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Type 2 Diabetes and Pancreatic Cancer – A Possible Reason

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1. Introduction

Nearly 80% patients of pancreatic cancer (PC) have impaired glucose metabolism, either frank diabetes or impaired glucose tolerance (IGT)[1, 2] and the majority of diabetes associated with PC is diagnosed either concomitantly or during the two years before the diagnosis of PC[3] Karmody and Kyle reported that PC was diagnosed within one year from the onset of the diabetes in 40 out of 51 patients (78.4%)[4] According to Gullo et al.[5], diabetes in patients with PC is frequently (56.1%) of the recent onset type and is presumably caused by the tumor. In this study, in 43.9% of the patients, the diagnosis of diabetes preceeded the diagnosis of PC by three years and in 37.2% it was five or more years. In a more recent study, the diagnosis of PC and diabetes was made concomitantly or shortly before the tumor diagnosis in 65% of the patients [6]. Presently, a number of investigators consider diabetes to be a clinical manifestation of PC rather than a risk factor for the disease. This view is in sharp contrat to views that diabetes is a predisposing factor for PC. In many retrospective case-control and prospective cohort studies, an association between longstanding diabetes and an increased rate of subsequent death from PC was indicated [7-9]. In recent studies, however, when the latency period between the onset of diabetes and PC was considered, the issue became muddled. In some studies, when the patients with short latency periods were excluded from the sample size, the relative risk of diabetes for PC was markedly diminished[3-5]. In another population-based case control study, a significant positive trend in risk for PC was detected (p=0.016) in patients with diabetes diagnosed ten or more years prior to cancer detection[10].The meta-analysis of more than 20 epidemiologic studies indicated the relative risk of PC for diabetics diagnosed at least five years prior to the diagnosis of cancer as 2.0[11]. In two prospective cohort studies with more than 20 years follow-up, an increased risk was found among subjects with high post-load plasma glucose levels [12, 13]. In the latter study, the risk was 2.2-fold higher for participants whose post-load glucose level was at least 200 mg/dl at baseline compared with those with the levels equal or less than 119 mg/dl. These studies evidently show that glucose intolerance may preceed the onset of PC rather than just being a consequence.

The major problem of such epidemiological studies is that the exact onset time of both diabetes and PC is obscure and insidious. The non-insulin-dependent diabetes mellitus may take more than seven years before it is clinically diagnosed [14]. The latency of PC is also unclear, and the development of some cancers seems to take ten years[15] or even longer[16]. Undiagnosed diabetes or PC may have proceeded for many years without diagnosis. For

these reasons, estimation of the exact duration needed to conclude that the diabetes occurred before or after the development of PC is difficult.

The incidental pancreatic cancer detection indicates that this cancer can remain silent for a considerable time or grow very slowly [17]. Nevertheless, the notion that diabetes is a predisposing factor for PC remains questionable. Data gained from experimental pancreatic cancer model unquestionably indicate that the IGT is associated with the development of pancreatic cancer. We performed the following study to understand reasons for the development of diabetes in pancreatic cancer.

Eight-week-old out bred Syrian Golden hamsters (SGH) of the Eppley colony were used. They were housed in the centralized Comparative Medicine Animal Facilities, an AAALAC International accredited animal facility, in plastic cages on corncob bedding (Bed-O-Cobs, The Anderson Cob Co., Maumee, OH) under standard laboratory conditions (temperature, 21 ± 2°C; humidity, 40±5%; light/dark cycle, 12 hr/12 hr; 10x air changes/hr). They were fed a commercial diet (Wayne Lab Blox, Allied Mills, Chicago, IL) and had free access to tap water. The maintenance and humane treatment of the animals followed the guidelines of the UNMC Animal Care and Use Committee.

For the determination of glucose metabolism, five randomly selected 8-week-old male SGH with an average weight of 100 g were treated with the potent pancreatic carcinogen, N-nitrosobis(2-oxopropyl)amine (BOP) at a dose of 10 mg/kg body weight once a weak for four weeks. The same number of animals served as controls. Ten weeks after the last BOP injection, at the time generally proliferative and hyperplastic lesions appears, glucose tolerance was determined in all hamsters as reported [18]. Following the test the pancreas of all hamsters was examined histologically.

