ORIGINAL ARTICLE

Pancreatic Adenocarcinoma, Multiple Insular-Ductualr Alterations and Malignant PPoma in a Patient Treated With Glp-1 Analogs

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ABSTRACT

Background According to numerous studies in the hamster pancreatic cancer model, islet cells are the most vulnerable to a carcinogen's insult and the induction of islet cell proliferation enhances the neoplastic process, however, the prevention of islet cell neogenesis inhibits it. In humans, however, discrepancies exist on the nature of the cancer progenitor cells. The recent discovery of islet cell expansion by GLP-1 analogs in type 2 diabetics offered a unique opportunity for comparative studies.

Materials and Methods The pancreatic tissue of a type 2 diabetic who was treated with Victoza and Byetta for 25 months and died about a year later from pancreatic cancer, was evaluated histologically and by immunohistochemistry. Results Differences were found relative to the patterns of the distribution of islets in the tissue derived from the ventral and dorsal anlage of the pancreas. In the glucagon-rich and PP-poor dorsal pancreas, most islets were well circumscribed and atrophic but many contained intrainsular ductules that were malignant in one case. In the PP-rich, glucagon-poor ventral pancreas, the islets were ill-defined, varied in size and shape, were much larger than in the control tissue, and in one area presented malignant growth. In the PP cell dominated area, islet cell transformation to normal, atypical and malignant ductular structures in various stages of differentiation were found, and remarkably in multiple sites. Conclusion This is the first report demonstrating that: 1) as in the hamster pancreatic cancer model, human islets also appear to present a source of pancreatic ductal neoplasms; and 2) the alterations seem to be related to the effects of GLP-1 analogs.

INTRODUCTION

No clues have emerged on the histogenesis of pancreatic cancer in humans. Induction of acinar cell lesions by chemical carcinogens in rodents led to the assumption that acinar cells are the primary targets of the carcinogens. Moreover, the partial ductal cell appearance of some of these tumors was considered evidence that ductal adenocarcinomas are the result of acinar cell to ductal cell transdifferentiation. This hypothesis contrasted with the virus- and carcinogen-induced primary adenocarcinoma in the Guinea fowl and Syrian Golden hamsters, respectively [1, 2]. In fact, in the hamster model, the initial step in neoplasia begins within the islets [1]. Numerous studies concerned with the role of islet cells in pancreatic carcinogenesis verified that any condition that stimulates islet cell proliferation and regeneration enhances, whereas the prevention of islet cell neogenesis inhibits pancreatic cancer formation [1]. In humans, however, such a possibility was doubted, as comparable studies in humans were absent. Recent findings of a massive proliferation of pancreatic islet cells in diabetic patients treated with the GLP-1 analog [3] provide an opportunity for comparative studies. With this in mind, we identified a case and were able to obtain the pancreas of a patient who was treated with Victoza and Byetta for 25 months and died from complications of pancreatic cancer a year later.

MATERIALS AND METHODS

The 66-year-old, type 2 insulin-dependent diabetic man, was treated with Victoza on September 20, 2009. For an unknown reason, the treatment was changed to Byetta on October 20, 2010 and continued until December 2011. Due to the patient's financial limitations, the treatment was switched back to insulin. No serious diseases of the patient were known until March 6, 2013, when invasive pancreatic adenocarcinoma was diagnosed and removed by Whipple operation two weeks later. After recovery from the surgery, the patient received adjuvant Gemcitabine for five cycles and insulin. He was re-admitted to the hospital in September 2013 with liver metastases and was treated with palliative FOLFRINOX until he expired in September 2014. An autopsy limited to the abdomen revealed “extensive carcinomatosis with a gelatinous reddish tan mass, necrosis and fibrosis throughout the abdomen with extreme difficulty locating the remnant of the pancreas.”
The pre-operative comprehensive metabolic panel showed hyper glucosemia, increased Anion Gap, decreased albumin and increased alanine and aspartamine transferase, alkaline phosphatase and total bilirubin. No hormonal profiles were measured. The post-Whipple metabolic panel showed almost the same abnormalities. Other values, including the estimated GFR, were within the normal range.

The pancreaticoduodenectomy specimen was a 4.8×4.2×3 cm mass that extended into the peri-pancreatic fat, within 0.4 cm of the distal pancreatic neck margin, and abutted the SMV/SMA vascular groove margins. Metastases were identified within one peri-pancreatic lymph node (stage PT3,N1). The pancreatic parenchyma in the region of the uncinate process was grossly unremarkable.

