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Glucagon-like Peptide-1 Receptor (GLP-1R) Expression in Normal and Neoplastic Human Pancreatic Tissues

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Abstract

Objectives—Studies have proposed pro-oncogenic effects of glucagon-like peptide-1 receptor (GLP-1R) agonists in the pancreas by promoting GLP1-R over-activation in pancreatic cells. However, the expression of GLP-1R in normal and neoplastic pancreatic cells remains poorly defined, and reliable methods for detecting GLP1-R in tissue specimens are needed.

Methods—We employed RNA *in-situ* hybridization to quantify *glp-1r* RNA in surgically resected human pancreatic specimens, including pancreatic ductal adenocarcinoma (PDAC), pre-invasive intraepithelial lesions (PanINs), and non-neoplastic ductal, acinar, and endocrine cells. A mixed-effect linear regression model was used to investigate the relationship between *glp-1r* signals and all cells, ordered by increasing grade of dysplasia.

Results—All cell types had evidence of *glp-1r* transcripts, with highest expression in endocrine cells and lowest in ductal cells. The slope of the fitted line was not significantly different from zero (0.07, 95% CI -0.0094, 0.244; $p=0.39$), suggesting that progression from normal cells to PDAC is not associated with a parallel increase in *glp-1r* RNA. A series of pairwise comparisons between all cell types with respect to their *glp-1r* expression showed no significant difference in *glp-1r* in cancer, PanIN, acinar and ductal cells.

Conclusions—Our study supports the lack of evidence for GLP-1R overexpression in PDAC.

Keywords

Glucagon-like peptide-1 receptor; incretins; pancreatic cancer; pancreatic intraepithelial neoplasia

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Introduction

Glucagon-like peptide-1 receptor (GLP-1R) agonists are incretin analogues that potentiate glucose-dependent insulin release following food intake¹. These drugs are being increasingly used for patients with type-2 diabetes, as they improve blood glucose control and weight loss, with low risk of hypoglycemia. Despite the favorable effects, a number of publications have questioned the safety profile of incretin analogs, raising concern that these drugs may induce pancreatic inflammation and carcinogenesis². A retrospective analysis of adverse event reports showed increased incidence of pancreatitis, thyroid cancer and pancreatic ductal adenocarcinoma (PDAC) in patients treated with incretin-based therapies². A similar study from Germany reported a significant association between the use of GLP-1R agonists and a diagnosis of PDAC³.

Incretin analogs have also been proposed to stimulate the growth of both the exocrine and endocrine pancreas, promoting subclinical pancreatic neoplasms. A recent autopsy study found an association between incretin use and increased volume of the exocrine and endocrine pancreas^{4, 5}, suggesting that these drugs may have proliferative effects. However, several limitations of the design of this have been highlighted including the small number of pancreas samples analyzed, and differences between the study group and control group with respect to key clinical characteristics, such as age, gender, and duration of diabetes⁶⁻⁹.

Thus far, preclinical studies have largely failed to demonstrate pathologic alterations in the pancreas induced by incretins¹⁰⁻¹². Nyborg et al¹³ recently reported studies on mice, rats and primates treated long-term with a GLP-1R agonist, and found no pathologic alterations in the exocrine pancreas. Similar results were reported by Gotfredsen et al.¹⁴ after extensive toxicological studies on the pancreas of primates treated with two different GLP-1R analogs. Another study reported the spontaneous formation of pancreatic ductal cell proliferation, atrophy and inflammation in normal and diabetic rats¹⁵, questioning the contribution of incretins to the genesis of pancreatic lesions. The lack of unequivocal recognition of which cell types express GLP-1R currently limits our understanding of pharmacologic actions of incretin analogs. Although several rodent and human studies have documented GLP-1R expression in pancreatic ductal cells, acinar and cancer cells and in pancreatic precursor neoplasms, known as pancreatic intraepithelial neoplasia (PanIN)¹⁶⁻¹⁹, data from multiple studies suggest that the methods used to assess GLP-1R tissue expression lack sufficient sensitivity and specificity based on evidence that the antibodies used in these studies either do not bind to GLP-1R by western blot, or also bind to other proteins isolated from cells that do not express *glp-1r* RNA^{6, 20}. For example, *glp-1r* protein expression in animal models has been reported in the heart, in both atria and ventricles²¹. However, subsequent investigations revealed that lack of *glp-1r* expression in the ventricles, questioning the reliability of the antibodies previously used^{22, 23}. Furthermore, Panjwani et al⁷ recently studied the action of GLP-1R agonists in atherosclerosis and liver steatosis, in a genetically modified murine model. Contrary to previous data, the authors could neither identify *glp-1r* mRNA transcripts in isolated macrophages or hepatocytes at RT-PCR, nor could they validate the sensitivity and specificity of three commercially available anti-*glp-1r* antibodies using Western Blot analysis and immunoprecipitation. Overall, there is insufficient evidence to conclude that there is an association between incretin-based therapies and PDAC, but the

existing literature has left open several important questions about the physiology of incretins, particularly with regard to GLP-1R localization in normal and neoplastic pancreas.

