Gang Huang
Editor

Nuclear Medicine in Oncology

Molecular Imaging and Target Therapy

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Preface

With the latest development of electronics and computer sciences, medical imaging has become a multidisciplinary clinical science characterized by the use of computed tomography (CT), magnetic resonance imaging (MRI), nuclear medicine, and ultrasound imaging. This book aims to provide professional suggestions on clinical study and practice, by using images from multiple clinical case studies, informing this ever-evolving specialty. This book mainly focuses on nuclear medicine and therapy, molecular imaging, and imaging-based therapy evaluation. It contains 20 chapters covering a variety of topics on medical physics and instruments, nuclear medicine, and its application in multiple diseases both in the areas of diagnosis and therapy.

In the early part of the book, a brief introduction of the ^{18}F-FDG positron emission tomography (PET) technique and its application in clinical cancer studies are given, which include breast cancers, non-small-cell lung cancers, lymphoma, genitourinary cancers, gastrointestinal cancers, head and neck cancers, melanoma, and pediatric cancers. This book then discusses the current applications of molecular imaging for various clinical studies proposed, including metabolic imaging, receptor imaging and therapy, immuno-imaging and therapy, imaging of apoptosis, imaging of gene expression, radioactive iodine imaging and therapy, neuroimaging and therapy, and cardiac imaging and therapy. The latter part of the book focuses on the latest research findings of the medical physics and the molecular imaging, highlighting the application of novel molecular probes in clinical studies and its potential impacts. As a component of medical imaging, nuclear medicine and molecular imaging could be used as an important approach in evaluating human physiological profiles, including assessing organic functions, metabolism, blood flow, receptor density, and gene expression at both molecular and functional levels. The modality of nuclear medicine and molecular imaging would also provide the quantitative analysis to help early disease diagnosis and to evaluate disease progression, prognosis, and therapy outcomes. These novel techniques could be incorporated with other reliable analytical data to reach the goal of precision and molecular medicine.

By presenting the latest findings in molecular imaging with validated clinical data, this book may serve as a useful tool for nuclear medicine physicians, nuclear radiologists, residents, and graduate students in nuclear medicine to help with their learning and teaching processes.

Special thanks are given to all the scholars and colleagues who contributed to the preparation of this book. Finally but not least, special gratitude to their families for their unfailing support in making this book a reality.

Shanghai, P. R. China — Gang Huang

Contents

Abbreviations

ACS	Acute coronary syndrome
BC	Bladder cancer
CABG	Coronary artery bypass grafting
CAD	Coronary artery disease
CAG	Coronary angiography
CHF	Congestive heart failure
CMR	Cardiovascular magnetic resonance
CTCA	Computed tomography coronary angiography
CVD	Cardiovascular disease
DTPA	Diethylenetriaminepentaacetic acid
ERNA	Equilibrium radionuclide angiocardiography
FDG	Fluorodeoxyglucose
FPRNA	First-pass radionuclide angiocardiography
GLUT1	Glucose transporter type 1
HLA	Horizontal long axis
IHD	Ischemic heart disease
LAD	Left anterior descending artery
LCX	Left circumflex artery
LDHA	Lactate dehydrogenase A
LEAP	Low-energy all-purpose
LEHR	Low-energy high-resolution
LM	Left main artery
LV	Left ventricular
LVEDV	End-diastolic volume of LV
LVEF	Ejection fraction of LV
LVESV	End-systolic volume of LV
LVSV	Stroke volume of LV
MCTs	Monocarboxylate transporters
MI	Myocardial infarction
MIBI	2-Methoxy-isobutyl-isonitrile
MPI	Myocardial perfusion imaging
MRCA	Magnetic resonance coronary angiography
MRI	Magnetic resonance imaging
MUGA	Multiple-gated acquisitions
PCI	Percutaneous coronary interventions
PTCA	Percutaneous transluminal coronary angioplasty
RCA	Right coronary artery
RCC	Renal cell carcinoma
RNA	Radionuclide angiocardiography
ROI	Region of interest
SA	Short axis
SCE	Severe cardiac event

SDS	Summed difference score
SRS	Summed rest score
SSS	Summed stress score
TLG	Total lesion glycolysis
VLA	Vertical long axis

About the Editor

Gang Huang is a Professor and the President of Shanghai University of Medicine & Health Sciences (SUMHS). He is also the Elected President of the Asia Oceania Federation of Nuclear Medicine and Biology; Dean of Asia School of Nuclear Medicine; Editor in Chief, *Chinese Journal of Nuclear Medicine and Molecular Imaging*; and the Predecessor President of the Chinese Society of Nuclear Medicine.

Glucose Metabolism Imaging

1

Liang Shi and Jianjun Liu

1.1 Glucose Metabolism

The metabolism of carbohydrates includes glycolysis, aerobic oxidation, pentose phosphate pathway, glycogen synthesis, and gluconeogenesis. Glucose metabolism has two major functions: providing energy for living organisms and supplying a huge array of metabolic intermediates for biosynthetic reactions [1].

1.1.1 Glucose Metabolism Pathway and Flux Analysis

Glycolysis is an anaerobic catabolic pathway in which a molecule of glucose is broken down into two molecules of lactate with the concurrent generation of two molecules of ATP. In the cytosol, glucose is initially converted into pyruvate, and then the latter is catalyzed into lactate by lactate dehydrogenase with concurrent regeneration of NAD^+ from NADH. Alternatively, pyruvate, as an important metabolic intermediate, harbors several potential fates. Pyruvate can then entrance into the mitochondria and join in the tricarboxylic acid (TCA) cycle to produce NADH and $FADH_2$. The mitochondrial electron transport chain (ETC) subsequently uses the electrons donated from these reducing agents to the complex V ATP synthase of the mitochondrial inner membrane with a generation of an additional 34 molecules of ATP per glucose. This process of glucose conversion to CO_2 and water with liberation of energy as the form of ATP is named as aerobic oxidation. The reactions and the enzymes related to TCA cycle are located in the mitochondrial matrix. TCA cycle acts as the final pathway for the oxidation of glucose. Pentose phosphate pathway supplies ribose 5-phosphate for biosynthesis of nucleic acid and NADPH for the synthesis of fatty acids, amino acids, cholesterol, etc. The process of glycogenesis occurs mainly in cytosol of the liver and skeletal muscle. Lactate, glycerol, pyruvate, glucogenic amino acid, and other noncarbohydrates can be transformed to glucose or glycogen mainly in the liver and kidney. This process is termed as gluconeogenesis. This pathway is essentially a reversal of glycolysis; however, there are three different energy barriers obstructing the reversal process.

Proliferating malignant cells prefer aerobic glycolysis pathway even in the presence of oxygen, whereas nonmalignant cells would choose mitochondrial respiration in an oxygen-rich environment. Glycolysis may provide additional biosynthetic precursors to support rapid proliferation of cancer cell. Tumor cells need glycolysis to support higher rates of nucleotide synthesis for DNA replication and RNA transcription, phospholipid synthesis for membrane production, and amino acid synthesis for protein translation. Thus, an increased flux through glycolysis is essential for the proliferation of most cancer cells, by providing additional energy in the form of ATP and metabolic intermediates derived from glucose for lipid, nucleotide, and protein biosynthesis. Accordingly, positron emission tomography (PET) scanning has been exploited in clinical practice, using fluorinated glucose analogs such as ^{18}F-deoxyglucose, to detect tumors with the shifting metabolism toward glycolysis.

Measurements of metabolites offer an opportunity to obtain steady-state data on the levels of all detectable metabolic reactions within the cell. Mass spectrometry (MS) or nuclear magnetic resonance (NMR) spectrometry with unlabeled metabolites or ^{13}C-labeled metabolites can be used to quantify this metabolic information. As fluctuant flux through any of its associated metabolic pathways can influence the source and exit of almost all the metabolic intermediates, using metabolite concentrations for extrapolation of flux information is difficult. However, adding a labeled standard for the metabolite of interest to make an absolute quantification of an unlabeled metabolite before MS analysis is a reasonable way. For example, decreased serine biosynthesis

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resulting from PKM2 silencing was identified by metabolite profiling [2]. Nowadays, using stable isotope-labeled glucose as the nutrient source to analyze glucose metabolic flux of cells may help to detect the metabolism characteristics of metabolic-related diseases [3]. Any of the six carbons within a glucose molecule can be replaced by stable isotope ^{13}C. [U-$^{13}C_6$]-glucose, which is replaced by ^{13}C at all six carbons, is often used to trace the glucose in the TCA cycle. [1, 2-$^{13}C_2$]-glucose can be used for glycolytic pathway metabolite measurements [4]. In addition, [U-$^{13}C_6$]-glucose cannot well define carbon flux through the pentose phosphate pathway. Instead, [1, 2-$^{13}C_2$]-glucose distinguishes metabolites that have gone through this pathway from those that have gone directly through glycolysis because it loses one ^{13}C during its passing through the pentose phosphate pathway. There is a competition between the labeled glucose and unlabeled glucose within the media for being absorbed into cells. Therefore, cells should be cultured in [U-$^{13}C_6$]-glucose- or [1, 2-$^{13}C_2$]-glucose-supplemented media for 24 h before metabolite extraction and flux analysis. The release of ^3H_2O from [5-^{3}H]-glucose is another way to obtain glycolytic flux [5]. Enolase catalyzes and removes a single tritium at C5 of glucose by a condensation reaction in the ninth step of glycolysis, with the production of ^3H_2O, which diffuses freely out of cell. A liquid scintillation counter is used to quantify the ^3H_2O in culture medium. Studies to track changes in glucose metabolic flux and its collateral anabolic pathways can reveal and quantify the metabolic alterations that underlie malignant cell proliferation. Glycolysis and other metabolic pathways that regulate tumor cell proliferation may represent valuable targets for therapeutic interventions and diagnostic procedures.

