SYSTEMATIC REVIEWS

Glucose metabolic phenotype of pancreatic cancer

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Abstract

AIM: To construct a global "metabolic phenotype" of pancreatic ductal adenocarcinoma (PDAC) reflecting tumour-related metabolic enzyme expression.

METHODS: A systematic review of the literature was performed using OvidSP and PubMed databases using keywords "pancreatic cancer" and individual glycolytic and mitochondrial oxidative phosphorylation (MOP) enzymes. Both human and animal studies investigating the oncological effect of enzyme expression changes and inhibitors in both an *in vitro* and *in vivo* setting were included in the review. Data reporting changes in enzyme expression and the effects on PDAC cells, such as survival and metastatic potential, were extracted to construct a metabolic phenotype.

RESULTS: Seven hundred and ten papers were initially retrieved, and were screened to meet the review inclusion criteria. 107 unique articles were identified as reporting data involving glycolytic enzymes, and 28 articles involving MOP enzymes in PDAC. Data extraction followed a pre-defined protocol. There is consistent over-expression of glycolytic enzymes and lactate dehydrogenase in keeping with the Warburg effect to facilitate rapid adenosine-triphosphate production from glycolysis. Certain isoforms of these enzymes were over-expressed specifically in PDAC. Altering expression levels of HK, PGI, FBA, enolase, PK-M2 and LDA-A with metabolic inhibitors have shown a favourable effect on PDAC, thus identifying these as potential therapeutic targets. However, the Warburg effect on MOP enzymes is less clear, with different expression levels at different points in the Krebs cycle resulting in a fundamental change of metabolite levels, suggesting that other essential anabolic pathways are being stimulated.

CONCLUSION: Further characterisation of the PDAC metabolic phenotype is necessary as currently there

are few clinical studies and no successful clinical trials targeting metabolic enzymes.

Core tip: Our systematic review constructs a global "metabolic phenotype" of pancreatic ductal adeno-carcinoma (PDAC) reflecting tumour-related metabolic enzyme expression. We show that the Warburg effect is consistently demonstrated, with the over-expression of glycolytic enzymes and lacate dehydrogenase to facilitate rapid adenosine-triphosphate (ATP) production from glycolysis. We also show that the Warburg effect on mitochrondial oxidative phosphorylation in PDAC is more varied and not solely focused on ATP production, but also to stimulate other anabolic pathways for the purposes of tumourigencity. The metabolic phenotype provides an overview essential to elucidating the pathological changes that occur in PDAC.

INTRODUCTION

Pancreatic cancer (PC), typically ductal adenocarcinoma (PDAC), is the 13th most common cancer[1] contributing to 250000 deaths annually worldwide and accounting for 3.6% of cancer deaths and 0.5% of all deaths[2]. Despite advances in diagnostic technology and treatment modalities, there has been no significant improvement over the last three decades, with five-year survival remaining below 7%. Recent progress in genetics has led to a renewed interest in the Warburg Effect described in 1956 by German physiologist Otto Warburg[3] who postulated that carcinogenesis was the result of "irreversible injuring of respiration". Eukaryotic cells utilise glycolysis to derive energy, where glucose is broken down over a series of enzymatic steps to produce adenosine-triphosphate (ATP) and pyruvate. In aerobic respiration, pyruvate is oxidised in the Krebs cycle to produce ATP and NADH (nicotinamide adenine dinucleotide hydride). Mitochondrial oxidative phosphorylation (MOP) then occurs *via* a series of redox reactions to generate more ATP from NADH. Overall, between 30 and 36 ATP are generated from 1 molecule of glucose. In the absence of an adequate oxygen supply, anaerobic fermentation occurs, reducing pyruvate to lactate and converting NADH into NAD^+ (nicotinamide adenine dinucleotide) for use in

further glycolysis reactions. The energy released per glucose molecule in anaerobic respiration is only 2 ATP; per mole, this is 18-fold less than aerobic respiration but at a much faster rate of several hundred times[4]. The ratio of MOP and anaerobic fermentation is reduced in cancer cells[5-7], such as the Henrietta Lacks (HeLa) cervical cancer cell line where approximately 80% of glucose uptake undergoes glycolysis, and only 5% enters the Krebs cycle[8]. Warburg proposed that this "morphological inferiority" would change highly differentiated cells into undifferentiated cells that can divide, grow and lead to cancer.

