

Glutamine Metabolism in Prostate Cancer

Xuguang Guo^{1,2,3,4}

Cite this article: Guo XG: Glutamine Metabolism in Prostate Cancer. *Ann Urol Oncol* 2024, 7: 14. <https://doi.org/10.32948/auo.2024.08.01>

Abstract

Prostate cancer (PCa) is the most common malignancy in the urinary system. Research suggest that prostate cancer is often accompanied by gene mutations and metabolic reprogramming during disease progression, leading to disease advancement, metastasis, and therapeutic resistance. During metabolic reprogramming, glutamine serves as a carbon and nitrogen source to replenish the tricarboxylic acid cycle driving tumor metabolism. Among all types of amino acids, glutamine is the most widely distributed and functionally diverse in the human body. In healthy and diseased states of the human body, immune cells metabolize glutamine at a rate similar to glucose. Glutamine release and utilization in circulation are primarily controlled by organs such as the intestines, liver, and skeletal muscles. Under high catabolism such as inflammation and cancer, glutamine can become essential molecule for metabolic function, but its availability may be impaired due to disrupted homeostasis of amino acids in inter-tissue metabolism. This research briefly reviews the metabolism of glutamine in prostate cancer.

Key words metabolic reprogramming, prostate cancer, glutamine metabolism, neuroendocrine, tricarboxylic acid cycle

1. Department of Clinical Laboratory Medicine, The Third Affiliated Hospital of Guangzhou Medical University, Guangzhou, 510150, China.

2. Department of Clinical Medicine, The Third Clinical School of Guangzhou Medical University, Guangzhou, 511436, China.

3. Key Laboratory for Major Obstetric Diseases of Guangdong Province, The Third Affiliated Hospital of Guangzhou Medical University, Guangzhou, 510150, China.

4. Key Laboratory of Reproduction & Genetics of Guangdong Higher Education Institutes, The Third Affiliated Hospital of Guangzhou Medical University, Guangzhou, 510150, China.

Correspondence: Xuguang Guo (Department of Clinical Laboratory Medicine, The Third Affiliated Hospital of Guangzhou Medical University, Guangzhou, 510150, China; Email: gysyngx@gmail.com)

Introduction

Cellular metabolism involves complex biochemical processes, including the consumption of carbohydrates, fatty acids, and amino acids [1]. These nutrients are the main sources for cellular energy homeostasis and macromolecule synthesis. Core metabolic pathways can be categorized into synthetic metabolism, catabolic metabolism, and waste generation, involving processes such as glycolysis, tricarboxylic acid cycle (TCA cycle) [2], glycogen breakdown, lipogenesis, and the urea cycle.

Unlike normal cells, cancer cells adjust their metabolic pathways to promote growth and survival, leading to different nutritional requirements. For example, the Warburg effect is a change in the metabolism of most neoplastic cells, allowing them to convert glucose into lactate even in the presence of sufficient oxygen, a process commonly known as aerobic glycolysis. This change was initially thought to be due to mitochondrial dysfunction leading to oxidative defects, but studies also show that the rapid uptake and metabolism of glucose enable cancer cells to limit its supply to multiple non-mitochondrial pathways, such as the pentose phosphate pathway, the ribose pathway required for producing ribose and reducing equivalents like (Nicotinamide adenine dinucleotide phosphate, NADPH) for reductive biosynthesis, and the hexose metabolic pathways essential for glycerol used in protein glycosylation and complex lipid synthesis [3, 4]. The Warburg effect may indirectly support synthetic metabolism while maintaining abundant glycolytic intermediates within cells, facilitating involvement in the pentose phosphate pathway and other intracellular biosynthetic pathways [5]. Although the mechanisms behind cancer cell metabolic changes and their impact on cancer behavior remain unclear, there is growing awareness of cancer cells' dependence on specific metabolic pathways and the potential for therapeutic development targeting these dependencies. Therefore, this review provides a brief overview of the glutamine metabolism in prostate cancer.

Metabolic characteristics of prostate cancer

Prostate cancer globally ranks as the second most common cancer in men. In China, the annual incidence rate of PCa is approximately 10.23 per 100,000, with a mortality rate of about 4.36 per 100,000. In recent years, the incidence of PCa in China has shown a rapid growth trend [6, 7]. The treatment strategy for PCa depends on serum PSA levels, pathological type, Gleason score, clinical TNM staging, as well as patient's age and overall health. The most effective treatment for prostate cancer is surgery, however advanced PCa, undergoes androgen deprivation therapy, radiotherapy, and targeted therapy [8]. Although these treatments initially achieve satisfactory efficacy, inevitably, prostate cancer cells will face the dilemma of developing resistance through various mechanisms, especially with single therapies targeting a single metabolic pathway, ultimately leading to castration-resistant prostate cancer (CRPC).

