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Anne Le *Editor*

The Heterogeneity of Cancer Metabolism

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The Heterogeneity of Cancer Metabolism

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This book is dedicated to my colleague and mentor, Dr. Edward Gabrielson.

Anne Le

Preface

Genetic alterations in cancer, in addition to being the fundamental drivers of tumorigenesis, can give rise to a variety of metabolic adaptations that allow cancer cells to survive and proliferate in diverse tumor microenvironments. This metabolic flexibility is different from normal cellular metabolic processes and leads to heterogeneity in cancer metabolism within the same cancer type or even within the same tumor.

In this book, the authors delve into the complexity and diversity of cancer metabolism and highlight how understanding the heterogeneity of cancer metabolism is fundamental to the development of effective metabolism-based therapeutic strategies. Deciphering how cancer cells utilize various nutrient resources will enable clinicians and researchers to pair specific chemotherapeutic agents with patients who are most likely to respond with positive outcomes, allowing for more cost-effective and personalized cancer treatment.

This book has three major parts:

Part I: Basic Metabolism of Cancer Cells
Part II: Heterogeneity of Cancer Metabolism
Part III: Relationship between Cancer Cells and Cancer-Associated Fibroblasts

This book is designed for cancer metabolism researchers, cancer biologists, and any other researchers, physicians, epidemiologists, health care professionals of various disciplines, policy makers, and marketing and economic strategists… It is also designed for teaching undergraduate and graduate students and researchers.

The metabolic pathways and their regulations mentioned in this book serve as examples to illustrate the heterogeneity of cancer metabolism and are noninclusive.

Dr. Anne Le is the corresponding author for all the chapters in this book. Email: annele@jhmi.edu.

Baltimore, MD, USA — Anne Le

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About the Editor

Anne Le studied at the Paris Descartes University, Cochin Port-Royal School of Medicine, in France where she obtained a Habilitation degree (https://en.wikipedia.org/wiki/Habilitation), the highest academic qualification a scholar can achieve in Europe. After her clinical training at Henri Poincaré University Hospital, Nancy, in France, she started her postdoctoral research fellowship at the Johns Hopkins University School of Medicine in 2007. Since 2011, Dr. Le has been an independent investigator who has yielded a number of contributions to the field of cancer metabolism, demonstrated by her publication record as a pioneer in the field. She has published in the best journals, such as *Cell Metabolism* and the *Proceedings of the National Academy of Sciences of the United States of America*. Dr. Le has been invited to present her work at several annual American Association for Cancer Research meetings, the most prestigious international meeting for cancer research scientists and professionals, as well as by the National Cancer Institute, and universities in France, Monaco, Japan, and Taiwan. Research media, such as Science Daily, American Association for the Advancement of Science (AAAS), Business Insider, ALN® Magazine, among many others, have written about her work. Dr. Le is highly respected and sought after for her strong proficiency in judging the work and ideas of her peers. She is regularly invited to serve on review panels by prestigious organizations such as the National Institutes of Health and the US Department of Defense. She is frequently asked by highly-cited scientific journals to review manuscripts submitted to their journals.

Part I
Basic Metabolism of Cancer Cells

Glucose Metabolism in Cancer

Sminu Bose and Anne Le

Key Points

- Tumor cells exhibit an upregulation in glycolysis, glycogen metabolism, and gluconeogenesis as opposed to normal cells.
- The metabolic reprogramming underlying the Warburg effect and other changes in glucose metabolism are driven by several oncogenes and tumor suppressors.
- Numerous therapies based on cancer metabolism have been developed but have yet to show success in clinical trials.

Keywords Glucose metabolism · Warburg effect · Glycogenolysis · Gluconeogenesis · Cancer metabolism

Abbreviations

3PO	3-(3-Pyridinyl)-1-(4-pyridinyl)-2-propen-1-one
AGL	Amylo-alpha-1, 6-glucosidase, 4-alpha-glucanotransferase
AKT	Also known as PKB, protein kinase B
ATP	Adenosine triphosphate
CP-320626	5-Chloro-N-[(2S)-3-(4-fluorophenyl)-1-(4-hydroxypiperidin-1-yl)-1-oxopropan-2-yl]-1H-indole-2-carboxamide
F1,6-BP	Fructose-1,6-bisphosphatase
F2,6-BP	Fructose-2,6-bisphosphate
FX-11	3-Dihydroxy-6-methyl-7-phenylmethyl-4-propylnaphthalene-1-carboxylic acid

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G1P	Glucose-1-phosphate
G6P	Glucose-6-phosphate
GBE	1,4-Alpha-glucan branching enzyme
GLUT	Glucose transporter
GSK2	Glycogen synthase kinase 2
GYS1	Glycogen synthase 1
HIF-1α	Hypoxia-inducible factor 1α
HK2	Hexokinase 2
LDHA	Lactate dehydrogenase A
mTOR	Mechanistic target of rapamycin
NAD	Nicotinamide adenine dinucleotide
PCK2	Phosphoenolpyruvate carboxykinase 2
PCK1	Phosphoenolpyruvate carboxykinase 1
PFK	Phosphofructokinase
PFKFB3	6-Phosphofructo-2-kinase/fructose-2,6-biphosphatase 3
PGM	Phosphoglucomutase
PI3K	Phosphoinositide 3-kinase
PPP	Pentose phosphate pathway
PPP1R3C	Protein phosphatase 1 regulatory subunit 3C
TCA	Tricarboxylic acid
TIGAR	TP53-induced glycolysis and apoptosis regulator
TP53	Tumor protein 53
UGP2	UTP:glucose-1-P uridylyltransferase 2
VHL	Von Hippel-Lindau

Introduction

Otto Warburg observed a peculiar phenomenon in 1924, unknowingly laying the foundation for the field of cancer metabolism. While his contemporaries hypothesized that tumor cells derived the energy required for uncontrolled replication from proteolysis and lipolysis, Warburg instead found them to rapidly consume glucose, converting it to lactate [1]. The significance of this finding, later termed the Warburg effect, went unnoticed by the larger scientific community at that time. The field of cancer metabolism lay dormant for almost a century awaiting advances in molecular biology and genetics which would later open the doors to new cancer therapies.

1 The Warburg Effect

1.1 Otto Warburg's Early Studies of Normal Cellular Respiration

Warburg began his forays into research studying the oxygen consumption of sea urchin eggs, finding that the rate of respiration increased several fold after fertilization. He went on to further describe two processes that were crucial to cellular glucose metabolism: respiration and fermentation [2].

Most differentiated cells metabolize glucose through the TCA cycle under aerobic conditions. They then undergo oxidative phosphorylation to generate ATP (between 32 and 34 ATP molecules per glucose molecule) [3] (Fig. 1). While glycolysis produces only two net molecules of ATP per one molecule of glucose, the majority of ATP production occurs during the TCA cycle and oxidative phosphorylation. During these latter steps of respiration, the pyruvate molecule produced in glycolysis undergoes a series of reactions in the presence of oxygen. Without the presence of oxygen, cells undergo fermentation or anaerobic glycolysis, shunting the resultant pyruvate molecules to lactate production.

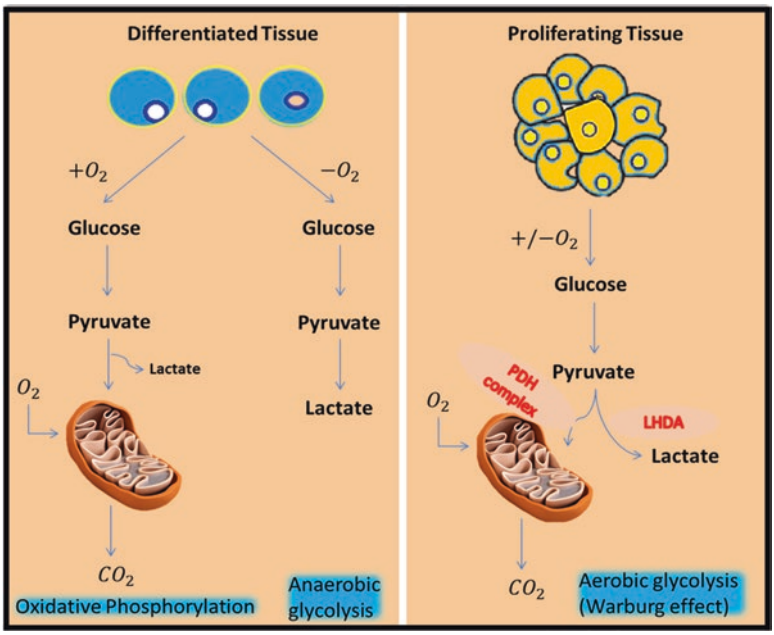

Fig. 1 The Warburg effect depicted in proliferating tissue (right) in contrast with normal respiration in normal differentiated tissue (left)

1.2 The Warburg Effect Is a Prominent Feature of Cancer Cell Metabolism

In 1927, Warburg studied the processes of respiration and fermentation in tumor cells. According to normal cellular respiration, glucose is converted to pyruvate and then enters the TCA cycle to undergo oxidative phosphorylation in the presence of oxygen. There should be minimal lactate production. However, in his in vivo and ex vivo studies, Warburg observed an increased glucose uptake and increased lactic acid production in tumor cells as compared to normal cells, even in the presence of oxygen [4]. This phenomenon, the metabolism of glucose to lactate despite the presence of adequate oxygen, is called the Warburg effect.

For Warburg, several questions remained unanswered, including why cancer cells would inefficiently shunt glucose to lactate production instead of to the TCA cycle, which would result in significantly higher ATP production. Warburg hypothesized that the lactate production in cancer cells was due to impairment of oxidative phosphorylation caused by mitochondrial damage [5].

There was debate surrounding this theory with disagreement arising particularly from Sidney Weinhouse, one of Warburg's contemporaries. Using isotope tracing, Weinhouse's experiments showed that the rates of oxidative phosphorylation in both normal cells and tumor cells are similar, suggesting that the mitochondria of tumor cells are intact [6]. Rather, tumor cells in oxygen-rich environments utilize both aerobic glycolysis and oxidative phosphorylation to sustain their rapid rates of proliferation. Only in hypoxic environments, such as the tumor core, do the rates of lactic acid production by anaerobic glycolysis overtake oxidative phosphorylation as the primary source of energy [7].

1.3 Metabolic and Genetic Reprogramming Underlying the Warburg Effect

With current advances in genetics and molecular biology, much of the past several decades of cancer research have been consumed by characterizing the genetic alterations which lead to the development of cancers. Warburg's question regarding the cause of upregulation of aerobic glycolysis in cancer remained unanswered. However, cancer cells need not only a genetic switch but also metabolic building blocks and an energy source to undergo rapid proliferation. The recognition of the importance of this energy source allowed for the resurgence of cancer metabolism as a field that is closely related to tumor genetics. It is now understood that the metabolic reprogramming underlying the Warburg effect is driven by several oncogenes and tumor suppressors.

Some of the identified oncogenes, namely, Akt, PI3K, Ras, and VHL, act via the protein hypoxia-inducible factor 1α (HIF-1α) [8], resulting in the non-hypoxic expression of HIF-1α. In normal cells, HIF-1α becomes stabilized in a

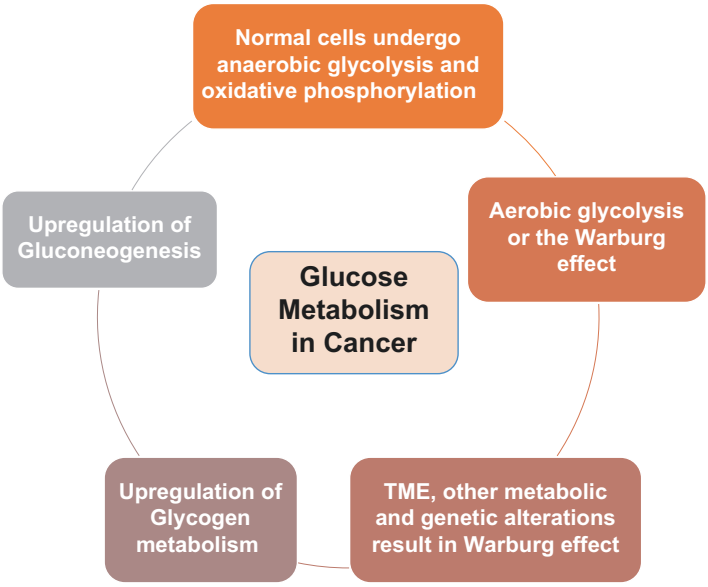

Fig. 2 Glucose metabolism in cancer

hypoxic environment to form a transcription factor involved in promoting glycolysis and suppressing oxidative phosphorylation [9]. HIF-1α, when present, upregulates the GLUT1 transporter to promote the retention of glucose inside cells in addition to upregulating hexokinase 2 (HK2), the enzyme which catalyzes the first committed step of glycolysis [10]. Typically, when oxygen is present, HIF-1α degrades in a concentration-dependent manner. In tumor cells, even in the presence of oxygen, high AKT and mTOR oncogenic activity promotes HIF-1α expression, leading to persistent transcription of the enzymes driving glycolysis and lactate production.

Oncogenic pathways have been found to work independently of HIF-1α to promote aerobic glycolysis as well, namely, the activation of oncogenes such as Myc, Ras, and Akt and the deactivation of tumor suppressors such as TP53 [8]. Like HIF-1α, Myc directly upregulates GLUT and HK2. The loss of TP53 function also upregulates GLUT expression. Additionally, TP53 deactivation indirectly leads to increased glycolysis. Without TP53 expression, TIGAR, a protein which causes shunting of glucose to the pentose phosphate pathway (PPP), is no longer upregulated, resulting in a greater flux of glucose through the glycolytic pathway [11].

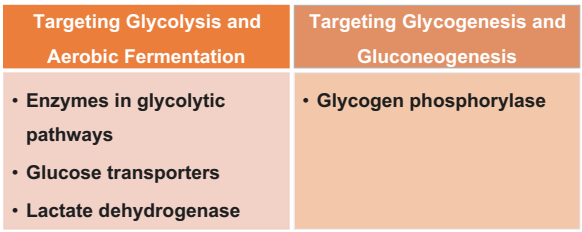

Fig. 3 Potentially viable targets within glucose metabolism for cancer therapy

2 The Role of Glycogen Metabolism and Gluconeogenesis in Tumor Growth

2.1 Glycogen Metabolism Is Upregulated in Several Cancers

Glycolysis is not the only component of glucose metabolism which plays a significant role in tumor growth. Glycogenolysis, the process by which glycogen is converted to glucose-1-phosphate (G1P) and then to glucose-6-phosphate (G6P) to enter the glycolytic pathway, provides another energy source for tumors in the face of nutrient stress (Fig. 2). Glycogen metabolism, although studied far less than glycolysis by cancer researchers, is upregulated in many cancer types including renal, breast, bladder, uterine, ovarian, skin, and brain cancers. The glycogen content of cancer cells has been demonstrated to be inversely proportional to the rate of replication [12]. Renal cell carcinoma which classically has clear cells on histology appears this way due to high glycogen content.

Advances in tumor genetics have again allowed for the characterization of tumor suppressor genes and oncogenes which have driven these changes in glycogen metabolism in tumor cells. The overexpression of the oncogene Rab25 has been demonstrated as a driver in increasing cellular glycogen stores via the AKT pathway [13]. In bladder cancer, the glycogen debranching enzyme AGL has been identified as a tumor suppressor. Additionally, deactivation of AGL leads to the accumulation of abnormal glycogen stores and promotes tumorigenesis in xenograft models [14].

Given this, Guo-Min Shen and colleagues studied glycogen metabolism in the setting of hypoxia in 2010. It was noted that glycogen accumulated in breast cancer cells after 24 and 48 hours of hypoxia due to HIF-1α induction of PPP1R3C, a glycogen synthase [15]. Later studies demonstrated that glycogen synthesis promotes cancer cell survival in the setting of hypoxic conditions [16]. Both glycogenolysis and glycogen synthesis enzymes appear to be upregulated by tumor cells with HIF-1α dependence including UGP2, PGM, GBE, GYS1, and PPP1R3C [17]. In vivo studies of suppression of glycogen synthase kinase 2 (GSK2) activity demonstrated a reduction in prostate tumor growth [18]. Glycogen metabolism is an important target of therapy given that cancer cells can utilize glycogen as an energy source even during nutrient deficiency due to poor angiogenesis [19].

2.2 Upregulation of Gluconeogenic Enzymes in Cancer

Gluconeogenesis is the process of generating glucose from carbon substrates which are not carbohydrates. There are two gluconeogenic enzymes which play important roles in cancer metabolism: phosphoenolpyruvate carboxykinase 1 (PCK1) and phosphoenolpyruvate carboxykinase 2 (PCK2). It has been demonstrated that TP53 inhibits both enzymes, meaning that the loss of TP53 upregulates these enzymes and gluconeogenesis [20]. It was also observed that the inhibition of mTOR in hepatocellular carcinoma and renal cell carcinoma cells directs the glycolytic flux towards lactate and gluconeogenesis with resultant tumor cell death via the downregulation of PCK1 [21].

3 Targeting Glucose Metabolism for Cancer Therapy (Fig. 3)

3.1 Therapies Targeting Glycolysis and the Warburg Effect

As discussed previously, over the latter half of the twentieth century, advances in molecular biology and the identification of oncogenes and tumor suppressors drew the attention of much of the anti cancer therapeutic efforts. It is true that genetic alterations drive uncontrolled replication in cancer cells, but it is important to recognize that a cancer cell is still dependent on nutrient availability. In the past two decades, there has been an upsurge in efforts to exploit the addiction of cancer cells to glucose and the Warburg effect [8]. Several enzymes in the glycolytic pathway have been targeted, some showing tumoricidal effects in vitro and in vivo. Unfortunately, there has been little clinical success given that glycolysis is crucial to the glucose metabolism of normal cells. The focus is targeting those elements of aerobic glycolysis which are more upregulated in cancer.

As mentioned previously, glucose transporters (GLUT1-4) are upregulated in tumor cells by Myc and HIF-1α. Previous attempts with small molecule inhibitors of GLUT1 have seen in vitro tumoricidal effects in a renal cell carcinoma cell line [22] and hepatocellular carcinoma cell line [23]. However, GLUT1 is a prevalent glucose transporter in normal cells as well, which would likely preclude clinical success. Homozygous Glut1 deletion is embryonically lethal in mice, and heterozygous deletion has caused impaired motor activity and seizures [24]. A GLUT1 inhibitor called silibinin failed to demonstrate any reduction in prostate-specific antigen in a phase I clinical trial [25].

Hexokinase phosphorylates glucose to glucose-6-phosphate in the first committed step of glycolysis. Hexokinase 2 (HK2) is mostly expressed in cancer cells and is the primary hexokinase to function in tumors, so it is another potential therapeutic target. Experiments in which HK2 was systemically deleted have shown to be well tolerated in mice [26]. A glucose analog that competitively inhibits G6P isomerase in order to inhibit the phosphorylation of glucose, 2-deoxyglucose, had a phase I

trial in combination with radiation therapy with good toleration in glioblastoma multiforme. However, a HK inhibitor called lonidamine failed to show any benefit in two phase III randomized trials [25].

Phosphofructokinase (PFK) is the enzyme which catalyzes the second committed step in glycolysis, the conversion of fructose-6-phosphate to fructose-1,6-bisphosphate (F1,6-BP). Although inhibiting PFK directly is not possible since it is crucial to glycolysis in normal cells, it may be feasible to target it indirectly. PFK is strongly allosterically activated by fructose-2,6-bisphosphonate (F2,6-BP). F2,6-BP is activated by another protein, PFKFB3, a target of HIF-1α. Attenuation of glycolysis was achieved in in vitro and in vivo studies with a small molecule PFKFB3 inhibitor called 3PO [27]. PFKFB3 inhibitors were also shown to reduce tumor angiogenesis [28].

In seeking a target that was more unique to cancer cell metabolism and central to the Warburg effect, Le et al. focused on lactate dehydrogenase A (LDHA) which reciprocally mediates the redox-coupled conversion between lactate with NAD+ and pyruvate with NADH [29, 30]. Elevated expression levels of LDHA are a hallmark of many types of tumors, including squamous head and neck cancer, colorectal cancer, and non-small cell lung cancer [30–33]. By perturbing the NADH/NAD+ ratio, a small molecular inhibitor of LDHA called FX-11 was shown to increase reactive oxygen species in tumor cells with subsequent cell death in not only in vitro studies but also pancreatic and lymphoma xenografts [34]. Several other LDHA inhibitors, such as gossypol, galloflavin, and N-hydroxyindole-based inhibitors, were tested in preclinical settings [34–38]. Among them, gossypol (AT-101), a non selective inhibitor of LDH, was tested in a phase I clinical trial targeting metastatic colorectal cancer (NCT00540722). Despite active investigations for developing LDH inhibitors, there is still a clinical need for highly selective and efficient LDH inhibitors, as gossypol shows off-target effects such as the inhibition of NADH-dependent enzymes (GAPDH) [36]. Although compounds targeting lactate metabolism have not yet been approved, it is clear that LDH-targeting strategies are promising approaches for cancer therapy.

3.2 Therapies Targeting Glycogenolysis and Gluconeogenesis

Significantly fewer therapies have been developed targeting glycogen metabolism or gluconeogenesis. Lee et al. inhibited glycogen phosphorylase in a pancreatic cell line with a compound called CP-320626 leading to tumor cell death with no effect on normal human fibroblasts [39]. Flavopiridol, another glycogen phosphorylase inhibitor, had safe and modest efficacy in clinical trials with prostate cancer, renal cell carcinoma, and colorectal carcinoma. However, flavopiridol is also a cyclin-dependent kinase inhibitor, so it is uncertain whether the anti tumor effects were purely from glycogen phosphorylase inhibition.

Conclusion

Currently, there are several challenges to metabolic cancer therapies. First, an understanding of the heterogeneity of metabolic phenotypes is only beginning to be established. Metabolic phenotypes likely vary based on tissue of origin, tumor microenvironment, primary versus metastatic tumors, and mutational differences. Second, there are limitations in translating in vivo mouse studies to clinical trials as is evidenced by the lack of success in advancing metabolic inhibitors through clinical trials up until this point. Third, there is the potential for metabolic inhibitors to be overcome by the adaptation of tumors to new energy sources. With renewed interest in cancer metabolism, the development of metabolic inhibitors will continue to grow, and it may be most effective to combine these therapies with other modalities of therapy in order to increase efficacy.

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Glutamine Metabolism in Cancer

Ting Li and Anne Le

Key Points

- Dysregulation of the TCA cycle and glutamine addiction are characteristic features of glutamine metabolism in cancers.
- Targeting glutamine metabolism in cancer includes inhibition of glutaminolysis by glutaminase inhibitors, combination therapy, knockdown of c-*MYC*, inhibition of GDH, depletion of glutamine supply, inhibition of glutamine uptake, and usage of glutamine analogs.
- Transaminase upregulation and targeting amino acid synthesis have potential for cancer therapy.

Keywords Glutamine metabolism · Glutamine addiction · Targeting glutamine metabolism · Transaminase upregulation · Targeting amino acid synthesis

Abbreviations

α-KG	α-Ketoglutarate
2HG	2-Hydroxyglutaric acid
ASS	Argininosuccinate synthetase
ECG	Epicatechin gallate
EGCG	Epigallocatechin gallate
ETC	Electron transport chain
FDG-PET	Fluorodeoxyglucose-positron emission tomography
FH	Fumarate hydratase

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GBM	Glioblastoma multiforme
GDH	Glutamate dehydrogenase
GLS	Glutaminase
GOT	Glutamic-oxaloacetic transaminase
GPT	Glutamic-pyruvate transaminase
HIF	Hypoxia-inducible factor
IDH	Isocitrate dehydrogenase
IDO	Indoleamine-2,3-dioxygenase
PEG	Poly(ethylene glycol)
PHD	Prolyl 4-hydroxylases
PLGA	Poly(lactic-co-glycolic acid)
PSAT	Phosphoserine aminotransferase
RCC	Renal cell carcinomas
SDH	Succinate dehydrogenase
SHMT	Serine hydroxymethyltransferase
TCA	Tricarboxylic acid
TDO	Tryptophan-2,3-dioxygenase

Introduction

Metabolism is the fundamental process for all cellular functions. For decades, there has been growing evidence with regard to the relationship between metabolism and malignant cell proliferation. Unlike normal differentiated cells, however, cancer cells have reprogrammed metabolisms in order to fulfill their energy requirements. These cells display crucial modifications in many metabolic pathways, including glucose transport, glutaminolysis which includes the tricarboxylic acid (TCA) cycle, the electron transport chain (ETC), and the pentose phosphate pathway (PPP) [1]. Since the discovery of the Warburg effect, it has been shown that the metabolism of cancer cells plays a critical role in cancer survival and growth. More recent research suggests that the involvement of glutamine in cancer metabolism is more significant than previously thought. Glutamine, a non essential amino acid with an amine functional group, is the most abundant amino acid circulating in the bloodstream [2]. This chapter will discuss the characteristic features of glutamine metabolism in cancers.

1 Characteristic Features of Glutamine Metabolism in Cancer

1.1 Dysregulation of the TCA Cycle

This section will focus on abnormalities within the TCA cycle, also known as the citric acid cycle or Krebs cycle (Fig. 1), that alter cancer cell metabolism. The TCA cycle is the central metabolic hub of the cell; it acts as a common pathway for the

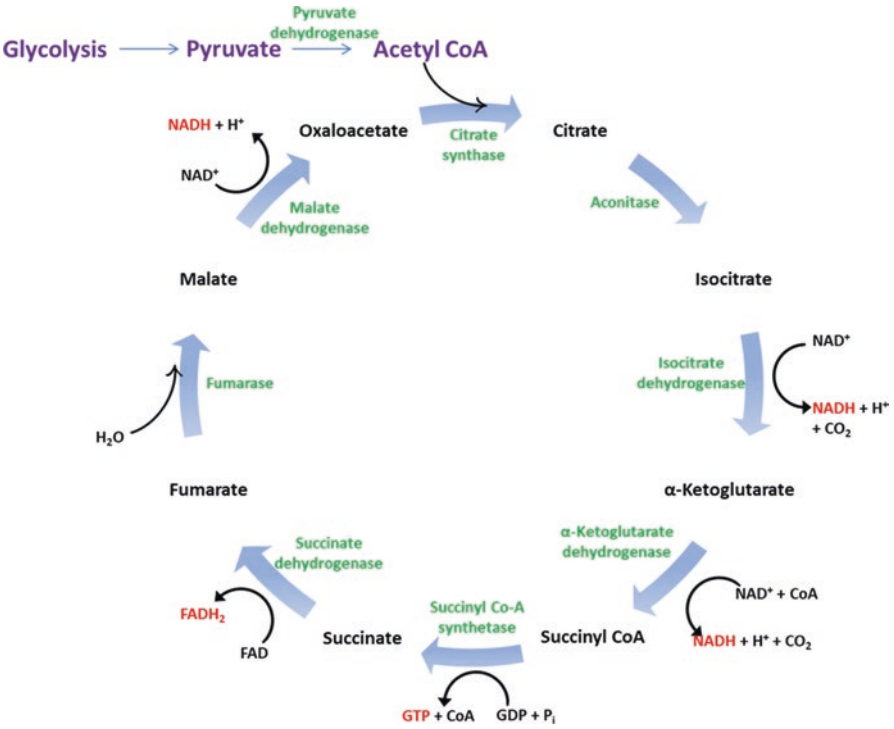

Fig. 1 Illustration of the TCA cycle with electron carriers in red, enzymes in green, and substrates in black

catabolism of many different sugars, fatty acids, and amino acids [3]. It also generates electrons that fuel oxidative phosphorylation by way of the ETC, a process that produces a majority of the energy used by normoxic cells [3].

Under aerobic conditions, pyruvate formed as a product of glycolysis goes through oxidative decarboxylation, a process that removes a carboxyl group and releases O_2 to form acetyl coenzyme A (acetyl-CoA), the typical starting molecule of the TCA cycle [4]. While glycolysis occurs in the extracellular matrix, the TCA cycle takes place within the mitochondrial matrix [5]. The steps of the TCA are as follows: (1) The condensation of oxaloacetate and acetyl-CoA to form citrate is catalyzed by citrate synthase. (2) The enzyme aconitase then converts citrate to isocitrate. (3) Isocitrate is further oxidatively decarboxylated by isocitrate dehydrogenase (IDH). (4) The resulting compound, α-ketoglutarate (α-KG), is transformed into succinyl-CoA (5) and then further converted to succinate by succinyl-CoA synthetase. (6) The succinate dehydrogenase (SDH) complex catalyzes the oxidation of succinate to fumarate. (7) Finally, fumarate is hydrated to malate by fumarate hydratase (FH), (8) and malate is then oxidized to oxaloacetate by malate dehydrogenase—initiating the cycle once again [6] (Fig. 1).

Mutations of TCA cycle genes have been linked to familial cancer types [6]. Recent research has found that mutations in the TCA cycle enzymes SDH, FH, and

IDH result in dysfunction of the TCA cycle and defects in mitochondrial metabolism in a wide range of human cancers [7, 8]. The SDH complex (also known as mitochondrial complex II) is the only membrane-bound enzyme of the TCA cycle and is constituted of four subunits: SDHA, SDHB, SDHC, and SDHD. SDHA and SDHB are catalytic subunits that protrude into the mitochondrial matrix, while SDHC and SDHD are anchored to the inner membrane [9]. The SDH enzyme plays an essential role in tumor suppression. Heterozygous mutations in SDH genes cause complete inactivation of the protein function and were found to be associated with hereditary paragangliomas and pheochromocytomas [10–12]. Tumors exhibiting SDH mutations are more aggressive and usually proliferate at a much faster rate than normal cells [9]. In addition to these cancers, a number of other neoplasms have been associated with mutations in SDH subunits, including renal cell carcinoma, neuroblastoma, gastrointestinal stromal tumors, thyroid cancer, and testicular seminoma [13–15].

Similar to SDH, FH mutations occur throughout the genome. Research has indicated an association between heterozygous FH mutations and uterine fibroids, hereditary leiomyomatosis, and papillary renal cell cancer [16]. Additionally, loss of the wild-type allele in these cancers results in the absence of FH enzymatic activity. FH acts as a tumor suppressor, and its reduced expression leads to the accumulation of the transcription factor hypoxia-inducible factor-1α (HIF-1α) [1, 17]. High levels of fumarate accumulate and act as oncometabolites, which often accumulate to result in dysregulation of cellular functions, in SDH- or FH-deficient cells [1]. Both accumulated succinate and fumarate are potent inhibitors of prolyl 4-hydroxylases (PHDs). PHDs are negative regulators of HIF-1α, a transcriptional factor that is upregulated under hypoxic conditions when tumor cells are deprived of adequate oxygen supplies. Impaired PHD activity leads to HIF-1α activation under normoxia, a condition known as pseudohypoxia [6]. Pseudohypoxia, in turn, facilitates tumor cell growth.

Similar to the metabolic consequences of SDH and FH mutations, mutations in the IDH enzyme often result in dysfunction of the TCA cycle. There are three isoforms of the enzyme IDH. IDH1 is found in the cytoplasm and peroxisomes; IDH2 and IDH3 are localized in the mitochondrial matrix. IDH3 is the primary form of IDH in the TCA cycle whose function is to convert isocitrate to α-KG. Genomic analysis has identified mutations in either IDH1 or IDH2 in the vast majority of grade II and III gliomas as well as glioblastoma multiforme (GBM) patient samples. The abnormal expression and activity of IDHs result in the loss of the enzyme's ability to catalyze isocitrate to α-KG and instead gaining a new ability to facilitate the NADPH-dependent reduction of α-KG to 2-hydroxyglutaric acid (2HG), an oncometabolite. Excess accumulation of 2HG, in turn, contributes to the formation of malignant gliomas [18]. The discovery that IDH1 and IDH2 mutations are nearly all localized to a single amino acid (codons R132 and R172, respectively) provides a promising biomarker for cancer diagnosis and possibly gene therapy [19, 20]. In addition, research has found that cells harboring IDH1-R132 and IDH2-R172 mutations in patients with acute myeloid leukemia gain the ability to convert α-ketoglutarate to 2HG [21].

1.2 Glutamine Addiction

In addition to glucose, proliferating cancer cells also rely on glutamine as a major source of energy and building blocks. They exhibit an increased dependency on other nutrients to feed the TCA cycle, which is typically glutamine. This condition is known as glutamine addiction. In fact, some tumor cells are so reliant that they have been reported to die in the absence of exogenous glutamine [22]. Glutamine is one of the most abundant nonessential amino acids (produced by the human body and thus not an essential part of the diet) in the bloodstream and contributes to virtually every biosynthetic pathway in proliferating cells. Moreover, it acts as a nitrogen donor in purine and pyrimidine synthesis as well as a precursor for protein and glutathione biosynthesis [23].

Since glutamine-derived α-KG fuels the TCA cycle, cancer cells can employ glutaminolysis to sustain the biosynthesis of many essential molecules. In renal cell carcinomas (RCC) that were either ETC or TCA cycle deficient, it was found that cancer cells relied on the reductive carboxylation of glutamine-derived citrate to produce acetyl-CoA and other precursors to TCA cycle metabolites. Acetyl-CoA is a necessary intermediate for the synthesis of lipids, and without it, cancer cells are not viable. Furthermore, TCA intermediates are needed to synthesize other essential cellular building blocks. Thus, cells can become utterly dependent upon glutaminolysis as a result of genetic alterations affecting oxidative mitochondrial function [24]. A study by Gameiro et al. found that the transcription factor HIF expression maintained a low level of intracellular citrate to maintain adequate lipogenesis. Therefore, the VHL-deficient RCC cells which constitutively express HIF-1α and/or HIF-2α became heavily dependent on glutamine for proliferation [25].

Glutamine addiction was also found to occur in glioma cells that possess a recurrent mutation of the enzyme isocitrate dehydrogenase 1 (IDH1). IDH1 normally catalyzes the conversion of isocitrate to α-KG, yet the mutant isoform instead converts α-KG into D-2-hydroxyglutarate which has been shown to inhibit cellular differentiation through epigenetic alterations [26]. Due to mutant IDH1 function, the glioma cells become increasingly dependent upon glutamine-derived α-KG production, thereby exhibiting glutamine addiction and reliance on GLS for survival. Inhibition of GLS suppressed the growth of glioma cells with IDH1 mutations by decreasing the availability of glutamine-derived α-KG [27].

Further evidence supports that the dependence of certain cancer cells on glutamine may be more profound than previously thought. Fluorodeoxyglucose-positron emission tomography (FDG-PET) scanning, a clinical imaging technique, can detect cancers based on areas of increased glucose uptake; however, some cancers are invisible to PET scans and are thus deemed PET negative. As cancer cells need an abundance of resources, these PET-negative cancers must be relying on alternative metabolic pathways for their primary source of energy [28]. Glutamine is likely an alternative fuel for these cancers, but glutamine-based tumor imaging agents are still being developed [29].

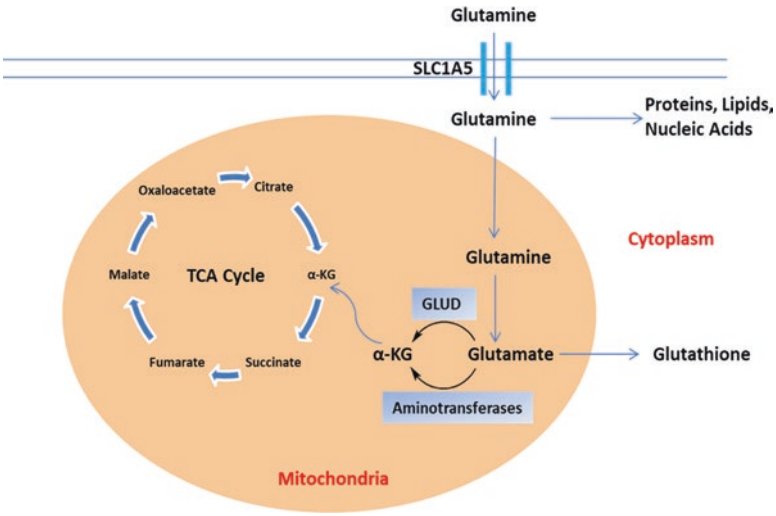

Fig. 2 Glutamine metabolism. Glutamine traverses the cell membrane via SLC1A5 and is hydrolyzed to glutamate and ammonia within the mitochondria. Glutamate, in turn, is transformed to α-KG, which acts as a TCA cycle intermediate

Cancer cells use precursors derived from TCA cycle intermediates to synthesize proteins, lipids, and nucleic acids. In order to maintain mitochondrial activity, tumor cells must compensate for lost TCA cycle intermediates caused by their metabolic diversions [30]. This process of replenishing metabolic intermediates is known as anaplerosis [31]. Glutamine provides mitochondrial anaplerosis because of its role as a nitrogen and carbon donor to the cell [30]. Glutamine traverses the cell membrane through amino acid transporters, ASCT2 and SN2 [32]. Once it enters the cytoplasm, glutamine is hydrolyzed to glutamate and ammonia (NH3) via glutaminase (GLS) (Fig. 2) [22].

Glutamate can be further catabolized through the TCA cycle or serve as a substrate for glutathione synthesis. It can be catalyzed by either glutamate dehydrogenase (GDH) or aminotransferases to ammonium and α-KG. Or glutamate can further be oxidized to glutathione by glutathione cysteine ligase. Glutathione is an antioxidant vital to a cell's immune defense, nutrient metabolism, and cellular functions [33]. It also plays an important role in the neutralization of mitochondrial reactive oxygen species (ROS). ROS are byproducts of oxygen metabolism abundantly present in the tumor microenvironment that promote tumor progression while invading healthy cells [34]. Recent studies have shown that the inhibition of glutamine metabolism results in increased ROS production, which could devastate cancer cells [35, 36]. Mitochondrial glutamine metabolism is a significant anaplerotic step in tumorigenesis. It is often enhanced in cancer cells along with increased levels of TCA cycle metabolites [30]. Thus, inhibiting glutaminase could effectively starve cancer cells of the glutamine essential to their survival [37].

2 Targeting Glutamine Metabolism for Cancer Therapy

Due to its central role in many cancers, glutaminolysis is becoming an increasingly prominent target for cancer therapy. Mammalian cells express two isoforms of glutaminase: kidney-type GLS1 and liver-type GLS2. GLS1 is more broadly expressed in normal tissue, while GLS2 is mainly present in the liver, brain, pituitary gland, and pancreas [38]. Both encode a mitochondrial glutaminase which catalyzes the hydrolysis of glutamine to glutamate [39, 40]. Studies have shown that *c-MYC* upregulates glutaminase importers and GLS1 expression and that p53 upregulates GLS2 expression [40–42].

Using stable isotope-resolved metabolomics (SIRM) studies, Le et al. also reported the persistence of glutamine oxidation via the TCA cycle under hypoxia. SIRM studies track metabolic transformations using stable isotope labeling and analyze metabolic products using nuclear magnetic resonance (NMR) and mass spectrometry (MS) at different time points. Using a human Burkitt lymphoma model P493 cell line carrying an inducible *MYC* vector, the group showed the coexistence of oxidative and aerobic glycolysis. Thus, inhibition of glutaminase induced oxidative stress and diminished ATP levels in hypoxic cells [36]. It was also found that glutamine metabolism supports cellular bioenergetics and redox homeostatic for proliferation under both aerobic and hypoxic conditions. P493 cells exhibited low glutathione levels and high ROS production under inhibition of glutaminase and hypoxia. Furthermore, glutamine-derived glutathione production was sustained under hypoxia as a coping method under high ROS levels [36]. These results suggest that glutamine metabolism, especially GLS, serves as a promising target for cancer therapy.