In this study we employed an RNA *in-situ* hybridization (ISH) assay to characterize the presence of *glp-1r* in pancreatic cell types using surgically resected human pancreas specimens that contained PDAC cells, premalignant intra-epithelial lesions (PanINs), as well as acinar and islet cells.

Research Design and Methods

This study was approved by the Johns Hopkins Medical Institutional Review Board. We utilized two tissue microarrays (TMAs) of normal and neoplastic archival formalin-fixed paraffin-embedded (FFPE) pancreatic tissues from patients who underwent resection of a primary pancreatic adenocarcinoma. The cases were selected only on the basis of having their neoplastic and normal tissues available on existing TMAs. There was no selection of cases based on any clinical criteria (such as diabetes) or pathological criteria other than having PDAC and PanIN tissues. We used tissues from cases that had undergone pancreaticoduodenectomy because these represent ~80% of the resections for pancreatic head tumors. The areas of normal pancreas selected for analysis were chosen to avoid as much as possible areas of chronic pancreatitis that can result from ductal obstruction by the tumor. TMA1 included representative cores of PDAC. TMA2 included representative cores of PDAC and PanIN lesions of all grades (PanIN-1-2-3) identified from the patients' resected pancreata. TMA methods are further described in ²⁴. After *in situ* hybridization, sufficient tissue was available for analysis from 32 patients. Each TMA also contained histologically normal pancreatic tissues from patients' resected pancreata. Matching normal tissue was available for most cases. Clinico-pathological data of the patients included were retrieved from the Johns Hopkins Surgical Pathology database.

In-situ hybridization was performed using RNAScope (v2.0 High-Definition-Red; Advanced Cell Diagnostics, Inc. Hayward, CA), an assay based on branched-chain amplification steps that allows for sensitive detection and quantification of RNA transcripts in tissue sections ²⁵. Four µm-thick tissue sections of TMA1 and TMA2 were deparaffinized, and ISH was performed manually in two consecutive experiments using positive and negative controls, in accordance with the manufacturer's recommendations. Representative tissue cores of non-neoplastic prostate and lymphoid tissues present in the TMAs were also evaluated as internal negative controls, as these tissues have been shown to have low or absent GLP-1R expression²⁶. Following ISH, every tissue core was digitally photographed to capture images of sufficient numbers of cells from each pancreatic cell type using an Olympus BX51 microscope equipped with an Olympus DP26 camera (Olympus America Inc. NY USA).

A pathologist with extensive experience in pancreatic pathology (HK) reviewed tissue cores to confirm the histological diagnosis and quantify hybridization signals. After using a training set of slides to gain experience in scoring, signals and nuclei were manually counted by two authors (MDM and HK) in an independent way using computer image analysis software ImageJ, v1.46 (available at <http://rsb.info.nih.gov/ij/index.html>). ISH signals were

calculated as signal-to-cell ratios: for each digital image, the total number of ISH signals counted for each cell lineage (cancer, PanINs, endocrine, acinar, ductal) was divided by the number of nuclei present in the same cell population. This counting procedure was applied on tissue cores hybridized for GLP-1R as well as for the DNA-directed RNA polymerase II subunit RPB1 (POLR2A), which we employed as positive control in order to assess the level of GLP-1R expression against a common housekeeping gene.

Since most PDAC cells are several-fold larger than normal pancreatic ductal epithelial cells, and RNA abundance is thought to be associated with cell size²⁷, we reasoned that large cells such as cancer cells might have more abundant mRNA transcript levels than smaller cells, such as non-neoplastic pancreatic ductal cells. We therefore employed a statistical model in which the calculated *glp-1r* signals in each cell type were corrected for cell size (further explained in the Statistical analysis section). To calculate cell size we measured the average cell area as a surrogate indicator of size. Cell area was calculated by multiplying the two largest diameters in each cell. Each cell type was measured in the same way.

To accurately measure cell area, we immunolabeled additional TMA slides for E-cadherin. E-cadherin was chosen because of its exclusive membrane localization. The staining allowed us to better visualize cell perimeter and obtain more precise measurements of cell diameters.

Immunohistochemistry (IHC) was performed using primary anti-E-cadherin rabbit monoclonal antibody (EP700Y, Cell Marque, Rocklin, CA), as previously described²⁴. After IHC, each core was photographed as described above for ISH. Measurements of the two largest cell diameters were taken using ImageJ software. A total of 50 images were evaluated for each cell type.

Statistical Analysis

Values are presented as median and range of the *in situ* hybridization/cell volume ratios and as mean and standard deviation (SD) of the log-transformed ratios, as well as total and mean number of cells analyzed. Two mixed-effects linear regression models were used to evaluate the association between ISH signal intensity and cell type, with log-ratios as the response and fixed effect terms for cell type, cell size and TMA. We included cell type using two approaches: in the first approach, cell types were ordered according to the pancreatic carcinogenesis model from no dysplasia to progressively higher grades of dysplasia (PanIN-1, PanIN-2, PanIN-3), to PDAC cells (Table 1), and the slope of the linear relationship calculated.