Normal prostate tissue and prostate tumors exhibit distinct metabolic profiles. Primary prostate cancer tends to increase oxidative phosphorylation while showing limited glycolysis. Fatty acid synthesis also plays an important role in early prostate tumor events and is associated with disease progression [9]. Increased glycolysis is a characteristic of CRPC. Studies indicate a tendency towards glycolysis in advanced PCa cells, with treatment-resistant PCa cells exhibit higher glucose consumption and lactate secretion compared to naive PCa cells [10, 11]. From a mechanistic standpoint, CD44 and Ataxia Telangiectasia Mutated (ATM), proteins involved in vascular dilation and genomic stability, are considered as key regulatory factors. Their alterations significantly impact the glucose metabolism of PCa. Li et al. [12] demonstrated

that specific expression of CD44 in neuroendocrine prostate cancer (NEPC) significantly increases the levels of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 4 (PFKFB4). PFKFB4 is one of the rate-limiting enzymes in the glycolysis pathway [13]. Xu et al. [10] demonstrated that ATM mutations (a common genetic event observed in recurrent PCa) upregulate the expression of lactate dehydrogenase-A (LDHA), which is a key enzyme converting pyruvate to lactate. Inhibition of CD44 increases the chemotherapeutic sensitivity of small-cell neuroendocrine cancer [12]. Similarly, targeting LDHA by disrupting the link between ATM alterations and LDHA activation may be a strategy for overcoming PCa resistance to poly (ADP-ribose) polymerase (PARP) inhibitors.

Metabolic characteristics of glutamine

Glutamine is an L- α -amino acid with five carbons, and its elemental composition includes carbon, hydrogen, oxygen, and nitrogen. Glutamine is an amino acid source of proteins, meaning that it accounts for 5% to 6% of the bound amino acids in proteins [14]. Glutamine contains two amino groups: the α -amino group and the amide group on its side chain, which make it effective as a nitrogen transporter and ammonia carrier (NH₃). In healthy individuals, the total amount of glutamine distributed throughout the body is approximately 70 to 80 grams [15]. Studies using isotope and pharmacokinetic techniques have shown that the endogenous production of glutamine ranges between 40 to 80 grams per day [16, 17]. The concentration of glutamine in blood plasma varies between approximately 500 to 800 μ M/L, comprising about 20% of the total free amino acid pool in the blood [14]. In tissues like the liver and skeletal muscle, glutamine levels are significantly higher than in plasma, making up about 40% to 60% of the total amino acid pool [18, 19]. In both plasma and tissues, glutamine levels are 10 to 100 times higher than those of any other amino acid, making it the most abundant amino acid in the body.

The body's glutamine concentration and availability are influenced by a delicate equilibrium between its synthesis, release, and uptake across various human organs and tissues. Specific organs such as the lungs, liver, brain, skeletal muscles, and adipose tissue exhibit distinctive activities of glutamine synthetase. Conversely, among 31 major tissues that heavily consume glutamine—including the intestinal mucosa, leukocytes, and renal tubular cells—three tissues possess notable levels of glutamine enzyme activity and cofactors that facilitate glutamine degradation. However, the liver may become a site of glutamine consumption under certain conditions, such as reduced intake of carbohydrates [19] and amino acids [20], high catabolic states, illness, and stress [21], leading to decreased glutamine synthesis particularly in the muscle. Some hormones such as glucocorticoids [22], thyroid hormones [23], growth hormone [24], and insulin [25] can regulate the activity of glutamine metabolism regulatory enzymes.

The intracellular enzymes involved in glutamine metabolism are primarily glutamine synthetase (GS) and phosphate-dependent glutaminase (GLS). GS facilitates the synthesis of glutamine from ammonia (NH₄⁺) and glutamate, requiring ATP as an energy source. In contrast, GLS breaks down glutamine, converting it back into glutamate and NH₄⁺ [26, 27] (**Figure 1**). GS predominantly resides in the cytoplasm, whereas GLS is primarily localized within the mitochondria. This segregation in cellular localization corresponds with their respective enzymatic roles. Glutamine synthetase produces glutamine to aid in the synthesis of cytoplasmic proteins and nucleotides, while GLS converts glutamine into glutamate. This process is crucial for the tricarboxylic acid cycle and the formation of the metabolic