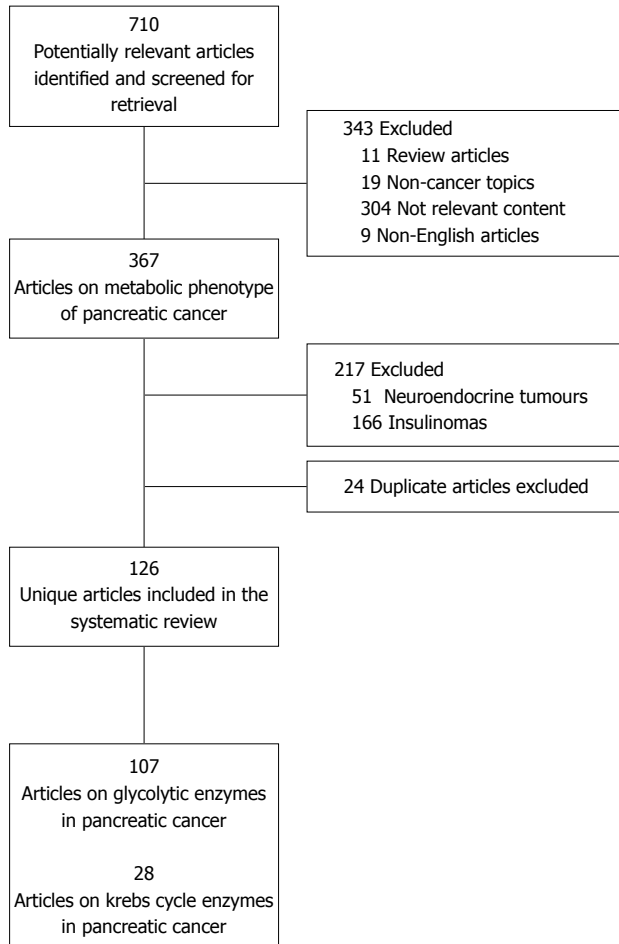Hypoxia is one stress factor in the tumour micro-environment that is thought to lead to this switch[9]. Hypoxia-inducible factor 1 (HIF-1) is an important regulator of cellular oxygen homeostasis[10], but is also up-regulated in many cancers, including pancreatic, gastric, lung, breast and hepatic cancers[11-14]. HIF-1 up-regulates most glycolytic enzymes, including hexokinase II, the first enzyme in the glycolysis pathway[15], and reduces MOP by up-regulating pyruvate dehydrogenase kinase I, responsible for inactivating the pyruvate dehydrogenase complex that subsequently stops pyruvate decarboxylation for entry into the Krebs cycle[16]. HIF-1 also up-regulates other genes including vascular endothelial growth factor (VEGF, a known promoter of tumour angiogenesis[17]) and the glucose transporter protein, Glut-1, facilitating glucose influx[11].

The Warburg Effect is likely a result of mutations in oncogenes and tumour suppressor genes with several pathways contributing to this "metabolic switch"[18]. This study undertakes a systematic literature review of changes in enzyme expression and the resulting metabolite levels in both the glycolytic and MOP pathways in PDAC in order to construct a 'metabolic phenotype' of this disease. New potential therapeutic targets can be identified within this phenotype for further study as novel treatments for PDAC.

MATERIALS AND METHODS

Literature search strategy
A systematic review of the literature was performed using OvidSP and the PubMed database. Search terms for individual glycolytic enzymes (hexokinase, phosphoglucose isomerase, phosphofructokinase, aldolase, triosephosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, phosphoglycerate mutase, enolase, pyruvate kinase and lactate dehydrogenase) and Krebs cycle enzymes (pyruvate dehydrogenase, pyruvate carboxylase, citrate synthase, aconitase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, succinyl-CoA synthase, succinic dehydrogenase, fumarase and malate dehydrogenase) were combined with key words "PC" and the Boolean "AND" operator (*e.g.*, "Hexokinase and *PC*"). Human and animal *in vivo* studies, as well as *in vitro* studies involving cell

Figure 1 Preferred reporting items for systematic reviews and meta-analyses flowchart of articles found and screened in the systematic review.

lines, were included. The initial search yielded 710 results, and after excluding review articles, non-cancer articles and those with non-relevant content, 367 articles were analysed. A further 217 articles describing pancreatic cancers of histology other than PDAC, such as carcinoid and other neuroendocrine tumours were excluded. Finally, duplicate articles (24) were identified and excluded. One hundred and twenty-six publications were identified as meeting the inclusion criteria of this systematic review looking at the metabolic phenotype of PDAC (Figure 1). Of these, 107 unique articles describe glycolytic enzymes (Table 1) and 28 unique articles describe the MOP pathway (Table 2) in PDAC.

Eligibility, data extraction and analysis
Studies describing the biochemical mechanisms involved in the physiological, onco-pathological or manipulation of metabolic pathways and/or individual metabolic enzymes in PDAC were included. For quality assurance, information was extracted following a predefined protocol from the text of each article, including changes in expression of metabolic enzymes and/or in the metabolic pathways and their overall effect on normal and PDAC phenotype. The effect on

cell viability and growth was also noted, particularly if a metabolic inhibitor was used, as was evidence implicating known oncogenic pathways. The data were collated (Tables 1 and 2) using Microsoft Excel (Microsoft, Richmond, United States) for analysis. A PDAC "metabolic phenotype" was constructed using these data (Tables 3-5), and the results presented as the hexose and triose stage of glycolysis, anaerobic fermentation and aerobic respiration (Krebs Cycle).

