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Diabetes Mellitus and Reprogrammed Glucose Metabolism in Pancreatic Cancer: Features for Clinical Translation

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Abstract

The reprogrammed metabolism of cancer cells underlies the shift of glucose energetics from the highly efficient oxidative phosphorylation to the less efficient aerobic glycolysis, the Warburg effect. This phenomenon, with the activation of the glutamine pathway, advantages survival and proliferation of pancreatic ductal adenocarcinoma (PDAC) cells, which live in an adverse hypoxic and nutrient restricted microenvironment. In PDAC, glucose metabolic alterations occur also at the whole organism, Diabetes Mellitus (DM) being diagnosed in approximately 60% to 80% of patients. The association between PDAC and DM is a dual face phenomenon, DM being both a risk factor for and a consequence of this tumor type. Data from epidemiology indicate that longstanding DM increases PDAC risk 1.5 to 2.0 fold, probably because of the pro-proliferative effects of hyperinsulinemia. By contrast early onset DM, i.e. diabetes diagnosed no more than two years prior to cancer diagnosis, is considered a consequence of PDAC. Secondary DM is due to complex interactions between tumor cells, tumor microenvironment and pancreatic endocrine cells. In this scenario the role of the inflammatory S100A8 calcium binding protein, matrix metalloproteinases, Vanin1 or amylin has been experimentally demonstrated. However, the efforts made to translate in the clinical practice any individual new poteantial biomarker failed, because none reached enough sensitivity and specificity to be considered a reliable biomarker to diagnose PDAC even in high risk subjects as those with new onset DM. Therefore the identification and clinical validation of new biomarkers remains a challenge for future studies.

Introduction

The glucose metabolism alterations present at both the cancer cell site and throughout the organism level in cancer patients are particularly evident in pancreatic ductal adenocarcinoma (PDAC), the fourth leading cause of cancer related deaths [1]. Glucose transporter GLUT1 over expression at the cancer cell site favours the uptake of glucose, the main source for cellular energetics, on which in PET imaging the use of the tracer ^{18}fluorodeoxyglucose is based [2]. In cancer cells glucose metabolism is reprogrammed and, even in the presence of oxygen, glucose is mainly processed in the cytosol to pyruvate, which largely escapes from the energy efficient Krebs cycle in the mitochondria [3]. This phenomenon, first described by Otto Warburg almost 100 years ago, and now known as Warburg's effect or "aerobic glycolysis", is considered one of the emerging hallmarks of cancer [4]. The clinical manifestation of altered glucose metabolism in the organism is diabetes mellitus, considered a risk factor for, and a consequence of, PDAC [5]. Although it is not known whether glucose metabolic alterations in the cancer cell, and in the entire organism, influence each other, it has been suggested that insulin and insulin-like growth factors play a part in cancer onset and progression [6,7].

Alterations in glucose metabolism at the cancer cell site

Although first described almost 100 years ago, renewed attention in the Warburg effect over the last few decades, has led to the definition of two main concepts:

1. Metabolic reprogramming is a feature of cancer cells contributing to proliferation and metastases [3,8];

2. Drugs targeting cancer metabolism might enhance the efficacy of chemotherapy [9].

In PDAC, cancer cells are dispersed within a hypovascular dense desmoplasia, which contributes to a hypoxic and nutrient deficient tumoral microenvironment. These features might limit the access of cancer cells to fuel and nutrients, indispensable for the biosynthesis of amino acids and nucleotides, required for cell proliferation. By reprogramming their metabolism, PDAC cells are

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Table 1: PDAC prevalence impacts on the positive (PPV) and negative (NPV) predictive values of biomarkers.