In a study by Wise et al., *c-MYC* expression was found to activate the transcription of key regulatory genes required for glutamine uptake and metabolism by selectively binding to the promoter regions of glutamine transporters ASCT2 and SN2. As a result, *c-MYC* induced reprogramming of mitochondrial metabolism by diverting glucose away from the TCA cycle and leaving cells susceptible to glutamine addiction to sustain anaplerosis. Moreover, *c-MYC*-transformed cells were found to be sensitive to GDH inhibitors. These results suggest that glutamine addiction may be a direct consequence of *c-MYC* activation [41]. Gao et al. found that *c-MYC* expression induced the expression of mitochondrial glutaminase in human P493 B lymphoma cells and PC3 prostate cancer cells by suppressing microRNAs miR-23a and miR-23b, which target the GLS 3′ untranslated region (UTR) seed sequence [1, 42]. Overall, these results may be exploited for cancer therapy using inhibitors of enzymes involved in glutamine metabolism or therapeutics that inhibit the transcriptional properties of *c-MYC*.

The *TP53* gene codes for a tumor suppressor protein known to trigger cell cycle arrest, apoptosis, or senescence in response to a variety of cellular dysfunctions, including DNA damage, oncogene activation, and hypoxia [43]. It is one of the most frequently mutated genes among all cancers. However, recent studies have discovered *TP53's* additional roles in regulating energy metabolism and antioxidant defense mechanisms [44, 45]. GLS2 is a *p53* target gene that plays an important role in mediating the tumor-suppressant properties of *p53*. GLS2 increases intracellular

levels of glutamate and α-KG, thus leading to enhanced mitochondrial respiration and ATP production. It also leads to increased cellular glutathione levels and thus decreased ROS levels [40]. Hu et al. demonstrated that *p53* increases GLS2 expression under both stressed and non-stressed conditions—enhancing glutamate levels, mitochondrial respiration rates, and glutathione levels, while decreasing ROS levels. It was also found that GLS2 expression is significantly decreased in liver carcinomas, while its over expression reduces cancer cell colony formation. Furthermore, the GLS2 gene promoter contains a *p53* consensus DNA-binding element whose expression is induced in response to oxidative stress [1, 40]. Hu's findings suggest that GLS2 may be a mediator to *p53*'s role in energy metabolism and antioxidant defense, ultimately contributing to its tumor suppression abilities.

Due to its crucial role in energy regulation and biosynthesis, targeting glutamine metabolism has the potential to affect a broad spectrum of cancers. In addition to GLS inhibition, the role of oncogenes and tumor suppressors in regulating glutamine metabolism makes it a promising venture for therapeutic strategies. However, while many drugs have been synthesized to target glutamine metabolism from its initial transport into the cell to its conversion to α-KG, most are still in preclinical stages (Table 1) [38].

2.1 Inhibition of Glutaminolysis by GLS Inhibitors

The most straightforward approach for targeting glutaminolysis is the inhibition of glutaminase (GLS), which catalyzes the hydrolysis of glutamine to glutamate. Inhibiting glutaminase can starve cancer cells by blocking the synthesis of

Table 1 Current therapeutic strategies and their mechanism of action in targeting glutamine metabolism

Classification	Drug
GLS inhibitors	CB-839
	• BPTES
	• 968
Glutamine depletion	L-Asparaginase
	Phenylbutyrate
ASCT2 inhibitors	Benzylserine
	GPNA
	γ-FBP
Glutamine mimetics	Acivicin
	Azaserine
	6-Diazo-5-oxo-L-norleucine (DON)
GDH inhibitors	R162
	EGCG
	ECG
Aminotransferase inhibitors	AOA

glutamate and thus prevent α-KG from feeding the TCA cycle. After NF-kB activates GAC, the slicing isoform of GLS1, via phosphorylation, NF-kB itself is activated by Rho GTPases. Alteration of Rho GTPases by small-molecule inhibitors showed a significant decrease in GAC activity in human breast cancer cells [46]. The decrease in GAC activity caused breast cancer cells to stop proliferating and reduced their ability to invade surrounding cells [46]. GLS-targeting studies are being actively investigated as potent therapeutic inhibitors, such as bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl)ethyl sulfide (BPTES) and CB-839 [35, 47–50]. The kidney isoform, GLS1, is found in many primary tumors [51], while the liver isoform, GLS2, is less often expressed in cancers. BPTES, which allosterically inhibits GLS1 by altering the conformation of the enzyme [48], has been shown to inhibit the growth of a variety of tumors. BPTES has been proven in many studies to inhibit cancer cell growth in vitro and slow tumor growth in xenografts in vivo [36]. While BPTES produces formidable results in vitro, higher concentrations are needed to achieve the same effect in vivo. Due to its low solubility, BPTES tends to precipitate at high concentrations, thus posing a challenge to the physiological delivery of the drug in clinical trials [52]. This challenge was recently solved by Elgogary et al. with an emulsification method that encapsulated BPTES into biodegradable nanoparticles coated with poly(ethylene glycol) (PEG) and poly(lactic-co-glycolic acid) (PLGA) to improve nanoparticle circulation time in the blood. This process enhanced the efficacy of BPTES in vivo by improving its solubility and increasing tumor drug exposure [35]. The effect is enhanced under hypoxic conditions, often inducing cancer cell death [36].

Furthermore, another GLS1 inhibitor named CB-839 is a highly potent allosteric inhibitor that has moved on to clinical trials (NCT02071862). It has proven efficacy in the treatment of triple-negative breast cancer [49].

Taken together, glutamine dependency of cancer cells may be a particular metabolic vulnerability of cancer, and glutaminolysis-targeting strategies could be a promising approach for glutamine-addicted cancer therapy.

2.2 Combination Therapy

The heterogeneity of cancer metabolism poses many challenges for potential drug therapies. Hence, the use of combination therapies to target multiple metabolic pathways to suppress tumor growth effectively may be optimal, especially in identifying cases that induce synthetic lethality, where two drugs induce cell death in combination, but not individually. Glutamine's role in cellular functions makes GLS inhibition an ideal candidate for combination therapy. In their study, Elgogary et al. found that a combination therapy of BPTES and metformin produced better results than monotherapy of either drug alone. Metformin is an FDA-approved drug for the treatment of type II diabetes that also inhibits glycolysis and glycogen synthesis. In this case, BPTES targets glutamine metabolism and metformin targets glucose

metabolism, resulting in an optimal reduction of tumor development [35]. Other treatments that are synthetically lethal with the inhibition of GLS include GLUT2q1 inhibition, mTOR inhibition, and ATF4 activation [38].

2.3 Knockdown of c-MYC

Niu et al. found that suppressing *c-MYC* expression resulted in reduced cell growth, colony formation, and tumor progression in nasopharyngeal carcinoma cell lines both in vitro and in vivo [53]. Using RNA interference (RNAi)-mediated silencing of the *c-MYC* gene, Zhang et al. showed that the down regulation of *c-MYC* induces apoptosis in vitro and suppresses the growth of colon cancer cells in vivo [54]. Along the same lines, research on RNAi-mediated knockdown of GLS expression is also underway as a promising potential target to glutamine metabolism [55].

2.4 Inhibition of Glutamate Dehydrogenase (GDH)

Inhibiting the oxidative deamination of glutamate to α-KG has devastating effects on cancer cells comparable to inhibiting glutaminolysis [56, 57]. This process is catalyzed by glutamate dehydrogenase (GDH). GDH can be inhibited by the preclinical compounds R162, epigallocatechin gallate (EGCG), and epicatechin gallate (ECG) [38, 58]. Using perifusion assays, Li et al. showed that EGCG and ECG blocked GDH activity by binding to the allosteric regulator ADP's binding site [59].

Furthermore, it was demonstrated that green tea polyphenols such as hexachlorophene, GW5074, and bithionol might inhibit GLDH function. These inhibitors work by restricting enzyme movement, either by forming ring barriers around the enzyme or by wedging between the enzyme's subunits. Currently, green tea polyphenols have been shown to inhibit lung, colon, and prostate adenocarcinoma growth in xenograft models [60]. These compounds also had significant effects on glioblastoma, colon, lung, and prostate adenocarcinoma cell proliferation [61]. Additionally, it was found that GLDH inhibition through siRNA resulted in a marked decrease in the proliferation of glioblastoma cells that were glutamine dependent [62].

2.5 Inhibiting the TCA Cycle by Depleting Glutamine and Asparagine

One of the earlier means of suppressing glutamine metabolism arose from reducing the amount of available glutamine itself. Ollenschläger et al. found that the abundance of glutamine in the body dropped precipitously by giving patients with acute

myeloid leukemia L-asparaginase. Asparaginase catalyzes the removal of the amide nitrogen from asparagine to form aspartic acid. The administration of asparaginase also dropped stored levels of glutamine [63]. When applied in culture, asparaginase inhibited cell growth and induced cell death in pancreatic cancer cells. The effect of asparaginase can largely be reversed through re introduction of small amounts of glutamine [64].

Studies of acute lymphoblastic leukemia indicate that asparaginase activity correlates to glutamine depletion in the bloodstream and improved treatment outcomes [65, 66]. Furthermore, cancer cells with a deficiency of asparagine synthase (ASNS), an enzyme that generates asparagine, such as acute lymphoblastic leukemia (ALL), must use asparagine from blood [67]. In 1979, Ertel et al. treated ALL patients with asparaginase, which exhausted the asparagine supply in the blood. This treatment reinduced remission in up to 60% of cases [68]. ALL can upregulate ASNS to restore intracellular asparagine levels and satisfy their asparagine demand [69]. However, some studies show that ASNS levels may not impact the sensitivity of ALL to asparaginase treatments in all cases [70]. The diverse metabolic phenotypes of malignant cells create many challenges for therapeutic strategies. It seems that a combination drug therapy targeting both asparagine and glutamine metabolism could be a promising treatment.

Another possible treatment in development is phenylbutyrate, a drug that lowers glutamine concentrations in the plasma. It is currently approved by the FDA and has shown clinical improvement in patients with hormone-refractory prostatic carcinoma and GBM [65, 71, 72].

2.6 Inhibiting Glutamine Uptake

The *c-MYC*-activated amino acid transporter ASCT2 (or SLC1A5) is upregulated in many cancers and involved in controlling glutamine uptake [41, 65]. High levels of ASCT2 are correlated with aggressive tumor growth and short survival time. ASCT2 inhibitors include benzylserine, GPNA, and γ-FBP [38]. Research shows that the inhibition of glutamine importers significantly slowed growth in human colon and lung cancer cells [73, 74].

2.7 Using Glutamine Mimetics

Another means of decreasing the availability of glutamine is the creation of glutamine analogs. Analogs such as 6-diazo-5-oxo-L-norleucine (DON) and acivicin did show cytotoxic effects against several tumor types, including leukemia and colorectal cancers; however, these analogs are no longer clinically available due to patient toxicity [75].

A few glutamine analogs, namely, acivicin, azaserine, and 6-diazo-5-oxo-L-norleucine (DON), have been extensively researched in an effort to inhibit glutamine metabolism. DON is a substrate analog of glutamine that binds to the active site of human kidney glutaminase to serve as an inhibition mechanism [76]. However, DON has not progressed into clinical trials due to concern regarding its lack of selectivity (unexpected inhibition of other glutamine-utilizing enzymes) and toxicity [75, 77, 78]. Similar to DON, acivicin and azaserine are also glutamine analogs that interrupt nucleotide synthesis by inhibiting aminotransferases to halt DNA transcription in tumor cells [55, 75]. All three analogs exhibit excessive side effects and toxicity that have prevented them from reaching clinical trials.

3 Transaminase Upregulation and Targeting Amino Acid Synthesis for Cancer Therapy

Another means of inhibiting glutaminolysis is to target alanine transaminase through L-cycloserine [79] or aspartate transaminase through the inhibitor amino oxyacetate [80] which could almost completely halt the growth of breast cancer in xenograft mice. What is truly promising is that there appears to be little to no toxicity in non neoplastic cells. The effectiveness of the inhibitor, combined with the lack of toxicity, makes inhibition of aspartate aminotransferase a potentially successful chemotherapeutic target.

Transaminases, also known as aminotransferases, are enzymes that catalyze reactions between amino acids and α-keto acids. Particularly, aminotransferases convert glutamate to α-KG without producing ammonia. Glutamate acts as a nitrogen donor in these transaminations. Alanine aminotransferase, also known as glutamic-pyruvate transaminase (GPT), and aspartate aminotransferase, also known as glutamic-oxaloacetic transaminase (GOT), are abundantly present in the liver and often serve as markers for liver toxicity. There are three aminotransferase pathways through which glutamate can be transformed to α-KG. These three paths of catalysis are GPT, GOT, and phosphoserine aminotransferase 1 (PSAT1)—each of which produces a different amino acid byproduct in addition to α-KG. As illustrated in (Fig. 3), GPT transfers nitrogen from glutamate to pyruvate to produce alanine and α-KG. GOT transfers nitrogen from glutamate to oxaloacetate to produce aspartate and α-KG. PSAT1 transfers nitrogen from glutamate to 3-phosphohydroxy-pyruvate to produce phosphoserine and α-KG [38]. PSAT1 is also involved in the serine synthesis pathway, which is essential for many breast cancers. Serine is essential for the synthesis of proteins necessary for cell proliferation. The PSAT1 expression has recently been demonstrated to be upregulated in many studies [38]. Possemato et al. found that serine pathway flux is augmented in some breast cancer cell lines and that suppression of PSAT1 inhibited proliferation of these cells in addition to causing significant reduction of α-KG [81]. In a study by Son et al., aspartate amino-

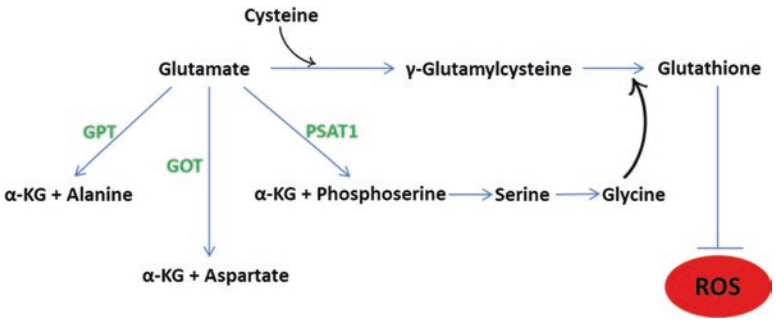

Fig. 3 Glutamate pathways through transamination. Glutamate can be catalyzed via three different pathways, GPT, GOT, and PSAT1, all of which yield α-KG and a different amino acid

transferases were demonstrated to be vital to maintaining redox homeostasis in PDAC cells. Furthermore, oncogenic mutant KRAS activity was found to upregulate the expression of aminotransferases, hence yielding high ROS levels and slowing tumor growth in vivo [56, 57]. Taken together, these works suggest that targeting the amino acid synthesis pathway may be another effective strategy for cancer therapy.

Apart from glutamine, many other amino acids play important roles in tumorigenesis, namely, arginine, tryptophan, serine, and glycine. Arginine is a precursor for the synthesis of proteins, urea, and various signaling molecules [82]. Although glutamine is considered a nonessential amino acid, many cancer cells that lack the enzyme argininosuccinate synthetase 1 (ASS1) are dependent upon arginine for proliferation. ASS1 catalyzes the conversion of citrulline into argininosuccinate in the arginine synthesis pathway. Loss or suppression of ASS1 in osteosarcoma cells results in depletion of arginine. Studies have shown that ASS1 acts as a tumor suppressor because cells with low ASS1 expression could not grow in an environment without arginine [83, 84].

Tryptophan is linked to the regulation of anti tumor immune responses [85]. Figure 4 shows that it can be degraded to kynurenine via two enzymes: indoleamine-2,3-dioxygenase (IDO) and tryptophan-2,3-dioxygenase (TDO). IDO activity commonly leads to suppression of the immune system [86]. Dendritic cells (immune system cells that present antigens to T cells) expressing IDO can limit tryptophan supply to T cells in the extracellular matrix, thus limiting T-cell response to tumor growth [87]. Studies have shown that mice transfected with IDO-induced cells developed large tumors and exhibited poor survival, while mice transfected with IDO-negative cells showed no signs of tumor development [88]. To further support this, immunohistochemical staining for IDO expression revealed a correlation between high IDO expression and low levels of immune cells $CD3^+$, $CD8^+$, and $CD57^+$ [89]. This, in turn, can be correlated with aggressive tumor progression and poor survival in cancer patients with high IDO expression.

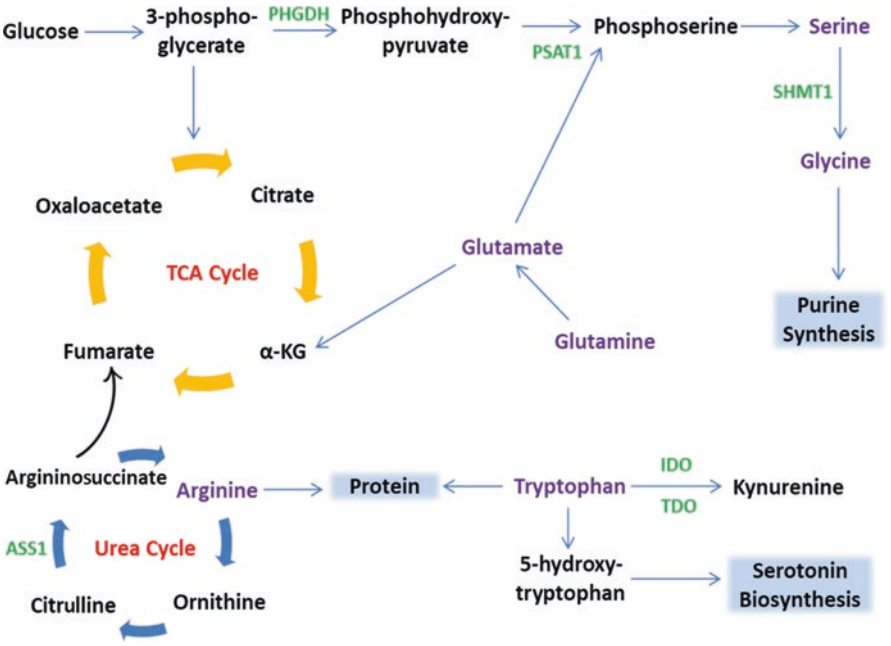

Fig. 4 Amino acid metabolic pathways. Cancers that lack ASS1, present in the arginine pathway, are dependent upon arginine. Tryptophan is linked to the regulation of antitumor immune responses. The serine and glycine biosynthesis pathways support purine and pyrimidine synthesis in proliferating cancer cells. Taken together, the illustrated pathways can be targeted for potential cancer therapy strategies

There are currently four IDO inhibitors under clinical development and more in preclinical testing [90]. In 2013, Beatty et al. studied the effects of the small-molecule IDO inhibitor INCB024360 treatment in 52 cancer patients. The drug was well tolerated by patients and successfully inhibited more than 90% of IDO activity when administered twice a day. Results show stable disease conditions in 30% of patients [91]. Because INCB024360 was well tolerated, it has the potential to be potent as either a monotherapy or part of combination therapy. Phase II clinical trials of this inhibitor are currently underway for patients with ovarian cancer and myelodysplastic syndrome. Combinatorial therapies with IDO inhibitors and cancer vaccines have also shown progress. A phase I study of indoximod, another IDO inhibitor, in combination with docetaxel, an antimitotic chemotherapy drug, showed stable or partially stable disease conditions in more than 50% of patients [92]. Other combinatorial therapies being tested in the clinic include INCB024360 and MK3475, an immune checkpoint inhibitor [90].

Other than IDO, cancer cells can also use TDO, an immunosuppressive enzyme, to avoid immune destruction. TDO is abundantly present in melanomas, bladder carcinomas, and hepatocarcinomas. Similar to IDO, the use of TDO inhibitors prevents the growth of TDO-expressing tumor cells [93]. There are several other

enzymes that cancer cells exploit for immune tolerance; hence, targeting tryptophan metabolism with combinatorial approaches may yield optimal therapies [84].

The serine and glycine biosynthesis pathways are interconnected. They both provide methyl groups for the one-carbon pool that supports purine and pyrimidine synthesis in proliferating cancer cells [94]. Research has unveiled that phosphoglycerate dehydrogenase (PHGDH), the enzyme that catalyzes the first reaction in the serine synthesis pathway, is highly upregulated in metastatic breast cancer and correlated to short patient survival times [95, 96]. The gene encoding PHGDH is also amplified in melanoma and breast cancer types [81]. In addition to PHGDH, serine hydroxymethyltransferase (SHMT) is also implicated in tumorigenesis. SHMT catalyzes the conversion of serine to glycine and is regulated by *c-MYC*, an oncogene that controls the transcription of 15% of human genes [84, 97, 98]. Glycine is a component of glutathione and is required for regulating cellular redox balance. It also fuels biosynthesis and sustains oxidative phosphorylation in mitochondria. Thus, glycine metabolism has been shown to promote rapid tumor proliferation [94, 99, 100]. In an attempt to block glycine biosynthesis, researchers are using antimetabolites (drugs that alleviate the effects of metabolites) methotrexate and pemetrexed to inhibit SHMT [94, 101]. Since serine and glycine are considered nonessential, their depletion can be tolerated in vivo. Maddocks et al. found that mice fed diets lacking serine and glycine showed a reduction in tumor sizes and survived longer than those fed diets containing the amino acids, indicating that diet regulation may be a potential therapy for investigation [102].

Many cancers become dependent on exogenous supplies of increased de novo synthesis of specific amino acids. This characteristic can be exploited for cancer therapies by depleting amino acid supplies, blocking uptake by transporters, and inhibiting biosynthetic enzymes. The identification of novel therapeutic strategies targeting amino acid pathways could allow for the emergence of new drugs and enhance the current therapeutic efficacy.

Conclusion

Glutaminolysis is a metabolic process that has been shown to play a critical part in a wide variety of cancers. As a result, glutamine metabolism is an important potential target for cancer therapy. The heterogeneity of cancer metabolism is pervasive. Just as only some cancers are dependent upon glucose for the TCA cycle, only some cancers will exhibit aberrant glutaminolysis. Even within a single patient, the cancer cells may exhibit vast differences in their dependence on metabolic fuel supplies. This implies that not all cancers will respond in the same manner, or to the same extent, to the inhibition of glutaminolysis. It is important to note that inhibiting glutaminolysis will have a better effect on cancers that display glutamine addiction. That being said, there is a huge potential for inhibition of glutaminolysis in cancers. As stated before, genetic alterations, as well as the tumor environment, can influence cancer's use of glutaminolysis. Developing and exploring glutaminolysis

inhibitors present a strategic course of action toward the goal of finding an effective treatment for the many glutamine-dependent cancers. Inhibitors of GLS, GLDH, or other key enzymes could be used in combination with standard chemotherapy treatments to increase their overall effectiveness.

Currently, the use of stable isotope-resolved metabolomics (SIRM) with NMR has been very effective in tracking and examining metabolite usage within certain cancer lines [48]. Increased efforts should be made in the future to use metabolomic technologies for the analysis of different cancers.

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The Heterogeneity of Lipid Metabolism in Cancer

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Key Points

- Fatty acid synthesis is upregulated in cancer.
- The mitochondrial citrate transporter protein (CTP) protects mitochondrial function in cancer.
- ATP citrate lyase (ACLY) is upregulated in cancer.
- Multifaceted effects of inhibiting acetyl-CoA carboxylase (ACC) in cancer.
- First fatty acid synthase (FAS) inhibitor TVB-2640 in clinical trials for cancer.
- What markers can predict cancer cell sensitivity to lipid synthesis inhibition?
- Tumor microenvironment impacts the sensitivity of cancer cells to lipid synthesis inhibitors.
- The efficacy of inhibiting cholesterol synthesis with adjuvant statins is variable.
- Fatty acid uptake is associated with metastasis.
- Targeting FAO for cancer therapy may be achieved by inhibiting carnitine palmitoyltransferase 1.
- Carnitine palmitoyltransferase 1 (CPT1) inhibitors are now in clinical trials.
- FAO occurs at the peroxisome where peroxisome proliferator-activated receptors (PPARs) act as ligand-activated transcription factors.

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Abbreviations

4-HNE	4-Hydroxy-nonenal
ω-3/6	Omega-3/6 fatty acid
ACC	Acetyl-coenzyme A carboxylase
ACLY	Adenosine triphosphate citrate lyase
ACSL3	Acyl-coenzyme A synthetase long-chain family member 3
ACSS2	Acyl-coenzyme A synthetase short-chain family member 2
AMPK	Adenosine monophosphate-activated protein kinase
ATP	Adenosine triphosphate
BMI	Body mass index
BTA	Benzene-tricarboxylate
CD36	Cluster of differentiation 36 protein
CTP	Citrate transporter protein
CoA	Coenzyme A
CPT1	Carnitine palmitoyltransferase 1
DNA	Deoxyribonucleic acid
DNLS	De novo lipid synthesis
EMT	Epithelial-mesenchymal transition
ERS	Endoplasmic reticulum stress
$FADH_2$	Flavin adenine dinucleotide
FAO	Fatty acid oxidation
FAS	Fatty acid synthase
FATP	Fatty acid transport protein
GBM	Glioblastoma multiforme
HFD	High-fat diet
HMGCR	3-Hydroxy-3-methylglutaryl-coenzyme A reductase
IDH	Isocitrate dehydrogenase
LD	Lipid droplet
LDL	Low-density lipoprotein
LPL	Lipoprotein lipase
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
PE	Phosphatidylethanolamine
PIP2	Phosphatidylinositol-4,5-bisphosphate
PUFA	Polyunsaturated fatty acid
ROS	Reactive oxygen species
SCD	Stearoyl-coenzyme A desaturase
TCA	Tricarboxylic acid
TG	Triglyceride
TME	Tumor microenvironment

Introduction

The study of cancer cell metabolism has traditionally focused on glycolysis and glutaminolysis. However, lipidomic technologies have matured considerably over the last decade and broadened our understanding of how lipid metabolism is relevant to cancer biology [1–3]. Studies now suggest that the reprogramming of cellular lipid metabolism contributes directly to malignant transformation and progression [4, 5]. For example, de novo lipid synthesis can supply proliferating tumor cells with phospholipid components that comprise the plasma and organelle membranes of new daughter cells [6, 7]. Moreover, the upregulation of mitochondrial β-oxidation can support tumor cell energetics and redox homeostasis [8], while lipid-derived messengers can regulate major signaling pathways or coordinate immunosuppressive mechanisms [9–11]. Lipid metabolism has therefore become implicated in a variety of oncogenic processes, including metastatic colonization, drug resistance, and cell differentiation [10, 12–16]. However, whether we can safely and effectively modulate the underlying mechanisms for cancer therapy is still an open question.

As discussed in previous chapters, inter- and intra-tumoral heterogeneity are major causes of treatment failure in clinical oncology because tumor subclones with either intrinsic or acquired resistance to therapy can be selected by Darwinian mechanisms and allowed to drive disease relapse [17–20]. An alarming number of parameters seem capable of inducing this diversity, including (epi)genetic lesions, microenvironmental constraints, stromal interactions, and treatment effects [21–23]. Perhaps unsurprisingly then, translational strategies targeting lipid metabolism have reported mixed or even diverging responses in preclinical models of cancer. These results hint at differential tumor cell dependencies on lipids, but we are far from understanding the extent to which this heterogeneity arises. Moreover, how this non uniformity of lipid metabolism undermines patient treatment is all the more unclear. To better understand the clinical potential of this emerging discipline, we will have to address both the spatial and temporal heterogeneity of cellular lipid metabolism.

Here, we provide a brief synopsis of novel findings on the lipid metabolism of cancer cells, with an emphasis on heterogeneity across and/or within tumors. Given the rapid pace of this field, we focus on central pathways involving fatty acid synthesis, uptake, and oxidation.

1 Fatty Acid Synthesis Is Upregulated in Cancer

Endogenous fatty acid synthesis is frequently upregulated in cancer because fatty acids can serve as substrates to produce lipid signaling molecules, modify protein function through lipidation, synthesize phospholipids for cell membranes, or store energy as triglycerides. The primary source of carbons for fatty acid synthesis in

cancer cells comes from glucose, which is broken down into acetyl-CoA then citrate in the mitochondria. The mitochondrial citrate transporter protein (CTP) carries citrate from the mitochondria to the cytosol. ATP citrate lyase (ACLY), a key enzyme of de novo fatty acid synthesis (DNLS), cleaves cytosolic citrate into acetyl-CoA and oxaloacetate. Cytosolic acetyl-CoA is used to form fatty acids. Hence, the localization of acetyl-CoA within a cell can determine its metabolic fate.

1.1 The Mitochondrial Citrate Transporter Protein (CTP) Protects Mitochondrial Function in Cancer

The Avantaggiati research group has extensively studied (CTP) and demonstrated that CTP plays an important role in preventing mitochondrial damage and preserving its function, such as in cellular bioenergetics [24]. The inhibition of CTP resulted in anti-tumorigenesis in vivo. Although the authors observed a decrease in fatty acid synthesis from glucose due to the suppression of CTP-dependent transport of citrate by a benzene-tricarboxylate analog (BTA), they believe this effect only played a partial role in tumor reduction because the total FA levels were not drastically affected. Moreover, CTP levels were associated with cancer aggressiveness [24].

1.2 ATP Citrate Lyase (ACLY) Is Upregulated in Cancer

ACLY was found to be elevated in many types of cancers, including breast [25], lung [26], liver [27], and bladder cancers [28]. Migita et al. found that ACLY expression was significantly higher in human lung adenocarcinoma samples as compared to normal lung tissue. It also correlated with stage, differentiation grade, and a poorer prognosis. ACLY inhibition arrested lung cancer cell growth in vitro and in vivo. ACLY knockdown compromised de novo lipogenesis, but intracellular lipids were increased, suggesting alternative mechanisms of lipid accumulation [26]. A study by Schlichtholz et al. similarly demonstrated an upregulation of ACLY, glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and citrate synthase, which are involved in fatty acid synthesis, in bladder cancer [28].

1.3 Multifaceted Effects of Inhibiting Acetyl-CoA Carboxylase (ACC) in Cancer

After ACLY produces cytosolic acetyl-CoA, the enzyme acetyl-CoA carboxylase (ACC) irreversibly converts acetyl-CoA into malonyl-CoA. Malonyl-CoA is required for fatty acid synthesis and elongation and negatively regulates β-oxidation of long-chain fatty acids by inhibiting the enzyme carnitine palmitoyltransferase 1

(CPT1) [29]. ACC exists as two isoforms (ACC1/2, genes *ACACA/B*) [30]. ACC1 is preferentially expressed in lipogenic cells, such as adipocytes [30]. The two ACC isoforms both catalyze the same reaction and can compensate for loss of function of an ACC isoform as malonyl-CoA levels only decrease in hepatocytes if both ACC1 and ACC2 are inhibited [31]. This demonstrates that inhibiting both ACC1 and ACC2 isoforms may be more efficacious than inhibiting either isoform alone for the treatment of cancer. ACC is now receiving greater attention as a therapeutic target against cancer because the formation of malonyl-CoA by ACC is the rate-limiting step of fatty acid synthesis.

The expression of ACC1 is highly enriched in breast [32], prostate [33], liver [34], and renal cancers [35]. Expression of ACC1 also increases with tumor grade in liver cancer, and its overexpression increases liver cancer cell viability while decreasing apoptosis [34, 36]. ACC1 expression is also prognostic for some cancers. High expression of ACC1 is correlated with worse survival in renal cancer [35]. Inhibition of ACC1 with siRNA reduced cell viability in breast [37] and liver cancers [36]. Furthermore, simultaneous inhibition of both ACC1 and ACC2 with a small chemical molecule or siRNA reduced tumor growth in prostate [38], brain [39], and pancreatic cancers [40].

While ACC inhibition appears to arrest the growth of certain cancer types, it has paradoxically been shown to promote breast cancer invasion and metastasis by promoting epithelial-to-mesenchymal transition (EMT) [41]. ACC-deficient hepatocytes are also more susceptible to diethylnitrosamine-induced hepatocellular carcinoma. ACC-deficient mice exhibited a reduction in hepatic lipogenesis, a decrease in glutathione, and an increase in NADPH [34]. Collectively, these preclinical studies demonstrate the duality of ACC inhibition: it could attenuate tumor growth in some cancer types, but it could also contribute to carcinogenesis or promote metastasis in others.

Long-term regulation of ACC occurs at the level of transcription, while short-term regulation of ACC occurs through allosteric binding and reversible phosphorylation. Short-term regulation allows ACC activity to rapidly adapt to the microenvironment. For instance, AMP-activated protein kinase (AMPK) can inactivate ACC via phosphorylation (p-ACC). Metformin is a widely prescribed first-line treatment for type 2 diabetes that activates AMPK. Preclinical studies in mice have demonstrated that metformin can reduce cancer growth, in part by increasing p-ACC levels [42, 43]. There are currently hundreds of clinical trials investigating whether metformin can be repurposed to treat cancer as adjuvant monotherapy or in combination. However, a potential adverse effect of metformin may be an increase in the metastasis of certain cancer types, given that both metastatic breast and lung tumors have increased levels of p-ACC1 [41]. Protein phosphatase 2A (PP2A) can reactivate p-ACC by dephosphorylation. The tumor suppressor known as breast cancer susceptibility gene 1 (BRCA1), which is deactivated primarily in breast and ovarian cancers, prevents dephosphorylation of p-ACC [44]. Cancers with loss-of-function mutations in BRCA1 have increased ACC activity due to less phosphorylation of ACC and thus may be more susceptible to ACC inhibition [44, 45].

1.4 First Fatty Acid Synthase (FAS) Inhibitor TVB-2640 in Clinical Trials for Cancer

A great number of studies have now documented an increase in the expression of lipogenic enzymes across several cancers. For instance, Szutowicz et al. revealed that the activity of citrate lyase, an important enzyme in lipogenesis, was elevated in breast carcinoma and fibrocystic disease, compared to healthy breast tissue [25]. As such, it stands to reason that key enzymes involved in de novo lipid synthesis could be potential targets for cancer therapy. One such enzyme is fatty acid synthase (FAS) encoded by the *FASN* gene [46].

FAS is a multienzyme protein complex that catalyzes the final reactions converting malonyl-CoA and acetyl-CoA into a saturated long-chain fatty acid composed of 16 carbons known as palmitic acid. Palmitic acid can be used as a precursor to produce lipid signaling molecules, modify protein function through palmitoylation, store energy as triglycerides, or form structural lipids for cell membranes. NADPH is the reducing agent for fatty acid synthesis, and 14 molecules of NADPH are used to synthesize each molecule of palmitic acid. The pentose phosphate pathway (PPP) generates NADPH through the oxidation of glucose into pentose sugars and ribulose-5-phosphate. Overexpression of FASN is usually accompanied by the over expression of enzymes in the pentose phosphate pathway to supply NADPH for fatty acid synthesis. Increased expression of FASN and PPP enzymes is associated with worse survival in renal and breast cancers [35, 47]. Increased FASN expression is also associated with tumor grade in prostate cancer [48]. Inhibition of FASN reduces cell proliferation and increases cell death in human breast [37, 43], prostate [49], and colon cancers [50]. FASN inhibitors can also be used in combination with chemotherapy taxane to improve anticancer efficacy [51]. Colorectal cancer metastasis is also mitigated by FASN inhibition in mice [50]. While inhibition of FASN reduces tumor growth and metastasis in most cancers, it also has been demonstrated to reduce survival rate in mice with lung cancer by increasing metastasis [52], demonstrating how FASN inhibition can sometimes worsen cancer outcomes as seen with ACC inhibition.

The FASN inhibitor TVB-2640 has been tested on cancer patients in clinical trials (Clinical Trial ID: NCT02223247). Inhibiting FASN did not result in severe side effects, and all mild side effects were reversible after discontinuation [53]. Moreover, side effects were not worsened by its combined application with the chemotherapy drug paclitaxel [53]. Monotherapy with TVB-2640 stabilized cancer progression in three out of six patients who had KRAS-driven non-small cell lung cancer [53]. There are now two clinical trials (phase II) testing the efficacy of TVB-2640 in combination with additional chemotherapy drugs for $HER2^+$ breast cancer (Clinical Trial ID: NCT03179904) and astrocytoma (Clinical Trial ID: NCT03032484). The third clinical trial is a phase I study investigating the pharmacodynamic effects of TVB-2640 in patients that require surgery for colon cancer (Clinical Trial ID: NCT02980029).

1.5 Which Markers Can Predict Cancer Cell Sensitivity to Lipid Synthesis Inhibition?

Phosphatidylinositol (3,4,5)-trisphosphate (PIP3) is produced by phosphoinositide 3-kinase (PI3K) activation, which is mutated in many types of cancers [54–56]. PI3K, which is a key regulator of phosphoinositide metabolisms, is considered a potential target in preclinical and clinical settings to suppress advanced solid tumors, including malignant glioma, NSCLC, and breast cancer [57] (NCT00485719, NCT00777699, NCT00704080, NCT00907205, NCT00600275, NCT00876109, and NCT00726583).

Two highly studied intracellular signaling pathways that oncogenes activate to drive tumorigenesis and increase expression of lipid synthesis enzymes are the PI3K/Akt/mTOR pathway and the RAS/MAPK pathway [58, 59]. Constitutive activation of the PI3K/Akt/mTOR pathway results from activation of receptor tyrosine kinases (RTKs), loss-of-function mutations in the tumor suppressors PTEN and TSC, or activating mutations in PIK3CA and AKT1 [60]. The RAS oncogene family includes HRAS, KRAS, and NRAS and can also activate the PI3K/Akt/mTOR pathway [61]. Cancer cells that are driven by the overactivation of the PI3K or RAS pathways are more susceptible to inhibitors that target lipid synthesis than cancers that are driven by pathways not associated with lipid synthesis regulation [62, 63]. Inhibitors of ACLY, ACC, and FASN have been shown to be efficacious in cancers with PI3K- and RAS-driven pathways [64–67]. Cancer cells that overactivate RTKs, such as epidermal growth factor receptor (EGFR, also known as HER) family members 1–4 and c-MET [68–70], are also sensitive to lipid synthesis inhibition because these RTKs activate PI3K and RAS. ACLY and ACC are efficacious in HER1- and HER2-driven cancers [39, 66, 71–73], and FASN inhibition is efficacious in HER1-, HER2-, and c-MET-driven cancers [68, 74, 75]. Additional oncogenic signaling pathways that may be susceptible to FASN inhibition are MYC, beta-catenin, and steroid-responsive tumors because FASN inhibition downregulates these pathways [64, 76, 77]. Cancers with a loss of function in BRCA1 and p53 are also sensitive to FASN and ACC inhibition [45, 67, 78]. Preclinical studies testing ACLY, ACC, and FASN inhibitors may reveal the oncogenes and RTKs that confer susceptibility to DNLS inhibition and guide the design of future clinical trials. To date, several mTOR/PI3K inhibitors, such as idelalisib, copanlisib, rapamycin, temsirolimus, everolimus, and ridaforolimus, have been approved by the FDA.

Nevertheless, not all cancers with oncogene-mediated overactivation of RAS and PI3K pathways appear to be susceptible to lipid synthesis inhibition. For instance, KRAS mutations correlated with FASN sensitivity in lung cancer cell lines but not in colon cancer cell lines [64], which demonstrates that cancer cell susceptibility to lipid synthesis inhibition is not always driven by oncogenes. In other studies, oncogenes conferred resistance to FASN inhibition. Hepatocytes that are transformed into malignant cancer cells by the overactivation of c-MET and Akt are susceptible to FASN inhibition, but hepatocytes that are transformed by the overactivation of

c-MET and Wnt/beta-catenin signaling are unresponsive to FASN inhibition [63]. The c-MET-/beta-catenin-driven cancer cells may be unresponsive because beta-catenin activation in hepatocytes reduces FASN expression and lipid synthesis [63, 79, 80]. Interestingly, while beta-catenin decreases lipid synthesis in hepatocytes, beta-catenin signaling can increase lipid synthesis in B-cell lymphoma. Beta-catenin-driven B-cell lymphoma is susceptible to FASN inhibition [81]. This demonstrates that cancer cell type is relevant to determining susceptibility to FASN inhibition since oncogenic signaling pathways can affect different phenotypes depending on cell. In order for oncogenes to be reliable markers of lipid synthesis sensitivity, it will be important to consider the cell type of the cancer being discussed.