Tissue Processing

Pancreatic tissue was fixed in 10% phosphate buffered formalin and processed for histology by conventional methods. Serial sections from the tissues that appeared to be intact were stained for H&E or processed for immunohistochemistry by Avidin-Biotin method using the following antibodies: anti-insulin (Cell Signaling Technology, Danvers, MA; 1:100 dilution, 45 minute incubation), anti-glucagon (Cell Signaling Technology, Danvers, MA; 1:100 dilution, 30 minute incubation), anti-chromogranin A (Ventana Medical Systems, Tucson, AZ, pre-diluted by manufacturer, 36-minute incubation), anti-PP (Abcam, Cambridge, MA; 1:1,200 dilution, 45-minute incubation), and the pre-diluted anti-CA 19-9 antibody [PA0424] from Leica Biosystems, Buffalo Grove, IL. Multiplex dual chromagen staining (insulin+glucagon, PPoma cases [4, 5, 6] (Figure 1f). These insulin-ductular complexes could be best differentiated and visualized with the dual staining with anti-PP and anti-CA19-9 antibodies (Figure 2a).

The proportion of the insular and ductular elements varied in different islets. In general, the more ductular cells, the less islet cells (Figures 2b-2d). In some areas, almost the entire islet was replaced by ductular elements. Indisputable evidence for the development of ductular elements from islet cells was the presence of intermediary cells, the ductular cells that contained traces of endocrine granules (Figures 2d-2f, 3a). With a decreasing number of islet cells, the ductular cells showed increasing hypertrophy, atypia and patterns consistent with in situ carcinoma (Figures 3a-3d). In such lesions, only a few or no islet cells were detectable (Figures 4a, 4b). The multiplicity of the lesion at different stages of islet cell transdifferentiation was extraordinary. In one area of about 6 mm in diameter, five such lesions were identified.

In a section taken from the peri-pancreatic tissue near the pancreas head, colonies of PP cells (Figure 4d) were detected within the connective tissue, presenting features consistent with malignant PPMa. The original site of the malignant tumor could not be visualized.

Some differentiated areas of the adenocarcinoma contained numerous endocrine cells that were identified as insulin and glucagon but not PP cells (Figure 4c), possibly implying that the tumor originated from the islets of the PP-cell poor ventral pancreatic tissue.

A remarkable observation was that the process of malignancy (normal-malignant) did not follow to the staging of PAN-IN classification but it seems to occur from the normal to the malignant phenotype in a linear fashion.

The material from the autopsy primarily contained necrotic tissue with small islands of scarred and inflamed exocrine pancreatic tissue without any specific abnormalities.
Figure 1. The patterns of the islets in the tumor-free areas of the pancreas.

(a) Multiple intrainsular ductules in the tumor-free area of the ventral pancreas expressing CA-19-9 as do the surrounding ductal and ductular cells (arrow). Note that the islet is well delineated. ABC method. Original magnification x 200. (b) Two well-circumscribed islets with several distended intrainsular ductules, some filled with mucinous material. The islet on the left was stained with anti-PP (black in color) and the one on the right with insulin (red) and glucagon (black). ABC method, Original magnification x 200. (c) A group of islets with round or ovoid form stained with anti-chromogranin A (CGA) in dark brown color. Several of these islets contain intrainsular ductular structures (blue in color). Scattered single or small groups of islet cells are in the surrounding tissue (dark brown spots). ABC method, Original magnification x 65. (d) At the region between the ventral and dorsal pancreas, malignant cells intermingled with CGA positive cells (dark brown) were found within a cystic islet. The islet cells in the middle of the cyst (in blue color) are mostly replaced by fibrotic tissue. ABC method, Original magnification x 100. (e) Distribution of islets composed primarily of PP cells (dark brown) show differences in size and shape. In contrast to the islets in the dorsal region, the islets are ill defined. The inset depicts several interconnected islets forming a horseshoe shape. The staining intensity of the cells with the anti-PP antibody was remarkably very strong. ABC method, Original magnification x 40. Inset, x 100. (f) A large islet in the ventral pancreatic tissue composed primarily of cells immunoreactive with anti-CGA (brown-to-dark brown). In the center of the islet there are a few ductules (in pink color) with hypochromatic nuclei. Some unstained cells are also present in the lower middle and the lower right of the islet. ABC method, Original magnification x 200.
Figure 2. Insulo-ductular complexes in the ventral pancreas. ABC method was used for immunostaining.