1.1.2 Biochemical of Glucose Metabolism

The initial procedure of glucose metabolism is glycolysis, in which glucose is converted to pyruvic acid. All the enzymes and ten reactions related to glycolysis locate in cytosol. This pathway is composed of three stages. Stage 1 is the conversion of glucose into fructose-1,6-bisphosphate (F-1,6-BP). This stage consists of three reactions with two phosphorylation and isomerization reactions. The meaning of first stage in glycolysis is to trap the glucose in the cell as well as generate a compound, which is readily cleaved into phosphorylated three-carbon units. In stage 2, F-1, 6-BP is cleaved into two three-carbon fragments. These resulting three-carbon units are readily interconvertible. The final stage is the generation of ATP during the oxidization of the three-carbon fragments to pyruvate.

Glucose enters cell through specific transport proteins, glucose transporters (GLUT). Then glucose is phosphorylated to form glucose 6-phosphate (G-6P), which cannot diffuse through the membrane because of its negative charges. At the same time, glucose is destabilized by the addition of the phosphoryl group, facilitating its further metabolism. Hexokinase (HK) catalyzes the transfer of the phosphoryl group donated from ATP to the hydroxyl group on carbon 6 of glucose. Then, glucose 6-phosphate is isomerized to fructose 6-phosphate (F-6P). This process is a conversion of an aldose into a ketose catalyzed by phosphoglucose isomerase which first opens the six-membered ring of glucose 6-phosphate, catalyzes the isomerization, and promotes the production of the five-membered ring of fructose 6-phosphate. F-6P is phosphorylated to fructose-1,6-bisphosphate (F-1,6-BP). This step is a key reaction of glycolysis and catalyzed by phosphofructokinase (PFK), an allosteric enzyme. This enzyme plays a crucial role in the integration of metabolism. A molecule of glucose consumes two molecules of ATP in stage 1. Then, in the second stage, fructose-1,6-bisphosphate is split into two kinds of three-carbon units, glyceraldehyde 3-phosphate (GAP) and dihydroxyacetone phosphate (DHAP), both of which can be converted to each other. Aldolase catalyzes this reaction. The reaction is readily reversible under intracellular conditions. The conversion of glyceraldehyde 3-phosphate into 1,3-bisphosphoglycerate (1,3-BPG) is catalyzed by glyceraldehyde 3-phosphate dehydrogenase. The third stage in glycolysis is the production of ATP from the phosphorylated three-carbon metabolites of glucose. The transfer of the phosphoryl group from the acyl phosphate of 1,3-bisphosphoglycerate to ADP is catalyzed by phosphoglycerate kinase. The products of this reaction are ATP and 3-phosphoglycerate. 3-Phosphoglycerate is then transferred into phosphoenol pyruvate (PEP) under the catalysis of mutase and enolase. Phosphoenol pyruvate releases a molecule of ATP and then transformed into pyruvate. This means that one molecule of glucose generated two molecules of pyruvic acid with the release of two molecules of ATP. Electrons generated in the process of GAP oxidation are accepted by NAD+, which guarantees the continuity of glycolysis.

There are three key enzymes which catalyze irreversible reactions: hexokinase (HK), phosphofructokinase (PFK), and pyruvate kinase (PK). The most important rate-limiting step of the glycolysis pathway is the reaction catalyzed by PFK. High ATP and citric acid concentration can inhibit PFK-1 activity, while AMP and fructose-2, 6-diphosphate can activate it. PFK is activated when cells are in the need of energy or synthetic ingredients. HK is inhibited by its product glucose-6-phosphate (G-6-P), which is increased when fructose phosphate kinase is inactive. PK activity is regulated by allosteric regulation. ATP and alanine act as allosteric inhibitors. So when the cells are in a low energy demand and produce much glycogenolytic intermediates, pyruvate kinase activity is high. PK can be phosphorylated to inhibit its activity.

1.1.3 Abnormal Glucose Metabolism and Related Diseases

Blood glucose balance is maintained by several factors, including hormone, enzyme, substrate system, etc. Hyperglycemia means that blood glucose level is more than 130 mg/dL, which is a major symptom of diabetes. Hypoglycemia is characterized by blood glucose level equal to or less than 50 mg/dL and the presence of clinical symptoms, such as confusion and aberrant behavior. Severe hypoglycemia can cause side effects on the brain, even leading to coma and brain death.

Fasting for a long time can cause liver glycogen synthesis and reduce peripheral tissue glucose consumption. The brain uses ketone bodies as a compensation source of glucose to provide energy during a chronic fasting. After long-term fasting and in type 2 diabetes, glucose cannot be fully utilized. This state is called insulin resistance. Insulin resistance can be caused by decreased ability of target tissue to respond to normal (or elevated) circulating concentrations of insulin. If the glycogen synthesis and glycogen degradation accelerate, muscle and adipose tissues glucose utilization rate is reduced, which results in extracellular glucose concentration and in turn leads to hyperglycemia. On the contrary, too much insulin secretion or insulinoma can increase peripheral glucose consumption and lead to hypoglycemia and coma if accompanied by glycogen synthesis inhibition.

Insulin plays an important role in the regulation of glucose balance. Insulin can inhibit glycogen synthesis in the liver and increase glucose uptake by skeletal muscle, cardiac muscle, and adipose tissue; therefore, it can decrease serum glucose level. On the contrary, if the body lacks insulin, catabolism hormone (adrenaline, cortisol, glucagon) will be dominant, which can cause the increased release of liver glucose and decreased glucose uptake by peripheral muscle and adipose tissue. For instance, in type 2 diabetes, disproportionate hepatic glucose output and peripheral glucose consumption can cause hyperglycemia.

Renal glucose metabolism also plays a great role in glucose balance and ranks only second to the liver. Glucose production of the proximal renal tubule and non-insulin-dependent glucose uptake of other nephrons are balanced. Renal generates much more glucose only in the conditions of lactic acidosis and decompensation of liver glucose synthesis.

Under the condition of hyperglycemia, the kidney has a key function of eliminating glucose from the cardiovascular system. Normally most glucose in the renal glomerular filtrate can be reabsorbed into the blood. If glucose concentration exceeds the renal glucose threshold, glycosuria occurs.

1.2 Glucose Metabolism and Tumor

1.2.1 Cancer (Tumor Suppressor) Gene and Glucose Metabolism

Compared to normal cells, malignant cells are associated with a higher glycolysis metabolic rate and higher lactate releasing rate, namely, Warburg effect [6], which was proposed by Otto Warburg in 1956. Many cancers frequently harbor a metabolic characteristic of enhanced glycolysis. It is known that activation of oncogenes and the inactivation of tumor suppressor genes constitute in vivo. These classical oncogenic genes also participate in the regulation of the Warburg effect.

The proto-oncogene *MYC* encodes the Myc transcription factor, which can bind DNA and alter gene expression. Accumulated evidence showed that Myc plays key roles in regulating cancer cell metabolism [7]. The *MYC* proto-oncogene is frequently overexpressed in over half of human cancers [8]. Myc activates many genes involving in cellular processes, including transcription, translation, chromatin modification, and protein degradation. In *Drosophila*, glucose activates insulin signaling, which activates TOR through PI3K/Akt pathway and suppresses FOXO. Myc is a downstream of TOR and FOXO signaling in response to nutrients. When glucose is abundant, Myc protein is rapidly increased by the activated TOR. Conversely, under fasting conditions, Myc expression is directly inhibited by derepressed FOXO [9]. In colon cancer cells, Myc activity was inhibited by FOXO3a which could induce Myc antagonist Mxi1 proteins. Thus, Myc seems to be a well evolutionary conserved nutrient sensor, which is critical in the process of utilizing extracellular nutritional substrates. Many glucose metabolic genes have been documented to be the downstream targets of Myc. Myc enhances genes encoding glucose transporters (GLUT) and hexokinase (HK), resulting in an increase of glucose uptake [10]. The expression of many other glycolytic genes can be activated by Myc. They are phosphoglucose isomerase, phosphofructokinase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, and enolase [10]. Using [U-$^{(13)}$C]-glucose as the tracer, Myc expression increased both glucose consumption and lactate production in a *MYC*-inducible human Burkitt lymphoma model, suggesting Myc overexpression increases overall glycolytic flux [11]. Myc also upregulates lactate dehydrogenase A (LDHA) which generates NAD+, a cofactor required for maintaining the high flux of glycolysis [12]. Moreover, oncogenic transcription factor Myc upregulates transcription of polypyrimidine tract binding protein (PTB) and heterogeneous nuclear ribonucleoproteins (hnRNPA1 and hnRNPA2). PTB, hnRNPA1, and hnRNPA2 promote mutually exclusive alternative splicing of the PKM pre-mRNA, ensuring a high PKM2/PKM1 ratio [13]. There are two distinct isoforms of pyruvate kinase:

PKM2 promotes aerobic glycolysis, whereas PKM1 favors oxidative phosphorylation. Thus Myc ensures high flux of glycolysis by regulating alternative splicing of the rate-limiting enzyme pyruvate kinase. Furthermore, overexpressed Myc, PTB, hnRNPA1, and hnRNPA2 levels are correlated with PKM2 expression level in human gliomas.

