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REVIEW

The enhancement of glycolysis regulates pancreatic cancer metastasis

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Abstract

Pancreatic ductal adenocarcinoma is prone to distant metastasis and is expected to become the second leading cause of cancer-related death. In an extremely nutrient-deficient and hypoxic environment resulting from uncontrolled growth, vascular disturbances and desmoplastic reactions, pancreatic cancer cells utilize "metabolic reprogramming" to satisfy their energy demand and support malignant behaviors such as metastasis. Notably, pancreatic cancer cells show extensive enhancement of glycolysis, including glycolytic enzyme overexpression and increased lactate production, and this is caused by mitochondrial dysfunction, cancer driver genes, specific transcription factors, a hypoxic tumor microenvironment and stromal cells, such as cancer-associated fibroblasts and tumor-associated macrophages. The metabolic switch from oxidative phosphorylation to glycolysis in pancreatic cancer cells regulates the invasion–metastasis cascade by promoting epithelial–mesenchymal transition, tumor angiogenesis and the metastatic colonization of distant organs. In addition to aerobic glycolysis, oxidative phosphorylation also plays a critical role in pancreatic cancer metastasis in ways that remain unclear. In this review, we expound on the intracellular and extracellular causes of the enhancement of glycolysis in pancreatic cancer and the strong association between glycolysis and cancer metastasis, which we expect will yield new therapeutic approaches targeting cancer metabolism.

Keywords Warburg effect · Mitochondrial respiration · Tumor microenvironment · Epithelial–mesenchymal transition · Metastatic niche · Hybrid metabolic phenotype

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Abbreviations

PDAC	Pancreatic ductal adenocarcinoma
EMT	Epithelial–mesenchymal transition
CAFs	Cancer-associated fibroblasts
OXPHOS	Oxidative phosphorylation
NOX	NADPH oxidase
mtDNA	Mitochondrial DNA
$\Delta\Psi m$	Membrane potential
PFK	Phosphofructokinase
ALDOA	Aldolase A
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
PGM	Phosphoglycerate mutase
ENO	Enolase
PKM	Pyruvate kinase muscle isozyme
LDHA	Lactate dehydrogenase A
TME	Tumor microenvironment
TGF	Transforming growth factor
IGF1R	Insulin-like growth factor 1 receptor
MAPK	Mitogen-activated protein kinase
PI3K/AKT	Phosphoinositide 3-kinase/protein kinase B

mTOR	Mammalian target of rapamycin
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
PDLCs	Patient-derived pancreatic cancer cell lines
PGC-1α	Peroxisome proliferator-activated receptor γ coactivator-1α
SDH	Succinate dehydrogenase
HK	Hexokinase
TCA	Tricarboxylic acid cycle
HIF	Hypoxia-induced factor
PPP	Pentose phosphate pathway
GOF mutp53	Gain-of-function of mutant p53
GLUT	Glucose transporter
AMPK	AMP-activated protein kinase
TIGAR	*Tp53*-induced glycolysis and apoptosis regulator
GAMT	Guanidinoacetate N-methyltransferase
GLS2	Glutaminase-2
ROS	Reactive oxygen species
PFKFB	6-Phosphofructo-2-kinase/ fructose-2,6-bisphosphatase
ACL	ATP citrate lyase
PDK	Pyruvate dehydrogenase kinase
PGI/AMF	Glucose-6-phosphate isomerase/Autocrine motility factor
VEGF	Vascular epidermal growth factor
TGFBI	Transforming growth factor beta-induced
FAK	Focal adhesion kinase
ECM	Extracellular matrix
EMT-TFs	EMT-TF
MMP	Matrix metalloproteinase
6-PGD	6-Phosphogluconate dehydrogenase
FGF	Fibroblast growth factor
GPR81	G-protein-coupled receptors 81
MCT	Monocarboxylate transporters
TAMs	Tumor-associated macrophages
CXCL	CXC chemokine ligand
CXCR	CXC chemokine receptor
ECs	Endothelial cells
CTCs	Circulating tumor cells
CSCs	Cancer stem cells
HSCs	Hepatic stellate cells
HMFs	Hepatic myofibroblasts
GOT	Glutamic-oxaloacetic transaminase

Introduction

Pancreatic ductal adenocarcinoma (PDAC) accounts for more than 85% of all malignant pancreatic exocrine tumors [1]. PDAC accounts for approximately 3% of all new cancer cases, and it is the fourth leading cause of cancer-related death in both males and females in the U.S. [2]. Over the past several decades, the survival rate for PDAC has shown poor improvement, in contrast to the steady improvement in the survival rate for most types of cancer [2]. Moreover, PDAC is expected to be the second most common cause of cancer-related death by 2030 in the U.S. [3]. The lack of clinically informative early diagnostic symptoms and biomarkers leads to less than 20% of patients being diagnosed at a stage amenable to resection [4]. Most patients with PDAC are diagnosed with distant metastasis. Although patients with resectable tumors have longer survival than patients with unresectable tumors, distant metastases still occur among a great majority [5].

Malignant cells proliferate indefinitely and are prone to distant metastasis, which requires both sufficient energy and biosynthetic precursors to fuel cell division, invasion and migration. However, hypovascularization in PDAC reduces the delivery of nutrients for biosynthesis into cancer cells and leads to an energy crisis. Nevertheless, tumor cells have a robust ability to survive in harsh environments by changing their energy metabolism, which is known as "metabolic reprogramming"; the most common example of this is enhanced glycolysis, which is characterized by increase in glucose uptake and the production of lactate and was initially described as the "Warburg Effect" [6]. This metabolic reprogramming is of great significance for the abnormal survival and growth of cancer cells, as it provides energy, macromolecular precursors and reducing equivalents [7]. Otto Warburg discovered the enhancement of glycolysis under normoxic conditions and hypothesized that respiratory injury led to aerobic glycolysis, which may be the origin of cancer. However, the enhancement of glycolysis can be induced by hypoxia or hypoxia-induced factor 1 (HIF-1), even in the presence of fully functioning mitochondria, and is present in different heterogeneous cancer cells depending on oxidation phosphorylation or glycolysis and in cancer-associated fibroblasts (CAFs) that also launch metabolic reprogramming to support cancer cell growth and metastatic dissemination [8, 9]. In fact, the coexistence of increased glycolysis and oxidative phosphorylation (OXPHOS) is observed in some cancer cells, and these metabolic phenotypes can be induced to mutually switch in response to drug or microenvironmental stimulation [10, 11].

Notably, to ensure its survival, PDAC is characterized by a high-glycolysis rate due to hypovascularization and the desmoplastic reaction, which create a nutrient-poor and highly hypoxic microenvironment [12, 13]. Glycolysis in PDAC supports the vigorous growth of tumor cells by generating large amounts of substrates and promoting invasion and migration via the interaction of glycolytic enzymes and actin, which most likely increases the supply of ATP more easily. Furthermore, important enzymes and intermediates of glycolysis can regulate PDAC metastasis through participating in signaling transduction or epigenetic regulation related

to epithelial–mesenchymal transition (EMT), angiogenesis and colonization [14–17]. This review aims to provide an overview of the metabolic plasticity of PDAC, the causes of enhanced glycolysis, and the mechanisms underlying the regulation by glycolysis of the invasion–metastasis cascade and to discuss the potential use of therapeutic strategies to target cancer metabolism. In addition, we also discuss the unclear role of OXPHOS in pancreatic cancer metastasis.

Promotion of pancreatic cancer by the high-glycolysis phenotype

Mitochondrial dysfunction promotes glycolysis

Decades ago, Otto Warburg theorized that cancer originates when a nonneoplastic cell adopts anaerobic metabolism as a means of survival after injury to its respiratory system [18, 19]. Warburg believed that irreversible oxidative phosphorylation (OXPHOS) injury or insufficiency led to aerobic glycolysis. Many studies have discovered abnormalities in mitochondrial DNA (mtDNA), proteomics, lipidomics and structures that compromise the ability of OXPHOS to provide enough energy, and they also revealed that glucose transporters and glycolytic enzymes were apparently upregulated (Fig. 1).

