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Gene Network Modeling of Cancer Metabolism

by

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ABSTRACT

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Abstract

Metabolism plays a crucial role in cellular behaviors and activities. The abnormal metabolism has been proposed to be one of the hallmarks of cancer. Unlike normal cells, cancer cells largely depend on glycolysis to produce energy even in the presence of oxygen, which is referred as the Warburg effect. Recent evidences, however, suggest that oxidative phosphorylation is also required for cancer progression. Yet, the underlying regulatory mechanism of these metabolic modes in cancer cells is still poorly understood. Here, we use the computational systems biology approach to establish a theoretical framework for modeling genetic regulation of cancer metabolism. According to experimental evidences, we built a network of both regulatory proteins and metabolites. The network was first coarse-grained to a three-component regulatory circuit composed of HIF-1, AMPK and ROS. Thereafter, we further explored the interplay between the circuit and the metabolic pathways, including glucose oxidation, glycolysis and fatty acid oxidation. By exploring the dynamics of the metabolic circuits, we show that, while normal cells have two stable steady states – an oxidative state (O: low HIF-1, high AMPK) and a Warburg state (W: high HIF-1, low AMPK), cancer cells open an additional hybrid state (W/O: high HIF-1, high AMPK) due to higher mitochondrial ROS production and lower HIF-1 degradation rate. The ‘W/O’ hybrid phenotype contributes to cancer metabolic heterogeneity and plasticity, thus allowing cancer cells to adapt to the microenvironment changes and to promote cell proliferation and metastasis. Based on the model, we investigated the effectiveness of possible cancer therapies targeting metabolism in reducing the metabolic plasticity and circumventing the hybrid state during the course of treatment. We also discuss the connection of the metabolic hybrid state to EMT and stemness of cancer cells.
I would like to thank my advisor, Dr. Jianpeng Ma, for his guidance, encouragement and continuous support to my study and research.

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My days spent on searching the literatures, setting up the models, deriving the equations and debugging the code during my whole graduate study would not have been as enjoyable without my current and former colleagues in Dr. Ma’s laboratory and Center for Theoretical Biological Physics: Tianwu Zang, Zhenwei Luo, Tianqi Ma, Dr. Xiaorui Chen, Qingqing Xie, Dr. Yufeng Gou, Dr. Fengyun Ni, Dr. Junqing Du, Dr. Chong Zhang, Yunxiang Liao, Dr. Yushao Cheng, Dong Chen, Dr. Jia Zeng, Bin Huang, Dongya Jia, Mohit Kumar Jolly and Dr. Fang Bai.

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<tbody>
<tr>
<td>AMPK</td>
<td>(Phosphorylated) AMP-activated Protein Kinase</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
</tr>
<tr>
<td>HIF-1</td>
<td>Hypoxia-inducible Factor 1</td>
</tr>
<tr>
<td>OXPHOS</td>
<td>Oxidative Phosphorylation</td>
</tr>
<tr>
<td>TCA</td>
<td>Tricarboxylic Acid Cycle</td>
</tr>
<tr>
<td>ETC</td>
<td>Electron Transport Chain</td>
</tr>
<tr>
<td>2DG</td>
<td>2-Deoxy-D-glucose</td>
</tr>
<tr>
<td>FTS</td>
<td>S-trans, trans-Farnesylthiosalicylic Acid</td>
</tr>
<tr>
<td>3BP</td>
<td>3-Bromopyruvate</td>
</tr>
<tr>
<td>Acetyl-CoA</td>
<td>Acetyl coenzyme A</td>
</tr>
<tr>
<td>PHDs</td>
<td>Prolyl hydroxylases</td>
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<tr>
<td>VEGF</td>
<td>Vascular Endothelial Growth Factor</td>
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Chapter 1

Introduction

As one of the leading causes of deaths, cancer has a huge impact on society worldwide. Therefore, tremendous efforts are being devoted to the cancer researches. Among the works, deciphering the complexity of cancer to the major underlying principles is especially important for better understanding the cancer biology and making the effective therapeutic strategies accordingly. In this chapter, we will have a look at the hallmarks of cancer. Specifically, we will have a deeper discussion on cancer metabolism, one of the hallmarks of cancer. In addition, an introduction about how to utilize gene regulatory network to study cancer will be presented in this chapter.

1.1. The Hallmarks of Cancer

Cancer cells differ from normal cells in many ways as they have the different control mechanisms governing cell activities. Particularly, normal cells grow and divide upon stimulation of growth factors under many controls. If the growth factors are
damaged, normal cells will stop growing until they are repaired, otherwise, the apoptosis will be induced. Besides, the number of times a normal cell can divide is limited. And normal cells are usually part of a tissue structure, thus lack of motility. In addition, normal cells mainly utilize mitochondria to process oxidative phosphorylation (OXPHOS) to gain energy. For cancer cells, all of these mechanisms are changed due to gene damages through acquired or somatic mutations. Despite of the various cancer cell genotype, there are several essential cellular alterations that collectively decide the malignant growth \textsuperscript{1}. Therefore, Hanahan and Weinberg proposed the eight hallmarks of cancer: sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis, reprogramming of energy metabolism and evading immune destruction \textsuperscript{1,2} (Fig 1.1).

\begin{center}
\includegraphics[width=\textwidth]{hallmarks.png}
\end{center}

\textbf{Figure 1.1 – The hallmarks of cancer and the enabling characteristics \textsuperscript{2}.}
Among these hallmarks, sustaining chronic proliferation is widely regarded as the most fundamental trait of cancer cells. Unlike the normal cells which carefully control the productions and cell cycles to maintain the homeostasis of the cell numbers, the cancer cells can acquire the growth signals autonomy, which enables its unlimited proliferations. There are various ways the cancer cells to get this special capability: they may produce growth factor ligands themselves, or they can deregulate the growth factor signaling pathways, for instance, the TGF-β signaling and PI3K signaling pathways. Yet, the precise identities and sources of the proliferative signals as well as the deregulatory mechanisms of cancer cells are poorly understood so far.

In addition to the autonomy acquiring growth signal capability, evading growth suppressors and resisting cell death are the other two hallmarks of cancer. It has been discovered that the RB and TP53 are two significant proteins which control the cell cycles in that they can either decide the cell growth-and-division cycle or initiate cell apoptosis. However, RB expression is lost in many cancer cells and about half of human cancers have the inactivated TP53 protein due to genetic mutations, which induced tremendous attention from cancer researchers. While the corresponding therapeutic strategies have been developed, new technologies being able to create more clinical benefits by targeting these hallmarks of cancer is in anticipation.

Enabling replicative immortality is of crucial importance for cancer cells. Instead of being able to pass through only limited number of cell growth-and-division cycles like normal cells, cancer cells have unlimited replicative potential. Accumulating evidences indicate that telomeres and telomerase play central role in acquiring the unlimited replication ability. Telomere is the end of a chromosome that protects this region
from recombination and degradation. Loss of either TTAGGG telomere repeats or telomere-binding factors can result loss of cell viability. Therefore, by protecting telomere length from being shortened, telomerase enables the continuous cell replication. A growing number of therapeutic strategies targeting telomerase have been developed for cancer treatment 15,17,18.

Pathological angiogenesis is another hallmark of cancer 19,20. Due to rapid and abnormal proliferations, cancer cells have large demands of nutrients and biomasses as well as urgent requirements to evacuating metabolic wastes such as carbon dioxide. Therefore, the abnormal angiogenesis is induced, which causes the normally quiescent vasculature to sprout new vessels. This process is also controlled by some gene regulators. One well recognized angiogenesis promotor is vascular endothelial growth factor (VEGF) 21, which is up-regulated by oncogene expression, a variety of growth factors, and hypoxia. As a hallmark of cancer, angiogenesis has become a target for cancer treatment 22.

It has been very clear that the formation of tumor is highly associated to the local invasion and distant metastasis of cancer cells, which is the sixth hallmark of cancer. Although this capability is developed by multi-steps, it is mainly regulated by the epithelial-mesenchymal transition (EMT) process 23. A set of transcriptional factors and microRNAs, such as SNAIL, SLUG, TWIST, ZEB and miR200 24, are involved in for regulating this process. Comprehensive researches have been done on the EMT process by both the experiments 25 and computational modeling 26,27, suggesting that it has crosstalk with many key cellular properties such as stemness, cell motility, cell–cell communication, metabolism, and resistance to apoptosis 28,29. Hence, further exploring
the mechanism of the invasion and metastasis and its impact to the other cellular activities remains a critical cancer research topic.

The reprogrammed metabolism is the seventh hallmark of cancer. Under the aerobic conditions, normal cells produce pyruvate via glycolysis in cytosol then transport them to mitochondria to produce ATP; while under the anaerobic condition, little pyruvate is transported to mitochondria and the glycolytic pathway is used to generate energy, with lactate as the end product. However, cancer cells favor the glycolytic pathway even in the presence of oxygen, which is referred as the aerobic glycolysis or Warburg Effect. Aerobic glycolysis plays the dominant role in cancer cells since it can produce ATP at a high rate and prepare biomass for rapid cell proliferation. Yet, recent studies show that the mitochondrial OXPHOS is also present in cancer cells depending on their stage and microenvironment. It has been shown that the metabolic reprogramming of cancer cells is regulated by the oncogenes (for example, RAS, SRC and MYC), transcription factors (for example, HIF), energy sensor genes (for example, AMPK) and metabolites (for example, ROS). Despite the importance of the interplay and regulation among these pathways, the reprogrammed metabolic mechanism is complex and elusive. We will study this hallmark of cancer in the following sections and chapters.

Evading immune destruction is another newly emerged hallmark of cancer. The association between immune responses and cancer development has been known for decades. Yet, the complex interplay between the two is ambiguous. It has been discovered that people with chronic inflammatory diseases or in the infectious conditions have an increased risk of cancer. Indeed, the long-term usage of anti-inflammatory
drugs such as aspirin and selective COX2 inhibitors is able to reduce cancer risks\textsuperscript{46}. However, it has also been revealed that the accumulative antibiotic use has a positive association with breast cancer\textsuperscript{47}. The seemingly contradictory facts are usually explained by the defects of the cancer immune systems, which remains controversial. How cancer cells evading the immune destruction and the mechanism of immune-tumor interplays need further explorations.

Despite the different regulatory mechanisms and roles they play of the hallmarks enumerated above, they are not independent in tumorous development. The acquisition of the hallmarks is facilitated by two consequential characteristics of cancer cells: the first one is the genomic instability and mutability, which promotes cell genetic alternations to drive tumor progressions; and the second one is the tumor promoting inflammation, which regulates the tumor microenvironment to enhance tumorigenesis and metastasis\textsuperscript{2} (Fig 1.1).

1.2. Cancer Metabolism: Beyond the Warburg Effect

Cells utilize multiple metabolic pathways for energy production and biosynthesis depending on the need of cellular functions and the availability of various metabolites. In the presence of glucose, cells typically uptake glucose and convert it into pyruvate inside the cytosol by glycolysis. Under normoxia condition (with a normal oxygen level), pyruvate is further transported into mitochondria where it undergoes OXPHOS to produce large amounts of ATP through the tricarboxylic acid (TCA) cycle and the electron transport chain (ETC) (Fig.1.2). While under hypoxia condition (with a low oxygen level), cells utilize anaerobic glycolysis instead, which converts pyruvate into
lactate, and produces ATP in a much faster but less efficient way (Fig.1.2). If fatty acids and oxygen are available, cells can also undergo fatty-acid oxidation (also called beta-oxidation)\(^{48}\). Besides, cells can also utilize glutamine oxidation\(^ {49}\) to produce energy and maintain cell survival during the pyruvate transport process\(^ {50}\).

Unlike normal cells, cancer cells largely depend on glycolysis to produce energy even in the presence of oxygen, which is referred as the Warburg effect\(^ {32}\) or aerobic glycolysis (Fig.1.2). Aerobic glycolysis is an aggressive metabolic phenotype in that it has the advantage to produce ATP at a high rate and prepare biomass for amino acids and fatty-acids synthesis that are required for rapid cell proliferation\(^ {32}\).

![Figure 1.2 – OXPHOS, anaerobic glycolysis, and aerobic glycolysis\(^ {32}\).](image)

In the presence of oxygen, normal cells (the left panel) first metabolize glucose to pyruvate via glycolysis, then completely oxidize most of the pyruvate in the mitochondria to CO\(_2\) during the process of OXPHOS. When oxygen is limited, cells can redirect the pyruvate generated by glycolysis away from mitochondrial OXPHOS by generating lactate (anaerobic glycolysis). This generation of lactate during anaerobic glycolysis results in less efficient but faster ATP production compared with OXPHOS. Cancer cells (the right panel) tend to convert most pyruvate to lactate regardless of the presence of oxygen (aerobic glycolysis or Warburg effect).
In cell metabolism, mitochondria is playing the central role in energy production, as the cellular respirations are happening in mitochondrial matrix. Originally, Warburg hypothesized that the aerobic glycolysis is caused by mitochondrial defects and suggested that it might be the central of cancer cell biology \(^{51}\). Since Warburg’s proposal, many researchers devoted efforts to find the defects of mitochondrial OXPHOS \(^{52}\). For example, the differences of mitochondrial down-regulatory enzymes in normal cells and tumor cells are studied \(^{53}\). However, none of the discoveries are adequate to fully explain the Warburg effect, and people found that the role of mitochondria in cancer cell is far more complex. It is not only related to energy production, but also related to the signaling through ROS production \(^{51,54}\). Moreover, it has been noticed that the mitochondrial ROS production \(^{51}\) as well as the mitochondrial OXPHOS in cancer cells are underestimated \(^{55}\).