Hypoxia happens in a number of physiological and pathophysiological processes, particularly when rapid tissue growth exceeds blood supply. For example, malignant tumor cells reside in a pathophysiological "hypoxic" environment. Hypoxia-inducible factors (HIFs) are the primary transcriptional regulators for cellular metabolic adaptation. Hypoxia-inducible factor-1(HIF1), as a master hypoxic regulator, plays a crucial role in glucose metabolism regulation in cancer cells. HIF-1 is able to bind to the hypoxia response element DNA sequence in mammalian cells cultured under reduced O_2 tension [14]. HIF-1 is essential to activate many glycolytic enzymes under hypoxic conditions. HIF-1 directly activates the gene encoding pyruvate dehydrogenase kinase 1 (PDK1) [15]. PDK1 inactivates the TCA cycle enzyme, pyruvate dehydrogenase (PDH), which catalyzes the conversion from pyruvate to acetyl-CoA. HIF-1 promotes a hypoxia-induced glucose metabolic switch from the mitochondrial TCA cycle to glycolysis to maintain ATP production. HIF-1 upregulates mRNA and protein expression of MCT4 (monocarboxylate transporter), which transports lactate out of cancer cells reducing intracellular lactic acidification [16]. HIF-1 induces the expression of glucose transporter 1, 3 (Glut1, Glut3), LDHA, which are associated with the increased glucose uptake and lactate production in tumors. Sodium-hydrogen exchanger NHE1 and carbonic anhydrase 9 (CA9) are frequently overexpressed in cancer cells, which are able to maintain both an alkaline intracellular pH and an acidic extracellular pH. Expression of the NHE1 and CA9 genes is also induced by HIF1. It has been observed that HIF-1 is accumulated in many cancers, including astrocytomas, superficial urothelial bladder carcinoma, breast cancer, cervical cancer, gastroesophageal adenocarcinomas, non-small cell lung cancer, and malignant melanoma. Furthermore, elevated HIF1 expression is associated with poor prognosis of tumor patients.

The *KRAS* oncogene encodes a 21-kDa small GTPase, which transforms between the inactive guanosine diphosphate-bound state and the active guanosine triphosphate-bound state. Recently, a number of studies have shown that the oncogene *KRAS* plays a prominent role in regulating cancer metabolism by reprogramming multiple metabolic pathways [17]. Oncogenic activation of *KRAS* can influence cellular morphology, survival, and proliferation by activating its downstream pathways, such as the MAPK and PI3K/AKT/mTOR pathways [18, 19]. It has been reported that KRAS signaling takes part in the modulation of aerobic glycolysis in several types of cancer. *KRAS*-driven cancers harbor great possibility to resist to therapeutic intervention. Mutation of KRAS happens in a variety of human tumors, especially frequently in colorectal cancer (CRC), pancreatic ductal cell carcinoma (PDCA), and non-small cell lung cancer (NSCLC). In CRC cell lines, KRAS and BRA*F* mutations increase the GLUT1 expression and glucose uptake. CRC cells with mutated *KRAS* or *BRAF* were able to survive long term in low-glucose culture environments, which suggests that enhanced glucose metabolic alteration induced by KRAS mutation could provide a significant survival advantage for tumor cells [20]. In retrospective studies with primary and metastatic CRCs, ^{18}F-FDG accumulation in CRC tissues of *KRAS*-mutant CRC patients was significantly higher than that of *KRAS* wild-type CRC patients [21]. It is suggested that FDG accumulation may reflect the mutational status of *KRAS* in CRC [22]. *KRAS* mutation occurs in >90% of pancreatic carcinoma cases. *KRAS* mutations play a great role in pancreatic malignant progression from intraepithelial neoplasia to invasive malignant tumor. Studies, using this *KRAS* ^{G12D}-driven PDCA mouse model, have shown that mutated *KRAS* maintains tumor growth by stimulating glucose uptake. Mutated *KRAS* enhances the expression of glucose transporter-1 (GLUT1) and several rate-limiting glycolytic enzymes, including hexokinase (HK2) and lactate dehydrogenase (LDH), which channels glucose intermediates into the non-oxidative pentose phosphate pathway (PPP) and hexosamine biosynthesis pathway (HBP). As a result, *KRAS* mutations promote protein glycosylation through HBP and ribose production through non-oxidative PPP [23]. Conversely, silencing either the HBP gene (*Gfpt1*) or non-oxidative PPP genes (*Rpia* or *Rpe*) results in a suppression of KRAS-dependent tumor growth in vivo, implying a potential therapeutic strategy. Hexokinases catalyze the first committed step of glucose metabolism. HK2 deletion in *KRAS*-driven NSCLC cells reduces glucose-derived ribonucleotide synthesis and inhibits the incorporation of glutamine-derived carbon into TCA cycle intermediates [24].

Tumor-suppressor protein p53 prevents cancer development through various mechanisms, including the induction of apoptosis, cell-cycle arrest, and the maintenance of genome stability. p53 inactivation induces Warburg effect by affecting HIF-1 and a number of glycolytic enzymes' function. In hypoxic conditions, p53 induces the expression of Ras-related associated with diabetes (RRAD), which blocks membrane localization of GLUT1, resulting in an inhibition of glycolysis. p53 decreases the rate of aerobic glycolysis and upregulates GLUT3 through the IKK-NF-kappaB pathway suppression of glycolysis [25]. Inactivation of tumor suppressor p53 activates HK2 to maintain a high glycolytic phenotype [26]. TP53-induced glycolysis and apoptosis regulator (TIGAR) is one of the downstream glycolytic targets of p53. TIGAR can lower fructose-2,6-bisphosphate protein levels in cells, resulting in a downregulation of glycolysis and a decrease in intracellular reactive oxygen species (ROS) levels by regulating PPP pathway [27]. The RING finger protein MDM2 is a transcriptional target of p53. As MDM2 also ubiquitinates the tumor suppressor

p53, p53 may regulate glycolysis by posttranscription via its target *MDM2*. Moreover, MDM2 gene amplification is observed in certain cancers.

1.2.2 Glucose Enzyme and Tumor

Tumor glucose metabolic change is a complex process, and metabolic enzymes are the direct executors following oncogene activation and tumor-suppressor gene inactivation [28]. The first step of glucose metabolism is to transport glucose across the plasma membrane, which is mediated by GLUT proteins. Many studies have reported that GLUT1 is upregulated in cancers, which directly enhances glucose metabolism. PI3K/Akt signaling functions as a master regulator for cancer cells to uptake glucose. PI3K/Akt signaling increases the expression of glucose transporter GLUT1 mRNA and promotes the translocation of GLUT1 protein from the endomembrane to the cell surface [29]. Besides the PI3K/Akt signaling module, other oncogenic stimuli also play a role in regulating the expression and translocation of GLUT1 protein. Oncogenic protein Ras has been reported to upregulate mRNA expression of GLUT1 and increase cellar glucose uptake.

HK, mediating the critical first reaction of glycolysis, catalyzes the phosphorylation of glucose to glucose-6-phosphate (G6P). G-6P may enter into either the glycolic pathway or the pentose phosphate pathway for glycogen synthesis in tumor cells. There are four mammalian isoforms of HK, designated as HK-1 to HK-4. HK-2 is predominantly expressed in cancer cells. Clinical studies have showed that the expression of hexokinase-2 protein is upregulated in a number of cancers, including breast, lung, and liver cancers. Studies have revealed that deletion of HK2 inhibits the tumor progression by shutting down glucose flux at the earliest step in glucose metabolism. HK2 interacts with voltage-dependent anion channel (VDAC), which is located in the outer membrane of mitochondria. This interaction promotes the production of glycolytic fuel through ATP generated from mitochondria. Combined expression of a plasma membrane glucose transporter GLUT1 and hexokinase (HK) provides a survival advantage for cells cultured in a growth factor-deprived medium.

It also has been well established that PFK, as the second rate-limiting enzyme, controls the glycolytic pathway by its allosteric regulation. The activity of PFKFB3 can be regulated by the oncogenic Ras signaling pathway. In breast cancer cells, PFK expression is increased by constitutive HER2 expression.

Downregulation of PFK expression leads to a reduction in glycolytic flux and suppresses tumor proliferation.