Disease prevalence	Biomarker Sensitivity	Biomarker Specificity	PPV	NPV
0.00013	0.90	0.90	0.0012	0.9999
0.03	0.90	0.90	0.2177	0.9966
0.00013	0.95	0.95	0.0025	0.9999
0.03	0.95	0.95	0.3701	0.9984
0.00013	0.99	0.99	0.0129	0.9999
0.03	0.99	0.99	0.7538	0.9997

enabled to support amino acids and nucleotide biosynthesis, thus deriving advantage from the adverse microenvironment. In cancer cells glucose uptake and glycolysis are favoured by the over expression of the glucose transporter GLUT1 and of a series of glycolytic enzymes, including lactate dehydrogenase (LDH, that converts pyruvate into lactate), hexokinase 2 (HK2, the first rate limiting enzyme of glycolysis) and pyruvate kinase M2 (PKM2, the final rate limiting enzyme of glycolysis) [2]. Even in the presence of oxygen, only a minimal part of pyruvate enters mitochondrial oxidative phosphorylation (OXPHOS), mainly being converted to lactate; this is due, at least in part, to the inactivation of pyruvate dehydrogenase (PDH, which converts pyruvate into acetyl-CoA for the TCA cycle). Lactate accumulates in the microenvironment and lowers pH, which induces the expression of matrix metalloproteinases, mainly MMP-2 and MMP-9 [3], while inhibiting the immune response [10], thus favouring the metastatic potential. Aerobic glycolysis is less efficient than OXPHOS in terms of energy supply: only four rather than 36 ATP moles per mole of glucose are produced. Energy supply by OXPHOS in cancer cells may be supported by the glutamine pathway, which also supports the biosynthesis of nucleotides, lipids and glutathione [11]. The metabolic reprogramming of cancer cells by means of aerobic glycolysis and glutaminolysis, appears to be closely correlated with the genetic landscape of cancer cells themselves. *KRAS* activating mutations, *TP53* loss of function and *MYC* over expression, frequently found in PDAC, regulate the Warburg's effect [2,9]. It has recently emerged that in tumours metabolic reprogramming is not restricted to cancer cells: this phenomenon, also known as the reverse Warburg effect, also involves stromal cells, such as cancer associated fibroblasts (CAFs). In pseudo-hypoxyc conditions, CAFs produce HIF-1 alpha, which promotes glycolysis with the production of lactate that further reduces pH, and glutamate, which might fuel cancer cells. This metabolic symbiosis also occurs between cancer cells and cancer stem cells [3], and between cancer cells and immune cells [12]. Intriguingly, elsewhere we observed a reverse Warburg effect in myoblasts, the magnitude of lactate production being correlated with PDAC-associated diabetes mellitus, suggesting that there is a link between alterations in glucose metabolism at the cancer cell site and in the whole organism [13].