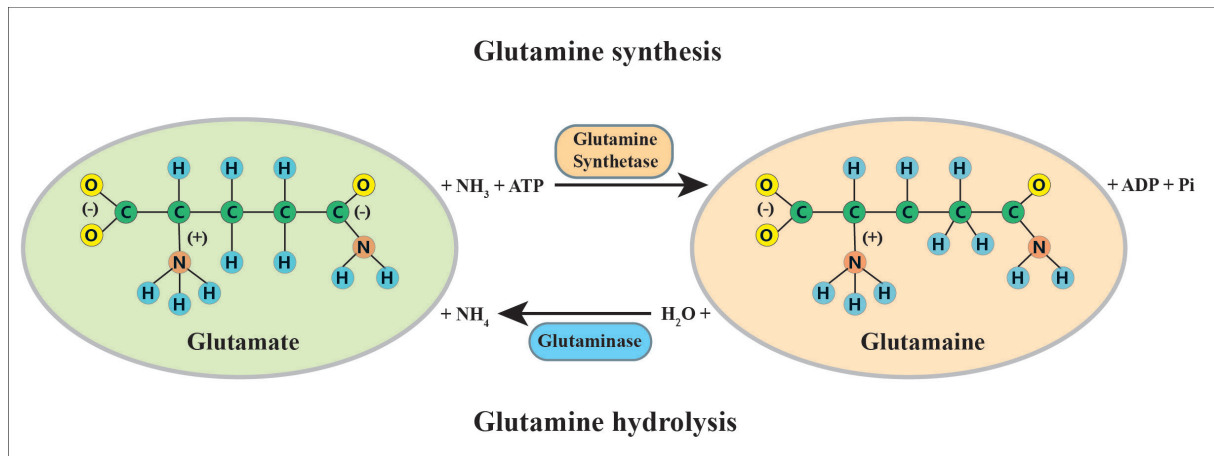


Figure 1. Possible mechanisms of obesity affecting prostate cancer.

intermediate 2-oxoglutarate [28]. The production of glutamine by GS depends on the availability of glutamate. Glutamate forms from 2-oxoglutarate and NH₄⁺ via glutamate dehydrogenase's catalytic activity and can also arise from the catabolic metabolism of alternative amino acids like branched-chain amino acids (BCAAs) and leucine [20, 29].

Studies have shown that branched-chain amino acids like leucine can readily transaminate with α -ketoglutarate to form glutamate. Glutamate can react with free NH₃, and with the help of glutamine synthetase, it is converted into glutamine. [30, 31]. The levels of glutamine in tissues and blood are influenced by the functioning of GS or GLS. During catabolic states like cancer [32], sepsis [33], infections [34], surgical procedures [35], traumatic injuries [36], and prolonged strenuous exercise [37], the body's ability to produce sufficient glutamine internally may be insufficient to meet its requirements. In such deficient circumstances, glutamine serves as a conditionally essential amino acid by stimulating elevated GLS expression and suppressing GS activity. It is important to note that although plasma glutamine levels decrease from 500-800 $\mu\text{mol/L}$ to 300-400 $\mu\text{mol/L}$, cells dependent on this amino acid actually show little impact on their proliferation and function [38]. Conversely, heightened tissue catabolism leads to diminished glutamine reserves in the body, particularly in muscles and the liver (**Figure 2**). The reduced glutamine levels in human tissues have systemic implications because this amino acid supplies nitrogen atoms necessary for synthesizing purines, pyrimidines, and amino sugars [39]. Continued high degradation of glutamine in these tissues can disrupt metabolic pathways and mechanisms that heavily depend on glutamine availability, resulting in immune suppression. The research indicates that bacterial infections (like *Escherichia coli*) can modify their metabolic processes and use glutamine to counteract the impacts of acid stress and copper toxicity [40]. As a result, bacterial pathogens can adjust and thrive by modifying essential antimicrobial strategies imposed by the host within fundamental metabolic pathways.