Although aerobic glycolysis is generally regarded as a dominant metabolism in cancer cells \(^{56,57}\), recent evidences suggest that mitochondrial OXPHOS is also present in cancer cells depending on their stage and microenvironment \(^{33,34,56,58,59}\). Indeed, the influence of mitochondria on cancer cells is well documented \(^{60-64}\), indicating that mitochondria are active in cancer metabolism. More importantly, it has been discovered that OXPHOS is required for cancer cells to become aggressive and invasive \(^{33,54,61-65}\). Study shows that some aggressive tumor cells, such as SiHa and HeLa, have not only oxidative respiration but also glycolysis due to stronger HIF-1 activation from lactate \(^{65}\). Moreover, the inhibition of the mitochondrial respiratory chain contributes to the reduction of multidrug resistance of slow cycling melanoma cells \(^{62}\). It has also been shown that the surviving pancreatic cancer cells after doxycycline withdrawal depend on OXPHOS and are highly sensitive to OXPHOS inhibitors \(^{61}\). Also, the supper-metastatic
tumor cells obtained by experimental selection in vitro (SiHa-F3 cells) and in vivo (B16F10 and B16-M1 to M5 tumor cells) have increased OXPHOS with higher ROS production 54. TGFβ1 treatment on non-small lung carcinoma A459 cells induces metastasis, and they were found to have decreased fatty acid synthesis and more oxygen consumptions 33. Besides, the inhibition of OXPHOS by graphene on multiple cancer cells, including breast cancer cells and hepatocellular carcinoma cells, can effectively inhibit tumor migrations and invasions 66. In the context of 4T1 mammary epithelial cancer cells, PGC-1α promotes metastasis through activating mitochondrial biogenesis and OXPHOS 34. The breast cancer cells can also shift from glycolysis to mitochondrial OXPHOS after radiation exposure to generate more ATP for surviving 67. The experimental evidences enumerated above are shown in Table 1.1.

<table>
<thead>
<tr>
<th>References</th>
<th>Cell Lines</th>
</tr>
</thead>
<tbody>
<tr>
<td>De Seadeleer et al., 2012 68</td>
<td>SiHa human cervix squamous cell carcinoma, WiDr human colorectal adenocarcinoma, FaDu human pharynx squamous cell carcinoma, HeLa human epithelial cervix cancer, and HCT116 human colorectal carcinoma cancer cells</td>
</tr>
<tr>
<td>Porporato et al., 2014 42</td>
<td>SiHa-F3 human cervix squamous cell carcinoma</td>
</tr>
<tr>
<td>LeBleu et al., 2014 34</td>
<td>4T1 mouse mammary adenocarcinoma, B16F10 mouse melanoma, MDA-MB-231 human mammary adenocarcinoma, and MDA-MB-435 human melanoma cell lines</td>
</tr>
<tr>
<td>Jiang et al., 2014 33</td>
<td>A459 non-small cell lung carcinoma cells</td>
</tr>
<tr>
<td>Zhou et al., 2014 66</td>
<td>MDA-MB-231 human metastatic breast cancer cells, HepG2 human hepatocellular carcinoma cells, PC3 human metastatic prostate cancer cells, B16F10 mouse metastatic melanoma cells and NIH-3T3 mouse embryonic fibroblast cells</td>
</tr>
<tr>
<td>Viale et al., 2014 61</td>
<td>Mice and human pancreatic cancer cells</td>
</tr>
<tr>
<td>Roesch et al., 2013 62</td>
<td>Melanoma cells</td>
</tr>
<tr>
<td>Lu et al., 2015 57</td>
<td>Breast cancer MCF-7, glioblastoma U87, colon cancer HCT116 cell lines</td>
</tr>
<tr>
<td>Xu et al., 2015 64</td>
<td>Human breast cancer cell lines (MDA-MB-231, MDA-MB-468, MDA-MB-453)</td>
</tr>
</tbody>
</table>

Table 1.1 – Experimental evidence on the requirement of OXPHOS for cancer metastasis
1.3. The Regulatory Network of Metabolism

Cellular metabolic processes are regulated by the signal transduction network, whose properties are determined by the network topology. To capture the global network of metabolism, genomic data and statistical analysis are used to integrate the pathways and regulatory nodes. These models are useful to map global network topologies but are limited in providing critical mechanistic insights. In contrast, coarse-grained approaches can identify patterns of co-regulation by compartmentalizing the global networks into modules, but they may blur the regulatory details within these modules. Nevertheless, constructing biological networks can proceed at different levels of resolution, depending on the available data, and the aims of the analysis.

There are two kinds of basic regulations in the regulatory networks: one is the activation (Fig.1.3A) and the other one is inhibition (Fig.1.3B). Usually, we use the arrow to represent the activation regulation (Fig.1.3A), and the line with a bar to represent the inhibition regulation (Fig.1.4A). The two regulations exist among the different genes, thus form the complex gene regulatory circuits/networks. Fig.1.3 shows some examples of the simplest circuit motifs involving two regulatory components. In Fig.1.3C, the regulatory components A and B activate each other, while in Fig.1.3D, they mutually inhibit each other. The circuit in Fig.1.3D is also known as toggle switch. The genes can also regulate themselves. For example, both A and B can inhibit themselves (Fig.1.3E) or activate themselves (Fig.1.3F). The circuit shown in Fig.1.3F is also known as toggle switch with self-activations. The different circuits have different dynamic and stability properties, which we will discuss in the following sections.
Figure 1.3 – The gene regulatory motifs.

The arrows represent positive regulations and the bars represent negative regulations. The blue and orange circles represent regulatory gene A and B, respectively. (A) A activates B; (B) A inhibits B, (C) A and B have mutual activations; (D) A and B have mutual inhibitions (toggle switch); (E) A and B both have self-inhibitions, and mutually activate each other; (F) A and B both have self-activations, and mutually inhibit each other (toggle switch with self-activations).

After an extensive literature analysis, we constructed a regulatory network of metabolism (Fig.1.4). The network features regulations of oxidative respiration and glycolysis by both genes and metabolites. Overall, the three metabolic pathways – aerobic glycolysis, glucose oxidation and fatty-acid oxidation inhibit each other \(^72\). In detail, aerobic glycolysis and glucose oxidation compete with each other as the same pyruvate molecules are used in both pathways. Moreover, both glucose and fatty-acid oxidations produce acetyl-CoA, the only fuel that enters into TCA cycle. However, excessive acetyl-CoA prevents pyruvate from becoming acetyl-CoA, and inhibits glycolysis and fatty-acid oxidation in cytosol. Thus, glucose and fatty-acid oxidations inhibit each other as well, which is also known as the Randle cycle \(^72\).
All the three metabolic pathways are regulated by both genes and metabolites. On one hand, fatty acid and glucose oxidation metabolic pathways are regulated by an energy sensor gene, AMPK \(^73-75\), and the glycolytic pathway is regulated by the hypoxia-inducible factor, HIF-1 \(^40,76,77\). On the other hand, these metabolic pathways are also regulated by metabolites such as ATP and ROS. HIF-1 is stabilized by both NOX-derived ROS (noxROS) from cytosol and OXPHOS-derived ROS from mitochondria (mtROS) \(^78-80\). Meanwhile, ROS induces phosphorylation and activation of AMPK \(^79,81-84\), yet excessive production of ATP by either metabolic pathway could block the AMPK activity.

**Figure 1.4 – The regulatory network of metabolism.**

The red ellipses are enzymes or regulatory proteins, the blue ellipses are EMT regulatory proteins, and green/yellow rectangles are metabolites and small molecules. The arrows represent positive regulations and the bars represent negative regulations. AMPK and HIF-1 play a central
role in regulating the different metabolic pathways, while the metabolic pathways regulate activities of AMPK and HIF-1 partially through ROS. The regulatory circuit of EMT is also coupled with the metabolism network through the interactions among AMPK, TWIST, TGF-β, SNAIL and HIF-1.

It is worth noting that the metabolic network is also coupled to the core EMT regulatory circuits via HIF-1 and AMPK. HIF-1 activates SNAIL transcription, a master regulator of EMT, while AMPK inhibits TGF-β expression, which is a major signaling pathway to promote EMT. Hence, EMT might be regulated by cell metabolism. Reversely, TWIST, which is up-regulated by SNAIL, inhibits AMPK activity. Meanwhile, excessive TGF-β can inactivate acetyl-CoA carboxylase (ACC) through SNAIL.

1.4. Mathematical Modeling of the Metabolic Network

Mathematical and computational models are useful tools that enable us to understand the complex processes via quantitative analysis. We model the dynamical behaviors of the gene regulatory network/circuit (Fig.1.4) by solving nonlinear differential rate equations. Typically, the deterministic rate equation for a protein or metabolite has a generic form:

\[ \frac{dX}{dt} = g_X \cdot G - X \cdot k_X \cdot K \]

Equation 1.1 – Generic deterministic rate equation.

Here, \( X \) represents the level of the protein or the metabolite. \( g_X \) is the basal production rate of \( X \), and \( k_X \) is the basal degradation rate of \( X \). \( G \) and \( K \) represent the effects of the regulations from any molecule in the circuit (including themselves) to the
production and the degradation of $X$, respectively. The functional form of both $G$ and $K$ are explained as follows.

Overall, we assumed that regulation of a component $Y$ to $X$ in the network/circuit could be described by the inhibitory and excitatory Hill functions:

The inhibitory Hill function (Fig.1.5A), $H^-$ is defined as

$$H^-(Y, Y^0, n_Y) = \frac{1}{1 + \left(\frac{Y}{Y^0}\right)^{n_Y}}$$

**Equation 1.2 – The inhibitory Hill function.**

The excitatory Hill function (Fig.1.5B), $H^+$ is defined as

$$H^+(Y, Y^0, n_Y) = \frac{Y^{n_Y}}{[1 + \left(\frac{Y}{Y^0}\right)^{n_Y}]}$$

**Equation 1.3 – The excitatory Hill function.**

Panel (A) shows the inhibitory Hill function (Eq.1.2): the number of protein/metabolite $X$ decreases when the number of $Y$ increases. Panel (B) shows the excitatory Hill function (Eq.1.3): the number of protein/metabolite $X$ increases when the number of $Y$ increases. Here, the threshold of $Y$ ($Y^0$) is 50, and the hill coefficient ($n_Y$) is 3.
In both Eq.1.2 and Eq.1.3, $Y^0$ is the threshold level of $Y$, $n_Y$ is the Hill coefficient. Both $H^-(Y, Y^0, n_Y)$ and $H^+(Y, Y^0, n_Y)$ are ranging from 0 to 1. The inhibitory Hill function can model the decreasing protein/metabolite level due to inhibition from $Y$, while the excitatory Hill function can model the increasing protein/metabolite level due to activation from $Y$.

Here, we use a nonlinear function that combines the inhibitory and excitatory Hill functions to describe the general regulation from $Y$ to $X$, namely the shifted Hill function,

$$H^s(Y, Y^0, \lambda_Y, n_Y) = H^-(Y, Y^0, n_Y) + \lambda_Y H^+(Y, Y^0, n_Y)$$

Equation 1.4 – The shifted Hill function.

Figure 1.6 – The shifted Hill functions.

Panel (A) shows the shifted Hill function with $\lambda_Y = 0.1$: the fold change of protein/metabolite $X$ is less than 1, and the number of $X$ decreases with the increasing number of $Y$. Panel (B) shows the excitatory Hill function with $\lambda_Y = 10$: the fold change of protein/metabolite $X$ is larger than 1, and the number of $X$ increases with the increasing number of $Y$. In both (A) and (B), the threshold of $Y$ ($Y^0$) is 50, and the hill coefficient ($n_Y$) is 3.
The shifted Hill function has the uniformed expression for modeling both the inhibitory and excitatory regulatory signaling. And the parameter, $\lambda_Y$, can represent the minimum/maximum fold change of $X$ due to the regulation of $Y$. Particularly, the minimum fold change $\lambda_Y < 1$ for the case of an inhibitory regulation (Fig.1.6A), and the maximum fold change $\lambda_Y > 1$ for the case of an excitatory regulation (Fig.1.6B). When $\lambda_Y = 1$, it means the number of $Y$ has nothing to do with the number of $X$.

When the production of $X$ is regulated by only one component $Y$, as shown above, $G = H^S$. Similarly, for regulations on the degradation, $K = H^S$. When the production of $X$ is regulated by two components $Y$ and $Z$, $G$ can be expressed as:

$$G = \begin{cases} H^s(Y, Y^0_X, \lambda_Y, n_Y)H^s(Z, Z^0_X, \lambda_Z, n_Z) & \text{Y and Z are independent} \\ C^{comp}(k_0, Y, Y^0_X, k_Y, n_Y, Z, Z^0_X, k_Z, n_Z) & \text{Y and Z are competitive} \end{cases}$$

**Equation 1.5 – The function representing multiple regulations.**

$H^s(Y, Y^0_X, \lambda_Y, n_Y)H^s(Z, Z^0_X, \lambda_Z, n_Z)$ is simply the production of two shifted Hill functions, which means that $Y$ and $Z$ independently regulate the generation of $X$. $C^{comp}(k_0, Y, Y^0_X, k_Y, n_Y, Z, Z^0_X, k_Z, n_Z)$ represents the competitive regulation from $Y$ and $Z$ to $X$, whose form is shown in the Method sections in the following chapters. Similar functional form also applies to the degradation regulation term $K$.