The presence of Insulin and glucagon assay in pancreatic juice and plasma was investigated in thirty 8-week-old male SGH with an average weight of 100g, who were treated with BOP as above. Thirty hamsters of the same age and weights were served as controls. In each group, the insulin content of pancreatic juice and plasma were assayed in 10 hamsters each at 12, 16 and 20 weeks after the last BOP injection as reported [19, 20].

From our tumor archieve, the pancreatic tissue of 30 SGH with tumors induced by BOP at a dose of 10 mg/kg body weight weekly for six weeks were examined immunohistochemically using antibodies to insulin, glucagon, somatostatin and PP with a multilabeling technique, developed in our laboratory [21]. Sixty seven surgically removed human pancreatic cancer specimens from our previous study [22] were also subjected to immunohistochemical examination for the expression of insulin, glucagon, somatostatin and PP as above.

The results of these experiments showed that at 10 weeks after BOP treatment plasma insulin level did not change compared to that in untreated control hamsters, whereas the glucose level increased significantly at 120 minutes (Fig. 1). Histologically focal or multi-focal ductal and ductular hyperplasia and in one hamster ductal in situ carcinoma were found. As in our previous studies, intrainsular ductular proliferation were found in all hamsters.

Insulin assay in pancreatic juice and plasma: As summarized in Table 1, although at week 12 the plasma insulin concentration did not differ from the control value, it decreased by one-half at week 16 but was significantly lower at week 20. On the contrary the level of insulin in pancreatic juice increased successively and was significantly decreased significantly and successively while juice insulin level conversely increased significantly by time (Table 1). The glucagon level in plasma did not change significantly at either point, whereas its level increased significantly and successively and was the highest at 20 week (Table 2).

Proliferative and hyperplastic ductal lesions were found at week 12 and 16 and a few in situ carcinomas and a microcarcinoma at week 20. A remarkably large number of endocrine cells (primarily β-cells) were found in hyperplastic ducts and in the microcarcinoma (Fig. 2), as well as some of hybrid type containing endocrine granules and mucin.
Immunohistochemically in all hyperplastic, premalignant and malignant lesions in human tissues, scattered or generally a large number of endocrine cells, composed primarily of insulin and glucagon, and less frequently somatostatin cells were found in the basal layer, but sometimes within the papillary fonts (Fig. 2). Insulin immunoreactivity was also present in the luminal content of hyperplastic ducts and malignant glands.

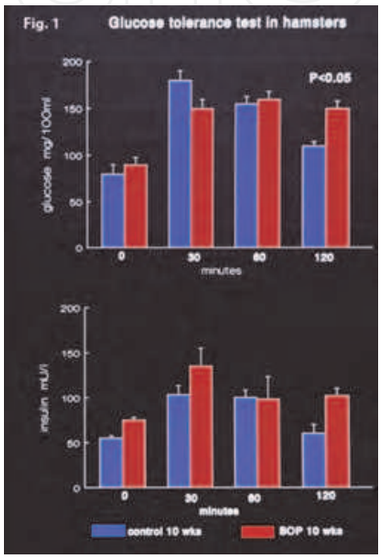

Fig. 1. Glucose tolerance test in SGH treated with BOP weekly for 6 weeks (red) and in untreated controls (blue)

Week	Plasma	Juice
12	6.07±1.96	4.28±1.40
16	3.02±1.67	5.07±2.05
20	2.52±0.12*	8.43±2.40*
*p<0.05 compared to control values		

Table 1. Insulin Levels (μU/ml)

Week	Plasma	Juice
12	0.62±0.07	25.8±4.40*
16	3.02±1.67	35.4±2.19*
20	2.52±0.12	39.4±3.10*
*p<0.001 compared to control values		

Table 2. Levels of Glucagon (ng/ml)