Alterations in Glucose Metabolism in the Whole Organism

Diabetes mellitus as a cause of PDAC – evidence from epidemiology

The association between diabetes mellitus and PDAC has been recognised for over 100 years. Diabetes mellitus or reduced glucose tolerance are diagnosed in the majority of PDAC patients, i.e. 50% and 30-40% of cases, respectively [14]. This high association rate was soon to give rise to the question as to whether diabetes mellitus was the cause or effect of PDAC. Epidemiological and experimental studies conducted to address this issue have led to the conclusion that diabetes mellitus is a modest risk factor for PDAC, which is rather a cause of diabetes mellitus. In 2005 in their meta-analysis, Huxley et al. [15] analysed 17 case-control and 19 cohort studies and reported pooled risk estimates for PDAC among diabetics of 1.94 (95% CI: 1.53-2.46) and 1.73 (95% CI: 1.59-1.88), respectively. On considering studies investigating the association between PDAC and the duration of pre-existing diabetes mellitus, the authors reported that the shorter the duration of diabetes, the higher the risk of PDAC, the relative risk being 2.05 (95% CI: 1.87-2.25) for a duration of four years or less, 1.54 (95%CI: 1.31-1.81) for a duration above five and equal to or less than 9 years, and 1.51 (95%CI: 1.16-1.96) for a longstanding history of diabetes (>=10 years). The magnitude of the overall increase in PDAC risk among diabetics and its decreasing trend proportionate to the duration of diabetes has been confirmed in two large pooled analyses of data from US and European case control studies [16,17]. The increased PDAC risk associated with diabetes mellitus appears to be independent from geographic location [18], the risk estimates reported for eastern Asia being close to those of Europe and US [adjusted hazard ratio: 1.54 (95% CI 1.39–1.71) in Taiwan and 2.1 (95% CI 1.3–3.5) in Japan] [19,20]. Epidemiological studies exploring the inverse relationship between the duration of diabetes mellitus and PDAC risk agree that early onset diabetes mellitus is probably a manifestation of PDAC rather than a pre-existing condition, while longstanding diabetes mellitus increases the risk of PDAC [15,17,18,21,22]. However, consensus has not been attained concerning the time limit distinguishing early onset from longstanding diabetes mellitus. This time limit ranges from one to four years across studies [15,18], although the majority of authors agree with a duration of less than two years in defining the early onset form [17,21,22]. The difficulty in defining this temporal limit might also depend on the following: a) when PDAC develops on a ground of pre-existing longstanding diabetes mellitus, the tumour might progressively decompensate metabolic control and, in this case, the switch time from non-neoplastic to neoplastic diabetes might be extremely difficult to define; b) PDAC arises from precursor lesions, such as pancreatic intraepithelial neoplasia (PanIN) or intraductal papillary mucinous neoplasia (IPMN), its evolution and progression following a stepwise model similar to that described for the polyp adenocarcinoma sequence in colon cancer [23]. PDAC cells accumulate a series of molecular aberrations. Some, namely *KRAS*, *CDKN2A*, *TP53* and *SMAD4*, are highly frequent across tumours while others are sporadic, thus accounting for the extremely high molecular heterogeneity of this tumour type [24,25]. The stepwise accumulation of genetic defects during tumour progression might underlie a stepwise worsening of tumour-associated diabetes mellitus the progression of which might follow the evolution of the tumour, its clinical manifestations occurring and diagnosis being made after variable periods of mild hyperglycemia. This suggestion is borne out by

Table 2: Proposed biomarkers for the diagnosis of PDAC in the selected population of patients with diabetes mellitus.

Biomarker	Positive/Total PDAC cases (Sensitivity)	Negative/Total diabetes cases (Specificity%)	Negative/Total controls (Specificity%)	Reference Nr.
CA 19-9	39/68 (57%) PDAC with new onset DM	1812/2295 (79%) new onset DM	NA	[57]
CA 19-9	43/80 (54%) PDAC with new onset DM	78/85 (92%) new onset DM	76/80 (95%)	[58]
CA 19-9	47/60 (78%) PDAC with DM	42/43 (98%) Type 2 DM	29/30 (97%)	[40]
Plasma IAPP	17/30 (57%)	23/23 (100%)	24/25 (96%)	[59]
Plasma IAPP	22/60 (36%) PDAC with DM	8/9 (89%)	104/107 (97%)	[60]
Combined blood mRNA expression of vanin 1 (VNN1) and matrix metalloproteinase 9 (MMP9)	23/24 (96%) PDAC with DM	NA	19/25 (76%)	[61]
Blood mRNA expression of MMP9	31/60 (52%) PDAC with DM	37/43 (86%) Type 2 DM	30/30 (100%)	[40]
Serum cysteamine (downstream molecules of VNN1)	8/18 (44%) PDAC with new onset DM	13/15 (87%) new onset DM	15/15 (100%)	[54]
Plasma adrenomedullin	16/30 (53%) PDAC with new onset DM	24/27 (89%) new onset DM	25/28 (89%)	[52]
Combined serum miR-483-5p, miR-19a, miR-29a, miR-20a, miR-24, and miR-25	47/50 (95%) PDAC with new onset DM	30/50 (60%) new onset DM	30/50 (60%)	[58]
Combined model with CA 19-9, Apolipoprotein A1 and complement C3	51/57 (90%) PDAC with DM	54/68 (80%) Chronic pancreatitis with DM (n=17) and Type 2 DM (n=51)	NA	[62]

findings from the Mayo Clinic demonstrating a progressive increase in blood glucose starting from 36 months prior to PDAC diagnosis [26], thus supporting the proposal of screening asymptomatic individuals using new-onset hyperglycemia and diabetes as a first filter to detect those at a higher risk of PDAC. However this detection calls for a reliable biomarker of pancreatic cancer-associated diabetes mellitus [27].