(a). An islet composed of PP cells (dark brown) is fragmented by numerous ductular elements (in pink color) occupying a large portion of the islet. CA-19-9 reactive elements are in black and the cytoplasm of the ductal and acinar cells are in pink color. A few small islets, some intermingled with ductular cells are seen at the upper right. ABC method, combined anti-PP and anti-CA19-9 antibodies. Original magnification. Original magnification x 200. (b). The insulo-ductular cell group (pink in color) has seemingly replaced major portions of the original islet cells (dark brown). Ductular cells are composed of a cuboidal cell with large nuclei with no atypia. CGA, ABC method. Original magnification. Original magnification x 200. (c). Several islets in the dorsal pancreas stained with anti-CG-A antibody (brown in color) contain small cystic-like ductules that replace a small or large portion of the islets. In the upper right islet, the lower portion of the islet is separated from the islet (arrow). Original magnification x 65. (d). Irregularly shaped ductular structures have substituted a major portion of the islet cells stained with anti-CGA antibody in dark brown color. Note that some of the ductular cells (intermediary cells) contain PP hormones (arrow). Differences are recognizable in the arrangement, size, and opacity of the ductular nuclei. A few unstained cells are seen in the center. Original magnification x 200. (e). Several cells of a ductule with large cuboidal and cylindrical cells present intermediary cells that contain various amounts of granules stained with anti-CGA in dark brown color. A group of islet cells stained dark brown are attached to the ductule. Another ductule with a few islet cells (dark brown) in its wall is in the upper right corner. ABC method. Original magnification x 400. (f). Two ductules with hyperplastic cells are composed of many intermediary cells. The chunk of islet cells attached to the lower ductule represent the remnant of an original islet. Anti-CGA, x Original magnification x 400.
Figure 3. Islet cells of the ventral pancreas stained with anti-PP antibody by ABC methods. 
(a). Several atypical ductular structures with pleomorphic cells and scattered PP cells (black) in between. Intermediary cells are seen in one area (arrow). Original magnification x 400. (b). A large ductule with pleomorphic and locally crowded nuclei imitating Ca in situ. There are several PP cells (black) attached to the ductule and seemingly present the basal layer of the duct. Original magnification x 400. (c). Cross sections of a duct with nuclear atypia and a mitotic figure (arrow; see also figure E) are encircled by a wall of inflammatory cells, typically seen around malignant lesions. Hyperplastic ductular cells are present in the surrounding area along with the remnants of islets (dark brown patches). Original magnification x 40. (d). Several variously shaped ductules with pleomorphic nuclei. Remnants of an islet (dark brown) are seen in the lower left corner. Original magnification x 400. (e). High power view of the lesion in Figure C presenting nuclear atypia and a mitotic figure (arrow). Original magnification x 400. (f). A twisted large ductules with irregularly arranged pleomorphic nuclei. No endocrine cells. Original magnification x400.

DISCUSSION

No clues have emerged on the histogenesis of pancreatic cancer in humans. While the histological appearance of pancreatic cancer suggests a ductal cell of origin, genetic studies have suggested that, under different experimental conditions, different pancreatic cell types may undergo malignant transformation [7, 8, 9, 10, 11, 12]. Accordingly, a range of different cell types have been proposed as putative pancreatic progenitor and cancer-initiating cells, including pre-existing acinar cells, pre-existing β-cells, pancreatic ductal cells, and cells expressing the mesenchymal marker nestin [11, 12].

The primary derivation of cancer cells from pancreatic islets, as verified by numerous studies.
in the hamster pancreatic cancer model [1], has not been generally accepted. In this model, the induced adenocarcinomas closely mimic the human pancreatic ductal adenocarcinomas in clinical, morphological, biological and genetic characteristics [1] initiates from the intra-insular ductules, the existence of which was already recognized by Bensley in 1911 [13]. Our studies confirmed the presence of intrainsular ductules also in the human pancreas [1] and presented evidence for the potential of hamster and human islet cells to readily transdifferentiate into ductal and other pancreatic and extra-pancreatic cells in vitro [14, 15, 16, 17, 18, 19, 20, 21] and in vivo [17]. Moreover, we have demonstrated that the treatment of cultured pure hamster islets in vitro by pancreatic carcinogen grow in vivo as ductal adenocarcinoma [16].

In our view, the potential of insular cell to ductal cell transdifferentiation and malignancy is linked to a combination of specific features, including: 1) the existence of a unique islet-acini microcirculation, where circulating blood preferentially and directly nourishes the islets [22, 23, 24], 2) the presence of a massive amount of growth factors produced by the islet cells (insulin and IGF-1), and 3) the existence of highly specialized drug-metabolizing enzymes within the islet cells of humans and other mammals [25, 26, 27, 28, 29]. Remarkably, some of the potent carcinogen-metabolizing enzymes are restricted to the islet cells [25, 26]. These anatomical, physiological and pharmacological characteristics seem to make the islet cells the primary target of blood-borne carcinogens leading to their malignant transformation via transdifferentiation to ductular cells. The rapid proliferation of these ductular elements is entertained by a massive amount of growth factors produced by the surrounding islet cells.

