_P P \quad \text{upregulation} \quad \text{downregulation}
\]
\[
\frac{\partial L}{\partial t} = D_L \Delta L + \nu_{LP} \frac{P^2}{\alpha_P + P} N - \mu_L L - \nu_{LO} L P_o \tag{4.1h}
\]
\[
\quad \text{random motion} \quad \text{upregulation} \quad \text{decay} \quad \text{uptake by } P_o \text{ cells}
\]
\[
\frac{\partial H}{\partial t} = D_H \Delta H + \nu_{HL} \frac{L^2}{\alpha_L + L} - \mu_H H + d_H + d_{HN} (1 - N) \tag{4.1i}
\]
\[
\quad \text{random motion} \quad \text{upregulation} \quad \text{decay} \quad \text{production} \quad \text{production}
\]

### 4.3 Results

The parameters used in the results shown here are given by Tables 4.1 and 4.2. The system evolves over time and its final output at time 50 is shown in Figures 4.1 - 4.2. The numerical results are similar to those in Chapter 3, with a striking spotted pattern in glycolysis and OXPHOS. Near the boundary, there is a very high level of glycolysis, with localized spots of high glycolysis surrounded by relatively lower levels. We also see this spotted pattern in PDK activity, lactate, and HIF. When the mock and dnLEF tumors were stained for HIF, we saw a spotted pattern in both, with the dnLEF tumor exhibiting larger and fewer spots (see Figure 4.3).
Figure 4.1: 2D simulation results for detailed Wnt-inhibitor model

Figure 4.2: 2D simulation results for detailed Wnt-inhibitor model (continued)
4.4 Discussion

Metabolic symbiosis has been observed in cancers for some time, but never before has this symbiosis been observed as a spatial pattern in the form of localized areas of glycolysis surrounded by areas undergoing higher levels of oxidative phosphorylation. In an effort to understand this metabolic pattern, we developed a mathematical model for the spatio-temporal dynamics in the population fraction of oxidative cells, glycolytic cells, Wnt, Wnt inhibitor, PDK activity, lactate, HIF, and a general nutrient term. The model is based on the Gierer-Meinhardt activator-inhibitor model, where we modeled Wnt after the activator, and a Wnt inhibitor (e.g., SFRP5) as the agent that inhibits production of the activator.

The results give us good qualitative agreement with our IHC data when we compare the numerical results for PDK activity with our phospho-PDH stains. We see the larger, fewer
spots and lighter background in the dominant negative LEF PDK results in comparison to mock. It was not possible to stain for lactate, but experimental data show that the cells and HIF follow the same spotted pattern, and the same change in the pattern from mock to dnLEF/dnTCF, and we therefore conclude that this model is sufficient to recapitulate qualitatively our experimental observations.

Importantly, this more detailed model gives similar qualitative results to the simpler model presented in Chapter 3 when the experimental results are compared to numerical results for PDK activity. Since PDK drove switching in the cells, we saw patterns in metabolism as well. The simpler model retains the most important elements of the larger model while producing similar results. In the Wnt signaling model discussed in Chapter 3, the positive feedback between Wnt and PDK (high PDK implies more $P_g$ cells; higher levels of $P_g$ cells imply more Wnt activity; more Wnt activity means increased PDK) has been distilled so that Wnt activity level is the effective switch rather than PDK. Because PDK was directing the switch in metabolism, we can use $P_g$ and $P_o$ as the effective patterned state to compare to the xenograft stains. Thus, the equations for PDK activity, HIF concentration, and lactate concentration can be removed from the system, with Wnt activity driving the switch rather than PDK, so that the remaining equations contain only most important elements of the model.