It is still a matter of debate whether PDAC originates from ductal or acinar precursor lesions. The commonly accepted morphological pathway of pancreatic carcinogenesis postulates a stepwise progression from ductal cells to intraepithelial lesions (PanINs) and eventually cancer²⁸. However, since experimental evidence suggests that PDAC cells may originate from an acinar progenitor cell²⁹⁻³¹, we included the expression levels of both acinar and ductal cells in our model.

In the second approach, indicator variables for each cell type were included in the models, yielding 15 pairwise comparisons between combinations of cell types. Both modeling approaches included a random intercept for each patient, to account for within-patient correlation across cell types. Results are expressed as the difference in log ratios between cell types, and as the ratio of means of the untransformed ratios, calculated by exponentiating the difference. All pairwise differences in signal-to-cell ratios between cell types were estimated, and the type-I error rate was controlled using a Bonferroni correction, resulting in p -values $< 0.05/15 = 0.003$ as a threshold for statistical significance. All analyses were performed using R version 3.0 (R Core Team-2014; R Foundation for Statistical Computing, Vienna, Austria, <http://www.R-project.org>).

Results

The clinicopathological characteristics of the 32 patients included are presented in Table 1. None of the patients were known to have been taking GLP-1R agonists.

Glp-1r mRNA signals were found in endocrine, acinar, ductal, PanIN and PDAC with variable levels of expression (Figure 1 and Table 2). No signals were observed in prostate or lymphoid tissues (Supplemental Figure 1), in agreement with previous data²⁶. To identify potential differences between ISH experiments, we compared mean signal-to-cell ratios of acinar cells, which was the most represented cell type, between TMA1 and TMA2 and found no significant difference ($p=0.68$, two-tailed t-test; data not shown).

Acinar cells were the most prevalent cell type with a median number of 1020 cells analyzed across all tissue cores. The least represented cell type were ductal cells (median number: 85), although we observed a considerable variability in the number of ductal cells across different tissue cores (Table 2). Overall, GLP-1R signal-to-cell ratios were < 0.5 in every cell type observed. Endocrine cells had the highest (0.196) and ductal cells had the lowest (0.01) median signal-to-cell ratio, whereas PanINs, acinar cells and PDAC cells showed intermediate values. We did not find any outliers with respect to GLP-1R signals of individual cells within any of the cell groups, nor did we not observe significant inter-patient variability with respect to signal to cell ratios within the various cell types.

In support of previous data³², PDAC cells were the largest cell type, with a mean value of $592 \mu\text{m}^2$, whereas ductal cells had a mean value: $265.8 \mu\text{m}^2$ and were the smallest cell type observed (Table 3). A significant difference in cell area (hereinafter referred to simply as “size”) was observed across cell types (1-way ANOVA, $p<0.001$). To examine the relative level of GLP-1R expression, we compared the signal-to-cell ratios calculated for islet, acinar, ductal and PDAC cells with the signal-to-cell ratios obtained from the positive control (POLR2A gene), in the same cell types (Welch two-sample t-test, Figure 2). The mean GLP-1R signal-to-cell ratio was 0.221 in endocrine cells, 0.097 in PDAC cells, 0.012 in ductal cells and 0.043 in acinar cells. The mean POLR2A signal-to-cell ratio was 0.333 in endocrine cells, 0.564 in PDAC cells, 0.273 in ductal cells and 0.338 in acinar cells. Analysis of endocrine cells showed no significant difference in the level of GLP-1R expression compared with POLR2A (95% Confidence Interval (CI): -0.259; 0.036, $p=0.122$). Expression of POLR2A was significantly higher than GLP-1R in cancer cells

(95% CI: -0.650, 0.284; $p < 0.001$), acinar cells (95% CI: -0.429, 0.160; $p = 0.001$) and ductal cells (95% CI: -0.330, 0.192; $p < 0.001$).

We next compared the level of GLP-1R expression in non-neoplastic acinar, endocrine and ductal pancreatic cells (Table 4). The GLP-1R signal-to-cell ratio was significantly higher in endocrine cells than in acinar and ductal cells ($p = 0.002$ and $p < 0.001$, respectively). The signal-to-cell ratios in acinar cells tended to be higher than the ratios in ductal cells, although the difference was not significant ($p = 0.061$).

The first mixed-effect linear regression modeling approach was used to investigate the relationship between *glp-1r* signal-to-cell log-ratios and cancer cells and their putative precursor lesions. In the regression model, which included acinar cells, ductal cells, all grades of PanIN and cancer cells, the slope of the fitted line was not significantly different from zero (Slope = 0.07; 95% Confidence Interval: 0.91 to 0.244, $p = 0.39$), suggesting that progression from normal pancreas cells (acinar or ductal cells) to PanINs and eventually PDAC is not associated with a significant increase in *glp-1r* expression.