Mitochondrial-to-nucleus retrograde signaling regulates tumor properties and can be triggered by altered mitochondrial functioning resulting from mtDNA mutations, mtDNA copy number alterations, mitochondrial respiratory chain complex defects and reactive oxygen species (ROS) production [20]. A reduction in mtDNA copy numbers causes a membrane potential ($\Delta\Psi m$) disruption that simulates Ca^{2+}/calcineurin signaling, activating a number of oncogenic factors and kinases such as IGF-1R, NF-kB, PI3 K and AKT that are associated with the upregulation of glycolytic enzymes [21]. Due to changes in mitochondria-generated ROS levels, mtDNA mutations could be induced and affect tumor cell metastasis [22]. Notably, an early study that sequenced the complete mtDNA genome in pancreatic cancer cells discovered a large increase in the intracellular mass of mtDNA mutations compared to that in normal cells [23]. Furthermore, 24 somatic mtDNA mutations and 18 somatic nuclear DNA mutations encoding mitochondrial and metabolic genes were identified, and the changes in the metabolic phenotypes of patient-derived pancreatic cancer cell lines (PDLCs) were correlated with mitochondrial dysfunction, including decreased oxygen consumption and an increased glycolysis rate [24]. In recent years, the role of mitochondrial uncoupling protein 2 (UCP2) in suppressing OXPHOS has been discovered, and this promotes aerobic glycolysis and facilitates the malignant progression of PDAC [25]. Moreover, it was found that NADPH oxidase (NOX), a specific enzyme regulating ROS production, was important for maintaining high glycolytic activity in PDAC cells with mitochondrial respiratory dysfunction. The suppression of NOX expression in PANC-1 cells decreased glucose uptake and lactate generation, which inhibited tumor growth in vivo [26].

Induction of PDAC by driver genes

Soon after the publication of Warburg's discovery, other cancer researchers criticized his hypothesis, because Warburg's theory did not address the role of tumor-associated mutations and the phenomenon of metastasis. It was hoped that the debate regarding respiratory impairment could be settled to move on to the field of molecular genetic changes in cancer cells in the later part of the twentieth century. However, by the twenty-first century, this issue emerged as a focal point once again. Robert A. Weinberg highlighted the essential role of genome instability in the hallmarks of cancer and acknowledged that metabolic reprogramming was an emerging hallmark associated with activated oncogenes [27]. Although aerobic glycolysis apparently impacts cancer cell survival, it is controlled by proteins involved in cellular programs involved in other core hallmarks of cancer. The Warburg effect (enhanced glycolysis) may constitute a certain phenotype driven by genomic alterations. Studies have shown that *Kras* and *Tp53* are the most important driver genes, with mutation rates of 98% and 50%, respectively, that play vital roles in PDAC development by controlling the regulatory processes or pathways involved in the G_1/S phase cell cycle transition, transforming growth factor β (TGF-β) signaling, DNA damage control and *Kras* signaling; however, they have also been revealed to regulate the "Warburg Effect" in recent years [28]. *Kras* mutation drives the uncontrolled proliferation of PDAC cells through activating downstream signaling pathways such as the MAPK and PI3 K–AKT–mTOR pathways, promotes local invasion and distant metastasis and plays a key role in regulating glucose utilization and anabolic metabolism [29, 30]. Oncogenic *Kras* promotes glycolysis through the stimulation of glucose uptake and regulates the glucose transporter (GLUT-1) and several rate-limiting glycolytic enzymes, including hexokinase 1 (HK1), HK2, phosphofructokinase 1 (PFK1) and lactate dehydrogenase A (LDHA), without affecting the glycolytic metabolites involved in the three carboxylic acid (TCA) cycles.

Moreover, hypoxia and HIF-1α also activate glycolytic enzyme expression and coordinate with mutant *Kras* to maintain cytosolic ATP generation, although the knockdown of HIF-1α has minimal impact on metabolic enzyme expression [30, 31]. In addition, oncogenic *Kras* activates the hexosamine biosynthetic pathway (HBP) to enhance the generation of the precursor moieties required for protein

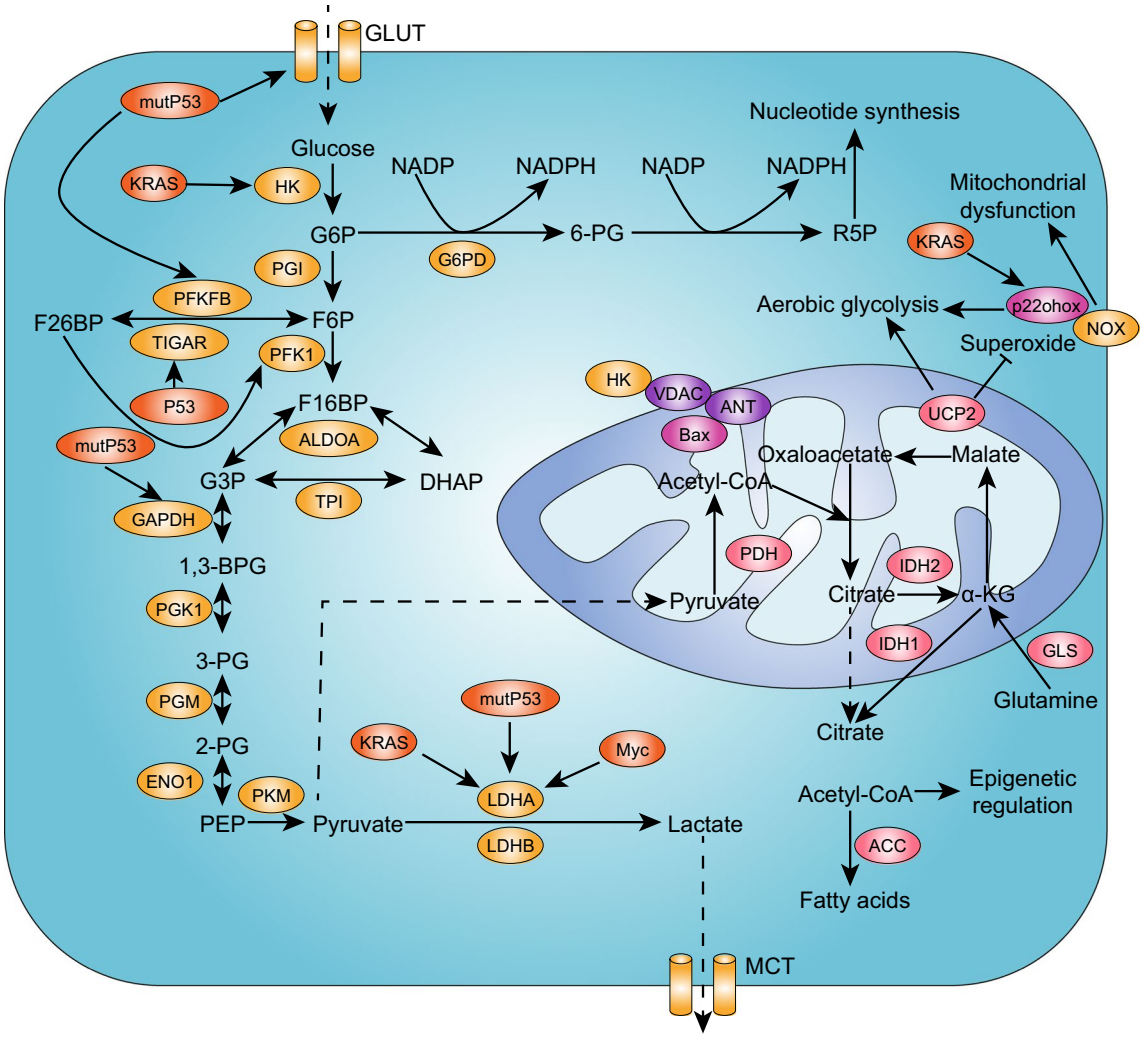


Fig. 1 Warburg effect in PDAC. The "Warburg effect" exists in the majority of tumors with enhanced glycolysis and lactate production under aerobic conditions, which was initially considered to be the result of mitochondrial dysfunction. PDAC cells present a high-glycolysis phenotype in which glucose uptake is increased and the glycolysis rate is accelerated, which is regulated by Kras, mutp53 and c-Myc, as a result of respiratory injury and the overexpression of glycolytic enzymes, which are marked in the yellow ellipses in this figure. The enhancement of glycolysis shunts more glucose into the pentose phosphate pathway and results in the accumulation of lactate in the microenvironment