RESULTS

Hexose stage of glycolysis
Hexokinase: Hexokinase (HK) is the first enzyme in glycolysis, and exists as 4 isoforms (HKI - Ⅲ, and glucokinase). It phosphorylates glucose into glucose-6-phosphate (G6P), and represents the rate-limiting step in glycolysis[19]. G6P is transported into mitochondria and immediately used for ATP production *via* glycolysis, or for nucleic acid synthesis *via* the pentose-phosphate shunt[20]. Three HK isoforms (Ⅰ, Ⅱ and Ⅲ) are competitively inhibited by 2-Deoxy-D-glucose, whereas 3-bromopyruvate (3-BP) selectively inhibits HK-Ⅱ[21].

Several studies have shown HK to be over-expressed in PDAC[22,23] with expression levels varying by tumour histology. Ductal tumours, for example, take up more glucose and express HK-Ⅰ and Ⅱ more than acinar variants[24]. Direct inhibition with 3-BP reduces cell survival and increase necrosis in PDAC[25,26] and Panc-1 cell lines[27]. Treatment of Panc-1 with 3-BP also affects cell signalling, significantly reducing the expression of the GTPase signal transduction KRas (Kirsten rat sarcoma) pathway, as well as the Akt (protein kinase B) and mTOR (mammalian target of rapamycin) pathways, to induce cell necrosis[27]. Inhibiting the mTOR pathway in Panc-1 using everolimus reduces the expression of HK-Ⅱ and glycolysis, which inhibits cell proliferation and induces apoptosis[28].

The up-regulation of HK is partly due to the HIF-1 pathway in hypoxic conditions[29]. An increase in extracellular glucose is responsible for increasing HIF-1 expression (and subsequently intracellular ATP) whilst inhibiting mitochondrial activity in MiaPaCa2 cells[30]. An interaction between insulin and HIF-1 has also been suggested[31], with type 2 diabetes mellitus associated with the development of PDAC in patients who have a particular HK Ⅱ (genotype R844K) or glucokinase (ICS1+9652C>T) variant[32,33]. Clinically, a high level of HK-Ⅱ expression is associated with longer survival, with different variants of HK Ⅱ (such as N692N) associated with differing clinical outcomes[33].

Phosphoglucose isomerase: Phosphoglucose iso-merase (PGI) reversibly catalyses glucose-6-phosphate to fructose-6-phosphate. Previously identified as the autocrine motility factor (AMF)[34], PGI binds to the gp78/AMFR receptor[35] to stimulate cell migration and metastasis[36]. Under hypoxic conditions, PGI expression

Table 1 Systematic review of the literature involving glycolytic enzymes and pancreatic ductal adenocarcinoma

Glycolytic enzyme	In vitro studies		In vivo/clinical studies	
	Ref.	Summary	Ref.	Summary
Hexokinase (HK)	[23-30,32,33,153-155]	Induced by hypoxia. Higher expression in PDAC than acinar cells. Suppresses mitochondrial ATP production	[31,153,156,157]	HK-2 expression suggests an unfavourable clinical outcome
Phosphoglucose isomerase (PGI)	[29,38-40,158]	Stimulates cell migration and metastatic potential. Induced by hypoxia. β-1 integrins are stimulated by PGI Upregulated in PDAC epithelia.	[39]	Over-expression contributes to a more aggressive PDAC phenotype
Phosphofructokinase (PFK)	[11,48]			
Aldolase	[49,52,54]	Overexpressed in PDAC. Induced by hypoxia. Delays apoptosis.	[52,53]	Highly expressed in PDAC where HIF-1α is constitutively expressed. Inhibition prolongs survival
Triosephosphate isomerase (TPI)	[55,56]	Overexpressed in PDAC		
Glyceradehyde-3-Phosphate Dehydrogenase (G3PD)	[27,50,55,57,58,61,159]	Overexpressed in PDAC. Increases PDAC metabolic activity, and disrupts downstream apoptotic capases	[50,58]	Increased expression. Possible biomarker candidate.
Phosphoglycerate kinase (PK)	[62-66,160]	Overexpressed in PDAC. Angiogenesis promoter	[63]	No significant increase in expression in a murine model, but fivefold increase in activity.
Phosphoglycerate mutase (PGM)	[67,68]	M-isoform is under-expressed and B-isoform is over-expressed in murine models		
Enolase	[55-58,69-83]	Overexpressed in PDAC. Promotes cell migration and metastasis. Biomarker candidate	[58,69,76,77,161-163]	Increased expression. Induced antibodies to enolase-alpha correlates to a better outcome
Pyruvate kinase (PK)	[108,109,117,121,122, 157,164-166]	Overexpressed in PDAC. Dimeric form favours synthesis; Tetrameric form favours energy production; PK-M2 used as a tumour marker	[110,112,115,119,120, 157,167,168]	M2 isoform levels correlate to tumour metastasis. HK-2 and M2 expression indicates an unfavourable clinical outcome
Lactate dehydrogenase (LDH)	[28,89,123-138,164, 169-179]	Overexpressed in PDAC. Down regulated by graviola	[153,180-183]	Down regulated by graviola to reduce tumorigenicity and metastasis

HIF: Hypoxia-inducible factor; PDAC: Pancreatic ductal adenocarcinoma.