PK is another glycolytic enzyme, which converts PEP into pyruvate in the final step of glycolysis. PK has two isoforms, PKM1 and PKM2. PKM2 is detected in embryonic tissues and many tumors, while PKM1 is expressed in normal adult tissues. Studies have found that mice injected with the PKM2-expressing cells showed a faster tumor growth rate and a larger tumor size than those injected with PKM1-expressing cells, which suggesting that PKM2 provides a growth advantage for tumor cells in vivo. Proteomic studies have showed that PKM2 constitutively shifted its active tetrameric structure by multiple additional posttranslational regulations, which leads to the regulation of pyruvate kinase activity. PKM2 activity is inhibited by the displacement of the activating cofactor fructose-1,6-bisphosphate followed by PKM2 directly binding to phosphotyrosine peptides. Phosphoproteomic study of PKM2 showed that phosphorylation disrupts its interaction with fructose-1,6-bisphosphate and inhibits the formation of the active tetrameric form. Glucose stimulated K305 acetylation of PKM2, and PKM2 activity is inhibited by subsequent autophagic degradation, which results in enhanced tumorigenicity. Immunohistological staining using anti-PKM2 antibodies revealed a strong staining of PKM2 in almost all kinds of solid tumor tissues [30]. Collectively, a variety of studies have shown that PKM2 promotes glucose metabolic flux and contributes to cancer cell proliferation.

Lactate dehydrogenase A (LDHA) plays a critical role in tumor development. It catalyzes the final step of the glycolytic pathway, to produce lactate and NADP from pyruvate and NADPH. LDHA, as the direct target gene of *MYC* and *HIF1*, promotes glucose uptake and lactate generation of cancer cells. A large number of studies have reported that silencing LDHA protein in tumor cells increases mitochondrial respiration, decreases the proliferation, and leads to cell death in both normal and hypoxic environments. It has been well known that many human tumors have higher LDHA levels than surrounding normal tissues. Because abolishing LDHA has no significant effect on normal tissue, it suggests that LDHA may be a promising therapeutic target in cancer.

G6PD catalyzes the formation of glucono-D-lactone-6-phosphate and NADPH via the oxidation of glucose-6-phosphate with NADP. Glucose-6-phosphate dehydrogenase (G6PD) is the rate-limiting enzyme in the pentose phosphate pathway. It has been reported that G6PD overexpression promoted fibrosarcoma growth in nude mice. Knockdown G6PD in melanoma cells decreases proliferation and promotes apoptosis. Clinically, G6PD is overexpressed in many human cancers, including breast, bladder, cervical, ovarian, and prostate cancer. Moreover, G6PD is an independent prognosis predictor in breast and gastric cancer.

Isocitrate dehydrogenase 1 (IDH1) and isocitrate dehydrogenase 2 (IDH2) mutations have been identified in several human cancers, such as low-grade glioma, cholangiocarcinoma, chondrosarcoma, and acute myeloid leukemia (AML). IDH1 or IDH2 with mutant alleles harbors an unusual neomorphic enzymatic function. Mutant IDH prefers to catalyze a-ketoglutarate to D-enantiomer of 2-hydroxyglutarate (2-HG), whereas the wild-type IDH catalyzes the converse reaction

from TCA cycle metabolite isocitrate to a-ketoglutarate. A prominent CpG island hypermethylation was observed in IDH-driven glioma, leukemia, and chondrosarcoma. Notably, normal hematopoietic cells or chondrocytes with a mutant IDH1 allele knock-in can show an aberrant expansion.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) catalyzes the reversible conversion of glyceraldehydes-3-phosphate (G-3-P) to 1,3-diphosphoglycerate in glycolytic metabolism. Recent studies show GAPDH is also a pro-apoptotic agent. Remarkably increased GAPDH in many human cancer types are often correlated with reduced survival. Several cancerous factors, such as HIF-1, p53, insulin, nitric oxide (NO), and acetylated histone, not only regulate GAPDH gene expression but also modulate its protein functions [31].

Phosphoglycerate mutase 1 (PGAM1) is a vital enzyme in the glycolytic pathway catalyzing the conversion of 3-phosphoglycerate (3-PG) to 2-phosphoglycerate (2-PG) [32]. It has been reported that PGAM1 is upregulated in diverse cancers, including lung squamous carcinoma, hepatoma, and cholangiocarcinoma. Ablation of PGAM1 results in blocking aerobic glycolysis and oxidative pentose phosphate pathway (PPP), which consequently inhibits cancer cell proliferation.

1.2.3 Glucose Metabolism and Targeted Therapy

Enhanced glycolysis is a metabolic characteristic of many cancers. It is confirmed that dysregulation of oncogenes and glycolytic enzymes is directly linked to the aerobic glycolysis and oxidative pentose phosphate pathway, which are essential to cancer cell proliferation. Thus, it is believed that the inhibition glycolysis pathway may be a promising therapeutic approach.

The transcription factors HIF-1 orchestrate multiple metabolic-related enzymes and promote tumor growth via enhancing glucose uptake and utilization in tumor cells. As a consequence, inhibition of HIF-1 is an attractive anticancer strategy. Many anticancer drugs being tested in clinical trials of cancer patients are now recognized as HIF-1 inhibitors. The expression of HIF-1α mRNA can be induced by topoisomerase 1 (TOP1), so that TOP1 inhibitors, for example, topotecan and irinotecan, downregulate HIF-1α expression. TOP1 inhibitors are being studied in cancer clinical trials to assess its anticancer effects. There are more HIF-1 inhibitors being tested in oncology clinical trials. HSP90 inhibitor ganetespib impairs HIF-1α stability. Digoxin, an antiarrhythmic drug, inhibits HIF-1α translation. Proteasome inhibitor bortezomib inhibits HIF-1α transactivation. Although many agents can inhibit cancer growth in a HIF-1-dependent way, preclinical and clinical studies about these agents have been stopped for toxicity or safety problems.

Some of the drugs that target glycolytic related oncogenes, enzymes, and transporters of glycolytic products are under clinical investigation. For example, silibinin (also known as silybin), one of the GLUT1 inhibitors, is being tested clinically. A novel representative inhibitor of GLUT1, WZB117, is a small-molecule inhibitor of *SLC2A1*. WZB117 decreases GLUT1 protein expression levels and represses activity of GLUT1. Its effect has been extensively tested in vitro and in vivo. WZB117 inhibits glucose uptake in cancer cells in a dose-dependent manner [33]. In vitro studies, WZB117 inhibits glucose transport of lung cancer A549 cell lines rapidly, starting 1 min after treatment. Furthermore, WZB117 significantly inhibited the proliferation of lung cancer cells by 50%, 48 h after treatment. However, WZB117 showed no such obvious effects in the NL20 noncancerous lung cell line. The levels of cyclins and phosphorylated retinoblastoma protein (RB1) of A549 cells were also blocked 6 h after WZB117 treatment, as well as prominent cell-cycle arrest and senescence within 24 h. Finally WZB117 treatment induced a necrosis within 48 h. WZB117 not only inhibited cancer cell growth in vitro study but also inhibited subcutaneously implanted tumor growth in a nude mouse mode. In in vivo studies, injection of WZB117 into nude mice for 10 weeks reduced the tumor growth of A549 cell xenografts by 70% when compared with grafts from mice mock treated [34]. In addition to testing GLUT1 inhibitor alone as an anticancer agent, researches are also performed to assess the effectivity of combining use of GLUT1 inhibition and other cancer therapeutics. WZB117 showed a stronger synergistic antitumor effect when administered in vitro along with the anticancer drug cisplatin or paclitaxel in lung cancer cell lines A549 and H1229 and breast cancer MCF7 cells than when they were tested alone. Another natural inhibitor of a GLUT1, phloretin, presented a better antitumor effect if administered in combination with daunorubicin. In in vitro study, combining use of inhibiting cisplatin with shRNA-GLUT1, which stably knock down GLUT1 protein expression, has a stronger antitumor effect in head and neck carcinoma cells under both normoxic and hypoxic conditions. Although these studies show promising effects of GLUT1 inhibitor in combination with other anticancer treatment, more researches are needed to explore the underlying mechanism for the synergistic antitumor activity in the combined therapy.

The important role of HK2 in glycolysis makes it an important therapeutic target for designing agents against cancer. 2-Deoxyglucose (2-DG) and 3-bromopyruvate (3-BrPA) are two well-known inhibitors of HK2. 2-Deoxidation-D-glucose (2-DG) is a kind of glucose analog and functions as a competitive inhibitor of HK2. 2-DG is phosphorylated by hexokinase (HK) to generate 2-DG-6-phosphate. The latter is not metabolized further, which inhibits phosphohexoisomerase and glucose-6-phosphate

dehydrogenase and finally reduces the output from glycolysis (ATP) and the pentose phosphate pathway. Systemic deletion of HK2 in breast and NSCLC cancer mouse models inhibited tumor initiation, implying HK2 as an attractive target for these tumors. Clinical trial has been initiated to evaluate the therapeutic effectiveness of using 2-DG in combination with other chemotherapeutics [35]. 3-Bromine pyruvate (3-BrPA) is a lactose analog. It directly inhibits the activity of HK2 in a particular way different from 2-DG by alkylation thiol in HK2. 3-Bromopyruvate (3-BrPA) was highly effective on the xenografts derived from CRC cells with mutated *KRAS* or *BRAF* [21]. 3-BrPA has been proposed as an anticancer agent as well as a chemosensitizer for use in combination with anticancer drugs [36]. Lonidamine, a small-molecule inhibitor, has been reported to inhibit HK activity. The interaction of HK and VDAC can be interrupted by various azoles and their derivatives, such as clotrimazole and bifonazole. Methyl jasmonate, a plant lipid derivative, is reported to impair the interaction of HK2 and VDAC. Therefore, HK2 seems to be a potential target for the therapeutic agent designing.