glycosylation and promote ribose biogenesis by enhancing the activity of the nonoxidative arm of the pentose phosphate pathway (PPP) [30]. Furthermore, $Kras^{G12D}$ mediates this metabolic reprogramming via downstream MAPK signaling pathways and the transcriptional control of c-Myc (Fig. 2). As an oncogenic transcription factor, c-Myc regulates cell growth, differentiation and malignant transformation. It has been demonstrated that c-Myc exerts a crucial effect on the glycolytic phenotype of PDAC and impacts its development [32]. Oncogenic *Kras* in pancreatic cancer inhibits the tumor suppressor FBW7, which negatively regulates glucose uptake by targeting the c-Myc/TXNIP axis [33]. The overexpression of the kinase IKKε in pancreatic cancer is associated with poor prognosis, and it can promote aerobic glycolysis in PDAC cells by promoting c-Myc accumulation in the nucleus by targeting related genes [34].

The tumor suppressor gene *Tp53* plays a central role in pancreatic tumor prevention. In addition to the loss of tumor suppression, the gain-of-function version of *p53* (GOF mutp53) generated through missense mutation can stimulate cancer cell growth by modulating a set of genes via its interaction with a number of transcription factors [35]. In particular, GOF mutp53 was found to stimulate the Warburg effect by promoting GLUT1 translocation to the plasma membrane that was mediated by the RhoA/ROCK/GLUT1 signaling pathways (Fig. 2) [36]. Through stabilizing the cytosolic localization of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) via regulation

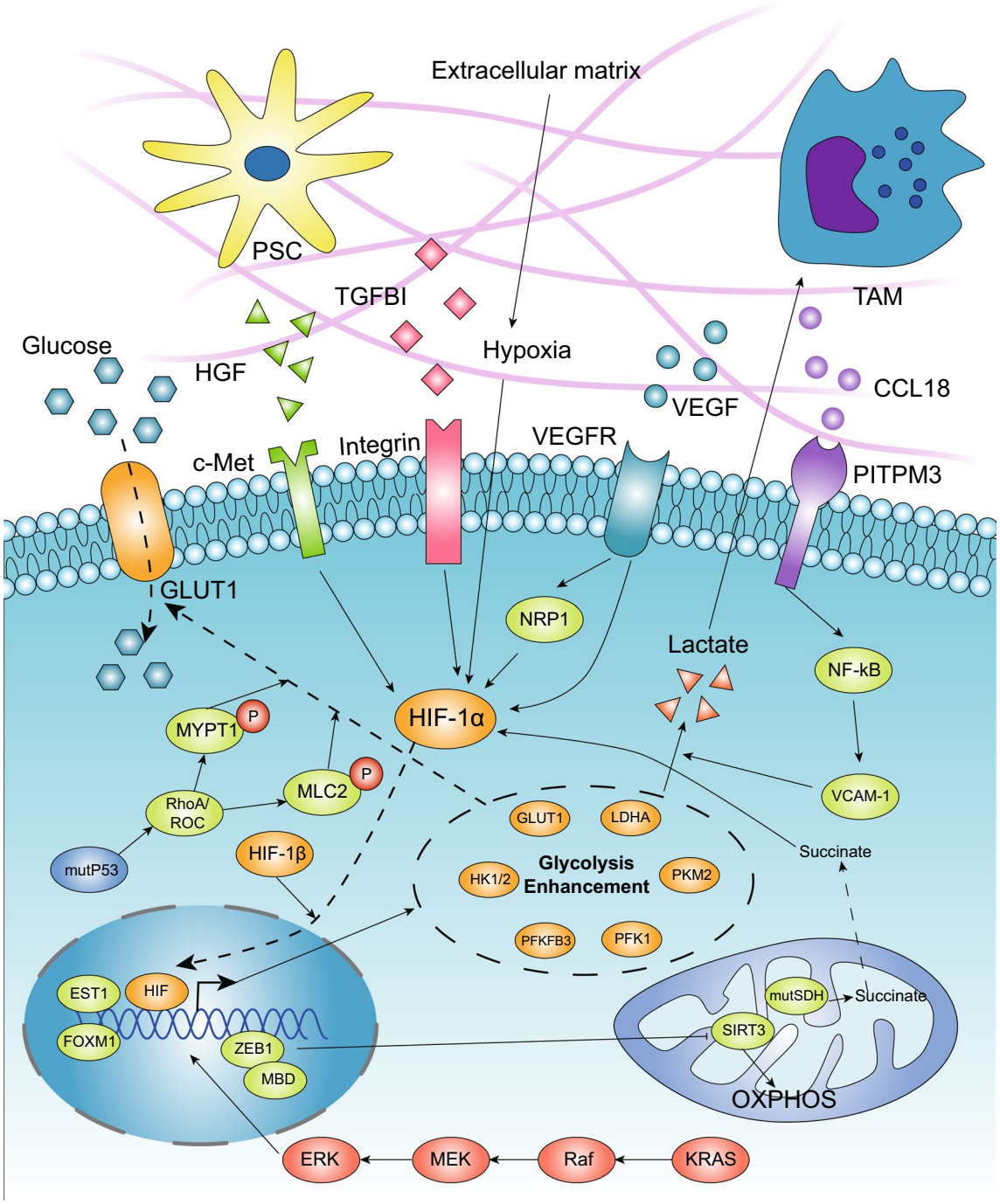


Fig. 2 Driver genes and TME promote the enhancement of glycolysis in PDAC. The high-glycolysis phenotype is considered to be an emerging hallmark associated with activated oncogenes. Mutant Kras can upregulate the expression of glycolytic enzymes via the Raf/MEK/ERK pathway, which is dependent on Myc and functions transcriptionally. GOF mutp53 also promotes glycolysis mainly by regulating GLUT1 translocation to the cytoplasmic membrane. In the pancreatic microenvironment, hypoxia is considered the main regulator that transcriptionally upregulates the expression of multiple glycolytic enzymes. SDH mutations leading to succinate accumulation can promote glycolysis through stabilizing HIF-1α. Additionally, stroma cells, such as TAMs and pancreatic stellate cells, secrete cytokines that interact with membrane receptors in cancer cells, and soluble factors in the TME, such as VEGF and TGFBI, promote glycolysis mainly via the HIF-1α and NF-kB pathways. ZEB1, known as an EMT regulator, can also promote glycolysis via its interaction with MBD1, which transcriptionally suppresses the expression of SIRT3

by Sirtuin1 (SIRT1) and avoiding its nuclear transport, GOF mutp53 also promotes glycolysis, which enhances the sensitivity of cancer cells to metabolic drugs [35]. For instance, tumors harboring mutant versions of *Tp53* exhibit increased apoptosis and reduced proliferation rates when exposed to FX11, a small-molecule inhibitor of LDHA [37]. In cancer cells without *Tp53* mutations, when glucose uptake reduces the level of p53, p53 is phosphorylated by AMPK to upregulate *Tp53*-induced glycolysis via Tp53-induced glycolysis and apoptosis regulator (TIGAR), SCO2 cytochrome c oxidase, guanidinoacetate N-methyltransferase (GAMT), and glutaminase 2 (GLS-2) and to downregulate the expression of phosphoglycerate mutase (PGM) to provide more ATP and reduce the level of ROS [38]. Thus, p53 can block ROS-induced apoptosis in cancer cells under nutrient stress; however, when *Tp53* mutations occur, cancer cells exhibit enhanced glycolysis that supports rapid proliferation.