Table 2 Systematic review of the literature involving Krebs cycle enzymes and pancreatic ductal adenocarcinoma

Glycolytic Enzyme	In vitro/In vivo/clinical studies	
	Ref.	Summary
Pyruvate dehydrogenase	[30,109,131,132,140,154,164,170,171,173,177, 179,180,184-188]	Inhibition of pyruvate dehydrogenase kinase (the activity of which is regulated by pyruvate dehydrogenase) stimulates the Krebs cycle and reverses the Warburg effect.
Pyruvate carboxylase	[141]	Over expressed in human and murine PDAC
Citrate synthase	[67,124,179]	Over expressed in PDAC. Activity of citrate synthase also higher in PDAC compared to normal tissue
Aconitase	[67]	Mitochrondrial isoform under-expressed
Isocitrate dehydrogenase	[67,158,189,190]	Regulated by HuR (an RNA-binding protein) in PDAC.
a-ketoglutarate dehydrogenase	[67,187]	Marginally increased in murine PDAC
Succinyl-CoA synthetase	[67]	Reduced expression
Succinic dehydrogenase	[67,128]	Over expressed in human and murine PDAC
Fumarase	[30]	HIF-1α increases fumarate by inhibiting distal mitochondrial metabolisms
Malate dehydrogenase	[50,64,67]	Over expressed in human and murine PDAC. Possible candidate biomarker

HIF: Hypoxia-inducible factor; PDAC: Pancreatic ductal adenocarcinoma.

is regulated in part by the HIF pathway[29,37] and has been found to be over-expressed in the Capan-2 cell line. PGI increases the metastatic potential of PDAC[38] and MiaPaCa-2 cells transfected with PGI grow more aggressively with an increase in tumour mass[39]. Down-regulation of E-cadherin expression

- a protein involved in cell adhesion - also occurs. HIF-1 expression in PDAC can be inhibited by 3-(5'-hydroxymethyl-2'-furyl)-1-benzyl indazole, which subsequently reduces PGI mRNA expression; this has the effect of reducing overall cell viability and increasing apoptosis rates[40,41]. Herceptin has

Table 3 Summary of changes in glycolysis in pancreatic ductal adenocarcinoma

Glycolytic enzyme	Encoding gene	Change in PDAC	Implicated pathways	Known inhibitors	Inhibitor effect in PDAC
Hexokinase			HIF	2-Deoxy-D-glucose	
Hexokinase I	10q22	Up	mTor		
Hexokinase II	2p13	Up	K-ras	3-BP[21]; Lonidamine, Everolimus	Reduced PDAC survival/induces PDAC necrosis
Hexokinase III	5q35.2	-	Akt		
Glucokinase	7p15.3-p15.1				
Phosphoglucose Isomerase (also known as Autocrine Motility Factor)	19q13.1	Up	HIF; Apoptosis	Insulin-like growth factor binding protein-3; Herceptin; 3-(5'-hydroxymethyl-2'-furyl)-1-benzyl indazole	Reduces overall cell viability and increases apoptosis rates
Phosphofructokinase			HIF	Aurintricarboxylic acid[191]	Inhibits fatty acid synthesis in rat hepatocytes
PFK-M (muscle type)	12q13.3	Down			
PFK-L (liver type)	21q22.3	Up			
PFK-P (platelet type)	10p15.3-p15.2	Up			
Aldolase			HIF	3-fluro-D-glucose; 4-fluro-D-glucose	
Aldolase-A	16p11.2	Up		3-[2-hydroxyethyl(methyl)amino]-2-quinoxalinecarbonitrile 1,4-dioxide	Reduces PDAC proliferation and tumour volume
Aldolase-B	5q22	Down			
Aldolase-C	17cen-q12	Up			
Triose Phosphate Isomerase	12p13	Up		2-phosphoglycolate; D-glycerol-1-phosphate[192]	
Glyceraldehyde Phosphate Dehydrogenase			HIF; p53	Iodoacetate[27]; gossypol[21]	Reduces cell survival and induces necrosis. No effect on K-ras
GAPDHS	12p13	Up			
GAPDHS (testes-specific)	19q13.12				
Phosphoglycerate Kinase			HIF	1,3-bisphosphoglycerate[193]	
PGK1	Xq13.3	Up			
PGK2	6p12.3	?			
Phosphoglycerate Mutase			p53	Inositol hexakisphosphate[194]	
PGM-B	1p31	Up			
PGM-M	4p14	Down			
Enolase			c-Myc	Sodium fluoride[21]; D-tartonate; 3-aminoenolpyruvate 2-phosphate[195]	
ENO1 (alpha)	1p36.2	Up			
ENO2 (gamma, neuronal)	12.p13	Up			
ENO3 (beta, muscle)	17pter-p11	-			
Pyruvate Kinase			Tyrosine kinase		
Isoform PK-M1 (liver/RBC)	1q21	-	Akt/c-Myc		
Isoform PK-M2 (muscle)	15q22	Up		L-phospholactate; M2-PK-binding peptide aptamers[196]	Anti-cancer effects in animal models
Dimeric form					
Tetrameric form					

HIF: Hypoxia-inducible factor; PDAC: Pancreatic ductal adenocarcinoma.