**Materials and Methods**

*Numerical Simulations*

Numerical simulations were performed in MATLAB, using a forward difference method for each time derivative. $P_o$, $P_g$, $W$, $W_I$, $P$, $L$, and $H$ equations were solved implicitly in centered diffusion terms. The nutrient equation was solved implicitly in uptake, decay, and
centered diffusion terms. No-flux boundary conditions were used for all fields except for the nutrient boundary which used a Dirichlet boundary condition. The initial condition for the $P_g$ cell population was a random distribution near the boundary of the domain. $W$ and $W_I$ initial conditions were a random distribution in the same locations where initial $P_g$ cells were located. Initial $N$ was 1 everywhere in the domain; results did not change qualitatively if $N$ was solved as a quasi-steady state equation. Initial conditions for $P_o$, $P_d$, $P$, $L$, and $H$ terms were 0.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Mock value</th>
<th>dnLEF value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_o$</td>
<td>Diffusion coefficient of oxidative cells</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>$D_g$</td>
<td>Diffusion coefficient of glycolytic cells</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>$D_W$</td>
<td>Diffusion coefficient of Wnt</td>
<td>0.01</td>
<td>0.035</td>
</tr>
<tr>
<td>$D_{W_I}$</td>
<td>Diffusion coefficient of Wnt inhibitor</td>
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<td>1.5</td>
</tr>
<tr>
<td>$D_P$</td>
<td>Diffusion coefficient of PDK activity</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>$D_L$</td>
<td>Diffusion coefficient of lactate</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>$D_H$</td>
<td>Diffusion coefficient of HIF</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>$D_N$</td>
<td>Diffusion coefficient of nutrient</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>$\tau_o$</td>
<td>Oxidative cell proliferation time</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>$\tau_g$</td>
<td>Glycolytic cell proliferation time</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>$\tau_{og}$</td>
<td>Switch time from OXPHOS to glycolysis</td>
<td>1/24</td>
<td>1/24</td>
</tr>
<tr>
<td>$\tau_{go}$</td>
<td>Switch time from glycolysis to OXPHOS</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>$\alpha_W$</td>
<td>Constant for Michaelis-Menten dynamics</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>$\kappa_W$</td>
<td>Rate of nonlinear Wnt production</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>$\kappa_{W_I}$</td>
<td>Rate of Wnt inhibitor production</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>$\mu_o$</td>
<td>Decay rate of $P_o$ cells</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>$\mu_g$</td>
<td>Decay rate of $P_g$ cells</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>$\mu_d$</td>
<td>Decay rate of $P_d$ cells</td>
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<tr>
<td>$\mu_W$</td>
<td>Decay rate of Wnt</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>$\mu_{W_I}$</td>
<td>Decay rate of Wnt inhibitor</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>$\mu_P$</td>
<td>Decay rate of PDK activity</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>$\mu_L$</td>
<td>Decay rate of lactate</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>$\mu_H$</td>
<td>Decay rate of HIF</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>$\mu_N$</td>
<td>Decay rate of nutrient</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>$\nu_{PW}$</td>
<td>Rate of PDK upregulation through Wnt</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>$\nu_{PH}$</td>
<td>Rate of PDK upregulation through HIF</td>
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<td>0.5</td>
</tr>
<tr>
<td>$\nu_{LP}$</td>
<td>Rate of lactate upregulation through PDK activity</td>
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<td>3000</td>
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<tr>
<td>$\nu_{HL}$</td>
<td>Rate of HIF stabilization through lactate</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>$\nu_P$</td>
<td>PDK activity upregulation through cells</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>$\nu_{LO}$</td>
<td>Rate of lactate uptake by $P_o$ cells</td>
<td>$10^5$</td>
<td>$10^5$</td>
</tr>
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<td>$d_H$</td>
<td>Constitutive HIF production</td>
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<td>0.5</td>
</tr>
<tr>
<td>$d_{H_{ni}}$</td>
<td>Rate of HIF stabilization due to low nutrient</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>$S_W$</td>
<td>Rate of Wnt production through cells</td>
<td>7.5</td>
<td>5.5</td>
</tr>
<tr>
<td>$a$</td>
<td>Constant of inhibition</td>
<td>$10^{-8}$</td>
<td>$10^{-8}$</td>
</tr>
<tr>
<td>$b$</td>
<td>Constant of inhibition by $W_I$</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Model parameters for mock and dnLEF/dnTCF simulations. Red text indicates changes from mock to dnLEF/dnTCF.
Table 4.2: Wnt-PDK-lactate-HIF model parameters (continued)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Mock value</th>
<th>dnLEF value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\gamma_P$</td>
<td>Sensitivity level of PDK switch functions</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>$\gamma_N$</td>
<td>Sensitivity level of nutrient switch function</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>$\nu_{NG}$</td>
<td>Uptake of nutrient by $P_g$ cells</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>$\nu_{NO}$</td>
<td>Uptake of nutrient by $P_o$ cells</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>$N_s$</td>
<td>Parameter for nutrient source</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>$P^*$</td>
<td>PDK activity level at which 50% of cells switch metabolism</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>$N^*$</td>
<td>Nutrient level below which cells will die</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>$N^*_g$</td>
<td>Nutrient level below which $P_o$ cells cannot switch to glycolysis</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>$\alpha_N$</td>
<td>Value of scaling function when $\int P_g = 0$</td>
<td>0.025</td>
<td>0.025</td>
</tr>
<tr>
<td>$S_x$</td>
<td>Horizontal length of spatial domain</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>$S_y$</td>
<td>Vertical length of spatial domain</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

Model parameters for mock and dnLEF/dnTCF simulations
Chapter 5

Summary and Conclusions

Through this work we hope to gain a better understanding of one of the hallmarks of cancer, reprogrammed metabolism [4]. One example of reprogrammed metabolism is the Warburg Effect, also referred to as aerobic glycolysis, whereby cells undergo glycolysis to metabolize glucose and other nutrients even in the presence of high oxygen levels [28]. This pathway is much less efficient than oxidative phosphorylation, which noncancer cells normally undergo in high oxygen levels, creating only 4 molecules of ATP versus 36 per molecule of glucose [26], but recent research suggests that this may in fact contribute a significant growth advantage, by allowing metabolic byproducts to support proliferation and biomass production [26], [17]. Lactic acid, a byproduct of glycolysis, dissociates into lactate and H+ ions. This is a growth advantage to the tumor because lactate is pro-angiogenic, thus increasing nutrient supply to the tissue [6]. It has also been proposed that Warburg metabolism is utilized because tumor cells have evolved to withstand the acidic environment, while normal cells cannot survive [2].