We also analyzed the expression data using a series of pairwise comparisons involving PDAC cells, all grades of PanIN, and non-neoplastic cells (ductal, acinar cells) to test for significant differences in their respective signal-to-cell ratios (second regression, Table 5). Acinar cells and PDAC cells showed higher *glp-1r* ratios than ductal cells ($p = 0.006$ and $p = 0.021$, respectively). However, these differences were not statistically significant after adjusting for multiple comparisons. There were no other statistically significant differences in any of the other comparisons. Specifically, we did not observe differences in ratios between different PanIN grades (PanIN-1 vs. PanIN-2, $p = 0.406$; PanIN-1 vs. PanIN-3, $p = 0.154$; PanIN-2 vs. PanIN-3, $p = 0.684$). Similarly, there was no significant difference in *glp-1r* expression between PDAC cells and any grade of PanIN, between PDAC cells and acinar cells, or between PDAC cells and ductal cells. Further more, we did not find any outliers with respect to *glp-1r* expression in the PDAC cases that might indicate GLP-1R overexpression in one or two of the cancers relative to the group overall or within the subgroup of patients that had been diagnosed with diabetes at the time of surgery.

Discussion

The most reliable evidence that a gene has oncogenic functions is finding activating mutations, gene amplification or fusions of the gene. This is further substantiated by the observation that selective inhibition of the gene or gene product can suppress its oncogenic phenotype. In the literature genes that are overexpressed in cancer cells are identified as oncogenes, particularly if the gene is thought to have growth-promoting functions.

GLP-1R agonists have been recently suggested to promote PDAC, due to the putative action of GLP-1R on cell growth and proliferation⁵. Thus far, there is no definitive evidence supporting this hypothesis. Moreover, reliable data regarding the expression of GLP-1R in normal and neoplastic pancreas remains elusive.

In this study we sought to determine if *glp-1r* expression is a feature of PDACs, PanINs and non-neoplastic pancreatic cells. We chose to employ RNA *in situ* hybridization to measure

gene expression because in contrast to the uncertain antigenic specificity of most commercially available antibodies^{6, 7, 20}, RNA probes for ISH have been reported to have excellent sensitivity and specificity, even in FFPE tissues^{33, 34}.

Given the relatively low level of GLP-1R expression in pancreatic islets reported by microarray and RNA sequencing studies^{35, 36}, we chose to assess gene expression in a quantitative manner. Although low levels of *glp-1r* transcripts were present in all cell types, endocrine cells expressed *glp-1r* at a significantly higher level than PDAC cells or any other cell type. Importantly, the level of expression in PDAC cells was not significantly different from that of normal pancreatic acinar or ductal cells or PanIN cells, which would be expected if GLP-1R were an important contributor to PDAC development or progression.

We would have expected that if GLP-1R were functioning as an oncogene promoting pancreatic carcinogenesis, at least some of the PDACs we tested would have had much higher levels of *glp-1r* expression than normal pancreas cells. However, we never observed PDAC cells expressing significantly higher levels of *glp-1r* than normal pancreas cells. Pancreatic expression of GLP-1R using immunohistochemistry and autoradiography was recently reported and found similar results to ours³⁷. These authors did not identify GLP-1R expression in pancreatic adenocarcinomas, although they did find low levels of GLP-1R expression in PanIN-1 and PanIN-2 lesions. Together, these findings and our results indicate that of the level of expression of *glp-1r* transcripts in normal and neoplastic pancreatic ducts may be below the level required for protein expression to be detected using techniques such as immunohistochemistry.

Interestingly, we also detected *glp-1r* expression in normal pancreatic acinar cells, confirming the findings recently reported by Waser et al³⁷. This observation may explain the occurrence of pancreatic gland hypertrophy arising from *glp-1r* agonist therapy described in animal models. Koehler et al, recently reported that *glp-1r* agonists increase protein, but not DNA expression or cell proliferation in pancreatic acinar cells³⁸.

Although the lack of *glp-1r* overexpression in PDAC cells suggests that direct effects of incretins on pancreatic cells may not be important in driving carcinogenesis, we cannot exclude effects related to systemic metabolic effects of these agents on the pancreas. Similarly, our findings do not preclude the possibility that PDAC cells could respond to GLP-1R agonists differently to normal pancreas cells without requiring gene overexpression.

Ultimately, the long-term outcome results of ongoing randomized trials will reveal if there is any significant effect of these drugs on the incidence of PDAC or pancreatitis. So far the initial results of randomized trials do not find evidence for an increase in the incidence of pancreatitis or PDAC associated with incretin use^{39, 40}.