Table 4 Summary of changes in anaerobic fermentation in pancreatic ductal adenocarcinoma

Glycolytic enzyme	Encoding gene	Change in PDAC	Implicated pathways	Known inhibitors	Inhibitor effect in PDAC
Lactate dehydrogenase				Oxamate	Inhibits PDAC growth
Lactate dehydrogenase A	11p15.4	Up	c-Myc; mTor	Aryl-substituted N-hydroxyindole-2-carboxylates; Everolimus (by blocking the mTor pathway[28])	Inhibits PDAC growth
Lactate dehydrogenase B	1p12.2-p12.1	Up			
Lactate dehydrogenase C	11p15.1	-			

PDAC: Pancreatic ductal adenocarcinoma.

been shown to inhibit the expression of PGI and potentiate the effects of other PGI inhibitors[42]. Beta-1 integrins (receptors that facilitate binding between neighbouring cells) are stimulated by PGI, up-regulating cell adhesion, invasion and metastasis possibly by a signalling pathway involving protein kinase C[43]. Beta-1 integrins have been shown to be highly expressed in several PDAC cell lines[44] which may explain its high metastatic potential. PGI has also been shown to regulate the expression of apoptotic

Glycolytic Enzyme	Encoding gene	Change in PDAC	Implicated pathways	Known inhibitors	Inhibitor effect in PDAC
Table 5 Summary of changes in aerobic respiration in pancreatic ductal adenocarcinoma					
Pyruvate dehydrogenase complex	11p13		HIF-1	Dichloroacetate (inhibits PDC regulator, pyruvate dehydrogenase kinase)	Stimulates Krebs Cycle and reverses Warburg effect; Reduces PDAC proliferation and viability
Pyruvate dehydrogenase				Stimulator: Lipoic acid	Reduces cancer cell viability
Pyruvate dehydrogenase α	Xp22.1	Down			
Pyruvate dehydrogenase β	3p21.1-p14.2	Down			
Pyruvate carboxylase	11q13.4-q13.5	Up/down[1]		Avidin	
Citrate synthase	12q13.2	Up		Succinyl-CoA	
Aconitase				Flouroacetate	
Aconitase 1 (soluble)	9p21.1				
Aconitase 2 (mitochondrial)	22q13.2	Down			
Isocitrate dehydrogenase					
ICD (soluble)	2q33.3				
ICD (mitochondrial)	15q26.1	Down			
Oxoglurate (α-ketoglutarate) Dehydrogenase	7p14-p13	Up			
Succinyl-CoA synthetase		Down			
Succinic dehydrogenase		Up/down[1]			
Fumarase	1q42.1			D-malic, trans-aconitic, citrate, glycine	
Malate dehydrogenase					
MD (soluble)	2p13.3				
MD (mitochondrial)	7cen-q22	Up			

[1]Under-expressed in animal PDAC. PDAC: Pancreatic ductal adenocarcinoma.

protease activating factor 1 (Apaf-1) and caspase-9 genes involved in apoptosis[45].

Phosphofructokinase: Phosphofructokinase (PFK) phosphorylates fructose-6-phosphate into fructose 1,6-bisphosphate[19]. It has been referred to as the "pacemaker of carbohydrate metabolism" as it also regulates a wide range of sugars, including fructose and galactose that can feed into glycolysis[21]. The activity of PFK is regulated by fructose-2,6-bisphosphate, which itself is regulated by 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatases, encoded by 4 genes (PFKFB1-4)[46]. PFKFB expression (particularly isoenzyme 3 which exhibits the highest kinase/bisphosphatase activity) is altered in lung, gastric and pancreatic cancers[11,47,48]. Hypoxia also up-regulates the expression of PFKFB-3 and -4 in Panc-1 cells *via* the HIF-1α dependent pathway[11].