Although the Warburg Effect was discovered decades ago and has shown prevalence in many cancers, not all cancer cells exhibit a completely glycolytic phenotype at all times. Cells can
shift to oxidative phosphorylation depending on various microenvironmental and temporal factors. A hypothesis of metabolic symbiosis was proposed by Sonveaux et al. [23], in which oxidative cells are located closer to vasculature and thus oxygen supply, and glycolytic cells are located away from blood vessels. Lactate, a byproduct of glycolysis, can be uptaken by the oxidative cells, thereby allowing glucose to diffuse toward the glycolytic cells, so that both populations are working together in a mutually beneficial relationship. This hypothesis suggests a gradient of low to high glycolysis with respect to distance from vasculature. Another example of metabolic symbiosis has been observed in cancer-associated fibroblasts (CAFs), which undergo aerobic glycolysis; the resulting lactate is used directly by tumor cells for OXPHOS [19].

In this work, we discussed yet another example of metabolic symbiosis. We presented experimental data for different xenograft SW480 colon cancer tumors: “mock” tumors (tumors in which Wnt was not interfered with); “dnLEF” or “dnTCF” tumors (tumors in which a dominant negative form of LEF1 or TCF1 were overexpressed; i.e., tumors in which Wnt signaling was interfered with); and “dnLEF + PDK” tumors (dnLEF tumors in which PDK was overexpressed). The mock and dnLEF/dnTCF tumors were shown to have a remarkable spatial relationship in the two metabolism programs of glycolysis and oxidative phosphorylation, in the form of a striking spotted pattern in immunohistochemical staining. The pattern appeared as localized areas of elevated phosphorylated PDH levels (“spots”), surrounded by relatively lower levels of pPDH. Since pPDH is a marker for PDK activity, which drives glycolysis in colon cancer [18], the spots can be considered to be areas of elevated glycolysis, with the surrounding areas undergoing relatively less glycolysis and more oxidative phosphorylation. This spotted pattern is evidence of a beautiful symbiosis between glycolytic and oxidative cells: Glycolytic cells localize into discrete clusters of cells, supplying lactate to surrounding oxidative cells, improving the cells’ ability to grow and proliferate.
The spots appeared in both mock and dominant negative tumors, but the pattern in the dnLEF and dnTCF tumors was significantly altered compared to mock: The spots were noticeably larger, fewer, and farther apart, and the background staining was lighter as well. Not only was there a pattern in metabolism in mock and dominant negative tumors, but a similar spotted pattern was also evident when the tumors were stained for markers for Wnt signaling (LEF1 or β-catenin), indicating that Wnt signaling plays a critical role in regulating this symbiotic relationship. The dnLEF + PDK1 tumors did not have a pattern and exhibited elevated levels of pPDH, as further evidence that PDK1 rescues glycolysis and is a crucial driver in Warburg metabolism in colon cancer [18].

We presented three reaction-diffusion models to describe the spotted pattern and the interactions among the two metabolic cell types, signaling pathways, nutrients, and growth factors. The glucose-lactate model in Chapter 1 was based on Gierer-Meinhardt’s activator-substrate model and described the spotted pattern in our data nicely, for mock, dnLEF/dnTCF, and dnLEF + PDK1 tumors, but a limitation was a labyrinthine pattern, rather than a spotted pattern, in some of the parameter sets we investigated. The labyrinth pattern could be explained by the possibility that IHC stains are not very sensitive and only capture the highest levels of glycolysis (“hot spots”) which is revealed as a spotted pattern (see Figure 2.32). Another limitation is that for patterns to form in the model, glucose must diffuse much farther range than lactate (by at least an order of magnitude). The diffusion coefficients for glucose and lactate have not been measured in xenograft tumors like those in our study, but in media in which they have been measured, it has been reported that their values are approximately on the same order of magnitude.

Therefore, due to these limitations, and since the glucose-lactate model has no connection to the Wnt signaling pathway, which is known to influence Warburg metabolism [18], we developed another model based on Wnt and a Wnt inhibitor. We built this model off the Gierer-Meinhardt activator-inhibitor equations where Wnt signaling is the activator that is
autocatalytic and produces its own inhibitor. It was found that this model is robust to parameter changes and was in good qualitative agreement with experimental data.