**1.5. Analysis of the Regulatory Network**

Nullclines are lines or curves in the space of the concentrations of molecules (Fig.1.7). The concept of nullclines is useful in analyzing the stability of a system. In a system described by differential equations, the nullclines are the curves obtained when
the concentrations of the molecules do not change with time (curves on which every point is the solution of corresponding differential equations that are equal to zero). In a graph with two nullclines, any intersection of the nullclines is an equilibrium point of the system (steady state, Fig.1.7). By examining the graphs of the nullclines, we can infer whether or not a system is multi-stable. The nullclines have to intersect in more than one place for the system to be multi-stable. If they only intersect in one place, there is only one single equilibrium point and the system cannot be multi-stable. If there are more than two intersections, the middle equilibrium points are often unstable saddle point(s). For example, Fig.1.7 shows the nullclines and steady states of the toggle switch and toggle switch with symmetric self-activations.

![Figure 1.7 – The nullclines and steady states.](image)

The nullclines for the toggle switch and the toggle switch with self-activations. Panel (A) shows the nullclines and steady states of the toggle switch. In this case, the circuit has two stable steady states (green circles with sold face) and one unstable steady state (saddle point, green circle with empty face), therefore, it is a bistable circuit. Panel (B) shows the nullclines and steady states of the toggle switch with self-activations. In this case, the circuit has three stable steady states (green circles with sold face) and two unstable steady states (saddle points, green circle with empty face), therefore, it is a tristable circuit.
The plot of the steady states of a circuit as a function of a parameter is called the bifurcation plot. Typically, the solid line segments represent the stable steady states, while the dashed line segments represent the unstable steady states. Usually, a system can have one or multiple steady states, depending on the changes of the parameter. Bifurcation plot is especially useful to show how an internal/external signal (represented by the corresponding parameter) affects the stability of a circuit. It can be also used to detect the sensitivity of the system behavior to the corresponding parameter. For example, Fig.1.8 shows an example in which an external input signal drives the change of multi-stability of a toggle switch.

**Figure 1.8 – Toggle switch driven by an external signal.**

(A) Phase space showing the nullclines for a specific value (80) of the input signal I when two stable states coexist. (B) Bifurcation diagram as a function of the input signal. The green solid lines represent the stable fixed points, while the magenta dotted line represents the unstable fixed points. Two cases are highlighted by the dotted vertical lines. When I = 80, there are two coexisting stable states (bistability). When I = 160, there are only single stable state (monostability). Green circles with light green face mark the stable fixed points, and the green circle with empty face marks the unstable saddle fixed point.
Chapter 2

Modeling the Genetic Regulatory Circuit of Metabolism

In this chapter, the full metabolic network will be reduced to a minimalist regulatory circuit composed of HIF-1, AMPK and ROS. We will explore the dynamics of this regulatory circuit, study the metabolic phenotypes and investigate the roles they play in the cells. In addition, the therapeutic strategies targeting cancer metabolism are studied by taking the different therapies as external signals to the circuit.

2.1. The AMPK:HIF-1:ROS Regulatory Circuit

Although the full metabolic network (Fig.1.3) includes most essential elements, it is too complicated to perform modeling analysis on the whole network. To capture the principles of how genes and metabolites modulate metabolism for both cancer and normal cells, we coarse-grained the full metabolic network to a minimalist regulatory circuit consisting of AMPK, HIF-1 and ROS (including noxROS and mtROS) (Fig.2.1).
These components were chosen, as they play critical roles in regulating the decision-making of both glycolysis and OXPHOS. Moreover, the reduced regulatory circuit captures the main features of the full network. As we will show later, the circuit is sufficient to explain important experimental observations of cancer metabolism.

**Figure 2.1 – The AMPK:HIF-1:ROS regulatory circuit.**

The red ellipses are HIF-1 and AMPK, and the yellow rectangles are noxROS and mtROS respectively. In this circuit, AMPK and HIF-1 are the master regulators of mitochondrial OXPHOS and glycolysis, respectively. HIF-1 has an indirect self-activation because of the interplay between the HIF-1 and glycolytic pathway. AMPK has an indirect self-inhibition because of the interplay between the AMPK and OXPHOS pathway. HIF-1 indirectly inhibits the mtROS production by up-regulating glycolysis and inhibiting OXPHOS. HIF-1 also promotes the noxROS production by activating NOX. AMPK inhibits mtROS by upregulating Trx through FOXO, and promotes mtROS by activating OXPHOS. ROS (including both mtROS and noxROS) stabilizes HIF-1 and activates AMPK.

In this AMPK:HIF-1:ROS circuit, AMPK and HIF-1 are directly associated with mitochondrial OXPHOS and glycolysis respectively. In response to various metabolic stresses, AMPK is activated through phosphorylation, and induces fatty acid and glucose metabolism. In particular, AMPK increases glucose uptake in mitochondria by increasing GLUT-4 translocation, and induces fatty acid oxidation by phosphorylating...
and inactivating the acetyl-CoA carboxylase (ACC), and activating PPARA and PGC-1. HIF-1 activates enzymes needed in the glycolytic pathway and induces glycolysis.

Besides, AMPK and HIF-1 regulate each other through mutual inhibition. AMPK down-regulates HIF-1 through inhibiting mTOR complex, and HIF-1 directly inhibits AMPK in a transcriptional manner. Also, AMPK activation leads to enhanced ATP production through OXPHOS, while excessive ATP inhibits the phosphorylation of AMPK. Therefore, AMPK has an indirect self-inhibition. In contrast, HIF-1 has an indirect self-activation via glycolysis, because the glycolytic products, such as pyruvate and lactate, stabilize HIF-1.

Moreover, AMPK and HIF-1 both highly interact with ROS. HIF-1 promotes noxROS production through targeting NOX, and inhibits mtROS production by decreasing OXPHOS and activating glycolysis. On the other hand, AMPK promotes mtROS production by OXPHOS, and increases mtROS scavenging through the AMPK-FOXO pathway that upregulates thioredoxin (Trx) expression. In cytosol, AMPK inhibits noxROS production by regulating NOX. ROS, including both mtROS and noxROS, stabilizes HIF-1α and activates AMPK, respectively.

The detailed information of the regulatory interactions of the full network and the reduced circuit is listed in Table 2.1.

<table>
<thead>
<tr>
<th>Regulations</th>
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<td>HIF-1 inhibits AMPK</td>
<td>ROS</td>
<td>Hwang et al., 2014</td>
<td>Caenorhabditis elegans</td>
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<td>AMPK inhibits HIF-1</td>
<td>ROS, Raptor, mTOR</td>
<td>Hwang et al., 2014 [92]</td>
<td>Caenorhabditis elegans, liver cells, human cervical epithelial adenocarcinoma cells</td>
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<td>Gwinn et al., 2008 [91]</td>
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<td>Harada et al., 2009 [94]</td>
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<td>HIF-1 self-activation</td>
<td>Glycolysis, Pyruvate, Lactate</td>
<td>Luo et al., 2011 [95]</td>
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<td>Lu et al., 2002 [96]</td>
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<td>De Saedeleer et al., 2012 [97]</td>
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<td>AMPK self-inhibition</td>
<td>ATP, Fatty acid oxidation</td>
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<td>Escherichia coli cells, skeletal muscle cells</td>
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<td>Lee at al., 2006 [90]</td>
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<td>Steinberg at al., 2014 [98]</td>
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<td>AMPK promotes mtROS</td>
<td>Fatty acid oxidation</td>
<td>Rosca et al., 2012 [99]</td>
<td>Kidney cortical tubules</td>
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<td>AMPK inhibits mtROS</td>
<td>FOXO3, Glutathione, Thioredoxin</td>
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<td>Human aortic endothelial cells, MEFs, HT1080, and HeLa cells</td>
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<td>Ameri et al., 2015 [101]</td>
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<td>Aon et al., 2012 [102]</td>
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<td>AMPK inhibits nox ROS</td>
<td>NADPH oxidases</td>
<td>Song et al., 2012 [103]</td>
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<td>Wang et al., 2010 [104]</td>
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<td>HIF-1 inhibits mtROS</td>
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<td>Lung cancer cells, UT-7/TPO cells</td>
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<td>Kiriito et al., 2009 [106]</td>
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<td>HIF-1 promotes noxROS</td>
<td>NADPH oxidase-2</td>
<td>Yuan et al., 2011 [107]</td>
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<td>Diebold et al., 2012 [108]</td>
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<td>mtROS activates AMPK</td>
<td>Mitochondrial metabolism, AMP/ATP</td>
<td>Lamberts et al., 2009 [83]</td>
<td>Rat heart cells, vascular smooth muscle cells</td>
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<td>Emerling et al., 2009 [84]</td>
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<td>Zhang et al., 2008 [109]</td>
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<td>mtROS stabilizes HIF-1</td>
<td>Oxygen, Mitochondria</td>
<td>Ma et al., 2013 [110]</td>
<td>Breast cancer cells, human immortalized hepatocyte cells, human lung cells</td>
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<td>Brunelle et al., 2005 [80]</td>
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<td>noxROS activates AMPK</td>
<td>H$_2$O$_2$</td>
<td>Zmijewski et al., 2010 [111]</td>
<td>Human embryonic kidney cells (HEK 293)</td>
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<td>noxROS stabilizes HIF-1</td>
<td>NOX PDH</td>
<td>Li et al., 2014 [93]</td>
<td>HL-7702 immortalized human hepatocyte cells</td>
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<td>HIF-1 activates SNAIL</td>
<td>Direct transcriptional regulation</td>
<td>Zhu et al., 2013 [112]</td>
<td>Human pancreatic cancer cell lines, human HCC cell lines HepG2 and SMMC-7721, human embryonic kidney cell line HEK293</td>
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<td></td>
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<td>Zhang et al., 2013 [86]</td>
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<tr>
<td>HIF-1 activates TWIST</td>
<td>Direct transcriptional regulation</td>
<td>Yang et al., 2008 [113]</td>
<td>Human hypopharyngeal carcinoma (FaDu), embryonic kidney (293T) cell lines, breast cancer (MCF-7), lung cancer (H1299) and tongue cancer (SAS) cell lines</td>
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<td>Regulations</td>
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<td>AMPK inhibits TGF-β</td>
<td>p300</td>
<td>Mishra et al., 2008(^{87})</td>
<td>Human primary mesangial cells (HMC), Hepatic stellate cells (HSC)</td>
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<td>TWIST inhibits AMPK</td>
<td>mTOR</td>
<td>Jin et al. 2012(^{88})</td>
<td>H1299 and A549 non-small cell lung carcinoma cells</td>
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<td>TGF-β elevate OXPHOS</td>
<td>Fatty acid synthesis Fatty acid oxidation</td>
<td>Jiang et al. 2015(^{33})</td>
<td>A459 non-small cell lung carcinoma cells</td>
</tr>
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</table>

Table 2.1 – Experimental evidences for the metabolic network and core circuit.

2.2. Methods

2.2.1. Mathematical Model for the Core AMPK:HIF-1:ROS Circuit

Here, we present the details about how we mathematically model the circuit. The metabolism circuit was modeled by the following deterministic rate equations:

\[
\dot{R}_{mt} = g_{r_{mt}}H^{s-}(A, A_{ar}^0, \lambda_{ar}, n_{ar})C_{r_{mt}}(r, g_h, h, h_{r_{mt}}^0, n_{h_{mt}}, A, A_{ar_{mt}}^0, n_{a_{mt}}) - k_{r_{mt}}R_{mt}
\]

\[
\dot{R}_{nox} = g_{r_{nox}}C_{r_{nox}}(g_0, h, h_{r_{nox}}^0, n_{h_{nox}}, g_{r_{nox}}^{h}, A, A_{ar_{nox}}^0, g_{r_{nox}}^{a}, n_{a_{nox}}) - k_{r_{nox}}R_{nox}
\]

\[
R = R_{mt} + R_{nox}
\]

\[
\dot{A} = g_AH^{s-}(R, R_{ra}^0, \lambda_{ra}, n_{ra})H^{s-}(h, h_{ha}^0, \lambda_{ha}, n_{ha})H^{s-}(A, A_{aa}^0, \lambda_{aa}, n_{aa}) - k_A A
\]

\[
\dot{h} = g_hH^{s-}(A, A_{ah}^0, \lambda_{ah}, n_{ah}) - k_h \cdot \dot{h} \cdot H^{s-}(h, h_{hh}^0, \lambda_{hh}, n_{hh})H^{s-}(R, R_{rh}^0, \lambda_{rh}, n_{rh})
\]

Equation 2.1 – The rate equations modeling the AMPK:HIF-1:ROS circuit.