Of note, protein expression or enzyme activity may be better predictors of susceptibility to lipid synthesis inhibitors than genetic markers. For example, mRNA expression of ACLY, ACC, and FASN may not correlate with protein expression and activity [64, 82]. Increased expression of FASN and ACC at the protein level can occur without an increase in mRNA expression by increased translation of FASN and ACC mRNA via mTOR signaling [82].

Metabolic profiling may be a valuable method for determining susceptibility to FASN inhibition. One study examined 38 pancreatic cancer cell lines and classified them as lipogenic or glycolytic depending on their metabolic profile, which was determined by the amount of lipogenic or glycolytic metabolites [83]. Glycolytic cancer cells were significantly more susceptible to glycolytic inhibitors than those that were lipogenic. However, lipogenic cancer cells were not significantly more susceptible to lipogenic inhibitors, such as FASN inhibition, than glycolytic cancer cells [83]. Only half of the lipogenic cancer cell lines were sensitive to FASN inhibition, suggesting that broad lipogenic profiling is not an accurate predictor of susceptibility to FASN. While no single marker is able to definitively predict which cancers are susceptible to lipid synthesis inhibition, using a combination of markers, such as cell type, oncogene mutations, expression/activity of lipid synthesis enzymes, and metabolic profiling, may provide a reliable means to identify cancers that are sensitive to lipid synthesis inhibitors.

1.6 Tumor Microenvironment Impacts the Sensitivity of Cancer Cells to Lipid Synthesis Inhibitors

As mentioned in the chapter “Different Tumor Microenvironments Lead to Different Metabolic Phenotypes,” TCA cycle activity is reduced under hypoxic conditions, which results in reduced citrate and acetyl-CoA production. However, cancer cells manage to generate acetyl-CoA for fatty acid synthesis by different mechanisms, including a reliance on glutamine for citrate synthesis and acetate for acetyl-CoA via acetyl-CoA synthetase (ACSS2). Evidence suggests that ACSS2 expression can be increased to maintain growth under microenvironmental stress, such as hypoxia [84].

Bensaad et al. showed that while DNLS is repressed in hypoxia, lipid droplet accumulation and fatty acid uptake proteins, such as fatty acid-binding protein 3 (FABP3) and FABP7, are induced by HIF-1α. Lipid synthesis was restored in cancer cells after reoxygenation or the removal of anti-angiogenic therapy [85]. Other studies have corroborated that hypoxic tumor cells may be extraordinarily dependent on fatty acid uptake compared to those in normoxia [86, 87]. However, this can be differentially driven by oncogenic mTORC1, Ras, and/or HIF-1α signaling [85–87]. Moreover, triple-negative breast cancer is reliant on lipid droplet-derived substrates for β-oxidation and ATP generation after hypoxia-reoxygenation, whereas GBM is more dependent on glycolytic pathways [85]. This implies that FA uptake is not a universal feature of hypoxic cancer cells; therefore, inhibiting FA uptake may be a strategy for targeting tumor cells in hypoxic microenvironments for certain types of cancers but not others.

The availability of metabolic nutrients can also greatly impact the susceptibility of cancer cells to inhibition of DNLS. FASN expression was observed to be highest at the edge of tumors, suggesting that DNLS is preferred in cancer cells that are vascularized and have access to oxygen and glucose [88]. Tumors in low-lipid environments increase de novo fatty acid synthesis and thus may demonstrate increased sensitivity to FASN inhibition. The fact that lipoprotein supplementation can override DNLS inhibition emphasizes the importance of nutrient availability and again the role of exogenous lipid uptake [89, 90]. The availability of glucose for glucose-dependent lipogenesis is also important for cancer cell sensitivity to ACLY inhibitors. Low-glucose environments result in cancer cells that are less susceptible to ACLY inhibition because cancer cells can use acetate instead of citrate to produce acetyl-CoA for DNLS [90, 91].

2 Targeting Fatty Acid Elongation

Once palmitic acid is produced by de novo lipid synthesis, it can be modified by having its fatty acid chain elongated. Elongation of fatty acids is important for creating lipid precursors that are involved in cellular signaling and for producing phospholipids of cell membranes. Fatty acids that consist of 16 carbons or more, such as palmitic acid, are elongated in the smooth endoplasmic reticulum, while fatty acids consisting of fewer than 16 carbons are elongated in the mitochondria. Elongation of fatty acids in the smooth endoplasmic reticulum is regulated by four enzymes. These enzymes elongate fatty acids by using malonyl-CoA. The first step is the rate-determining reaction regulated by the enzyme β-ketoacyl-CoA synthase (elongase). There are seven types of elongases in humans known as ELOVL1–7. ELOVL7 was identified to be overexpressed in prostate cancer, and feeding mice a diet high in long- and very-long-chain fatty acids increased growth of ELOVL7-expressing tumor cells [92]. Meanwhile, inhibiting ELOVL7 with siRNA attenuated prostate cancer growth [92]. ELOVL1 is another elongase implicated in cancer growth. ELOVL1 was observed to be overexpressed in breast

cancer, and inhibition of ELOVL1 with siRNA reduced breast cancer cell viability in some cell lines [37].

While inhibiting elongases appears to be therapeutic for cancer, inhibiting ACC1 as described previously may be a more promising therapeutic strategy because ACC1 inhibition reduces both fatty acid synthesis and fatty acid elongation, while elongase inhibition only targets elongation. ACC1 inhibition can reduce fatty acid elongation by decreasing the availability of malonyl-CoA for fatty acid elongation [93]. This is suggested by a study in which silencing of ELOVL1 with a silencing efficiency of 70–80% decreased cell viability by greater than 50% in one breast cancer cell line while silencing ACC1 with a lower silencing efficiency of 30% decreased cell viability by greater than 50% in two breast cancer cell lines [37]. Patients who have neoadjuvant chemotherapy-resistant breast cancer have been associated with increased expression of fatty acid elongation proteins in the mitochondria [94]. Whether inhibiting mitochondrial fatty acid elongation is therapeutic against cancer remains to be determined.

3 The Efficacy of Inhibiting Cholesterol Synthesis with Adjuvant Statins Is Variable

Another anabolic pathway associated with lipid metabolism is the mevalonate pathway which synthesizes cholesterol. Cholesterol is a major component of cell membranes, influencing membrane fluidity and function. It also forms detergent-resistant microdomains called lipid rafts that coordinate the activation of signal transduction pathways. The enzyme 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) catalyzes the rate-limiting step of cholesterol synthesis. Increased expression of HMGCR and other cholesterol synthesis enzymes is associated with reduced survival rate in breast cancer [95]. HMGCR is the target for a class of cholesterol-lowering drugs called statins. Numerous epidemiological studies have demonstrated that patients who use statins have a reduced risk of cancer and cancer mortality [96–98]. This has raised the question as to whether statins can improve treatment outcomes in cancer patients. There are many clinical trials currently investigating if statins can be prescribed to reduce the progression of cancer.

Results from preclinical studies suggest that the efficacy of statins can be predicted based on the status of gene expression, such as that of HMGCR [99]. Breast cancer cells with overactive HER2 are also sensitive to statins because HER2 signals through the RAS pathway [100]. On the other hand, estrogen receptor-positive breast cancer cells appear to be less responsive to statins. *MYC* is another transcription factor that regulates cholesterol synthesis. Cancers with overactive *MYC* have been observed with increased expression of HMGCR and sensitivity to statins [101, 102]. Statins have also been shown to reduce metastasis in colon and ovarian cancer and selectively induce apoptosis in cancer cells [103, 104]. Along with monotherapy of statins being efficacious in preclinical studies, statins are also efficacious in combination therapy for their role in increasing sensitivity to radiation therapy [105].

The ability of statins to bind to HMGCR greatly affects their efficacy. Genetic variations in HMGCR have been found to modify the therapeutic effect that statins have on colorectal cancer [106]. A single-nucleotide polymorphism (SNP) in the HMGCR–statin-binding domain reduced the protective association between statins and colorectal cancer. An in vitro experiment demonstrated that the SNP in the HMGCR gene reduced the ability of statins to inhibit HMGCR and cholesterol synthesis. The anticancer activity of statins is also dependent on the ability of statins to enter cancer cells. For instance, pravastatin was found to inhibit tumor growth preferentially in cancers that expressed sodium-independent organic anion transporter protein-1B1 (OATP1B1), such as liver cancer, because this transporter is necessary for cellular uptake of pravastatin [107].

While preclinical studies have provided promising results for statins, clinical trials have not been as successful. A phase II clinical trial demonstrated that combining the statin simvastatin with the chemotherapy drug afatinib did not improve treatment efficacy compared to using afatinib in monotherapy [108]. Two additional phase II clinical trials found that statins were unable to resensitize cancers harboring KRAS activating mutations to the chemotherapy drugs cetuximab and panitumumab [109, 110].

4 Fatty Acid Uptake Is Associated with Metastasis

As discussed previously, enhanced lipogenesis is a frequent alteration of lipid metabolism in cancer cells, and therapeutic potential is promising. However, studies show that this strategy can be undermined by the supplementation of exogenous fatty acids, suggesting that extracellular lipids in the microenvironment may functionally substitute for endogenously derived FA [111]. After all, the scavenging of circulating nutrients is another hallmark of cancer cell metabolism [112]. Recently, it was reported that tumors, including those of breast and liposarcoma cancer, may rely on extracellular lipolysis in addition to lipogenesis to fuel cellular lipid requirements [113]. Lipoprotein lipase (LPL) is a rate-limiting enzyme of this mechanism, hydrolyzing circulating triglyceride-rich lipoprotein, such as very low-density lipoproteins and chylomicrons, into free FAs and monoacylglycerol molecules. Free FAs are then imported into cells by FA transporters such as cluster of differentiation (CD36) or those of the fatty acid-binding protein (FABP). Both LPL and CD36 expression have been associated with aggressive cancers, including HCC and PDAC, and negatively correlated with patient prognosis [114–117], but how this phenomenon varies among tumors and whether it can be inhibited for therapeutic effect remain uncertain.

An estimated 90% of all cancer-related deaths are attributed to metastasis, but the detailed mechanisms of metastasis remain unclear [118]. Recently, metastasis was associated with enhanced lipid metabolism [14, 119, 120]. One study identified an altered gene signature associated with fatty acid uptake (e.g., CAV1, CD36) in metastatic tumors across cancer types [121]. Moreover, this genetic signature had a

significant effect on patient survival rates, suggesting prominent roles of extracellular fatty acids specifically on metastatic progression. Corroborating this is a recent report describing abnormally high expression of CD36 in metastasis-initiating oral cancer cells [12]. Treating orthotopic xenografts with CD36-neutralizing antibodies inhibited metastasis initiation. These studies suggest that tumor cells of high metastatic potential have an outsized need for FA uptake, compared to those displaying less aggressive phenotypes. Interestingly, however, an earlier study observed up to a 100-fold *lower* expression of CD36 in breast cancer cells of high metastatic potential compared to less aggressive counterparts [122]. This inconsistency may be due to alternate mechanisms of CD36 related to cell adhesion [123]. Nevertheless, these data support the overarching concept of asymmetrical CD36 expression and fatty acid uptake even within cancers of the same type.

5 Fatty Acid Oxidation Encompasses a Diverse Set of Molecular Mechanisms

Lipids are important for cancer proliferation not only because of their ability to provide structural support in the cell membrane but also because they can be broken down to provide energy. Lipids can be catabolized after cellular uptake via the β-oxidation pathway, also known as fatty acid oxidation (FAO). FAO has not been examined as thoroughly as glycolysis or glutaminolysis, but recent advances have shed light on the role of FAO in cancer cells. Recently, lipids were also identified as a carbon source for nucleotide synthesis and histone acetylation in nonmalignant cells, and emerging evidence suggests that these mechanisms are relevant to tumor cells as well [124, 125]. The tumor microenvironment is often depleted of nutrients like glucose, so cancer cells often rely on FAO to generate ATP. Lipids are energetically dense molecules that cancer cells can exploit as an alternative source of energy. FAO yields ATP and NADPH, which support cellular energetics and redox homeostasis, respectively. Several studies have demonstrated that certain malignancies such as those in prostate, breast, lung, and B-cell lymphoma heavily depend on FAO for growth and survival [126–128]. Similarly, acetate is a 2-carbon fatty acid that is avidly oxidized in tumors including GBM [129, 130].

5.1 Targeting FAO for Cancer Therapy May Be Achieved by Inhibiting Carnitine Palmitoyltransferase 1

Inhibition of the FAO pathway could prevent cancer progression. An example of this strategy is the inhibition of carnitine palmitoyltransferase 1 (CPT1), which is the rate-limiting enzyme of FAO. CPT1 is a membrane protein that removes an acyl from a fatty acyl-CoA and attaches the acyl to carnitine. This results in the formation of palmitoylcarnitine and thereby facilitates the shuttling of fatty acids such as

palmitate into the mitochondrial matrix for FAO [124]. There are three subtypes of CPT1. CPT1A is expressed throughout several tissue types, but CPT1B is restricted mostly to muscle tissue. In physiological settings, all isoforms are inhibited by malonyl-CoA, but due to the greater binding efficiency of CPT1A to malonyl-CoA, CPT1A is found to be the isoform with the greatest capacity to perform the rate-limiting step of FAO [131]. The third and final isoform of CPT1 is CPT1C, which is normally found only in the brain [132]. However, many cancers also express CPT1C [133]. CPT1C is thought to confer resistance to oxidative stress in many tumors. CPT1C promotes resistance to rapamycin, an mTOR pathway inhibitor [133].

Physiologically, it is crucial to note that successful inhibition of CPT1 is dependent on the source and location of the malonyl-CoA. Malonyl-CoA produced via acetyl-CoA carboxylase 1 (ACC1) is localized in the cytosol and thus will not inhibit CPT1. The malonyl-CoA produced via the mitochondrial ACC2 enzyme, however, is capable of this inhibitory action. Thus, the relative concentrations of acetyl-CoA to malonyl-CoA can influence whether the cell is in a state of FAS or FAO [134]. AMP-activated protein kinase (AMPK) inhibits both ACC1 and ACC2 and in doing so increases reactive oxygen species (ROS). The increase in ROS leads to depletion of NADPH and produces oxidative stress on the cell, eventually leading to death [8]. This finding is in accordance with other studies that have noted the role of AMPK activation in cancer states. For example, metformin exerts anticancer effects and activates AMPK, but in tumors lacking CPT1C, the effect of metformin is less pronounced. This suggests that the action of metformin on AMPK is upstream of its effect on CPT1C [133].

The upregulation of CPT1 in several cancer types makes it a potential therapeutic target [131]. However, this upregulation does not appear to be a universal feature of all tumors as demonstrated by a recent study showing that, in clear cell renal cell carcinoma (ccRCC), transcriptional repression of CPT1A is mediated by hypoxia-inducible factors (HIF-1α and HIF-2α) [135]. However, conflicting reports regarding the role of HIFs in FAO have also emerged, and one may speculate that this is again due to the heterogeneity of metabolism across cancer subtypes. Although HIFs are known to inhibit FAO, one study performed in liver cancer cells determined that HIF-1 also decreases ROS levels and maintains redox homeostasis, thereby promoting cell proliferation [136]. This effect is thought to be mediated by the action of HIF-1 on medium- and long-chain acyl-CoA dehydrogenases (MCAD and LCAD, respectively). This study further pointed to correlations between decreased LCAD expression and patient mortality rates [136]. Thus, we see that the precise role of HIFs varies across cancer types, and as such therapies targeting products in HIF-related pathways may need to be tailored to specific cancers to maximize their impact.

5.2 CPT1 Inhibitors Are Now in Clinical Trials

As far as pharmacological interventions for FAO are concerned, some CPT1 inhibitors are in development for other conditions such as diabetes [137]. Therefore, the possibility of repurposing them for cancer therapy is an intriguing possibility

requiring further clinical trials [137]. One CPT1 inhibitor, etomoxir, has been difficult to advance through clinical trials due to its toxicity. A clinical study examining etomoxir in healthy adults found elevated levels of transaminases in the livers of some patients, and the study had to be terminated early [138]. The issue with etomoxir arises from its inability to distinguish CPT1 across tissue types. However, it has been applied to preclinical studies of breast cancer, where an interesting degree of heterogeneity has been noted. In one study, etomoxir was compared across two cancer lines derived from a triple-negative breast cancer (TNBC). One line expressed high amounts of the oncogenic transcription factor MYC, whereas the other expressed low amounts of MYC. In the highly expressed line, application of etomoxir decreased levels of ATP, and this effect was not observed in the low-expressed line [128]. Furthermore, this effect was observed in no other breast cancer subtypes besides TNBC. This provides further evidence of the ways in which cancer heterogeneity should be appreciated and exploited for the development of viable treatments.

5.3 FAO Occurs at the Peroxisome Where Peroxisome Proliferator-Activated Receptors (PPARs) Act as Ligand-Activated Transcription Factors

FAO also occurs in peroxisomes. Oxidation at the peroxisome is restricted to very-long-chain fatty acids. The peroxisome breaks these very long chains into smaller chains which may then be further oxidized in the mitochondria. Peroxisomes are built via peroxins, the products of the Pex genes. So far, 3 of the 30 known peroxins, Pex3, Pex16, and Pex19, have been shown to be necessary for proper peroxisome assembly [139]. One of these peroxins, Pex19, was shown in a series of experiments involving the transition to malignancy in prostate cancer. Monocarboxylate transporter 2 (MCT2) is upregulated in prostate cancer and, like other MCTs, serves to facilitate the transport of lactic acid in glycolytic tumors. Immunoprecipitation experiments demonstrated that colocalization of MCT2 with peroxisomes was strongest at disease initiation and decreased as metastasis increased; furthermore, colocalization was absent in nonmalignant prostate cancer lines [140].

Other components of peroxisomes are the peroxisome proliferator-activated receptors (PPARs). Three PPARs (PPARα, PPARγ, PPARβ/δ) are known and have been described as ligand-activated transcription factors [141]. These differ predominantly in tissue distribution, and their exact functions in cancer remain ambiguous. It has been shown that PPARs are key regulators that integrate lipid metabolism and inflammation [142]. Furthermore, the PPARs have been directly implicated in a host of cancers as well as in cancer-related processes including carcinogenesis and chemoresistance [143, 144].

The theme of heterogeneity persists within the various PPARs and across species. For example, long-term PPARα agonism in rodents leads to the development of liver cancer. Interestingly, PPARα is expressed at lower levels in human liver relative to rodent liver, and as such PPARα agonism does not lead to liver cancer in human samples [143, 145]. PPARβ/δ displays tissue-wide distribution. One of its functions is to reduce oxidative stress, such as in breast cancer [146]. However, it is expressed ubiquitously and has been shown to be involved in many cancer types, particularly in cancers of hypoxic environments, such as breast, colon, lung, and ovarian cancers, as well as chronic lymphocytic leukemia [143]. Its precise role remains controversial, but it appears that PPARβ/δ may play a role as a lipid-activated mediator of an anti-inflammatory response. Like PPARβ/δ, mystery surrounds PPARγ. Although it may be coded for by four mRNAs (*PPARG1* through *PPARG4*), PPARγ1 and PPARγ2 are responsible for most PPARγ physiological action [143]. PPARγ1 mRNA is found ubiquitously, whereas PPARγ2 mRNA is restricted to adipocytes [147]. Some, but not all, PPARγ agonists induce apoptosis in cancer cells and have also been reported to induce terminal differentiation. Targets of PPARγ include many genes involved in the cell cycle and apoptosis in tumors, such as p53 and PTEN. The increasing characterization of PPARγ as a biomarker in cancer led some to speculate that it may be utilized in screens [148]. Together, the PPARs constitute an area of research that may prove critical in our understanding of tumor development and treatment.

The many aspects of peroxisomal signaling further convey the diversity of lipid signaling across many types of cancers. Abnormalities within the peroxisomes themselves or at PPARs can alter the efficacy of the critical lipid signaling that cells rely on. Further research, particularly in the form of genomic analyses, will be useful in harnessing this heterogeneity in personalized medicine approaches.

Conclusion

Therapeutic strategies targeting lipid metabolism are now in various stages of clinical development, and one approach worth highlighting is the “repurposing” of drugs from cardiology [149]. As emphasized, we urge caution on the significance of heterogeneity in cancer lipid metabolism as we translate basic science into clinical applications. Drug combinations have become a cornerstone against refractory and heterogeneous tumors, so the question now is *how* to combine treatment options for maximum safety and efficacy [150–152]. Going forward, systems biology and bioinformatics will likely become essential tools for integrating various levels of -omic data [153, 154]. Dissecting the spatial and temporal heterogeneity of lipid metabolism with these tools will likely accelerate the tailoring of clinical care according to patient-specific signatures, as envisioned by precision medicine (Fig. 1).

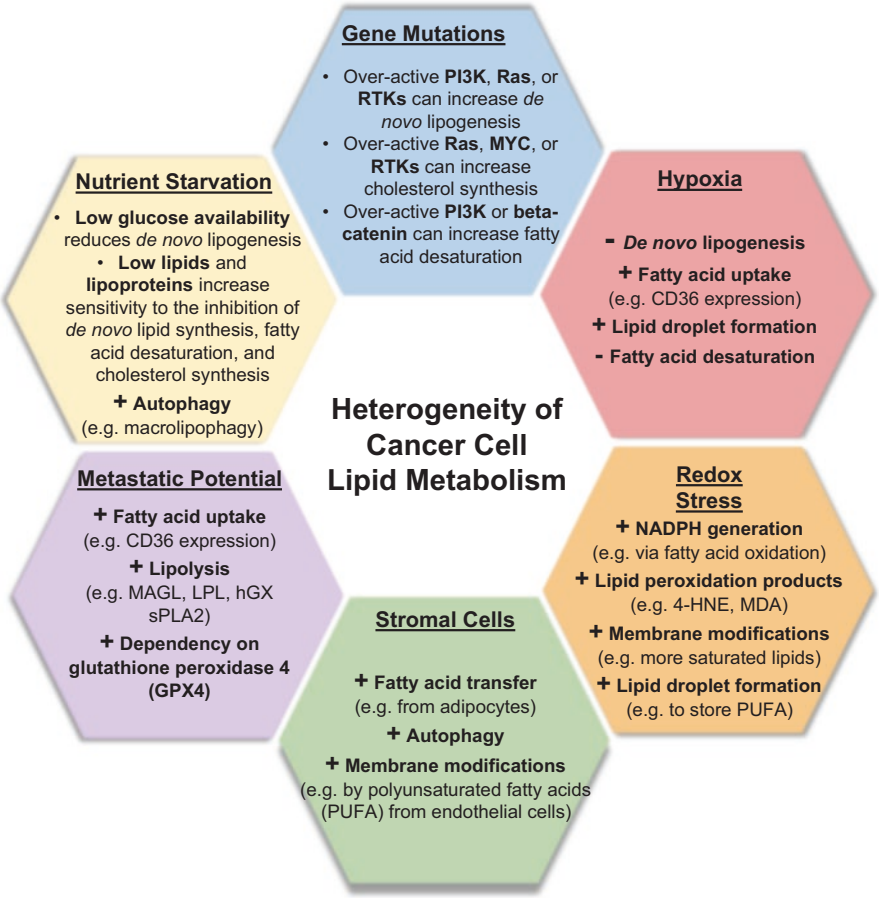

Fig. 1 Factors that can contribute to spatial and temporal heterogeneity in cancer cell lipid metabolism

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Part II
Heterogeneity of Cancer Metabolism

The Multifaceted Metabolism of Glioblastoma

Addison Quinones and Anne Le

Key Points

- Glioblastoma (GBM) can be categorized into different subtypes based on diverse metabolic profiles.
- Characteristic genomic alterations lead to transformed metabolism.
- Synergistic therapies are beneficial to combat dynamic adaptations of glioblastoma metabolism.
- Advanced-grade brain tumors exhibit distinct metabolic profiles compared to lower-grade tumors.

Keywords Glioblastoma · Metabolic profile · Glutamine metabolism · IDH1 mutation · mTOR signaling · Cysteine catabolism

Abbreviations

2HG	2-Hydroxyglutarate
aKG	Alpha-ketoglutarate
BPTES	Bis-2-(5-Phenylacetamido-1,3,4-thiadiazol-2-yl)ethyl sulfide
CDKN2A	Cyclin-dependent kinase inhibitor 2A
CDO	Cysteine dioxygenase
CSA	Cysteine sulfinic acid
EGFR	Epidermal growth factor receptor
GABRA1	Gamma-aminobutyric acid type A receptor alpha-1

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GBM	Glioblastoma
GDH	Glutamate dehydrogenase
GLS	Glutaminase
GSH	Glutathione
HK	Hexokinase
IDH	Isocitrate dehydrogenase
M2	Macrophage 2
MAX	Myc-associated factor X
mTOR	Mechanistic target of rapamycin or mammalian target of rapamycin
NEFL	Neurofilament light
NF1	Neurofibromatosis type 1
p53	Phosphoprotein 53
PDGFRA	Platelet-derived growth factor receptor alpha
PDH	Pyruvate dehydrogenase
PI3K	Phosphoinositide 3-kinase
PTEN	Phosphatase and tensin homolog
ROS	Reactive oxygen species
RTK	Receptor tyrosine kinase
SLC12A5	Solute carrier family 12 member 5
SYT1	Synaptotagmin 1
TCA	Tricarboxylic acid
TET	Ten-eleven translocation

Introduction

Glioblastoma multiforme (GBM) develops on glial cells and is the most common, as well as the deadliest, form of brain cancer [1]. As in pancreatic cancers, distinct combinations of genetic alterations in GBM subtypes induce a multiplicity of metabolic phenotypes, which explains the variability of GBM sensitivity to current therapies targeting its reprogrammed metabolism. Therefore, it is becoming imperative for cancer researchers to account for the metabolic heterogeneity within this cancer type before making generalized conclusions about a particular drug's efficacy against all cancers of that type. GBMs can be classified initially into two subsets consisting of primary and secondary GBMs, and this categorization stems from cancer development. GBM is the highest grade of gliomas, which includes glioma I, glioma II, glioma III, and glioma IV (GBM). Secondary GBM develops from a low-grade glioma to advanced stage cancer, while primary GBM provides no signs of progression and is identified as an advanced stage glioma from the onset. The differences in prognosis and histology correlated with each classification are normally negligible, but the demographics of individuals affected and the accompanying genetic/metabolic properties show distinct differentiations [2].

1 GBM Classifications Based Upon Metabolic Profile

Previously, tumors had been classified based on histological and structural similarities without accounting for clinical disparities among them [3]. More recently, tumor classification has shifted toward a more molecular and genetic basis in combination with phenotypic information. This new-era classification allows practitioners to differentiate between biologically similar cases, allowing for more precise treatment and prognosis when encountering distinct mutant variants [3]. Phenotypic information must be used in accordance with genotypic data to determine tumor type and grade differentiations and to account for the rare occurrences when the phenotype differs from the usual criteria accompanying the defined genotype [4].

A recent study identified four gene expression subtypes of GBM: mesenchymal, classical, proneural, and neural (Fig. 1). The mesenchymal subtype is characterized by high mutation rates of the tumor suppressor genes: neurofibromatosis type 1 (NF1), phosphatase and tensin homolog (PTEN), and phosphoprotein 53 (p53). Following aggressive treatment, mesenchymal groups frequently display substantial increases in length of survival [5]. Classical GBM is defined by focal epidermal growth factor receptor (EGFR) mutation events in much larger frequencies than in the other three subtypes while containing zero mutations of the most altered gene in GBMs: p53. Similar to the mesenchymal subtype, classical groups tend to show the greatest survival rates of all the subtypes when subjected to aggressive treatments [5]. The proneural subtype carries mutations of p53, platelet-derived growth factor receptor alpha (PDGFRA), and isocitrate dehydrogenase-1 (IDH1) [5]. IDH1 and PDGFRA mutations can result in irregular cell/tumor growth. Proneural patients are characteristically younger than other subtype patients and have longer survival, but their survival remains constant whether they are exposed to aggressive treatment or not. The neural subtype is categorized based on the overexpression of neurofilament

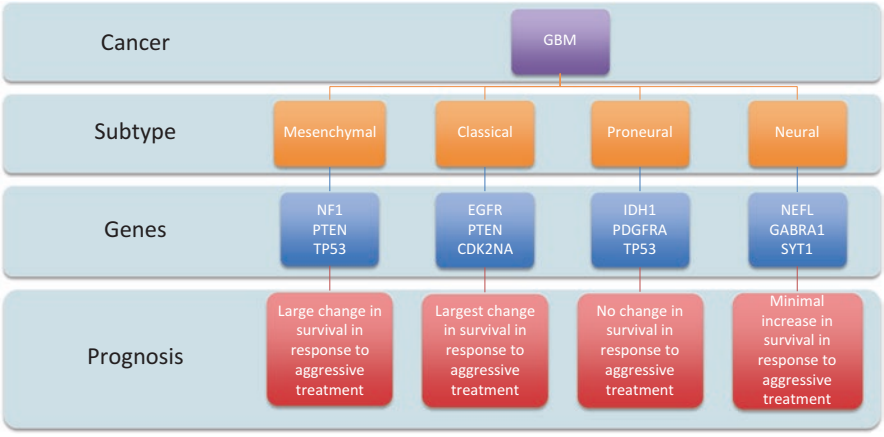

Fig. 1 Subtypes of glioblastoma, including the major genes altered, and effect on prognosis following treatment

light (NFL), gamma-aminobutyric acid type a receptor alpha-1(GABRA1), synaptotagmin 1 (SYT1), and solute carrier family 12 member 5 (SLC12A5) neural markers. The gene expressions present within the neural subtype have been determined to be the most similar to normal brain tissue and are weakly characterized. Furthermore, studies have demonstrated that classical and mesenchymal subtypes have a better response to therapy and better prognosis compared to the proneural subtype [5]. Data suggests average efficacy of treatment in the neural subtype, but it is not as effective as treatments of classical and mesenchymal subtypes [5]. These unique genetic alterations leading to subtype classifications result in different metabolic profiles of cancers depending on the specific genes altered. Their effects on metabolism will be discussed in the next section.

Glioblastomas are also divided into different groups based on the World Health Organization (WHO) classification system. Wild-type IDH accounts for 90% of diagnosed GBMs, which have a high correlation to primary GBM, especially in elderly patients. IDH-mutant GBMs occur in about 10% of patients and have a higher correlation to secondary glioblastomas, which are glioblastomas progressing from previous lower-grade gliomas. The final classification is reserved for cases in which a complete IDH evaluation cannot be completed. With all the heterogeneity that exists within glioblastomas, the classifications are constantly changing, and the variants and patterns must frequently be updated to keep up with the evolving characteristics.

The complex ecosystems created by human cancers, including the phenotype, genotype, and epigenetic states that are presented in patients, create a large range of intratumoral heterogeneity of metabolism [6–10]. In a study by Patel et al., single-cell RNA sequencing was used to create a profile for 430 cells harvested from 5 diverse glioblastomas. These cancers were categorized on the basis of oncogenic signaling, proliferation, complement/immune response, and hypoxia. Variability between singular tumors is extremely evident, which leads to different stages, expressions, and outcomes for therapeutic strategies [11]. What has made therapy more difficult is the existing intratumoral heterogeneity within individual gliomas. Distinct cells contained within the same tumor can present with different mutations and phenotypic or epigenetic states, resulting in different subtypes being found in different cells of the same tumor. These variances within the same tumor ultimately lead to the inefficiency of treatment and cancer recurrence. Thus, these studies suggest that synergistic treatments will be the direction of new therapeutic strategies [11].

2 Genomic Alterations Lead to Distinct Cancer Cell Metabolisms

As previously mentioned, GBMs can be classified according to their genetic and metabolic profiles. Genetic mutations are the causes for deviance of metabolism from the status quo. As genes are overexpressed, inactivated, or mutated, it leads to downstream effects occurring throughout the whole cell. These downstream effects

are fluctuations from a normal brain cell's metabolism disrupting cellular function. These disruptions can be identified and investigated to potentially develop therapeutic strategies, which is the current goal of cancer metabolism research.

2.1 NF1 Mutations Result in Unregulated Growth Signaling

There has been speculation that GBM subtypes do not remain stagnant during disease progression nor while being bombarded with varying treatments [12]. A study by Wang et al. investigated phenotypic plasticity and genetic drivers behind the evolution of proneural, classical, and mesenchymal subtypes. Samples were collected from varying gliomas at the time of diagnosis as well as at the first onset of GBM recurrence, and genetic profiles were created to establish their molecular subtypes for comparison. After analysis, 50 of 91 (55%) samples had their expression subtypes remain constant. After recurrence, the quantity of proneural and mesenchymal subtypes had increased, and the quantity of classical subtypes had decreased. There was no direct correlation observed between proneural and mesenchymal subtypes, as expected. The intratumoral heterogeneity of the initially collected samples was taken into account, and the samples with the lowest purity were typically the groups to undergo a transition of subtype. The mesenchymal subtype was discovered to have a large association with both the tumor-promoting M2 macrophage gene and the deactivation of NF1 [12]. This suggests a pathway linking the loss of function of NF1 to promoting macrophage/microglia recruitment and invasion of the tumor microenvironment, leading to a poorer prognosis for patients afflicted with mesenchymal subtype expression factors [12]. High-grade gliomas containing altered NF1 frequently have an associated deactivation of cyclin-dependent kinase inhibitor 2A (CDKN2A) that inhibits Ras-mediated growth signaling, allowing NF1 to be labeled as another tumor suppressor gene in the central nervous system. As a consequence of losing NF1 function, Ras activity stimulates Ras effectors (PI3K, PAK, RAF, ERK1/2), increasing the proliferation of astrocytes, contributing to GBM growth [13].

2.2 IDH1 Mutations Lead to Oncometabolite Production and Glutamine Addiction and Act as a Prognostic Marker

A study by Dang et al. demonstrated that mutations in IDH1 give rise to a novel function of this enzyme, which produces (R)-2-hydroxyglutarate (2HG) from alpha-ketoglutarate (aKG) (Fig. 2) [14]. It was reported by Struys et al. that "2HG" in fact does not exist [15]. The compound has an asymmetric carbon atom that leads to L-2HG and D-2HG, which are both regular endogenous metabolites in all bodily fluids. Routine analytical methods measure the sum of these two metabolites, which creates problems because IDH1 mutations solely result in increased levels of

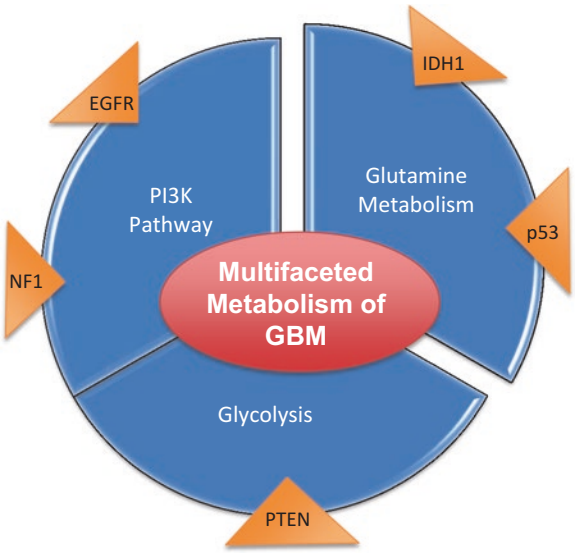

Fig. 2 Genomic alterations (indicated in orange triangular panels) leading to changes in specific pathways (indicated by blue sections) that contribute to growth and survival of GBM

D-2HG. Therefore, an increase in L-2HG could yield false positives, and miniscule increases of D-2HG may be missed. This is why analytical methods able to distinguish L-2HG from D-2HG must be used [15, 16]. It was determined that D-2HG is not only found in glioblastoma but has also been found to be sufficient in promoting several other types of cancers, such as leukemia, through mutations in a homolog to IDH1 and IDH2 [17, 18]. D-2HG retains a structure similar to that of aKG, thus inhibiting enzymes from binding to aKG. 2HG inhibits aKG-dependent histone demethylases and occupies the active site of CeKDM7A, which is where aKG usually binds. 2HG also interferes with 10–11 translocation (TET) family interactions, which promotes hyper-methylation, triggering cancer proliferation and preservation [18]. These mutations render cancer cells addicted to glutamine as aKG, which feeds into the tricarboxylic acid (TCA) cycle after the conversion of glutamine to glutamate via glutaminase (GLS). Thus, glioblastoma cells with IDH1 mutations are more sensitive to GLS inhibition by BPTES, a small-molecule inhibitor of GLS, as compared to their wild-type counterparts [19]. The reduction of proliferation was modest (20%), and it induced no apoptosis of malignant cells. Bis-2-(5-phenylacetamido-1,3,4-thiadiazol-2-yl)ethyl sulfide (BPTES) is a selective glutaminase inhibitor. Metabolic dependence on aKG was confirmed through rescue experiments showing that the supplement of an aKG reduced the impact of BPTES on proliferation hindrance. However, wild-type IDH1 and IDH1 mutants were equally sensitive to glutamine deprivation, suggesting there are different downstream effectors active when considering inhibition of glutamine conversion versus inhibition of glutamine uptake. Metabolomics analysis provided interesting findings that 2HG levels remained constant between the IDH1-mutant BPTES-treated group and the IDH1-mutant non-treated group. However, the glycolytic intermediate levels increased and TCA cycle intermediates had decreased levels in the group treated

Table 1 Data analysis and statistics between wild-type versus mutant IDH1 patient demographics, prognoses, and genetic alterations

	Mutant IDH1	Wild-type IDH1
Primary GBM cases	14	363
Secondary GBM cases	22	8
Median age	47.9 years	60.6 years
Survival	27.1 months	11.3 months
TP53 mutant	81%	26.6%
EGFR mutant	6.5%	35%

with BPTES when compared to the non-treated group. The increase of glycolytic intermediates and decrease of TCA cycle intermediates can be attributed to compensatory mechanisms attempting to maintain aKG levels by reallocating glutamine from the TCA cycle to glycolysis [19]. The message from this specific example is that the mutations of IDH1 form a subset of glioblastomas that exhibit a shift toward glutamine-dependent energy pathways. These pathways allow tumor cells to best utilize glutamine and its products in order to produce ATP as a fuel source for biosynthetic pathways, an ability that is particularly advantageous when glucose is scarce. GLS inhibition and methods for augmenting inhibition could serve as a potential therapeutic target, but they will most likely need to be used in conjunction with other treatment regiments to combat the dynamic properties of cellular metabolism [19].

Unexpectedly, in a study by Nobusawa et al., individuals with secondary glioblastoma who possessed IDH1 mutations had a more favorable prognosis [20]. Histologically, primary and secondary glioblastomas are identical; thus, clinical data is relied upon to determine their subdivision because they occur in patients with different ages and develop through different pathways [21, 22]. Through statistical analyses, it was determined that there was a positive correlation between IDH1 mutations accompanied by p53 mutations, diagnosis of secondary glioblastoma, longer median survival, and younger patient age [23]. WT IDH1 genes correlated with older patients, shorter median survival, and higher EGFR amplification. Secondary glioblastomas make up a smaller fraction of the disease compared to primary, but of the secondary cases sampled, a mutant IDH1 affected the majority, while a vast minority of primary glioblastomas were affected (Table 1). For the cases originally diagnosed as secondary, which did not harbor an IDH1 mutation, and the cases originally diagnosed as primary, which contained an IDH1 mutation, further analysis showed that these cases were likely misdiagnosed. The “primary” diagnosed patients with mutant IDH1 had every characteristic of secondary glioblastoma, while the opposite was true for “secondary” glioblastomas with WT IDH1. These discrepancies suggest incorrect diagnoses for gliomas that may have started at a low grade then progressed quickly making them look like a primary or higher-grade glioma with some progression disguising it as secondary. Nobusawa et al. accurately identified a reliable signature marker for secondary glioblastoma and a more favorable outcome [20].