Characteristics and roles of glutamine metabolism in prostate cancer progression

Interestingly, despite most glucose being diverted from the TCA cycle into lactate fermentation in advanced PCa, mitochondrial activity remains highly sustained. This fact prompts us to explore another readily available metabolite responsible for maintaining cellular TCA cycle. Amino acid metabolism plays a crucial role

in the development of prostate cancer, where some amino acids are converted into precursors needed for building blocks such as glucose, lipids, and nitrogen-containing metabolites like purines and pyrimidines, used in nucleotide synthesis. Unlike other solid tumors, prostate cancer relies more on amino acid metabolism and biosynthesis of carboxylation reactions rather than energy production [41]. Glutamine is the second most abundant amino acid in blood after glucose, playing diverse roles in energy generation and macromolecular synthesis [42]. Importantly, catalyzed by glutaminase-1 (GLS1), glutamine serves as a carbon source to aid the TCA cycle, sustain cellular energy, and provide NADPH for synthesizing nucleotides, proteins, and lipids. Compared to hormone-sensitive PCa, CRPC tends to rely more on glutamine and utilizes more amino acids to support cell proliferation. This apparent glutamine dependency is attributed to the differential expression of two subtypes of GLS1: kidney-type glutaminase (KGA) and glutaminase C (GAC). KGA is the predominant variant in primary PCa, whereas GAC is the more efficient subtype, predominant in castration-resistant PCa. Interestingly, KGA is an isoform regulated by the androgen receptor (AR), whereas GAC is not. Therefore, during hormone therapy, the activity of KGA is suppressed due to AR inhibition, leading GAC to become the primary subtype of GLS1. This helps tumor cells evade hormone therapy by increasing their dependence on glutamine rather than androgens. In treatment, the GLS1 inhibitor CB-839 exhibits strong inhibition of GAC, leading to tumor regression independent of AR-targeted therapy [11]. Subsequent studies have shown that the carbon skeleton of glutamine can substitute glucose to enter mitochondria, maintaining the operation of the TCA cycle in CRPC cells. This provides energy for tumor cells, making glutamine an important metabolic nutrient for late-stage androgen-independent PCa [43]. In addition, GLS1 is a crucial rate-limiting enzyme that mediates the entry of glutamine into the TCA cycle. It removes the amino group from glutamine, retains the remaining five-carbon skeleton, and gradually converts it into α -ketoglutarate, thereby participating in the tricarboxylic acid cycle. Inhibiting the enzyme activity of GLS1 effectively blocks the breakdown and utilization of glutamine, reduces mitochondrial function, and thereby slows down the progression of androgen-independent prostate cancer [11].

Glutamine's chemical structure not only includes a carbon chain skeleton composed of five carbon atoms but also incorporates two nitrogen atoms. The glutamine metabolism pathway mediated by GLS1 described earlier actually refers specifically to the