Longstanding diabetes mellitus preceding PDAC is not a single entity, but a highly complex and heterogeneous disease. The heterogeneity is due to differences in comorbidities, medications, compensation, and in some cases, exposure to diabetogenic and/or carcinogenetic environmental factors. The metabolic syndrome, the main comorbidity of diabetes mellitus to have been investigated, is a complex of inter-related co-existing conditions, mainly insulin resistance and diabetes, hypertension, dyslipidemia and obesity. Epidemiological studies exploring the role of diabetes mellitus as a risk factor for PDAC while taking other components of the metabolic syndrome into account have confirmed that diabetes mellitus has an independent role, but have also highlighted an increased body mass index as a risk factor for PDAC [20,28]. Co-existent metabolic syndrome components appear to enhance the risk of PDAC [29]. Available treatments for diabetes mellitus mainly include insulin and oral antidiabetic drugs. Since it targets hepatocytes, adipocytes and muscle cells, insulin is the main glucose-regulating hormone. However, in other normal and transformed cell types this drug has a relevant pro-proliferative and pro-survival effects through direct or indirect effects, which include enhancing of insulin-like growth factor I activity [6]. It is therefore more than likely that diabetes mellitus increases PDAC risk because of hyperinsulinemia due to insulin resistance and/or insulin therapy. However, although some epidemiological studies support the assumption that insulin treatment increases PDAC risk among diabetics two to fivefold [16,22], other studies do not [30]. In a few epidemiological studies focusing on patients with type 1 diabetes mellitus, invariably treated with insulin, PDAC risk (RR: 2.0, 95% CI: 1.37-3.01) was similar to that of patients with type 2 diabetes mellitus [31], thus suggesting that insulin treatment in case of endogenous insulin deficiency has a neutral effect on the risk of PDAC. The protective or carcinogenetic effects of oral antidiabetics is still widely debated, even if treatment with metformin appears to slightly decrease, while sulfonylureas appear to increase, PDAC risk (30). A poor glycemic control in diabetics might be regarded as a potentially relevant risk factor for PDAC since chronic hyperglycemia can induce an increased tumour cell proliferation and migration by enhancing the release of the chemokine CXCL12 from stromal pancreatic stellate cells [32]. Although the specific aim of these studies was not to investigate how poor glycemic control impacts on PDAC risk, in a population of male smokers fasting glucose was shown to correlate with PDAC risk [33], and in their dose response meta-analysis Liao et al. [34] found a linear dose-response relation between fasting blood glucose concentration and the rate of PDAC, every 0.56 mmol/L increase in fasting blood glucose being associated with a 14% increase in the rate of pancreatic cancer. Fasting glucose is, however, an imperfect index of the glycemic control, glycated haemoglobin (HbA_{1c}) being a much more reliable tracer of long-term glucose exposure. The pre-diagnostic levels of HbA_{1c} are also positively correlated with PDAC risk with a linear trend across increasing quartiles [35]. While in type 1 diabetes mellitus, insulin is lacking as a consequence of beta cell destruction, in type 2 diabetes mellitus hyperinsulinemia frequently occurs as a consequence of insulin resistance. Epidemiological studies have been conducted to investigate whether or not insulin resistance is the main predisposing factor for PDAC in diabetics. Stolzenberg-Solomon et al. used the HOMA-IR formula {[fasting insulin (mIU/L) x fasting glucose (mmol/L)]/22.5} to estimate insulin resistance and their findings indicate that an increased HOMA-IR is a risk factor for PDAC, this risk appearing to be greater when insulin resistance is diagnosed more than ten years before cancer. Wolpin et al. [33] used plasma proinsulin levels as a marker of peripheral insulin resistance in their study, which confirmed that insulin resistance was associated with an almost 2.5-fold increase in the risk of PDAC, the risk being even greater (3.6-fold) when insulin resistance was detected more than 10 years before cancer diagnosis [35]. In their study, Michaud et al. provided further evidence of the role of insulin resistance in increasing PDAC risk. These Authors found that elevated post-prandial C-peptide, a fragment enzymatically released from proinsulin in equimolar concentrations with insulin, enhances the PDAC risk 4.24-fold [36]. Hyperinsulinemia should therefore be considered one of the factors involved in PDAC carcinogenesis, in line with its pro-survival and pro-metastatic effects [6,7,37].