Furthermore, another study by Labussiere et al. showed that individuals with IDH1-mutant tumors lived longer than those who had wild-type IDH1 tumors, despite all tumors being of the same grade [24]. Another classical function of IDH1 is to support oxidative carboxylation of isocitrate to aKG coupled with the reduction of NADPH (Fig. 2), allowing NADPH to promote further reduction of glutathione (GSH), a crucial antioxidant [25, 26]. When glioblastomas have a mutant IDH1, they lose normal enzymatic function, lowering production of aKG and NADPH while increasing 2HG [14]. The surge of 2HG increases oxidative stress present in cancer cells and the accumulation of reactive oxygen species (ROS) which encourages tumor cell growth [27]. However, inflicted damage by ROS is limited by increased GSH creating a paradox. An IDH1 mutation encourages tumor progression through ROS evolution but also counteracts that damage by introducing elevated levels of GSH [28]. These conclusions suggest the need for further analyses on the mechanistic links between metabolic phenotype and clinical outcome.

2.3 EGFR Mutations Shift Cancer Cells Toward a Glycolytic Phenotype and Permit Survival Under Glucose-Deprived Conditions

Mutations in EGFR provide an additional example of genetic alterations that lead to changes in cancer cell metabolism [29]. In their study, Babic et al. revealed an activating EGFRvIII mutation, which causes an intracellular increase in heterogeneous nuclear ribonucleoprotein (hnRNP) A1 splicing factor. This upregulation, in turn, promotes the splicing of Myc-associated factor X (MAX), a partner protein of Myc, which ultimately results in the expression of genes that encode for glycolytic enzymes, a glycolytic phenotype in aggressive glioblastoma tumors, and shorter patient survival time [29]. In SF188 glioblastoma, high amplification of Myc activates glutamate dehydrogenase (GDH), enabling cancer cell survival under glucose deprivation [30]. GDH, an enzyme necessary for the conversion of glutamate to aKG for uptake into the TCA cycle, is upregulated in the absence of glucose. This upregulation allows glioblastoma cells to maximize the use of glutamine and thus contributes to the growth and proliferation of neoplastic cell growth in the absence of glucose [30].

Growth factor signaling pathways are responsible for cellular metabolism, proliferation, and environmental adaptation [31, 32]. The growth factor signaling pathways are heavily dependent upon regulation from receptor tyrosine kinases (RTKs), showing that genetic mutations in RTKs lead to variable progression and growth of tumors stemming from the changes in the signaling pathways such as EGFR [33]. Furnari et al. used mouse models in correlation with clinical samples wherein the mouse growth factor signaling pathways were genetically modified to match clinical samples [34]. The corresponding GBMs were determined to be histologically identical, indicating the importance of RTK alterations in the progression of GBMs. In a study of 251 patient-derived GBMs comprised mainly of de

novo GBMs (95%), there were alterations to RTKs in 66% of the samples, and the dominant alteration was to EGFR. This lesion was usually accompanied by other PI3K activation, alteration, and deletion of CDKN2A. EGFRvIII^{+} cells had a higher proliferation rate with less cell death in xenograft models when using EGFR-targeted therapies and showed increased glycolysis to fulfill the energy demand [32, 33, 35, 36]. Further studies are required to evaluate mechanisms utilized by GBMs to sustain growth based on their environment.

2.4 PTEN Mutations Lead to High Rates of Glycolysis, Facilitating Survival in Harsh Microenvironments

In a study by Wolf et al., GBM with loss of PTEN activity demonstrated high expression of the glycolytic enzyme hexokinase 2 (HK2), a major facilitator of aerobic glycolysis in GBM, providing survival and proliferative advantage in harsh tumor microenvironments [37]. HK2 is expressed in basal levels in adipose and skeletal tissue, but it is not expressed in normal brain tissue, which typically expresses HK1. Inhibition of HK2, without interfering with HK1 function, by siRNA led to a reverse of the Warburg effect to oxidative glucose metabolism, which ultimately led to impaired tumor growth. Also, HK2 inhibition sensitized GBM cells to multiple treatments including the following: (1) temozolomide, the current chemotherapeutic for GBM treatment; (2) radiation; and (3) hypoxia-induced apoptosis. Therefore, high HK2 expression predicted poorer overall survival [37]. These findings again support the genetically evolved metabolic heterogeneity in cancer cells.

2.5 p53 Mutations Result in Activation of the Warburg Effect

Tumor suppressor gene p53 has been identified as a gene commonly mutated in many cancers, including GBM cancer [38]. p53 serves to initiate cell cycle arrest and apoptosis when the cell is subjected to stressors including hypoxia, hyperproliferative signals, nutrient deprivation, and DNA damage [39, 40]. These stressors can be indicative of inchoate tumor cells in the microenvironment, resulting in activation of p53 to inhibit initiation, progression, and replication of oncogenic mutations. Mutant p53 genes typically lead to complete inactivation of p53, which is characterized by a higher malignancy of cancer through greater rates of metastasis, genetic instability, and cellular differentiation [41–43]. Novel functions of p53 have emerged showing its potential to regulate cellular metabolism. A signature feature of cancer is its reliance on the Warburg effect. p53 has shown to counter this oncogenic hallmark by activating synthesis of cytochrome oxidase 2 to incite oxidative phosphorylation and inhibiting glycolysis by repressing glucose transporters (SLC2A1, SLC2A4). Thus, the Warburg effect is more profound when p53 is

inactivated [44–46]. Recently, there have been contradictory studies showing that the impact of p53 as a tumor suppressant is not as compelling as originally suggested. These studies found evidence to support the claim that cell cycle arrest and apoptosis, as a result of DNA damage, are not large contributors to tumor suppression depending on the cancer tissue examined [38]. These findings have yet to be discovered when discussing GBM development, but further examination is necessary.

3 Multiple Facets of GBM Metabolism Due to Cancer Cell Adaptation

Cancer cells have a higher consumption rate of glucose when compared to their benign counterparts. As glucose is processed through the TCA cycle, glutamine is produced as one of the intermediates. Glutamine serves as the major contributor to cell growth and energy production after it is processed by GLS into glutamate and GDH into aKG. For this reason, GLS inhibition has become a popular therapeutic strategy to treat cancer patients. The mechanistic target of rapamycin (mTOR) is a protein kinase that promotes oncogenic signaling through the phosphoinositide 3-kinase (PI3K) pathway, which in turn promotes cancer growth [47]. This has also made mTOR a popular target for cancers using PI3K as a major pathway [48]. mTOR has been identified as a primary factor in downstream signaling for EGFR-mutant GBM, which is resistant to kinase inhibitors [49]. In a study by Tanaka et al., they found that mTOR-targeted treatments affected glutamine catabolism, increasing GLS expression, which is already highly expressed in GBM patients. mTOR-targeted treatments (by rapamycin or PP242) limited cell proliferation, glucose usage, and lactate production [48]. However, it was ineffective in promoting cell death. Following these results, Tanaka et al. performed an experiment in which the U87 and EGFRvIII GBM cells were subjected to glutamine deprivation through compound 968 (GLS inhibitor) and then treated with PP242, which was seen as the more effective mTOR-targeted treatment. Results showed that when used in combination, the GLS- and mTOR-targeted therapies yielded a synergistic effect triggering enhanced tumor cell death compared to when either treatment was applied individually [48]. This combined treatment was then tested on normal human astroglial cells, and the results revealed that the treatment did not cause any normal cell death to occur. This synergistic treatment was then tested on GBM xenograft models of U87 and EGFRvIII GBM samples. The treatment resulted in 80% shrinkage of tumors and a sixfold increase in cell death from mTOR treatment alone. To determine the effects of the drug on body systems and motor function, the same treatment was applied to normal mice. There were no changes in body weight, motor function, or cell morphology, indicating that this has the potential to be developed into an effective treatment for mTOR-targeted resistant GBM cancers [48]. Studies have exhibited the effectiveness of treating an altered metabolism with therapies directed at its specific metabolic profile.

4 Benefits of Combined Therapy

Among the many struggles in treating cancer, tackling its inherent metabolic heterogeneity is a major obstacle. Metabolic pathways relevant to GBM have been established, but those pathways are dynamic, and cancer cells alter their metabolism as their environments change. When a pathway is hit and deactivated, the ability of a cancer cell to work around it contributes to the complexity of treatments. After multiple pathways are knocked out, a cancer cell's metabolism will eventually be cornered with nowhere to turn. This is the strategy employed by Tanaka et al. when combining therapies inhibiting mTOR and GLS to limit cancer cell proliferation [48]. As the PI3K is inactivated by mTOR-targeted treatments, GBM switches to higher expressions of GLS to rescue it from apoptosis [48]. Combining the mTOR treatment with GLS inhibition essentially traps certain GBM cells so that their metabolism cannot shift pathways to encourage cell survival. Heterogeneity creates problems when determining treatment because different metabolic profiles result in differences as to how cancer metabolism will change in response to treatment. These synergistic treatments are beneficial because they can work together when attempting to target different pathways, but the challenge remains to affect only the cancerous cells while not having a detrimental impact on benign cells.

5 Advanced Brain Tumors (GBM) Display Distinct Metabolic Profile as Compared to Lower-Grade Tumors

Similar to IDH1 mutations, which can distinguish between primary and secondary GBM, another novel metabolic pathway has been identified in high-grade tumorigenesis involving cysteine catabolism which is not highly activated in lower-grade tumorigenesis [50]. The traditional pathway of cysteine begins with a simultaneous efflux of glutamate and influx of cystine, which is then reduced to cysteine and converted to GSH when combined with glutamate and glycine. GSH functions as a central nervous system antioxidant increasing cancer cell survival when subjected to redox stress and hypoxia [51]. Prabhu et al. investigated an alternative pathway of cysteine, which results in an accumulation of cysteine sulfinic acid (CSA) through the regulatory enzyme cysteine dioxygenase-1 (CDO1). When comparing grade 2 gliomas, there was a 23-fold increase in the relative accumulation of CSA in GBM, ranking it as the highest of any metabolite. This increase of metabolite concentration correlated with a higher expression of the CDO1 enzyme in GBM when evaluated using Western blot analysis. The buildup of CSA inhibited cellular respiration and decreased both oxidative phosphorylation and ATP production. CSA modulates mitochondrial function through inhibition of pyruvate dehydrogenase (PDH). PDH functions as a channel enzyme controlling the rate at which glycolysis occurs, and this enzyme was inhibited in a dose-dependent manner when treated with CSA using established GBM cell lines (U251). It was determined that GBM proliferation was only repressed when tested in vivo, proving that further

investigation is needed to determine how this alternative pathway of cysteine catabolism contributes to GBM tumorigenesis [50]. The analysis was conducted to uncover the impacts PDH modulation had on tumorigenesis in GBM samples. It was determined that PDH phosphatase expression regulated PDH activity as a result of Ras-mediated signaling. When the impairment of PDH was reversed, it inhibited tumor growth, making this pathway a possible therapeutic target to treat GBM in the future.

Conclusion

Glioblastoma is the most common and deadliest form of brain cancer in humans. Its poor prognosis and unreliable diagnosis are a result of its intricate heterogeneity and evolutionary characteristics. Experts have made substantial progress in characterizing this cancer with the use of improved technologies; most recently, there has been a spotlight on the use of metabolomics to discover its underlying molecular mechanisms. As more data and results are obtained to determine how different glioblastomas function and why they function that way, more therapeutic strategies can be developed to treat patients individually with respect to their genotypic and phenotypic profiles.

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The Intricate Metabolism of Pancreatic Cancers

Felipe Camelo and Anne Le

Key Points

- Oncogenic *KRAS* regulates glucose and glutamine metabolism in pancreatic cancer cells.
- MUC1 overexpression leads to increased glucose metabolism.
- p53 functions predict the sensitivity of pancreatic cancer tumors to glycolytic inhibition.
- Targeting alpha-ketoglutarate dehydrogenase function by CPI-613 to slow mitochondrial metabolism.
- The antidiabetic drug, metformin, targets pancreatic cancer stem cells.
- Combined therapy is used to target pancreatic metabolism heterogeneity.

Keywords Pancreatic ductal adenocarcinoma · *KRAS* mutation · Glucose metabolism · Glutamine metabolism · Combined therapy

Abbreviations

ASP	Aspartate
EGFR	Epidermal growth factor receptor
GLS	Glutaminase
GLUD1	Glutamate dehydrogenase 1

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GLUT	Glucose transporter
GOT1	Glutamic-oxaloacetic transaminase 1
HIF-1α	Hypoxia-inducible factor 1-alpha
HK2	Hexokinase 2
KRAS	Kirsten rat sarcoma viral oncogene homolog
LDH	Lactate dehydrogenase
MCT	Monocarboxylate transporter
OAA	Oxaloacetate
PDAC	Pancreatic ductal adenocarcinoma
PFK1	Phosphofructokinase 1
TCA	Tricarboxylic acid cycle

Introduction

Currently, approximately 95% of pancreatic cancers are pancreatic ductal adenocarcinoma (PDAC), which is the most aggressive form and the fourth leading cause of cancer death with extremely poor prognosis [1]. Poor prognosis is primarily attributed to the late diagnosis of the disease when patients are no longer candidates for surgical resection [2]. Cancer cells are dependent on the oncogenes that allow them to proliferate limitlessly. Thus, targeting the expression of known oncogenes in pancreatic cancer has been shown to lead to more effective treatment [3]. This chapter will discuss the complexity of metabolic features in pancreatic cancers. To be able to fully comprehend the heterogeneous nature of cancer metabolism, we need to take into account the close relationship between cancer metabolism and genetics. Gene expression varies tremendously, not only among different types of cancers, but also within the same type of cancer among different patients. Cancer metabolism heterogeneity is often prompted and perpetuated not only by genetic mutations in oncogenes and tumor suppressor genes but also by the innate diversity of the tumor microenvironment. Much effort has been focused on elucidating the genetic alterations that correlate with disease progression and treatment response [4]. However, the precise mechanism by which tumor metabolism contributes to cancer growth, survival, mobility, and aggressiveness represents a functional readout of tumor progression.

1 Oncogenic *KRAS* Regulates Metabolism in Pancreatic Cancer Cells (Fig. 1)

1.1 Oncogenic KRAS Regulates Glutamine Metabolism

A cancer cell's specific metabolic adaptations in nutrient uptake and biosynthesis have been linked to a particular genetic mutation. The *KRAS* (Kirsten rat sarcoma) oncogene homolog is a known regulator of glutamine metabolism among other

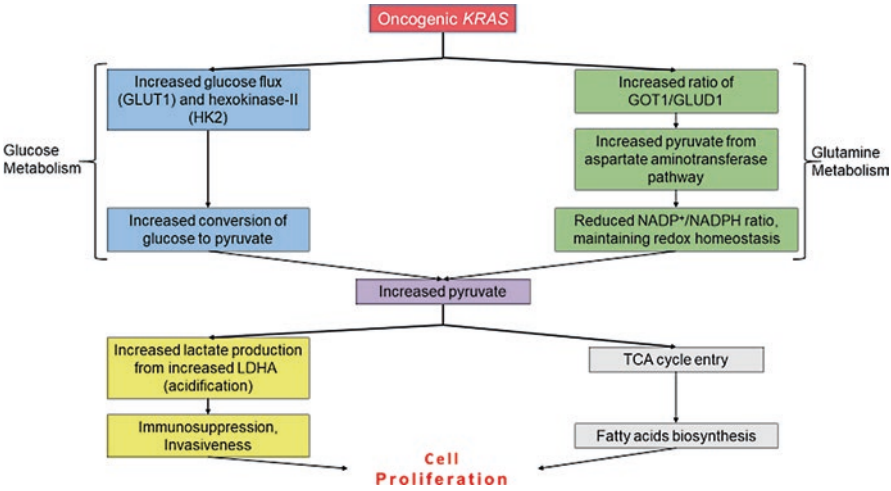

Fig. 1 Oncogenic KRAS regulates glutamine and glucose metabolism in PDAC

intermediary metabolic pathways that renders cancer cells addicted to glutamine [5–7]. A range of mutations in the *KRAS* oncogene occurs in over 90% of PDAC [8, 9].

Normally, glutamate feeds into the TCA cycle after being converted to alpha-ketoglutarate in the mitochondrion via glutamate dehydrogenase 1 (GLUD1). A study by Son et al. showed that KRAS regulated the reprogramming of glutamine metabolism through transcriptional regulation of key metabolic enzymes of transaminase reactions which, in turn, determine PDAC tumor growth. Notably, they concluded that PDAC cells greatly depend on these reactions for redox homeostasis. Given that this pathway is nonessential in normal cells, the unique importance of this pathway in PDAC suggests novel approaches to therapy in treating PDAC [6]. *KRAS* mutation led to the reprogramming of glutamine metabolism, which was partially due to increased aspartate aminotransferase or Glutamic-oxaloacetic transaminase 1 (GOT1) expression and decreased GLUD1 expression. The change in the ratio of expression of GOT1 and GLUD1 shunts glutamine flux through the aspartate aminotransferase pathway. Furthermore, they demonstrated that GOT knockdown failed to impair growth in several normal cell lines. According to Lyssiotis et al., the observation that the glutamine metabolism pathway is downstream of mutant *KRAS* serves as an explanation for the distinct glutamine dependency of pancreatic cancer. Not only do their results yield novel targets for pancreatic cancer therapy, but they also suggest that inhibiting glutamine metabolism in pancreatic cancer therapies may synergize with therapies that increase ROS [7].

1.2 Oncogenic KRAS Regulates Glucose Metabolism

The *KRAS* oncogene is also known to contribute to the glucose metabolism in pancreatic cancer cells via upregulation of glucose uptake and diversion of glucose into the hexosamine biosynthesis pathways [10]. Oncogenic *KRAS* controls the diversion of glycolytic intermediates into ribose biosynthesis pathways via upregulation of the non-oxidative pentose phosphate pathway (PPP), a pathway that is fundamental to nucleic acid synthesis and thus cancer cell proliferation [10]. Expression of GLUT1 (glucose transporter-1), hexokinase-II (HK2), and LDHA that catalyzes the reaction of pyruvate to lactate is greatly enhanced by *KRAS* in pancreatic tumor cells [10]. Subsequently, glycolytic flux, the production of lactate from glucose, was high in *KRAS*-mutant tumors. It is of note that these alterations are not nearly as pronounced in the stromal cells of these tumors which are able to uptake the lactate generated by tumor cells and use pyruvate dehydrogenase (PDH) to convert the lactate back to pyruvate in order to fuel the TCA cycle [11, 12]. Yun et al. found that cells with mutated *KRAS* undergo the Warburg effect and survive in low-glucose environments compared to cells with wild-type *KRAS* due to the fact that *KRAS* upregulated GLUT1 [13]. This suggests that *KRAS* mutation is involved in the altering of a cancer cell's bioenergetics that is seen in most PDAC tumor cells, which take advantage of altered metabolic pathways to successfully proliferate and grow.

2 Other Alternative Metabolisms in Pancreatic Cancer

2.1 MUC1 Overexpression Leads to Increased Glucose Metabolism

A study by Chaika et al. revealed that the overexpression of transmembrane protein MUC1 led to elevated glucose metabolism and related activities, such as increased glucose uptake and lactate production resulting from increases in GLUT1 expression and LDHA expression, respectively. These metabolic effects are particularly pronounced under hypoxic conditions, which are associated with the stabilization of hypoxia-inducible factor 1-alpha (HIF-1α), a transcription factor for many genes involved in regulating glucose uptake, through the overexpression of MUC1 [14]. Pancreatic cancer cells that do not overexpress MUC1 have a reduction in lactate and glycolytic intermediates. Overall, the overexpression of MUC1 is capable of influencing glucose metabolism, the elevation of amino acid metabolism, and the TCA cycle, all of which are important in the biosynthesis of cellular building blocks, and thus tumorigenesis. The signaling pathway associated between MUC1 and HIF-1α plays an important role in the facilitation of tumor growth and metastasis, serving as a potential target for manipulation in the treatment of diseases reliant upon these proteins [14].

2.2 p53 Functions Predict the Sensitivity of Pancreatic Cancer Tumors to Glycolytic Inhibition

The heterogeneity of metabolic alterations within the same cancer types is best illustrated by a recent study by Rajeshkumar et al. They showed that PDAC's sensitivity to the same metabolic inhibition could vary drastically from one tumor to another, depending on the specific tumor's genetic status and unique metabolic phenotype [15]. More specifically, they uncovered that responses to LDHA inhibition by the small-molecule FX11 were determined by a tumor's p53 status, a tumor suppressor gene that is largely inactivated in many cancers [16]. Within the same PDAC type, tumors with wild-type TP53 demonstrated resistance to FX11, while those with mutant TP53 exhibited sensitivity in the form of increased apoptosis, reduced proliferation, and attenuated tumor growth. Their data show that FX11 specifically reduces pyruvate to lactate conversion by LDHA *only* in the *TP53*-mutant tumor, suggesting LDHA inhibition as a possible therapeutic target to reduce TP53-mutant tumor growth. Resistance in *TP53*-WT tumors is thought to result from reduced dependence on glucose, as corroborated by their data showing higher levels of TIGAR, a p53-inducible protein that lowers glycolytic flux [17]. This study supports not only growing evidence for variable metabolic phenotypes across cancer types but also within cancers of the same type. From a clinical perspective, this insight emphasizes the importance of metabolic phenotypes in pancreatic cancer sub-characterization in order to pair drug therapies according to phenotypic sensitivity for a more selective and personalized treatment.

3 Suggested Therapy (Fig. 2)

The *KRAS* gene may be a solution to this type of disease since *KRAS* appears to have a prominent role in the metabolic rewiring of PDAC tumors and plays critical roles in PDAC pathogenesis [9]. While oncogenic *KRAS* alters the PDAC cell's metabolism, it requires the cancer cell to become dependent on the oncogenic *KRAS* to continue proliferation [18]. This is known as oncogene addiction, in which the cancer cell becomes dependent on the activity of the oncogene for survival and proliferation [3]. Since *KRAS* mutations are found in a majority of PDAC cancer cells and *KRAS* regulates cancer cell's metabolism, targeting these regulations for cancer therapy is an approach that researchers are taking [18].

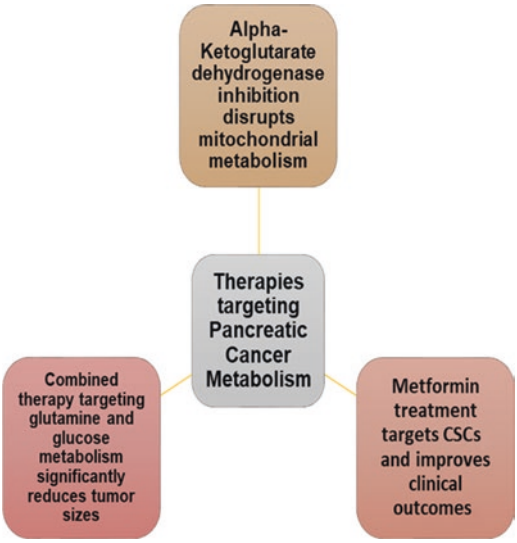

Fig. 2 Overview of therapeutic options targeting pancreatic cancer metabolism

3.1 Alpha-Ketoglutarate Dehydrogenase Function by CPI-613 to Slow Mitochondrial Metabolism

Drugs have been developed to target mitochondrial metabolism in cancers [18]. One of these drugs is CPI-613, an inhibitor of cancer-specific mitochondrial energy metabolism. The drug causes tumor cell apoptosis, necrosis, and autophagy by selectively targeting alterations in mitochondrial enzyme activities and redox status [19]. CPI-613 is a small molecule that attacks alpha-ketoglutarate dehydrogenase in tumor cells through a redox process [20]. The drug is known to simultaneously attack multiple essential components of tumor cell regulation [20]. However, the exact mechanism is not well understood. CPI-613 has been recognized to be effective against various types of cancers [21], including patients with metastatic pancreatic cancer [19]. CPI-613 used in combination with modified FOLFIRINOX (mFOLFIRINOX) in patients with metastatic pancreatic cancer demonstrated better survival, but since this phase I study was not designed to determine the efficacy of adding CPI-613 to mFOLFIRINOX, the results should be interpreted with caution. Nevertheless, Alistar et al. have obtained encouraging results from the phase I studies and are currently performing a randomized phase II trial to compare FOLFIRINOX against mFOLFIRINOX with CPI-613. These results suggest that targeting mitochondrial metabolism holds enormous potential in combating pancreatic cancer.

3.2 *Antidiabetic Drug, Metformin, Targets Pancreatic Cancer Stem Cells*

Recent studies have shown that tumorigenic cancer stem cells (CSCs), a highly chemoresistant subclass of PDAC, are strongly dependent on oxidative metabolism [22, 23]. Retrospective analysis showed that oral administration of metformin in patients with type 2 diabetes was associated with reduced risk of developing PDAC [24] along with a better outcome for patients that had established PDAC [25]. More recently, it has been discovered that metformin targets pancreatic CSCs but not the differentiated progenies (non-CSCs) [22]. *KRAS* targeting has resulted in tumor shrinkage but fails to kill all the CSCs [26]. Viale et al. established that dormant tumor cells that survived oncogene ablation were shown to have high sensitivity to oxidative phosphorylation inhibitors [26]. Lonardo et al. uncovered that metformin uniformly reduced ATP levels in adherent cells and sphere cells from CSCs, but not in non-CSCs [23]. Although the mechanism for metformin in CSCs is largely unknown, what is known is that metformin slowly accumulates in the mitochondria and directly inhibits complex 1 (NADH dehydrogenase) in the electron transport chain, affecting oxidative phosphorylation [23]. Therefore, a potentially strong therapeutic strategy to manage pancreatic cancer is the combined targeting of the *KRAS* pathway and mitochondrial respiration [26].

3.3 *Combined Therapy to Target Pancreatic Metabolism Heterogeneity*

Combination therapy to target multiple metabolic pathways in pancreatic cancer has been demonstrated as a favorable therapeutic solution. Elgogary et al. found that targeting glutamine metabolism using the glutaminase inhibitor bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl)ethyl sulfide (BPTES) encapsulated in nanoparticles effectively shrinks pancreatic cancer tumor size and slows proliferation [27]. They also found, using metabolomics technologies, that the tumor cells remaining after glutaminase inhibition were dependent on glycolysis and glycogen synthesis. Elgogary et al. continued the study by adding both BPTES nanoparticles and metformin to target both glutamine and glucose metabolisms in pancreatic cancer cells. They discovered that the combined therapy provided enhanced efficacy that inhibited tumor growth significantly more compared to the single treatment of BPTES or metformin alone. This highlights the fact that there is great heterogeneity in pancreatic cell metabolism since targeting only glutamine metabolism did not kill all the pancreatic cancer cells, but targeting both glutamine and glucose metabolisms reduced the tumor growth of the cells with considerably larger efficacy than targeting either glutamine or glucose metabolism alone. This has been observed in pancreatic cancer cells, but more clinical trials must be done in order to see if combination therapy can assist in pancreatic cancer patient survival.

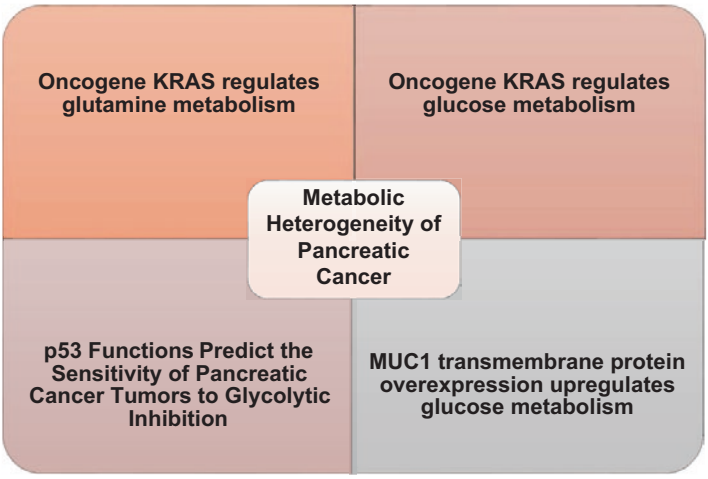

Fig. 3 Overview of pancreatic cancer metabolic heterogeneity

Conclusion

Pancreatic ductal adenocarcinoma is the fourth leading cause of cancer death in the United States and is expected to be the second largest by 2030 [28, 29]. The deadliness of this disease can be attributed to its metabolic heterogeneity which developed through cancerous evolution (Fig. 3). With that in mind, the investigation of PDAC within the past few years has been exponentially increasing with improved technology and research methods that allow us to understand these intricate mechanisms better. Exploration of more aspects of a pancreatic cell enables scientists and clinicians to better target multiple facets of a pancreatic cell, resulting in more effective therapeutic methods.

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Breast Cancer Metabolism

Jessica Tan and Anne Le

Key Points

- Aberrant metabolic pathways present in breast cancer contribute to breast cancer heterogeneity.
- Differences in glycolytic upregulation among breast cancer subtypes can be attributed to GLUT expression.
- Choline metabolism in breast cancer is strongly associated with tumor grades.
- Different roles of estrogen in estrogen metabolism and ER binding promote breast cancer tumorigenicity.
- Metabolic profiling of breast cancers can be used for clinical breast cancer diagnosis and prediction of recurrence or metastasis.

Keywords Breast cancer · Estrogen receptor status · Metabolic fingerprint · Choline metabolism · Estrogen metabolism · Serine biosynthesis · Glycolytic upregulation

Abbreviations

3HP	3-Phosphohydroxypyruvate
3PG	3-Phosphoglycerate
αKG	Alpha-ketoglutarate
CK	Choline kinase
COMT	Catechol-O-methyltransferase

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D-2HG	D-2-hydroxyglutarate
E2	17b-Estradiol
ER	Estrogen receptor
GLUT	Glucose transporter
GSTP	Glutathione S-transferase P
HER2	Human epidermal growth factor receptor 2
PCho	Phosphocholine
PHGDH	Phosphoglycerate dehydrogenase
PR	Progesterone receptor
PSAT1	Phosphoserine aminotransferase 1
PSPH	Phosphoserine phosphatase
PtdCho	Phosphatidylcholine
TCA	Tricarboxylic acid
TNBC	Triple-negative breast cancer

Introduction

Despite advances in screening, therapy, and surveillance that have improved survival rates, breast cancer is still the most commonly diagnosed cancer and the second leading cause of cancer mortality among women [1]. Breast cancer is a highly heterogeneous disease rooted in a genetic basis and reflected in clinical behavior. The diversity of breast cancer hormone receptor status and the expression of surface molecules has guided therapy decisions for decades; however, subtype-specific treatment often yields diverse responses due to varying tumor evolution and malignant potential. Although understanding the mechanisms behind breast cancer heterogeneity is still a challenge, available evidence suggests that studying its metabolism has the potential to give valuable insight into the causes of these variations, as well as viable targets for intervention.

1 Aberrant Metabolic Pathways Present in Breast Cancer Contribute to Breast Cancer Heterogeneity (Fig. 1)

In order to sustain tumorigenic proliferation, cancer cells exploit diverse metabolic pathways. The diversity of hormone receptors present within breast cancer cells is classified into different subtypes. Breast cancers with hormone-positive receptors such as estrogen (ER) and progesterone (PR) receptors rely on their respective hormones for growth. Patients with $HER2^{+}$ breast cancer have overexpression of human epidermal growth factor receptor 2 (HER2). Patients negative for all three receptors are considered to have triple-negative breast cancer (TNBC)—the most heterogeneous molecular profile. This diversity, in turn, reflects the different metabolic

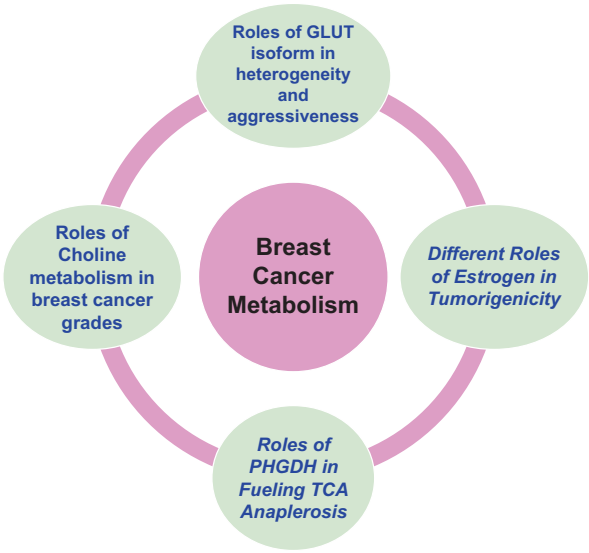

Fig. 1 Non-inclusive summary of the different aspects contributing to metabolic heterogeneity seen in breast cancer

phenotypes of breast cancer. Explained later on, some of these core metabolic aberrations may have fundamental effects on breast cancer tumorigenicity and offer rationale behind the aggressiveness of certain subtypes.

Tumor evolution results in the reprogramming of cell metabolism in order to adapt to support cell proliferation. Certain mutations in oncogenes and tumor suppressor genes are hypothesized to cause metabolic reprogramming within different breast cancer subtypes. Although several mutations are commonly seen in breast cancers, they appear in different combinations which are reflective of their diverse metabolic behaviors. For example, mutations in *BRAF*, *KRAS*, and *HRAS* were found to be metabolic regulators of TNBC [2]. These genetic alterations are known to regulate glutamine metabolism, which renders cancer cells dependent on glutamine [3–7]. A study by Martinez-Outschoorn et al. uncovered that loss-of-function mutations in the *BRCA1* tumor suppressor gene resulted in the production of hydrogen peroxide and oxidative stress in epithelial breast cancer cells and stromal fibroblasts [8]. This loss of function also causes elevated expression of monocarboxylate transporter 4 to shuttle L-lactate out of cells. Furthermore, in cancer-associated fibroblasts, the loss of caveolin-1 is attributed to mutations of BRCA1, and is also associated with elevated production of ROS and increased glycolysis in stromal cells, both of which play a fundamental role in tumorigenesis. Mutations in *BRCA1* are marked by high rates of proliferation and substantial cellular inflammation. This study suggests that antioxidant agents present promising therapies for this subtype of breast cancer.

1.1 Differences in Glycolytic Upregulation Among Breast Cancer Subtypes Can Be Attributed to Glucose Transporter (GLUT Expression)

First postulated by Otto Warburg in 1927 [9] and firmly established in the literature henceforth, a hallmark of cancer malignancy is an upregulation in aerobic glycolysis even in the presence of oxygen, known as the Warburg effect [10]. Breast cancer tumors are no exception to the Warburg effect; however, there are variations in glycolytic rates and metabolite-related protein expression among breast cancer subtypes that correlate with tumor aggressiveness.

Previous studies in vitro have first observed that noninvasive breast cancer cell lines showed a significantly lower rate of glucose intake compared to more aggressive, metastatic cells [11]. Higher rates of glucose uptake are accompanied by altered gene expression and translation of metabolism-related proteins as well. Glucose transporter (GLUT) expression has been studied extensively in breast cancer. GLUTs are integral transmembrane proteins that facilitate glucose delivery across the plasma membrane. It is thought to be a rate-limiting step that controls the amount of glucose accessible to the cell [12]. Studies have shown that different isoforms of GLUTs have been detected and/or overexpressed in breast cancer cells. Different GLUT expression patterns are found to be associated with different pathological grades and tumor aggressiveness in patient-derived samples. Choi et al. discovered that GLUT1, one of the isoforms of the GLUT family, had the highest expression in the TNBC subtype and in tumors with high histologic grade [13]. This increased rate of glycolysis subjects the cell to intracellular lactic acidosis—leading to cell death. Interestingly, the same group showed that TNBC had the highest expression of carbonic anhydrase IX, an enzyme that prevents acidosis and provides it with an acid-resistant phenotype [13]. This suggests that aggressive breast cancer subtypes adopt metabolic phenotypes able to suppress apoptosis. GLUT1 overexpression has also been linked to invasiveness in breast cancer [14].

The link between metabolic reprogramming and protein expression offers an adaptive advantage that contributes to a level of aggression specific to certain subtypes of breast cancer, making them characteristically resilient and harder to treat, such as TNBC.

1.2 Choline Metabolism in Breast Cancer Is Strongly Associated with Tumor Grades

The deregulation of choline metabolism and elevated levels of choline-containing compounds are frequently observed in breast cancer progression [15–18]. Choline plays the important role of supplying methyl groups through its metabolism and is essential for the cellular structure as a precursor of phospholipids. Choline metabolism in breast tissue is distributed between two central pathways: (1) the

biosynthesis of phosphatidylcholine (PtdCho) known as the Kennedy pathway and (2) the oxidation to betaine, a methyl group donor in many methylation reactions. A study by Katz-Brull et al. revealed that breast cancer cells exhibited a higher choline transport rate compared to normal breast cells—a majority converted to phosphocholine (PCho) through the Kennedy pathway while ~25% was oxidized to betaine [16]. Although levels of phospholipid-related metabolites are enhanced in general breast cancer, significantly higher levels of PCho were found to be associated with ER^- tumors and the more aggressive histologic grade 3 tumors [19]. Because of this, choline-containing compounds have often been seen as biomarkers for breast tumor malignancy. Oncogenic expression of choline kinase (CK), the enzyme responsible for the conversion of choline to PCho, has been responsible for elevated levels of PCho in breast cancer cells [16]. Furthermore, CK also showed a strong association with high histologic grade and ER^- subtypes [20]. For this reason, CK has been an attractive antitumor target for subsequent studies. Whether or not choline metabolism represents an agent of disease progression or merely a marker for transformation has still not been defined. CK inhibitors blocking choline metabolism have shown promising antitumor results. A study by Rodríguez-González et al. discovered that blocking the enzyme had no effect in normal cells but disrupted phospholipid production in tumor cells—resulting in apoptosis due to cytotoxic ceramide accumulation, the simplest class of sphingolipids [21]. Advancing toward clinical applications, the CK inhibitor TCD-717 is currently undergoing clinical trials as a chemotherapy drug with promising results [22].

1.3 Different Roles of Estrogen in Estrogen Metabolism and ER Binding Promote Breast Cancer Tumorigenicity

Endogenous estrogens and their metabolism have been linked to breast carcinogenesis especially in postmenopausal women [23]. 17b-Estradiol (E2), the main estrogen in breast tissue, acts as both a ligand for ER and a substrate in metabolism—both roles contributing to estrogen as a carcinogen. The mechanism of estrogen carcinogenesis is a combination of ER signaling and estrogen metabolism.

ERs, when activated, are responsible for the mediation of many downstream signaling pathways that function as transcription factors promoting cancer development [24]. In addition, ER signaling interacts with growth factor receptors and other signaling molecules to promote growth and antiapoptotic signals [25]. ER activation has also been shown to promote downstream reprogramming in choline metabolism, an aberration in breast cancer [26].