6-Phosphofructo-2-kinase (PFKFB3) is also a key regulator of glycolysis. PFKFB3 catalyzes the synthesis of fructose-2,6-bisphosphate (F26BP), which is an activator of 6-phosphofructo-1-kinase, a key step of glycolysis. A small-molecule inhibitor of PFKFB3, 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3PO), suppresses PFKFB3 activity, reduces glucose uptake, and decreases the intracellular concentration of Fru-2,6-BP, lactate, and ATP. Then, one small molecule, 1-(4-pyridinyl)-3-(2-quinolinyl)-2-propen-1-one (PFK15), which is selected from synthesized 73 derivatives of 3PO, exhibits a strong activity against recombinant PFKFB3 [37]. PFK15 was further used in preclinical trials to evaluate its antimetabolic, antineoplastic, and pharmacokinetic properties in vitro and in vivo. PFK15 induces apoptosis, suppresses the glucose uptake and growth of Lewis lung carcinomas, and has adequate pharmacokinetic properties in syngeneic mice. PFK15 inhibits the growth of human cancers in xenograft model mice.

Pyruvate is an attractive target for anticancer therapy because of its dual functions in both ATP generation and biosynthetic reactions. TT-232, a structural somatostatin analog, has been studied in clinical trials. TT-232 inhibits PKM2 dimerization, which leads to its activity decrease. In addition, TT-232 also promotes nuclear translocation of PKM2 resulting in a caspase-independent cell death [38].

LDHA inhibition for anticancer treatment is a safe therapeutic target because a complete abolishment of LDHA protein in humans by hereditarily deleting *LDHA* gene only induces non-life-threatening and a few cases of exertional myoglobinuria. 3-Dihydroxy-6-methyl-7-(phenylmethyl)-4-propylnaphthalene-1-carboxylic acid (FX11) is a small and specific inhibitor of LDHA. FX11 blocks cellular energy metabolism via inhibition of glycolysis; thereby targeted tumor types are those with glycolysis metabolic phenotype. In addition to inhibition of glycolysis, FX11 also elevates oxygen intake and ROS generation and induces apoptosis and cell death in P493 human lymphoma B cells in vitro. The xenograft growth rate of P493 lymphoma B cells and P198 human pancreatic cancer cells in mice is significantly reduced by FX11 treatment. Besides FX11, other molecules agents, such as galloflavin and *N*-hydroxyindole, have been identified to inhibit LDHA. Although initial efficiency of these novel antitumor agents has been revealed, more studies are needed to investigate their underlying mechanisms for cancer therapy.

The Warburg effect also generates lactate followed by preferential use of the glycolytic pathway [12]. Overexpressed MCT1 in cancer and disrupting lactate transport indicate a promising approach for therapeutic targeting. AZD3965 is a selective MCT1 inhibitor that exhibits growth-inhibitory activity in small cell lung cancer tumor xenografts. Phase I and progressing to phase II clinical trials for AZD3965 have been initiated in the UK.

Dichloroacetate (DCA), a pyruvate analog, can stimulate pyruvate dehydrogenase which promotes the conversion of pyruvate into acetyl-CoA, significantly shifting the metabolic flux in glycolytic cancer cells with impaired mitochondrial activity and increasing mitochondrial apoptosis. Clinical studies have been performed in patients with glioma to verify the efficacy of DCA and its adverse effects.

Although almost all cancer cells prefer glycolysis pathway, mitochondrial metabolism is also essential to some types of tumors. Therefore, mitochondrial metabolic products or enzymes offer a variety of attractive targets for anticancer therapy. To date, a number of drugs that interfere with oxidative phosphorylation (OXPHOS) are being investigated as anticancer drugs. A key example is metformin, which interferes with OXPHOS by inhibiting NADH-coenzyme Q oxidoreductase (complex I). Metformin can reduce gluconeogenesis in the liver and is recognized as an important drug for the type 2 diabetes mellitus treatment. Some epidemiological studies have found metformin treatment reduced risk of cancer in patients of diabetes, which remains controversial. As thus, the use of metformin as an anticancer agent has been deeply studied, and metformin has showed anticancer effect activity in clinical trials. Metformin treatment at physiological doses induced apoptosis prominently in breast cancer cells in vitro. This effect is more significant under low-glucose concentration conditions. Clinical trials have supported the anticancer effects of metformin in patients with breast, prostate, and endometrial cancer. Metformin is widely assessed in many clinical trials of non-diabetic cancer patients.

The following table contains the inhibitors that target glucose metabolism (Table 1.1).

Table 1.1 Glucose metabolic inhibitors

Target protein	Agent	Mechanism	Development stage	Observations
Transcription factors				
HIF-1	Irinotecan, topotecan	• TOP1 inhibitor • Downregulates HIF-1α mRNA expression	Clinical studies	Anticancer effect but with toxicity or safety problems
	Ganetespib	• HSP90 inhibitor • Impairs HIF-1α stability	Preclinical, clinical studies	
	Digoxin	Inhibits HIF-1α translation	Preclinical, clinical studies	
	Bortezomib	• Proteasome inhibitor • Inhibits HIF-1α transactivation	Preclinical, clinical studies	
Glycolysis				
GLUT1	Silibinin	GLUT1 inhibitor	Clinical studies	
	WZB117	• SLC2A1 inhibitor • Decreases GLUT1 protein expression levels • Inhibits the proliferation of lung cancer cells • Induces tumor cells necrosis	Preclinical, clinical studies	In vitro, presented a better effect if administered in combination with cisplatin or paclitaxel in lung cancer
	Phloretin	GLUT1 inhibitor	Preclinical, clinical studies	Better antitumor effect with daunorubicin
HK2	2-DG	• Glucose analog • HK2 competitive inhibitor • Inhibits tumor initiation	Preclinical, clinical studies	
	3-BrPA	• Lactose analog • Inhibits the activity of HK2	Preclinical, clinical studies	• Anticancer agent • Chemosensitizer
	Lonidamine	Inhibits HK activity	Preclinical, clinical studies	
	Clotrimazole, bifonazole	Interrupts the interaction of HK and VDAC	Preclinical, clinical studies	
	Methyl jasmonate	Impairs the interaction of HK2 and VDAC	Preclinical, clinical studies	
PFKFB3	3PO,PFK15	• PFKFB3 inhibitor • Reduces glucose uptake • Induces apoptosis	Preclinical, clinical study	• Antimetabolic • Antineoplastic • Pharmacokinetic
LDHA	FX11, galloflavin, *N*-hydroxyindole	• LDHA inhibitor • Inhibits glycolysis, elevates oxygen intake and ROS generation • Induces apoptosis	Preclinical study	
TCA cycle				
Pyruvate	TT-232	• Structural somatostatin analog • Inhibits PKM2 dimerization • Promotes nuclear translocation of PKM2	Clinical study	Leads caspase-independent cell death
PDK1	DCA	• Pyruvate analog • Stimulates pyruvate dehydrogenase • Promotes mitochondrial apoptosis	Clinical study	
Lactate				
MCT1	AZD3965	• Selective MCT1 inhibitor • Inhibits growth activity	Phase I, phase II clinical study	
Mitochondrial metabolism				
Mitochondrial complex I	Metformin	• Mitochondrial complex I inhibitor • Interferes with OXPHOS • Reduces gluconeogenesis in the liver	Preclinical, clinical study	• An important drug for the type 2 diabetes mellitus • Clinical trials have supported the anticancer effects in patients with breast, prostate, and endometrial cancer

1.3 ^{18}F-FDG PET/CT Scanning

1.3.1 Glucose and ^{18}F-Fluorodeoxyglucose (^{18}F-FDG)

^{18}F-FDG is a small-molecule probe with a structural similarity to glucose. ^{18}F, a kind of positron emission radioisotope, can substitute the second position hydroxyl group to make ^{18}F-FDG (Fig. 1.1). ^{18}F-FDG enters the tumor cells by using the same facilitated transport mechanism as glucose via cell surface glucose transporter proteins, such as GLUT1 and GLUT3. Once inside the cell, ^{18}F-FDG is phosphorylated by hexokinase into ^{18}F-FDG-6-phosphate (^{18}F-FDG-6-PO_4). Phosphofructokinase and other enzymes, which further metabolize glucose-6-phosphate, cannot use ^{18}F-FDG-6-PO_4 as a substrate; and phosphorylation increases the molecular polarity, so ^{18}F-FDG-6-PO_4 could not diffuse back out of the cell. As thus, ^{18}F-FDG can accumulate in the cell.