In the hamster model, the carcinogenic process is initiated with the formation of new ductular structures or the expansion of pre-existing ductular structures within and around the islets [1], similar to the lesions found in this study and presented in Figures 1a, 1b. These intrainsular ductular cells undergo malignant transformation and occupy the islets, many of which become cystic, similar to the lesions in Figure 4c. The gradual transformation event of islet cells to benign and malignant ductular cells is substantiated by the presence of intermediary cells (cells

Figure 4. (a). A portion of a duct with abnormal epithelial cells and pleomorphic nuclei, a lesion consistent with Ca in situ. A portion of the seemingly separated duct in the lower middle area is encircled by PP cells (black), ABC method, Original magnification x 400. (b). A cross section of a duct with malignant epithelium surrounded by islets of different size and shape stained with anti-PP antibody (black), ABC methods, Original magnification x 200. (c). Malignant glands in well-differentiated area of adenocarcinoma containing numerous insulin and glucagon cells at the base of the epithelium (black), ABC methods Original magnification x 200. (d). Large, irregular PP-cell aggregates, consisting with the term PP-oma within the connective tissue attached to the uncinate process. No intimate connection between this lesion and pancreatic tissue could be found, ABC method Original magnification x 100.
of ductal phenotype harboring endocrine granules) within the islet-ductular cell complexes (Figures 2d-2f, 3a).

Of particular interest were the differences in the patterns of the described transdifferentiation process in the tissue from the ventral and dorsal pancreas. In the dorsal pancreas, where islets are usually well defined and seem to be pseudo-encapsulated, proliferating ductular cells and their secretion cause cystic islets and provide a breeding ground for cancer cells exposed to a high concentration of insulin and IGF-1 [30, 31]. In the ventral pancreas, however, where islets are borderless, the transdifferentiated cells splinter the islets gradually and replace them completely. The demonstration of insulin and glucagon but not PP cells in the well-differentiated area of the cancer, could point to their origin from the dorsal pancreas. Future focused studies could clarify whether the identification of cancer-associated endocrine cells in tumors can differentiate cancers derived from the ventral or dorsal pancreas.

A number of experiments in the hamster model concerned with the prevention of pancreatic cancer [1] demonstrated that the induction of islet cell neogenesis and stimulation of islet cell replication by pharmaceutical drugs or diets significantly enhances the carcinogenic process, while prevention of islet cell replication and nesidioblastosis significantly inhibit it [1, 32, 33, 34, 35, 36, 37, 38]. It was contemplated that the same situation may apply to the human cases based on the epidemiological results linking the increase of pancreatic cancer in obese people, who have enlarged pancreatic cells [39, 40, 41, 42]. However, the possibility of direct stimulation of human islet cells were missing until the emergence of a recent study demonstrating a massive expansion of islet cells in type 2 diabetics treated with GLP-1 analogs [3]. The alterations described in that publication were almost identical to those observed at the early stages of carcinogenesis, as presented in a recent publication [43].

Whether the lesions observed in the presented case are linked to the treatment of the patient with Victoza and Bayetta is presently difficult to answer, as the treatment (12 months with Victoza and 13 months with Byetta) ceased about a year earlier. Morphologically, the reported expansion of insulin and glucagon cells in the pancreas of the incretin-treated patients were not seen in the tumor-free tissue of the dorsal pancreas of this case. On the contrary, the islets in this region of the pancreas were atrophic and the patient was diabetic. However, the presence of intrinsaular ductular structures in several areas (Figures 1a-1c) that have never been reported in the literature and not observed by us in the pancreas of normal and diabetics [44] is a hallmark of carcinogenesis in the hamster model [1] and hints to the effects of the drugs. Is it possible that genetic alteration caused by the drugs triggered islet-ductular cell transdifferentiation that perpetuated even after their cessation? The intrinsaular malignant cells in Figure 1D, which is a characteristic finding in carcinogen-treated hamsters but has never been reported in the human pancreas, could be a positive answer to this question. The extraordinary multiplicity of the lesion at different stages of islet cell transdifferentiation also is in line with this possibility. Since these lesions have never been reported in the pancreas of diabetic patients, the role of diabetes as an etiological factor appears very unlikely. Whether or not the invasive pancreatic cancer of the patient arose from one of these foci or by their coalescence remains an unanswered question.