As a substrate, the metabolism of estrogen through the 4-hydroxylation pathway produces specific catechol estrogens and estrogen quinones known to be carcinogenic. Estrogen is hydroxylated by cytochrome P450 enzymes and shuttled into three main pathways depending on the three different carbons hydroxylated by catechol-O-methyl transferase (COMT): C2, C3, and C16. The catechol estrogens (2-OH E1, 2-OH E2, 4-OH E1, 4-OH E2) are either methylated by catechol-O-

methyltransferase (COMT), thereby reducing its mutational potential, or converted to damaging catechol estrogens. 4-OH catechol estrogen, when oxidized to a reactive estrogen quinone, leads to DNA damage by forming unstable DNA adducts between adenine and guanine nucleotides [27, 28]. Mutations caused by this mechanism have the potential to initiate breast cancer or increase cancer risk. In contrast, metabolites formed through the 2-OH pathway form stable DNA adducts and are anticarcinogenic—dubbing the 2-OH metabolites as "the good estrogen" in some cases [29]. Protective mechanisms such as estrogen quinone conjugation with glutathione via glutathione S-transferase P (GSTP) help lower the risk of cancerous mutations by detoxifying the estrogen quinones [27]. However, estrogenic imbalances lead to competition between the pathway forming the unstable DNA adducts and the detoxification of its cancer-promoting substrates [27]. Accordingly, hormone therapy for breast cancer has targeted ER^+ subtypes with drugs such as tamoxifen, which acts as a competitive inhibitor that prevents estrogen from binding to ER. Another important class of drugs inhibits aromatase, an important rate-limiting enzyme that converts androgens to estrogen to lower estrogen levels in the body.

1.4 PHGDH Overexpression in Serine Biosynthesis Fuels TCA Anaplerosis

Serine biosynthesis has been shown to be an essential pathway for breast tumorigenicity in specific subsets of breast tumors. Using RNAi-based loss-of-function screening, Possemato et al. identified phosphoglycerate dehydrogenase (PHGDH) in breast cancer with enhanced protein levels in 70% of aggressive ER^- subtypes [30]. PHGDH catalyzes the committed-limiting step that oxidizes 3-phosphoglycerate (3PG) to 3-phosphohydroxypyruvate (3HP) substrates in the serine synthesis pathway. Enhanced PHGDH expression was associated with increased serine synthesis and glutamine uptake. Suppression of PHGDH expression led to a significant decrease in cell proliferation but did not affect intracellular serine levels; instead, researchers found a resulting drop in alpha-ketoglutarate (αKG), an output of the serine pathway [30]. Based on available evidence, it is hypothesized that in cells with overexpression of PHGDH, the serine synthesis pathway plays an important role in tricarboxylic acid (TCA) cycle anaplerosis—supplying αKG to support cell proliferation [30]. In addition, suppression of phosphoserine aminotransferase 1 (PSAT1) and phosphoserine phosphatase (PSPH) enzymes downstream in the serine pathway inhibited cell proliferation in PHGDH-enhanced cell lines as well [30]. Subsequent studies have revealed that in addition to 3PG oxidation, PHGDH also catalyzes the reduction of αKG to D-2-hydroxyglutarate (D-2HG) [31], an established oncometabolite [32, 33]. D-2HG acted as a competitive inhibitor of αKG-dependent dioxygenases, resulting in aberrations in histone methylation and DNA hypermethylation [34]. High levels of D-2HG and N-acetyl-aspartate were found to accumulate preferentially in ER^- and basal-like tumors which may contribute to their aggressive phenotypes [33]. In vitro experiments revealed that accumulation of D-2HG increased

cell proliferation and inhibited apoptosis [33]; however, the oncogenic effects of 2HG in breast cancer still need to be defined. Because of its deregulated expression and oncogenic effects, PHGDH is considered a promising target for therapy in cell lines that have PHGDH overexpression. Although a preliminary PHGDH inhibitor has been recently developed [35], PHGDH-targeted therapy is still in its infancy.

Using a novel computational method, Jerby et al. contributed further evidence that the metabolic profiles of ER^+ and ER^- subtypes are vastly different [36]. Stoichiometric analysis revealed serine metabolism to be coupled to glutamine uptake [36]. ER^+ tumors exhibit a stronger preference for glutamine biosynthesis and secretion than ER^- tumors [36]. In addition, their model identified ER^+ phenotypes as having more capacity to convert glucose to lactate than ER^- tumors. Due to higher rates of serine metabolism, ER^- subtypes are rationalized to preferably divert 3PG toward serine metabolism via PHGDH to exploit alternative pathways for glutaminolysis [36]. In addition, a high MYC overexpression and low thioredoxin-interacting protein expression, an inhibitor of glucose utilization, was found to be a characteristic gene signature of TNBC and no other subtypes [37].

2 The Clinical Applications of Metabolic Profiling

Metabolic profiling has garnered much research interest within the past decade. Although the mechanisms behind breast cancer transformation have not been firmly established, changes in tumor evolution have been investigated through metabolic variation. The exploitation of these metabolic signatures has the potential to improve clinical results through diagnosis confirmation, early detection, and prediction of disease progression (Fig. 2).

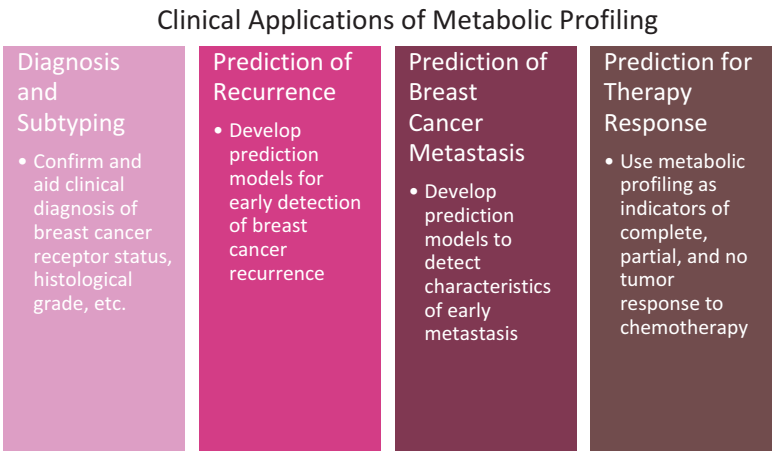

Fig. 2 Summary of the potential clinical applications of metabolic profiling for breast cancer

2.1 Breast Cancer Diagnosis and Subtyping in Metabolomics

Studies have used metabolic profiling for the general diagnosis of breast cancer—using different techniques to build prediction models that distinguish specific metabolic fingerprints of breast cancer hormone receptor status, histologic grade, and axillary lymphatic spread [38–40]. Jove et al. used a combination of random forest classification and multivariate statistics to identify combinations of metabolites that were used to distinguish breast cancer plasma samples from healthy control samples [38]. On the other hand, Huang et al. sought out a model more tolerant of breast cancer heterogeneity by following metabolic pathways rather than metabolite-based biomarkers for early diagnosis of breast cancer [39]. Other studies have used metabolic profiling to build models to distinguish breast cancer stage [40] and levels of malignancy [41].

2.2 Metabolic Profiling as a Strategy for Prediction of Recurrence in Breast Cancer

Recurrence after initial therapy causes significant morbidity and mortality. Current methods for detecting recurrences such as medical imaging and serum tumor markers are not considered specific enough to be routinely recommended; therefore, there is still much room for improvement. A combination of NMR and MS analysis and multivariate statistics has been used to explore potential metabolic profiles sensitive to cancer recurrence [42]. Asiago et al. developed a prediction model built upon 11 biomarkers that correctly detected 55% of patients with breast cancer recurrence an average of 13 months prior to their clinical diagnosis [42]. Although there is vast room for improvement on more specific and accurate models for early detection of recurrence, metabolic profiling of serum can be viewed as a promising noninvasive method for breast cancer surveillance.

2.3 Metabolic Fingerprinting in Breast Cancer Metastasis

Oakman et al. identified a preliminary metabolic fingerprint from patient serum that detected early and metastatic disease in breast cancer patients. In their study, higher levels of phenylalanine, glucose, proline, lysine, and N-acetyl cysteine and lower values of lipids composed the metabolic profile of metastatic individuals [43]. Jobard et al. used similar serum NMR analysis to identify metabolic profiles between localized and metastatic breast cancer. They found eight statistically significant elevations of metabolite biomarkers in metastatic disease: histidine, acetoacetate, glycerol, pyruvate, N-acetyl, mannose, glutamate, and phenylalanine [44]. Although there are differences in biomarkers between the two studies, it is of note that the same trends of variation in glucose concentration and lowered lipid levels

were seen between early and metastatic breast cancer [44]. Defining an accurate metabolic fingerprint specific across all metastatic breast cancers is a challenge due to the variability and high mutational load of metastatic disease. Under changing tumor microenvironments, metastatic breast cancer cells readily switch between glycolysis and oxidative phosphorylation [45]. The metabolic plasticity of breast cancer metastasis may contribute to the inconsistencies of biomarkers across different tumors. However, studies attempting to identify these metabolic patterns provide greater insight into the general characteristics of advanced diseases.

2.4 Prediction of Response to Therapy

Metabolic fingerprinting has also been used to predict responses to therapy and drug resistance. Using a combination of NMR and LC-MS, Wei et al. were able to identify four altered metabolites (threonine, glutamine, isoleucine, and linolenic acid) to be indicators of adjuvant chemotherapy response within breast cancer [46]. A prediction model derived from these metabolic markers was able to distinguish between complete, partial, and no tumor response to chemotherapy in a neoadjuvant setting using patient samples [46]. The model was able to correctly identify 80% of patients whose tumors did not show a complete pathologic response to chemotherapy [46].

Collectively, these studies highlight the potential impact of metabolic profiling on the integration of metabolomics into clinical practice. Further advancements in profiling could improve diagnosis and early detection or at least offer confirmation in the treatment of breast cancer, quickly and at low cost. Although most of the prediction models and metabolic phenotypes presented in these studies are in their preliminary stages, improvements could make way for more individualized treatments specific to each patient.

Conclusions

Metabolomics serves as an important utility by offering a perspective that represents the net interactions between the tumor, host, and environment and within the tumor itself. The metabolic nuances across different breast cancer subtypes and treatment timelines can be taken advantage of when thinking about potential prognostic markers, prediction models, and mechanisms involved with breast cancer.

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Non-Hodgkin Lymphoma Metabolism

Brian James Kirsch, Shu-Jyuan Chang, and Anne Le

Key Points

- Aggressive lymphomas exhibit the Warburg effect.
- Lactic acidosis is a result of overproduction of lactate and leads to a fatal prognosis.
- Mutation of p53 helps cancer cells survive glutamine deprivation.
- PI3K regulates fatty acid synthesis (FAS) in primary effusion lymphoma (PEL) and other B-NHLs.
- AMPK regulates NADPH balance for fatty acid oxidation as a means of supplementing the TCA cycle.
- PRPS2 couples protein and nucleotide biosynthesis to drive lymphomagenesis.
- mTOR activation promotes fatty acid synthesis (FAS).
- *MYC* regulates cancer cell metabolism under glucose-deprived and hypoxic conditions.
- HIF-1 acts as a regulator in hypoxia adaption and the related metabolic change.

Keywords Heterogeneous malignant lymphomas · Lactic acidosis · Aerobic glycolysis · Glutamine metabolism · Fatty acid metabolism · Gene expression · mTOR signaling

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Abbreviations

^{13}C MRS	^{13}C magnetic resonance spectroscopy
2-DG	2-Deoxyglucose
acetyl-CoA	Acetyl coenzyme A
AMPK	5′AMP-activated protein kinase
ATP	Adenosine triphosphate
BCR	B-cell receptor
B-NHL	B-cell non-Hodgkin lymphomas
DLBCL	Diffuse large B-cell lymphoma
FAO	Fatty acid oxidation
FAS	Fatty acid synthesis
FASN	Fatty acid synthesizing enzyme
FDG-PET	18F-deoxyglucose positron emission tomography
FL	Follicular lymphoma
HIF-1	Hypoxia-inducible factor-1
LDH	Lactate dehydrogenase
mTOR	Mammalian target of rapamycin
mTORC	Mammalian target of rapamycin complex
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NHLs	Non-Hodgkin lymphomas
OAA	Oxaloacetate
OXPHOS	Oxidative phosphorylation
PDK1	Pyruvate dehydrogenase kinase, isozyme 1
PEL	Primary effusion lymphoma
PI3K	Phosphatidylinositol-3-kinase
POX/PRODH	Proline dehydrogenase
PRPS2	Phosphoribosyl-pyrophosphate synthetase 2
TCA	Tricarboxylic acid
tFL	Transformed follicular lymphoma
VEGF	Vascular endothelial growth factor

Introduction

Non-Hodgkin lymphomas (NHLs) are a heterogeneous group of lymphoid neoplasms with differing biological characteristics. About 90% of all lymphomas in the United States originate from B lymphocytes, while the remaining originate from T cells [1]. The treatment of NHLs depends on neoplastic histology and the stage of the tumor, which will indicate whether radiotherapy, chemotherapy, or a combination is the best suitable treatment [2]. The American Cancer Society describes the staging of lymphoma as follows: Stage I is lymphoma in a single node or area. Stage II is when that lymphoma has spread to another node or organ tissue. Stage III is when it has spread to lymph nodes in two sides of the diaphragm. Stage IV is when the cancer has

significantly spread to organs outside the lymph system. Radiation therapy is the traditional therapeutic route for localized follicular and mucosa-associated lymphomas. Chemotherapy is utilized for the treatment of large cell lymphomas and high-grade lymphomas [2]. However, treatment of indolent lymphomas remains problematic as the patients often have metastasis for which no standard approach exists [2].

Follicular lymphoma (FL), a form of non-Hodgkin lymphoma, is the second most common form of B-cell lymphoma and remains incurable in the majority of cases despite recent advances, including anti-CD20 antibodies (Rituxan) and kinase inhibitors (ibrutinib) [3]. Following an indolent phase, 50% of patients suffer from disease transformation to an aggressive form of lymphoma (transformed FL; tFL) [4]. This dramatic switch in disease behavior typically culminates in rapid deterioration and patient demise. Accordingly, much effort has been focused on understanding the genetics of transformation and has resulted in the identification of key genetic lesions (e.g., *MYC* activation, loss of p53, cell cycle controls, activation of NFKB (TNFAIp3/A20)) [5–8]. However, exactly how tumor metabolism, which is altered by these genetic lesions, contributes to disease aggressiveness is not known, and therefore the metabolic changes that occur during FL transformation are poorly understood. We need to understand the biological and metabolic changes upon disease transformation to develop effective prevention and treatment strategies.

Moreover, malignant cells have metabolic adaptations supporting bioenergetics, biosynthesis, and redox in response to the development of the tumor microenvironment [9]. Metabolic heterogeneity is present in the tumor microenvironment, where concentrations of key resources are spatially (localization) and temporally (stage of the diseases) variated [9]. Cancer metabolism is influenced by the tumor localization and the vascularization status. Cancer cells can uptake nutrients and oxygen from the blood supply, which results in the production of ATP via aerobic oxidative phosphorylation and enhance anabolic pathways, supporting rapid cell proliferation.

In this chapter, we will describe the intricacies of NHLs' metabolism resulting from alterations in gene expressions which subsequently lead to poor prognosis.

1 Lymphoma Metabolism Exhibits Multifaceted Characteristic Features Which Are Correlated to Poor Prognosis

1.1 Aggressive Lymphomas Exhibit the Warburg Effect

As described in the previous chapters, the drastic increase in glucose uptake of cancer cells is a feature of the distinctive metabolic rewiring known as the Warburg effect. In more recent times, researchers have taken advantage of this metabolic shift to clinically detect localized glucose uptake of cancer cells using 18F-deoxyglucose positron emission tomography (FDG-PET). High-grade NHL patients and intermediate-grade NHL patients with poor prognoses showed a high accumulation of FDG [10, 11].

Primary effusion lymphoma (PEL) exhibits high glycolytic activity due to its hypoxic environment. This form of lymphoma requires aggressive treatment, but no standard therapy exists [12]. PEL is, however, highly sensitive to glucose withdrawal and glycolysis inhibitors, such as 2-deoxyglucose (2-DG) [12]. In this situation, the distinctive metabolic phenotype, glucose dependency, offers hope of a novel treatment.

Cancer cells exhibiting the Warburg effect avidly take up glucose. Upon glucose uptake, cancer cells favor the conversion of glucose-derived pyruvate to lactate. Recent reports showed that NHL patients had elevated plasma lactate and lactate dehydrogenase (LDH) levels, which were linked to poor survival rates [13–15]. Taken together, the characteristic metabolic features of the Warburg effect offer diagnostic tools as well as relevant therapeutic targets.

1.2 Lactic Acidosis Is a Result of Overproduction of Lactate and Leads to a Fatal Prognosis

Following the Warburg effect, lactic acidosis can occur when lactate homeostasis is disproportioned, due to overproduction and/or underutilization. Lactic acidosis is divided into two categories: type A and type B. Type A results from poor oxygenation in the tissue. Type B occurs in normoxic tissue as a result of a cause other than oxygenation, such as a drug or toxin [16]. Type B lactic acidosis is the result of alteration of glycolytic processes and their effects on redox [17, 18]. Type B lactic acidosis is present in many human malignancies, but notably in lymphomas and leukemias [16, 17, 19–21]. Once cancers exhibit type B lactic acidosis, these cases show a poor prognosis and outcome if not immediately treated [16].

Another notable cause of lactic acidosis is thiamine deficiency, a discernible characteristic connected to type B lactic acidosis. Thiamine is a cofactor that contributes to the conversion of pyruvate into acetyl-CoA via pyruvate dehydrogenase. When malignant cells exhibit thiamine deficiency, pyruvate heavily converts to lactate [22, 23]. Subsequently, thiamine deficiency leads to lactic acidosis (Fig. 1).

2 Genetic Alterations Lead to Different Metabolic Phenotypes in NHL (Fig. 2)

NHL often have abnormal activation of mTORC1 that reprograms multiple metabolic pathways, including nucleotide synthesis, amino acid synthesis, fatty acid synthesis, and glutaminolysis. Additionally, *MYC* is also an important trigger for inducing many genes correlated with anabolic growth, including transporters and enzymes involved in glycolysis, mitochondrial biogenesis, fatty acid synthesis, and glutaminolysis [24–26]. *MYC* is a gene involved in cellular proliferation whose dysregulation was found in B-cell lymphomas [27]. Reprograming by transcription

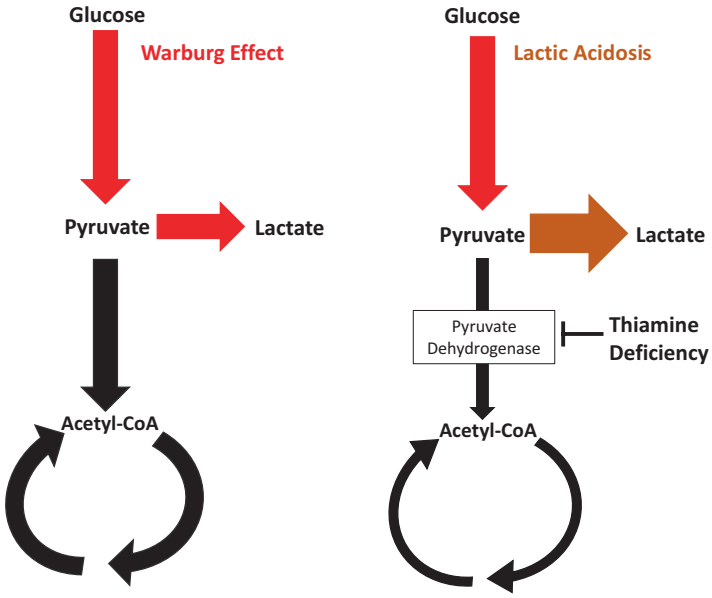

Fig. 1 The Warburg effect and lactic acidosis. Some cancer cells are known to produce lactate, an effect known as the Warburg effect (*red*). Lactic acidosis can result from unregulated lactate buildup (*orange*). One notable cause is thiamine deficiency which inhibits acetyl-CoA production

factors such as *MYC* and hypoxia-inducible factor 1 (HIF-1) in malignant tissues allows them to better survive the tumor microenvironmental alterations [28, 29]. These genes can influence each other; for instance, mTOR can also activate HIF-1 expression even under normoxic states [30] (Table 1).

2.1 Mutation of p53 Helps Cancer Cells Survive Glutamine Deprivation

As described in the previous chapter, many cancers depend on glutamine for bioenergy, redox homeostasis, and DNA synthesis, which are essential requirements for cancer survival. Many therapeutic strategies target cancer's glutamine dependency. However, these treatments do not always have the intended impact, as many cancers are resistant to treatment. One such example is that of TP53, a protein responsible for tumor suppression, and its mutant form [31]. Specifically, in lymphoma cell lines, Tran et al. reported that mutp53 proteins can directly bind to the promoters of p53-target genes that regulate the cell cycle, which leads to cell cycle arrest and helps cancer cells survive in glutamine deprivation conditions [31]. Cancer cells expressing mutp53 proteins are able to survive the metabolic stress of glutamine deprivation in poorly vascularized tumor microenvironments, whereas p53-deficient cells and wtp53-expressing cells experience impaired proliferation and

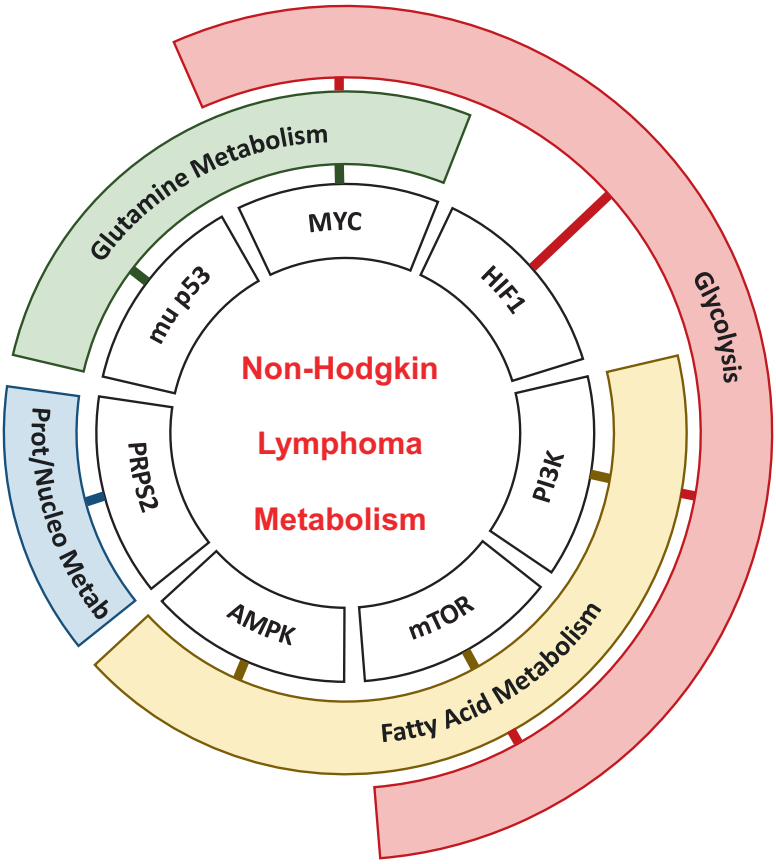

Fig. 2 Genetic alterations lead to different metabolic phenotypes in NHL. Various lymphomas show these alterations in gene expression and the resulting changes in metabolism

Table 1 Genetic alterations lead to different metabolic phenotypes in NHL

Gene	Metabolic effect	Lymphoma type
Mutant p53	Allows cells to survive glutamine deprivation	B- and T-cell lymphoma
PI3K	Regulated glycolysis and FAS	Primary effusion lymphoma
mTOR	Enhances glycolysis and FAS	B-cell lymphoma
AMPK	Regulates FAO to support TCA	Diffuse large B-cell lymphoma
PRPS2	Drives protein and nucleotide synthesis	B-cell lymphoma
MYC	Regulates glycolysis, TCA, glutamine, and proline metabolism	B-cell lymphoma
HIF-1	Promotes glycolysis, particularly in hypoxia	B-cell lymphoma

increased cell death [31]. The resistance to glutamine deprivation in mutp53-expressing malignant cells allows these cells to survive in metabolically restrictive environments.

2.2 PI3K Regulates Fatty Acid Synthesis (FAS) in Primary Effusion Lymphoma (PEL) and Other B-NHLs

While many lymphomas rely on glucose to produce lactate and energize their metabolism, this is not always the case. Dysregulation of cell metabolism in primary effusion lymphoma (PEL), an aggressive type of B-cell lymphoma, increased not only aerobic glycolysis but also fatty acid synthesis [32]. By using ^{14}C-labeled glucose, Bhatt et al. showed that PEL creates more lipids from glucose compared to primary B cells. Furthermore, these cells were sensitive to both an inhibitor of fatty acid synthase, C75, and an inhibitor of glycolysis, 2-DG. Interestingly, each of these inhibitors affected both glycolysis and fatty acid synthesis (FAS) [32].

Previous work of Bhatt et al. and others shows that PEL cells are known to show high activity of phosphatidylinositol 3-kinase (PI3K), AKT, and mTOR, genes related to proliferation and survival as well as glycolysis [33–35]. In their more recent work, Bhatt et al. showed that inhibiting PI3K by LY294002 decreased not only glycolytic flux but also the incorporation of ^{14}C glucose into lipids [32]. We see that in PEL, glucose was important for not only providing energy but also acetyl-CoA for lipid synthesis. This illustrates that lymphoma's metabolism is complex and functions on different axes.

2.3 AMPK Regulates NADPH Balance for Fatty Acid Oxidation as a Means of Supplementing the TCA Cycle

Jeon et al. showed that AMPK orchestrates NADPH consumption (by FAS) and production (from fatty acid oxidation (FAO)) in lymphoma to support ATP synthesis, redox homeostasis, and biosynthesis responses under low glucose environments [36]. By doing so, AMPK decreases pentose phosphate pathway activity and increases FAO [36].

Diffuse large B cell lymphoma (DLBCL), a common lymphoma, utilizes FAO to greatly support energy production and growth [37]. Fatty acids provide fuel for oxidative phosphorylation (OXPHOS), increased glutathione levels, and attenuated oxidative stress [37]. The DLBCL with OXPHOS is aggressive and resistant to ibrutinib, an inhibitor of B cell receptor (BCR) survival signaling [38, 39]. More research into the combination of fatty acid oxidation targeting drugs and the B-cell receptor could provide potential therapeutic approaches for patients with DLBCL [37, 40].

2.4 PRPS2 Couples Protein and Nucleotide Biosynthesis to Drive Lymphomagenesis

One of the current mainstay chemotherapeutic strategies involves targeting one-carbon metabolism in malignant cancers. This strategy reduces the production of nucleotides and ATP as well as altering redox. Individual drugs often inhibit the metabolism of folate, nucleotides, and most notably thymidine [40, 41]. Key enzyme targets of nucleic acid synthesis include dihydrofolate reductase, thymidylate synthase, adenine/adenosine deaminase, and DNA polymerase/ribonucleotide reductase [40–42]. As indicated by Cunningham et al., nucleotide biosynthesis is coupled to protein biosynthesis by a critical enzyme, phosphoribosyl-pyrophosphate synthetase 2 (PRPS2), which specifically promotes increased nucleotide biosynthesis in *MYC*-driven lymphoma. In these lymphomas, PRPS2 may be an effective anticancer target, and there may exist other similar enzymes utilized by oncogenes [43].

2.5 mTOR Activation Promotes Fatty Acid Synthesis (FAS)

mTOR activation during nutrient abundance enhances aerobic glycolysis and lipid synthesis, which is mediated by the SREBP group by inducing the transcription of the fatty acid synthesizing enzyme (FASN) [44]. FASN exists at notable levels in liver and at lower levels in other tissues, but cancerous tissues express excessive FASN which has identified it to be a metabolic oncogene [45–47].

Bhatt et al. showed a significant difference in the metabolic profiles of primary B cells and those of human B-cell non-Hodgkin lymphomas (B-NHL) including PEL. Poor-prognosis PEL and other B-NHLs exhibit high levels of aerobic glycolysis and fatty acid synthesis (FAS). Both PEL and other B-NHLs were sensitive to the FAS inhibitor, C75, compared to primary B cells [32]. This suggests that the different types of malignant lymphomas can be distinguished by the rate of fatty acid biosynthesis, which may have the potential for targeted therapy against these aggressive lymphomas [32].

In PEL, rapamycin treatment improves the survival time in the in vivo model by inhibiting autocrine signaling and the vascular endothelial growth factor (VEGF) [35, 48]. Shestov et al. revealed that inhibition of mTOR has impacts on flux of the glycolysis, pentose phosphate pathway, and the TCA cycle [49].

2.6 MYC Regulates Cancer Cell Metabolism Under Glucose-Deprived and Hypoxic Conditions

MYC is considered to be a co-regulator in glycolysis and mitochondrial respiration [50–54]. Using stable isotope-resolved metabolomics, Le et al. explored the metabolic alterations that occur in the oncogenic transcription factor c-*MYC*-inducible

human Burkitt lymphoma model P493 cell line under aerobic and hypoxic conditions as well as glucose deprivation. They found a coexistence of oxidative and aerobic glycolysis. They also documented the prominent contribution of glutamine to the TCA cycle of proliferating cells and that hypoxic cancer cells continue to oxidize glutamine for cell growth and survival. Furthermore, this study uncovered that glutamine metabolism alone can sustain the TCA cycle for cell growth and survival in the absence of glucose [55]. This glucose-independent pathway reflects the dependence of cancer cells on metabolic reprogramming, allowing for the proliferation and survival of cancer cells under the harsh hypoxic and nutrient-deprived conditions of the tumor microenvironment [56].

MYC also regulates proline metabolism as found by Liu et al. [57]. They found that proline dehydrogenase (POX/PRODH), the first enzyme in proline catabolism, was suppressed by *MYC* through upregulating miR-23b*. This study provided a deeper understanding of tumor metabolism while enabling the development of novel therapeutic strategies.

2.7 HIF-1 Acts as a Regulator in Hypoxia Adaption and the Related Metabolic Change

HIF-1 activity is enhanced by mTOR-altered metabolism and promotes glycolysis as a hypoxia-adaptive transcriptional program. The HIF-1 and HIF-2 heterodimers respond to and are stabilized by hypoxia, resulting in metabolic changes [58]. Of these two heterodimers, HIF-1 is a critical component involved in tumor metabolism that upregulates glucose transporters, glycolytic enzymes, and pyruvate dehydrogenase kinase, isozyme 1 (PDK1), an enzyme which prevents pyruvate from entering the TCA cycle [30]. Qiao et al. demonstrated that malignant lymphomas exhibit constitutive expression of HIF-1α. This expression is mediated by nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and ionizing radiation treatment of lymphoma showed increased NF-κB activation and noteworthy HIF-1α levels. This indicates that supplemental treatment targeting HIF-1α in combination with radiation therapy of lymphoma cells could potentially improve patient outcomes [59].

Conclusion

The therapeutic challenges in malignant lymphomas include chemoresistance, radiation tolerance, and multidrug resistance. A novel therapeutic strategy depends on the metabolic phenotypes of aggressive lymphomas; however, many metabolic phenotypes exist. The malignant cells show different ways of altering catabolism and enhancing anabolism for rapid cell proliferation in order to adapt to the tumor microenvironment. The metabolic differences occur in many lymphomas; therefore, understanding and learning about these differences can lead to new targets for therapy, both individually or in combination with other treatments.

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The Metabolism of Renal Cell Carcinomas and Liver Cancer

Tu Nguyen and Anne Le

Key Points

- Different oncogenic mutations lead to different metabolic phenotypes in renal cell carcinomas (RCC).
- Loss of the von Hippel-Lindau (VHL) tumor suppressor gene results in metabolic alterations including aerobic glycolysis in RCC.
- Fumarate hydratase mutations result in an increase in aerobic glycolysis in RCC.
- Different oncogenic mutations lead to different metabolic phenotypes in primary liver cancer.
- MYC and MET mutations regulate glucose and glutamine differently in primary liver cancer.
- Glucose metabolism increased by acetylated phosphoglycerate kinase 1 (PGK1) leads to the promotion of cancer cell proliferation and tumorigenesis in the liver.

Keywords Renal cell carcinoma · Primary liver cancer · Metabolic phenotypes · Glucose metabolism · Glutamine metabolism · Oncogenic heterogeneity

Abbreviations

α-KG	α-Ketoglutarate
ACC	Acetyl-CoA carboxylase
AMPK	AMP-activated protein kinase
ATP	Adenosine triphosphate

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ccRCC	Clear-cell renal cell carcinoma
CL	Cardiolipin
COX5B	Cytochrome C oxidase subunit 5B
DEN	Diethylnitrosamine
ePC	Ether-type phosphatidylcholine
ePE	Ether-type PE
ERRα	Estrogen-related receptor A
FASN	Fatty acid synthase
FH	Fumarate hydratase
G6PH	Glucose-6-phosphate dehydrogenase
GLS2	Glutaminase 2
Glu	Glutamine
GLUT1	Glucose transporter 1
GLUT2	Glucose transporter 2
HCC	Hepatocellular carcinoma
HIF	Hypoxia-inducible factor
HIF-1α	Hypoxia-inducible factor 1-alpha
HK2	Hexokinase 2
LCSCs	Liver cancer stem cells
LDHA	Lactate dehydrogenase A
LRH-1	Liver receptor homolog 1
Me1	Malic enzyme 1
MIR21	MicroRNAs-21
mTORC1	Mechanistic target of rapamycin complex 1
NADPH	Nicotinamide adenine dinucleotide phosphate
Non-LCSCs	Non-liver cancer stem cells
PE	Phosphatidylethanolamine
PGK1	Phosphoglycerate kinase 1
PGLS	6-Phosphogluconolactonase
PI3K	Phosphatidylinositol-3 kinases
PTEN	Phosphatase and tensin homolog deleted in chromosome 10
RCC	Renal cell carcinoma
ROS	Reactive oxygen species
SM	Sphingomyelin
STF-31	4-[[[[4-(1,1-Dimethylethyl)phenyl]sulfonyl]amino]methyl]-N-3-pyridinyl-benzamide
TALDO	Transaldolase
TKT	Transketolase
TSC2	Tuberous sclerosis 2
VEGFR	Vascular endothelial growth factor receptor
VHL	Von Hippel-Lindau tumor suppressor gene

Introduction

According to data from the American Cancer Society, cancer is one of the deadliest health problems globally. Annually, renal cell carcinoma (RCC) and liver cancer cause more than 100,000 and 800,000 deaths worldwide, respectively [1–4], creating an urgent need to develop effective therapeutic treatments to increase patient survival outcomes. New therapeutic treatments are expected to address a major factor contributing to cancer's resistance to standard therapies: oncogenic heterogeneity. Because gene expression can vary tremendously among different types of cancers, different patients of the same tumor type, and even within individual tumors, various metabolic phenotypes can emerge, making single-therapy approaches insufficient. This heterogeneity translates into changes in the landscape of metabolic enzymes and biomolecules within both the cancer cell and tumor microenvironment. Novel strategies targeting the diverse metabolism of cancers aim to overcome this obstacle, and though some have yielded positive results, it remains a challenge to uncover all of the distinct metabolic profiles of RCC and liver cancer. Nonetheless, the metabolic-oriented research focusing on these cancers has offered different, fresh new perspectives, which are expected to contribute heavily to the development of new therapeutic treatments.

1 The Heterogeneity of Renal Cell Carcinoma Metabolism

Renal cell carcinoma (RCC), or hypernephroma, is the most common type of kidney cancer in adults responsible for approximately 90–95% of all cases. RCC originates from the network of convoluted tubules of the nephron [5] and consists of diverse histological subtypes, each with unique sets of metabolic rearrangements that can be traced to gene alterations [6, 7]. These genomic abnormalities provide cancer cells with the advantageous abilities to adapt to the limitations of their microenvironments and meet the demands of rapid and deleterious cell division.

1.1 Different Oncogenic Mutations Lead to Different Metabolic Phenotypes in RCC

1.1.1 Loss of the von Hippel-Lindau (VHL) Tumor Suppressor Gene Results in Metabolic Alterations Including Aerobic Glycolysis in RCC

Loss of the von Hippel-Lindau tumor supressor gene is the most prominent genetic alteration in RCC, commonly associated with over 80% of clear-cell renal cell carcinoma (ccRCC) tumors [8, 9]. The protein product of *VHL* facilitates the degradation of hypoxia-inducible factor (HIF) [10]. Thus, the loss of *VHL* leads to the

accumulation of HIF-1α and the constitutive activation of hypoxia-inducible genes even in oxygenated conditions. This includes the enhanced expressions of *GLUT2, HK2,* and *LDHA*, which are key to the metabolic shift towards aerobic glycolysis in tumors with this genotype [11, 12]. In fact, enhanced HIF-1α activity is thought to mediate the Warburg effect in RCC [13, 14]. In addition, pentose phosphate shunt dependence, increases in glutamine transport, and fatty acid production all have been documented as *VHL*-associated metabolic alterations in ccRCC [15]. The increased activity of the pentose phosphate pathway plays a significant role in protecting cancer cells from oxidative stress since this pathway generates NADPH and allows for the maintenance of glutathione levels [14]. The Consortia of The Cancer Genome Atlas Research Network revealed that upregulation of pentose phosphate pathway genes (G6PH, PGLS, TALDO, and TKT), fatty acid synthesis genes (ACC and FASN), and PI(3)K pathway enhancing genes (MIR21) correlated with worse survival, while upregulation of AMPK complex genes, multiple Krebs cycle genes, and PI(3)K pathway inhibitors (PTEN, TSC2) correlated with better survival [15].

The current standard frontline therapies for metastatic RCC are largely VEGFR inhibitors, such as sunitinib and sorafenib. However, about 20–30% of patients do not respond to these therapies and among those, nearly all become resistant [16]. Recent work by Gameiro et al. found that loss of *VHL* rendered RCC cells sensitive to glutamine deprivation [17]. In line with this finding, they found that systematic treatment with glutaminase inhibitors suppressed ccRCC growth both in vitro and in vivo. Other metabolic-targeting therapies for RCC include mitochondrial inhibition by auraptene [18] and GLUT1 inhibition by STF-31 [19].

1.1.2 Fumarate Hydratase Mutations Result in an Increase in Aerobic Glycolysis in RCC

Studies by Tong et al. indicated that RCC cells carrying mutated fumarate hydratase, a TCA cycle enzyme, demonstrated metabolic changes that were distinct from other genetically defined RCC, such as an increase in aerobic glycolysis, and advanced tumorigenicity. Thus, fumarate hydratase-deficient kidney cancer has low oxygen consumption rates as well as low complex I activities [20, 21]. In addition to having a glycolytic shift, FH-deficient kidney tumors and cell lines from patients with hereditary leiomyomatosis and RCC also exhibited decreased levels of AMP-activated protein kinase (AMPK), a key metabolic regulator. Glycolytic upregulation allows cells to adapt to growth demands by generating NADPH, acetyl-CoA, and precursors for ribose, protein, and fatty acid biosynthesis through reduced AMPK signaling. LDHA inhibition has shown promise against FH-deficient RCC cells in vitro and in vivo [22]. Metformin, an antidiabetic medication, was reported to activate AMPK and inhibit RCC growth in vitro and in vivo [23].

1.2 Metabolic Signatures of RCC

To compensate for the high demands of energy and biosynthetic macromolecules for proliferation, RCC cells deviate their metabolic phenotypes from that of normal renal cells to satisfy the demands. This leads to the different metabolic signatures of RCC cells as compared to normal renal cells. One of the metabolic signatures presenting in RCC cells found by Saito et al. is the decrease in the level of phosphatidylethanolamine (PE) in RCC cells as compared to normal cells [24]. PE is one of the most abundant glycerophospholipids in eukaryotes and plays crucial roles in autophagy, cell division, and protein folding and acts as a precursor of other protein syntheses [25–28]. Furthermore, the study shows that increased PE, which is induced by ethanolamine, inhibits RCC cell proliferation [24]. The low levels of PE inhibit cell apoptosis, which explains why the downregulation of PE benefits RCC cells. The study also found that increases in ether-type PE (ePE) and ether-type phosphatidylcholine (ePC) are associated with ccRCC metastasis [24]. Moreover, another metabolic feature of RCC is the decrease of sphingomyelin (SM), an essential component of the plasma membrane that regulates the formation of lipid microdomains through interacting with cholesterol and glycerophospholipids [24, 29]. A high level of SM was reported to make the cells more vulnerable to apoptosis [30].