Diabetes mellitus as a consequence of PDAC – clinical and experimental evidence

The concept that early onset diabetes mellitus is a consequence of PDAC is supported not only by the epidemiological observations described in the previous section, but also by the clinical observation that overt diabetes mellitus or reduced glucose tolerance is found in more than 60% of patients at PDAC diagnosis [38-40], and that diabetes mellitus ameliorates after surgical removal of the tumor [41,42]. This last finding, furthermore, argues against the simple hypothesis that pancreatic cancer-associated diabetes mellitus is due to cancer-related islet cells destruction and supports the hypothesis that PDAC induces diabetes through the release of diabetogenic molecules, which might cause peripheral insulin resistance and/or impaired insulin release from beta-cells, both of which have been found in PDAC patients [43-45]. Another clinical issue concerns the impact of diabetes mellitus on the prognosis of patients with PDAC. Fasting glucose levels are positively associated with the overall cancer-related mortality [46], and survival after surgical removal of PDAC was shown to be significantly affected by uncontrolled longstanding severe hyperglycemia [47].

PDAC-associated diabetes mellitus and islet cell dysfunction

Several research groups, ours included, have thrown light on the pathophysiological mechanisms underlying PDAC-associated diabetes mellitus, which is due to a complex interplay between tumor and stromal-derived molecules, pancreatic endocrine cells and insulin targeted peripheral tissues/organs. The key player molecules in this process appear to be matrix metalloproteinases and the calcium binding protein, S100A8, a 10 kDa protein belonging to the family of S100 Ca^{2+} binding EF hand type proteins [48], which form homo- and hetero-complexes, S100A9 being the main binding partner of S100A8. The resulting S100A8/A9 heterodimer, also known as calprotectin, is normally produced and released by polymorphonuclear and mononuclear cells. The extracellular S100A8/A9 complex acts as a ligand for different receptors, including RAGE and TLR4. In PDAC, high S100A8 expression is found in the stromal compartment when tumor cells express the tumor suppressor gene *SMAD4*, while, when *SMAD4* is lost, S100A8 is no longer expressed by stromal cells, but by cancer cells [49]. This inverse relationship between *SMAD4* and S100A8 expression is further supported by findings made "in vitro": when pancreatic cancer cells without *SMAD4* expression, but with S100A8 expression are forced to express *SMAD4* by transfection, they lose their ability to express S100A8 [50]. The numerous biological effects of S100A8 in PDAC include epithelial to mesenchymal transition and the *SMAD4*-dependent inhibition, or activation, of pro-survival and pro-metastatic intracellular signalling pathways such as NF-κB, AKT and mTOR [51]. But S100A8 can also induce the expression of MMP8 and of MMP9 by inflammatory mononuclear cells [40]. Intriguingly, S100A8 is a substrate for metalloproteinases, which catalyse the release of the N-terminal 14 aminoacid peptide from the entire molecule; this, in turn, alters intracellular calcium fluxes and renders beta-cells insensitive to glucose stimulation, leading to a reduced insulin secretion, a potential cause of PDAC-associated diabetes mellitus [40,50]. It has also been demonstrated that glucose stimulated insulin secretion is reduced by adrenomedullin, a pluripotent hormone overexpressed in PDAC [52]. This hormone shares homology with amylin or islet amyloid polypeptide (IAPP), which is co-secreted with insulin by beta-cells at a constant ratio in the normal pancreas, while in the presence of PDAC-conditioned media, the IAPP/insulin molar ratio increases [53]. IAPP has also been found to reduce arginine stimulated insulin, glucagon and somatostatin release, and might play a part in determining islet dysfunction in PDAC patients [43]. It has been observed that beta-cell proliferation impairment with apoptosis induction is dependent on the enzyme overexpressed in PDAC, vanin 1 (VNN1), which hydrolyzes pantetheine and produces Vitamin B5 and cysteamine [54].