In conclusion, we find that normal and neoplastic pancreatic cells express low levels of *glp-1r* transcripts. Our results suggest that PDAC cells or their precursor lesions do not overexpress *glp-1r* compared to non-neoplastic pancreatic cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Campbell JE, Drucker DJ. Pharmacology, physiology, and mechanisms of incretin hormone action. *Cell Metab.* 2013; 17:819–837. [PubMed: 23684623]
2. Elashoff M, Matveyenko AV, Gier B, et al. Pancreatitis, pancreatic, and thyroid cancer with glucagon-like peptide-1-based therapies. *Gastroenterology.* 2011; 141:150–156. [PubMed: 21334333]
3. Arzneimittelkommission der Deutschen Ärzteschaft: ÑAus der UAW-Datenbanki Pankreaskarzinome im Zusammenhang mit Exenatid (Byetta Æ). *Dtsch Arztebl International.* 2011; 108:1080.
4. Butler AE, Campbell-Thompson M, Gurlo T, et al. Marked expansion of exocrine and endocrine pancreas with incretin therapy in humans with increased exocrine pancreas dysplasia and the potential for glucagon-producing neuroendocrine tumors. *Diabetes.* 2013; 62:2595–604. [PubMed: 23524641]
5. Butler PC, Elashoff M, Elashoff R, et al. A critical analysis of the clinical use of incretin-based therapies: Are the GLP-1 therapies safe? *Diabetes Care.* 2013; 36:2118–2125. [PubMed: 23645885]
6. Pyke C, Knudsen LB. The glucagon-like peptide-1 receptor--or not? *Endocrinology.* 2013; 154:4–8. [PubMed: 23267050]
7. Panjwani N, Mulvihill EE, Longuet C, et al. GLP-1 receptor activation indirectly reduces hepatic lipid accumulation but does not attenuate development of atherosclerosis in diabetic male ApoE(-/-) mice. *Endocrinology.* 2013; 154:127–139. [PubMed: 23183176]
8. Heine RJ, Fu H, Kendall DM, et al. Comment on: Butler et al. Marked expansion of exocrine and endocrine pancreas with incretin therapy in humans with increased exocrine pancreas dysplasia and the potential for glucagon-producing neuroendocrine tumors. *Diabetes* 2013;62:2595-2604. *Diabetes.* 2013; 62:e16–7. [PubMed: 24065798]
9. Engel SS, Golm GT, Lauring B. Comment on: Butler et al. Marked expansion of exocrine and endocrine pancreas with incretin therapy in humans with increased exocrine pancreas dysplasia and the potential for glucagon-producing neuroendocrine tumors. *Diabetes* 2013;62:2595-2604. *Diabetes.* 2013; 62:e18. [PubMed: 24065799]
10. Koehler JA, Harper W, Barnard M, et al. Glucagon-like peptide-2 does not modify the growth or survival of murine or human intestinal tumor cells. *Cancer Res.* 2008; 68:7897–7904. [PubMed: 18829546]
11. Kissow H, Hartmann B, Holst JJ, et al. Glucagon-like peptide-1 (GLP-1) receptor agonism or DPP-4 inhibition does not accelerate neoplasia in carcinogen treated mice. *Regul Pept.* 2012; 179:91–100. [PubMed: 22989472]
12. Engel SS, Round E, Golm GT, et al. Safety and tolerability of sitagliptin in type 2 diabetes: pooled analysis of 25 clinical studies. *Diabetes Ther.* 2013; 4:119–1145. [PubMed: 23700194]
13. Nyborg NC, Molck AM, Madsen LW, et al. The human GLP-1 analog liraglutide and the pancreas: evidence for the absence of structural pancreatic changes in three species. *Diabetes.* 2012; 61:1243–1249. [PubMed: 22338093]
14. Gotfredsen CF, Molck AM, Thorup I, et al. The Human GLP-1 Analogs Liraglutide and Semaglutide: Absence of Histopathological Effects on the Pancreas in Nonhuman Primates. *Diabetes.* 2014; 63:2486–2497. [PubMed: 24608440]