In addition to the metabolic signatures of RCC reported by Saito et al., the study by Catchpole et al. found that the upregulation of fatty acid levels was potentially linked to the metastatic stage of the malignancy [31]. The high fatty acid levels were the result of an increase in de novo fatty acid synthesis and/or decrease in fatty acid oxidation which often occurred during invasive and metastatic stage of RCC [31, 32]. Another compound with high concentration, α-tocopherol, was found in RCC and ovarian cancer to protect tumor cells against oxidative stress [31, 33]. Metabolic profiling using metabolomics technologies has revealed different key metabolic phenotypes of RCC and identified potential targets for new therapeutic treatments for RCC patients.

2 The Heterogeneity of Liver Cancer Metabolism

Liver cancer is the second leading cause of cancer-related deaths [3, 4]. Hepatocellular carcinoma (HCC), a malignant tumor with hepatocellular differentiation, accounts for approximately 90% of the total number of primary liver cancer cases [34, 35]. Most patients with HCC are diagnosed at advanced stages, and the current effective treatments for these patients are limited. Nevertheless, if HCC patients are diagnosed in an early stage, the tumors can be resected or ablated. However, these patients often experience recurrence after resection/ablation [35, 36].

Strategies to increase patient survival outcomes involve therapies exploiting the metabolic vulnerabilities of cancer cells. However, within the tumor microenvironments, the alterations in metabolic pathways, resulting from the combinational

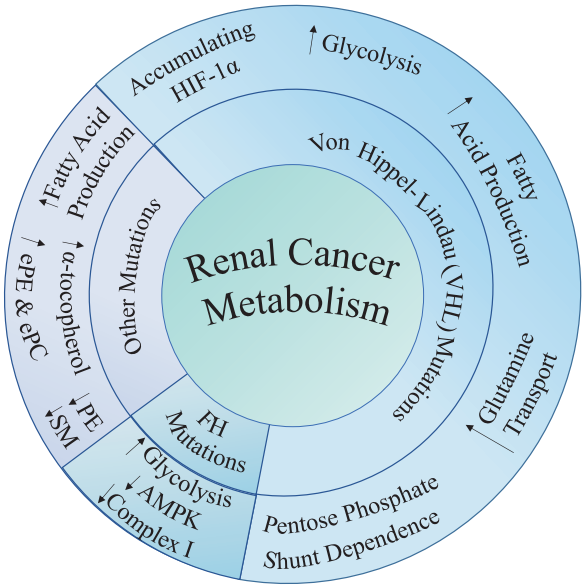

Fig. 1 The heterogeneity of renal cell carcinoma metabolism

effect of genetic, epigenetic, and transcriptomic variations [37, 38], occur frequently to accommodate the high energy demands of tumor growth. Consequently, the complexity of the heterogeneity of altered cancer metabolism leads to resistance in therapeutic cancer treatments [39, 40]. Additionally, different patients also exhibit different forms of liver cancer that correspond to genetic differences [37, 41–45]. Given the genetic and metabolic complexities of HCC, identifying core metabolic pathways utilized by the tumors to drive metabolic phenotypic plasticity of this neoplasm will substantially contribute to the development of effective metabolic therapies.

2.1 Different Oncogenic Mutations Lead to Different Metabolic Phenotypes in Primary Liver Cancer

2.1.1 *MYC* and *MET* Mutations Regulate Glucose and Glutamine Differently in Primary Liver Cancer

Yuneva et al. found that the reprogramming of glucose and glutamine metabolism was different depending on the activation of the *MYC* oncogene or the *MET* proto-oncogene even within a specific liver cancer type [46]. They found an increased uptake and catabolism of glucose in primary liver cancer as compared to normal liver in both *MET*- and *MYC*-induced liver tumors. However, *MYC*-induced liver

tumors exhibited the Warburg effect in which these tumors produced significantly high levels of lactate, a phenotype not observed in *MET*-induced tumors [46]. This study suggests that within the same cancer type, cells exhibit diverse genetic abnormalities that result in diverging and distinct metabolic manifestations. These numerous and remarkably pliant alterations appear to be essential for meeting the variety of demands of cell proliferation which include ATP production, biosynthesis of cellular building blocks, ROS detoxification, and degradation of the extracellular matrix scaffolding to allow for angiogenesis and thus tumorigenesis.

Tumor cell metabolism of the same tissue type has been shown to depend on the identity of the genetic mutations. While *MYC*-induced mouse liver tumors exhibit enhanced glutamine and glucose metabolism, accompanied with an increase in lactate production and Krebs cycle intermediates, *MET*-induced mouse liver tumors are found to consume glucose as a means of synthesizing glutamine [46]. Thus, it is reasonable to conclude that these two genes dictate radically opposite roles of glutamine, a central player in cancer metabolism. This fact illustrates, once again, that cancer metabolism can be determined by the nature of the genetic alterations and that the ways in which the metabolism is altered across different tumors can be extremely substantial. This helps to explain how the same tissue in different patients, in this case, can lead to different genetic alterations and metabolic phenotypes, thus substantiating the potential role of heterogeneity, even in a single tumor in a single tissue.

2.1.2 Liver Receptor Homolog 1 (LRH-1) Regulates Mitochondrial Glutamine Metabolism

The study led by Xu et al. has revealed the crucial role of LRH-1 in regulating mitochondrial glutamine metabolism, which eventually leads to the production of NADPH through a noncanonical glutamine pathway. Specifically, the study found that the regulation of Me1, an enzyme that catalyzes the conversion of malate to pyruvate to produce NADPH through a noncanonical glutamine pathway [47], is dependent on LRH-1 [48].

Furthermore, using $^{13}C_5$-labeled glutamine, the study highlighted the essential role of LRH-1 in promoting the production of glutamate from glutamine via controlling GLS2 (mitochondrial glutaminase 2) [48]. Consequently, the production of α-ketoglutarate from glutamate activates the mechanistic target of the rapamycin complex 1 (mTORC1) signaling pathway [48], a regulator of cell growth metabolism including proteins, lipids, and nucleotides [49]. Due to its pivotal role in the production of the reductive biosynthetic product NADPH and the activation of mTORC1 signaling pathway through glutamine metabolism, LRH-1 promotes cell proliferation. Thus, loss of LRH-1 prevents DEN-induced liver carcinogenesis [48].

2.1.3 Glucose Metabolism Increased by Acetylated Phosphoglycerate Kinase 1 (PGK1) Leads to the Promotion of Cancer Cell Proliferation and Tumorigenesis in Liver

Compared to normal liver cells, cancerous liver cells and cancer cells in general need a much greater amount of energy to fuel their proliferation. One of the ways to satisfy these high demands of energy is to adjust the energy-yielding pathways accordingly to produce energy in the most efficient manner. Therefore, deciphering different energy-enhancing mechanisms in cancers has attracted a lot of attention because having a better understanding of these mechanisms provides strategies to advance therapeutic treatments for cancers. Similarly, the study led by Hu et al. was trying to elucidate to the role of phosphoglycerate kinase 1 (PGK1), an enzyme catalyzing the conversion of 1,3-bisphosphoglycerate to 3-phosphoglycerate yielding one ATP molecule, in glycolysis, cell proliferation, and tumorigenesis. The formation of acetylated PGK1 at position K323 is required to activate PGK1 [50]. Activated PGK1, in turn, regulates cancer cell metabolism. Specifically, acetylated PGK1 enhances the production of energy in the form of ATP more rapidly [50]. Ultimately, the acetylation of PGK1 at K323 promotes liver tumorigenesis. With this understanding of how PGK1 K323 acetylation functions in liver cancer, the emergence of new effective therapeutic treatments using PGK1 as a therapeutic target for patients with liver cancer is promising.

2.2 Metabolic Differences Between Liver Cancer Stem Cells (LCSCs) and Non-Liver Cancer Stem Cells (Non-LCSCs)

Given their metabolic heterogeneity, LCSCs are able to adapt to many different environments, which causes therapeutic resistance to many available treatments for HCC. Understanding the metabolism of LCSCs is crucial not only for improvement of currently available treatments but also for paving a new path for developing other therapeutic treatments targeting the revealed metabolic pathways. In an effort to elaborate on the understanding of the metabolism of LCSCs, Hur et al. found the following differences in the metabolism of LCSCs as compared to non-LCSCs. The increased proliferation of LCSCs can be explained based on the metabolome analysis which reveals the higher presence of essential metabolites that are either resulting from highly activated catabolism or acting as substrates to promote other energy-yielding processes. Specifically, the study found higher concentration of lactate, the final product from glycolysis, citrate, succinate, and several amino acids such as aspartate, glutamate, isoleucine, leucine, phenylalanine, tyrosine, and valine in LCSCs as compared to non-LCSCs. Moreover, they identified that *MYC*, a known regulator of glycolytic metabolism [51, 52], was highly expressed in LCSCs as compared to non-LCSCs, and this resulted in increasing the amount of energy available for the rapid proliferation of the cancer cells [53]. This study also found that the activation of fatty acid oxidation in LCSCs was less active

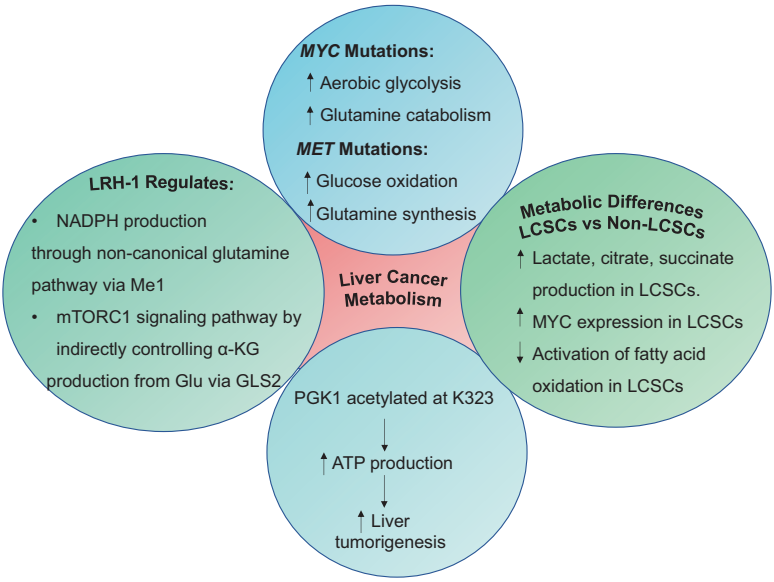

Fig. 2 The heterogeneity of liver cancer

than that in non-LCSCs [53]. Consequently, the production of NADPH produced from fatty acid oxidation contributing oxidative phosphorylation to generate ATP was less for LCSCs. Nevertheless, the assessment of three genes—COX5B, ATP5α, and ERRα—involved in oxidative phosphorylation showed no difference between LCSCs and non-LCSCs [53]. This means that LCSCs must have utilized more glycolysis to produce ATP to satisfy the demands of energy for their rapid proliferation.

Conclusion

Given the complex heterogeneity and intricate evolutionary characteristics of RCC and liver cancers, the increase in resistance rate to current therapies has emerged as a main obstacle that many studies focusing on these two types of cancers have been trying to overcome. Among the different methods available to tackle the problem, metabolomics-based approaches serve as powerful strategies—allowing researchers to uncover metabolic profiles of different cancers. In addition, using metabolomics technologies to track a variety of metabolites in cancers offers researchers a better picture of the interactions that occur within the tumor microenvironments. Understanding the heterogeneity of cancer metabolism will pave a new path for the development of metabolism-based therapies to increase the outcome of cancer therapy.

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Different Tumor Microenvironments Lead to Different Metabolic Phenotypes

Marjorie Justine Antonio and Anne Le

Key Points

- Cancer cells adapt to changes in nutrient and oxygen availability by adopting alternative metabolic pathways.
- Fatty acid oxidation in cancer cells is a survival mechanism of action to glucose deprivation.
- Lipid scavenging is utilized to enable cancer cells to survive periods of tumor regression.
- Distinct, and often complementary, metabolic processes operate concurrently within a single tumor.

Keywords Tumor microenvironments · Metabolic phenotypes · Fatty acid oxidation · Metabolic processes · Heterogeneity of cancer

Abbreviations

α-KG	α-ketoglutarate
ACC	Acetyl-CoA carboxylase
AMPK	AMP-activated protein kinase
ATP	Adenosine triphosphate
CAF	Cancer-associated fibroblasts
Cav-1	Caveolin-1
ETC	Electron transport chain

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FABP4	Fatty acid-binding protein 4
FASN	Fatty acid synthase
HBx	Hepatitis B virus X protein
HCC	Hepatocellular carcinoma
hMSCs	Human mesenchymal stem cells
KRAS	Kirsten rat sarcoma viral oncogene homolog
NADPH	Nicotinamide adenine dinucleotide phosphate
SCD1	Stearoyl-CoA desaturase 1
TME	Tumor microenvironment

Introduction

The beginning of the twenty-first century offered new advances in cancer research, including the expansion of the knowledge about the tumor microenvironment (TME). Because TMEs provide the niches in which cancer cells, fibroblast, lymphocyte, and immune cells reside, they play a key role in cancer cell development, differentiation, survival, and proliferation. Throughout cancer progression, the TME constantly evolves, causing cancer cells to adapt to the new conditions. The heterogeneity of cancer, evidenced by diverse proliferation rates, cellular structure, metabolism, and gene expression, presents challenges for cancer treatments despite the advances in research. This chapter discusses how different tumor microenvironments lead to specific metabolic adaptations which drive cancer progression.

1 The Tumor Microenvironment

The TME, the environment surrounding the cancer cells, is a heterogeneous mixture of immune cells, endothelial cells, secreted materials from cells and their organelles, and fibroblasts [1] (Fig. 1). Within this miniscule niche, the tumor survives in seemingly hostile conditions—hypoxia, nutrient deficiency, and necrosis—thanks to metabolic reprogramming. The question is: How does a tumor's microenvironment offer advantages for cancer cell survival under such conditions?

Hanahan and Weinberg suggest that there are six general characteristics of cancerous cells important for advancements toward malignant growth: (1) self-sufficiency in growth signals, (2) insensitivity to anti-growth signals, (3) evasion from apoptosis, (4) limitless replication potential, (5) sustained angiogenesis, and (6) tissue evasion and metastasis [2]. Despite the diversity of outcome in tumor progression, these same capabilities are shared by most, if not all, tumor types. Moreover, these features develop differently in various tumor types through distinct mechanisms and multistep tumorigenesis: genomic instability in cancer cells and

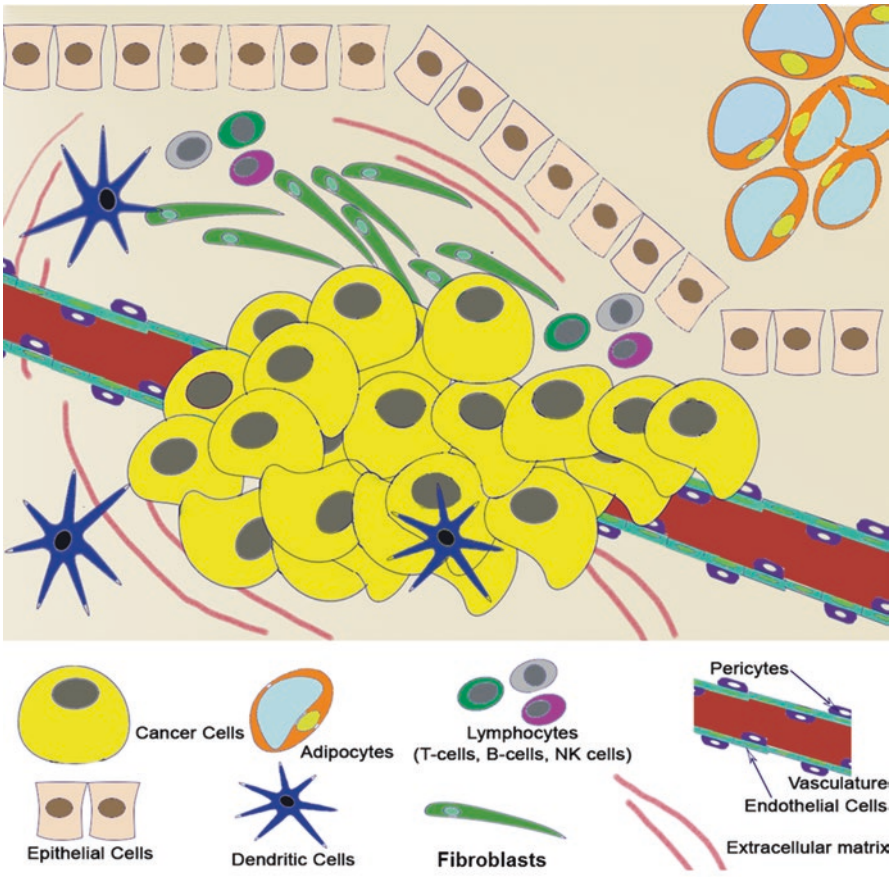

Fig. 1 The tumor microenvironment is composed of several components such as lymphocytes, adipocytes, fibroblasts, and dendritic cells

tumor-promoting inflammation [3]. The hallmarks of cancer provide further insight into potential opportunities for early interventions for cancer treatment.

Among their basic needs, cancer cells require rapid ATP generation, biosynthesis of macromolecules, and maintenance of cellular redox status [4]. The insidious nature of cancer cells does not stop at their determination to live but also extends to the factors that sacrifice adjacent living tissue to propagate cancerous cells. Tumors create alternate pathways for nourishment and, most importantly, survival.

The differences in cancer origin and stage of progression ultimately lead to the heterogeneity of cancer and the corresponding components involved in cancer metabolism.

2 Different Tumor Microenvironments Lead to Different Metabolic Phenotypes

2.1 Cancer Cells Adapt to Changes in Nutrient and Oxygen Availability by Adopting Alternative Metabolic Pathways (Fig. 2)

The harsh tumor microenvironment, hypoxia, low pH, and low nutrient concentrations are key characteristics in determining metabolic phenotypes. Various studies have demonstrated that cancer cells adapt to changes in nutrient and oxygen availability by adopting alternate metabolic pathways in order to continue providing the energy and macromolecules needed for cell proliferation. These pathways include fatty acid oxidation, lipid scavenging, alternative cellular respiration pathways, and mechanisms adopted by cancer cells under hypoxia and normoxia [5–8].

The nutrient- and oxygen-poor internal conditions of TMEs incite cancer-friendly metabolic changes to help survival in these harsh environments [9]. Under hypoxic conditions, oxidative phosphorylation or other aerobic reactions are limited. This state disrupts the redox balance and affects cell signaling. An increase in the levels of reactive oxygen species (ROS) leading to damaged lipids, proteins, and DNA is defined as oxidative stress [10]. Due to decreased oxygen tension, hypoxic cells depend on anaerobic glycolysis for energy production, while their low oxygen supply allows ATP production via oxidative phosphorylation [11]. In TMEs that demonstrate elevated levels of oxidative stress, nutrients that can lead to cancer cell

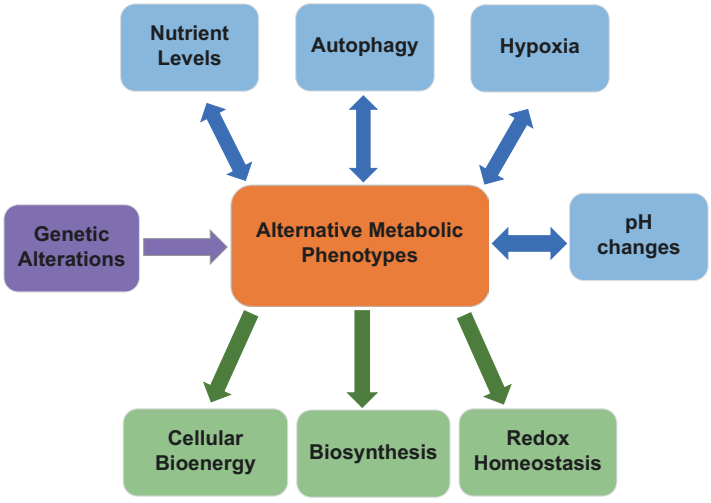

Fig. 2 The fundamental concept of how the tumor microenvironment (blue) leads to different metabolic phenotypes. Genetic alterations also contribute to the metabolic phenotype. The metabolic phenotype then propels bioenergetics, biosynthesis, and redox reactions in the tumor cells to taking place

proliferation are released [11, 12]. For example, breast cancer growth is attributed to the TME, which reacts to oxidative stress leading to the production of ROS [12, 13]. Similarly, a study by Le et al. found that there was an increase in ROS production in response to oxidative stress under hypoxia [14]. Thus, it can be concluded that cancer cells become dependent on glutamine for bioenergetics and redox homeostasis as a way to survive in hypoxia [14].

Extracellular acidity is another crucial component of the TME [15]. When cancer cells undergo anaerobic glycolysis in hypoxia, lactic acid levels increase, causing the TME's extracellular pH (pH_e) to diminish. This reaction generates an acidic TME [15]. Tumors that have an acidic TME have been shown to display more malignant phenotypes. Rofstad et al. treated melanoma cells with an acidic medium resulting in increased melanoma cells metastasizing to the lungs in mice [16]. The results seen in the study suggest that lower pH_e can cause malignant cell growth.

The heterogeneity of nutrient and oxygen supply and uptake within individual tumors, in conjunction with the evidence of the adaptive prowess of cancer cells in response to differing conditions, illustrates that cancers are composed of many different cells that are each capable of employing distinct metabolic pathways to supply energy and fuel biosynthesis as a means of maintaining tumorigenesis. Thus, the local TME holds the determining factors by which metabolic adaptation is acquired [7, 8, 11, 12].

The hypoxic conditions lead to pathways that would only be present due to the alterations made necessary by metabolic stress. Other cells responded to glucose deprivation by requiring less energy to survive or utilized alternative compounds to take glutamine's place in the tricarboxylic acid (TCA) cycle. However, different cancer cells take varying initiatives in order to survive, further exemplifying the heterogeneity of cancer metabolism.

2.2 Fatty Acid Oxidation Is Used as a Survival Response to Glucose Deprivation

Cancer cells employ fatty acid oxidation as a means to survive in response to glucose deprivation [5, 6]. Fatty acid oxidation is utilized by tumor cells to produce ATP as an energy source [6, 17]. Over twice the amount of ATP per mole of oxidation of glucose can be made under mitochondrial fatty acid oxidation [6]. Due to harsh TME conditions, for example, lacking nutrition, cancer cells adapt different metabolic phenotypes, such as transitioning from glycolytic to fatty acid oxidation phenotype [5, 17]. The lack of nutrition also enhanced both fatty acid synthesis and lipid droplet biogenesis to propel lipid oxidation for the maintenance of energy levels.

In a study conducted by Wang et al., the roles of the hepatitis B virus X protein (HBx) in hepatocellular carcinoma (HCC) adaption to metabolic stress were investigated. Wang et al. found that HBx activated fatty acid oxidation in glucose withdrawal [5]. HBx facilitated fatty acid oxidation under glucose deprivation,

maintaining nicotinamide adenine dinucleotide phosphate (NADPH) and adenosine triphosphate (ATP) homeostasis. HBx held dynamic equilibrium, mobilizing and oxidizing lipids to meet demands for ATP [5]. These results suggest that HBx plays a key role in maintaining redox and energy levels by activating fatty acid oxidation, a necessary part of HCC cell survival under metabolic stress.

Most cancer cells synthesize de novo fatty acids during normoxia without nutrition deprivation [6, 17]. Fatty acid synthesis is a crucial step for tumor cell survival [17]. Cancer cells synthesize de novo fatty acids in order to keep up with proliferation and energy production through fatty acid oxidation. Acetyl-CoA carboxylase (ACC) and fatty acid synthase (FASN) are essential enzymes in de novo fatty acid synthesis. Acidic and hypoxic environments induce FASN expression in cancer cells, which is an observable phenotype in a variety of human cancers [17].

2.3 Lipid Scavenging Is Utilized to Enable Cancer Cells to Survive Periods of Tumor Regression

Under hypoxic conditions, oncogenic KRAS (Kirsten rat sarcoma viral oncogene homolog) regulate lysophospholipids to replenish lipids for growth. The inhibition of stearoyl-CoA desaturase 1 (SCD1), which catalyzes the bypassing of saturated de novo fatty acids into lipids, was resistant in KRAS-derived tumor cells because of their adaption of lipid scavenging [6]. The increase in protein synthesis and decrease in lipid desaturation ultimately resulted in cell death [18, 19]. During tumor regression, cancer cell survival is made possible by fatty acid oxidation and other oxidative mitochondrial pathways. As demonstrated by KRAS-driven pancreatic cancer, tumor regression caused by kinase inhibitors or KRAS withdrawal resulted in inhibited oxidative respiration in tumor cells [20]. Lipid scavenging is an alternative pathway to gain fatty acids in hypoxic and Ras-driven cancer cells and can fulfill the requirements for cell monounsaturated fatty acids [21]. The reduction of the need for de novo fatty acid synthesis is attributed to the increase in fatty acids being brought into the TME. Ras-driven cancer cells become immune to SCD1 inhibition, demonstrating the hypoxic metabolic phenotype [21].

According to Ackerman and Simon, adipocytes within TMEs play a key role in increasing lipolysis and secreting fatty acids for energy production contributing to an aggressive growth phenotype [22]. Lipids produced from adipocytes were given to ovarian cancer cells in order to help tumor growth. These findings suggest that TMEs where adipocytes are key players in tumor growth by supplying fatty acids [23]. Moreover, this study uncovered fatty acid-binding protein 4 (FABP4) as a potential target for cancer therapy.

2.4 Persistence of Glutamine Oxidation Under Hypoxic and Glucose Deprivation Conditions

As established in previous chapters, the tricarboxylic acid (TCA) cycle is a crucial part of producing energy and biosynthesis [24]. However, how hypoxic TME's influence the TCA cycle is still being investigated. Le et al. determined how hypoxic conditions could influence glutamine metabolism [14]. Their study showed that when deprived of glucose and oxygen, B-cell lymphoma exhibit an addiction to glutamine where glutaminolysis is employed with a glucose-independent TCA cycle to fuel cell proliferation [14]. In this scenario, the glucose-independent TCA cycle was supported by glutamine. Similarly, hypoxic cells use glutamine to generate citrate from α-KG (α-ketoglutarate) in response to a reduced supply of glucose-derived citrate [14]. Collectively, these findings offer a cautionary note that therapeutic strategies targeting cancer metabolism should consider the metabolic heterogeneity in hypoxic cancer cells, particularly the non-Warburg cells that have so far been underrepresented in the cancer metabolism literature.

3 Nutrient Utilization Can Predict a Tumor's Metabolic Dependencies In Vivo [25]

As described by Sir Hans Kornberg, anaplerosis is the reloading of metabolic intermediates in the TCA cycle, which is a crucial part of energy production and the biosynthetic pathways. Glutamine and glucose both contribute to TCA anaplerosis in non-small cell lung cancer (NSCLC) cells [25]. A study by Davidson et al. found that glucose is a carbon source of the metabolites in the TCA cycle, which is needed for tumorigenesis.

For continuous proliferation, cancer cells must maintain the necessary precursors of biosynthetic pathways, glutamine being a major substrate for anaplerosis in many cancer cells [7]. For example, both hypoxic and normoxic renal cell carcinomas with a mutation in the VHL tumor suppressor gene sustain lipogenesis by reducing α-KG, derived from glutamine, to acetyl-coA, which then allows them to utilize the glucose-independent TCA cycle as a means of energy production [7, 8]. On the other hand, when glutaminase is inhibited, the breakdown of glutamine is prevented and some cancer cells employ pyruvate carboxylase and use glucose-derived pyruvate as a substitute for glutamine to fuel anaplerosis [7].

Similarly, a study by Cheng et al. demonstrated that "glutamine-addicted" cells fulfilled anaplerosis by the catalyzation of pyruvate carboxylase [7, 26]. It was found that the glutamine-addicted cells utilized glucose-derived pyruvate for anaplerosis when glutaminase (GLS) was silenced. The data from this study supports the model of pyruvate carboxylase's role in cancer cell resistance against GLS inhibition or glutamine deprivation. Cells such as a hepatocellular carcinoma cell line, Huh-7, use pyruvate carboxylase as a primary mechanism to resist against treatment of glutamine metabolism inhibition [7].

3.1 Inhibition of mTORC1 Decreases Energy Consumption for Cancer Cell Survival

mTORC1 (mammalian target of rapamycin complex 1) is a protein that translates the cell's TME into a growth phenotype through its control of autophagy and fatty acid oxidation. The inhibition of mTORC1 represses the AMPK-dependent activation of TSC1/2 (tuberous sclerosis proteins) as a result of the withdrawal of glucose [8]. When energy consumption is reduced, oxaloacetate (OAA) or methyl pyruvate (MP) can be substituted for glutamine and still be able to maintain ATP levels and prevent cell death. The TSC-mTORC1 pathway balances energy supply and demand in a way that leads to a reduction of the energy needed to survive [8]. Choo et al. demonstrated that, under glucose deprivation, a decrease in anabolic reactions occurred in order to prevent cell death. As shown with the decrease of energy consumption, the balance kept the cancer cells alive, through the dependence of TSC1/2 cells on glutamate dehydrogenase-dependent glutamine metabolism. The results found in this study support that tumor cells under stress create alternative pathways out of necessity. With glucose or glutamine metabolism inhibition, the potential treatment of TSC-deficient tumors may be possible.

3.2 Cancer Cells with Functionally Defective Mitochondria Employ Glutamine-Dependent Reductive Carboxylation as an Alternative to Normal Oxidative Metabolism

In normal cells, mitochondria play vital roles in regulating metabolic pathways and physiological states of the cell: they generate cellular energy, monitor cellular redox, and initiate cellular apoptosis. However, through investigation of mitochondria in cancer cells, it has become evident that mutations in mitochondrial genes correlate with tumorigenesis and metabolic adaptability [27]. Mitochondria in cancer cells subjected to hypoxia respond by releasing metabolites and proteins regulating metabolic pathways [27].

Cancer cells with functionally defective mitochondria employ glutamine-dependent reductive carboxylation as an alternative to normal oxidative metabolism. Oxidative metabolism is favored in cells with normal mitochondria and provides the acetyl-CoA needed for lipogenesis and other metabolites of the TCA cycle, which serve as precursors of other biosynthetic pathways. Even in cells with altered mitochondrial function, the glutamine-dependent reductive metabolism still allows for the formation of these necessary metabolic precursors [28]. The glutamine-dependent reductive pathway permitted glutamine to support cancer cell growth [28]. TCA cycle reactions were reversed in the glutamine-dependent reductive pathway, which led to cancer cell proliferation, replenishing TCA intermediates despite the presence of functionally defective mitochondria.

4 Distinct, and Often Complementary, Metabolic Processes Operate Concurrently Within a Single Tumor

The particular alternative metabolic pathways adopted by cancer cells are associated with specific genetic alterations that allow the cancer cells to make certain enzymes. The production of these enzymes allows cancer cells to use the available nutrients in their microenvironment to fuel cell survival and proliferation. For example, genetic alterations that result in the deactivation of caveolin-1 (Cav-1) expression lead to autophagy and aerobic glycolysis [29]. Subsequently, lactate, glutamine, and other metabolites that fuel biosynthesis are synthesized and exported to initiate oxidative metabolism in neighboring cancer cells [29].

Other studies have revealed that distinct, and often complementary, metabolic processes operate concurrently within a single tumor. Hypoxic breast cancer cells and stromal cells in the TME have exhibited a mutualistic relationship employing complementary metabolic processes [30]. When subjected to hypoxia, breast cancer cells demonstrate an increase in lactate secretion. The elevation in lactate concentration in the TME results in the migration of specific stromal cells called human mesenchymal stem cells (hMSCs) toward hypoxic tumor cells. These hMSCs, along with stromal cancer-associated fibroblasts (CAFs), consume the newly produced lactate and convert it to pyruvate, to be used in the TCA cycle. Lactate consumption by stromal cells serves two purposes: the breakdown of lactate serves as an energy source for the proliferating cancer cells, and the conversion of lactate to pyruvate, and ultimately to α-KG in the TCA cycle, prevents acidification of the TME [30].

Another example of this phenomenon of cancer microenvironments pairing metabolic processes is evident in ovarian cancers. Adipocytes in breast cancer microenvironments employ lipolysis to release fatty acids which provide energy to fuel rapidly proliferating ovarian cancer cells [23]. Within one region of the TME, two different types of cells undergo vastly different, yet complementary, metabolic processes in order to fuel tumorigenesis, thus demonstrating the heterogeneity of cancer metabolism.

Conclusion

As cancer cells seek to survive, alternate metabolic pathways adapt to different TME stresses. These adaptations, often through genetic alterations or coordination with other metabolic processes, exemplify how precisely the TME can alter metabolic characteristics. With the advancements in research into TMEs and the considerable number of novel metabolic pathways, there is a tremendous opportunity for uncovering new therapeutic targets and creating treatments that target TMEs. The heterogeneity of cancer metabolism is evident in genetic mutations in oncogenes and tumor suppressor genes, as well the diversity of the TME.

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The Intratumoral Heterogeneity of Cancer Metabolism

Karim Nabi and Anne Le

Key Points

- Heterogeneity is a hallmark of cancer.
- Clonal evolution theory and cancer stem cell theory explain tumor subpopulation growth.
- Intratumoral metabolism heterogeneity follows intratumoral genetics alterations.
- Epigenetics alterations lead to intratumoral metabolism heterogeneity.
- Intratumoral metabolic adaptation and heterogeneity are due to the intemperate conditions of the tumor microenvironment.
- Spatial and temporal heterogeneity provides survival advantages to tumors.
- Metabolic profile-targeted therapeutics can result in successful clinical outcomes.

Keywords Intratumoral heterogeneity · Metabolism · Genetic and metabolic adaptation · Angiogenesis · Hypoxia

Abbreviations

aKG	Alpha-ketoglutarate
CAF	Cancer-associated fibroblasts
BPTES	Bis-2-(5-phenylacetomido-1,3,4-thiadiazol-2-yl)ethyl sulfide
CSC	Cancer stem cell

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DTC	Disseminated tumor cells
EC	Endothelial cells
FBP1	Fructose-1,6-bisphosphatase 1
FBP2	Fructose-1,6-bisphosphatase 1
FH	Fumarate hydratase
GLUT-1	Glucose transporter type 1
HIF-1α	Hypoxia-inducible factor-1α
LDHA	Lactate dehydrogenase A
OXPHOS	Oxidative phosphorylation
PET	Positron emission tomography
PKM2	Pyruvate kinase muscle isoform 2
SDH	Succinate dehydrogenase
TCA	Tricarboxylic acid
VEGF	Vascular Endothelial Growth Factor

Introduction

Cancer is one of the deadliest diseases in the world, especially within the past few decades, causing over half a million deaths a year in the USA only [1]. Despite recent advances made in the field of cancer biology and the therapies that have been developed, it is clear that more advances are necessary for us to classify cancer as curable. The logical question that arises is simple: Why, despite all the technologies and medical innovations of our time, has a cure eluded us? This chapter will shed light on one of cancer's most impactful attributes: its heterogeneity and, more specifically, the intratumoral heterogeneity of cancer metabolism. Simply put, what makes cancer one of the deadliest known diseases is its ability to change and adapt. Cancer cells' rapid evolution, coupled with their irrepressible ability to divide, gives them the advantage over our immune systems. In this chapter, we will delve into the complexities of this adaptability and the vital role that metabolism plays in the rise and progression of this heterogeneity.

1 Multiple Theories Explain Cancer's Heterogeneous Nature

In any observable tumor, there is much more than meets the eye. In the carcinogenic environment, we can observe a microcosm of the theory of evolution at play. While Darwin's theory was proposed to explain the evolution of species due to slow cumulative changes that arise from natural selection, cancer cells, driven by their genetic instability and high reproductive rates, develop in a fraction of our lifetime, leading to dangerous and unpredictable outcomes. The genetic instability associated with cancerous cells gives rise to a plethora of downstream metabolic phenotypes. These phenotypes offer cancerous cells one of the most valued assets in their battle for

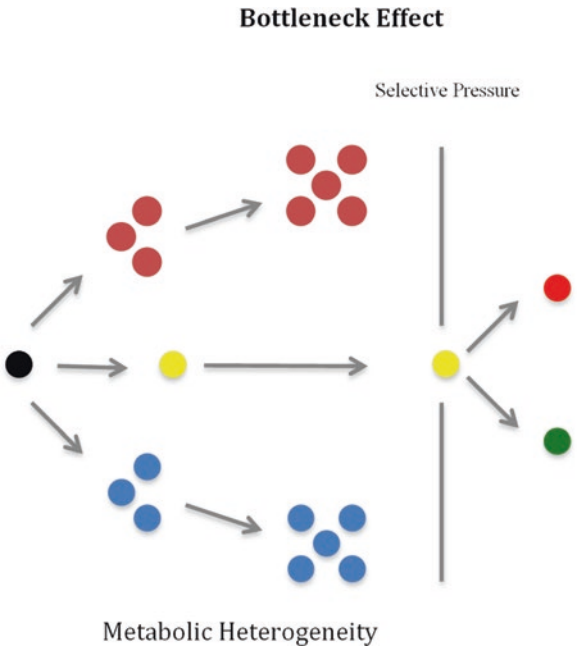

Fig. 1 Bottleneck effect in tumors. Metabolically different subclones of a tumor, each represented by a different color. Survival and growth rates of subclones depend on the various selective environmental pressures applied such as blood supply shortage, low oxygen levels, and drug treatment

survival: their metabolic diversity, which can explain why it is so difficult to find effective therapies for most cancers.

The explanation of intratumoral heterogeneity using the theory of evolution provides a solid basis for understanding why and how tumors possess this medley of metabolic phenotypes. Tumors have different genetic and metabolic phenotypes due to different environmental pressures such as vascularization, oxygen supply, and other factors such as drug treatments. While certain subpopulations with defined metabolic phenotypes may be sensitive to a suitable metabolic inhibitor, other subclones with different metabolic phenotypes may well be resistant to that drug. This explains why patients may become unresponsive to second-round treatment after an initial successful first round in which most of the tumor was targeted by treatment but small subpopulations were not [2, 3]. These selective pressures promote the survival and propagation of genetically and even epigenetically diverse subclones that lead to the downstream array of metabolic phenotypes in each subclone (Fig. 1).

It is important to mention another emerging theory, namely, the cancer stem cell (CSC) theory, which challenges the previously mentioned clonal evolution theory. The clonal evolution theory claims that genetically and metabolically distinct subpopulations arise from a previously larger population of cancer cells due to the expansion of the population, genetic diversification, and selection of certain subclones over others. On the other hand, the CSC theory states that a significant source of heterogeneity in cancer cells is due to CSCs, which are undifferentiated and have high rates of division. These cancer stem cells possess largely variable metabolic phenotypes through their differentiation into different types of cells [4]. They are

also capable of differentiating into metabolically and functionally diverse subclones within a single tumor. Moreover, they are usually resistant to many therapeutic methods due to their undifferentiated state. This fact is supported by findings suggesting that more differentiated cancer stem cells tend to lead to better prognoses due to their decreased tumorigenic potential [5]. In fact, the mechanism behind many therapies for cancer patients induces differentiation of CSCs. The origin of these CSCs ranges from tumor cells that acquired stem cell properties to differentiated stem cells that simply accumulated mutations that turn them into CSCs [6].