1.3.2 ^{18}F-FDG PET/CT Scanning

^{18}F-FDG PET/CT scanning is a molecular imaging technique that uses a radioactively labeled glucose (^{18}F-FDG) with PET/CT to visualize fundamental molecular and biochemical processes of glucose in the body. Preference to glycolysis pathway is the most common phenotype of malignant tumor energy metabolism disorder. ^{18}F-FDG PET/CT imaging can show the malignant tumors with high level of glycolysis. ^{18}F-FDG PET/CT imaging analysis is normally performed by two methods: visually qualitative interpretation and/or using quantitative parameters. Standardized uptake value (SUV) is the most commonly used quantitative analytical parameter in clinical practice. The SUV allows comparisons of ^{18}F-FDG uptake to be made between the target tissues and normal tissues. PET/CT imaging shows the glucose uptake and distribution in body organs, tissues, and cells. Cortical gray matter, nuclei, and thalamus in the basal ganglia and the cerebellar gray matter have more intense uptake of FDG; white matter and ventricular system are associated with less intense or absent FDG uptake. Uptake in the palatal tonsils, adenoids, and brown fat can be shown. Mild-to-moderate uptake within parotid gland, submandibular gland, and thyroid gland is also a physiological finding. There would be an intense uptake in the eye muscles, facial muscles (vocal cords, masseter, lingualis), and neck muscles (sternocleidomastoid and prevertebral muscles) due to exercise or stress. The uptake of FDG within the heart is extremely variable from low to high glucose uptake level under different physiological conditions; there is a mild uptake in mediastinum due to a large amount of blood in great vessels. Normal lung tissue uptake is low due to being filled with a large amount of gas; hilar lymph node uptake is a common finding especially in the old; and mild physiologic uptake is often seen in the thymus which is not completely degraded, secretory mammary gland and normal esophagus. The uptake within the stomach and intestines can be variable, and the distribution of the imaging agent is consecutive and consistent with the outline of digestive tract. The liver usually shows diffused uptake from mild to moderate levels, with a clear boundary; the spleen also has a diffused uptake of ^{18}F-FDG but a little lower uptake level than that of the liver. ^{18}F-FDG is filtered through the kidney and could not be reabsorbed by the renal tubule. Therefore, the kidneys, ureters, and bladder have intense uptake of ^{18}F-FDG (urinary retention). The prostate normally shows little uptake. There is a variable uptake in the uterus and ovaries due to the women menstrual cycle.

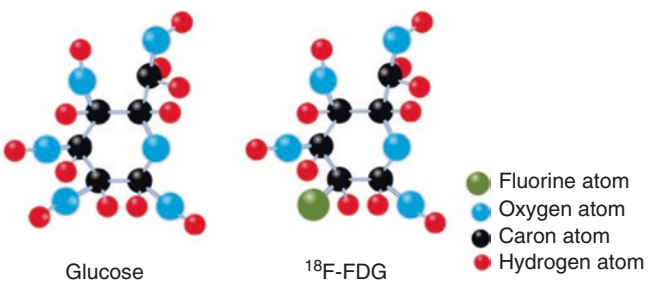

Fig. 1.1 Chemical structure of glucose (left) and 18F-FDG (right)

1.3.3 Clinical Utility of ^{18}F-FDG PET/CT Scanning

Nowadays, ^{18}F-FDG PET/CT has been widely used to detect malignant tumors; to make differential diagnosis, clinical stage, and prognosis; and to monitor therapeutic response. The uptake of imaging agent ^{18}F-FDG into the malignant tissue is related to the tumor histologic type and differentiation grade, etc. Most of the common tumors, such as squamous cell carcinomas, colorectal cancer, and malignant lymphoma, avidly uptake ^{18}F-FDG. Some benign tumors such as thyroid papillary adenoma, parotid gland tumor (Warthin's tumor, polycrystal adenomas), adenomatous polyposis coli, pastel adenoma, and leiomyoma may also demonstrate high ^{18}F-FDG uptake in PET/CT imaging. Increased ^{18}F-FDG uptake also occurs in acute inflammation caused by various causes, such as surgery, radiotherapy, or infection; granulomatous inflammation, such as sarcoidosis, fungal disease, or tuberculous disease; and chronic inflammatory diseases such as ulcerative colitis and systemic lymphadenopathy. These nonmalignant tumor diseases can cause potential confusion in clinical application, and other imaging information or pathological examinations are required to make a differential diagnosis. In clinical practice, ^{18}F-FDG PET/CT scanning also can be used in neurology, cardiology, neuropsychology, and psychiatry.

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Clinical Utility of PET/CT in Breast Cancer Management and Targeted Therapy

2

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2.1 Introduction

Breast cancer is currently the most prevalent malignant disease affecting women's health. Breast cancer is a highly heterogeneous tumor, comprising multiple entities associated with distinctive histological and biological features, clinical presentations, and responses to therapy. In addition to traditional treatment approaches, such as surgery, radiotherapy, endocrine therapy, and chemotherapy, targeted therapy is another emerging approach for breast cancer treatment. With the breakthrough in molecular biology and pharmacology research, new targeted drugs have been continuously applied in clinic and have achieved good clinical results.

2.1.1 Epidemiology

Breast cancer is the leading cause of cancer-related death for women in both developed and developing countries [1]. In 2012, 1.7 million women were diagnosed with breast cancer, and 522,000 died from it at the same year [1]. From 1989 to 2012, breast cancer death rates decreased by 36% in the United States [2]. But in China, the incidence of breast cancer is still slowly rising [3].

According to the study by Lei Fan [3] from China, breast cancer is now still the most common cancer in Chinese women. With more than 1.6 million people are diagnosed with breast cancer and 1.2 million people die every year in China, accounts for 12.2% of all newly diagnosed cancers and 9.6% of all cancer deaths worldwide [3]. There is still a long way for China in the prevention and treatment of breast cancer.

There are regional differences in the incidence of breast cancer, and urban areas are higher than rural areas. The average age at diagnosis of breast cancer in China is 45–55 years old, younger than Western women.

Some risk factors are thought to contribute to the development of breast cancer. Currently, recognized risk factors include early age at first menstruation, late childbearing or not at all, older age, prior history of breast cancer, family history, obesity, lack of physical exercise, drinking alcohol, smoking tobacco, hormone replacement therapy during menopause, and ionizing radiation. Genes are also thought to be a major factor in 5–10% of cases [4], including BRCA1 and BRCA2, among others.

2.1.2 Molecular Subtypes and Gene Expression Tests in Breast Cancer

Most of the malignant breast tumors are adenocarcinomas, usually called as breast cancer. Breast cancer is a highly heterogeneous tumor, comprising distinctive entities associated with its own clinical, histological, molecular, and genic characteristics.

At the gross histopathology level, breast cancer can be divided into carcinoma in situ, and invasive carcinoma depended on whether tumor breaks through the basement membrane or not. According to the growth location of tumors in the breast, it can be divided into ductal carcinoma and lobular carcinoma (such as intralobular carcinoma in situ, ductal carcinoma in situ).

In clinic, infiltrating ductal carcinomas are most common, accounting for 70–80% of invasive breasts, followed by infiltrating lobular carcinoma accounting for 5–10%. In addition, there are some rare types of invasive breast cancer histology, such as tubular, mucinous, and medullary carcinoma. Compared with infiltrating ductal carcinomas, infiltrating lobular carcinomas tend to be multicentric and/or bilateral. Inflammatory breast cancer and Paget's disease are two specific types of breast cancer, with particularly pathological and clinical features.

Over the years, new molecular diagnostic technology have been studied, which aims to find new biomarkers to

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better describe and distinguish entities. The development of new biomarkers provides clinicians with a reference for diagnosis of breast cancer, risk stratification, staging, treatment options, and finally helps to achieve precise and individualized treatment for patients.

According to the characteristics of immunohistochemistry (IHC), breast cancer can be divided into three major subtypes: tumors expressing the estrogen receptors (ERs), progesterone receptors (PRs), and human epidermal growth factor receptor 2 (HER2) breast cancer. The remaining group is commonly referred to as triple-negative breast cancer (TNBC) due to lack of expression of ERs, PRs, and HER2. TNBC itself contains many different entities that have been identified through gene expression tests.

The seminal discovery of breast cancer research over the last two decades was the description of the intrinsic breast cancer subtypes. Perou et al. [5] analyzed gene expression patterns of breast cancer employing microarray-based gene expression profiling, identifying four major intrinsic gene signatures: luminal, triple-negative/basal-like, HER2-positive-enriched, and normal-like. Subsequent studies led to subdivision of luminal tumors into luminal A and luminal B subgroups. Table 2.1 demonstrates the classification of these subtypes and the specific immunohistochemical expression patterns [6].

Of mammary carcinogenesis, perhaps the most extensively studied are BRCA1, BRCA2, and TP53 genes. These are associated with a high risk of developing breast cancer in carriers, and hence they are referred to as high penetrance genes. However, it should be noted that among breast cancer patients with a strong family history, only 40% are thought to be caused by the above three genes [7]. This suggests that in the remaining 60% of cases, apart from sporadic breast cancers, other genetic pathways are likely involved.

In recent years, five novel gene expression prognostic tests [8] for breast cancer have been developed: MammaPrint, MapQuant Dx, Oncotype DX, PAM50, and Theros Breast Cancer Index. The development of multigene-based prognostic tests is not only to add prognostic and predictive information to conventional biomarkers but to provide more reliable and reproducible techniques than the IHC-based assays, which in turn reduces the technical errors and subjective interpretation [9].

Table 2.1 Breast cancer intrinsic subtypes with prevalent immunohistochemical profiles [6]

Intrinsic subtype	Immunohistochemistry
Luminal A	ER- and/or PR-positive, HER2-negative with Ki-67 < 14%
Luminal B	ER- and/or PR-positive, HER2-negative with Ki-67 ≥ 14%
	ER- and/or PR-positive, HER2-positive with any Ki-67
HER2-enriched	ER- and PR-negative, HER2-positive
Basal-like	ER- and PR-negative, HER2-negative

In spite of their demonstrated efficacy, there are still large regional differences in the application of these tests, probably reflecting variations in economies, health systems, and physician training. Therefore, in many hospitals, immunohistochemical evaluation remains the primary method for the classification of breast cancer.