15. Chadwick KD, Fletcher AM, Parrula MC, et al. Occurrence of spontaneous pancreatic lesions in normal and diabetic rats: a potential confounding factor in the nonclinical assessment of GLP-1-based therapies. *Diabetes*. 2014; 63:1303–1314. [PubMed: 24222349]
16. Gier B, Matveyenko AV, Kirakossian D, et al. Chronic GLP-1 receptor activation by exendin-4 induces expansion of pancreatic duct glands in rats and accelerates formation of dysplastic lesions and chronic pancreatitis in the Kras(G12D) mouse model. *Diabetes*. 2012; 61:1250–1262. [PubMed: 22266668]
17. Busch SJ, Hoffmann P, Sahota P, et al. Studies in rodents with the dipeptidyl peptidase-4 inhibitor vildagliptin to evaluate possible drug-induced pancreatic histological changes that are predictive of pancreatitis and cancer development in man. *Diabetes Obes Metab*. 2013; 15:72–76. [PubMed: 22882290]
18. Matveyenko AV, Dry S, Cox HI, et al. Beneficial endocrine but adverse exocrine effects of sitagliptin in the human islet amyloid polypeptide transgenic rat model of type 2 diabetes: interactions with metformin. *Diabetes*. 2009; 58:1604–1615. [PubMed: 19403868]
19. Nachnani JS, Bulchandani DG, Nookala A, et al. Biochemical and histological effects of exendin-4 (exenatide) on the rat pancreas. *Diabetologia*. 2010; 53:153–159. [PubMed: 19756486]
20. Drucker DJ. Incretin action in the pancreas: potential promise, possible perils, and pathological pitfalls. *Diabetes*. 2013; 62:3316–3323. [PubMed: 23818527]
21. Ban K, Noyan-Ashraf MH, Hoefer J, et al. Cardioprotective and vasodilatory actions of glucagon-like peptide 1 receptor are mediated through both glucagon-like peptide 1 receptor-dependent and -independent pathways. *Circulation*. 2008; 117:2340–2350. [PubMed: 18427132]
22. Kim M, Platt MJ, Shibasaki T, et al. GLP-1 receptor activation and Epac2 link atrial natriuretic peptide secretion to control of blood pressure. *Nat Med*. 2013; 19:567–575. [PubMed: 23542788]
23. Wohlfart P, Linz W, Hubschle T, et al. Cardioprotective effects of lixisenatide in rat myocardial ischemia-reperfusion injury studies. *J Transl Med*. 2013; 11:84. [PubMed: 23537041]
24. Hong SM, Li A, Olino K, et al. Loss of E-cadherin expression and outcome among patients with resectable pancreatic adenocarcinomas. *Mod Pathol*. 2011; 24:1237–1247. [PubMed: 21552209]
25. Wang F, Flanagan J, Su N, et al. RNAscope: a novel in situ RNA analysis platform for formalin-fixed, paraffin-embedded tissues. *J Mol Diagn*. 2012; 14:22–29. [PubMed: 22166544]
26. Korner M, Stockli M, Waser B, et al. GLP-1 receptor expression in human tumors and human normal tissues: potential for in vivo targeting. *J Nucl Med*. 2007; 48:736–743. [PubMed: 17475961]
27. Schmidt EE, Schibler U. Cell size regulation, a mechanism that controls cellular RNA accumulation: consequences on regulation of the ubiquitous transcription factors Oct1 and NF-Y and the liver-enriched transcription factor DBP. *J Cell Biol*. 1995; 128:467–483. [PubMed: 7532171]
28. Hruban RH, Goggins M, Parsons J, et al. Progression model for pancreatic cancer. *Clin Cancer Res*. 2000; 6:2969–2972. [PubMed: 10955772]
29. Morris, JPt; Cano, DA.; Sekine, S., et al. Beta-catenin blocks Kras-dependent reprogramming of acini into pancreatic cancer precursor lesions in mice. *J Clin Invest*. 2010; 120:508–520. [PubMed: 20071774]
30. Rooman I, Real FX. Pancreatic ductal adenocarcinoma and acinar cells: a matter of differentiation and development? *Gut*. 2012; 61:449–458. [PubMed: 21730103]
31. Habbe N, Shi G, Meguid RA, et al. Spontaneous induction of murine pancreatic intraepithelial neoplasia (mPanIN) by acinar cell targeting of oncogenic Kras in adult mice. *Proc Natl Acad Sci U S A*. 2008; 105:18913–18918. [PubMed: 19028870]
32. Jones JB, Song JJ, Hempen PM, et al. Detection of mitochondrial DNA mutations in pancreatic cancer offers a “mass”-ive advantage over detection of nuclear DNA mutations. *Cancer Res*. 2001; 61:1299–1304. [PubMed: 11245424]
33. Schalper KA, Velcheti V, Carvajal D, et al. In situ tumor PD-L1 mRNA expression is associated with increased TILs and better outcome in breast carcinomas. *Clin Cancer Res*. 2014; 20:2773–2782. [PubMed: 24647569]

34. Vassilakopoulou M, Togun T, Dafni U, et al. In Situ Quantitative Measurement of HER2mRNA Predicts Benefit from Trastuzumab-Containing Chemotherapy in a Cohort of Metastatic Breast Cancer Patients. *PLoS One*. 2014; 9:e99131. [PubMed: 24968015]
35. Hall E, Dayeh T, Kirkpatrick CL, et al. DNA methylation of the glucagon-like peptide 1 receptor (GLP1R) in human pancreatic islets. *BMC Med Genet*. 2013; 14:76. [PubMed: 23879380]
36. Bramswig NC, Everett LJ, Schug J, et al. Epigenomic plasticity enables human pancreatic alpha to beta cell reprogramming. *J Clin Invest*. 2013; 123:1275–1284. [PubMed: 23434589]
37. Waser B, Blank A, Karamitopoulou E, et al. Glucagon-like-peptide-1 receptor expression in normal and diseased human thyroid and pancreas. *Mod Pathol*. 2015; 28:391–402. [PubMed: 25216224]
38. Koehler JA, Baggio LL, Cao X, et al. Glucagon-like peptide-1 receptor agonists increase pancreatic mass by induction of protein synthesis. *Diabetes*. 2015; 64:1046–1056. [PubMed: 25277394]
39. Scirica BM, Bhatt DL, Braunwald E, et al. Saxagliptin and cardiovascular outcomes in patients with type 2 diabetes mellitus. *N Engl J Med*. 2013; 369:1317–1326. [PubMed: 23992601]
40. White WB, Cannon CP, Heller SR, et al. Alogliptin after acute coronary syndrome in patients with type 2 diabetes. *N Engl J Med*. 2013; 369:1327–1335. [PubMed: 23992602]

Figure 1.

Representative images showing *glp-1r* hybridization in various pancreatic cell types: representative PDAC tissue section, showed at 20× (a) and 100× (b) magnification. The solid arrows in panel b indicate occasional signals observed. PanIN-3 sections are shown in panel c (40×) and d, (100×). The solid arrow indicates occasional staining. Islet cells show

prominent staining (solid arrows), whereas acinar cells show intermediate staining (dashed arrow) (panel e, 40×). Absent staining is visualized in ductal cells (f, 100×).