Fructose bisphosphate aldolase: Fructose bisphosphate aldolase (FBA) splits fructose 1,6-biphosphate into glyceraldehyde-3-phosphate (G3P) and dihydroxyacetone phosphate. Three different isoenzymes exist (A, B and C) and are encoded by 3 different genes[21]. FBA-A is overexpressed in PDAC and pancreatic cystadenoma[49], and proteome analysis of murine PDAC has also shown an overexpression of FBA-C[50]. Overexpression of FBA delays apoptosis, as does the addition of the end product G3P, by suppressing caspase-3 activity[51]. Under hypoxic conditions, PDAC cells expressing HIF-1 also express FBA-A and Glut-1 (glucose transporter 1) more highly, making the cell more resistant to apoptosis[52]. The hypoxic cytotoxin 3-[2-hydroxyethyl(methyl)amino]-2-quinoxaline-carbonitrile 1,4-dioxide inhibits the expression of FBA-A together with HIF-1, which subsequently reduces the proliferation of PDAC cells *in vivo* and reduced tumour volumes in models of *in vitro* murine cancer[53]. Suppression of HIF-1 also reduces the overexpression of FBA-A in PDAC and subsequently reduces *in vivo* tumorigenicity[54].

Triose phosphate isomerase: Triose phosphate isomerase (TPI) reversibly isomerises dihydroxyacetone into G3P. TPI is overexpressed in pancreatic cancer in several studies[55,56], but no correlation, however, is found between TPI-1 expression and tumour staging.

Triose stage of glycolysis
Glyceraldehyde phosphate dehydrogenase: Glyceraldehyde phosphate dehydrogenase (GAPDH) reversibly catalyses G3P into 1,3-biphosphoglycerate, and is over-expressed in PDAC[50,55,57,58], as well as other adenocarcinomas such as prostate[59] and breast[60]. GAPDH and Glut transporter over-expression is thought to be partially responsible for an increase in PDAC metabolic capacity[61]. Inhibition with iodoacetate on Panc-1 cells reduces survival and induces necrosis[27] but interestingly, did not significantly reduce the induction of signalling pathways K-ras, Akt and mTOR, as seen with 3-BP.

Phosphoglycerate kinase: Phosphoglycerate kinase (PGK) reversibly dephosphorylates 1,3-biphosphoglycerate into 3-phosphoglycerate, and is over-expressed in human PDAC[62]. In contrast, a study

involving hamster PDAC cell lines demonstrated no overall increase in expression levels, but did show a significant fivefold increase in PGK activity[63]. Clinically, its use as a diagnostic marker has been suggested due to its high antibody reactivity and high accuracy in distinguishing between cancer and non-cancer[64-66].

Phosphoglycerate mutase

Phosphoglycerate mutase (PGM) reversibly catalyses 3-phosphoglycerate into 2-phosphoglycerate, and exists as two isoenzymes (M and B). Expression levels of PGM-M have been found to be under-expressed, and PGM-B over-expressed, in rat PDAC[67]. PGM expression is regulated by p53[68].

Enolase

Enolase reversibly catalyses 2-phosphoglycerate into phosphoenolpyruvate, and is over-expressed in PDAC, particularly α-enolase[55-58,69-72]. It is also seen in pancreatic atypical hyperplasia[73] and intraepithelial neoplasia[74]. Its presence on the PDAC cell surface allows the promotion of cell migration and metastasis, but also induces a strong T-cell response leading it to be recognised as a tumour antigen[69,75,76]. As such, an increase in circulating enolase autoantibodies has been observed[76]. Animal studies have also shown good humoral and cellular immune responses against PDAC following inoculation with an α-enolase-encoded plasmid[77]. The resulting IgG response resulted in slower tumour progression and significantly improved survival. Several case reports have also clearly shown an over-expression of γ-enolase in serous microcystic adenomas[78,79], and solid-papillary[80], solid-pseudopapillary[81-86], solid-cystic papillary[87-102], and serous cystic[103,104] pancreatic tumours as well as PDAC[71].

Pyruvate kinase

Pyruvate kinase (PK) is the last enzymatic step in glycolysis, dephosphorylating phosphoenolpyruvate into pyruvate and converting ADP to ATP. Pyruvate then fuels the Krebs cycle. PK exists as 2 isoforms (M1 and M2); PK-M2 expressed exclusively in cancer cells (favouring anaerobic respiration) whereas the PK-M1 is predominately expressed in normal cells (favouring increased oxidative phosphorylation)[50,105,106]. Expression of the oncogenic tyrosine kinase receptor pathways inactivate PK to disrupt the pathway between glycolysis and MOP to perpetuate the Warburg effect[107]. Furthermore, two forms of PK-M2 have been identified - a tetrameric form favouring ATP production and a dimeric form that channels glucose into synthesis. The hypoxic and acidified tumour microenvironment staved of glucose favours the dimeric form of PK-M2[108]. Inhibition of the Akt/c-Myc (myelocytomatosis) pathway inhibits the activity of PK-M2 to down-regulate glycolysis[109]. TLN-232/CAP-232 (amino acid peptides targeting M2-PK) has also been shown to have anti-

cancer effects in animal models, and clinical trials are underway to ascertain its effectiveness in pancreatic cancer[21].