Here, we followed the computational modeling approach presented by Lu et al.\(^{24}\).

In the equations, \(R_{mt}, R_{nox}, A, h\) represent the levels of mtROS, noxROS, AMPK
(phosphorylated form) and HIF-1 respectively; \( g_{r_{mt}} \), \( g_{r_{nox}} \), \( g_a \), \( g_h \) are the synthesis rate of mtROS, noxROS, AMPK and HIF-1 respectively; \( k_{r_{mt}} \), \( k_{r_{nox}} \), \( k_a \), \( k_h \) represent the degradation rates of mtROS, noxROS, AMPK and HIF-1 respectively. We used the shifted Hill-function \( H^s+ \) for excitatory regulations, \( H^s− \) for inhibitory regulations, and \( C^c \) for competitive regulations by two genes/metabolites.

In Eq.2.1, the shifted Hill function \( H^s \) and the competitive regulations \( C^c \) are expressed by the following equations:

\[
H^s(X, X_0, \lambda, n) = \frac{1+\lambda \left(\frac{X}{X_0}\right)^n}{1+\left(\frac{X}{X_0}\right)^n}, \quad \begin{cases} H^s− \equiv H^s & \text{if } \lambda < 1 \\ H^s+ \equiv H^s & \text{if } \lambda > 1 \end{cases}
\]

**Equation 2.2 – The shifted Hill functions.**

\[
C^c_{r_{mt}}(\gamma, g_n, h, h^0_{r_{mt}}, n_{r_{mt}}, A, A^0_{ar_{mt}}, n_{a_{mt}}) = \frac{\gamma \left( g_n + \left( \frac{A}{A^0_{ar_{mt}}} \right)^{n_{a_{mt}}} \right)}{1 + \left( \frac{h}{h^0_{r_{mt}}} \right)^{n_{r_{mt}}} + \left( \frac{A}{A^0_{ar_{mt}}} \right)^{n_{a_{mt}}}}
\]

\[
C^c_{r_{nox}}(g_0, h, h^0_{r_{nox}}, n_{r_{nox}}, g^h_{r_{nox}}, A, A^0_{ar_{nox}}, g^a_{r_{nox}}, n_{a_{nox}}) = \frac{g_0 + g^h_{r_{nox}} \left( \frac{h}{h^0_{r_{nox}}} \right)^{n_{r_{nox}}} + g^a_{r_{nox}} \left( \frac{A}{A^0_{ar_{nox}}} \right)^{n_{a_{nox}}}}{1 + \left( \frac{h}{h^0_{r_{nox}}} \right)^{n_{r_{nox}}} + \left( \frac{A}{A^0_{ar_{nox}}} \right)^{n_{a_{nox}}}}
\]

**Equation 2.3 – The competitive modeling function.**

As described in Eq.2.2, \( H^s+ \) and \( H^s− \) correspond to the shifted Hill functions with \( \lambda > 1 \) and \( \lambda < 1 \) respectively. Eq.2.3 represents the competitive model to describe
the competitive regulations from AMPK and HIF-1 to mtROS and noxROS. The parameters for this model are shown in Appendix A.

2.2.2. Mathematical Model for Therapeutic Treatment Simulation

We also investigate the dynamic behavior of the AMPK:HIF-1:ROS core circuit in response to various treatment strategies. Specifically, we modeled the treatment strategies by the administration of metformin, 3BP, hyperbaric oxygen, the combination of metformin and 3BP as well as the combination of metformin and the hyperbaric oxygen. Here, we describe how we mathematically model the effects of the therapies to the circuit.

First, we consider the hyperbaric oxygen therapy. The hyperbaric oxygen mainly influences the HIF-1 degradation by directly binding to Prolyl hydroxylases (PHDs), which are crucial for HIF-1α hydroxylation. However, ROS (such as H$_2$O$_2$), can decrease the binding of oxygen to PHDs, thus stabilizes the HIF-1. Therefore, we can simplify the chemical reactions as following:

\[ n_{oh}O_2 + \text{HIF-1} \xrightarrow{t_{oh}} \text{HO} \]

\[ n_{rh}\text{ROS} + \text{HIF-1} \xrightarrow{t_{rh}} \text{HR} \]

\[ [\text{HIF-1}] + [\text{HO}] + [\text{HR}] = 1 \]

Where HO represents the HIF-1 that binds to oxygen, and HR represents the HIF-1 that binds to ROS. Here, we assumed the sum of the HIF-1 related products is constant.

We further assume that \( \frac{t_{oh}}{t_{O^+}} = [O_2]^{n_{oh}} \), \( \frac{t_{rh}}{t_{R^+}} = [R^0]^{n_{rh}} \). At equilibrium, we get
\[
\frac{[O_2]}{O_2^0}^{n_{oh}} = \frac{[HO]}{[HIF-1]}
\]

\[
\frac{[R]}{R_0}^{n_{rh}} = \frac{[HR]}{[HIF-1]}
\]

Therefore, we have \( [HIF-1] + \left( \frac{[O_2]}{O_2^0} \right)^{n_{oh}} [HIF-1] + \left( \frac{[R]}{R_0} \right)^{n_{rh}} [HIF-1] = 1 \). Hence, under the influence of oxygen, the dynamics of HIF-1 degradation can be expressed as:

\[
\frac{d[HIF-1]}{dt} = -k_h C(k_1, k_2, k_3, R, O, R_h^0, O_h^0, n_{rh}, n_{oh}, R_0) [HIF-1]
\]

Equation 2.4 – The degradation rate equation of HIF-1 under the treatment of hyperbaric oxygen.

In which, \( C(k_1, k_2, k_3, R, O, R_h^0, O_h^0, n_{rh}, n_{oh}) = \frac{k_1 + k_2 \left( \frac{[R]}{R_h} \right)^{n_{rh}} + k_3 \left( \frac{[O_2]}{O_h^0} \right)^{n_{oh}}}{1 + \left( \frac{[R]}{R_h} \right)^{n_{rh}} + \left( \frac{[O_2]}{O_h^0} \right)^{n_{oh}}} \), \( k_h \) is the basal degradation rate of HIF-1, \( k_1, k_2, k_3 \) together represent the contributions of the oxygen and ROS to HIF-1 degradation.

Hence, the rate equation of HIF-1 under hyperbaric oxygen treatment can be expressed as:

\[
\hat{h} = g_h H^{s-}(A_{ah}, A_{ah}^0, \lambda_{ah}, n_{ah}) - k_h \cdot h \cdot H^{s-}(h_{hh}, h_{hh}^0, \lambda_{hh}, n_{hh}) \cdot \alpha \cdot C(k_1, k_2, k_3, R, O, R_h^0, O_h^0, n_{rh}, n_{oh})
\]

Equation 2.5 – The rate equation of HIF-1 in response to hyperbaric oxygen.

Here, \( \alpha \) is a constant parameter, which is chosen so that Eq.2.5 is equivalent to the rate equation of HIF-1 in Eq.2.1. Therefore, \( \alpha \cdot C(k_1, k_2, k_3, R, O, R_h^0, O_h^0, n_{rh}, n_{oh}) = \)
\( H^{s-}(R, R_{rh}^0, \lambda_{rh}, n_{rh}) \) for any level of ROS. As a result, there are only two non-redundant parameters in \( \alpha, k_1, k_2 \) and \( k_3 \).

Secondly, we consider the metformin-based therapy. Metformin not only activates AMPK but also inhibits HIF-1 in an AMPK-independent way \(^{117}\). Therefore, we use the shifted Hill function \( H_{ma}^{s+} \) to model the AMPK generation and \( H_{mh}^{s+} \) to model the HIF-1 degradation under the influences of metformin. In addition, it has been shown that metformin can also induce more mtROS production by changing the mitochondrial potential \(^{118}\). Hence, we model this effect by increasing the mtROS production rate by an amount that is proportional to the level of metformin.

\[
\dot{A} = g_a H^{s+}(R, R_{ra}^0, \lambda_{ra}, n_{ra}) H^{s-}(h, h_{ha}^0, \lambda_{ha}, n_{ha})
\cdot H^{s-}(A, A_{aa}^0, \lambda_{aa}, n_{aa}) H^{s+}(M, M_{ma}^0, \lambda_{ma}, n_{ma}) - k_a A
\]

**Equation 2.6** – The rate equation of AMPK in response to metformin therapy.

\[
\dot{h} = g_h H^{s-}(A, A_{ah}^0, \lambda_{ah}, n_{ah}) H^{s-}(M, M_{mh}^0, \lambda_{mh}, n_{mh}) - k_h
\cdot h \cdot H^{s-}(h, h_{hh}^0, \lambda_{hh}, n_{hh}) H^{s-}(R, R_{rh}^0, \lambda_{rh}, n_{rh})
\]

**Equation 2.7** – The rate equation of HIF-1 in response to metformin therapy.

\[
(g_{r_{mt}})_{met} = g_{r_{mt}} + \alpha_{met} \cdot [Metformin]
\]

**Equation 2.8** – The mtROS generation rate in response to metformin therapy.

Last, we consider the 3BP (or 2DG) therapy. Since the 3BP acts on the enzymes to reduce the glycolysis, which decreases the strength of HIF-1 self-activation, we simply modeled the effects of 3BP by

\[
(\lambda_{hh})_{3BP} = \lambda_{hh} + \alpha_{3BP} \cdot [3BP]
\]
**Equation 2.9 – The maximum foldchange of HIF-1 due to self-activation under the influence of 3BP therapy.**

Here, \((\lambda_{hh})_{3BP}\) is the maximum fold change due to HIF-1 self-activation under the influence of 3BP (or 2DG), and \(\alpha_{3BP}\) is a constant representing the extent of the glycolysis reduction.

The parameters involved in the treatment modeling are shown in Appendix B.

**2.3. Results**

**2.3.1. The Metabolic Circuit Allows Multiple Cell Phenotypes**

We performed stability analysis of the circuit with two parameter sets that correspond to normal and cancer cells respectively. In our model, cancer cells differ from normal cells in two aspects. First, cancer cells have higher mtROS production due to reprogrammed mitochondria \(^{54}\). Second, cancer cells have more stable HIF-1 because less oxygen is available to each cell due to abnormally rapid proliferation. Therefore, compared to normal cells, in the modeling, cancer cells produce more mtROS in response to the AMPK activation (represented by \(\gamma\) in Eq.2.1, maximum fold change of mtROS by the AMPK activation), and lower HIF-1 degradation rate (represented by \(k_R\) in Eq.2.1).
Figure 2.2 – The nullclines of the normal cells and cancer cells.

The nullclines and steady states in the phase space of AMPK and HIF-1. The red line shows the nullcline of $dh/dt = 0$, and the blue line shows the nullcline of $dA/dt = 0$ (see Eq.2.1). The green solid dots denote stable fixed points (steady states) and the green empty circles denote unstable fixed points. Each stable steady state is associated with a cell metabolic phenotype. For normal cells (panel A), the circuit allows a Warburg state, denoted by ‘W’, and an oxidative state, denoted by ‘O’. For cancer cells (panel B, higher mtROS production and lower HIF-1 degradation), the circuit allows an additional hybrid state, denoted as ‘W/O’.

Our simulations show that normal cells have two stable steady states, which correspond to the high HIF-1 and low AMPK state, and the low HIF-1 and high AMPK state (Fig.2.2). We associate them to the Warburg effect (W) and oxidative respiration (O), respectively (‘W’ state and ‘O’ state in Fig.2.2A). This result is consistent with the fact that, while cells usually use glucose oxidation to produce energy, they switch to glycolysis during anaerobic exercises. Next, we performed analysis on the cancer cells, which have larger $\gamma$ and lower $k_h$, as we discussed above. Interestingly, we found that cancer cells have a new hybrid state with both high AMPK and high HIF-1 (‘W/O’ state in Fig.2.2B) in addition to the ‘W’ and ‘O’ states. In the hybrid state, the levels of AMPK and HIF-1 are both high because the cancer cells have high level of ROS that
both stabilizes HIF-1 and activates AMPK. The existence of the hybrid metabolic phenotype means that cancer cells have the flexibility to utilize both glycolysis and mitochondrial OXPHOS. The result is consistent with the observations that, although the glycolytic metabolic pathway generates as much as 40% to 75% ATP in cancer cells, mitochondrial respiration is still an important source for tumors’ energy demands 54,56,58,65,66,119,120.

We further performed the parameter sensitivity analysis by randomizing the parameters used in our model. We randomized all parameters (except for $\gamma$ and $k_h$) by 25%, from which we obtained 20,000 set of parameters for both normal cells and cancer cells. We show that, both normal cells and cancer cells have the similar probabilities to stay in the ‘W’ and ‘O’ phenotypes, but they are have quite different probabilities to stay in the hybrid state. While there is only a small probability (2.13%) to observe the hybrid state in normal cells (Fig.2.3A), there is a much larger probability (13.19%) to observe the hybrid state in cancer cells (Fig.2.3B). The result is consistent with the nullcline analysis above, which suggests the robustness of our model.