Tp53-induced glycolysis and apoptosis regulator (TIGAR) suppresses glycolysis and can be upregulated by p53, which directly binds to its promoter at the BS2 site [39]. Although TIGAR expression has been found to be upregulated in most human PDAC tissues, it was decreased in *Tp53*-mutated tumors in a patient-derived tumor xenograft (PDTX) mouse model [37, 40]. In addition, p53 was demonstrated to decrease 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 4 (PFKFB4) expression, which plays an important role in glycolysis by binding to its promoter and mediating transcriptional repression via histone deacetylases in multiple cancer cell lines. Cancer cells deficient in p53 are highly dependent on the function of PFKFB4 [41]. In addition, p53 can transcriptionally repress paraoxonase 2 (PON2), and mutant *p53* in PDAC cells increases PON2 expression, which activates GLUT1-mediated glucose transport and is necessary for anoikis resistance [42].

Regulation of glycolytic enzyme expression and activity

In fact, due to the lack of sufficient evidence of mitochondrial metabolism alteration, enhanced glycolysis is now believed to be the main alteration in metabolism of tumors and a hallmark of PDAC with multiple overexpressed glycolytic enzymes (Table 1) [43]. Within pancreatic cancer cells, there are multiple levels of regulation controlling the expression and activity of glycolytic enzymes to impact the glycolysis rate that involve genome stability, transcriptional regulation and posttranslational modification.

The alteration of mitochondrial function and the abnormal accumulation of metabolites promote the enhancement of glycolysis through affecting the nuclear genome by activating HIF-dependent pathways and histone modification [20]. The accumulation of succinate due to the mutation of succinate dehydrogenase (SDH) can promote glycolysis via the stabilization of HIF-1α, which promotes the expression of multiple glycolytic enzymes [44]. Intramitochondrial acetyl-CoA and oxaloacetate combine to form citrate, which is transported out of the mitochondria and converted to acetyl-CoA by ATP citrate lyase (ACL). In addition to being utilized for fatty acid synthesis, acetyl-CoA is also an important source of acetyl for histone acetylation, which results in global transcriptional upregulation and S phase progression. The downstream transcriptional targets of ACL-mediated histone acetylation include GLUT4, HK2, PFK1 and LDHA. The regulation of these metabolic enzymes controls aerobic glycolysis in cancer cells [38, 45]. Thus, there must be unknown mechanisms that regulate glycolytic enzymes through epigenetic changes to enhance glycolysis in cancers.

Table 1 Glycolytic enzymes are overexpressed in various types of cancer

Glycolytic enzyme	Organs with overexpression	Promote metastasis	References
Hexokinase 2 (HK2)	Colon, brain, tongue, liver, pancreas	Colon, brain, tongue, liver, pancreas	[135, 136]
Lactate dehydrogenase A (LDHA)	Lung, ovary, pancreas	Lung, ovary, pancreas	[32, 50, 137–139]
Phosphoglucose isomerase/Autocrine motility factor (PGI/AMF)	Breast, pancreas	Skin, brain, breast, fibroblast, pancreas	[15, 140–147]
6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3)	Lung, bone marrow, colon, nasopharynx, stomach, breast, brain, pancreas	Nasopharynx, stomach, breast, pancreas	[56, 57, 60, 82, 83, 148–150]
6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB4)	Stomach, prostate, liver, bladder, breast, pancreas	Prostate, breast	[57, 58, 60, 151–153]
TP53-induced glycolysis and apoptosis regulator (TIGAR) [attenuates glycolysis]	Colon, brain, breast, bone marrow, nasopharynx, lung, pancreas	Nasopharynx, lung	[40, 154–160]

Multiple glycolytic enzymes are highly expressed in malignancies in many organs and metastatic subclones, including PDAC. In addition to the glycolytic enzymes listed above, ENO1, PKM2 and ALDOA are overexpressed in PDAC and also promote metastasis

In addition to c-Myc, there are many transcription factors, such as EST1 and FOXM1, that have been known to regulate PDAC invasion and metastasis and can promote glycolysis in pancreatic cancer by binding to the promoters of glycolytic enzyme genes, including HK1, PFKFB3, LDHA and GLUTs [46, 47]. After their expression, the posttranslational modification of glycolytic enzymes can change their enzymatic activity to regulate glycolysis. For example, PFKFB3 can be modified by phosphorylation under energy crisis conditions and as a result of di-methylation and acetylation to enhance cancer cell glycolysis [48, 49]. Li et al. elucidated the mechanism by which cisplatin induced the acetylation of PFKFB3 at lysine 472 (K472) to impair its nuclear localization signal (NLS), thereby causing its accumulation in the cytoplasm; this facilitated the phosphorylation of PFKFB3 by AMPK, leading to PFKFB3 activation and enhanced glycolysis [49]. In addition, LDHA is overexpressed in PDAC cells because of the degradation of lysine 5 (K5)-acetylated LDHA. Acetylation at K5 decreased the LDHA levels to inhibit glycolysis and the migration of PDAC cells [50].