Several studies have shown an increase in serum PK-M2 in patients with metastatic PDAC[110], and other pancreatic conditions such as chronic pancreatitis[111]. Its presence has also led suggestions that it should be used as a tumour marker for diagnosis, prognosis[112] and surveillance[113,114] in both pancreatic and other gastrointestinal cancers[115-117], particularly in combination with another marker, such as Ca19-9[118] or CEA[119], to increase its specificity value[120]. Surprisingly, PK-M2 over-expression is not found immunohistochemically in premalignant or PDAC tissue samples[64,121]. It is also weakly expressed in some cell lines such as SK-PC-1[122].

Anaerobic fermentation - lactate dehydrogenase

Lactate dehydrogenase (LDH) reversibly catalyses pyruvate to lactate, and is a target gene of the c-Myc regulator[123]. There are five isoforms, with LDH-A being the primary and over-expressed isoform in PDAC[124-128], including cell lines Capan-1[129] and SW-1990[130]. Mass spectrometry studies on LDH from PDAC have shown differential methylation to the LDH from normal ductal cells[131]. PDAC LDH-A acetylation, which normally inhibits LDH-A and prepares it for lysosomal degradation is also reduced[132]. Forced expression and inhibition of LDH-A increases and reduces the rate of growth respectively[133]. The activity of LDH-A and subsequent lactate production can be inhibited by blocking the mTor pathway using everolimus. The transcription factor Forkhead box protein M1 (FOXM1) over-expresses LDH-A to increase tumorigenicity[134].

Other inhibitors of LDH-A, such as derivatives of aryl-substituted N-hydroxyindole-2-carboxylates, have also been shown to inhibit the growth of PDAC[135]. Oxidative stress and a subsequent reduction in ATP may be responsible for this inhibition[136]. Lactate in the PDAC microenvironment has also been shown to be immunosuppressive by directly inhibiting Natural Killer cells and preventing an innate response to tumour cells[137]. Clinically, a raised serum LDH levels can also be a poor prognostic indicator in PDAC[138].

Aerobic respiration - Krebs cycle

The pyruvate dehydrogenase complex (PDC) consists of pyruvate dehydrogenase (PDH), dihydrolipoyl transacetylase and dihydrolipoyl dehydrogenase, and is the first enzymatic reaction that converts pyruvate into acetyl Co-A for the Krebs cycle. PDC itself is regulated and inhibited by pyruvate dehydrogenase kinase (PDK). Three isoenzymatic forms of PDK have been found in eukaryotic cells, with PDK2 being the major form that regulates PDC[139]. Inhibition of PDK by dichloroacetate in Panc-1 cells has been shown to stimulate metabolism via the Krebs cycle and away

from glycolysis, with the effect of reducing Panc-1 proliferation and viability[140].

Expression levels of other Krebs cycle enzymes are also changed in PDAC (Table 5). Malate dehydrogenase has been shown to be over-expressed in both human[64] and animal PDAC[50] cell lines, as has succinic dehydrogenase in mice PDAC[128], and pyruvate carboxylase[141]. The activity of citrate synthase was found to be higher in PDAC tissue than in normal pancreatic tissue by up to 20%[67]. The expression levels of mitochondrial citrate synthase and α-ketoglutarate dehydrogenase were found to be marginally increased in recent rat PDAC mRNA microarray studies by Yabushita *et al*[67]; pyruvate carboxylase, aconitase, isocitrate dehydrogenase, succinic dehydrogenase, succinic-CoA ligase and malate dehydrogenase expression were found to be reduced. Transcriptomic profiling from the same dataset also showed an increase in anaerobic glycolysis and nucleotide degradation and a reduction in Krebs cycle activity.

There is evidence that the mitochondrial oxidative phosphorylation (MOP) pathway can be used as a therapeutic target[142,143]. In glucose-limiting conditions, MOP inhibitors have been shown to be cytotoxic to Panc-1 cells. Momose *et al*[144] report that treatment with efrapeptin F (a fungal toxin that acts as a potent ATPase inhibitor) was cytotoxic to Panc-1 cells if they were cultured in nutrient-deficient media and, importantly, in glucose limiting conditions. Panc-1 cells grown in nutrient-deficient media were found to have reduced levels of ATP, and were sensitive to MOP inhibitors, suggesting that mitochondrial oxidative phosphorylation contributes to intracellular ATP production.