Figure 2.3 – The parameter sensitivity analysis.
The distribution of the stable steady states for the normal cell (panel A) and cancer cell (panel B). The color bar on the right side represents the number of proteins in the logarithm (log2) scale. Among all the models, the probabilities of seeing the ‘W’ state, the ‘W/O’ state and the ‘O’ state for normal cells are 49.02%, 2.13% and 48.84%, respectively (panel A). The probabilities of seeing the ‘W’ state, the ‘W/O’ state and the ‘O’ state for cancer cells are 36.16%, 13.19% and 50.42%, respectively (panel B). There is also a low glycolysis and low OXPHOS state (with 0.23% probability) for cancer cells (panel B).

2.3.2. The Hybrid Metabolic Phenotype for Cancer Cells

We argue that cancer cells in the ‘W/O’ hybrid state have more advantages in supporting their survival, proliferation and metastasis. First, cells in the hybrid state have higher metabolic plasticity to better adapt to various microenvironments, such as hypoxia and acidic conditions. It is due to the fact that these cells are flexible to use available nutrients, such as glucose and fatty acids to produce energy. Second, the hybrid state allows cancer cells to efficiently produce energy by both OXPHOS and glycolysis, meanwhile to use lactate and pyruvate, the byproduct of glycolysis, to generate biomass for the need of cell proliferations. Third, cells in the hybrid state can modulate ROS at a moderate level, so that cells can take advantage of ROS signaling to promote metastasis meanwhile avoid excessive DNA damage to the cells. Here, ROS scavenging is achieved by pyruvate and NADPH, the byproducts of glycolysis and fatty-acid oxidation, respectively. In summary, cancer cells with the hybrid metabolic phenotype have advantage in multiple aspects over cells in either the ‘W’ or the ‘O’ phenotypes. This could explain why cancer cells with the hybrid ‘W/O’ phenotype are more aggressive and invasive. Therefore, we propose that the efficacy of metabolic drugs could be evaluated by targeting the hybrid metabolic phenotype.
2.3.3. The Role of mtROS Production and HIF-1 Stability in Cancer Metabolism

To better understand the effect of mtROS production and HIF-1 stability on the dynamic behavior of the AMPK:HIF-1:ROS circuit, we plotted a two-parameter bifurcation diagram of the number of stable steady states with respect to the parameters \( \gamma \) and \( k_h \), as shown in Fig.2.4A. For different values of these two parameters, cells can reside in one of seven phases. Each phase corresponds to a particular situation in which one or several different metabolic phenotypes can coexist. More specifically, the possible phases are: 1. phases with only one phenotype, (‘W’), (‘O’) and (‘W/O’); 2. phases in which two phenotypes coexist, (‘W’, ‘O’), (‘W’, ‘W/O’) and (‘O’, ‘W/O’); 3. phases in which three phenotypes coexist, (‘W’, ‘W/O’, ‘O’). As illustrated in the diagram, normal cells typically correspond to the lower right region of the phase space, where only the ‘O’ or ‘W’ states are accessible. Cancer cells typically correspond to the top left region of the phase space, where the hybrid ‘W/O’ state exists. The diagram also shows that \( \gamma \) plays more important roles than \( k_h \) in determining the existence of the hybrid state, which is consistent with our view that the hybrid state is mainly resulted from high level of ROS.

We further examined the response of the AMPK:HIF-1:ROS circuit to changes in either \( \gamma \) (Fig.2.4B) or \( k_h \) (Fig.2.4C) by one-parameter bifurcation diagrams. In Fig.2.4B, we show that, when the rest parameters remain unchanged, the increase of \( \gamma \) shifts the cells from bistability (with two stable steady states) to tristability (with three stable steady states) and gives rise to the hybrid metabolic state, ‘W/O’, in addition to ‘W’ and ‘O’ phenotypes. This is because, once again, higher production of mtROS by mitochondria stimulates both the AMPK activation and the HIF-1 stabilization. In Fig.2.4C, we show...
that, when the rest parameters remain fixed, the decrease of $k_h$ shifts the cells from the monostable phase (‘O’), to the bistable phase (‘O’, ‘W’), then to the tristable phase (‘O’, ‘W/O’, ‘W’), to the bistable phase (‘O’, ‘W’) again, and finally to the monostable phase (‘W’). The reason for these changes can be understood as follows. At a high rate of HIF-1 degradation (high $k_h$), the level of HIF-1 is low, therefore the glycolysis process is inhibited. As a result, the OXPHOS becomes the dominant metabolic mode. While at a low rate of $k_h$, HIF-1 accumulates, and glycolysis becomes the dominant metabolic mode. Only when $k_h$ is in a certain intermediate range, HIF-1 and AMPK can be both at high levels, which corresponds to the hybrid ‘W/O’ state. In summary, the bifurcation analysis indicates that the transitions from normal cell to cancer cell are highly associated with the mtROS production and HIF-1 stability.
Figure 2.4 – Bifurcation diagrams of the AMPK:HIF-1:ROS circuit with respect to the maximum fold change of mtROS by AMPK activation ($\gamma$) and the HIF-1 degradation rate ($k_h$).

(A) Two-parameter bifurcation diagram of the metabolic circuit, as the function of $\gamma$ and $k_h$. The circuit allows different phases for different values of $\gamma$ and $k_h$. Each phase (represented by different colors) allows a different combination of coexisting metabolic phenotypes. For example, the yellow area is the phase allowing three possible metabolic phenotypes ('W', 'W/O', 'O'). (B) One-parameter bifurcation diagram for the circuit driven by the parameter $\gamma$, when $k_h$ is fixed at 2.5 hour$^{-1}$. The corresponding trajectory in panel A is shown by the purple line. (C) One-parameter bifurcation diagram for the circuit with respect to the parameter $k_h$, when $\gamma$ is fixed at 8.0. The corresponding trajectory in panel A is shown by the red line. In both panel B and panel C, the blue, green, and magenta solid lines represent the phenotype ‘O’, ‘W/O’, and ‘W’, respectively. The red dashed line represents the unstable states. Different background colors represent regions for different phases, in consistent with panel A. The black dashed vertical lines highlight the normal cells (left line in panel B, right line in panel C) and the cancer cells (right line in panel B, left line in panel C).

2.3.4. Modeling Therapeutic Strategies Targeting Cancer Metabolism

Studies have shown that targeting cancer cellular metabolism is a promising strategy for fighting against cancer (a list of evidences shown in the Table 2.2). Certain metabolic drugs have been shown to be effective in treating cancers in some cases. These drugs, for example, S-trans, trans-Farnesylthiosalicylic Acid (FTS), 2-Deoxy-D-Glucose (2DG), 3-Bromopyruvate (3BP), metformin and 5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR), typically target glycolysis or mitochondrial OXPHOS. Studies also suggested that combinations of different drugs could sometimes be more effective. For example, administrating the metformin and 2DG together can induce massive ATP depletion in cancer cells and further trigger other cellular activities to induce cell death, such as autophagy or p53-dependent apoptosis. Often, metabolic drugs work well in some cases but not in the others, and the underlying mechanisms are not completely understood. Here we propose that these therapeutic strategies might be effective partly because they target the hybrid metabolic phenotype of
cancer cells. An effective treatment strategy could be achieved by shifting the metabolic phenotype from the hybrid state to the other states of the cancer cells. By doing so, the drugs might sensitize the cancer cells, therefore, some other therapies, such as chemotherapy, can be more effective in killing the cancer cells, but not the normal cells.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Effects to metabolic genes/pathways</th>
<th>Cell Lines</th>
</tr>
</thead>
<tbody>
<tr>
<td>AICAR</td>
<td>Activating AMPK</td>
<td>Cervix, prostate, liver</td>
</tr>
<tr>
<td>Metformin</td>
<td>Activating AMPK, inhibiting HIF-1, promoting ROS production</td>
<td>Breast cancer</td>
</tr>
<tr>
<td>FTS</td>
<td>Inhibiting HIF-1</td>
<td>Mice pancreatic tumor</td>
</tr>
<tr>
<td>2DG</td>
<td>Inhibiting glycolysis</td>
<td>Bovine aortic endothelial cells, human umbilical vein endothelial cells, Colon carcinoma cell lines, Mice hepatocellular carcinoma</td>
</tr>
<tr>
<td>3BP</td>
<td>Inhibiting glycolysis</td>
<td>Mice hepatocellular carcinoma, Colon carcinoma cell lines</td>
</tr>
<tr>
<td>Tiron</td>
<td>Scavenging ROS</td>
<td>Calu-6 human pulmonary adenocarcinoma cell line</td>
</tr>
<tr>
<td>mitoTEMPO</td>
<td>Scavenging mtROS</td>
<td>B16F10 murine melanoma tumor cells</td>
</tr>
<tr>
<td>FTS+2DG</td>
<td>Inhibiting HIF-1 and glycolysis</td>
<td>Human pancreatic cancer cell line</td>
</tr>
<tr>
<td>Metformin+2DG</td>
<td>Inhibiting glycolysis, Activating AMPK, promoting ROS production</td>
<td>LNCap and P69 cells, human gastric and esophageal cell lines</td>
</tr>
</tbody>
</table>

**Table 2.2 – Experiments on cancer treatment targeting metabolism.**

Hereby, we evaluated the dynamic behavior of the AMPK:HIF-1:ROS circuit for various treatment strategies, each of which has its own regulatory mechanism to the circuit. In particular, the hyperbaric oxygen therapy can effectively reduce the hypoxia and accelerate degradation of HIF-1; 3BP, similar to 2DG, targets the glycolytic enzymes such as glucose transporters (GLUTs) and hexokinase (HK), thus effectively reduces the cellular glycolysis; metformin activates AMPK, inhibits ETC Complex-1, and inhibits
mTOR in an AMPK independent manner, which further inhibits HIF-1. The effective circuit diagrams for each case are shown in Fig.2.5. Therefore, the model allows us to calculate the steady states and simulate the time course of the levels of AMPK, HIF-1 and ROS. We are especially interested in how different treatments drive cell phenotypic transitions among different metabolic states.

### 2.3.5. Treatment Efficacy Depends on the Initial Metabolic State of Cancer Cell

In our current model of cancer cells, the metabolic circuit allows three stable states – the ‘W’, the ‘W/O’ and the ‘O’ states. As a first step, we evaluated the effects of various treatment strategies to the tristability of the metabolic circuit by the bifurcation analysis. Each of the panel of Fig.2.5A-D shows the bifurcation of the levels of AMPK with respect to the level of the drugs for each treatment. The results show that these treatment strategies can shift the cancer metabolism from the tristable phase to the monostable phase (‘O’) by increasing the dose level. This may explain the efficacies of these therapies in certain antitumor studies. Meanwhile, the detailed dynamic behaviors of metabolic modes depend on the initial conditions of the cancer cells. For example, for patients whose cancer cells are initially at the ‘W’ state, the cancer cells could transit to the ‘W/O’ state by the administration of metformin (the first upward arrow in Fig.2.5C), which increases metabolic plasticity and cause the cancer cells to be more aggressive and invasive. Yet, if the initial state is the hybrid ‘W/O’ state, the cancer cells could be driven to the ‘O’ state by metformin (the second upward arrow in Fig.2.5C), which would be beneficial to the patient. Thus, our modeling provides an explanation of the double-faced effects of AMPK (a target of metformin) in cancer treatment. We argue that
overlooking the metabolic hybrid state in cancer treatment and heterogeneity among patients’ metabolic states might cause unexpected outcomes.

Next, the combinatorial effects of two treatment methods were evaluated by a 2D bifurcation diagram (Fig.2.5E), which shows a 2D bifurcation with respect to the levels of hyperbaric oxygen therapy (x-axis) and metformin (y-axis). The red lines and arrows illustrate several different strategies to administrate the two therapies, starting from the (‘W’, ‘W/O’, ‘O’) phase. In this case, all of the three therapeutic strategies, as illustrated by r1 (metformin only), r2 (hyperbaric oxygen therapy only) and r3 (both) in Fig.2.5E, can effectively drive cells out of the phases that allow the hybrid state. The particular 2D bifurcation can also be applied to another group of patients whose cancer cells are initially at the (‘W’) phase. If the patients are treated with metformin only (line b1), the cells switch from the (‘W’) phase to the (‘W’, ‘W/O’) phase first, then to the (‘W’, ‘W/O’, ‘O’) phase, and eventually to the (‘W/O’, ‘O’) phase, all of which allow the hybrid state. Therefore, the administration of metformin alone could be risky for the patients in this case, as it cannot reduce cancer metabolic plasticity. Here, metformin inhibits ETC but induces beta-oxidation, therefore inducing higher mtROS production, which drives cancer cells into the hybrid phenotype.
Figure 2.5 – The bifurcation of the AMPK:HIF-1:ROS circuit with respect to the levels of the external signals for different therapies.