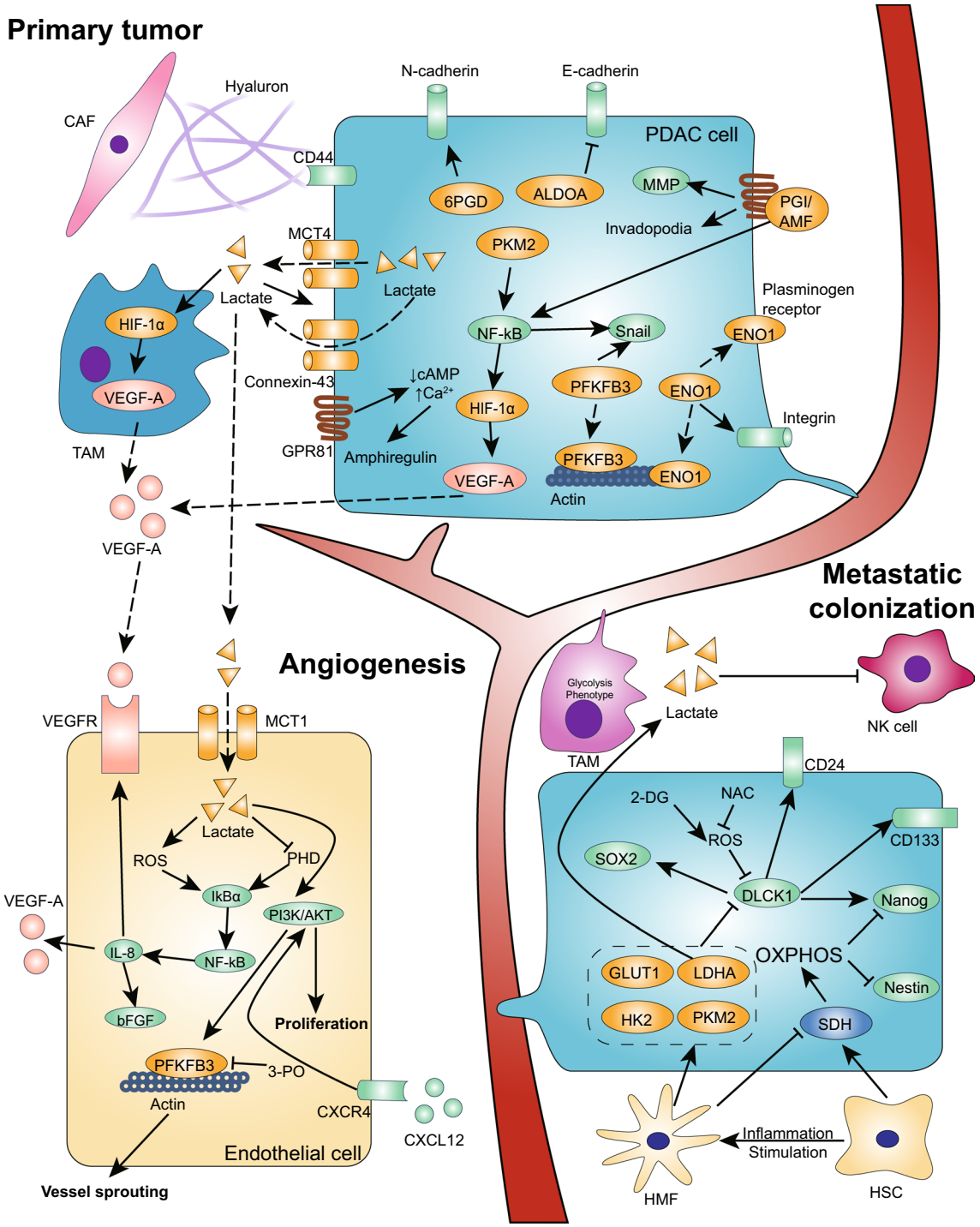
Pancreatic cancer microenvironment

The interaction of stromal, immune, and malignant cells creates a tumor microenvironment (TME) that imposes physical pressure, oxidative stress, nutrient deprivation, competition, hypoxia, and immune surveillance on cancer cells [51]. Notably, PDAC creates a strong desmoplastic reaction and low vascular density, which reduces the deliverability of nutrients and oxygen and results in enhanced glycolysis and lactate deposition [12]. Hypoxia can induce the transcription of various genes involved in anaerobic metabolism, angiogenesis and metastasis, which is considered the main inducer of the glycolytic switch in cancer. A lack of oxygen leads to reduced ATP levels, which reduces the inhibition of glycolytic enzymes [52]. In addition, hypoxia activates HIF-1α, which regulates the expression of many glycolytic enzymes via its binding to hypoxia-response elements, and HIF-2α also responds to hypoxic conditions to promote glycolysis (Fig. 2) [53]. In PDAC cells, hypoxia promotes the expression of pyruvate dehydrogenase kinase (PDK1), LDHA, pyruvate kinase muscle isozyme (PKM2), glucose-6-phosphate isomerase/autocrine motility factor (PGI/AMF) and HK2, which is mediated by HIF-1α [54, 55]. As important enzymes involved in the glycolysis process, PFKFB3 and PFKFB4 are highly expressed in various types of cancer, including pancreatic cancer, when induced by hypoxia or HIF-1α [56–60]. However, the deletion of HIF-1α does not impact the expression of glycolytic enzymes [54]. In tumor tissues from PDAC patients, TIGAR expression was shown to be increased, but hypoxic conditions could not induce its overexpression [40]. Moreover, TIGAR can translocate to the outer mitochondrial membrane, to bind to and activate HK2 during hypoxia or activate HIF-1α, thus regulating glycolysis and cell apoptosis [61].

PDAC stromal cells comprise pancreatic stellate cells, various leukocytes and endothelial cells, such as cancer-associated fibroblasts (CAFs) and tumor-associated macrophages (TAMs), in the TME that can be activated to release growth factors and cytokines to impact cancer cell metabolism (Fig. 2). TAMs (M2) highly express CCL18 to interact with PITPNM3 in PDAC cells and activate the NF-kB signaling pathway, which induces the overexpression of VCAM-1 and promotes aerobic glycolysis to increase the secretion of lactate and promote the conversion of M0 macrophages to M2 macrophages; this creates a feedback loop facilitating tumor survival [62]. Pancreatic stellate cells in tumors secrete hepatocyte growth factor (HGF), which activates its cognate receptor c-MET to regulate YAP translocation and HIF-1α stabilization, enhance the expression of HK2 and promote glycolytic metabolism in PDAC cells [63].

The TME comprises malignant cells, stromal cells and extracellular components such as VEGF, transforming growth factor beta-induced (TGFBI) and extracellular matrix (ECM). In addition to promoting angiogenesis, vascular epidermal growth factor (VEGF) stimulation was reported to lead to metabolic transition from mitochondrial OXPHOS to glycolysis in PDAC cells via HIF-1α upregulation [64]. Transforming growth factor beta-induced (TGFBI) is an ECM-interacting protein associated with an invasive phenotype in cancer and is highly expressed in PDAC lesions versus nonneoplastic pancreatic lesions. Secreted TGFBI could bind to integrin and activate the focal adhesion kinase (FAK) signaling pathway, which promotes the stabilization of HIF-1α and results in increased glycolysis [65]. In addition, the presence of bioavailable copper in pancreatic TME has been associated with tumor growth and metabolism. When using copper chelation, cancer cell proliferation was suppressed with the attenuation of OXPHOS and reduced ATP levels, despite the enhancement of glycolysis. Therefore, glycolysis activation in PDAC may reflect in part low copper bioavailability in the TME [66].

It is noteworthy that a subset of aerobic PDAC cells can use the lactate derived from stroma to fuel the TCA cycle for cell proliferation, resulting in less dependence on glucose metabolism. This process was described as “the reverse Warburg effect”, because a glycolytic phenotype in stromal cells such as CAFs supports adjacent cancer cell survival with increased OXPHOS activity and invasive ability [67–69]. Hence, stromal cells that make up the majority of pancreatic tumors can facilitate cancer cell growth and progression by promoting a glycolytic phenotype and supporting mitochondrial metabolism in oxidative PDAC cells. Therefore, targeting the stroma of PDAC seems to be a promising anti-cancer strategy for use in the future.



Glycolysis regulates the PDAC metastasis cascade

The metastasis process in malignant tumor cells consists of several steps, known as the invasion–metastasis cascade, that include invasion, intravasation, extravasation and colonization in distant organs. Regarding the regulation of the metastatic behaviors of PDAC in the cascade involving EMT, angiogenesis and distant colonization, recent studies have predominantly focused on the fields of genome instability, noncoding RNA, exosomes and TME [70–74]. However, an increasing number of studies have reported the association between the Warburg effect and glycolysis and malignant behaviors in PDAC in recent years. The Warburg effect can benefit pancreatic cancer cells by providing energy, macromolecular precursors and reducing equivalents, which