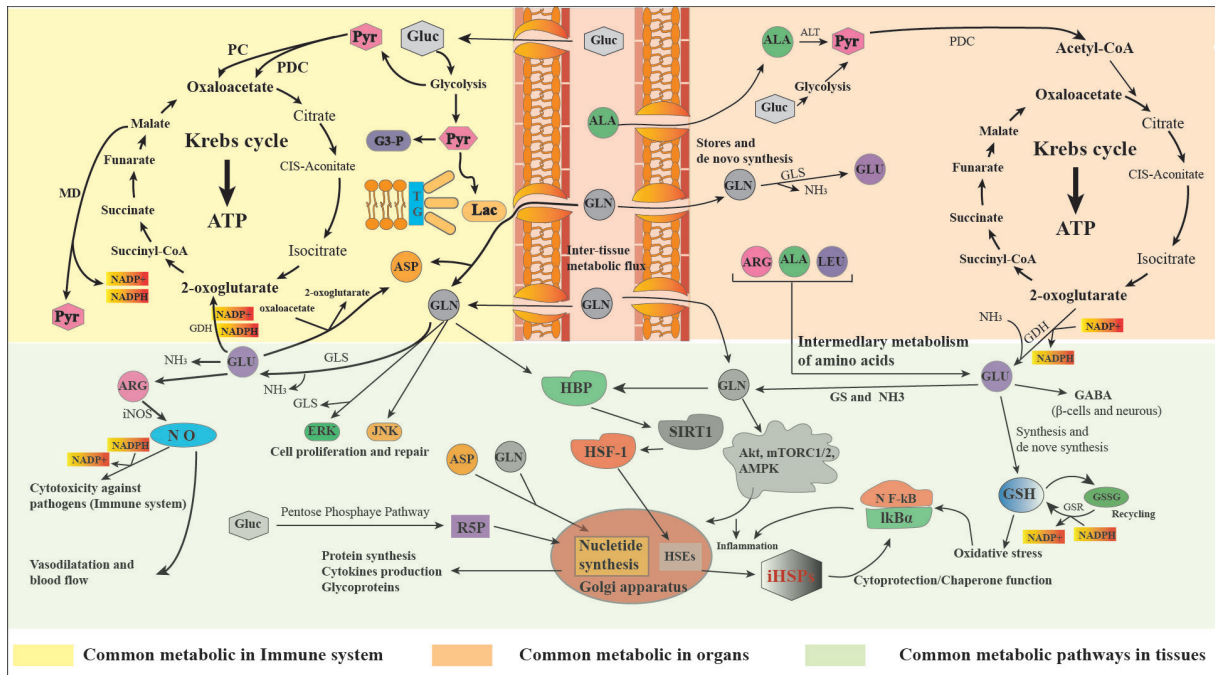


Figure 2. Clinical interventions and preventive measures.

carbon metabolism of glutamine. In other words, through the catalytic action of GLS1, glutamine is gradually converted into α -ketoglutarate, which then enters the TCA cycle. However, in addition to this, the metabolism of glutamine also includes nitrogen metabolism pathways involving two nitrogen atoms. Specifically, during carbon metabolism, the nitrogen elements of glutamine gradually detach from the carbon chain. However, these nitrogen elements are not merely excreted outside the cell as waste products such as free amines. Instead, they are more involved in the biochemical synthesis of numerous important nitrogen-containing compounds, such as nucleotides and other non-essential amino acids [44, 45]. Therefore, the metabolic network of glutamine is actually regulated by both its carbon and nitrogen metabolic pathways. These two pathways cooperate with each other to ensure the comprehensive utilization of glutamine by tumor cells.

The active metabolic pathways rely on the support of highly active key enzymes. In pyrimidine synthesis, three crucial enzymes are involved: carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase (CAD), along with dihydroorotase dehydrogenase (DHODH) and glutamic oxaloacetic transaminase (GOT) [43]. Bioinformatics analysis revealed that, compared to early primary PCa, only the expression level of CAD is significantly elevated in CRPC samples, while the other two key enzymes (DHODH and GOT) show no significant changes in comparison between primary PCa and CRPC samples. Similar results were confirmed in experiments with in cell lines representing CRPC exhibit higher levels of CAD protein. These early experimental results suggest that CAD is likely the most crucial enzyme determining the activity of the pyrimidine synthesis pathway in CRPC.

Although the specific metabolic pathways and final products may vary, multiple studies indicate that in tumor cells, the carbon and nitrogen metabolism of glutamine cooperate closely and are inseparable. More importantly, this co-operation ensures that when one metabolic pathway is obstructed, metabolic flux can be

rerouted through another pathway to fulfill the biological function, ultimately achieving tumor cell's demand for glutamine. Wang et al. noted that under hypoxic conditions, tumor cells markedly enhance their use of glutamine carbon chains. Simultaneously, the nitrogen atoms of glutamine participate in the synthesis pathway of nucleotides, not as final products but are secreted extracellularly in the form of intermediate products such as lactate. This indicates that the carbon and nitrogen metabolic pathways of glutamine can mutually regulate under different environmental conditions to achieve the most suitable metabolic steady state [46]. Kodama et al. found that during tumor development, the carbon pathway of glutamine closely interacts with another nucleotide metabolic pathway - purine metabolism. The rate-limiting enzymes in both pathways, GLS1 and phosphoribosyl pyrophosphate amidotransferase (PPAT), maintain a dynamic equilibrium. When this balance is disrupted, the metabolic flux of glutamine shifts towards the more efficient pathway, ensuring continued utilization of glutamine by tumor cells [47]. Considering that the nitrogen metabolic pathway of glutamine likely compensates to maintain the consumption of glutamine by CRPC cells when the carbon metabolic pathway of glutamine is inhibited, theoretically, simultaneously inhibiting both the carbon and nitrogen metabolic pathways of glutamine can maximally restrict the utilization of glutamine by tumor cells. This approach can cut off the material source of energy and nutrients for tumor cells, thereby enhancing the therapeutic effect of CRPC.