We also performed diffusive instability analysis for the Wnt signaling model to help examine the parameter space and to guide us in choosing a parameter set that would result in good qualitative agreement with our results. Through the analysis we determined the eigenvalue and wavenumber of given sets of parameters, which was useful in two ways: to determine whether we could expect a pattern (by the magnitude of a positive eigenvalue), and, if a spotted pattern were to emerge, to help determine the number of spots (by the wavenumber). We found that the instability analysis was consistent with numerical results. We also used it to help in determining the necessary parameter changes for replicating the pattern change from mock to dnLEF/dnTCF (fewer, larger spots and lighter background), by choosing parameter sets that yielded positive eigenvalues with smaller wavenumbers.

We used this model to predict what Wnt and inhibitor changes were necessary to understand the altered pattern in the dominant negative compared to mock stains. Our model predictions were that not only is overall Wnt signaling decreased in the dnLEF/dnTCF tumors, but surprisingly, both Wnt and Wnt inhibitor signaling spread farther out (i.e., diffusion coefficients increased). Thus, the effects of dnLEF/dnTCF overexpression are more complex than a simple decrease of Wnt throughput in the nucleus. The model predicted that the cell cluster size increase could be caused by the existence of an extracellular soluble factor that manipulates and crosstalks with the stability of Wnt. These observations led to further biological experiments testing for expression of various Wnt diffusers and inhibitors, where it was found that indeed several Wnt diffusers and inhibitors (including SPOCK2, SFRP5, and GPC4) were overexpressed in dominant negative tumors compared to mock tumors.

The implications of these validated predictions are threefold: It is a validation of the mathematical model and is a meaningful example of how modeling can point to a mechanism and make predictions that can be tested experimentally. Examples like these are invaluable
in promoting research in the field of mathematical biology and applied mathematics. Second, it provides evidence that the reaction to Wnt interference is to spread the Wnt signal, which is counterintuitive and was not known before the model predictions were made. Finally, it implies a relationship between Wnt diffusion regulators and metabolic activity in tumors, which is a new area of tumor biology that should be funded and explored.

In Chapter 3 we also simulated different treatment programs that could disrupt metabolic symbiosis. In simulations directly targeting either $P_o$ or $P_g$ cells, it was found that attacking glycolytic cells made the tumor more sensitive to death. We therefore chose to model therapies aimed at glycolytic cells. Because there are no known drugs that directly target $P_g$ cells, we considered drugs that attack them indirectly, by blocking Wnt signaling (through either Wnt ligands or $\beta$-catenin), or by inhibiting PDK. We considered the drugs dichloroacetic acid (DCA), which inhibits PDK and pushes metabolism towards OXPHOS; IWP2, which inhibits Wnt signaling by targeting Wnt ligands; and XAV939, which inhibits Wnt signaling by reducing $\beta$-catenin. We simulated DCA by decreasing the time to switch from glycolysis to OXPHOS. IWP2 was modeled by decreasing the diffusion coefficient of Wnt. Since XAV939 blocks $\beta$-catenin, which is similar to how the dominant negative tumors behave, we simulated it by making similar parameter changes: decreasing the constitutive Wnt signaling rate $S_W$ and increasing the diffusion of Wnt and the Wnt inhibitor linearly with respect to the decrease in $S_W$ (i.e., linear with respect to the change from mock to dnLEF parameters).

Our model predicts that a combination of these drugs (either DCA and IWP2 or DCA and XAV939) has the potential to be highly effective. We simulated these drugs by applying these therapies for a fixed time to a developed, patterned tumor, and observed that they were more effective in eradicating the tumor than applying only one of these therapies. Whether Wnt ligands or $\beta$-catenin is targeted can depend on the type of cancer to be treated.
The mathematical model in Chapter 3 is a model of minimal complexity. Since there are other factors and pathways involved in cancer metabolism, we also developed a Wnt-PDK-lactate-HIF model, which was similar to the Wnt signaling model, but included additional equations for the interactions with PDK, lactate, and HIF. It was found that this model yields similar qualitative results to the model in Chapter 3. We therefore concluded that the Wnt signaling model is sufficient to produce the desired results and contains the most essential elements to understand and capture the interplay between metabolism, nutrients, and Wnt activity, to make predictions for the mechanisms that cause the altered metabolic pattern in the tumors in which Wnt signaling was interfered with, and to investigate treatment programs \textit{in silico}.