◂**Fig. 3** Enhancement of glycolysis promotes PDAC metastasis. In the invasion–metastasis cascade in PDAC cells, glycolytic enzymes and lactate facilitate the process in three main ways. (1) Glycolysis promotes the EMT program by upregulating EMT-TFs, downregulating E-cadherin, and increasing MMP secretion and cytoskeleton remodeling. PFKFB3, ALDOA, ENO1, PKM2 and PGI/AMF can function in cytoplasmic, nuclear and extracellular forms. (2) Glycolysis promotes angiogenesis by increasing VEGF and amphiregulin secretion via a self-induced pathway and the regulation of stromal cells, which is dependent on the accumulation of lactate in the TME. Additionally, glycolytic enzymes such as PKM2 can be secreted into the blood stream to interact with receptors in the EC membrane to promote EC proliferation and migration. In addition, enhanced glycolysis in ECs also promotes vessel sprouting to facilitate angiogenesis. (3) Glycolysis promotes PDAC cell metastatic colonization. Glycolytic TAMs promote the extravasation of CTCs. In a distant microenvironment, the enhancement of glycolysis in metastatic PDAC cells maintains stemness through low ROS levels. In the hepatic microenvironment, many HSCs promote the elevated expression of SDH and enhance OXPHOS activity, reducing the self-renewal ability of pancreatic cancer metastatic cells via the reduced expression of Nanog and Nestin. In contrast, HMFs promote the downregulation of SDH and activate glycolysis, which promotes cancer cell proliferation and the formation of visible metastases. In addition, the secretion of lactate from PDAC cells reduces NK cell cytotoxicity, which promotes metastatic colonization

promote cancer cell growth and inhibit apoptosis [7, 30]. Notably, overexpressed glycolytic enzymes and enhanced glucose anabolism promote metastasis in PDAC cells (Table 1) [14, 15]. The overexpression of glycolytic enzymes leads to the deposition of lactate in the TME, which can be transported out of PDAC cells through MCTs and connexin-43 to not only serve as an energetic fuel for oxidative PDAC cells but also promote invasion and metastasis as a signaling molecule [69, 75, 76]. Here, we summarize the mechanisms by which glycolysis regulates the metastasis of PDAC cells in three aspects: EMT, angiogenesis and distant colonization. Robert A. Weinberg considered the deposition of cancer cells in the lymphatic system as a surrogate marker revealing the extent of dissemination [77]. Therefore, the discussion of metastasis mainly focuses on the vascular route through which cancer cells reach and survive in the secondary site.

Regulation of EMT

EMT confers to epithelial cells the neoplastic properties of dissemination and the ability to degrade components of the ECM, which is orchestrated by a series EMT-inducing transcription factors (EMT-TFs) [77]. In contrast to Snail and Twist1, which had no effect on PDAC metastasis, Zeb1 was found to have a crucial impact on the formation of lesions and tumor metastasis in a KPC mouse model [78, 79]. EMT programs seem to be triggered by signals, including TGF-β, Wnts and certain interleukins, from nearby tumor-associated stroma. However, the influence of somatic mutations acquired during primary tumor formation and that of the metabolic reprogramming in cancer cells on the activation and expression of EMT programs remains unclear.

Hypoxic PDAC cells with a high level of glycolysis exhibit a "mesenchymal phenotype" [80]. Glycolytic enzymes activate and maintain EMT programs mainly through regulating the expression of EMT-TFs. The mechanism by which PGI/AMF promotes cancer metastasis is mediated by its binding to gp78, a 78-kDa seven-transmembrane glycoprotein that activates the invasion–metastasis cascade through various intracellular effectors. The overexpression of PGI/AMF leads to the downregulation of E-cadherin expression associated with the upregulation of Snail expression, contributing to the aggressive phenotype of human pancreatic cancer (Fig. 3) [15]. In addition, PGI/AMF can promote the relocalization of RhoA and Rac1, small GTPase proteins regulating the cytoskeleton that are associated with the formation of filopodia and lamellipodia. Moreover, PGI/AMF increases the expression of the integrins a2b3 and a5b1 to regulate cell adhesion, stimulates matrix metalloproteinase (MMP2) activity and promotes MMP3 expression via the MAPK signaling pathway to promote the invasion of malignant tumor cells [81].

TGF-β1 was demonstrated to increase glycolysis by inducing PFKFB3 expression and mediating the invasion of PANC-1 cells via PFKFB3, which could regulate the expression of Snail [82]. In squamous cell carcinoma cells, PFKFB3 could be detected in invadopodia and lamellipodia, although most PFKFB3 was localized in the nuclei of tumor cells [83]. Thus, cytoplasmic PFKFB3 may promote cancer cell mobility through interacting with actin, stimulating protrusion formation and supplying ATP for migration. Upon TGF-β treatment, the expression of aldolase A (ALDOA) was found to increase the most significantly among the glycolysis genes. Furthermore, the high expression of ALDOA and low expression of E-cadherin were found in highly metastatic pancreatic cancer tissue samples, and the overexpression of ALDOA indicated a poorer prognosis for pancreatic cancer patients. In PANC-1 cells, the silencing of ALDOA decreased proliferation and metastasis, increased E-cadherin levels, and decreased the levels of N-cadherin and vimentin [84]. Conversely, lactate metabolism can promote TGF-β2 expression, which induces glioma cell migration by enhancing the production and activation of MMP-2 and b1-integrin [85]. Therefore, TGF-β promotes cancer cell glycolysis, which in turn upregulates its expression, forming a feedback loop. Intriguingly, during pancreatic cancer cell EMT progression, TGFβ-1 treatment was found to induce mitochondrial dysfunction simultaneously [86].

Alpha-enolase (ENO1), a well-known glycolytic enzyme, also acts as a plasminogen receptor on the cell surface, promoting metastatic cancer invasion via interacting cytoskeletal proteins such as F-actin and tubulin. After the

knockdown of ENO1 in pancreatic cancer cells, their adhesion to fibronectin and collagen I/IV decreases, and their adhesion to vitronectin increases. Importantly, shENO1 strongly represses the expression of the integrin alpha v/beta 3 complex, which is involved in binding these extracellular matrices to induce cancer cell invasion (Fig. 3) [87]. In addition, cancer cells secrete lactate into the microenvironment, which can induce the secretion of hyaluronan by cancer-associated fibroblasts [88]. Hyaluronan deposition is usually higher in malignant tumors and promotes tumor progression via the interaction of hyaluronan/RHAMM/CD44 [89], which affects adhesion functions, rearranges the cytoskeleton, increases the activity of ECM-degrading enzymes and promotes angiogenesis toward the metastatic lesion. Graviola, also known as the tropical tree *Annona muricata,* can decrease the expression of GLUT1, HK2 and LDHA by inhibiting the NF-kB/HIF-1α signaling pathway, which is correlated with the attenuation of PDAC cell migration characterized by the disruption of microtubule dynamics and the reduction of MMP9 [90]. This indicates a potential therapeutic approach to inhibit the metastasis of PDAC cells via the targeting of the regulatory system of glycolysis.

The activity of the metastatic pathway can be further increased by epigenetic alterations. For example, in distant metastases in PDAC, Oliver G observed increased glucose uptake, elevated lactate secretion and enhanced PPP activity. The enhanced PPP activity induced broad enrichment of H3 K27ac over that of CDH2/N-cadherin in distant metastatic subclones compared to normal cells or regional PDAC cells. Treatment with 6AN, an inhibitor of 6-phosphogluconate dehydrogenase (6-PGD) with no effects on glutamine metabolism, could reverse this reprogrammed histone modification [14].

In summary, glycolytic enzymes play vital roles in pancreatic cancer EMT and metastasis. However, few studies have reported the impact of EMT regulators, such as ZEB1, on glycolysis, which was found to promote aerobic glycolysis in pancreatic cancer cells by transcriptionally suppressing the expression of SIRT3 via its interaction with methyl-CpG binding domain protein 1 (MBD1) [91]. Thus, the association between EMT and metabolism in PDAC requires more research.

Promotion of angiogenesis

Angiogenesis is new blood vessel formation derived from preexisting endothelial cells [92]. The early steps in the metastatic cascade include invasion and migration into tissues and the circulatory system. Therefore, an "angiogenic switch" is always activated and remains on during tumor progression, causing quiescent vasculature to sprout new vessels and facilitating the expansion of neoplastic growth and cancer cell metastasis. A variety of angiogenic factors contribute to new vessel formation, such as members of the well-known VEGF-A and fibroblast growth factor (FGF) family [27]. Hypoxia can promote angiogenesis by directly inducing VEGF-A overexpression or enhancing glycolysis in cancer cells. PKM2 depletion in pancreatic cancer cells results in impaired tumor growth and angiogenesis in vivo. The hypoxic environment could induce the translocation of PKM2 to the nucleus, and PKM2 regulated HIF-1α-induced VEGF secretion in an NF-kB/p65-dependent manner (Fig. 3) [16]. Additionally, PKM2 can be secreted by cancer cells to promote endothelial cell (EC) proliferation, migration and adhesion to the ECM via the PI3 K/AKT and Wnt/β-catenin signaling pathways in prostate and colon cancer [93].