Metabolic characteristics of neuroendocrine prostate cancer

In prostate cancer, there are abundant luminal cells and a small number of neuroendocrine (NE) cells. Luminal cells express abundant AR (Androgen Receptor) protein and are sensitive to androgen deprivation therapy (ADT); In contrast, NE cells, lacking AR and other luminal markers, show no response to ADT. Therefore, androgen deprivation therapy can only restrain the

growth of luminal cells and has almost no therapeutic effect on NE cells. What's worse, long-term ADT can promote the differentiation and proliferation of NE cells, causing some cases of CRPC to transform into highly malignant NEPC [48]. In prostate cancer, AR protein acts as an intracellular receptor. It can bind to androgens such as testosterone and regulate the expression of related genes within the nucleus. The AR protein is essential in the development and treatment of prostate cancer, as many prostate cancer cells rely on its signaling pathway for their growth and proliferation [49, 50]. In addition, a small subset of NEPC originates directly from NE cells. NEPC is characterized by weakened AR signaling, increased expression of neuroendocrine lineage markers (including chromogranin A, neuron-specific enolase, and synaptophysin), and purely or mixed small cell histological features [51]. In NEPC, enhanced glycolysis and increased glutamine uptake further increase the production of pyruvate and acetyl-CoA [52]. Furthermore, enhanced glycolysis and lactate production/secretion mediated by monocarboxylate transporter 4 are the most significant and clinically relevant metabolic features of NEPC [53]. These studies collectively indicate that the differentiation of NEPC relies on metabolic reprogramming to supply the necessary epigenetic changes. This characteristic of tumor heterogeneity suggests that a combined approach using androgen deprivation therapy targeting luminal cells and specific inhibitors targeting NE cells, such as G protein-coupled receptor CXCR2 (C-X-C motif chemokine receptor 2) inhibitors, can more comprehensively kill various types of tumor cells in CRPC [54]. Hence, utilizing various treatment modalities concurrently for advanced prostate cancer proves to be a highly efficient.

Conclusion and future perspective

Prostate cancer exhibits specific metabolic characteristics. In contrast to normal prostate tissue, where the TCA cycle is inhibited, early-stage prostate cancer activates this cycle, resulting in a heightened reliance on oxidative phosphorylation. Metabolic reprogramming plays a crucial role in the development of prostate cancer, particularly glutamine, which plays a key role in the transition to CRPC. In tumor cells, the carbon and nitrogen metabolic processes of glutamine cooperate closely with each other. This close cooperation ensures that when one metabolic pathway is obstructed, metabolic flux can be diverted through another pathway to fulfill the biological function, ultimately meeting the tumor cell's demand for glutamine. This phenomenon leads to the development of glutamine dependency in CRPC. Research on prostate cancer metabolism contributes to a deeper understanding of the complexity of prostate cancer metabolism. Targeting combined therapies aimed at different metabolic pathways may potentially delay disease progression in prostate cancer and improve patient prognosis.

Understanding of tumor cell metabolism, especially glutamine metabolism, is rapidly advancing. Significant progress has been made, yet many details remain elusive. Given the interplay of numerous interconnected metabolic pathways, tumor cells may trigger compensatory mechanisms to evade metabolic suppression. Given the highly intra-tumoral and inter-tumoral heterogeneity of prostate cancer, future research on metabolic changes between tumor cells and the tumor microenvironment (immune cells and stromal cells), as well as the interactions of different cellular components in different patients, will lead to more effective, precise, and personalized treatment approaches to assist a large number of patients in need.

It is noteworthy that the current research on the complexity and authenticity of metabolic characteristics primarily relies on cellular biology. However, it has the following limitations: (1). Culture conditions such as oxygen concentration in the

laboratory-aided studies are vastly different from those in real tumors; (2). Geometric structure of cell culture is often conducted in two-dimensional layers, which cannot simulate the three-dimensional, dynamic heterogeneity of the tumor; (3). Composition and concentration of metabolites in cell culture media differ significantly from those in human blood or the tumor microenvironment; (4). Rapid growth and proliferation of cells in vitro also affect the translation of results. To overcome these technical obstacles, efforts are being made to develop new research models that closely mimic real-life conditions, thereby providing more reliable metabolic results. For example, organoid models are receiving widespread attention because they are generated from pluripotent or adult stem cells and possess the ability to simulate organ regeneration ex vivo. Animal models such as genetically engineered mouse models and patient-derived xenografts are also of great interest in cancer research and drug development. Since mice harbor almost all genes identical to humans, these models are suitable for metabolic experiments such as isotope tracing and metabolic imaging detection. In addition, because the human metabolic network encompasses thousands of enzymes, metabolites, and interactions, comprehensive analysis requires integration of various omics data.

Acknowledgements

None.

Ethical policy

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. Approval from institutional ethical committee was taken.

Availability of data and materials

All data generated or analysed during this study are included in this publication.

Author contributions

XG searched academic literature, wrote the draft manuscript, supervised the review writing progress and approved the final manuscript submission.

Competing interests

Authors report no conflict of interest.

Funding

None.

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