(A) Bifurcation of the AMPK level in response to the oxygen level in the hyperbaric oxygen therapy. The normal condition of oxygen level is 5%. (B) Bifurcation of the AMPK level in response to the 3BP level. The hypothetic-drug 1 represents the hypothetic signal with opposite effect of 3BP. (C) Bifurcation of the AMPK level in response to the metformin level. The hypothetic-drug 2 represents the hypothetic signal with the opposite effect of metformin. (D) Bifurcation of the AMPK level in response to the level of a combined 3BP and metformin therapy. The hypothetic-drug 3 represents the effect of the hypothetic therapy with opposite effect of a combination of 3BP and metformin. In Fig.5A-D, the blue, green and magenta solid lines represent the phenotype ‘O’, ‘W/O’ and ‘W’, respectively. The red dashed lines represent the unstable steady states. The dashed lines with arrows indicate the transitions between two phenotypes. Different background colors represent different regions corresponding to different
phases in panel E and F. (E) Two dimensional bifurcation diagram of the circuit in response to the treatment signal of metformin (y-axis) and hyperbaric oxygen (x-axis) therapies. The red lines and blue dashed lines with arrows illustrate three different treatment strategies for the patients with cancer cells in the ('W', 'W/O', 'O') phase, and three different treatment strategies for the patients with cancer cells in the ('W') phase respectively: the line r1 or b1 is the treatment with metformin only; the line r2 or b2 is the treatment with hyperbaric oxygen only; the line r3 or b3 is the treatment with a combination of both hyperbaric oxygen and metformin. (F) Two dimensional bifurcation diagram of the circuit in response to the treatment signal of metformin (y-axis) and 3BP (x-axis) therapies. In panel E and F, different colors represent the corresponding phases that allow different combinations of coexisting phenotypes (as denoted by words in the panels).

Similar bifurcation analysis is also performed for the combinatorial effects of both 3BP and metformin (Fig.2.5F). Again, for the patients with cancer cells in the ('W') phase, using metformin alone (b1 in Fig.2.5F) cannot reduce metabolic plasticity, while for the patients with cancer cells at the ('W', 'W/O', 'O') phase, using metformin alone (r1 in Fig.2.5F) is effective to drive cells to the ('O') phase, thus reduces the metabolic plasticity.

2.3.6. A Comparison of Possible Metabolic Therapies

As already shown above in the bifurcation analysis, metformin is less effective compared to the hyperbaric oxygen therapy and 3BP. The inefficiency of metformin is caused by the production of mtROS by the drug. The effectiveness of various treatment strategies was further evaluated by simulating the time course of the levels of AMPK, HIF-1 and ROS, and the transitions among different metabolic phenotypes. Here we illustrate our analysis by showing the results for patients whose cancer cells (with normal function of TCA and ETC) are initially at the ‘W’ state in the phase of ('W', 'W/O', 'O'). We simulated the treatments by adding time-dependent signals (representing the administration of the drugs) to the metabolic circuit. In Fig.2.6, we show an example of the metformin-based therapy for cancer cells. Starting from the ‘W’ state, AMPK is first
activated in response to the therapy, then HIF-1 is decreased in response to the high AMPK. Note that the simulated level of AMPK response to metformin well agrees the experimental data (Ref 136, Fig.4A), on the condition that we did not fit the model parameters to the experimental data. The result confirms that our parameters in the modeling are reasonably chosen. At the end of the treatment, the trajectory converges to the steady state nearby. Here, the cells transit from ‘W’ state to ‘W/O’ state, which increases metabolic plasticity and might promotes metastasis.

**Figure 2.6 – Assessment of metformin therapy with the AMPK:HIF-1:ROS model.**

(A) Dynamical trajectory in the phase plane, where the x-axis is AMPK (nM) and the y-axis is HIF-1(nM). Nullclines are shown in light blue (AMPK) and light red (HIF-1) solid lines, and the steady states are shown as solid green circles (stable) and empty green circles (unstable). The black, red, navy and brown lines with arrows represent different directions of the treatment trajectories on the phase plane. (B) The therapy signal. Metformin therapy is used at t = 10h, and last until 500h, the dose is 250uM, which is the same dose used in the experiment of Ref. 136. (C) Time evolution of the concentrations of the activated AMPK and HIF-1. The blue line represents
AMPK and the red line represents HIF-1. The dashed vertical line separates the regions correspond to the phenotype ‘W’ (left side) and ‘W/O’ (right side). (D) The beginning part (from \( t = 0h \) to \( t = 90h \)) of Fig.2.6B and Fig.2.6C. (E) Experimental data of the activity of AMPK in response to metformin (data from Ref. 136).

In a typical simulation, we observed cells initially remain in the ‘W’ state, later switch to the ‘W/O’ hybrid state, and eventually reach to the ‘O’ state if the therapy is administrated long enough (Fig.2.7). We consider treatment to be more effective if the cells reach to the ‘O’ state earlier and stay in the hybrid state for a shorter period. Our results show that treatments using hyperbaric oxygen therapy (Fig.2.7A), 3BP (Fig.2.7B) and metformin (Fig.2.7C) have different efficacies in inducing cancer cell phenotype transitions. Particularly, under the treatment of metformin, cells quickly transit to the hybrid ‘W/O’ state, and stay in this state for a much longer time than they are under the treatment of the other therapies (Fig.2.7C). In contrast, HIF-1 inhibitors (such as hyperbaric oxygen) or glycolysis blockers (such as 3BP and 2DG) take a longer time to make the cells transit from the ‘W’ state to the ‘W/O’ state, but take much less time to transit from the ‘W/O’ state to the ‘O’ state (Fig.2.7A-B). It is worth noting that the combined therapy that targets both glycolytic pathway and mitochondrial respiration is more effective if they are simultaneously administered in the same day (Fig.2.7D-E). Yet, if the drugs are administered alternatively in different days, no obvious improvement is observed (Fig.2.8). The modeling predictions are consistent with some experimental and clinical studies. For example, it has been shown that in human gastric and esophageal cell lines, the administration of both metformin and 2DG (whose effect is similar to 3BP) is more effective than the administration of each drug alone \(^{118}\). We argue that the combined therapy is effective due to its capability in reducing the cancer metabolic plasticity.
2.3.7. Therapies Targeting Glycolysis and OXPHOS Have Distinct Time Dynamics

One of the interesting findings of the time-course simulations is that therapies targeting glycolysis inhibition have distinct dynamics from the therapies promoting OXPHOS. As shown in Fig. 2.7, although both strategies eventually switch the cell phenotype from the ‘W’ state to the ‘O’ state, the levels of AMPK and HIF-1 are much higher during the course of the AMPK-based treatment (Fig. 2.7C) than that of the HIF-1-based treatment (Fig. 2.7A-B). Therefore, the AMPK-based treatment could increase the risk of inducing the metabolic plasticity of cancer cells. This new finding could also explain the dark face of AMPK, which is considered in some cases as a tumor promoter 41,135.
Figure 2.7 – Assessment of different therapeutic strategies with the AMPK:HIF-1:ROS model.

(A) hyperbaric oxygen therapy; (B) 3BP; (C) metformin; (D) combined therapy of both hyperbaric oxygen and metformin; (E) combined therapy of both metformin and 3BP. In both D and E, the two drugs are administered simultaneously. In each figure, the left diagram shows the dynamical trajectory in the phase plane, the two axes are AMPK (nM) and HIF-1(nM). The light red line shows the nullcline of $dh/dt = 0$, and the light blue line shows the nullcline of $dA/dt = 0$ (see Eq.2.1). The steady states are shown as solid green circles (stable) and empty green circles (unstable). The black, red, navy and brown lines with arrows represent the trajectories of the treatment on the phase planes. The diagrams on the upper right show the levels of drugs administered every day. Hyperbaric oxygen therapy is presented in light blue (panel A); 3BP is presented in light red (panel B and E) and metformin is presented in light green (panel C and D). The diagrams on the lower right show the time evolutions of the levels of the activated AMPK (blue line) and HIF-1 (red line). The dashed vertical lines separate the regions correspond to the states ‘W’ (left), ‘W/O’ (middle) and ‘O’ (right), respectively.
2.3.8. Treatment Simulation Gives Hints on Optimal Daily Dose and Treatment Duration

We further compared the different treatment strategies by evaluating the final outcomes for patients under different therapies with different daily doses and lengths of the whole therapy. We evaluated the patients’ outcomes by simulating dynamics of the metabolic circuit both during and after the treatment. We observed that, although different daily dose levels lead to slightly different outcomes in making phenotypic transitions, the difference is much larger for treatments with different drugs (Fig. 2.9). Among all the treatment strategies, the metformin-based therapy (group B) is least...
efficient in driving cells to the ‘O’ state after the treatment (boundary of green and blue), and the cells are more likely to be at the ‘W/O’ state for a large range of treatment durations (green bar). In contrast, the hyperbaric oxygen-based (group A) and 3BP-based (group C) therapies are more effective in that both reduce the required length of the treatment for inducing the cells to the ‘O’ state (Fig.2.9), and reduce the duration when cells are in the ‘W/O’ state (Fig.2.7A-B). Interestingly, the combined therapies (group D for the combination of hyperbaric oxygen-based and metformin-based therapies, group E for the combination of 3BP-based and metformin-based therapies) does not reduce the required length of the treatment (Fig.2.9), but they further reduce the duration when cells are in either the ‘W’ or the ‘W/O’ state (Fig.2.7D-E). Note that the length of the green bar is long for both of the combined therapies due to the following reason. Because of slow relaxation of the regulatory genes, even though the cell phenotype quickly reaches to the ‘O’ state under the influence of the therapies, it could return back to the hybrid state if the therapy is stopped too early. Therefore, an effective therapy requires additional sessions even when the cancer cells has transited to the ‘O’ state.
After a complete session of a treatment (x-axis) of certain length (y-axis), cells could eventually stay in the ‘W’ state (magenta), the ‘W/O’ state (green), or the ‘O’ state (blue). There are five groups of treatment strategies, each of which contains three different doses of the same therapy. Group A: hyperbaric oxygen therapy (15%, 20% and 25% from the left to the right); group B: metformin-based therapy (300μM, 350μM and 400μM from the left to the right); group C: 3BP-based therapy (250μM, 300μM, 350μM from the left to the right); group D: combined therapy using both hyperbaric oxygen (10%, 15% and 20% from the left to the right) and metformin (250μM, 300μM, 350μM from the left to the right); group E: combined therapy using both 3BP and metformin (both are 250μM, 300μM and 350μM from the left to the right).

2.4. Discussions

In this study, we established a theoretical framework for modeling genetic regulation of cancer metabolism. According to the existent data, a network was constructed that features the regulation of mitochondrial OXPHOS and glycolysis by both regulatory protein and metabolites. We further coarse-grained the network into a minimalist regulatory circuit that composed of HIF-1, AMPK and ROS. Even though we made several simplifications, the reduced circuit is still sufficient to capture the regulations of both glycolysis and OXPHOS, as well as the major differences in
metabolism between normal and cancer cells. Since the regulatory links in the reduced circuit are supported by multiple experimental evidences coming from different cell lines (Table.2.1), our circuit model is expected to be robust for studying the common behavior of cancer metabolisms and their regulations.

Normal cells typically use either OXPHOS or glycolysis at a time depending on the availability of energy sources, because of tight regulations of the metabolic circuit and the competition among different metabolic modes. Cancer cells, however, kidnapped the same gene regulatory circuit of metabolism for their advantage. Because of high energetic and oxidative stresses, cancer cells can be in a hybrid metabolic phenotype with both glycolysis and OXPHOS. Since cells in the ‘W/O’ hybrid state have plasticity in using both glycolysis and oxidative respiration, they could have advantage in survival over cells in the other phenotypes. The model also explains the phenomenon of oxygen shock\textsuperscript{137}. Cells that largely rely on glycolysis are exposed to large amount of oxygen when they reach the blood vessel. These cells could switch the metabolic phenotype to the ‘W/O’ state, which further induces metastasis\textsuperscript{34,138}.

Thus, we propose to design metabolic therapies by taking into account the hybrid metabolic phenotype (W/O). A putative strategy is to drive cancer cells away from the hybrid states, meanwhile preventing cancer cells from switching into the hybrid states. Guided by this assumption, we evaluated the effectiveness of several cancer therapeutic strategies by modeling. In details, we simulated the dynamic behavior of cancer cells driven by an external signal that represents the effects of metabolic therapies, such as those that target glycolysis or OXPHOS. We found that different therapeutic strategies have differentiated effects. For example, the therapy using metformin is more likely to
drive cells into the hybrid metabolic phenotype during the treatment than the therapies using glycolysis blockers, such as 3BP and 2DG. Our model also provide an explanation of why some combined therapies are more effective \(^{118,126}\), because these therapies are efficient in driving the cells out of the hybrid state. It is worth noting that treatment efficacy also highly depends on the initial metabolic states of the cancer cells. Thus, we think it is important to monitor the metabolic states of the cell during the treatment, so that the metabolic plasticity could be confined.

In summary, our model provides insights into the regulatory mechanism of cancer metabolism beyond the Warburg effect. The hybrid metabolic mode predicted by our model brings a new perspective on how metabolic switches promote tumorigenesis and cell invasiveness. We hope this work could be helpful to the design of improved cancer therapeutic strategies that target metabolism.
Modeling the Interplay between the Genetic Regulatory Circuit and the Metabolic Pathways

In last chapter, we showed the study of a minimalist metabolic circuit consisting AMPK, HIF-1 and ROS. In this chapter, we will further explore a more refined model for the investigation of the interplay between the genetic regulatory circuit and the different metabolic pathways.