High levels of glycolysis and mitochondrial dysfunction in cancer cells result in the accumulation of lactate, succinate and a reduction in β-hydroxybutyrate, and these molecules are reported to interact with specific cell-surface G-protein-coupled receptors to promote tumorigenesis and progression [94]. G-protein-coupled receptors 81 (GPR81) are upregulated in most cancers, including PDAC, and are associated with tumor growth and metastasis [75, 95]. Lactate released in the microenvironment can activate GPR81 to promote the proliferation and overexpression of monocarboxylate transporter 1 (MCT1), MCT4 and CD147. GPR81 activation also enhances angiogenesis via the increased secretion of the pro-angiogenic factor amphiregulin, which is involved in the PI3 K/Akt pathway, coupled to decreased cAMP (Fig. 3). In addition, lactate secreted by glycolytic cancer cells induces TAMs to express VEGF dependent on HIF-1α and promotes the conversion of M0 macrophages to M2 macrophages, which in turn enhances aerobic glycolysis in PDAC cells [62, 96]. Additionally, endothelial cells can take up lactate through MCT1, which independently triggers the NF-kB/IL-8 (CXCL8) pathway to promote human colorectal and breast cancer angiogenesis by increasing the expression of VEGF-A, VEGFR-2 and bFGF; this, in turn, is mediated by elevated ROS and the suppression of prolyl hydroxylase 2 (PHD2) [97].

The glycolytic phenotype of endothelial cells can promote tumor angiogenesis. The knockdown of PFKFB3 could suppress cultured endothelial cell proliferation and migration, which can be rescued by the addition of lactate [98]. It has been reported that PFKFB3 can be activated by the CXCL12/CXCR4-induced phosphorylation of PI3 K/Akt to promote microvascular sprouting [99]. The targeting of PFKFB3 to suppress cancer metastasis has already been used in clinical trials [100, 101]. De Bock et al. elucidated the mechanism by which PFKFB3 promoted vessel sprouting in HUVECs. PFKFB3 gene deletion in ECs caused vascular defects in vivo, and the knockdown of PFKFB3 impaired the formation of lamellipodia. Interestingly, PFKFB3 silencing or overexpression did not alter gene expression in tip or stalk cells. PFKFB3 was enriched

along with F-actin in membrane ruffles at the leading front of lamellipodia and was confirmed to interact with actin. In addition, PFKFB3 knockdown decreased ATP consumption and reduced protein synthesis, which led to the suppression of hypermetabolism induced by vessel sprouting [102]. Thus, in the hypoxic TME in PDAC, glycolytic cancer cells and stromal cells, including TAMs and endothelial cells, synergistically promote angiogenesis that helps cancer cells hematogenously spread.

Metastatic colonization

In the bloodstream, circulating tumor cells (CTCs) will be challenged by shear forces, the innate immune system and oxidative stress. However, interactions with platelets and neutrophils can protect CTCs from killing by immune cells and promote their capability for extravasation by enhancing the cancer cell EMT program [103–106]. Oxidative stress limits distant metastasis, and treatment with the antioxidant N-acetyl-cysteine (NAC) can promote the survival of CTCs and increase the metastatic burden in vivo [107]. Therefore, the enhancement of glycolysis can help CTCs to metastasize via increase in antioxidant capacity. To colonize distant organs, CTCs must overcome many obstacles to infiltrate distant organs, avoid immune defenses, settle in supportive niches and eventually overtake host tissues [108]. Although it has been shown that glycolysis promotes the distant metastasis of PDAC cells in vivo, the mechanism underlying metastatic colonization is only just starting to be understood, and studies on metabolic regulation have been rare [109].

To induce metastatic colonization, CTCs first need infiltrate the distant tissues, which partly depend on their mesenchymal cell properties that can be enhanced by glycolysis. Additionally, macrophages can contact CTCs to pull them across capillary walls in the lungs. Pancreatic cancer cell-conditioned macrophages were found to promote angiogenesis and augment CTC extravasation. The inhibition of TAM glycolysis with competitive inhibitors of hexokinase II (HK2) and 2-deoxyglucose (2DG) blocked pro-metastatic functioning [110]. Thus, the glycolytic phenotype of CTCs and the regulation of the intravascular microenvironment by CTCs could synergistically promote PDAC cell migration across the vessel wall.

Metastatic cells depend on supportive niches, including premetastatic niches, perivascular niches, ad hoc niches and native stem cell niches, to survive in distant tissues. Metabolic reprogramming has been shown to be involved in premetastatic niche-promoted tumor metastasis, such as that observed in colorectal tumor-initiating cells, and increased lysine catabolism was found to facilitate survival in the liver [108]. When cancer cells infiltrate distant organs, most of them will die due to immune surveillance in the new challenging microenvironment, for example, NK cells, which are enriched in liver, suppress metastatic cell survival [108]. However, tumor-derived lactate plays an immunosuppressive role that facilitates metastatic cell survival, and LDHA-deficient PanO2 cell-injected mice show significantly higher cytotoxic activity in their NK cells, suppressing the dissemination of cancer cells [111]. Additionally, bone marrow neutrophils with immunosuppressive activity exhibit elevated glycolysis and more spontaneous chemokine-induced migration to distant tissue, which aids in early cancer cell dissemination [112]. The liver is one of the most frequently infiltrated organs by distant metastatic PDAC cells [113]. A hepatic microenvironment with hepatic stellate cells (HSCs) has a negative impact on metastatic cancer cells that present the elevated expression of succinate dehydrogenase (SDH) and high OXPHOS activity, leading to a dormant stage with reduced CSC activity and proliferation capability. However, the presence of liver inflammation during the transdifferentiation of HSCs to hepatic myofibroblasts (HMFs) promotes the downregulation of SDH and increased glycolysis, which reactivates the dormant metastatic cancer cells and fosters their proliferation and self-renewal capabilities, thus facilitating the development of visible metastases [114].

In addition, there are glycolytic enzymes that can be secreted by cancer cells into circulation, such as LDHA and PKM2, which can function as extracellular activators to promote metastasis [93]. We hypothesize that glycolytic enzymes in the circulation can be transported to distant organs to interact with ECs, facilitating their proliferation and supporting metastatic cancer cell survival.

Multiple metabolic signaling pathways, such as the PI3 K-AKT, MAPK, HIF and NF-kB pathways, support metastatic cell growth and survival, and these pathways are mostly driven by cancer-associated genes that also regulate glycolysis and are considered to increase stem cell capacity via the upregulation of CD133, CD24, Nanog and Sox2 [17]. A proteomic analysis of pancreatic stem cells revealed that PANC-1 CSCs were characterized by the upregulation of glycolysis [115]. However, in a subset of pancreatic CSCs, the enhancement of glycolysis mediated by MYC reduced their stemness, while high OXPHOS activity preserved full CSC functionality; however, the glycolytic capacity of CSCs facilitate their survival during the energy crisis induced by metformin [11]. Additionally, cancer cells can obtain support through physical contact with stromal cells. Pancreatic stellate cells secrete hepatocyte growth factor (HGF) which promotes HK2 expression in PDAC cells through HGF/c-MET/YAP/HIF-1α signaling, enhances stem cell potential via the overexpression of Nanog, Oct-4, and Sox-2 and promotes stemness [63].

