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Pancreatic cancer-associated diabetes mellitus: An open field for proteomic applications

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Abstract

Background: Diabetes mellitus is associated with pancreatic cancer in more than 80% of the cases. Clinical, epidemiological, and experimental data indicate that pancreatic cancer causes diabetes mellitus by releasing soluble mediators which interfere with both beta-cell function and liver and muscle glucose metabolism.
Methods: We analysed, by matrix-assisted laser desorption ionization time of flight (MALDI-TOF), a series of pancreatic cancer cell lines conditioned media, pancreatic cancer patients' peripheral and portal sera, comparing them with controls and chronic pancreatitis patients' sera.
Results: MALDI-TOF analysis of pancreatic cancer cells conditioned media and patients' sera indicated a low molecular weight peptide to be the putative pancreatic cancer-associated diabetogenic factor. The sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis of tumor samples from diabetic and non-diabetic patients revealed the presence of a 1500 Da peptide only in diabetic patients. The amino acid sequence of this peptide corresponded to the N-terminal of an S-100 calcium binding protein, which was therefore suggested to be the pancreatic cancer-associated diabetogenic factor.
Conclusions: We identified a tumor-derived peptide of 14 amino acids sharing a 100% homology with an S-100 calcium binding protein, which is probably the pancreatic cancer-associated diabetogenic factor.

Keywords: Pancreatic cancer; Diabetes mellitus; MALDI-TOF; SDS-PAGE; Hepatocytes; Myoblasts

1. Introduction

Patients with pancreatic cancer have a dismal prognosis, and despite the availability of surgery and chemo-radiotherapy, the 5-year survival does not

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exceed 20% [1,2]. This rapidly evolving neoplasia significantly reduces the performance status of patients not only because it rapidly metastasizes to the loco-regional lymph nodes and distant organs, but also because it is associated with severe cachexia and glucose metabolic alterations [3–6]. It has been demonstrated that pancreatic cancer cells overexpress the glucose transporter GLUT1, which promotes an increased glucose uptake, and increases cellular proliferation and invasiveness, possibly by enhancing MMP-2 expression [7]. However, the main glucose metabolic alteration occurring in pancreatic cancer is represented by diabetes mellitus, which is diagnosed in more than 80% of the patients with this tumor [3,4]. In a series of 119 pancreatic cancer patients hospitalized in the Department of Medical and Surgical Sciences from 2000 to 2004, 71 (60%) were found to have diabetes mellitus, diagnosed on the basis of more than two fasting glucose levels above 126 mg/dl or on the basis of OGTT results, while 37 (31%) had reduced glucose tolerance. The remaining 11 patients (9%) had normal glucose tolerance, in agreement with findings made by Isaksson et al. [8].

Pancreatic cancer-associated diabetes has the following features: (1) it is usually of recent onset, being identified within a few months of the tumor diagnosis [3,4,9]; (2) the hormonal alterations found can be encompassed by the broad spectrum found both in type I and type II diabetes mellitus. In fact, fasting serum insulin levels may be significantly reduced, as occurs in type I diabetes mellitus, but they may also be normal or increased, as occurs in type II diabetes mellitus [3]; (3) the response of beta cells to physiological stimuli, such as glucagon or a meal, is usually impaired [10]; (4) peripheral insulin resistance is often found [11]; and (5) glucose tolerance ameliorates or even returns to normal after tumor resection [4,11]. Clinical data thus indicate that there is a strong association between pancreatic cancer and diabetes mellitus, and that the tumor is the cause of the glucose metabolism impairment found. However, the question of whether diabetes mellitus is caused by, or is a cause of, pancreatic cancer is still a widely debated issue, since different epidemiological studies conducted have yielded contradictory data. Findings made in a case–control study which enrolled 720 cases and 720 matched controls from Italy appear to support the hypothesis that pancreatic cancer causes diabetes mellitus [9]. However, findings made in another case–control study, from the United States, which enrolled 514 cases and 2047 matched controls favour to the hypothesis that diabetes mellitus is a risk factor for pancreatic cancer [12]. In another prospective cohort study made in the United States, involving 35,658 participants who had an oral glucose tolerance test (50 g) and a 25-year follow-up, it was estimated that the risk of pancreatic cancer in men is about twofold the risk in women, if the plasma glucose level after load is above 11.1 mmol/l [13]. Although it cannot be ruled out that diabetes mellitus may enhance the risk of pancreatic cancer, several experimental studies suggest that pancreatic cancer alters glucose metabolism, thus favoring the onset of diabetes, by altering both beta-cell function and glucose metabolism in peripheral tissues.