2.1.3 Targeted Therapy for Breast Cancer

Targeted therapy is a new treatment method in addition to the four traditional treatments of surgery, radiotherapy, endocrine therapy, and chemotherapy. With the deepening of pharmacology and molecular biology research, many molecular targets have been identified, and breakthroughs have also been made in the research and application of new targeted drugs.

Generally, according to the mechanism of action, targeted drugs can be classified into two categories. One of the categories acts on tumor cells, such as antihuman epidermal growth factor receptor 2 (HER2), PI3K/AKT/mTOR inhibitor, CDK4/6 inhibitor, and Poly (ADP-ribose) polymerase (PARP) inhibitor. Another category acts on the microenvironment, such as angiogenesis inhibitors.

2.1.3.1 Anti-HER2 Targeted Therapy

Human epidermal growth factor receptor 2 (HER-2) is not expressed in normal tissues but is overexpressed in tumor tissues. Twenty to thirty percent of breast cancer patients found HER-2 gene overexpression, and the high expression of HER-2 is closely related to the occurrence, development, prognosis, and metastasis of breast tumors. This type of breast cancer is usually highly invasive and has a poor prognosis.

According to the molecular mechanism, drugs targeting HER2 are mainly divided into three categories: Category 1 is a monoclonal antibody, including trastuzumab and pertuzumab, which specifically binds to the extracellular region IV or II of the HER2 receptor and inhibits HER2 receptor activation; Category 2 is a small molecule tyrosine-kinase inhibitor, representing the drug lapatinib, which reversibly interacts with the epidermal growth factor receptor (EGFR) and adenosine triphosphate (ATP) site of HER2 receptor tyrosine kinase regions and inhibit its tyrosine kinase activity; Category 3 is a monoclonal antibody. The representative drug is an antibody-conjugated drug, such as trastuzumab emtansine (TDM1), which is a combination of trastuzumab and anti-tubulin chemotherapeutic drugs through a disulfide bond. It can selectively bring chemotherapeutic drugs into cancer cells and enhance the induction of cancer cell apoptosis.

Numerous studies have shown that trastuzumab is the basic treatment for HER2-positive breast cancer, whether using trastuzumab alone or in combination with chemotherapy drugs, it can bring survival benefits to patients. However, in clinical practice, 70% HER2-positive breast cancer is resistant to trastuzumab, and almost all patients are relayed for resistance during treatment

[10]. How to overcome trastuzumab resistance has become a key issue to be addressed in anti-HER2 targeted therapy.

Several large clinical studies have demonstrated that trastuzumab combined with chemotherapy can reduce the risk of disease recurrence and death in patients with HER2-positive early breast cancer, significantly improving patient outcomes.

2.1.3.2 Anti-angiogenic Targeted Therapy

Angiogenesis is the main cause of tumor growth and metastasis. Therefore, the treatment of targeted angiogenesis is also one of the important strategies. Currently, anti-angiogenic drugs for breast cancer include bevacizumab (targeting vascular endothelial growth factor, VEGF), ramucirumab (targeting vascular endothelial growth factor receptor 2, VEGFR 2), and multiple target of sorafenib and sunitinib. The application of anti-angiogenic targeted drugs in the treatment of advanced breast cancer is controversial. The treatment of anti-angiogenic targeted drugs in breast cancer needs to be further explored. In the future, it is necessary to find a therapeutic target and optimize the benefit population.

2.1.3.3 PI3K/AKT/mTOR Pathway Inhibitor

The PI3K/AKT/mTOR pathway plays an important role in the development of breast cancer. On the one hand, it is downstream of the HER2 pathway, and the activation of the PI3K/AKT/mTOR pathway is involved in the resistance mechanism of trastuzumab; on the other hand, it also interacts with the estrogen receptor (ER) signaling pathway and is involved in the pathogenesis of endocrine therapy resistance.

Everolimus is an inhibitor of mTOR target protein, and a large number of studies have demonstrated that everolimus reverses the activity of the aromatase inhibitor by inhibiting the activity of the PI3K/AKT/mTOR pathway. For postmenopausal women with advanced breast cancer, patients with aromatase inhibitor treatment failure, use of other endocrine drugs in combination with everolimus will become a new strategy to reverse endocrine therapy resistance.

2.1.3.4 Other Targeted Therapy

There are still some other targeted drugs which had been developed, such as cell cycle inhibitors CDK4/6 and PARP inhibitors. CDK4/6 is a representative cell cycle blocker. Phase II study showed that the CDK4/6 inhibitor palbociclib combined with letrozole versus single-agent letrozole for the treatment of postmenopausal ER-positive, HER2-negative advanced breast cancer patients, PFS significantly benefited (20.2 month vs 10.2 months, $P < 0.001$) [11]. In 2015, the FDA approved palbociclib combined with letrozole as an initial regimen for the treatment of postmenopausal ER-positive, HER2-negative advanced breast cancer. For postmenopausal women with advanced breast cancer, patients who failed aromatase inhibitor therapy, use of other endocrine drugs in combination with everolimus will become a new strategy to reverse endocrine therapy resistance.

PARP mainly affects the repair of damaged DNA, resulting in the accumulation of damaged DNA, and ultimately induces tumor cell apoptosis. Currently, iniparib, olaparib, and veliparib are representatives of PARP inhibitors and are undergoing relevant clinical trials.

With the development of molecular biology, breast cancer has entered the era of targeted therapy. As new targeted drugs continue to be developed, prospective studies are needed to determine predictive outcomes, further optimizing the benefit population and maximizing the efficacy of targeted therapy.

2.2 Clinical Utility of ^{18}F-FDG PET/CT in Breast Cancer

The application of PET/CT has been extensively studied in the management of patients with breast cancer, but not applied as a clinical routine in the diagnosis of primary breast cancer. PET/CT cannot replace the sentinel node biopsy in the diagnosis of breast cancer clinically. However, for the detection of supraclavicular, mediastinal, and internal mammary metastatic lymph nodes, PET/CT performs better than other imaging methods. Lymph nodes in these areas may be easily missed in routine CT and MRI study. In the detection of distant metastases, PET/CT has a better accuracy in detecting lytic bone metastases compared to bone scintigraphy. PET/CT is recommended in clinic when advanced-stage disease is suspected and conventional modalities are inconclusive. For the monitoring of locoregional recurrence, PET/CT has a high sensitivity and specificity. Numerous studies support the role of PET/CT in prediction of response to neoadjuvant radiation or chemotherapy. With further research on the treatment planning and evaluation of patients with breast cancer, the role of PET/CT can be further extended.

2.2.1 The Heterogeneity and ^{18}F-FDG Uptake in Breast Cancer

Breast cancer is a highly heterogeneous tumor disease. It is well-known that the glycolysis activity of tumor cells affects ^{18}F-FDG uptake. In general, glycolytic rates of cancer cells are correlated with HIF-1a and c-Myc expression resulting in considerable variability in glycolytic activity. Researches have already shown that the extent of ^{18}F-FDG uptake in breast cancer is affected by a variety of factors.

A larger primary tumor, a positive axillary lymph node status, and higher TNM stage were all significantly associated with a higher SUVmax [12]. The uptake of ^{18}F-FDG is also correlative with the pathological types and cell phenotype of breast cancer. Infiltrating ductal carcinoma has higher ^{18}F-FDG uptake than infiltrating lobular carcinoma even for the same size tumors. The higher ^{18}F-FDG accumulation also correlates with the higher histological grade and the higher expression of

the proliferation marker Ki-67 [13, 14]. ER negativity, PR negativity, HER2 positivity, and high Ki-67 expression were also significantly correlated with a higher SUVmax. Basu et al. [15] and Kitajima et al. [12] found that tumors with a triple-negative phenotype had a higher FDG uptake. Breast cancers with a p53 mutation were repeatedly shown to be associated with poorer prognosis. Several studies [14, 16, 17] demonstrated the positive correlation between FDG uptake and p53 status, but another study by Buck A [18] showed that there was no correlation between these two indexes.

2.2.2 Detection and Differentiation of Primary Breast Cancer

Both mammography and ultrasound are most commonly used imaging methods in detection, differential diagnosis, the measurement of tumor size, and extent of breast cancer. MR has a high soft tissue resolution and has shown high sensitivity and specialty in the several aspects mentioned above. In addition, MR may find some additional breast cancer lesions, which may do not be displayed on other conventional imaging. Due to the many advantages of MR, it is increasingly being used in clinical practice.