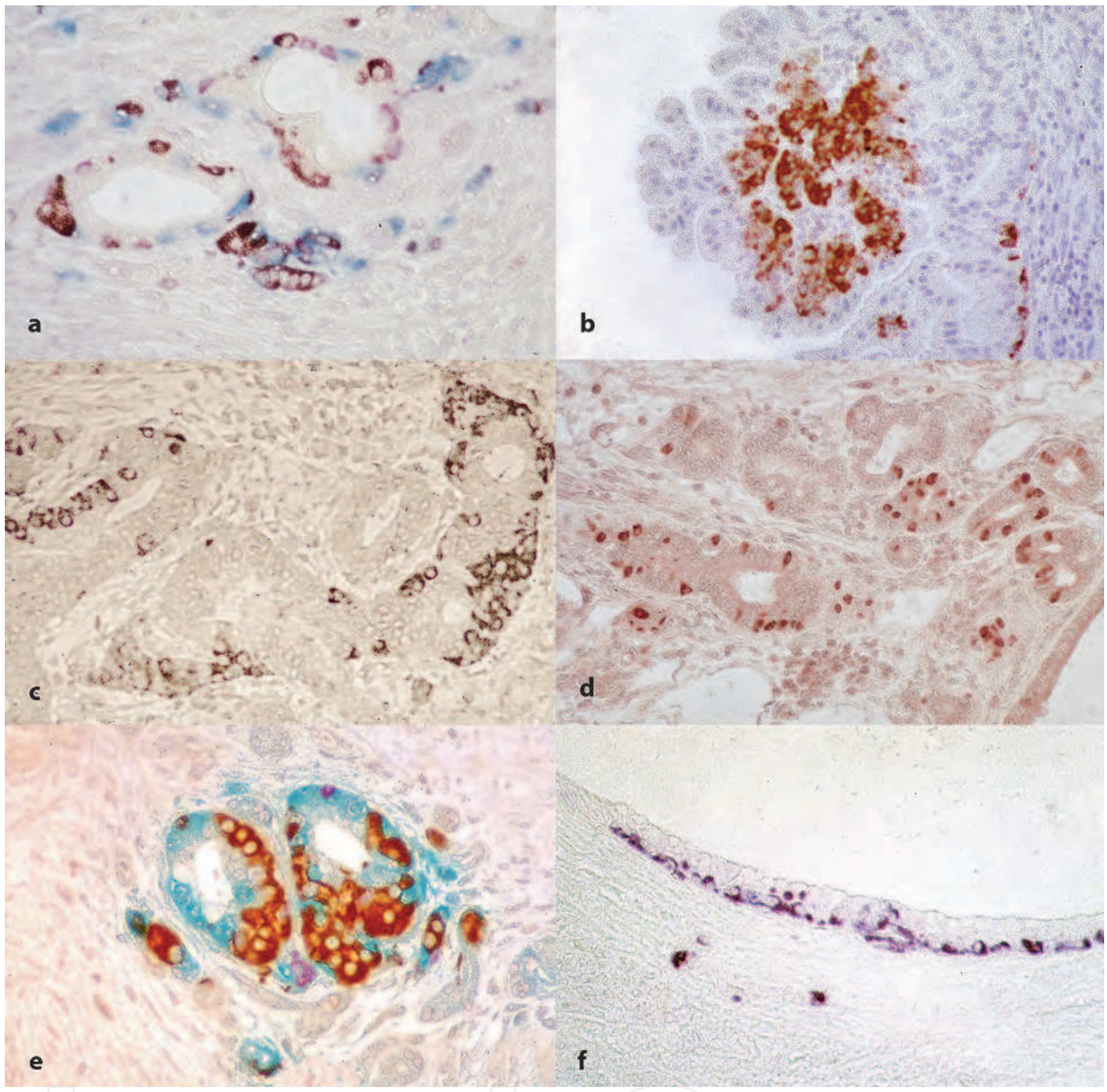

Fig. 2. Endocrine cells in pancreatic cancer. a) hamster adenocarcinoma. Insulin (brown), glucagon (blue), somatostatin (red). Multilabeling technique x65. B) Insulin cells in papillary fond and in the basal layer of the malignant epithelial cells of a human intraductal cancer. x50. c) insulin cells in human pancreatic cancer. x50; d) Insulin cells in proliferative ductal lesion in a hamster. x50; e) a large number of endocrine cells in human pancreatic cancer. insulin (blue); glucagon (brown); somatostatin (red). Multilabeling technique x50), f. endocrine cells covering the whole length of the basal layer of a tumor in a patient. X45.