All mathematical models are, by definition, an abstraction. In this work, we hope to understand tumor proliferation and metabolism, but our models represent an idealization and simplification of a complex system, and there are many more factors that play a role in tumor dynamics that have not been discussed here. Wnt clearly has a strong influence on the pattern, since the spots change drastically when it is interrupted, but it is possible that there are other contributors directing the spotted pattern. Additionally, since many of our parameter values are unknown, we had to make several assumptions for them. We do not know how our xenograft tumor results would translate to tumors in human subjects. The mock LEF1 and pPDH spots are similar in distribution and shape, but in dnLEF and dnTCF tumors the $\beta$-catenin spots did not line up with pPDH spots, an observation which should be studied in more depth.

Further directions for the Wnt signaling model include incorporating additional effects such as oxygen and a more detailed model of angiogenesis. More experiments can be conducted testing Wnt diffusers and inhibitors (including CDC42, LCN7, SPOCK2, DKK4, SFRP5). Trials testing the combination therapy programs described above in living tumors could also be performed. It is hoped that this investigation and subsequent studies will bring us to
a more complete understanding of cancer metabolism, and to the development of new and effective methods of treatment for this disease.
Bibliography


Appendix A

Glucose-Lactate Model

Nondimensionalization

The notation in this appendix is for one spatial dimension, but the method is the same for two dimensions. Let us first nondimensionalize the glucose equation. Define $t' = \frac{t}{T}$, $x' = \frac{x}{\ell}$, $P'_o = \frac{P_o}{P^*}$, $P'_g = \frac{P_g}{P^*}$, $L' = \frac{L}{\bar{L}}$ and $G' = \frac{G}{\bar{G}}$, where $T$ and $\ell$ are time and length scales respectively, $P^*$ is a characteristic value of cell density, and $\bar{L}$ and $\bar{G}$ are characteristic values of lactate and glucose levels.

This gives

$$\frac{\bar{G} \partial G'}{T \partial t'} = \frac{D_G \bar{G}}{\ell^2} \frac{\partial^2 G'}{\partial x'^2} - \kappa_G \bar{G} P'_o P^* + R - \kappa_{NG} G' \bar{G} \bar{L}^2 \bar{L}^2 P'_g P^* - \nu G' \bar{G}$$

Dividing by $\nu \bar{G}$, we have
\[
\frac{1}{T \nu} \frac{\partial G'}{\partial t'} = \frac{D_G \partial^2 G'}{\ell^2 \nu \partial x'^2} - \frac{\kappa_o}{\nu} G'o' P^* - \frac{R}{\nu G} G'L^2 \bar{T}^2 P'_g P^* - G'
\]

Then, introducing the time and length scales \( T = \frac{1}{\nu}, \ell^2 = \frac{D_G}{\nu}, \) and defining \( \bar{G} = \frac{R}{\nu}, \)

\[
\bar{L}^2 = \frac{\nu}{\kappa_{NG} P^*};
\]

and \( \kappa'_o = \frac{\kappa_o P^*}{\nu}, \) we get the following nondimensionalized equation for \( G: \)

\[
\frac{\partial G'}{\partial t'} = \frac{\partial^2 G'}{\partial x'^2} - \kappa'_o G'o' P^* + 1 - G'L^2 P'_g - G'
\]

Next, to nondimensionalize the lactate equation, we have

\[
\frac{L \partial L'}{T \partial t'} = \frac{D_L \partial^2 L'}{\ell^2 \partial x'^2} - \kappa_L L'L'o' P^* + \kappa_{NL} G'\bar{G} L^2 \bar{T}^2 P'_g P^* - \mu L' \bar{L}
\]

Dividing by \( \nu \bar{L}, \) we have

\[
\frac{1}{T \nu} \frac{\partial L'}{\partial t'} = \frac{D_L \partial^2 L'}{\ell^2 \nu \partial x'^2} - \frac{\kappa_L}{\nu} L'L'o' P^* + \frac{\kappa_{NL} G'\bar{G} L^2 \bar{T} L^2 P'_g P^*}{\nu} - \frac{\mu}{\nu} L'
\]

Defining \( D'_L = \frac{D_L}{D_G}, \kappa'_L = \kappa_L \frac{P^*}{\nu}, \kappa'_{NL} = \kappa_{NL} \bar{G} \frac{P^*}{\nu}, \) and \( \mu' = \frac{\mu}{\nu}, \) we get

\[
\frac{\partial L'}{\partial t'} = D'_L \frac{\partial^2 L'}{\partial x'^2} - \kappa'_L L'L'o' P^* + \kappa'_{NL} G'L^2 P'_g - \mu' L'
\]

which is the nondimensionalized lactate equation.