Cancer is further complicated by the fact that the different sources of heterogeneity, namely, CSC-derived heterogeneity, evolution-derived heterogeneity, and heterogeneity related to environmental factors, can all coexist at once [7]. This makes it a much more arduous feat to eradicate all subclones within any given tumor and then leads to the following question: Why do cancer cells employ various biological processes, even within a single tumor from a single patient? The ultimate advantage of intratumoral heterogeneity of cancer cell metabolism is that it confers an ability to survive and proliferate within the tremendously variable, and often harsh, tumor microenvironment. The diversity of the tumor microenvironment—characterized by areas of poor oxygenation, acidity, sparse nutrients, or growth factors—is the challenge that cancer cells must overcome in order to achieve the goals of survival and continued cell proliferation.

How do these diverse metabolic phenotypes arise? We know that the different microenvironments in any given tumor provide different selective pressures that lead to the propagation of specific advantageous mutations in each respective cancer subclone. We also know that CSCs can contribute to the heterogeneous aspect of a tumor by providing differentiated subpopulations with variegated genetic expressions and regulated metabolic pathways. These changes provide a variety of proteins and, most importantly, enzymes necessary to effectively convert locally available nutrients into energy and useful products suited for each microenvironment to obtain what they require for the production of a specific metabolic phenotype for each subclone.

2 Intratumoral Metabolism Heterogeneity Follows Intratumoral Genetic Alterations

The intricate relationship between genetics and metabolism in cancer is arguably the main reason the diverse metabolic phenotypes within a given tumor can arise. All of the genetic changes, if occurring in different regions of a tumor, can lead to a diverse array of differently regulated metabolic processes in a tumor.

Genetic alterations, which are often the result of a response to the tumor microenvironment, are the means by which cancer cells are able to produce the enzymes necessary to effectively convert locally available nutrients into energy and useful products to achieve their goals. Oxygen and nutrient supply vary across individual

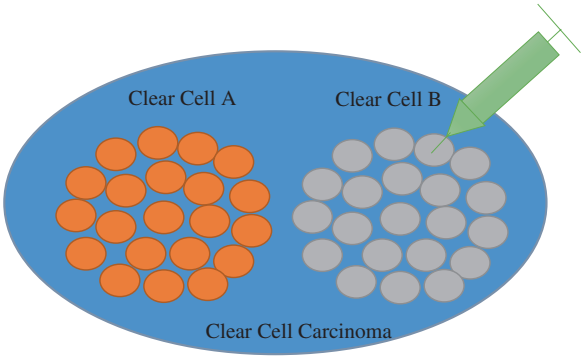

Fig. 2 Clear cell carcinoma tumor (shown in blue) with subclones (shown in orange and gray). The orange subclone consists of clear cell A cells (associated with good prognoses), and the gray subpopulation consists of clear cell B cells (associated with poor prognoses). Single biopsies taken from one population may indicate misleading prognoses

tumors. Thus, intratumoral gene expression is diverse, and it is this heterogeneity that allows cancer cells to adapt to the diverse and taxing conditions of the tumor microenvironment. These adaptations in nutrient uptake and biosynthesis, which have been linked to particular genetic mutations, must follow from gene expression in cancer cells. As such, the enzymes produced are the proximate cause of the adoption of alternative metabolic pathways, which contribute to the cancer cells' successful survival and growth.

In light of the evidence of intratumoral genetic heterogeneity, along with the fact that changes in cancer cell metabolism are consequences of alterations in gene expression, cancer metabolism must be vastly diverse across a single tumor. A recent study notes the coexistence of various genetically different subclones in advanced tumors, challenging the previously held notion that a dominant subclone usually appears in a given tumor [8]. Furthermore, based on the expression of 110 genes, another study showed that different subpopulations in one clear cell carcinoma were classified as clear cell A (associated with good prognosis) and B (associated with poor prognosis) [8, 9]. These results emphasize not only how varied gene expression within a single tumor can be but also the need to accurately use prognostic markers due to the different genotypes within each subclone, as not doing so could potentially lead to erroneous prognoses (Fig. 2).

In breast carcinomas, another study has shown that intratumoral genetic diversity is also widely prevalent within tumor cell populations, which were composed of stem cell-like or more differentiated cell populations due to their expressing different clusters of differentiation, antigens expressed on the cell surface of cells [10]. These subpopulations were further found to exhibit highly heterogeneous genetic composition, implying different biological and metabolic functions and, most likely, different responses to treatments [10].

3 Epigenetics Alterations Lead to Intratumoral Metabolism Heterogeneity

It is important to note that not all heterogeneity arises from genetic alterations. New studies point to the importance of epigenetics' role in tumor heterogeneity. Epigenetics studies have recently uncovered increased methylation in promoters of a variety of important genes in tumor progression such as tumor suppressor genes [11]. Along with other findings showing similar roles of epigenetics in cancer evolution, these results display the importance of epigenetics and its potential role in cancer evolution and heterogeneity. In a study published in Scientific Reports, we can observe an example of the effect of epigenetic intratumoral heterogeneity [12]. This study revealed that in a given glioblastoma tumor, 40% transcriptional heterogeneity was observed in a gene encoding a DNA repair enzyme: O6-methylguanine DNA methyltransferase (MGMT). Furthermore, 14% of heterogeneity was attributed to the methylation levels of the promoter of that gene, whose methylation status has been used for clinical purposes as a marker that correlates with therapeutic response [12]. However, these variations in expression across a single tumor pose a threat to the effectiveness of this clinical marker. In addition to the genetic heterogeneity observed, researchers and clinicians need to keep in mind the variability displayed on an epigenetic level across a single tumor. Therefore, it is fair to keep in mind the potential effect epigenetics could have on metabolism, as seen in the study regarding FBP1 and FBP2 methylation status, not only in normal cells but also in those of tumorigenic origin and even different subclones within the same tumor.

In another study by Okegawa et al., the characterization of kidney tumors revealed distinct metabolic profiles in different regions of the same tumors [13]. The study identified two distinct tumor clusters, MC1 and MC2, where MC2 displayed upregulated pyruvate metabolism which was confirmed using isotope tracing in tumor slices. This suggests that pyruvate metabolism may be a potential therapeutic target due to some clones' reliance on it. However, genetic differences between subpopulations did not match the metabolic profile of such subclones, suggesting that factors other than genetics, such as epigenetics or otherwise, may play a role in developing distinct metabolic phenotypes.

4 Intratumoral Metabolic Adaptation and Heterogeneity Is Due to the Intemperate Conditions of the Tumor Microenvironment

Now we take a closer look at how a tumor can metabolically adapt to its ever-changing environment. These adaptations also reflect an evolutionary advantage in cancer cells and give rise to the heterogeneity found in cancer. As a tumor grows in size, it develops hypoxic regions that are beyond the diffusion limits of oxygen in existing vasculature. Tumor hypoxia, in addition to its role in the mutation of

oncogenes and tumor suppressor genes, plays a major role in the overexpression of HIF-1α (hypoxia-inducible factor-1α) in cancer. HIF-1α is part of a heterodimeric protein that acts as a transcriptional regulator for many genes involved in angiogenesis, erythropoietin production, cell survival, and much more. While HIF-1α usually degrades quickly under normal conditions, degradation is suppressed in hypoxic environments. Therefore, HIF-1α upregulates the expression of genes that code for adaptive metabolic changes, switching cancer cell metabolism from oxidative phosphorylation to glycolysis, increasing the conversion of glucose to glycogen as a glucose reservoir, and using glutamine as the major substrate for fatty acid synthesis [14, 15]. Furthermore, HIF-1 directly transactivates lactate dehydrogenase A (LDHA) expression in hypoxia [15], which explains how hypoxia further accentuates glycolysis [3, 16].

In order for the tumor to metastasize and grow beyond a few millimeters, angiogenesis is necessary [17]. HIF-1α also upregulates the expression of genes that code for angiogenesis. One of the more notable genes is the gene encoding pro-angiogenic vascular endothelial growth factor (VEGF), which induces the proliferation of endothelial cells (ECs), a key process in angiogenesis [18–20]. Surprisingly, several studies found that ECs mainly rely on glycolysis rather than oxidative phosphorylation (OXPHOS) despite the ideal location of ECs that promotes their function as an endothelium and in maintaining vascular barrier homeostasis and bioenergetics [21–26]. Similar to cancer cells, ECs choose aerobic glycolysis over OXPHOS due to their rapid growth, which is necessary to fulfilling the demands of forming new blood vessels [21]. Reducing glycolysis by silencing its stimulator phosphofructokinase-2/fructose-2,6-bisphosphatase 3 (PFKFB3) decreased angiogenesis [21]. Moreover, PFKFB3-deficient ECs display poor vessel growth in several in vivo models of angiogenesis. VEGF, in turn, also promotes glycolysis through the upregulation of glucose transporter type 1 (GLUT-1) which facilitates glucose uptake [27].

As evident, the tumor microenvironment is tremendously dynamic and diverse concerning nutrient and oxygen supply, both spatially and temporally within a single tumor. Temporal variations of the partial pressure of oxygen within a specified region of the tumor, referred to as intermittent or cyclic hypoxia, occur in different regions throughout the tumor [28]. The occurrence of cyclic hypoxia is attributed to variations in red blood cell flux, which is thought to be a result of changes in blood flow resistance that arise from angiogenesis and other structural changes to the vasculature [28, 29]. Regions of the tumor with adequate vasculature are much more resistant to intermittent hypoxia than regions with insufficient vasculature [30]. Although reduced oxygenation to either select regions or the entire tumor can induce hypoxia, an increase of equal magnitude in the oxygen consumption is disproportionately more effective at inducing hypoxia [31–35]. These variations in oxygen and nutrient delivery, as well as in oxygen consumption within a tumor, are fundamental to the pervasive metabolic heterogeneity exhibited by cancer cells among different types of cancers, patients with the same cancer type, and most notably within a single tumor from any given patient.

5 Metabolic Heterogeneity Leads to Unpredictable Outcomes

Now that we have a basic background on how the various metabolic phenotypes in a given tumor arise and the different processes driving it, we can take a look at some specific examples and cases of intratumoral metabolic heterogeneity.

5.1 Spatial Heterogeneity Provides a Survival Advantage to Tumors

For a long time, it was believed that cancer cells' major metabolic footprint was the Warburg effect, which dictates that cancer cells undergo glycolysis to produce lactic acid even in the presence of oxygen, a process termed aerobic glycolysis. Although the Warburg effect is still relevant, it recently became clear that the metabolic phenotypes of cancer cells are far more varied and intricate. In a recent study published by Le et al. [36] in *PNAS*, the identification of genetic variability within the same tumor also revealed distinct metabolic profiles of each cell subpopulation within a given tumor. In addition to the hypoxia-inducible factor (HIF) positive and/or cycling cells (Warburg-effect-displaying cells), they found that the population that was HIF-negative and non-cycling cells expressed a distinct set of genes with increased expression of mitochondrial genes as compared to other subpopulations. This subpopulation respires under hypoxia, supported by the fact that it had the highest oxygen consumption rate and mitochondrial capacity. The non-cycling and HIF-negative subclone was able to produce a tumor when purified and injected as a xenograft. This points to the importance of understanding how cancer metabolism allows for tremendous metabolic variegation.

Hypoxic cells can also coexist with aerobic cells, those that undergo oxidative phosphorylation, in a commensal manner. Hypoxic cells provide lactate that can be converted to pyruvate in the aerobic cells, which use it to run the TCA cycle and undergo oxidative phosphorylation [37]. These aerobic cells are oxygenated through their proximity to a nearby blood supply. Therefore, they can survive in this manner and are more suited to doing so than hypoxic cells. However, in addition to these two types of cancerous cells, Le et al. recently uncovered the existence of a non-Warburg metabolic phenotype in B lymphoma cells that undergo hypoxic respiration by activating the TCA cycle through glutamine oxidation [3]. By oxidation of glutamine, this allows glutamine to be used as a source for running the TCA cycle and enables the decrease of reliance on glucose as a primary fuel source for cancer cells. This revelation once again supports the existence of a diverse metabolic phenotype in any given tumor.

The metabolic nature of cancer is muddled. Not only do some cancer subclones form commensal relationships with each other, but cancer cells can also form similar relationships with cancer-associated fibroblasts (CAFs). CAF cells are a sub-

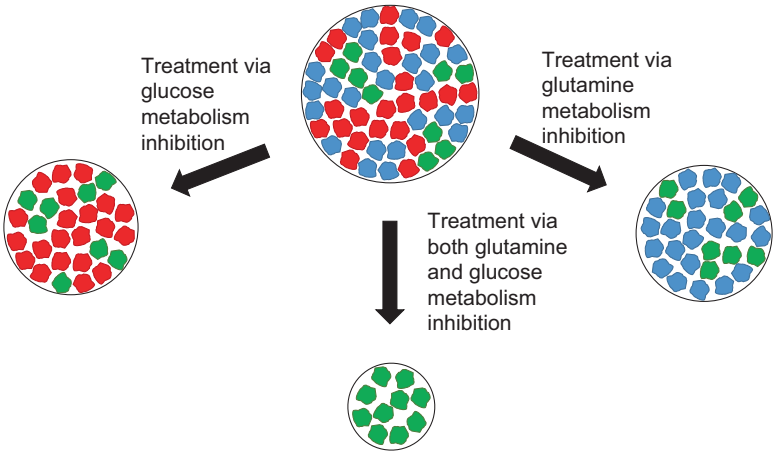

Fig. 3 Depiction of combined therapy effects in cancer metabolism. Depicted is a tumor in vivo containing different subpopulations of cancer cells (glycolytic cells presented as blue, glutamine-dependent cells presented as red, and green cells represent other metabolic pathway-dependent cells)

population of cells that reside within the tumor microenvironment and support the proliferation and growth of tumor cells. By providing lactate and other ketone bodies, acidic compounds that can form acetyl-CoA in a reversible manner, and receiving reactive oxygen species that promote glycolytic metabolic pathways, CAFs establish a fundamental relationship with adjacent cancer cells [38]. CAFs are also involved in the maintenance of an acidic extracellular environment, providing suitable conditions for optimum cancer cell growth [38].

Elgogary et al. present another case of spatial metabolic heterogeneity. Pancreatic tumors were targeted by Bis-2-(5-phenylacetomido-1,3,4-thiadiazol-2-yl)ethyl sulfide (BPTES), a glutaminase 1 (GLS1) inhibitor, which was encapsulated in a nanoparticle to enhance drug delivery [39]. The drug decreased tumor sizes, but metabolic analysis revealed that surviving tumors were shown to have relied on glycolysis and glycogen synthesis instead. Thus, further combination therapy of BPTES and metformin, a drug frequently used to treat diabetes by blocking glucose synthesis, further reduced tumor size. These results support the prevailing consensus that different metabolic phenotypes in any given tumor require specific therapeutic actions based on each subclonal phenotype (Fig. 3).

As previously noted, hypoxia has been found to play an important role in the development of heterogeneous phenotypes in cancer cells. In a recent study published in Nature Cell Biology, Fluegen et al. investigated the fate of disseminated tumor cells (DTCs). They revealed that these post-hypoxia DTCs were found to carry an array of upregulated genes, such as dormancy (NR2F1, DEC2, p27) and hypoxia (HIF1α) genes in addition to the GLUT1 gene [40]. This dormant subpopulation which evades many chemotherapies, as the authors of the paper note, could explain relapse and inferior survival rates. As a result, heterogeneity in can-

cer metabolism comes in a variety of forms, and the same factor, in this case, hypoxia, can come into play through a different approach depending on every scenario.

While the different aspects of cancer metabolism may seem to intertwine neatly, the relationship between these parts is far more complex. For instance, while glutamine utilization in the TCA cycle is heavily linked with low oxygen consumption and hypoxia, the latter can sometimes occur independently of the former. Thus, these pathways may overlap when intracellular lactate causes an increase in glutamine uptake and metabolism. However, anaplerosis (pyruvate conversion into oxaloacetate), which is an alternative use of pyruvate in hypoxic conditions, can sometimes lead to the conversion of glucose to glutamate, taking away glutamine's role as the glutamate provider needed to run the Krebs cycle [41].

Despite our tendency to separate different metabolic pathways and to assign rigid pathways to cancer metabolism, it must be noted that different pathways often cross-talk and that the correlative nature of many metabolic pathways does not necessarily point to a causative relationship.

5.2 Temporal Heterogeneity Provides Cancer with Short-Term Adaptive Capabilities

As discussed earlier, tumors tend to evolve rapidly and produce dissimilar subclones through their interaction with the microenvironment. There exists a similar sort of evolution in single cancer cells: a form of temporal heterogeneity. Cancer cells are also astoundingly plastic regarding their metabolism. For example, they can switch their mitochondrial energy source between glutamine and glucose through the utilization of different transcriptional factors that encode enzymes required for each respective metabolic pathway. Cancer cells achieve this kind of plasticity through a variety of mechanisms. Posttranslational modifications allow a quick and immediate response to changes in the environment, which could be useful in the sense that blood supply changes can be very rapid. Slower modifications do also exist, such as genetic and epigenetic modifications.

An example of such posttranslational modifications, and an illustration of cancer's remarkable plasticity, is seen once again in hypoxic cancer cells. Hypoxic cancer cells increase the transcription of pyruvate kinase muscle isoform 2 (PKM2), an enzyme responsible for the final nonreversible step in glycolysis, the conversion of phosphoenolpyruvate to pyruvate. This is achieved as the first intron of the PKM2 gene contains a hypoxia response element that is a target for HIF-1α. PKM2 is produced through the alternative splicing of the precursor mRNA PKM and is controlled by c-Myc. As such, high PKM2 levels are correlated with poor survival rates in signet ring cell gastric cancers. This upregulation of PKM2 helps cancer cells dedicate most of their glucose towards lactate production quickly and efficiently under hypoxic conditions [42]. This allows cancer cells to switch their metabolic profiles quickly and efficiently when faced with varying environmental conditions.

6 Tailored Clinical Applications and Therapies Targeting Metabolic Pathways Can Lead to Better Clinical Outcomes

Given the different tumor microenvironments, the diversity of their metabolism and their genetic and epigenetic composition, various techniques have been developed to visualize the different tumor microenvironments in a given tumor. These imaging techniques have further propelled us in the search for effective cancer therapies targeting different cancer cell metabolisms that can be specialized and tailor-made for every different microenvironment.

The most successful methods currently used to identify different tumor microenvironments include positron emission tomography (PET) and computed tomography (PET-CT) scans. FDG-PET (^{18}F-fluorodeoxyglucose-positron emission tomography) images of individual cervical tumors have revealed varying levels of glucose consumption across different regions of a single tumor [43]. The variation of glucose consumption within a tumor has been associated with increased expression of Glut (Glucose transporter)-1, Glut-3, and HK-II, the first key enzyme of glucose metabolism [44]. PET scans can be used to identify hypoxic microenvironments through the use of isotopically labeled ^{8}F-fluoromisonidazole (FMISO), which is injected and taken up by cells through passive diffusion. In the absence of oxygen, FMISO accumulates in cells to generate an image of the hypoxic regions within a tumor [45]. PET scans can also be used to measure various tumor microenvironments based on other variables such as the partial pressure of oxygen and many more [46].

Intratumoral metabolic heterogeneity in cancer can also serve as a useful tool for prognosis. In a study done by Mena et al., intratumoral metabolic heterogeneity was measured across 105 patients with oropharyngeal squamous cell carcinoma, along with either SUV (standardized uptake values of glucose) or MTV (metabolic tumor volume). These measurements were shown to have effective capacities as prognostic markers ($p = 0.026$ and 0.022, respectively), with higher levels indicating poorer prognoses, supporting the notion that the more diverse metabolic phenotypes exist within a tumor, the more arduous a task it becomes to eradicate all different subclones in the tumor [47].

Besides prognostication, increased knowledge of cancer's heterogeneous metabolic nature, and specifically its intratumoral heterogeneity, can enable specific targeting of subclones in a single tumor and has resulted in a surge in specific tailor-made cancer therapies. One of the earliest hallmarks of cancer was its ability to uptake increased amounts of glucose, through the utilization of many GLUT transporters. Cancer cells are also capable of metabolizing glucose at much quicker rates than normal cells. Consequently, this has resulted in increased research addressing the production of commercial GLUT transporter inhibitors and the transporter isoform-specificity of inhibition [48].

Other drugs target the hypoxic pathways of cancer cells, such as topotecan which inhibits hypoxia-inducible factor 1 (HIF-1) transcriptional activity and HIF-1alpha protein accumulation in hypoxia-treated U251 human glioma cells, a transcription

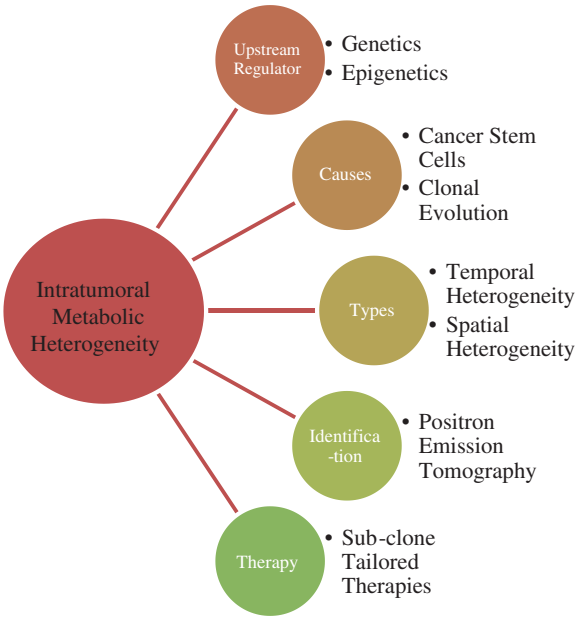

Fig. 4 Various factors associated with intratumoral metabolic heterogeneity

regulator of the previously discussed hypoxia-inducible factor, HIF-1α [49]. This has caused increased interest in mRNA regulating agents that target HIF-1α. Many drugs have followed with variable success that act by blocking mRNA transcription of the HIF-1α gene. Recent research has also provided many other pathways in cancer metabolism that can be targeted with effective results. For example, lactate dehydrogenase A (LDHA), an enzyme involved in the generation of lactate from glucose in Warburg-effect-displaying cells, has also been found to be a suitable target for effective tumor reduction through small molecule inhibition. Decreased expression of LDHA through small molecule inhibition elevated oxidative stress levels and ultimately resulted in cell death and tumor volume reduction [50]. Other methods targeting HIF-1α and hypoxia have been formulated through the integration of different therapies to each specific tumor microenvironment, making complete cancer recession very promising. Again, it is vital to realize the complex nature of cancer metabolism and the need for specific therapies to be directed at individual metabolic phenotypes in order to see effective responses in patients (Fig. 4).

Conclusion

Despite the challenges, there is much hope in the field of cancer therapies. The recently discovered and understood aspects of cancer's metabolic heterogeneity, including its intricate interactions with CAF cells, its exchanges between its distinct

subclones, and its impressive plasticity, promise to greatly advance this field. The importance of accounting for intratumoral heterogeneity in any given tumor has never been as widely understood as it is now. The latest findings we have discussed in this chapter give us a more solid understanding of cancer complexities, which we can seek to translate into effective and strategic therapies in the near future.

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Part III
Relationship between Cancer Cells and Cancer-Associated Fibroblasts

Metabolic Relationship between Cancer-Associated Fibroblasts and Cancer Cells

Christos Sazeides and Anne Le

Key Points

- Cancer-associated fibroblasts undergo the reverse Warburg effect and provide cancer cells with glycolytic metabolites.
- The interaction between cancer cells and CAFs helps cancer cells manage the Warburg effect.
- Loss of stromal Cav-1 is a biomarker of poor prognosis in breast cancers.
- CAF-derived exosomes (CDE) can reprogram the metabolic pathway of cancer cells.
- CAFs augment cancer addiction to glutamine and its metabolically-relevant consequences.
- Alanine secreted by pancreatic stellate cells supports tumor metabolism.

Keywords Cancer-associated fibroblasts · CAF-derived exosomes · Glutamine metabolism · Hypoxia-inducible factor-1 · Reverse Warburg effect

Abbreviations

α-SMA	α-Smooth muscle actin
CAF	Cancer-associated fibroblast
Cav-1	Caveolin -1
CDE	CAF-derived exosomes
EMT	Epithelial-mesenchymal transition

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FASN	Fatty acid synthase
FH	Fumarase
HIF-1	Hypoxia-inducible factor-1
LDHA	Lactate dehydrogenase A
MCT	Monocarboxylate transporter
NF	Normal fibroblasts
PDAC	Pancreatic ductal adenocarcinoma
PKM2	Pyruvate kinase isozymes M1/M2
PSC	Pancreatic stellate cells
ROS	Reactive oxygen species
SDH	Succinate dehydrogenase
TCA	Tricarboxylic acid
TGF-β	Transforming growth factor beta
TME	Tumor microenvironment

Introduction

Cancer-associated fibroblasts (CAFs), a major component of the tumor microenvironment (TME), play an important role in cancer initiation, progression, and metastasis. Recent findings have demonstrated that the TME not only provides physical support for cancer cells, but also directs cell-to-cell interactions (in this case the interaction between cancer cells and CAFs). As cancer progresses, the CAFs also co evolve—transitioning from an inactivated state to an activated state. The elucidation and understanding of the interaction between cancer cells and CAFs will pave the way for new cancer therapies [1–3].

The TME is a heterogeneous environment consisting of fibroblasts, tumor-associated macrophages, adipocytes, an extracellular matrix, and mesenchymal stem cells [4]. The exact composition of each stroma varies depending on cancer and tissue type. To add to this variation, there is heterogeneity even within the CAF population itself. Different CAFs express different markers and influence stromal pro-tumorigenic capacity and cancer progression in diverse ways [5, 6].

CAFs, unlike normal fibroblasts (NF), are not passive bystanders. They possess similar characteristics to myofibroblasts, the fibroblasts responsible for wound healing and chronic inflammation, such as the expression of α-smooth muscle actin (α-SMA) [7, 8]. Regarded in a similar light, cancer might be considered a wound that cannot be healed. CAFs can originate from the activation and differentiation of quiescent fibroblasts, bone marrow-derived mesenchymal stem cells, and epithelial, and endothelial cells [9].

The interaction of the TME, specifically among CAFs with cancer, is incontrovertible. The effect of CAFs on cancer is dependent on cancer type and stage. The production and secretion of growth factors, chemokines, cytokines, metabolites, and extracellular matrix aid in the recruitment of various cell types, such as pericytes and endothelial cells, facilitating angiogenesis and bestowing chemoresistant properties to the cancer cells. In this chapter, we aim to discuss the properties and

characteristics of CAFs, their importance in cancer progression, and how they can be targeted for cancer therapy [10, 11].

As mentioned in the chapter "Different Tumor Microenvironments Lead to Different Metabolic Phenotypes," Hanahan and Weinberg [12] have identified six hallmark capabilities of cancer cells: (1) self-sufficiency in growth signals, (2) insensitivity to anti-growth signals, (3) evading apoptosis, (4) limitless replicative potential, (5) sustained angiogenesis, and (6) tissue invasion and metastasis. The exact mechanisms by which the TME can influence cancer and lead to the acquisition of those hallmark capabilities are not yet fully understood. However, there is growing evidence suggesting that the manipulation of signal transduction pathways in cancer cells, CAFs, and altered metabolic pathways may play a role in the transformation process [13–18].

1 CAFs Undergo the Reverse Warburg Effect and Provide Cancer Cells with Glycolytic Metabolites

As mentioned in previous chapters, cancer cells undergo a phenomenon known as the Warburg effect, an increase in aerobic glycolysis to produce ATP even while in normoxic conditions (normoxia or normal oxygen levels). Warburg initially attributed this phenomenon to malfunctioning mitochondria, forcing the cancer cells to rely on glycolysis for energy production. Pyruvate and lactate, the two end products of glycolysis, were believed to be secreted by the hypoxic core of the tumor through monocarboxylate transporters (MCT4) for the adjacent oxygenated cancer cells to uptake (via MCT1) and utilize as substrates for the TCA cycle [19–21].

Recent studies, however, have revolutionized the way scientists view the TME, especially the cross talk between CAFs and tumor cells and the effect of this cross talk on metabolism. The Warburg effect, a phenomenon initially believed limited to cancer cells, has also been observed in the fibroblasts surrounding the cancer cells. To distinguish this CAF-related phenomenon from its cancer cell-related counterpart, Pavlides et al. named it the reverse Warburg effect [17]. Caveolin-1 (Cav-1) is a TGF-β type I receptor kinase inhibitor, and the loss of Cav-1 expression causes a myofibroblastic phenotype. By using Cav-1(−/−) fibroblasts, Pavlides et al. induced myofibroblastic differentiation to mimic CAFs. With the use of proteomics, they identified 25 proteins that were overexpressed when Cav-1 was suppressed. Eight of those proteins were glycolytic enzymes (Table 1), including M2-type pyruvate kinase and lactate dehydrogenase A [17]. Those two enzymes are known to play a crucial role in the Warburg effect [22, 23]. Additionally, two enzymes involved in oxidative stress, peroxiredoxin 1 and catalase, were overexpressed under normoxic conditions, which indicates an increase of reactive oxygen species (ROS) in Cav-1(−/−) fibroblasts. Hypoxia-inducible factor-1 (HIF-1) is a transcription factor that responds to low oxygen concentrations. Under high levels of ROS, HIF-1 is stabilized. Subsequently, HIF-1, a regulator of all glycolytic enzymes, as well as glucose transporters, GLUT1 and GLUT3, induces aerobic glycolysis [17, 23].

Table 1 Glycolytic enzymes upregulated in Cav-1(−/−) mammary stromal fibroblasts. All eight enzymes lead to the overproduction of pyruvate and lactate, which are then secreted in the medium for adjacent cancer cells to uptake and utilize as energy source

Glycolytic and metabolic enzymes	Metabolic reaction involved
M2-type pyruvate kinase	Phosphoenolpyruvate → pyruvate
Phosphoglycerate kinase I	Glycerate-1,3P2 ↔ glycerate-3P
Lactate dehydrogenase A	Lactate ↔ pyruvate
Fructose-bisphosphate aldolase A	Fructose-1,6P2 ↔ glyceraldehyde-3P
Glycerol 3-phosphate dehydrogenase 2	Dihydroxyacetone-P ↔ glycerol-3P
Enolase I	Glycerate-2P ↔ phosphoenolpyruvate
Triosephosphate isomerase I	Fructose-1,6P2 ↔ dihydroxyacetone-P
Phosphoglycerate mutase	Glycerate-3P ↔ glycerate-2P

Table 2 Enzymes being overexpressed by pancreatic cancer cells and their function

Enzymes	Function
Monocarboxylate transporter-1 (MCT1)	Plasma membrane transporter to transport *pyruvate* and *lactate* into the cell (mitochondrial): pyruvate and lactate → (cytosolic) pyruvate and lactate
Fumarase or fumarate hydratase (FH)	(F-hydration) fumarate ↔ malate (R-dehydration) Cytosolic isoenzyme: metabolism of amino acids and fumarate Mitochondrial isoenzyme: Krebs cycle
Succinate dehydrogenase (SDH)	Couples the oxidation of succinate to fumarate with the reduction of ubiquinone to ubiquinol at the mitochondrial membrane

A similar study performed by Shan et al. provided further evidence to support the reverse Warburg hypothesis. In this study, pancreatic-associated fibroblasts expressed elevated levels of the glycolytic enzymes LDHA and PKM2, as well as the MCT4 transporter responsible for lactate secretion. Additionally, they observed that when pancreatic cancer cells were exposed to CAF conditioned media, they underwent enhanced aerobic activity causing an observable enlargement of the mitochondria. Furthermore, pancreatic cancer cells significantly increased expression of MCT1, FH, and SDH (Table 2). The overexpression of those enzymes further indicated the existence of a metabolic coupling between CAFs and cancer cells [24].

2 The Interaction Between Cancer Cells and CAFs Helps Cancer Cells Manage the Warburg Effect

Even though the extra-tumoral high lactate concentration produced by CAFs is crucial for the progression of cancer, high intracellular lactate concentration causes a dramatic drop in the pH, which, if left untreated, results in the death of the cell. Interestingly, experimental research revealed a few mechanisms by which cancer cells manage the Warburg effect [25–28]. Cancer cells overexpress a Na+/H+

transporter, NHE1, that pumps H+ out of the cell and Na+ into it, therefore neutralizing this decrease in pH caused by lactic acid [26]. Under hypoxic conditions, cancer cells overexpress carbonic anhydrase 9, CA9, which is responsible for the conversion of carbon dioxide to bicarbonate to neutralize increased acidity [27]. Certain cancer cells also overexpress MCT4, the transporter involved in secreting lactate out of the cell. By doing this, if intracellular cancer lactate concentration goes too high, some of it can be secreted to prevent pH from dropping too low [28]. Cancer cells adjacent to autophagic CAF upregulate TP53-induced glycolysis and the apoptosis regulator (TIGAR). TIGAR is capable of protecting cancer cells against oxidative stress by inhibiting autophagy and apoptosis while simultaneously shifting cells towards oxidative phosphorylation and away from aerobic glycolysis [29]. Finally, several antioxidant enzymes, such as peroxiredoxin-1, have been observed to be upregulated in certain cancer cells [2]. It is likely that as more experiments are performed involving the tumor microenvironment and cancer cells, more evasion mechanisms will be elucidated.

3 Loss of Stromal Cav-1 Is a Biomarker for Poor Prognosis in Breast Cancers

The importance of Cav-1 in transdifferentiating normal fibroblasts into myofibroblasts is well established. Recent experiments have shed light on the complex mechanisms by which cancer cells modulate their environment and manage to down regulate Cav-1 expression in fibroblasts. Cav-1 inhibits TGF-β type I receptor kinase. The lack of Cav-1 expression in the Cav-1(−/−) null skin fibroblasts can induce a myofibroblastic phenotype. One of the most widely known tumor-derived factors involved in the activation of CAFs is TGF-β1 [1, 30, 31]. Interestingly, in the absence of CAFs, TGF-β itself in cancer cells has no direct effect on cancer proliferation and survival [31].

It is believed that cancer-derived TGF-β acts in a paracrine manner and causes the downregulation of Cav-1α in CAFs. This event results in the overexpression of ROS by CAF cells that can act both in an autocrine and paracrine fashion, stimulating themselves and nearby fibroblasts to acquire a myofibroblastic phenotype. ROS inhibit prolyl hydroxylase (PHD) from targeting the transcription factor HIF-1α for degradation [32–34]. As a result, HIF-1α gets stabilized and translocated into the nucleus causing the overexpression of autophagy genes (BNIP3, BNIP3L, CTSB, or ATG16L1) which compete with Beclin-1 [35]. Beclin-1 then acts as a mitophagy/autophagy factor causing the dysfunction of mitochondria and thus the increase of ROS, acting on a positive feedback loop [36, 37]. Additionally, TGF-β also causes the upregulation of BNIP3, BNIP3L, CTSB, and ATG16L1, all of which can induce mitophagy/autophagy and therefore shift the cell away from oxidative phosphorylation and towards aerobic glycolysis. BNIP3, BNIP3L, and CTSB increase lactate production whereas ATG16L1 increases ketone production. Lactate and ketones can then be utilized by cancer cells to enhance tumor growth [38]. TGF-β, therefore,

promotes tumorigenesis via CAF metabolism, and specifically, TGF-β in fibroblasts leads to upregulated mitochondrial activity of cancer cells and tumor growth [31].

The rapid proliferation of cancer, without a significant increase in vascularization, limits oxygen availability for normal fibroblasts, thus creating a hypoxic environment that forces the cells to undergo metabolic changes [1]. Hypoxia results in the stabilization of HIF-1α which, as described previously, is a very important transcription factor for genes involved in autophagy, mitochondrial biogenesis, and general energy homeostasis [29]. Furthermore, under normoxia, IκB inhibits NFκB, a key inducer of autophagy, by sequestering the nuclear localization signal of NFκB, therefore rendering it inactive in the cytoplasm [39]. However, hypoxic conditions activate IκBK which targets IκB for degradation by phosphorylation and therefore promotes the activation of NFκB [40]. Even though the exact mechanism by which NFκB is able to direct autophagy is unclear, it is believed that this transcription factor upregulates the expression of certain inflammatory cytokines, such as IL-6, IL-8, IL-10, and TNFα [41, 42]. These inflammatory mediators are able to induce autophagy independent from each other [41, 42]. Finally, NFκB also binds to the HIF-1α promoter and

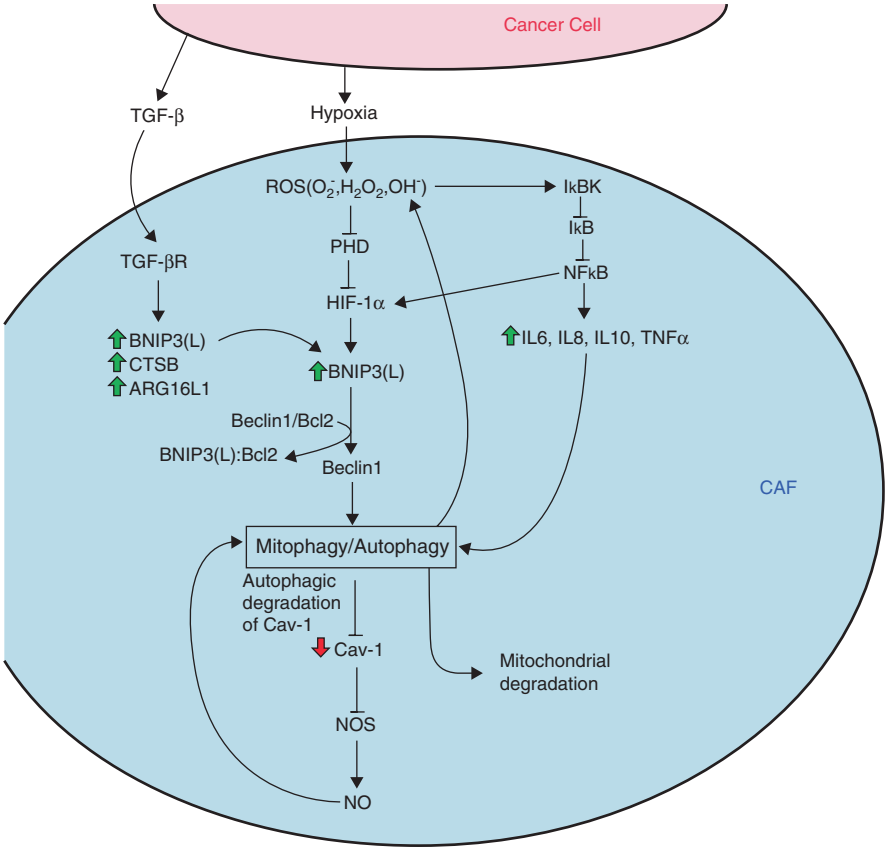

Fig. 1 Cancer-induced autophagic degradation of Cav-1α

results in its upregulation [43] (Fig. 1). Hypoxia- and TGF-β-induced autophagy cause the lysosomal degradation of Cav-1, as well as mitochondrial dysfunction and degradation, leading to a highly glycolytic state in CAF cells. Cav-1α normally inhibits nitric acid synthase and prevents the accumulation of nitric oxide (NO). In the absence of Cav-1α, NO accumulates and inhibits cytochrome c oxidase, causing mitochondrial uncoupling and thus rendering mitochondria susceptible to mitophagy [44]. This results in high amounts of lactate, pyruvate, ketone bodies, glutamine, and free fatty acids [2, 24, 45] that can be utilized by adjacent cancer cells.

The aforementioned oxidative stress and hypoxia derived from Cav-1 loss lead to mitochondrial dysfunction and degradation. Mitochondrial dysfunction causes the premature reaction of electrons with oxygen leading to the generation of ROS, such as O_2^-, H_2O_2, and OH^- [2]. ROS induce oxidative stress, stabilizing HIF-1α and inhibiting NFκB in a positive feedback manner.

The fact that TGF was not able to stimulate a significant increase in angiogenesis and vascularization suggests that the growth stimulated by CAF cells depends on the paracrine supply of high energy molecules such as lactate, pyruvate, ketone bodies, amino acids, and fatty acids [46, 47].