Similar to the findings in the hamster tissue, insulin, glucagon and, less frequently, somatostatin cells were found in hyperplastic, but in extremely large numbers in well differentiated malignant glandular structures in human PC. In many areas the number of endocrine cells exceeded the number of malignant cells in glandular structures (Fig. 2). Remarkably, in the lumen of several malignant glands, many cells and debris immunoreactive with anti-insulin were identified (Fig. 3).

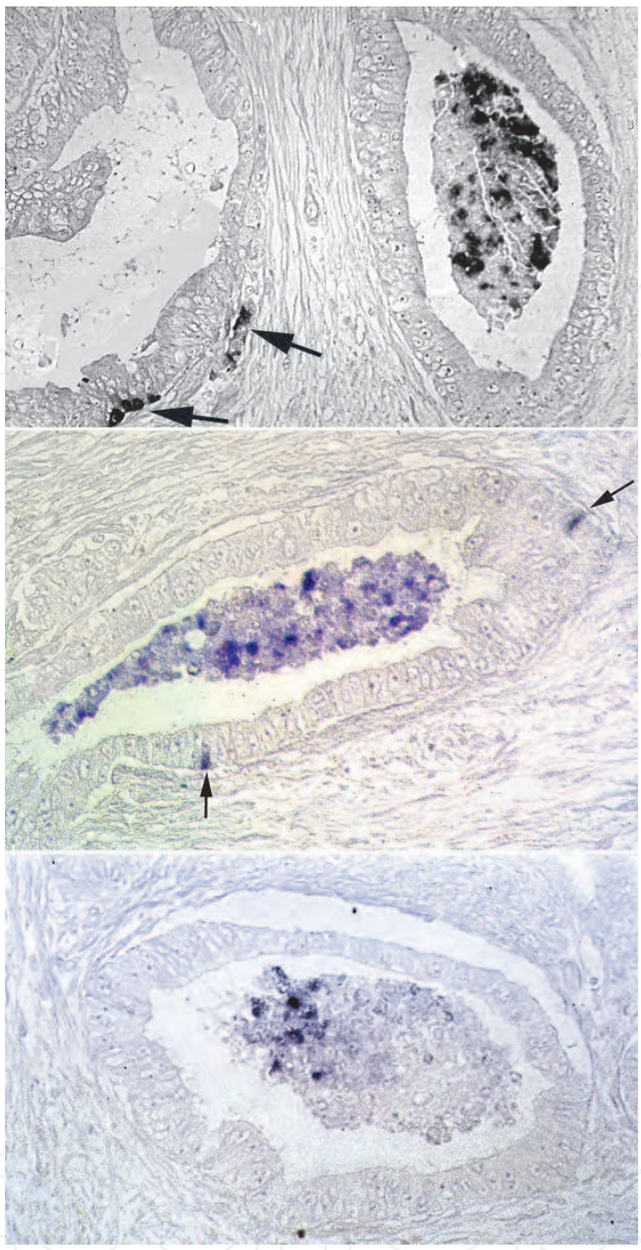

Fig. 3. Cells and debris immunoreactive with anti-insulin in the lumen of malignant gland structures. β cells are also present in the base of the epithelium (arrows). X65

Experimental models have opened avenues for studying areas of research that are impossible to obtain from humans. Among the existing pancreatic cancer model, SGH has provided the most relevant model for translational research. Morphologically, the wide spectrum of human pancreatic cancer, including the rare tumors, is reproducible in this species. Most human pancreatic cancer-associated antigens, including blood group antigens, as well as carbohydrate antigens are also expressed in cancers induced in hamsters. Most genetic mutations, methylation or deletions found in human pancreatic cancer, including the K-*ras*, $p16^{INK4A}$, DPC4/SMAD4, DCC and FHIT are also detected in hamster pancreatic

cancer[25]. Remarkably, the deletion of chromosome Y, frequently found in human pancreatic cancer [23, 24] occurs also in the hamster tumor. Moreover, among other existing PC models, including transgenic mouse models, the hamster model is the only model that shows insulin secretion abnormality, occurring in over 80% of PC patients.