3.1. The Regulatory Network Coupling the Genetic Regulatory Circuit and the Metabolic Pathways

During the AMPK:HIF-1:ROS modeling, two major simplifications are made in the coarse-grained model. First, we lumped together both the glucose oxidation and fatty-acid oxidation while they might need to be separately considered in a more detailed
model. Second, many of the regulatory links in the circuit are indirect. For example, we modeled the self-activation of HIF-1, because HIF-1-induced glycolysis converts glucose into lactate as the end product, which in return stabilizes HIF-1 \(^{68,95}\). Another important indirect regulation is the activation from AMPK to mtROS. AMPK activates mitochondria and promotes fatty acid and glucose uptake \(^{91}\), thus promoting the glucose oxidation and fatty acid oxidation \(^{73}\), which enhance the production of mtROS \(^{139}\). Therefore, to better understand the genetic regulatory mechanisms and the metabolic heterogeneities, a network involves the individual metabolic pathways as well as the more direct regulatory links is in demand.

Here, we investigate the network coupling the AMPK:HIF-1:ROS regulatory circuit, glucose oxidation, fatty acid oxidation and glycolysis pathways, and the metabolites (Fig.3.1). We keep all the regulatory links which do not involve the mitochondrial activities in the minimalist model. Particularly, AMPK and HIF-1 are mutually inhibited \(^{92}\). In addition, AMPK inhibits while HIF-1 promotes noxROS generation \(^{108}\). Yet, comparing with the previous model, we investigate the more direct regulations from the three different metabolic pathways to AMPK, HIF-1 and mtROS. Particularly, the HIF-1 self-activation, AMPK self-inhibition and the activation from AMPK to mtROS are substituted with the regulations from glycolysis to HIF-1, ATP to AMPK and production of mtROS in the OXPHOS processes. Besides, we include glucose and acetyl-CoA here, because both the glucose oxidation and glycolysis take the pyruvate produced from glucose as the metabolic resource. Acetyl-CoA, as the only fuel entering into the TCA cycle, is the important common intermediate product of both glucose oxidation and fatty acid oxidation \(^{140}\). All of the three metabolic pathways
produce ATP. And mtROS is produced in both glucose oxidation and fatty acid oxidation since they both involve ETC processes. In return, ATP regulates the circuit by preventing AMPK from over activated. ROS, including both mtROS and noxROS, activate both HIF-1 and AMPK.

**Figure 3.1 – The interplay of the genetic regulatory circuit and the glycolysis, glucose oxidation, fatty acid oxidation pathways.**

G1 represents the glucose oxidation, G2 represents the glycolysis, and F represents the fatty acid oxidation. G and C represent metabolic resource glucose and the intermediate product acetyl-CoA. Both the glucose oxidation and glycolysis take the pyruvate produced from glucose as the metabolic resource. And acetyl-CoA, as the only fuel entering into the TCA cycle, is the common intermediate product of both glucose oxidation and fatty acid oxidation. All of the three metabolic pathways produce ATP, and there are ROS generations during glucose oxidation and fatty acid oxidation processes. In return, ATP regulates the circuit by inhibiting AMPK. ROS (mtROS and noxROS), activate both HIF-1 and AMPK.
3.2. Methods

Mathematically, we use the shifted Hill functions to model the regulatory links between the different nodes (proteins, metabolites and metabolic pathways) in the network.

For the genetic regulatory part, we inherit the methods used in the previous chapter and have the rate equations as below:

\[
\dot{R}_{mt} = g_{r_{mt}} H^s(A, A^0_{ar}, \lambda_{ar}, n_{ar}) \cdot (\gamma_{g1} G_1 + \gamma_f F) - k_{r_{mt}} R_{mt}
\]

\[
\dot{R}_{nox} = g_{r_{nox}} C_{R_{nox}}^{comp}(g_0, h, h_{h_{nox}}^0, n_{h_{nox}}, g_1, A, A_0, g_2, n_{a_{nox}}) - k_{r_{nox}} R_{nox}
\]

\[
R = R_{mt} + R_{nox}
\]

\[
\dot{A} = g_a H^{s+}(R, R^0_{ra}, \lambda_{ra}, n_{ra}) H^s(h, h_{ha}^0, \lambda_{ha}, n_{ha}) H^s-(ATP, ATP^0_{aa}, \lambda_{aa}, n_{aa}) - k_a A
\]

\[
\dot{h} = g_h H^{s-}(A, A^0_{ah}, \lambda_{ah}, n_{ah}) - k_h \cdot h \cdot H^s-(G_2, G^0_{gzh}, \lambda_{gzh}, n_{gzh}) H^s-(R, R^0_{rh}, \lambda_{rh}, n_{rh})
\]

**Equation 3.1 – The rate equations modeling the genetic regulatory circuit.**

Eq.3.1 is different from Eq.2.1 in that the mtROS production is directly decided by the two OXPHOS pathways, i.e., the glucose oxidation and fatty acid oxidation. And the activation of AMPK is negatively regulated by the level of ATP. Also, HIF-1 is directly stabilized by glycolysis. In Eq. 3.1, the \( \gamma_{g1} \) and \( \gamma_f \) are the parameters represent the level of mtROS production by glucose oxidation and fatty acid oxidation respectively. The two parameters have the fixed ratio 2/9 because the ratio of acetyl-CoA entering into
ETC in the two processes is fixed at this ratio. Therefore, we can use one parameter $\gamma$ to represent the mtROS production in OXPHOS.

Since the chemical reactions in the metabolism processes are much faster than the genetic regulations, we assume the metabolites (glucose, acetyl-CoA) and the pathways are in the equilibrium states at the certain level of AMPK and HIF-1. Therefore, we use the following deterministic equations to represent the level of each of them.

First, the threshold of glucose (represented by $G_0$) and the threshold of acetyl-CoA (represented by $C_0$) are determined by the level of HIF-1 and AMPK. Particularly, both HIF-1 and AMPK can enhance the glucose uptake, and AMPK can activate mitochondrial respiration, which increase the production of acetyl-CoA.

$$G_0 = g_{h,g} H_s^+(h, h^0_{hg}, \lambda_{hg}, n_{hg}) + g_{a,g} H_s^+(A, A^0_{ag}, \lambda_{ag}, n_{ag})$$

$$C_0 = c_{ac} H_s^+(A, A^0_{ac}, \lambda_{ac}, n_{ac})$$

**Equation 3.2 – The threshold of glucose and Acetyl-CoA under the influences of AMPK and HIF-1.**

Where $h^0_{hg}$, $A^0_{ag}$ are the threshold of HIF-1 and AMPK in activating glucose uptake threshold, respectively. And $\lambda_{hg}$, $\lambda_{ag}$ are the corresponding maximum glucose threshold foldchanges, $n_{hg}$ and $n_{ag}$ are the corresponding Hill coefficients. Similarly, $A^0_{ac}$ is the threshold of AMPK in activating acetyl-CoA threshold. $\lambda_{ac}$ and $n_{ac}$ are the maximum foldchange of acetyl-CoA threshold and Hill coefficient respectively.
The glucose utilized in the glucose oxidation pathway is under the following regulations:

\[ G_1 = g_1 H^{s-}(G, G_{gg1}^0, \lambda_{gg1}, n_{gg1}) H^{s-}(C, C_{cg1}^0, \lambda_{cg1}, n_{cg1}) \]

\[ G_2 = g_2 H^{s+}(h, h_{hg2}^0, \lambda_{hg2}, n_{hg2}) H^{s-}(G, G_{gg2}^0, \lambda_{gg2}, n_{gg2}) \]

\[ F = f_0 H^{s-}(C, C_{cf}^0, \lambda_{cf}, n_{cf}) H^{s+}(A, A_{af}^0, \lambda_{af}, n_{af}) \]

*Equation 3.3 – The glucose and acetyl-CoA utilized in the glucose oxidation, glycolysis and fatty acid oxidation.*

Here, \( G_1 \), \( G_2 \), \( F \) represent the glucose oxidation, glycolysis and fatty acid oxidation, respectively. The negative regulations from the total number of glucose (represented by \( G \) ) and total acetyl-CoA (represented by \( C \) ) to the corresponding metabolic pathways are used because the number of glucose or acetyl-CoA shared by these metabolisms cannot be over promoted. The activate effects come from the fact that the threshold of the total numbers of glucose and acetyl-CoA are promoted by HIF-1 and AMPK respectively, which is resulted from the two genes’ regulations to mitochondria. Besides, glycolysis is activated by HIF-1 directly and fatty acid oxidation is activated by AMPK directly.

Since the total number of glucose is only shared by glycolysis and glucose oxidation, and the total acetyl-CoA is shared by glucose oxidation and fatty acid oxidation, there are the following restrictions:

\[ G = G_1 + G_2 \]

\[ C = 2 * G_1 + 9 * F \]
Equation 3.4 – The total glucose and acetyl-CoA.

Here, 2 Acetyl-CoA is produced in 1 glucose oxidation process, and 9 acetyl-CoA is produced in one fatty acid oxidation process, in which we assumed the average Carbon atom contained in each fatty acid is 18. Therefore, the ratio of mtROS produced in these two OXPHOS pathways is also the same as the ratio of acetyl-CoA produced (2:9), because mtROS is generated due to electron leakages during ETC. The corresponding ATP production is:

\[ ATP = 29 \times G_1 + 2 \times G_2 + 106 \times F \]

Equation 3.5 – The total ATP produced by the three different metabolisms.

By solving the deterministic/rate equations, we can get details of the dynamics of the network. Importantly, we can quantitatively get the steady states, stability information and time dynamics of the metabolic network.

3.3. Results

3.3.1. The Network Allows the Hybrid Metabolic Phenotype

We performed the stability analysis of the network with two sets of parameters that correspond to normal cells and cancer cells respectively. In our model, the cancer cells differ from normal cells in two aspects: first, cancer cells have higher mtROS production due to mitochondrial potential change and functional switch; second, cancer cells have more stable HIF-1 because less oxygen is available to each cell due to abnormally rapid proliferation. Therefore, compared to normal cells, in the modeling, cancer cells produce more mtROS in the OXPHOS processes (represented by \( \gamma \) as mentioned above), and have lower HIF-1 degradation rate (denoted by \( k_h \)).

Our simulations show that normal cells have two stable steady states, which correspond to the high HIF-1 and low AMPK state, and the low HIF-1 and high AMPK
state (Fig.3.2A). As it shows in Fig.3.3, cells in the high HIF-1 and low AMPK state mostly utilize glycolysis to produce energy, while cells in the low HIF-1 and high AMPK state mostly rely on the OXPHOS. Therefore, we associated the two states to the Warburg effect (‘W’ state in Fig.3.2A) and oxidative respiration (‘O’ state in Fig.3.2A), respectively. This result is consistent with the fact that, while cells usually use glucose oxidation to produce energy, they switch to glycolysis during some specific conditions, such as anaerobic exercises. Also, we performed analysis on the cancer cells, which have larger $\gamma$ and lower $k_h$, as we discussed above. Interestingly, we found that cancer cells have a new hybrid state with both high AMPK and high HIF-1 (‘W/O’ hybrid state in Fig.3.2B) in addition to the ‘W’ and ‘O’ states. In the hybrid state, the levels of AMPK and HIF-1 are both high because the cancer cells have high level of ROS that both stabilizes HIF-1 and activates AMPK. Cancer cells in hybrid ‘W/O’ state have both high glycolysis and OXPHOS (Fig.3.3B), which enables higher cellular metabolic flexibility, thus promoting cancer cell proliferations. Moreover, as we will show it later, the hybrid state is associated with higher metabolic heterogeneity, which benefits cancer progression and metastasis.
Figure 3.2 – The nullclines of the normal cells and cancer cells.

The nullclines and steady states in the phase space of AMPK and HIF-1. The red line shows the nullcline of $dh/dt = 0$, and the blue line shows the nullcline of $dA/dt = 0$ (see Eq.3.1). The green solid dots denote stable fixed points (steady states) and the green empty circles denote unstable fixed points. Each stable steady state is associated with a cell metabolic phenotype. For normal cells (panel A), the circuit allows a Warburg state, denoted by ‘W’, and an oxidative state, denoted by ‘O’. For cancer cells (panel B, higher mtROS production and lower HIF-1 degradation), the circuit allows an additional hybrid state, denoted as ‘W/O’.

Figure 3.3 – Association of the steady states to the metabolic pathways.

ATP produced by the different metabolic pathways in the ‘W’, ‘W/O’ and ‘O’ states. The blue, green and red bars represent the ATP produced by glucose oxidation, glycolysis and fatty acid oxidation, respectively. Panel (A) shows the ATP productions of the normal cells (Fig.3.2A). In the ‘W’ state, the ATP is mainly produced by glycolysis, while in the ‘O’ state, the ATP is mainly produced by OXPHOS. Similarly, panel (B) shows the ATP productions of the cancer cells (Fig.3.2B). In the ‘W’ state, the ATP is mainly produced by glycolysis, while in the ‘O’ state, the ATP is mainly produced by OXPHOS. In the hybrid ‘W/O’ state, the cells have high ATP productions from both glycolysis and OXPHOS.

We further performed the parameter sensitivity analysis by randomizing the parameters used in our model. We randomized all parameters (except for $\gamma$ and $k_h$ (see Methods)) by 25%, from which we obtained 15000 set of parameters for both normal cell and cancer cell. While there is only a small probability (1.05%) to observe the hybrid state in normal cells (Fig.3.4A), there is a larger probability (4.38%) to observe the hybrid state in cancer cells (Fig.3.4B). The randomization result shows that our model is
efficient to capture the core features of the physiology of cancer metabolism and is robust to distinguish the normal cells and cancer cells.