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Figure 2.

Comparison between signal-to cell-ratios observed for GLP-1R and POLR2A in endocrine (a), PDAC (b), acinar (c), and ductal cells (d) (Welch two-sample t-test).

Table 1
Clinicopathological characteristics of the patients whose pancreatic tissues were analyzed for GLP1R expression.

TMA	Patient	Age at surgery	Gender	Diabetes*	Diabetes therapy	Type of surgery	Grade of invasive cancer	Stage	Cell types analyzed
TMA 1	PC-1	54	M	N		PPPD	G2	T3N1M0	cancer, ductal, endocrine, acinar
	PC-2	56	M	Y	Insulin	Whipple	G3	T3N0M0	cancer, endocrine, acinar
	PC-3	72	F	N		PPPD	G2	T2N0M0	ductal, endocrine, acinar
	PC-4	54	F	N		PPPD	G3	T3N1M0	cancer, ductal, acinar
	PC-5	75	F	N		PPPD	G2	T1N0M0	cancer, ductal, acinar
	PC-6	57	M	Y	Metformin	PPPD	G3	T3N1M0	cancer, ductal, acinar
	PC-7	53	M	Unknown		Whipple	G2	T3N1M0	cancer, ductal, acinar
	PC-8	52	M	N		PPPD	G3	T3N1M0	cancer, ductal, acinar
	PC-9	69	F	N		Whipple	G2	T3N1M0	cancer, ductal, acinar
	PC-10	51	F	N		PPPD	G3	T3N1M0	cancer, ductal, acinar
	PC-11	66	M	Y	Unknown	PPPD	G2	T3N1M0	cancer, ductal, acinar
	PC-12	67	M	N		PPPD	G1	T3N0M0	cancer, ductal, acinar
	PC-13	63	M	Y	Insulin	PPPD	G2	T3N1M0	cancer, endocrine, acinar
	PC-14	74	M	N		PPPD	G3	T3N1M0	cancer, ductal, acinar
TMA 2	PC-15	65	M	N		PPPD	G2	T2N0M0	PanIN-1, PanIN-3, ductal acinar
	PC-16	74	M	Y	Unknown	PPPD	G2	T3N1M0	ductal, acinar
	PC-17	61	F	Y	Insulin	PPPD	G2	T3N1M0	ductal, PanIN-1, acinar
	PC-18	80	F	N		PPPD	G3	T3N0M0	ductal, PanIN-1, PanIN-2, acinar
	PC-19	68	M	N		PPPD	G2	T3N1M0	ductal, PanIN-1, cancer, acinar
	PC-20	56	M	N		PPPD	G3	T2N1M0	ductal, PanIN-1, endocrine, acinar
	PC-21	64	F	N		PPPD	G3	T3N1M0	ductal, PanIN-1, endocrine, acinar
	PC-22	69	M	N		PPPD	G2	T3N1M0	ductal, cancer, acinar
	PC-23	68	M	N		PPPD	G2	T3N1M0	ductal, PanIN-2, PanIN-3, acinar
	PC-24	56	F	N		PPPD	G3	T3N1M0	ductal, PanIN-1, PanIN-2, PanIN-3, acinar
	PC-25	76	M	N		PPPD	G2	T3N1M0	ductal, PanIN-3, cancer, acinar
	PC-26	42	M	N		Whipple	G3	T3N1M0	ductal, PanIN-1, acinar

TMA	Patient	Age at surgery	Gender	Diabetes*	Diabetes therapy	Type of surgery	Grade of invasive cancer	Stage	Cell types analyzed
	PC-27	80	F	N		PPPD	G2	T3N1M0	ductal, PanIN-1, PanIN-2, PanIN-3, acinar
	PC-28	63	F	N		Whipple	G2	T3N1M0	ductal, PanIN-1, PanIN-2, acinar
	PC-29	73	F	N		PPPD	G2	T2N1M0	ductal, PanIN-3, cancer, acinar
	PC-30	55	M	N		Whipple	G3	T3N1M0	ductal, PanIN-3, acinar
	PC-31	79	M	N		PPPD	G2	T3N1M0	ductal, PanIN-1, acinar
	PC-32	68	M	N		PPPD	G2	T3N1M0	ductal, PanIN-3, acinar

TMA: Tissue Microarray; Whipple: standard pancreaticoduodenectomy; PPPD: pylorus-preserving pancreaticoduodenectomy; N: no; Y: yes.

* Refers to long-standing diabetes mellitus.

Table 2

Summary statistics of the signal-to-cell values, by cell type. The median (range) of the ratios and the mean (SD) of the log-transformed ratios are shown. The median (range) of the number of cells, and the mean (SD) cell size, by type, are also shown.