The oxidative cell equation becomes
\[
\frac{P^* \partial P'_o}{T} \frac{T}{\partial t'} = D_o P^* \partial P'_o \ell^2 + \frac{1}{\tau_o} \left(1 - P'_o - P'_g \right) P'_o P^* + \frac{1}{\tau_{go}} \chi_L(L'L) P'_g P^* - \frac{1}{\tau_{og}} \chi^*_L(L'L) \chi_G(G'G) P'_o P^*
\]

Dividing by \(\nu P^*\), the equation simplifies to

\[
\frac{1}{\nu T} \frac{T}{\partial t'} = \frac{D_o}{\nu \ell^2} \partial P'_o \partial x'^2 + \frac{1}{\nu \tau_o} \left(1 - P'_o - P'_g \right) P'_o + \frac{1}{\nu \tau_{go}} \chi_L(L'L) P'_g - \frac{1}{\nu \tau_{og}} \chi^*_L(L'L) \chi_G(G'G) P'_o
\]

Define \(D'_o = \frac{D_o}{D_G}\), \(\frac{1}{\tau'_o} = \frac{1}{\nu \tau_o}\), \(\frac{1}{\tau'_{go}} = \frac{1}{\nu \tau_{go}}\), and \(\frac{1}{\tau'_{og}} = \frac{1}{\nu \tau_{og}}\). Then we have

\[
\frac{\partial P'_o}{\partial t'} = \frac{D'_o}{D_G} \partial P'_o \partial x'^2 + \frac{1}{\tau'_o} \left(1 - P'_o - P'_g \right) P'_o + \frac{1}{\tau'_{go}} \chi_L(L'L) P'_g - \frac{1}{\tau'_{og}} \chi^*_L(L'L) \chi_G(G'G) P'_o
\]

Recall that we use

\[
\chi_L(L) = \frac{1}{2} [1 + \tanh(\gamma_L(L - L^*_1))]
\]

\[
\chi^*_L(L) = \frac{1}{2} [1 - \tanh(\gamma_L(L - L^*_2))]
\]

and

\[
\chi_G(G) = \frac{1}{2} [1 + \tanh(\gamma_G(G - G_{\text{min}}))]
\]

Redefining \(\gamma'_L = \gamma_L L\), \(L^*_1' = \frac{L^*_1}{L}\), \(L^*_2' = \frac{L^*_2}{L}\), \(\gamma'_G = \gamma_G G\), and \(G'_{\text{min}} = \frac{G_{\text{min}}}{G}\), where now
\[ \bar{\chi}_L(L') = \frac{1}{2} \left[ 1 + \tanh(\gamma'_L(L' - (L^*_1)')) \right] \]

\[ \bar{\chi}_L^*(L') = \frac{1}{2} \left[ 1 - \tanh(\gamma'_L(L' - (L^*_2)')) \right] \]

and

\[ \bar{\chi}_G(G') = \frac{1}{2} \left[ 1 + \tanh(\gamma'_G(G' - G'_{\text{min}})) \right] \]

we have

\[ \frac{\partial P'_o}{\partial t'} = D'_o \frac{\partial P'_o}{\partial x'^2} + \frac{1}{\tau'_o} \left( 1 - P'_o - P'_{g} \right) P'_o \left( 1 - \frac{1}{\tau_{og}} \bar{\chi}_L(L') P'_g - \frac{1}{\tau_{go}} \bar{\chi}_L^*(L') \bar{\chi}_G(G') P'_o \right) \]

The glycolytic cell equation becomes

\[ \frac{P^* \partial P'_g}{T} = \frac{D_g P^*}{\ell^2} \frac{\partial^2 P'_g}{\partial x'^2} + \frac{1}{\tau_g} \left( 1 - P'_o - P'_{g} \right) P'_g P^* \left( 1 - \frac{1}{\tau_{go}} \chi_L(L'T) P'_g P^* + \frac{1}{\tau_{og}} \chi_L^*(L'T) \chi_G(G'T) P'_o P^* \right) \]

Dividing by \( \nu P^* \), we get

\[ \frac{1}{\nu T} \frac{\partial P'_g}{\partial t'} = \frac{D_g}{\nu \ell^2} \frac{\partial^2 P'_g}{\partial x'^2} + \frac{1}{\nu \tau_g} \left( 1 - P'_o - P'_{g} \right) P'_g - \frac{1}{\nu \tau_{go}} \chi_L(L'T) P'_g + \frac{1}{\nu \tau_{og}} \chi_L^*(L'T) \chi_G(G'T) P'_o \]

Defining \( D'_g = \frac{D_g}{\bar{D}_G} \) and \( \frac{1}{\tau'_g} = \frac{1}{\nu \tau_g} \), the nondimensionalized glycolytic cell equation is
\[
\frac{\partial P_g'}{\partial t'} = D_g \frac{\partial^2 P_g'}{\partial x'^2} + \frac{1}{\tau_g'} (1 - P_o' - P_g') P_g' - \frac{1}{\tau_{g'o}} \chi_L(L')P_g' + \frac{1}{\tau_{og'}} \chi_L(L') \chi_G(G') P_o'
\]