There is currently no information about the long-term effects of the incretins on pancreatic islets. The results of the clinical trials list at least eight cases of pancreatic cancer in incretin-treated diabetics that were not considered as side effects of the drugs, based on the short interval between the treatment and cancer diagnosis [45]. According to the assessment report for GLP-1 based therapies 25July 2013 EMA/474117/2013, in clinical trials, a few cases have been reported for some products. Although the data currently available from clinical trials do not indicate an increased risk for pancreatic cancer with these medicines, cases of pancreatic cancer have been reported in the post-marketing setting. A cumulative review of the cases has been undertaken and the majority (19 out of 29) had a time to onset of less than six months, a period considered too short to suggest a causal relationship.

The role of GLP-1 analogs in pancreatic cancer varies widely between the studies and based on the findings, multi-district litigation (MDL) were established. Some trials, including SAVOR-TIMI (of saxagliptin) and EXAMINE (of alogliptin), found no difference between dipeptidyl peptidase 4 (DPP-4) inhibitor treatment and placebo with regard to pancreatitis or pancreatic cancer [46]. In a recent study, pancreatic cancer rate was 9.39% vs. 2.61% for population controls, with an adjusted odds ratio of 3.6, suggesting that these drugs are strongly associated with pancreatic cancer [47]. In a more recent investigation 0.3% of patients receiving Liraglutide had pancreatic cancer compared to 0.1% in the placebo group, giving a p value short of a significance (P<0.6) [48]. Obvious reason for the lack of consistency in the incidence of pancreatic cancer in incretin treated patients is the missing autopsy procedure are performed in these patients, who clinically did not present any symptoms of pancreatic cancer and, hence remained false-negative.

The side effects of the incretins given for a short time and the lasting effects after their cessation are unknown. Although proliferation of insulin and glucagon cells has been identified as the hallmark of the drugs in humans [3], no report exists on their effects on the PP cells. The malignant PP cell tumor in the present study could well be related to the effect of the drugs on these cells as well. Future, more detailed studies could clarify the issue. Nevertheless, the present experimental and clinical data are not sufficient to rule out the late adverse effects of these drugs on pancreatic islets. It is conceivable that while withdrawal of the drug normalizes the structure of the pancreatic tissue in part, but leaves some transformed cells behind. Although based on a known long latency of pancreatic cancer this risk is
trivial, it could explode the already existing premalignant lesions, particularly in type 3 diabetics. It must be pointed out that hyperplastic in situ carcinomas and adenocarcinomas occur in some cases as incidental lesions and are unrelated to the cause of death [49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61]. These “silent” cancers are vulnerable to rapid growth in any condition leading to increased insulin and IGF-1 production. Support for this view is the increased pancreatic weight and ductal/ductular hyperplasia in incretin-treated animals and patients [3]. The reported and registered short latency of pancreatic cancer in clinical trials, mentioned previously is in-line with this likelihood. It could be argued that the alterations seen in this case could represent one of the incidental cases. However, such an impressive alteration of islets at different stages of transdifferentiation and in association with pancreatic and endocrine neoplasm has never been reported or observed. Moreover the concomitant occurrence of pancreatic adenocarcinoma and endocrine tumors is extremely rare [49]. Adenocarcinoma and PPoma were not seen in any of the reported cases. The induction of PP cell and insulin cells in the duodenal mucosa of a patient treated with incretin [62] justifies the notion that these drugs affect the synthesis of hormones also in extrapancreatic tissue as they do in the thyroid glanlds [63].

CONCLUSION

This is the first report demonstrating that: 1) as in the hamster pancreatic cancer model, human islets also appear to present a source of pancreatic ductal neoplasms, and 2) the alterations seem to be related to the effects of GLP-1 analogs. Given the >20 million known patients with type 2 diabetes in the United States alone, and the numerous GLP-1–based drugs, either available now or in the final stages of development, the potential impact of the adverse effects of this class of drugs is considerable. The heart of the problem with this assessment is the lack of data from the pancreas of incretin-treated diabetics, in the same fashion as Butler and associates did [3]. The ideal study to answer the question definitively would involve large-scale pathohistological examination of the pancreas of deceased type 2 diabetics treated with incretins, comparative results of the treatment of type 2 and type 3 diabetics, databases from multiple countries, providing enough data on new users as well as prevalent users in order to eliminate bias from the duration of use.

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Conflict of Interest

The authors declare that they have no conflict of interest.