^{18}F-FDG PET/CT can be used for detection and visualization of the primary tumor. However, due to the limited resolution of PET scanners and the influence of some breast cancer pathological characteristics (e.g., low ^{18}F-FDG uptake in high-grade cancer and/or in lobular cancer), PET has poor sensitivity for detection of small lesions. In a study by Avril et al. [19], while PET imaging detected 92% of pT2 lesions (>20 mm, but <50 mm) (see Fig. 2.1), only 68% of pT1 lesions (<20 mm) were detected. And 65% of lobular carcinomas had false-negative results, compared with ductal

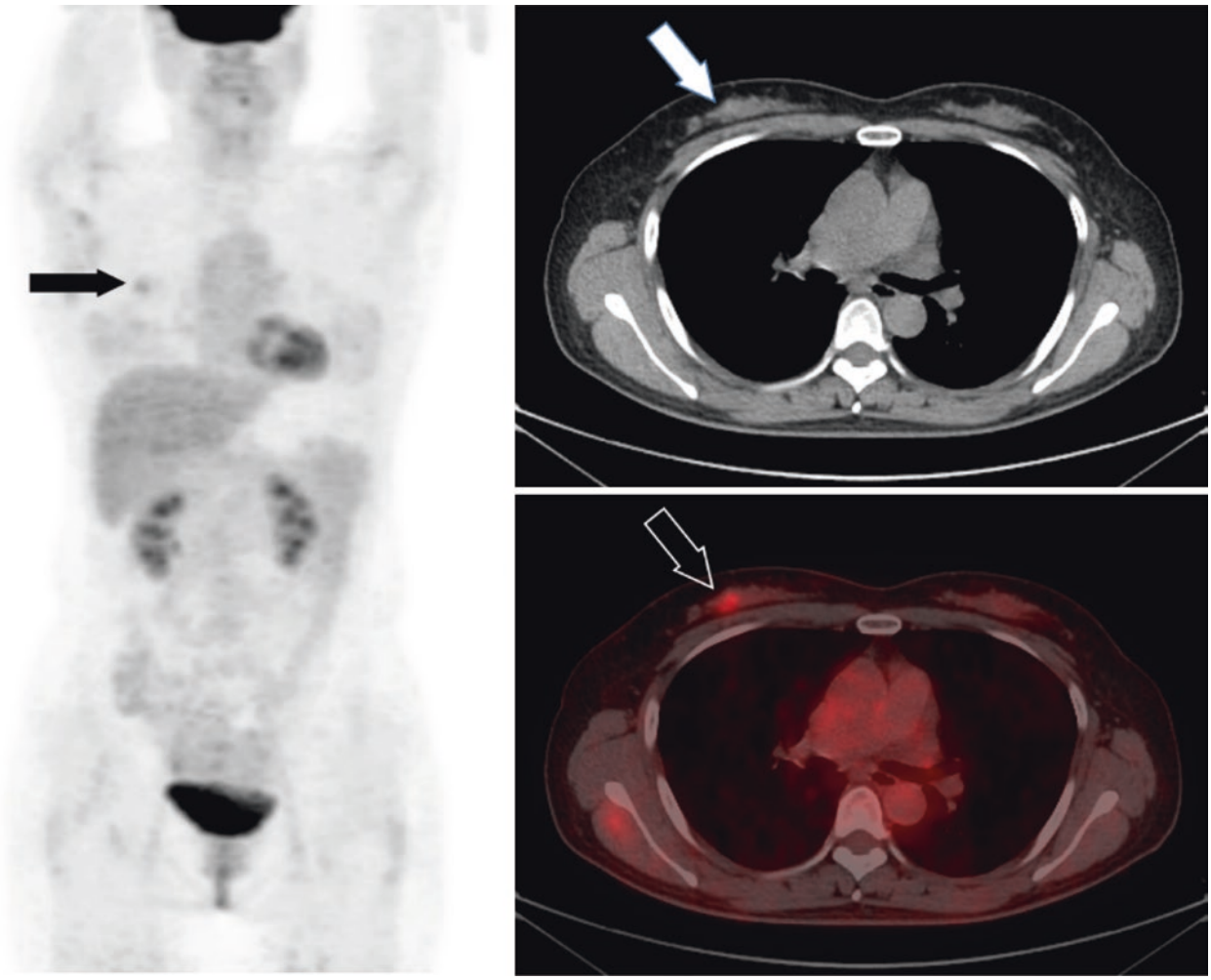

Fig. 2.1 A 56-year-old woman with suspicious left lung adenocarcinoma (*no increased activity*) undergoing ^{18}F-FDG PET/CT for tumor staging. An incidental ^{18}F-FDG focus (*black solid arrow*) was seen in the upper and medial quadrant of the right breast on coronal maximum intensity projection (MIP) PET image and fuse imaging (*white hollow arrow*), pathologically confirmed as invasive ductal adenocarcinoma with a maximum diameter of 2.6 cm. No obvious abnormality was found at CT image (*white solid arrow*)

carcinomas (24% false-negative) [20]. Studies have also shown that FDG-PET has poor sensitivity for submillimeter breast cancer lesions, and the sensitivity of detection is less than 50% [21, 22]. As mentioned above, infiltrating lobular carcinoma is more likely to be missed.

Positron emission mammography (PEM) is a breast-dedicated PET device, which with a high spatial resolution (even less than 2 mm) has showed promising results. It has dramatic improvements of sensitivity and specificity for detecting breast cancer lesions (especially for small lesions), compared to conventional whole-body PET. In the study of Kalinyak et al. [23], 109 primary invasive breast cancers (the average size 1.6 ± 0.8 cm) were enrolled. They found that the detection rates obtained with PEM and conventional PET/CT were 95% and 87%, respectively ($p < 0.029$). A meta-analysis [21] that evaluated eight studies comprising 873 breast lesions (the size ranged from 0.1 to 10 cm) showed a pooled sensitivity of 85% (95% CI, 83–88%) and a specificity of 79% (95% CI, 74–83%) on a lesion basis, using FDG PEM in women with suspected breast malignancy. Another report by Lima et al. [24] also showed similar results. A total of 80 lesions (the size ranged from 0.4 to 11.2 cm, mean 2.6 cm, included 76 breast cancers and 4 benign lesions) were enrolled; 63/76 breast cancer lesions was detected by PEM (the C-shape scanner); the lesion-based sensitivity was 83% (63/76), and this was increased to 90% (63/70) after excluding lesions outside the field of view.

A multicenter comparative study [25] determined the efficacy of PEM and DCE-MRI on ipsilateral pre-surgery planning, including 388 patients who undertook MRI and PEM, showed that a total of 116 malignant lesions were found after surgery, 61 of 116 malignant lesions (53%) were raised by MR for suspicious malignancy; 47 of these (41%) were raised as suspicious on PEM ($P = 0.04$), and only 24 lesions (21%) were raised as suspicious on conventional imaging. This result demonstrates that MR is superior to PEM in detecting breast cancer lesions.

Some benign lesions of the breast may also show the concentrated FDG uptake and sometimes are not easy to be differentiated from breast cancer. The following review will help us understand the uptake of breast lesions. The meta-analysis [26] reviewed the significance of incidental FDG uptake (from whole-body PET) in breast; the pooled risk of malignancy of incidental FDG uptake was 48% (95% CI, 38–58%), and the pooled risk of malignancy of incidental FDG uptake with histological examination was 60% (95% CI, 53–66%).

False-positive uptake caused by benign lesions had been reported, such as breast fibrocystic disease, fibroadenomas, papilloma, silicone leakage, fat necrosis, inflammatory, and infectious diseases. Breast fibrocystic disease usually does not exhibit very high FDG uptake, and higher FDG uptake generally indicates higher risk of malignancy. Lobulated contour, crab-like edge, ill-defined, and clustered granular calcification are typical morphological characteristics of breast cancer. In contrast-enhanced MR imaging, enhanced patterns of breast cancer, such as time-signal curves, contribute to the differentiation of benign diseases and breast cancer.

In addition, it has been reported that dual-time-point PET/CT imaging helps to increase the specificity and to better differentiate primary breast cancer from benign tumors or inflammatory processes [27, 28]. However, its usefulness has not yet been demonstrated in large series.

2.2.3 Initial Staging

Accurate and reliable initial staging is the premise for determining breast cancer treatment options and the basis for prognosis assessment. Once breast cancer is diagnosed, staging of the breast cancer must be performed. Currently, the TNM staging system (Tables 2.2 and 2.3) is widely used in clinic. Current evidence suggests that ^{18}F-FDG PET/CT has a good performance for patients with clinical stage IIB and higher stage, and its performance is not affected by the breast cancer cell phenotype, tumor grade, and patient's age.

2.2.3.1 T Staging

The size of the tumor lesion, tumor's relationship with the adjacent structure, and whether tumor are multicenter lesions are the important indicators for breast cancer T staging, which is an especially important consideration for planning of optimal breast conservation surgery.

Duo to the low spatial resolution of PET, PET remains inadequate for accurately defining the size and involved rang of breast cancer. To a certain extent, CT imaging in PET/CT can make up for this shortcoming. However, despite a high-density resolution of CT, small breast cancers (especially those in dense breasts) are often not well displayed by CT. When the breast cancer lesion grows to a certain size, CT can show its advantages in the measurement of lesion size and in judging the relationship between breast cancer and intercostal muscles and ribs.

US and mammography have relatively high sensitivity for the detection of small breast cancer lesions, high accuracy for measuring lesion size, and are easy to use and low in cost. But the size and extent of breast cancer are frequently underestimated by mammography and ultrasound.

MR can clearly display the contour of the lesion, so that accurate measurement of the lesion size can be performed, and determine the relationship between the lesion and the adjacent tissue. As it was reported in the study [28], FDG PET had less sensitivity than dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) in the determination of the delineation of the primary tumor and in screening