In summary, dropping the primes and overbars, the nondimensionalized system is

\[
\frac{\partial P_o}{\partial t} = D_o \frac{\partial P_o}{\partial x^2} + \frac{1}{\tau_o} (1 - P_o - P_g) P_o + \frac{1}{\tau_{g'o}} \chi_L(L)P_g - \frac{1}{\tau_{og}} \chi^*_L(L) \chi_G(G) P_o
\]

\[
\frac{\partial P_g}{\partial t} = D_g \frac{\partial^2 P_g}{\partial x^2} + \frac{1}{\tau_g} (1 - P_o - P_g) P_g - \frac{1}{\tau_{g'o}} \chi_L(L)P_g + \frac{1}{\tau_{og}} \chi^*_L(L) \chi_G(G) P_o
\]

\[
\frac{\partial L}{\partial t} = D_L \frac{\partial^2 L}{\partial x^2} - \kappa_LLP_o + \kappa_{NL} G^2 P_g - \mu L
\]

\[
\frac{\partial G}{\partial t} = \frac{\partial^2 G}{\partial x^2} - \kappa_o G P_o + 1 - GL^2 P_g - G
\]
Appendix B

Image Processing of Phospho-PDH Stains
Figure B.1: Mock contours. Each image is at 20x magnification, with dimensions approximately $440 \times 330\mu m^2$. The left column shows stained images; the right column shows stained images with contour outlines drawn around each spot.
Figure B.2: dnLEF contours. Each image is at 20x magnification, with dimensions approximately $440 \times 330 \mu m^2$. The left column shows stained images; the right column shows stained images with contour outlines drawn around each spot.
Figure B.3: dnTCF contours. Each image is at 20x magnification, with dimensions approximately $440 \times 330\mu m^2$. The left column shows stained images; the right column shows stained images with contour outlines drawn around each spot.
Table B.1: **Number of spots in IHC stains**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Number of spots</th>
</tr>
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<tbody>
<tr>
<td>Mock</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>89</td>
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<tr>
<td>dnLEF</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>37</td>
</tr>
<tr>
<td>dnTCF</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>44</td>
</tr>
</tbody>
</table>

Number of spots in analyzed 20x images (spots are based on the contours above), each with field approximately $440 \times 330\mu m^2$. Three mock images, four dnLEF images, and three dnTCF images were analyzed. Number of spots is consistently higher in mock experiments than in dominant negative LEF or dominant negative TCF experiments.
Appendix C

Parameter Ranges for Wnt Signaling Model

Table C.1 lists the set of parameters we investigated in order to test our Wnt signaling model for robustness. The parameters that are missing from this table were explored in Chapter 3, so their effects are already understood and are not included here. We varied each parameter one by one, varying between the minimum and maximum values listed in the table, and kept all other values the same as the mock values in Table 3.2 to ensure that a pattern would emerge that was qualitatively consistent with our mock stains. Next, we made the same changes from mock to dnLEF as presented earlier (increased the diffusion coefficients for $W$ and $W_I$ and decreased $S_W$) to see if we still obtained qualitatively similar answers as the dnLEF/dnTCF experiments. In this way we determined that our model is robust to a large range of values, and the results we obtained from the parameters presented in the main part of this paper can be considered characteristic of the system.
Table C.2 summarizes the effects of parameters on the final metabolic pattern. Note that these tests are done only within the pattern-forming parameter space, i.e., if the value of a given parameter does not yield a pattern, that value is ignored. For the parameter $b$, we get fewer spots with a lower value of $b$ (contrary to what we expect). When $\kappa_W$ is decreased in mock, $P_g$ increases overall and number of spots in turn decreases ($P_g$ is too close to 1 for a spot to emerge). When it is increased, $P_g$ decreases and number of spots decrease. When $\kappa_W$ is decreased in dnLEF, $P_g$ increases overall and the number of spots increases. When $\kappa_{W_I}$ is decreased in mock, $P_g$ decreases overall and the number of spots in turn decreases. When it is increased (it can only increase up to 1.25 from default value of 1, or patterns will not form), $P_g$ looks similar to the results from the base case default parameters. If $D_N$ is too small, no pattern will form. ($N$ must diffuse far enough from the boundary.) $D_N = 1000$ (from the default value of 100) yields no qualitative difference in the final spotted pattern. If $\nu_{NO}$ or $\nu_{NG}$ is too large, no pattern will form (sufficient nutrient is necessary for spots). If $N_s$ is low, the cells proliferate more slowly (more time is needed for the center of the domain to fill up). The spotted pattern is not qualitatively different. The parameters that affect proliferation of cells simply slows or hastens the rate of proliferation; if enough time is allowed, the cells will proliferate into the entire domain and will have a similar pattern to our default plots at the end of the simulation.
### Table C.1: Variables