DISCUSSION

This detailed overview demonstrates that the metabolic phenotype of pancreatic cells is altered in PDAC where the Warburg effect increases glucose utilisation to fuel the pathological metabolic requirements of neoplastic cells. Enzymes at almost every stage of glycolysis are over-expressed. The rate at which glucose is transported into cells is also increased, and it has long been known that an over-expression of glucose transporters (particularly subtypes Glut-1 and Glut-3 in PDAC[145]) is associated with cancer progression and poor tumour prognosis[145-148]. Glut-1 expression in pancreas neoplasia correlates to tumour size and histological grading, from low grade PanIN 1A dysplastic lesions which are devoid of Glut-1, to PDAC where most cases showed some degree of GLUT-1 expression[149]. Serous cystadenomas consistently exhibit Glut-1 expression[150]. With such differences in expression between normal and cancerous tissue, the Glut family represents a potential therapeutic target. 2-Deoxyglucose, a metabolic inhibitor of Glut, hexokinase and phosphoglucose isomerase, inhibits growth by as much as 59% in Panc-1 cells and over

95% in MIAPaCa-2 after 2 d of treatment[151]. A similar but less profound effect was seen when the cells were treated with oxamate, a competitive metabolic inhibitor of lactate dehydrogenase at the final step of glycolysis. The reduction of cell proliferation is more profound under hypoxic conditions, where Glut-1 expression levels were increased and were more sensitive to inhibition.

The enzyme changes in metabolic phenotype of PDAC is complex, with variable changes in expression levels. Altering expression levels of HK, PGI, FBA, enolase, PK-M2 and LDA-A with metabolic inhibitors have shown a favourable effect on PDAC, thus identifying these as potential therapeutic targets.

To date, there are no clinical trials involving metabolic inhibitors and PDAC. There is, however, progress in using metabolic inhibitors in cell types other than PDAC which show a translation to *in vivo* treatment. Ko *et al*[148] have reported the use of 3-BP as an anticancer drug tested on the highly glycolytic hepatocellular cancer cell line AS-30D, where it was shown to rapidly and completely deplete intracellular ATP levels whilst not affecting levels in normal hepatocytes. Cell viability was ultimately affected, dropping to 10% with the ATP depletion. The *in vivo* effects of 3-BP in an animal model confirmed this anti-cancer effect. More recently, Ko *et al*[152] published a case study of a 16 year old male with primary hepatocellular cancer treated with 3-BP. The patient responded to treatment with tumour destruction (confirmed by computed tomography) and went on to survive 2 years. Clearly, the use of metabolic inhibitors as a treatment for cancer is at an early stage. By "blocking" glycolysis, there will be a global and unpredictable effect on all cellular functions, and present as an obstacle when translating *in vitro* cell line research into clinical practice. By using a metabolic phenotype to identify pathologically over-expressed enzymes, a more targeting approach can differentiate a patient's normal enzymes to that of PDAC.

In summary, the Warburg Effect has been long been recognised to occur in cancer cells, and describes a "metabolic switch" in the way cells use glucose to produce ATP. This overview highlights the extensive changes that in PDAC to produce a distinct metabolic phenotype. This phenotype has potential clinical correlates in that distinct components may be amenable to therapeutic manipulation.

COMMENTS

Background

Pancreatic cancer, typically ductal adenocarcinoma (PDAC), is an insidious cancer with poor outcomes that has seen no significant improvement over the last three decades. Recent progress in genetics has lead to a renewed interest in the Warburg effect, which describes the pathological switch from mitochondrial phosphorylation to glycolysis for rapid ATP production, thus presenting new potential therapeutic targets. The primary aim of this review is to provide a comprehensive overview of the metabolic phenotype of PDAC, particularly where metabolic inhibitors have shown a favourable response. The second aim is to identify steps in the metabolic pathways where there is little or

no research evidence to show its oncological importance.

Research frontiers

The Warburg effect was described in 1956 by German physiologist Otto Warburg who postulated that carcinogenesis was the result of "irreversible injuring of respiration". Data from high throughput techniques, such as genomic and metabolomic studies, have shown the Warburg effect is much more complex and extends to biomass synthesis for tumour proliferation.

Innovations and breakthroughs

There have been numerous studies looking at individual metabolic enzymes and the effect of inhibiting these enzymes on tumour cell physiology. Retrieved manuscripts concerning the metabolic enzyme and their role in PDAC were reviewed by the authors, and the data extracted to construct an overall "metabolic phenotype".

Applications

The metabolic phenotype of PDAC illustrates the role of individual enzymes, and the known effects on PDAC. Inhibiting certain enzymes - hexokinase, phosphoglucose isomerase, aldolase, pyruvate kinase and lactate dehydrogenase - has been shown to interfere with PDAC cell function. The review also highlights areas (and thus unidentified therapeutic targets) in the pathways, particularly in mitochondrial oxidative phosphorylation, where there is little research into the effects of metabolic inhibition.

Terminology

The Warburg effect describes the pathological switch from mitochondrial phosphorylation to glycolysis for rapid ATP production. The change in enzyme expression at certain points of these pathways facilitating the Warburg effect may be potentially exploitable as a therapeutic target.

Peer-review

Simply "blocking" major pathways such as glycolysis in an *in vitro* cell line environment may yield successful in reducing cell growth or tumourigenicity, but would also have a non-specific effect on normal cells leading to as yet unknown toxic effects. This presents a challenge of using such broad inhibitors in an *in vivo* or clinical setting. A detailed metabolic phenotype is therefore useful in identifying and targeting specific oncological changes and so would theoretically have less effect on normal cells.

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