**Figure 3.4 – The parameter sensitivity analysis.**

The distribution of the stable steady states for the normal cell (panel A) and cancer cell (panel B). The color bar on the right side represents the gene expression level in the logarithm (log2) scale. Among all the models, the probabilities of seeing the ‘W’ state, the ‘W/O’ state and the ‘O’ state for normal cells are 35.95%, 1.05% and 50.08%, respectively (panel A). The probabilities of seeing the ‘W’ state, the ‘W/O’ state and the ‘O’ state for cancer cells are 30.14%, 4.38% and 59.19%, respectively (panel B). There is also a low glycolysis and low OXPHOS state (with 0.23% probability) for normal cells (panel A).

### 3.3.2. The Roles of mtROS Production and HIF-1 Stability in Cancer Metabolism

We further examined the response of the metabolism network to changes in either $\gamma$ (Fig.3.5A) or $k_h$ (Fig.3.5B) by one-parameter bifurcation diagrams. In Fig.3.5A, we show that, when the rest parameters remain unchanged, the increase of $\gamma$ shifts the cells from monostability (with one stable steady state) to bistability (with two stable steady states), then to tristability (with three stable steady states) and gives rise to the hybrid metabolic state, ‘W/O’, in addition to ‘W’ and ‘O’ phenotypes. This is because the higher mtROS generation by mitochondrial OXPHOS enables both AMPK activation and HIF-1 stabilization. In Fig.3.5B, we show that, when the rest parameters remain...
fixed, the decrease of $k_h$ shifts the cells from the monostable phase (‘O’), to the bistable phase (‘O’, ‘W’), then to the tristable phase (‘O’, ‘W/O’, ‘W’), to the bistable phase (‘O’, ‘W’) again, and finally to the monostable phase (‘W’). The reason for these changes is the feedback regulation between HIF-1 and AMPK. At a high rate of HIF-1 degradation (high $k_h$), the level of HIF-1 is low, therefore the glycolysis process is inhibited. As a result, AMPK level is high and the OXPHOS becomes the dominant metabolic mode. While at a low rate of $k_h$, HIF-1 accumulates, and glycolysis becomes the dominant metabolic mode. Only when $k_h$ is in a moderate range, HIF-1 and AMPK can be both at high levels, which corresponds to the hybrid ‘W/O’ state. Therefore, the bifurcation analysis indicates that the transitions from normal cell to cancer cell are highly associated with the mtROS production and HIF-1 stability.

**Figure 3.5** – The bifurcation diagrams of the network with respect to the mtROS production by OXPHOS ($\gamma$) and the HIF-1 degradation rate ($k_h$).

(A) One-parameter bifurcation diagram for the circuit driven by the parameter $\gamma$, when $k_h$ is fixed at 2.5 hour$^{-1}$. (B) One-parameter bifurcation diagram for the circuit with respect to the parameter $k_h$, when $\gamma$ is fixed at 50. In both panel A and panel B, the blue, green, and magenta solid lines represent the phenotype ‘O’, ‘W/O’, and ‘W’, respectively. The red dashed line represents the unstable states. Different background colors represent regions for different phases. The black dashed vertical lines highlight the normal cells (left line in panel A, right line in panel B) and the cancer cells (right line in panel A, left line in panel B).
3.3.3. Cancer Cells Have Higher Plasticity to Utilize Different Metabolisms

Metabolic heterogeneity has significant impact on tumor growth, invasion, and treatment outcomes\textsuperscript{142}. Therefore, we further explore the metabolic heterogeneity from the dynamics of our current network. First, we randomly chose 1500 states from both the cancer cells and normal cells (as described above), and observe the contributions of ATP from the three different metabolic pathways (Fig.3.6). We show that, both normal cells and cancer cells have the high glycolysis and low OXPHOS state as well as the high OXPHOS and low glycolysis state. This is because the glycolysis and OXPHOS are regulated by HIF-1 and AMPK respectively. Moreover, we show that their OXPHOS state is heterogeneous because the cells have the flexibility to utilize either glucose oxidation or fatty acid oxidation depends on the metabolic resources and cellular activity demands. Note that comparing to the normal cells, cancer cells have an obvious larger probability to stay in the state that involving high glycolysis and high OXPHOS, which has more flexibility to utilize glycolysis, glucose oxidation and fatty acid oxidation (Fig.3.6B). The results, once again, indicates the significance of the hybrid state to cancer cells.
Figure 3.6 – Metabolic heterogeneity of normal cells and cancer cells.

The distribution of ATP produced by the different metabolic pathways for normal cells and cancer cells. Panel (A) and (B) show the ATP distributions of the normal cells (1500 randomly selected cells represented in Fig.3.4A) and cancer cells (1500 randomly selected cells represented in Fig.3.4B), respectively.

3.3.4. The Hybrid State Promotes Metabolic Heterogeneity

We further analyzed the distributions of the three different metabolic pathways in corresponding to the ‘W’, ‘O’ and the hybrid ‘W/O’ states (Fig.3.7). We show that, the cells in the ‘W’ state can only use anaerobic glycolysis as the energy resource (Fig.3.7A). In contrast, the cells in the ‘O’ state can only use OXPHO. But they are flexible to choose either glucose oxidation or fatty acid oxidation. Interestingly, the cells corresponding to the ‘W/O’ state have much scattered distribution in the metabolic spaces (Fig.3.7C), which indicates higher metabolic heterogeneity. This means these cells have more flexibility to utilize the combinations of the three different metabolic pathways. The metabolic plasticity of the cells in the hybrid state allows cells to better adapt to the microenvironment and utilize the available resources, which benefits the survivor of the cancer cells. The result is also in consistent with the experimental results that showing cancer cells, especially the metastasis cells, needs the OXPHOS besides the glycolysis.
Figure 3.7 – Metabolic heterogeneity of the cancer cells in the ‘W’, ‘W/O’, and ‘O’ state.

The distribution of ATP produced by the different metabolic pathways in the ‘W’, ‘W/O’ and ‘O’ states. Panel (A) (B) and (C) show the ATP distributions of the cells in the ‘W’ state (blue circles), ‘W/O’ state (green circles) and ‘O’ state (red circles) (the cells corresponding to the left cluster, middle cluster, and right bottom cluster in Fig.3.4B), respectively. Panel (D) shows the overlap of the previous three panels. Cells in the ‘W’ state (A) can only rely on the glycolysis, while the cells in the ‘O’ state (B) can utilize the different combination of the glucose oxidation and fatty acid oxidation. The cells in the ‘W/O’ state, however, can utilize the various combination of the three different metabolic pathways, thus have higher metabolic plasticity.

3.4. Discussions

In this study, we established a metabolic network that features the interplay between the metabolic gene circuit and the metabolic pathways. In this model, the genetic regulatory circuit that composed of HIF-1, AMPK and ROS is directly coupled to
the glycolysis, glucose oxidation and fatty acid oxidation pathways. In addition to showing the major differences in metabolism between normal and cancer cells, it is also capable to capture the regulations among the genes, metabolites as well as the pathways. Therefore, modeling this network is especially useful for investigating the metabolic heterogeneity of cancer cells. Since the regulatory links in the network are supported by multiple experimental evidences coming from different cell lines (Table 2.1), our model is expected to be robust for studying the common behavior of cancer metabolisms and their regulations.

As the minimalist metabolic circuit showed in last chapter, this more confined model confirms that cancer cells, can hijack the normal cells’ gene regulatory circuit of metabolism for their advantage. Due to high energetic and oxidative stresses, cancer cells can be in a special hybrid metabolic phenotype with both glycolysis and OXPHOS. The cells in the ‘W/O’ hybrid state have plasticity to utilize both glycolysis as well as different kinds of oxidative respirations, thus increase the metabolic heterogeneity to support cell survival and tumor metastasis. On one hand, the Warburg Effect of cancer cells involves excess glucose consumption and acid production. On the other hand, the abnormal rapid proliferations and metastasis induce oxygen accessibility changes. Both of these could lead complex microenvironment situations. Therefore, enhanced metabolic heterogeneity can contributes the survivor of cancer cells, because it enables the cancer cells utilize the more suitable combination of metabolisms to adapt to the microenvironments and further invade the surrounding normal cells, whose metabolic phenotypes are of less plasticity.\textsuperscript{142}
In consist with the analysis in the last chapter, according to our model, the metabolic plasticity is mainly caused by the high level of mtROS in cancer cells. One might think that a putative strategy for cancer treatment is to inhibit ROS. Indeed, it has been observed that the invasiveness of certain cancer cells could be suppressed by neutralizing mitochondrial ROS. Some ROS scavengers have been tested in clinical trials but their efficacies are still unclear. It could be because that potential of several scavengers to get into mitochondria is limited. Another possible explanation is that, the ROS scavengers reduce the ROS levels of certain cancer cells and make them less subject to DNA damage and apoptosis. Meanwhile cells utilize intermediate levels of ROS to facilitate their cell signaling, which further benefits cancer cells. Therefore, one has to be cautious when using metabolic treatments that directly target the level of ROS.

Our model also explains the connection of cancer metabolism to cancer metastasis and cell stemness. As shown in Fig.1.4, both HIF-1 and AMPK are linked to the genes and/or signaling molecules that regulate EMT, suggesting that EMT decision making could be coupled to the phenotypic switches of cancer metabolism. The network suggests that, in the ‘W’ state, due to the high level of HIF-1 and low level of AMPK, SNAIL and TWIST are both activated. Therefore, the ‘W’ state might be associated with the mesenchymal state, and the ‘O’ state is associated with the epithelial state. Interestingly, the network suggests that cells in the hybrid ‘W/O’ state could also be in the hybrid E/M state, which have been shown to be invasive and metastatic. Similarly, cells of the hybrid ‘W/O’ state have an intermediate level of OCT4, which is associated with high stemness. Interestingly, normal stem cells have been found to
typically use glycolysis while differentiated cells use OXPHOS, which is consistent with our model of normal cells\textsuperscript{144}. Moreover, cancer stem cells have been found to require mitochondrial OXPHOS together with glycolysis\textsuperscript{148}, which is consistent with our model of cancer cells. Hence, cancer stem cells might benefit from acquiring metabolic plasticity.

In summary, we have developed a robust metabolic regulatory network for studying cancer metabolic plasticity and heterogeneity. Our model provides insights into the regulatory mechanism of cancer metabolism beyond the Warburg effect. The hybrid metabolic mode predicted by our model brings a new perspective on how metabolic switches promote tumorigenesis and cell invasiveness. We hope this work could be helpful to the design of improved cancer therapeutic strategies that target the different metabolic pathways.
References


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Appendix A

Parameters for modeling the AMPK:HIF-1:ROS regulatory circuit.

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**ROS**

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<td>Hill coefficient for HIF-1 activation</td>
</tr>
</tbody>
</table>

* $\gamma$ and $k_h$ are different for normal cells and cancer cells, which has been discussed in details in the main text part (see Methods). The values in the table are corresponding to the cancer cells. For normal cells, the values of these two parameters are 3, 0.3/min respectively.
## Appendix B

Parameters for modeling the therapeutic treatments.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
<th>Unit</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hyperbaric Oxygen</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_2$</td>
<td>0.2</td>
<td>-</td>
<td>HIF-1 foldchange due to ROS</td>
</tr>
<tr>
<td>$k_3$</td>
<td>1.8</td>
<td>-</td>
<td>HIF-1 foldchange due to oxygen</td>
</tr>
<tr>
<td>$n_{o,h}$</td>
<td>2</td>
<td>-</td>
<td>Hill coefficient for HIF-1 inhibition</td>
</tr>
<tr>
<td>$O_{h}^0$</td>
<td>5</td>
<td>%</td>
<td>Threshold for AMPK activation</td>
</tr>
<tr>
<td><strong>Metformin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\lambda_{ma}$</td>
<td>2</td>
<td>-</td>
<td>Foldchange for AMPK activation</td>
</tr>
<tr>
<td>$\lambda_{mh}$</td>
<td>0.5</td>
<td>-</td>
<td>Foldchange for HIF-1 inhibition</td>
</tr>
<tr>
<td>$n_{ma}$</td>
<td>2</td>
<td>-</td>
<td>Hill coefficient for AMPK activation</td>
</tr>
<tr>
<td>$n_{mh}$</td>
<td>2</td>
<td>-</td>
<td>Hill coefficient for HIF-1 inhibition</td>
</tr>
<tr>
<td>$M_{ma}^0$</td>
<td>300</td>
<td>μM</td>
<td>Threshold for AMPK activation</td>
</tr>
<tr>
<td>$M_{mh}^0$</td>
<td>300</td>
<td>μM</td>
<td>Threshold for HIF-1 inhibition</td>
</tr>
<tr>
<td>$\alpha_{met}$</td>
<td>0.025</td>
<td>/μM</td>
<td>Influence on mtROS maximum foldchange</td>
</tr>
<tr>
<td><strong>3BP</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>$\alpha_{3BP}$</td>
<td>0.0005</td>
<td>/μM</td>
<td>Influence on HIF-1 foldchange due to self-activation</td>
</tr>
</tbody>
</table>