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mock value</th>
<th>dnLEF value</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\kappa_W$</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>50</td>
<td>$\kappa_W = 2$: no pattern in dnLEF</td>
</tr>
<tr>
<td>$\kappa_{Wi}$</td>
<td>1</td>
<td>1</td>
<td>0.1</td>
<td>1.25</td>
<td>$\kappa_{Wi} = 1.5$: no pattern in dnLEF</td>
</tr>
<tr>
<td>$a$</td>
<td>$10^{-8}$</td>
<td>$10^{-8}$</td>
<td>$10^{-16}$</td>
<td>0.75</td>
<td>$a = 1$: no pattern in dnLEF</td>
</tr>
<tr>
<td>$b$</td>
<td>1</td>
<td>1</td>
<td>0.1</td>
<td>1.75</td>
<td>$b = 2$: no pattern in dnLEF</td>
</tr>
<tr>
<td>$\tau_{og}$</td>
<td>1/24</td>
<td>1/24</td>
<td>1/85</td>
<td>1/20</td>
<td>$\tau_{og} = 1/18$: no pattern in dnLEF; $\tau_{og} = 1/90$: almost all $P_g$; not enough spots in mock</td>
</tr>
<tr>
<td>$\mu_o$</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>40</td>
<td>$\mu_o = 50$: no pattern in dnLEF</td>
</tr>
<tr>
<td>$\mu_g$</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>100</td>
<td>Did not try larger than 100</td>
</tr>
<tr>
<td>$\mu_d$</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>100</td>
<td>Did not try larger values</td>
</tr>
<tr>
<td>$\alpha_W$</td>
<td>1</td>
<td>1</td>
<td>$10^{-8}$</td>
<td>1.5</td>
<td>Numerical error if $\alpha_W = 0$; no pattern in dnLEF if $\alpha_W = 2$</td>
</tr>
<tr>
<td>$\mu_W$</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>$\mu_W = 1$, dnLEF: pattern in Wnt but none in $P_g$ (100% $P_g$); $\mu_W = 1.5$, dnLEF: slight pattern in $P_g$ (almost 100% $P_g$); $\mu_W = 1.75$, mock: slight pattern in $P_g$ (almost 100% $P_g$; but fewer spots than in dnLEF); $\mu_W = 2.25$, dnLEF: all terms $\to 0$</td>
</tr>
<tr>
<td>$\mu_{WI}$</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>20</td>
<td>$\mu_{WI} = 2.75$, dnLEF: no pattern; $\mu_{WI} = 20$, mock: almost 100% $P_g$, 3 “spots”</td>
</tr>
<tr>
<td>$\nu_{NG}$</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>25</td>
<td>$\nu_{NG} = 30$: no pattern in dnLEF</td>
</tr>
<tr>
<td>$\nu_{NO}$</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>20</td>
<td>$\nu_{NO} = 25$: no pattern in dnLEF</td>
</tr>
<tr>
<td>$\mu_N$</td>
<td>0.1</td>
<td>0.1</td>
<td>0</td>
<td>1</td>
<td>$\mu_N = 1.5$: no pattern in dnLEF</td>
</tr>
<tr>
<td>$N_s$</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>10</td>
<td>$N_s = 10$: nutrient essentially constant throughout domain in dnLEF and mock</td>
</tr>
</tbody>
</table>

Robustness to parameter changes: The minimum and maximum values listed in this table indicate the range of tested values for which we still see the same characteristic phenotype changes in our model. This means that from mock to dnLEF, we observe fewer, larger spots farther apart from each other.
Table C.2: **Parameter variation**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_o$, $D_g$, $D_d$, $\mu_o$, $\mu_g$, $\mu_d$, $a$, $\nu_{NO}$, $\nu_{NG}$, $\mu_N$</td>
<td>Cell diffusion, decay rates, inhibition constant, uptake rates</td>
<td>No discernible effect</td>
</tr>
<tr>
<td>$\tau_o$, $\tau_g$, $N_s$, $D_N$</td>
<td>Proliferation rates, nutrient source, diffusion</td>
<td>Proliferation of cells at early times</td>
</tr>
<tr>
<td>$\tau_{go}$, $\tau_{og}$</td>
<td>Switching times</td>
<td>Overall $P_o$ and $P_g$ levels</td>
</tr>
<tr>
<td>$D_W$, $\mu_W$</td>
<td>Wnt diffusion and decay</td>
<td>Size of spots</td>
</tr>
<tr>
<td>$S_W$</td>
<td>Constitutive Wnt activity</td>
<td>Background level of Wnt</td>
</tr>
<tr>
<td>$\kappa_W$, $\kappa_{WI}$</td>
<td>Production rates</td>
<td>Number of spots and overall $W$ and $W_I$ levels</td>
</tr>
<tr>
<td>$D_{WI}$, $\mu_{WI}$, $b$</td>
<td>Inhibitor diffusion, decay, inhibition constant</td>
<td>Number of spots</td>
</tr>
</tbody>
</table>