PDAC-associated diabetes mellitus and impaired glucose metabolism in peripheral tissues

Muscle, liver and fat cells are the principal targets of insulin and glucagon, the two main hormones regulating glucose homeostasis. By binding its receptor, insulin favours glucose entry and storage as glycogen in target cells, while glucagon, the counter regulatory hormone, has the opposite effect, inducing glyogenolysis and glucose extrusion. The Insulin Receptor (IR), a tetrameric structure made up of two alpha and two beta subunits, binds insulin through its alpha chains and triggers intracellular signalling by the tyrosine kinase activity of its beta chains. The analysis of the IR signalling cascade in skeletal muscle tissue from PDAC patients has demonstrated that insulin binding, tirosin kinase activity of the IR and the content of the insulin receptor binding substrate 1 (IRS1) did not change with respect to control tissue samples, while the phosphatidylinositol 3-kinase (PI3-K) activity, glucose transport and glycogen synthase activity were impaired in pancreatic cancer patients [43,45]. We demonstrated that pancreatic cancer cells impair glycolysis of both muscle and liver cells through the activity of a low molecular weight tumor product that favours the metabolic shift of glucose from oxidative phosphorylation to aerobic glycolysis (lactate accumulation) and, in liver cells, to triglyceride biosynthesis through the accumulation of the intermediate D-1,2-diacylglycerol [13,55].

Biomarkers of PDAC-associated diabetes mellitus

Experimental studies have been performed to verify the pathophysiology of PDAC-associated diabetes mellitus and to identify any potential tumor derived molecule involved in causing islet cell and or peripheral glucose metabolic alterations, the end point being to identify a biomarker able to distinguish between the presence or absence of PDAC in patients with new onset diabetes mellitus. When exploring emerging biomarkers in this setting, a careful consideration should be made of the disease prevalence, since it significantly impacts on positive and negative predictive values for any given combination of sensitivity and specificity of the studied biomarker. The prevalence of PDAC varies between unselected and selected populations: in the whole asymptomatic population, its prevalence appears almost equal to its incidence (13/100000 per year, 0.013%) [56], being 230 fold lower than that reported among the selected population of asymptomatic patients with diabetes mellitus (almost 3%) [57]. Based on prevalence, the positive and negative predictive values of a biomarker with a given sensitivity and specificity are reported in Table 1. PDAC prevalence impacts on the positive (PPV) and negative (NPV) predictive values of biomarkers. PDAC prevalence in the general population (0.00013) and among patients with new onset diabetes mellitus (0.03) were considered to calculate PPV and NPV of biomarkers with 90, 95 and 99% sensitivity and specificity. Negative predictive value (NPV): Sensitivity x (1-Prevalence)/Specificity (1-Prevalence) + (1-Sensitivity) x prevalence. Positive predictive value (PPV): Sensitivity x Prevalence/ Sensitivity x Prevalence + (1-Specificity) x (1-Prevalence) Clearly, in

the unselected general population, positive findings of a biomarker with a very high sensitivity and specificity (99%) are due to PDAC in about 1/100 cases, thus supporting the notion that PDAC screening of the general population is not recommended. By contrast, in selected patients, a biomarker with a sensitivity and specificity of 95% could allow the identification of a potentially relevant number of cases, i.e. 37 PDAC out of 100 patients with positive results. It is also clear that sensitivity and specificity should be at least 90% to limit over-diagnosis and over-use of invasive diagnostic procedures. The biomarkers suggested for diagnosing PDAC in the selected population of patients with new-onset diabetes mellitus, include the established CA 19-9 marker as well as emerging new potential biomarkers, such as proteins, peptides, microRNA and mRNA, the detailed description of which also in terms of sensitivity and specificity is reported in Table 2. Overall none of the proposed biomarkers is superior to the established marker CA 19-9 in terms of sensitivity and specificity for PDAC diagnosis in patients with diabetes mellitus. Moreover, since none attain a sensitivity and specificity of at least 90%, their use cannot be supported in clinical practice.

In conclusion, the efforts made to translate in the clinical practice new potential biomarkers of PDAC-associated diabetes mellitus have failed, due to low sensitivity and specificity. Therefore the identification and clinical validation of new biomarkers remains a challenge for future studies.

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