	Median (Range)	Mean (SD)	Median (Range)	Mean (SD)
Endocrine Cells	0.196 (0.094, 0.359)	-1.649 (0.484)	126.5 (26, 190)	439.1 (49.8)
Ductal Cells	0.01 (0, 0.063)	-4.775 (1.239)	125.5 (50, 778)	265.8 (64.9)
PanIN-1A Cells	0.013 (0, 0.092)	-4.95 (1.633)	85 (50, 1991)	332.7 (72.9)
PanIN-1B Cells	0.019 (0.009, 0.133)	-3.707 (0.934)	412 (113, 1141)	368.6 (104.5)
PanIN-2 Cells	0.021 (0.011, 0.05)	-3.82 (0.597)	339 (36, 522)	493.8 (209.2)
PanIN-3 Cells	0.052 (0.003, 0.148)	-3.461 (1.249)	260 (72, 762)	547 (139.4)
Cancer Cells	0.062 (0, 0.186)	-2.95 (1.211)	321 (60, 1062)	592 (233.5)
Acinar Cells	0.029 (0.003, 0.103)	-3.484 (0.862)	1020 (219, 1351)	478 (78.7)

Table 3

Summary statistics of size calculations in the cell types analyzed.

	Cancer	Acinar	Ductal	PanIN-1	PanIN-2	PanIN-3	Endocrine
Min.	300.4	251.5	178.4	175	132.9	259	200
Mean	551.4	487.4	338.2	375.5	430.4	591.5	400
Max.	1278.4	780.8	900.3	720.5	710.3	1260.2	809.7

Values are expressed in μm^2 ; Min.: minimum area, Max: maximum area.

Table 4

Results from a mixed-effects linear regression model with the log-ratios as the response and fixed effect terms for cell type, cell size and TMA. Only acinar, ductal and endocrine cells are included. Random effects include a random intercept for each patient. The results are shown in two different ways: as the difference in the log ratios between cell types, and also as the ratio of the means of the untransformed ratios (calculated by exponentiating the difference).

	Difference in log ratios	95% CI	Ratio of means	95% CI	<i>p</i>
Acinar v. Ductal	0.99	(0.004, 1.982)	2.70	(1.004, 7.257)	0.061
Endocrine v. Ductal	2.73	(1.53, 3.936)	2.73	(4.618, 51.213)	0.00018
Endocrine v. Acinar	1.74	(0.764, 2.718)	5.70	(2.147, 15.15)	0.002

Table 5

Results from a mixed effects linear regression model with the log-ratios as the response and fixed effect terms for cell type, cell size and TMA. Random effects include a random intercept for each patient. The results are shown in two different ways: as the difference in the log ratios between cell types, and also as the ratio of the means of the untransformed ratios (calculated by exponentiating the difference).

	Difference in log ratios	95% CI	Ratio of means	95% CI	P
Acinar v Ductal	1.039	(0.343, 1.735)	2.826	(1.409, 5.669)	0.0048
Cancer v Ductal	1.2	(0.267, 2.133)	3.32	(1.306, 8.44)	0.014
Endocrine v Ductal	2.718	(1.613, 3.823)	15.15	(5.018, 45.741)	1.00E-05
PanIN-1 v Ductal	0.36	(-0.366, 1.086)	1.433	(0.694, 2.962)	0.335
PanIN-2 v Ductal	0.855	(-0.271, 1.981)	2.351	(0.763, 7.25)	0.142
PanIN-3 v Ductal	1.111	(0.115, 2.107)	3.037	(1.122, 8.224)	0.033
Acinar v Cancer	-0.162	(-0.895, 0.571)	0.85	(0.409, 1.77)	0.667
Endocrine v Cancer	1.518	(0.391, 2.645)	4.563	(1.478, 14.083)	0.01
PanIN-1 v Cancer	-0.841	(-1.819, 0.137)	0.431	(0.162, 1.147)	0.097
PanIN-2 v Cancer	-0.345	(-1.52, 0.83)	0.708	(0.219, 2.293)	0.566
PanIN-3 v Cancer	-0.089	(-1.091, 0.913)	0.915	(0.336, 2.492)	0.862
Acinar v PanIN-3	-0.073	(-0.943, 0.797)	0.93	(0.389, 2.219)	0.87
Endocrine v PanIN-3	1.607	(0.344, 2.87)	4.988	(1.411, 17.637)	0.015
PanIN-1 v PanIN-3	-0.752	(-1.744, 0.24)	0.471	(0.175, 1.271)	0.143
PanIN-2 v PanIN-3	-0.256	(-1.46, 0.948)	0.774	(0.232, 2.581)	0.678
Endocrine v Acinar	1.68	(0.636, 2.724)	5.366	(1.889, 15.241)	0.0025
PanIN-1 v Acinar	-0.679	(-1.421, 0.063)	0.507	(0.241, 1.065)	0.078
PanIN-2 v Acinar	-0.184	(-1.228, 0.86)	0.832	(0.293, 2.363)	0.731
Endocrine v PanIN-2	1.863	(0.477, 3.249)	6.443	(1.611, 25.765)	0.011
PanIN-1 v PanIN-2	-0.495	(-1.615, 0.625)	0.61	(0.199, 1.868)	0.39
PanIN-1 v Endocrine	-2.359	(-3.517, -1.201)	0.095	(0.03, 0.301)	0.00018