4 CAF-Derived Exosomes (CDE) Can Reprogram the Metabolic Pathway of Cancer Cells

Much research has been focused on exosomes secreted by cancer cells, while little is known about exosomes secreted by CAFs. Zhao et al., with the use of isotopologue tracing, showed that CAF-derived exosomes (CDE) are taken up by cancer cells in a *KRAS*-independent mechanism and are, indeed, capable of reprogramming the metabolic activity of pancreatic and prostate cancer cells [48]. They demonstrated how CDE can sustain the rapidly dividing cancer cells under hypoxic conditions or when the normal oxidative mitochondrial function has been disabled. Additionally, the presence of CAF-derived exosomes can rescue prostate and pancreatic cancer cells from starvation by providing de novo metabolites, such as amino acids (Table 3). This suggests that there is constant communication between the tumor cells and the adjacent fibroblasts, where both constantly coevolve [48].

4.1 CDE Contain miRNA That Downregulate Oxidative Phosphorylation of Cancer Cells

CAF-derived exosomes contain amino acids, fatty acids, pyruvate, lactate, miRNA, and many other compounds. miRNAs are essential in regulating gene expression [48]. Zhao et al. showed that miRNAs present in CDE are capable of down regulating all 109 OXPHOS-related genes in cancer cells. As shown in Table 4, the 17 most abundant miRNA present in those exosomes target one or more OXPHOS genes. Therefore, cancer cells must rely on alternative metabolic pathways to maintain their rapid proliferation [48].

Table 3 Amino acids present in various CDEs

Amino acid	CDE from prostate CAF	CDE from pancreatic CAF
Alanine	Yes	Yes
Anserine		Yes
Arginine	Yes	Yes
Asparagine	Yes	
Citrulline	Yes	
Cysteine		Yes
Glutamic acid	Yes	Yes
Glutamine	Yes	Yes
Glycine	Yes	Yes
Histidine	Yes	Yes
Isoleucine		Yes
Leucine	Yes	Yes
Lysine	Yes	Yes
Methionine		Yes
Ornithine	Yes	Yes
Phenylalanine		Yes
Phosphoserine	Yes	
Proline	Yes	Yes
Serine	Yes	Yes
Threonine	Yes	Yes
Tryptophan		Yes
Valine	Yes	Yes

Table 4 The 17 most abundant miRNAs present in CDE and their respective target genes

Exosomal miRNA	OXPHOS gene silenced
miR-302d-3p	UQCRFS1
miR-29b-3p	NDUFA10, ATP5G1, ATP6V1A, ATP5G3
miR-22-3p	ATP6V1A
miR-155-5p	ATP5G3
miR-25-3p	NDUFS4
miR-29a-3p	ATP5G1, ATP6V1A, ATP5G3
miR-23a-3p	NDUFA10, NDUFS4, ATP6V0E2, NDUFA2
miR-21-5p	ATP5L, ATP5G2
miR-16-5p	ATP5G3
miR-222-3p	ATP6V1A

4.2 *Effect of CDE on Glycolysis and TCA*

With the use of GC-MS and $^{13}C_6$-glucose, Zhao et al. identified that glucose from CDE was the main glycolytic substrate for cancer cells. This was evident due to the increase in labeled glycolytic metabolites, lactate and pyruvate, present in both pancreatic and prostate cancer cells, and the reduced amount of non-labeled pyruvate and lactate (Fig. 2). Additionally, they further identified that the labeled metabolites

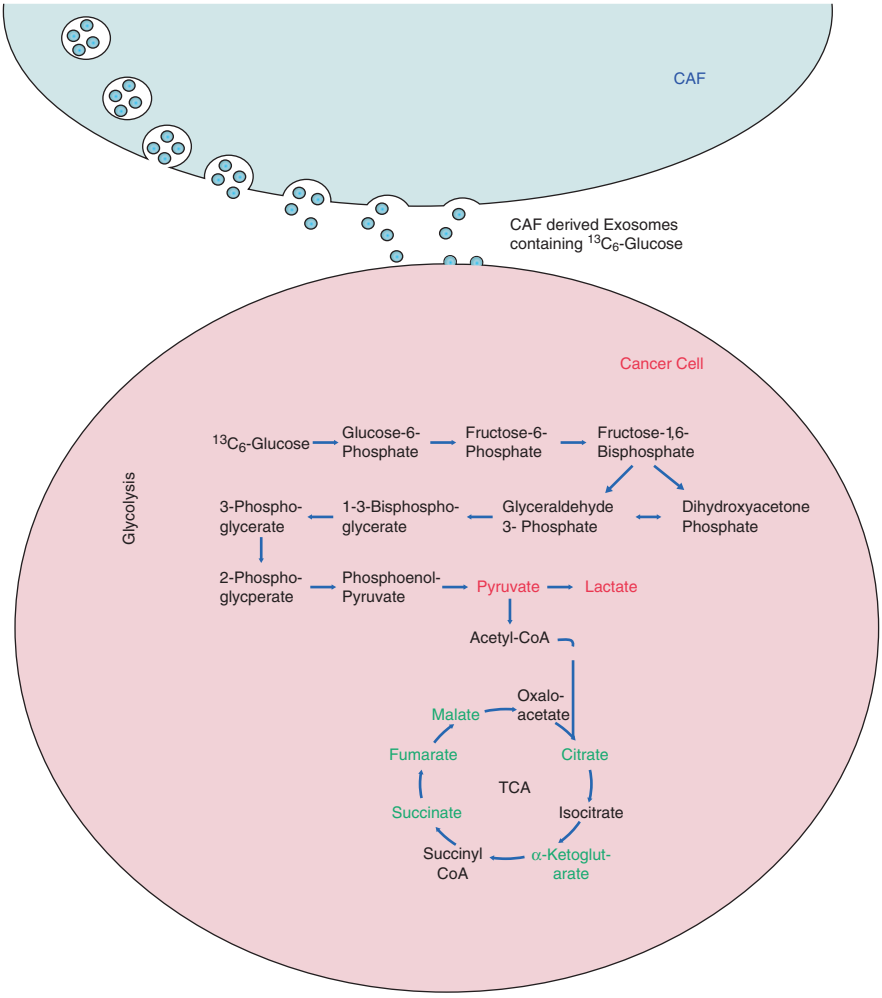

Fig. 2 CDE-derived glucose is mainly used in cancer cell glycolysis and, to a lesser extent, the TCA cycle. Compounds in red letters represent the compounds found in cancer cells present in high concentrations resulting from CDE-derived glucose. Compounds in green represent the compounds found in cancer cells present in low concentrations from CDE-derived glucose

involved in TCA cycle (citrate, α-ketoglutarate, fumarate, and malate) were found in significantly lower concentrations. Therefore, the increase of labeled glycolytic metabolites and the decrease of labeled TCA metabolites suggest that glucose provided by CDE is primarily used in glycolysis, not in mitochondrial oxidative phosphorylation [48].

4.3 CDE Glutamine Undergoes Mainly Reductive Metabolism That Also Results in Aberrant Lipogenesis

Glutamine is another major carbon source for the TCA cycle and a nitrogen source for protein synthesis [49, 50]. Zhao et al. identified the contribution of CDE-derived glutamine to the TCA cycle using U-$^{13}C_5$ glutamine isotopologue tracing [48]. Under both normoxic and hypoxic conditions, glutamine can enter the oxidative metabolic pathway and produce oxaloacetate, which then combines with acetyl-CoA to form citrate, a fatty acid precursor. Additionally, under hypoxic conditions, glutamine enters the reductive metabolic pathway generating citrate by reducing α-ketoglutarate [51]. As shown in (Fig. 3), citrate is further reduced to fumarate and then malate. The presence of M5 citrate, M3 fumarate, and M3 malate in high concentrations suggests that cancer cells mainly rely on the reductive glutamine metabolism when the normal mitochondrial functioning of the cell is disrupted. Additional evidence to support this is the decreased M4/M5 citrate ratio. M4 is derived from the oxidative pathway of glutamine, whereas M5 from the reductive pathway, and therefore this reduced ratio confirms the glutamine-reductive pathway [48]. Furthermore, a major component and requirement for cell proliferation is lipogenesis, the generation of fatty acids for cell membranes [52] (Table 3).

Zhao et al. also showed that exposure to CDE resulted in increased acetate contribution and simultaneously decreased pyruvate contribution to lipogenesis. This event suggests that the main source of carbon for acetyl-CoA upon exposure to CDE is the reductive carboxylation pathway and not the oxidative glucose pathway. Finally, metabolic analysis of CDE revealed significant amounts of stearate and palmitate that can be directly utilized by the cancer cells for lipid synthesis [48]. It is worth mentioning that FASN (fatty acid synthase) expression has been found elevated in numerous types of cancer [53]. Even though there is still no direct link between CAFs and the overexpression of FASN, this could be the result of the coevolution of stroma and cancer. However, more research is required before conclusions can be drawn.

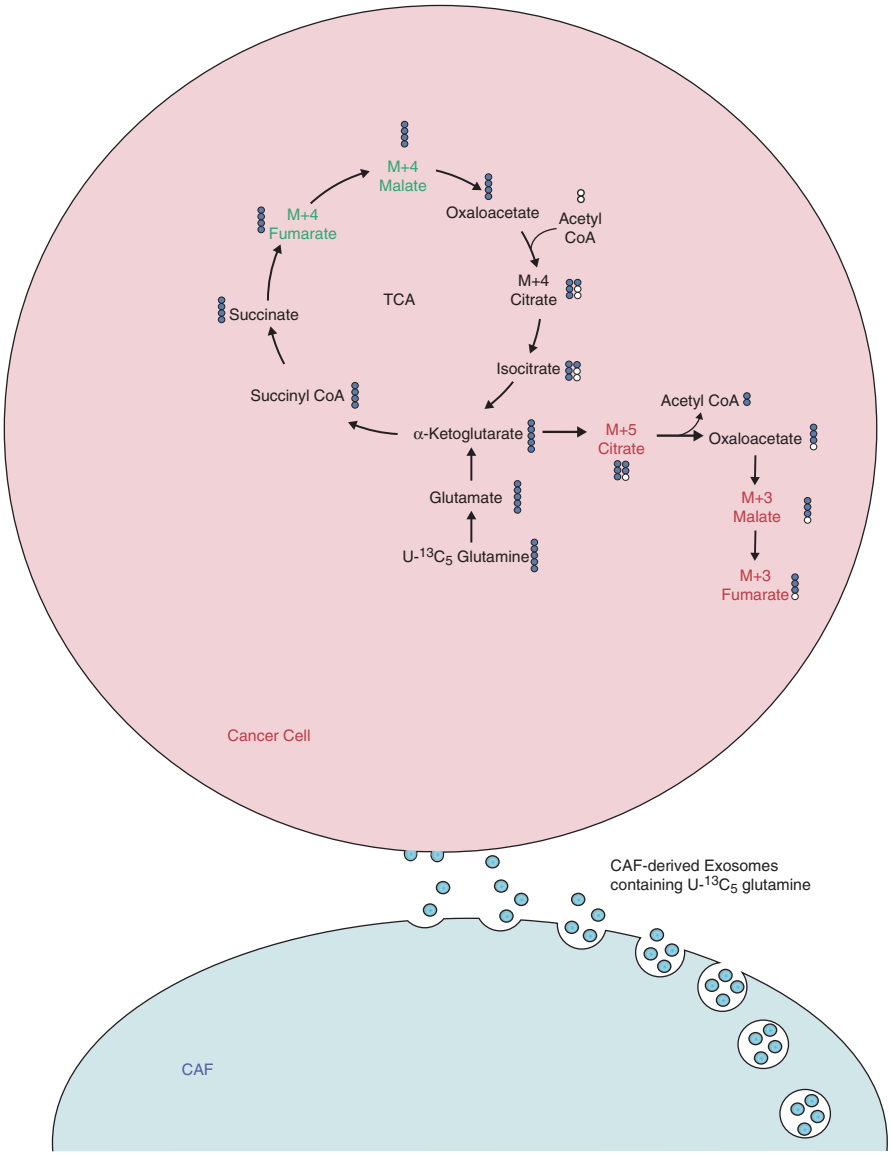

Fig. 3 Oxidative and reductive glutaminolysis. Blue circles represent labeled carbons (C13), and white circles represent non-labeled carbons (C12). Glutamine derived from CDE is fully labeled, and its subsequent pathway (oxidative or reductive) in cancer cells was analyzed

5 CAFs Augment Cancer Addiction to Glutamine and Its Metabolically Relevant Consequences

As mentioned earlier, there exists a constant coevolution between cancer cells and the TME. Cancer cells stop directing glucose into the TCA cycle and, instead, use glucose for the production of nucleotides. Consequently, cancer cells start relying on other carbon sources for oxidative phosphorylation, particularly on glutamine derived from CAF cells. However, during glutaminolysis, specifically during the conversion of glutamine to glutamate and then to α-ketoglutarate (which enters the TCA cycle), ammonia is released as a by-product [54]. Ammonia is a diffusible compound and an inducer of autophagy. This has detrimental effects on the surrounding stroma, as it causes autophagy in the adjacent CAFs. CAFs, subsequently, undergo autophagy and further release glutamine to be metabolized by the cancer cells. Therefore, a positive feedback loop exists between the cancer's addiction to glutaminolysis and CAF conversion/autophagy [55, 56].

6 Alanine Secreted by Pancreatic Stellate Cells Support Tumor Metabolism

In another experiment, Sousa et al. discovered that myofibroblast-like pancreatic stellate cells (PSC) secreted alanine and were able to support pancreatic cancer metabolism [57]. Among the 200 metabolites analyzed, only alanine and aspartate followed the desired motif:

1. Increased amounts of metabolite in PSC medium.
2. Decreased amounts of metabolite in PSC medium when exposed to pancreatic cancer cells (PDAC).
3. Increased amounts of metabolite in PDAC after exposure to PSC medium.

Furthermore, kinetic studies showed that alanine was secreted even more rapidly than lactate. In fact, PSC-derived alanine does not contribute to the production of glycolytic intermediates or alter the NAD+/NADH ratio, but rather, it gets transaminated to pyruvate, providing additional substrates for the TCA cycle. This intermediate contribution to the TCA cycle subsequently increases oxygen consumption [57]. Alanine-derived pyruvate enters the TCA cycle, in the mitochondria, and contributes predominantly to the generation of citrate (23–46% among different PDAC cell lines) and isocitrate. To a lesser extent, it also contributes to the generation of malate, fumarate, aspartate, and glutamate. Alanine, therefore, fuels mitochondrial metabolism without affecting glycolysis. Alanine-derived citrate is then transported from the mitochondria to the cytosol for lipogenesis. Metabolite tracing showed that alanine significantly contributed to the generation of palmitate and stearate, more than 20% and 10% of the total concentrations, respectively [57].

In the presence of alanine, glucose enters the serine biosynthetic pathway in PDAC cells and produces serine and glycine. Serine and glycine can then be used in the biosynthesis of nucleic acids. Under nutrient-deprived conditions, the entry of glucose into the serine biosynthetic pathway is more evident. This suggests that in cases of glucose-deprived conditions, alanine can take over aerobic respiration by providing TCA intermediate metabolites, and subsequently, glucose can then enter different metabolic pathways, such as the serine biosynthetic pathway [57].

The induction of alanine secretion by PSC cells for PDAC cells to uptake is a two-way intra-tumoral cross talk. PDAC cells initially stimulate PSC to undergo autophagy and thus the release of alanine. PSC-derived alanine is then taken up by PDAC cells to contribute to metabolic pathways. In nutrient-rich conditions, the PSC autophagic alanine secretion has minimum effect on PDAC proliferation. However, in nutrient-deprived conditions, PSC autophagic alanine secretion can significantly rescue and promote the growth of PDAC cells. This effect mainly occurs during early stages of cancer development. Interestingly, autophagy does not influence the proliferation rate of PSCs themselves [57].

7 Reciprocal Communication Is Essential for Cancer Progression

The importance of KRAS in supporting heterocellular communication was demonstrated by Tape et al. [25]. When pancreatic ductal adenocarcinoma (PDAC) cells were exposed to homocellular conditions, mitochondrial functioning decreased, and superoxide concentrations increased. However, when PDAC cells were exposed to heterocellular conditions (i.e., co-cultured together with CAFs), mitochondrial functioning was restored, and superoxide concentrations were well regulated. These results suggest that heterocellular and reciprocal communication between CAFs and cancer cells are essential for the progression of cancer. In this experiment, PDAC cells with the *KRAS* mutation initially stimulated the surrounding CAF cells to undergo metabolic and cellular changes. Reciprocal stimulation between CAFs and PDAC cells prevents cancer cell mitochondrial dysfunction and superoxide production. The exact signals involved in this dialogue between CAFs and PDAC cells are still unclear, and further research is required to unravel this mechanism [25].

Conclusion

As cancer research progresses, the significance of the tumor microenvironment in cancer progression is better elucidated. Tumor cell-derived TGF-β causes the lysosomal targeting of fibroblastic Cav-1, inducing a myofibroblastic phenotype (activated form). Additionally, the increased oxygen consumption of tumor cells with no significant increase in vascularization induces hypoxia and oxidative stress, causing the stabilization of HIF-1α and inhibition of IκB in CAFs. Stabilized HIF-1α

induces autophagy and mitophagy. Subsequently, CAFs rely on glycolysis for energy, producing a high amount of lactate, ketone bodies, glutamine, and fatty acids, which are then secreted and taken up by the surrounding tumor cells.

In the case of PDAC, it was evident that the secretion of alanine by PSC (myofibroblast-like pancreatic stellate cells) was sufficient to rescue tumor cells in low-nutrient environments. It was noted that alanine, and not glucose, was used in the TCA cycle. This allowed glucose to enter the serine biosynthetic pathway to generate nucleic acids, further contributing to the rapid tumor cell proliferation.

CAF contribution to cancer progression does not end here. Exosomes derived from CAFs (CDE) contain a variety of miRNAs responsible for downregulating genes involved in OXPHOS and therefore contribute to the reprogramming of the metabolic activity of tumor cells. CDE also contains de novo metabolites that enable the rapidly dividing tumor cells to survive in low-nutrient conditions. For example, CDE-derived glucose is primarily used in glycolysis and nucleotide biosynthesis and not in the TCA cycle. Whereas CDE-derived glutamine undergoes reductive metabolism and generates acetyl-CoA for lipogenesis. However, during glutamine metabolism, ammonia, a diffusible autophagy factor, is produced as a by-product. CAFs that are stimulated by ammonia undergo autophagy and in turn further release more glutamine to be metabolized by cancer cells in a positive feedback loop.

It is clear that cancer should not be regarded as an individual entity anymore, but rather it should be viewed within the context of its microenvironment. The understanding of the extent of stromal impact on cancer metabolism and progression can provide new targets for cancer therapy.

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Targeting Metabolic Cross Talk between Cancer Cells and Cancer-Associated Fibroblasts

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Key Points

- Considering metabolic cross talk between CAFs and cancer as a metabolic target for cancer therapy
- Targeting the reverse Warburg effects via disruption of the "lactate shuttle" by MCT1/MCT 4 inhibitors
- Blocking the function of CAFs, which promote cancer cell growth, by metformin
- Using metformin to inhibit glycolysis of CAFs
- Targeting glutaminolysis by blocking the glutamine uptake of cancer cells from CAFs
- Targeting ketone bodies and ketosis in CAFs
- Targeting fatty acid metabolism, as a nutrient reservoir for cancer cell growth, from cancer-associated adipocytes (CAAs)

Keywords Cancer-associated fibroblasts · Cancer-associated adipocytes · Tumor microenvironment · Metabolism · Metabolites · Cancer therapy

Abbreviations

βOHB	β-Hydroxybutyrate
ACAT1	Acetyl-CoA acetyltransferase
ACC	Acetyl-CoA carboxylase
ACCA	Alpha-cyano-4-hydroxycinnamic acid

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ACLY	ATP citrate lyase
ASCT2	Alanine, serine, cysteine-preferring transporter 2
BDH1	3-Hydroxybutyrate dehydrogenase 1
CAAs	Cancer-associated adipocytes
CAFs	Cancer-associated fibroblasts
DAG	Diacylglycerol
ECM	Extracellular matrix
EGF	Epidermal growth factor
FASN	Fatty acid synthase
G3P	Glycerol-3-phosphate
GLS	Glutaminase
HGH	Human growth hormone
HMG-CoA	3-Hydroxy-3-methylglutaryl-CoA
HMGCS2	3-Hydroxy-3-methylglutaryl-CoA synthase 2
HSP60	Heat-shock protein 60
LPA	Lysophosphatidic acid
MCT1	Monocarboxylate transporter 1
MCT4	Monocarboxylate transporter 4
MMP	Matrix metalloprotease
OXPHOS	Oxidative phosphorylation
PA	Phosphatidic acid
PI3K	Phosphoinositide 3-kinase
PIP3	Phosphatidylinositol (3,4,5)-trisphosphate
PTEN	Phosphatase and tensin homolog deleted on chromosome 10
TAG	Triacylglycerol
TCA	Tricarboxylic acid
TME	Tumor microenvironment

Introduction

Although tumorigenesis has classically been regarded as a genetic disease of uncontrolled cell growth, the importance of the tumor microenvironment (TME) is continuously emphasized by the accumulating evidence that cancer growth is not simply dependent on the cancer cells themselves [1, 2] but also dependent on angiogenesis [3–6], inflammation [7, 8], and the supporting roles of cancer-associated fibroblasts (CAFs) [9, 10]. After the discovery that CAFs are able to remodel the tumor matrix within the TME and provide the nutrients and chemicals to promote cancer cell growth [11], many studies have aimed to uncover the cross talk between cancer and CAFs. Moreover, a new paradigm in cancer metabolism shows how cancer cells act like “metabolic parasites” to uptake the high-energy metabolites, such as lactate, ketone bodies, free fatty acid, and glutamine from supporting cells, including CAFs and cancer-associated adipocytes (CAAs) [12, 13]. This chapter provides an overview of the metabolic coupling between CAFs and cancer to further define the therapeutic options to disrupt the CAF-cancer cell interactions.

1 Overview of the Metabolism of CAFs in Solid Tumors

Pathological analysis shows that CAFs either locate the tumor margin or infiltrate the tumor mass, indicating that CAFs and cancer cells are physically and functionally connected to each other [14, 15]. Of note, other than their locations, the physiological roles of CAFs depend on the existence of neighboring cancer cells [16], leading Madar et al. to propose a new concept: A "CAF state" instead of "cell type" [17].

The experimental approach to study cancer metabolism is difficult due to the dynamic and rapid metabolic influx/efflux of heterogeneous cancer cells. However, it is clear that the reprogramming of energy metabolism is one of the hallmarks of cancer [18]. Thus, researchers seek to identify the metabolic vulnerabilities of cancer cells and exploit them for therapy. Cancer-friendly fibroblasts are the most abundant noncancerous cells in solid tumors, and they promote cancer cell growth and induce chemotherapy resistance [19]. Unfortunately, the underlying mechanism of how CAFs help tumor cell growth remains unclear. However, the recent progress of metabolic technology, including stable isotope-resolved metabolomics, NMR-based metabolomics, and fluorescence-activated cell sorting [20–23], is deepening our insight into the metabolic cross talk between CAFs and cancer cells in the context of metabolic alterations. Using these advanced technologies, CAF-cancer cell interactions were investigated in various types of cancers, including breast cancer, prostate cancer, ovarian cancer, lymphomas, non-small cell lung cancer, and head and neck cancers [19]. For instance, CAF produces and releases lactate to the TME while cancer cells simultaneously utilize lactate for mitochondrial oxidative phosphorylation (OXPHOS) in order to produce ATP efficiently and rapidly (Fig. 1). Interestingly, not only do CAFs produce those metabolites to help cancer cells grow, but cancer cells also release epidermal growth factor (EGF) to educate CAFs to enhance the production and secretion of leptin which eventually leads to tumor progression [24]. Additionally, tumor cells express pro-inflammatory genes, including NF-κB and IL-1, so that normal fibroblasts can be guided by cancer cells to become pro-inflammatory CAFs [25] (Fig. 1).

Moreover, several studies have identified CAF gene expression profiles, including certain extracellular matrix (ECM) components and several matrix metalloproteases (MMP2, MMP11, and MMP14). This suggests that ECM biosynthesis and remodeling are one of the critical features of interplay between CAFs and cancer [26–28]. For instance, in ovarian and small cell lung cancers, many ECM genes remarkably elevate their expression levels in chemotherapy-treated cancer cells to induce chemoresistance [29, 30]. Additionally, CAFs bypass anti-VEGF treatment by activating the platelet-derived growth factor C (PDGF-C) pathway so that tumor cells resist the inhibition of angiogenesis by anti-VEGF treatment [31] and stroma cells mediate RAF inhibitor resistance in BRAF-mutant melanoma through human growth hormone (HGH) secretion [32]. Even though gene expression profiles provide a method to predict the risks of metabolic coupling between cancer and CAFs, gene expression level does not always correlate with metabolic changes. Therefore, measuring the metabolite levels in a sample could be a more accurate method to predict the risk of metabolic interplay.

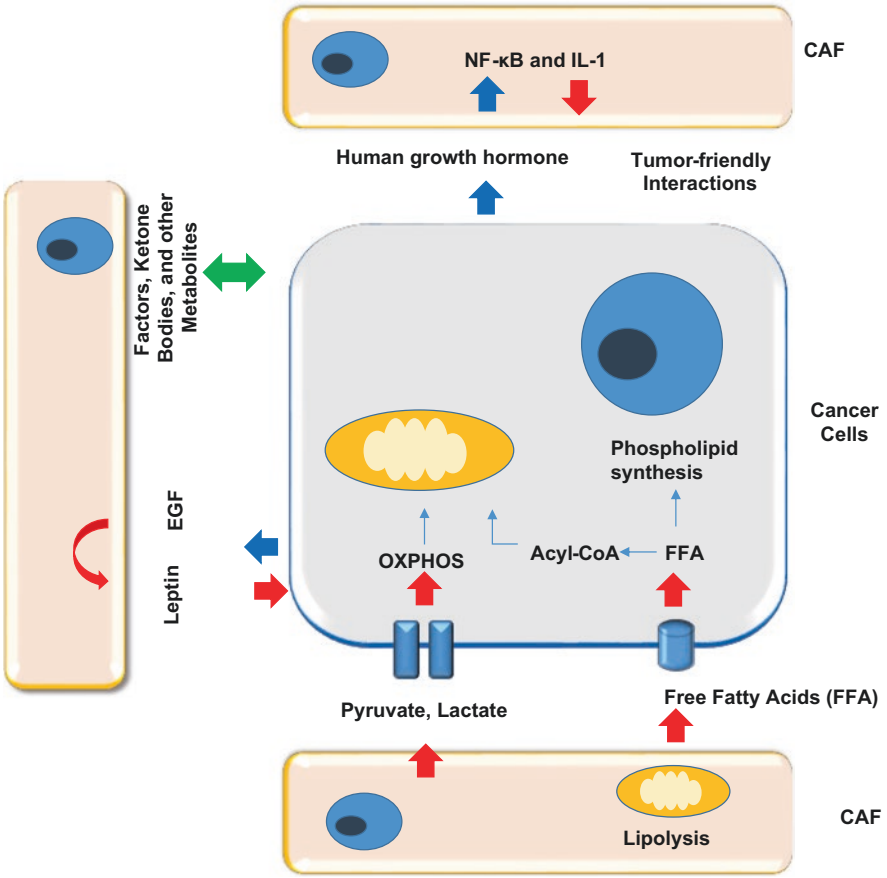

Fig. 1 Overview of the CAF metabolism in solid tumors

2 Targeting the Metabolic Exchanges between CAFs and Cancer Cells

2.1 Targeting the Reverse Warburg Effect via Disruption of the "Lactate Shuttle" by MCT1/MCT 4 Inhibitors

Glycolysis, the process of converting glucose to pyruvate, is an essential metabolic pathway to produce energy in the form of ATP in cells. In the 1920s, however, Otto Warburg found that cancer cells preferably produce energy by converting glucose to lactic acid, even in aerobic conditions, to generate ATP rapidly. This is known as the Warburg effect [33].

Interestingly, it was suggested that the reverse Warburg effect was the result of fibroblast cells secreting lactate/pyruvate and epithelial cancer cells simultaneously

uptaking the energy-rich metabolites to utilize in the tricarboxylic acid (TCA) cycle and promote energy production for their growth [34]. In this hypothesis, cancer cells firstly guide the normal stromal cells to become CAFs, providing a tumor-friendly microenvironment to activate tumor growth. Next, lactate from CAFs is directly fed to the cancer cells as fuel for OXPHOS after the conversion of lactate into pyruvate [35]. Accordingly, the expression levels of glycolytic enzymes, such as lactate dehydrogenase, pyruvate kinase isozymes M2, and monocarboxylate transporter 4 (MCT4), are elevated in the CAFs of breast and lung cancers [19, 36]. Of note, lactate plays an important role in generating energy for the brain and heart [37–40] and serves as an energy interplay shuttle between stromal cells and various types of cancer cells [36, 40, 41]. In this scenario, cancer cells and surrounding CAFs can communicate with each other through direct cell-to-cell contact by releasing an exosome packaged with CAF-produced metabolites [42]. This coincides with neovascularization, inflammatory cell infiltration, and extensive remodeling of extracellular matrix in TME [43]. Evidence supporting the "lactate shuttle" in human cancers [13, 44, 45] further shows that lactate can directly transfer from CAFs to adjacent tumor cells under the premise that (1) CAFs overexpress the transmembrane monocarboxylate transporter 4 (MCT4) for lactate efflux from CAFs to cancer cells [44], (2) cancer cells overexpress monocarboxylate transporter 1 (MCT1) for lactate influx within cancer cells [36, 46], and (3) cancer cells finally utilize lactate as fuel for producing ATP via the TCA cycle [47–49] (Fig. 2). Of note, MCT1 and MCT4, the main transporters of lactate, are key modulators for lactate homeostasis [50]. The elevated expression levels of genes involved in the lactate shuttle system, including high expression levels of MCT4, are associated with poor

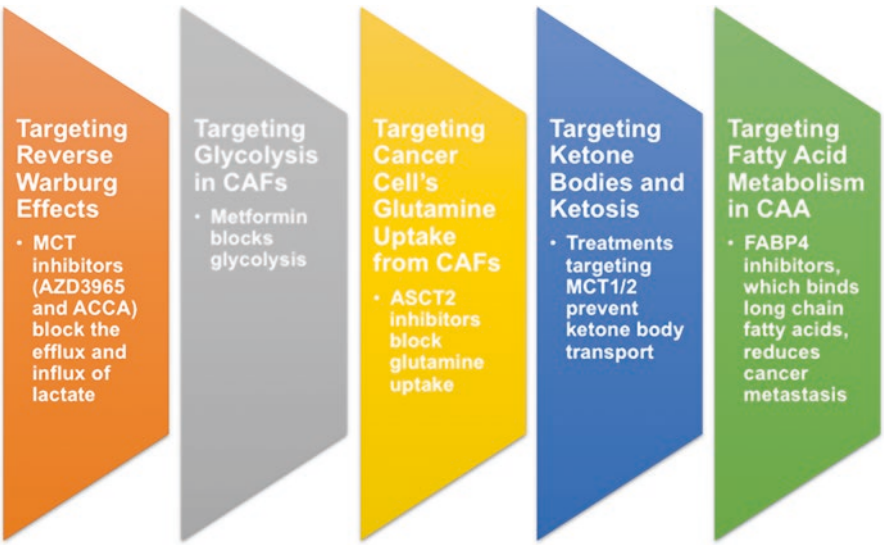

Fig. 2 Therapies targeting metabolic cross talk between cancer cells and cancer-associated fibroblasts

prognosis in prostate, pancreas, and triple-negative breast cancer [50–52]. Consequently, accumulating evidence suggests that MCT1 and MCT4 transporters could be promising targets for cancer therapy.

Over the last decade, there has been significant progress in understanding the roles of the TME in tumorigenesis and the development of effective strategies for cancer therapy. In order to disrupt the metabolic bridge in CAF-tumor interactions using glycolysis and lactate metabolism inhibition, three potential strategies have been proposed. First, elevated expression of the lactate transporter MCT1 in cancer cells is a potential target for blocking cellular uptake of two types of mitochondrial fuels, such as ketone bodies and lactate [53]. MCT1 and MCT2 inhibitors can block the influx and efflux of lactate, produced by either CAFs or cancer cells. Thus, due to the rapid accumulation of lactate and protons within cancer cells by inhibiting lactate influx/efflux, rapid acidification can occur in cancer cells and the TME, resulting in lactic acidosis [54–56]. For instance, alpha-cyano-4-hydroxycinnamic acid (ACCA), a MCT inhibitor, not only inhibits lactic acid efflux in glycolytic gliomas but also disrupts redox hemostasis and enhances radiosensitivity [57, 58]. AZD3965, a MCT1 inhibitor, is currently being tested in a phase I clinical trial in solid tumors, including lymphoma, prostate cancer, and gastric cancer (NCT01791595). However, there are concerns over the alternative effects of MCT1 inhibitors which includes the blockage of the lactate transport in the muscles, gastrointestinal tract, and liver [59, 60].

2.2 Blocking the Function of CAFs by Metformin

Metformin, a drug that has been widely used for Type II diabetes treatment, has found new applications as an anticancer drug for its glucose-targeting effects. Metformin activates the AMPK pathway and simultaneously inhibits cancer cell growth through the inhibition of glycolysis by facilitating the trafficking of glucose transporters 1 and 4 [61, 62]. Recent studies have also shown the therapeutic potential of metformin in blocking the function of CAFs [63]. In other words, metformin is sufficient to reverse the effects of CAFs on cancer cell growth [63]. Thus, this provides a rationale for why metformin is actively being tested in multiple clinical trials in solid tumors and lymphoma (NCTNCT00659568, NCT00881725, NCT00984490, and NCT00909506).

3 Targeting the Glutamine Uptake of Cancer Cells from CAFs

Glutamine addiction is a physiological phenomenon where cancer cells rely on the presence of exogenous glutamine to be used as an intermediate in the TCA cycle and as a nitrogen donor for nucleotide and amino acid synthesis [64, 65]. It was

recently revealed that CAFs produce and release glutamine, while cancer cells uptake and reutilize glutamine from the TME as an alternative carbon and nitrogen source [42, 66]. This explains why glutamine transporters (ASCT2 and SLC38A5) are usually overexpressed in breast and prostate cancers [67–69]. Of note, an alanine, serine, cysteine-preferring transporter 2 (ASCT2) mediates the uptake of glutamine, an essential amino acid in triple-negative basal-like breast cancer [69], so ASCT2 inhibitors, such as benzylserine and L-γ-glutamyl-*p*-nitroanilide, have been shown to inhibit glutamine uptake and cell growth in melanoma and endometrial carcinoma [70–72]. Additionally, FDA-approved tamoxifen and raloxifene also suppress estrogen receptor-negative breast cancer growth by inhibiting glutamine uptake [73].

4 Targeting Ketone Bodies and Ketosis in CAFs

Ketone bodies such as acetoacetate, β-hydroxybutyrate (βOHB), and acetone are produced by fatty acid metabolism in the liver [74]. Liver hepatocytes convert fatty acids into ketones and release ketone bodies into the bloodstream, especially under starvation conditions. Consequently, ketolysis, a process of conversion of ketone bodies into acetyl-CoA, additionally feeds into the TCA cycle or OXPHOS to generate ATP [75]. Recent studies have shown that CAFs secrete ketone bodies, and cancer cells utilize them as energy sources [66, 74, 76]. Furthermore, Bonuccelli et al. observed that ketone bodies, especially βOHB, serve as a more powerful energy source for cancer cell growth in comparison to lactate [77]. Genes associated with ketolysis and ketogenesis in CAFs, including 3-hydroxybutyrate dehydrogenase 1 (BDH1) and 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2), were overexpressed [78, 79]. Specifically, BDH1 catalyzes the conversion of acetoacetate to βOHB. HMGCS2, a family of the 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase, generates HMG-CoA [75, 80]. In contrast to the gene expression profile of the surrounding epithelial cells, cancer cells themselves have upregulated gene expression associated with ketone reutilization (acetyl-CoA acetyltransferase, ACAT1) and mitochondrial biogenesis (heat-shock protein 60, HSP60) [78]. Moreover, ketone can be a source of lactate and pyruvate, because acetone—an end product of ketosis—can be metabolized to lactate and pyruvate [81, 82]. Taken together, this suggests that the ketone bodies produced by CAFs can serve as an energy fuel for tumor growth and have further implications as a potential therapeutic target for cancer therapy. Furthermore, ketone bodies, including βOHB, are transported by the monocarboxylate transporters (MCT1 and MCT2) which also transports them across the blood-brain barrier [80, 83]. Accordingly, treatments targeting MCT1 and MCT2 are currently being tested in phase I clinical trials in solid-tumor cancers (NCT01791595). Thus, the MCT1 and MCT2 inhibitors may effectively block the transport of lactate and ketone bodies, both generated by CAFs (Fig. 2).

5 Targeting Fatty Acid Metabolism, as a Nutrient Reservoir for Cancer Cell Growth, from Cancer-Associated Adipocytes (CAAs)

CAAs play an important tumorigenic role in fatty acid metabolism in the TME. For instance, omental adipocytes promote the migration and invasion of ovarian cancer cells to the omentum [84, 85]. It is known that omental adipocytes generate free fatty acids (FFAs) that are further transferred to cancer cells to generate ATP via β-oxidation (Fig. 1). Therefore, in order to utilize FFAs, a subset of cancer cells overexpresses the fatty acid-binding protein 4 (FABP4), which plays a key role in fatty acid transport in ovarian and breast cancer metastasis [12, 84, 86] (Fig. 1). It has been shown that a FABP4 inhibitor, which binds long-chain fatty acids, reduces metastasis of prostate cancer and regulates fatty acid production in ovarian and prostate cancer [84, 87]. Because adipocytes are a major component of the TME in breast and ovarian cancer, it may be a rationale for FABP4's effectiveness in those cancers [84, 88]. Accumulation of fatty acids in the TME could serve as a nutrient reservoir for cancer cell growth during nutrient deprivation. Taken together, stromal catabolites, such as free fatty acids and phosphoinositides, promote tumor growth and act as a chemoattractant to metastasizing cancer cells in the omentum.

Conclusion

The TME is comprised of cancer cells, CAFs, immune cells, vasculature, and other supporting cells. Of those, CAFs are one of the key regulators of tumorigenesis, given that (1) CAFs firstly produce and release high-energy metabolites to the TME, (2) cancer cells uptake those metabolites through the membrane transporters, and (3) cancer cells simultaneously utilize those metabolites for OXPHOS to produce ATP as an alternative energy source. Within the TME of solid tumors, heterogeneous cancer cells and CAFs interact by transferring their metabolites, ketone bodies, amino acids, cytokines, growth factors, and fatty acids, which reciprocally facilitate the growth of cancer cells. Moreover, CAFs provide not only a structural matrix for providing a tumor-friendly microenvironment to cancer cells but also nutrients for cancer cells. As such, metabolic interplay between CAFs and cancer cells is considered as an area of vulnerability among cancer cells given that (1) cancer cells release a human growth hormone (HGH) and induce pro-inflammatory gene expression in CAFs, (2) CAFs produce and release high-energy metabolites to the TME, and (3) cancer cells uptake those metabolites to utilize energy for cancer cell growth. Therefore, it is widely accepted that CAF-mediated metabolism plays a key role in tumorigenesis and that targeting the metabolic cross talk between cancer cells and CAFs can serve as potential targets for cancer therapy. Consequently, researchers have made continuous efforts to exploit areas of metabolic vulnerability by targeting for (1) glycolysis and lactate metabolism, (2) glutaminolysis, (3) ketone bodies and ketosis, and (4) fatty acid metabolism.

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