Based on these similarities, the model offered a useful tool to understand the existing controversial view on the association between type 2 diabetes and PC. Although SGH are not prone to diabetes, under certain diet they present pre-diabetic condition such as peripheral insulin resistance, which, as in humans, can be controlled by Metformin[26]. Because in this model initial alteration occurs within the islets by formation of intrainsular ductular structures, which progressively leads to the development of cancer that destroys the islet [27], studies were performed to explore the effect of such alteration on glucose metabolism. Ahrén and Andrén-Sandberg, were the first to demonstrate the abnormality in glucose tolerance occurs at the very early stages of carcinogenesis[18]. In their study, where the glucose tolerance and insulin secretion during the development of pancreatic cancer was examined, the glucose tolerance and glucose-stimulated insulin secretion were found to be normal at 6, 12, and 18 weeks after start of carcinogen treatment compared with age-matched saline-injected controls. By contrast, after 24, 30, and 42 weeks, at which time proliferative and neoplastic lesions develop, an exaggerated plasma-glucose response and a concomitant impaired plasma-insulin response occurred during the glucose infusion ($P < 0.05$). Hence, it was conclude that the development of pancreatic cancer in this model is accompanied by glucose intolerance and impaired insulin secretion, and that these effects occur concomitantly with the development of cancer. In the present study using the same experimental protocol confirmed the development of IGT (Fig. 1). At the time of proliferative and hyperplastic ductal lesions.

Reasons for examining the pancreatic juice during pancreatic cancer development was based on our findings of insulin-like and growth hormone-like substances in pancreatic juice of untreated hamsters[19]. Literature search revealed that this discovery was not unique to hamsters and has been reported in pancreatic juice of humans and laboratory species [28-34] supporting the assumption for the existence of an insuloacinar portal system to regulate exocrine pancreatic functions by islet hormones[35]. The question on the mechanism of whether insulin enters into the ductal system by passive or active mechanism has remained illusive. Since CCK is a powerful stimulant for the release of insulin and somatostatin[34], the greater effect of CCK infusion in eliciting a higher concentration of immunoreactive insulin and somatostatin in the pancreatic juice[34] indicated that the hormones in the juice derive directly from the islets to the ductules[29]. This view is further supported by our electron microscopical findings that revealed the likelihood of the direct route of insulin into the ductal lumen, as insulin granules were found in large numbers in peri ductal and peri vascular spaces, suggesting that insulin secretion occurs into both blood vessels and ductal lumen[36]. Hence, It appears that in the normal condition pancreatic hormones is secreted into blood vessels and ductal system possibly in a controlled defined proportion. The physiological importance of insulin secretion via ductal system as a potent growth factor for gastrointestinal epithelium has been discussed by us elsewhere[36].

As stated earlier, the initial development of ductular structures within the islets with gradual proliferation, malignant alteration and gradual replacement of the islets [27]suggested alteration in insulin secretion. The development of mixed ductular-insular structures, suggesting intimate communication between islet cells and ductal cells prompted us to

examine the pancreatic juice of hamsters during tumor development. In a study, we measured the concentrations of insulin, glucagon, somatostatin, and islet amyloid polypeptide (IAPP) in plasma and secretin-stimulated pancreatic juice at 12 and 27 weeks after the treatment of hamsters with BOP[20]. At 12 weeks after BOP, plasma glucagon levels were significantly increased. An exaggerated plasma-glucose response were observed at 27 but not at 12 weeks after BOP. Plasma IAPP concentrations, but not glucagon or somatostatin, were elevated at 27 weeks. Tissue concentrations of IAPP were substantially reduced in BOP-treated hamsters at 27 weeks. The study, nevertheless, showed that islet hormone changes accompany the early development of pancreatic tumors in this model. Thus, the hormone changes and apparent insulin resistance resemble the metabolic changes found in humans with pancreatic cancer[20].