2. Beta-cell function

In patients with pancreatic cancer, the C-peptide response to glucagon or a test meal is impaired [10]. Furthermore, increased circulating levels of islet amyloid polypeptide (IAPP) have been described in patients with pancreatic cancer-associated diabetes mellitus [14,15]. This peptide, which co-localizes and is co-secreted with insulin by pancreatic beta cells, inhibits insulin secretion and decreases insulin sensitivity and, in the early 1990s, IAPP was therefore suggested to be the pancreatic cancer-associated diabetogenic factor [14]. However, since then, this hypothesis has not been further confirmed, although studies on IAPP secretion in vitro and in vivo confirmed that there is an altered beta-cell function in the presence of pancreatic cancer, with isolated islets incubated in vitro with pancreatic tumor cell conditioned media being stimulated to secrete IAPP rather than insulin [16,17]. Yet these results are contradicted by findings made in vivo: in pancreatic cancer patients, glucose was found to stimulate IAPP secretion in the absence of diabetes, while the release of this peptide in the bloodstream was found to be significantly reduced in the presence of diabetes [18]. It may therefore be concluded that pancreatic cancer alters beta-cell function by reducing insulin secretion after stimulation and by enhancing IAPP secretion. Moreover, these effects are probably caused by soluble mediators released by tumor cells.