OXPHOS activity in PDAC metastasis

Although we consider the enhancement of glycolysis to be the dominant metabolic alteration in malignant tumors, OXPHOS activity also plays critical roles in some cancer cells due to metabolic heterogeneity [116, 117]. However, the role of OXPHOS in the regulation of PDAC metastasis remains unclear and complicated. Due to the dysregulation of mitochondrial function in the glycolysis phenotype, we can indirectly conclude that OXPHOS may be negatively associated with PDAC metastasis. A study investigating mitochondrial function during EMT in pancreatic cancer cells found that TGFβ-1 treatment resulted in a high mtDNA copy number, increased intrinsic ROS stress and decreased the ΔΨm, suggesting that mitochondrial dysfunction may play a role in PDAC progression [86]. In liver metastases, dormant cancer cells could reacquire CSC potential along with the enhancement of glycolysis when HSCs transdifferentiated into HMFs, which was induced by the hepatic inflammatory microenvironment [114].

However, many studies have also demonstrated the role of OXPHOS in promoting PDAC metastasis. Coculture of CAFs and pancreatic cancer cells could enhance the migration and invasion abilities of the latter, which could be eliminated by MCT1-specific inhibitors [69]. Myoferlin, a ferlin family member protein overexpressed in PDAC, was reported to promote pancreatic cancer cell metastasis by enhancing OXPHOS activity [118]. Moreover, it was found that high OXPHOS activity could maintain CSC functionality; in contrast, enhanced glycolysis repressed stemness via increased MYC expression, which can promote glycolytic gene expression and suppress PGC-1α [11]. In another study, a subpopulation of dormant tumor cells surviving *Kras* ablation was found to rely on mitochondrial respiration, show high sensitivity to OXPHOS inhibitors and exhibit a decreased dependence on glycolysis [116]. For other types of cancer, such as breast cancer, cervical carcinoma and melanoma, many studies have demonstrated the association between increased OXPHOS and metastatic properties [20, 119]. Valerie et al. systematically reported the effect of PGC-1α on the promotion of breast cancer metastasis via mediating mitochondrial biogenesis and OXPHOS activity [119].

In summary, we believe that pancreatic CSCs obtain energy mainly through OXPHOS, whereas non-CSCs rely more on aerobic glycolysis. However, many factors can impact the fate of pancreatic cancer cells. The nuclear genetic background, including *Kras* mutations and MYC expression, restrict metabolic plasticity [11, 116]. Additionally, the number of mtDNA mutations, lipid utilization and the microenvironment collectively influence the metabolic phenotype of PDAC to maintain high OXPHOS activity, facilitating PDAC growth and metastasis [10, 69].

Therapeutic strategies for targeting glycolysis

The glycolytic phenotype has been widely demonstrated to promote tumor growth and metastasis, suggesting that it is a potential therapeutic target, and the limitation of metabolic processes or glycolytic enzymes must be examined and confirmed to be safe. Until now, only the glycolytic inhibitor PFK158 targeting PFKFB3 has been tested in a clinical trial, which confirmed it to be safe and provide a clinical benefit in late-stage PDAC patients [120, 121]. In addition, combining PFK158 with immunotherapeutic agents may result in additional clinical benefits due to the immunosuppressive role of PFKFB3 in cancer cells. HK2 has potential value as a target of anti-cancer therapy, such as treatment with 3-bromopyruvate (3BP), a HK2 inhibitor that was reported to suppress pancreatic cancer growth and progression in animals compared with gemcitabine [122]. Additionally, systematic HK2 deletion in adult mice reduced the tumor burden of lung cancer without adverse physiological consequences [123]. Thus, clinical trials of HK2 inhibitors in patients with PDAC should be considered. The targeting of the Warburg effect with LDHA inhibitors proved to be effective, particularly under hypoxic conditions, and could reduce the expression of MMP and $CD133^+$ to suppress pancreatic cancer metastasis and exhibited synergistic cytotoxic activity with gemcitabine [124]. In addition, pancreatic tumors with *Tp53* mutations exhibit a therapeutic response when treated with FX11, a small-molecule inhibitor of LDHA [37, 125]. In addition, ECs exhibited a high level of glycolysis during vessel sprouting, and targeting ECs has recently emerged as a novel approach for cancer therapy. For instance, treatment with the PFKFB3 inhibitor 3PO at a low dose can tighten the vascular barrier and induce vessel maturation to reduce cancer cell metastasis in orthotopic pancreatic mouse models [101].

Targeting glycolytic enzymes seems to be successful for cancer therapy and has been shown to work in animal models. However, such inhibitors have not been used in the clinic, even in clinical trials, suggesting that more potential enzymes need to be identified, especially those that function against cells with the mutation of specific genes such as *Tp53* and *Kras*.

Future perspectives

Disturbances in carbohydrate metabolism in pancreatic cancer have been studied for more than 70 years and have received the most attention in investigations of the crosstalk

between metabolic regulation and signaling. We have summarized the causes of the switch to a high-glycolysis phenotype in pancreatic cancer and its role in cancer metastasis. However, cancer cells have many energy resources involving glucose, lipid and glutamine metabolism that can also promote the progression of PDAC [126–131]. In contrast to the dependence of cancer cells on glutamate dehydrogenase, which converts glutamine-derived glutamate into α-ketoglutarate, PDAC cells rely on the transaminases GOT2 and GOT1 for metabolism to promote the conversion of aspartate to OAA, malate and then pyruvate, which supports PDAC cell growth by maintaining the redox balance. Therefore, there must be a great deal of complex crosstalk among the metabolic processes of different energy sources that cooperatively regulate the malignant behaviors of PDAC.

In addition to aerobic glycolysis, OXPHOS also plays a critical role in cancer growth, metastasis and therapy resistance. It is worth noting that cancer cells with higher OXPHOS activity, which supports CSC survival and metastasis, showed no decrease in glycolytic activity and even exhibited a dependence on glycolysis that was promoted by therapy resistance or an inflammatory state, indicating the important role played by the hybrid metabolic phenotype [11, 114, 119]. Jia et al. demonstrated that metastatic TNBC cells could acquire a stable hybrid metabolic phenotype and that repressing either of the metabolic phenotypes would activate the fine-tuning genes (HIF-1 and AMPK) of the other. Only combining glycolysis inhibition and FAO inhibition could lead to a major reduction in both the proliferation and clonogenic potential of breast cancer cells [10]. Additionally, the LDHA inhibitor GEN140 could not suppress the growth of pancreatic cancer cells that utilized OXPHOS rather than glycolysis unless the compounds targeting OXPHOS, such as phenformin, were combined [132]. Overall, due to the enriched metabolic plasticity of cancer cells, future therapeutic strategies might consider targeting the hybrid glycolysis/OXPHOS phenotype and eliminating the capability of cancer cells for metabolic phenotype transition to improve cancer treatment outcomes.

The high-glycolysis phenotype of PDAC favors cancer cell metastasis by promoting EMT, angiogenesis and metastatic colonization. In addition to their cytosolic and nuclear roles in regulating metastasis, glycolytic enzymes can regulate metastasis through multiple pathways as extracellular nonenzymatic regulators [15, 16, 61, 93]. The secretory mechanisms of glycolytic enzymes may be associated with exosomes or microvesicles, and this requires further investigation. Additionally, the metabolic reprogramming of endothelial cells also contributes to cancer growth and metastasis significantly. Glycolysis in ECs benefits metastasis mainly due to the rapid increase in the energy supply, the massive increase in lactate production during pro-angiogenic signaling, and the binding of glycolytic enzymes to actin in invadopodium to directly supply ATP and decrease ROS-mediated apoptosis [133]. The investigation of metabolic factors in tumor ECs is expected to be a fruitful direction for the elucidation of tumor metabolism.

Finally, it is worth noting that metabolic factors can regulate PDAC metastasis by modifying the epigenetic status of cells, which is suggested by studies in which no metastasis-specific driver mutations were identified [14, 134]. Hence, reversible epigenetic modifications induced by enzymatic catalysis can be easily targeted for cancer therapy.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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