In the present study, a dramatic increase of insulin and glucagon levels was found in pancreatic juice of hamsters during pancreatic carcinogenesis indicating an alteration in islet function causing a shift in insulin secretion. Indeed, in both humans and hamsters islet cells during cancer development undergo alterations, including transdifferentiation of hormone producing cells into duct-like elements[23] that could explain the reduction in the level of plasma insulin. The level of insulin in pancreatic juice, however, is maintained and further increased by another event. In the hamster model and in human pancreatic cancer subjects as well, normal ducts, but more often altered ducts and especially malignant glandular structures show a large number of endocrine cells immunoreactive to ani-insulin and anti-glucagon antibodies, embedded within the epithelium ([27], and Fig 2). In some cases, the number of endocrine cells exceeded the number of the malignant epithelial cells (Fig 2). Within the malignant epithelium as well as in the lumen of several malignant glands many cells immunoreactive with anti-insulin were identified (Fig. 3). The finding suggested than insulin-containing cells, produced by malignant glands, are expelled into the glandular lumen, as are malignant cells, and thus into the pancreatic juice. Remarkably, we could not find any study examining the presence of hormones in pancreatic juice of pancreatic cancer patients, although, as stated earlier, endocrine hormones in pancreatic juice has been recognized many years ago in patients without pancreatic cancer.

The above information along with our current results let us conclude that the induction of proliferative and malignant lesions in the pancreas is associated with IGT, which also occurs in the majority of human cases. In fact, a study in Japan has shown that the incidence of IGT in PC patients was dependent on the size of cancer; in patients with tumor size >2.1 cm, the incidence of IGT was 56%, in tumors of 2.0 cm in size, it was 36%, in those with 1.1-2 cm, it was 39% and in 7 patients with tumors <1 cm, it was 28%. In the latter case, the abnormality was the only clinically detectable abnormality [37]. Remarkably, the determination of IGT has not yet been adapted as a routine test in the United States possibly awaiting additional validating data to be presented.

2. Conclusion

In SGH the development of PC is associated with IGT, as has also been suggested in humans. 2) In the normal pancreas, insulin is also secreted into pancreatic juice, both in humans and in animals. 3) The reduced level of insulin in blood but its increased concentration in pancreatic juice during pancreatic carcinogenesis is likely responsible for the IGT and diabetes, and 4) the development of IGT, along with hypersecretion of insulin in pancreatic juice could present early marker for PC.

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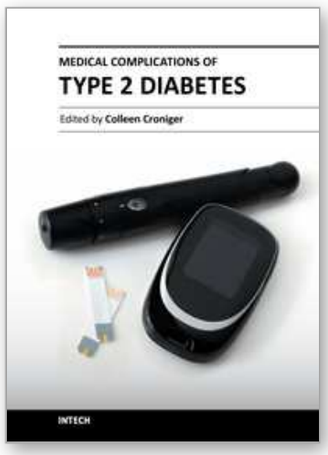

Medical Complications of Type 2 Diabetes

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Obesity and type 2 diabetes are increasing worldwide problems. In this book we reviewed insulin secretion in both healthy individuals and in patients with type 2 diabetes. Because of the risk associated with progression from insulin resistance to diabetes and cardiovascular complications increases along a continuum, we included several chapters on the damage of endothelial cells in type 2 diabetes and genetic influences on endothelial cell dysfunction. Cardiovascular complications occur at a much lower glucose levels, thus a review on the oral glucose tolerance test compared to other methods was included. The medical conditions associated with type 2 diabetes such as pancreatic cancer, sarcopenia and sleep disordered breathing with diabetes were also discussed. The book concludes with several chapters on the treatments for this disease offering us hope in prevention and successful alleviation of the co-morbidities associated with obesity and type 2 diabetes.

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