3. Glucose metabolism in peripheral tissues

Glucose is a fundamental energy supply for all cell types: the net energy balance deriving from the catabolism of one glucose molecule is by 34 ATP molecules: 2 from glycolysis and 32 from mitochondrial oxidative phosphorylation. This fuel is so important that its circulating concentration is maintained within a restricted range by an exact homeostatic balance. The liver and the skeletal muscle mass are mainly involved in glucose homeostasis, due to the ability of liver and skeletal muscle cells to store and release glucose in response to the organism's needs by the synthesis and breakdown of glycogen. These processes are strictly regulated by insulin and the counter-regulatory hormones, mainly glucagon. A reduced peripheral insulin sensitivity has been described in pancreatic cancer patients with diabetes, as well as a reduced muscle glycogen accumulation secondary to a reduced glycogen synthase and the enhanced activity of glycogen phosphorylase enzymes [11,19]. Elsewhere it was demonstrated by us in vitro that pancreatic cancer cell conditioned media reduce glycolysis in isolated and perfused rat hepatocytes [20,21]. When these cells are incubated with pancreatic cancer cell conditioned media, they internalize glucose as control hepatocytes, but, unlike in controls, only a minor quantity of glucose is converted into lactate, being shifted into the triglyceride synthesis pathway, as evidenced by the accumulation of 1,2-diacylglycerol. As demonstrated by ourselves and other authors, glucose metabolism is also altered in skeletal muscle cells [22,23]. When myoblasts are conditioned by pancreatic cancer cell derived media, an enhanced glucose transformation into lactate is found. The enhanced glucose transformation into lactate by pancreatic cancer conditioned myoblasts is greater in tumors from patients with diabetes than in tumors from those with normal glucose tolerance [23]. The glucose metabolic alterations found in the muscle cells of patients with pancreatic cancer-associated diabetes mellitus appear to occur independent of insulin signaling: insulin receptor binding, insulin receptor tyrosine kinase activity, insulin receptor messenger RNA, and insulin receptor substrate-1 content. GLUT-4 and GLUT-4 mRNA were found to be normal in skeletal muscles from patients with pancreatic cancer-associated diabetes mellitus [19]. Yet alterations have been described in the activities of enzymes involved in glycogen synthesis and degradation, with glycogen synthase being reduced and glycogen phosphorylase activities being increased, thus having a net effect characterized by reduced glycogen synthesis and increased glycogen breakdown. The alterations in glucose metabolism found in myoblasts prepared with pancreatic cancer conditioned media are accompanied by the altered expression of several genes, some of which are over-expressed while others are down-regulated [23]. The expression levels of genes encoding for glycolytic enzymes (hexokinase 1, GSK3A, glycogen phosphorylase, pyruvate kinase, enolase 3, aldolase A, GAPDH, phosphofructokinase, pyruvate dehydrogenase, isocitrate dehydrogenase 2, glycogenin, isocitrate dehydrogenase 3 gamma, succinyl CoA synthetase, succinate dehydrogenase, malate dehydrogenase 1, phosphoglycerate mutase 2) were not altered by pancreatic cancer conditioned media. Yet tumor conditioned media were found to cause: (a) an overexpression of genes involved in protein synthesis (18%), protein catabolism (18%), RNA processing (14%), cell cycle control (23%), and energy metabolism (27%); and (b) a down-regulation of genes involved in protein synthesis (27%), muscle development and contraction (33%), vesicle transport (13%), signal transduction and oxidation (20%), and energy metabolism (7%). Two genes in particular, found to be over-expressed in pancreatic cancer conditioned myoblasts, might be linked to an altered glucose metabolism: propionyl CoA carboxylase and isocitrate dehydrogenase 3 beta (IDH3B). Propionyl CoA carboxylase converts propionyl CoA derived from methionine, isoleucine, valine, and unpaired fatty acids into succinyl CoA, which enters the TCA cycle. This may cause an acceleration of the TCA cycle, which, in turn, would lead to a reduction in muscle glycogen synthesis while ATP and NADH accumulation inhibits pyruvate dehydrogenase, and this might explain lactate accumulation. IDH3B converts oxaloacetate into phosphoenolpyruvate, which may be utilized for glucose synthesis. These data might indicate that glucose metabolic alterations encountered in pancreatic cancer conditioned myoblasts occur secondary to alterations in mitochondrial energy metabolism, rather than to alterations in cytoplasmic glycolysis.

4. Identification of the pancreatic cancer-associated diabetogenic factor

In vitro studies indicate that the effects of pancreatic cancer on beta cells, hepatocytes, and myoblasts are probably mediated by soluble factors. When pancreatic cancer cell lines conditioned media are fractionated according to different molecular weight ranges, the metabolic effects of the entire media are reproduced by the fractions with a low molecular weight (less than 10,000 Da) [21,23]. Elsewhere, in order to better identify the molecular weight of this product, we analysed by matrix-assisted laser desorption ionization time of flight (MALDI-TOF) a series of pancreatic cancer cell lines conditioned media, pancreatic cancer patients' peripheral and portal sera, comparing them with non-conditioned media and control or chronic pancreatitis patients' sera [24,25]. Two fragments were identified in four pancreatic cancer cell lines, which were absent in the control medium: their *m*/*z*, corresponding to the molecular mass, were 1874 and 2030 respectively. Of the range of low molecular weight peptides detected in patients' sera, only a few (those at *m*/*z* 2030 and 2727) were also detected in at least one pancreatic cancer cell line conditioned media (Table 1). Among the numerous peptides identified in pancreatic cancer patients' peripheral sera, only that at *m*/*z* 2030 was correlated with the presence of diabetes mellitus. We therefore suggested this peptide to be the pancreatic cancer-associated diabetogenic factor [24]. The pattern of low molecular weight peptides in the portal sera of pancreatic cancer patients is different from that found in the peripheral sera and only one peptide, at *m*/*z* 5002, was found to be positively correlated with diabetes mellitus [25]. The 5002 *m*/*z* peptide, present in pancreatic cancer patients' peripheral sera and in the vast majority of control subjects, was not correlated with the presence of diabetes mellitus. The 5002 *m*/*z* peptide produced by the neoplastic pancreas may act on liver cells, altering their glucose metabolism. A liver-derived degradation product of this peptide, with a lower molecular weight, might be released into the bloodstream, thus reaching muscle cells, where it may interfere with their glucose metabolism.

Table 1
Main low molecular weight peptides found by MALDI-TOF in sera from control subjects (CS), patients with pancreatic cancer (PC), and chronic pancreatitis (CP)

m/*z*	CS (*n* = 10)	PC (*n* = 14)	CP (*n* = 9)
1240	7 (70%)	6 (43%)	3 (33%)
1327	0	0	3 (33%)
2013	6 (60%)	12 (86%)	7 (78%)
2030	1 (10%)	6 (43%)	3 (33%)
2134	0	4 (29%)	3 (33%)
2298	0	0	3 (33%)
2308	0	3 (21%)	0
2399	5 (50%)	12 (86%)	6 (66%)
2413	0	8 (57%)	1 (11%)
2517	0	4 (29%)	4 (44%)
2586	0	6 (43%)	1 (11%)
2727	0	11 (79%)	3 (33%)
3143	0	8 (57%)	5 (55%)
3597	0	8 (57%)	1 (11%)
3813	0	10 (71%)	3 (33%)
4068	0	6 (43%)	4 (44%)
4218	0	10 (71%)	3 (33%)
5002	4 (40%)	14 (100%)	8 (89%)

The numbers and percentages (in parentheses) of positive cases are reported. The *m*/*z* 2030 and 2727 peptides were also found in pancreatic cancer cell lines conditioned media, but not in nonconditioned media.

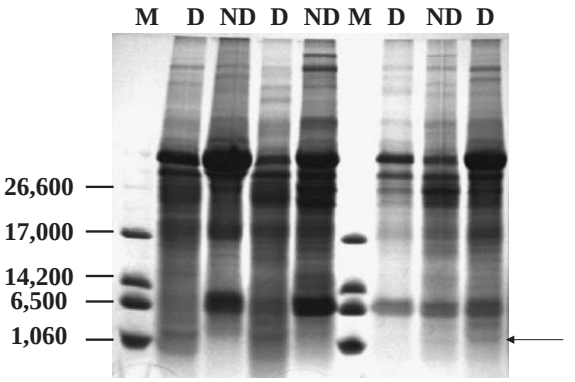

Fig. 1. SDS-PAGE of pancreatic cancer tumor samples obtained from patients with (D) or without (ND) diabetes mellitus. M = molecular weight marker. The arrow indicates a peptide band of about 1500 Da evident in patients with, not in those without, diabetes mellitus.

The sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis of cancer tissues revealed a protein band of about 1500 Da in samples from diabetic patients which were not present in non-diabetics (Fig. 1). Protein sequencing revealed that this 14 amino acid peptide was the N-terminal of an S-100 calcium binding protein [26–29]. When the synthetic peptide was incubated with cultured myo-

blasts, it caused an inhibition of lactate accumulation and a reduced glucose utilization, thus suggesting that it probably plays a significant role in the pathogenesis of pancreatic cancer-associated diabetes mellitus.

In conclusion: (i) diabetes mellitus might be a risk factor for pancreatic adenocarcinoma, but pancreatic carcinoma causes diabetes mellitus; (ii) the pancreatic cancer-associated diabetogenic factor, a low molecular weight peptide (<10,000 Da), acts on liver and muscle cells, and probably on beta cells; (iii) while the pancreatic cancer-associated diabetogenic factor may alter the activity of glycolytic enzymes, it does not appear to alter their transcription. Proteins involved in mitochondrial function, vesicle transport, and trafficking may be alternative targets of the effects of the pancreatic cancer-associated diabetogenic factor; (iv) we recently identified a tumor-derived peptide of 14 amino acids sharing a 100% homology with an S-100 calcium binding protein, which alters glucose metabolism in myoblasts and which is probably the pancreatic cancer-associated diabetogenic factor.

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