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Insulin-like growth factor-1 receptor expression and disease recurrence and survival in patients with resected pancreatic ductal adenocarcinoma

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Abstract

BACKGROUND—Insulin-like growth factor-1 receptor (IGF1R) signaling is important in pancreatic ductal adenocarcinoma (PDAC) biology, but little is known regarding IGF1R expression and patient characteristics and outcomes.

METHODS—In 365 patients with resected PDAC we evaluated IGF1R protein expression using immunohistochemistry on whole-slide sections and *IGF1R* genomic status using next-generation sequencing. Associations of IGF1R expression, measured by H-scores incorporating staining intensity and proportion of positive tumor cells, with disease-free survival (DFS) and overall survival (OS) were evaluated in 317 and 321 patients, respectively, using Cox regression adjusting for known prognostic factors.

RESULTS—Higher IGF1R expression in tumor cells was associated with worse DFS comparing highest vs. lowest expression tertiles (median DFS, 10.8 vs. 16.1 months; adjusted hazard ratio [HR], 1.73; 95% CI, 1.24–2.44; P_{trend}=0.002) and worse OS (median OS, 17.4 vs. 25.8 months; HR, 1.39; 95% CI, 1.00–1.92; $P_{trend=}$0.046). The association between high IGF1R expression and reduced DFS was identified primarily among patients with a preoperative body mass index ≥25 kg/m^2 (HR, 4.27; 95% CI, 2.03–8.96, comparing extreme tertiles; $P_{interaction}$=0.032). *KRAS*-mutant tumors had greater IGF1R expression, and IGF1R expression in tumor epithelium was inversely correlated with that in stromal cells. Mutations in *IGF1R* were infrequent, and no overt loss of function alterations were identified. Higher IGF1R expression was modestly associated with higher gene copy number (Pearson correlation coefficient=0.26, P<0.001).

CONCLUSIONS—Higher IGF1R protein expression was associated with worse patient outcomes in resected PDAC.

IMPACT—IGF1R expression in PDAC represents a potential biomarker to guide patient selection for more aggressive, multi-drug regimens in the adjuvant setting.

Keywords

IGF1R; pancreatic adenocarcinoma; survival; biomarker; immunohistochemistry

INTRODUCTION

Pancreatic cancer is the third leading cause of cancer-related mortality in the United States.(1) Even among patients with potentially curable, localized disease, the rate of subsequent mortality from recurrent cancer is high, with 5-year OS rates up to 25–30%.(2,3) Surgical resection combined with perioperative systemic chemotherapy offers the best opportunity for cure;(4–6) however, the survival benefit of more aggressive multi-drug regimens must be counterbalanced against the elevated toxicity of such regimens. It is therefore critical to

identify biomarkers that inform risk of disease recurrence and death after cancer resection in order to enable better therapy selection.

The insulin-like growth factor (IGF) pathway is a complex signaling system that has been implicated in driving pancreatic ductal adenocarcinoma (PDAC) tumorigenesis and progression.(7–11) Central to this pathway is IGF1R (IGF-1 receptor), a membrane-associated receptor tyrosine kinase that is broadly expressed in normal human tissue. Upon binding to its ligands IGF-1, IGF-2, and insulin, IGF1R drives activation of downstream mitogenic and apoptotic pathways.(12–14) Experimental evidence suggests that IGF signaling, mediated by IGF1R, contributes to tumor invasiveness, disease recurrence, and resistance to chemotherapy in PDAC.(7–11) Some of these interactions are likely mediated by autocrine as well as paracrine signaling that may be a consequence of reciprocal interactions between tumor cells and non-neoplastic cells in the tumor microenvironment.(7–11,15–18) While prior studies have shown that tumors with high IGF1R expression are associated with worse survival, limited availability of clinicopathologic and correlative data in these studies precluded investigation into the mechanisms by which IGF signaling may influence patient outcomes.(19,20) Notably, IGF1R activity may be especially relevant in subgroups of patients with obesity or diabetes mellitus, both known risk factors for PDAC, which are associated with a hyperinsulinemic state that may drive IGF1R activity via endocrine signals.(10,21–23) Taken together, these data indicate that IGF1R activity may be associated with adverse clinicopathological features and poor outcomes for PDAC patients.

In this study, we assessed expression of IGF1R protein in tumor and stromal cells using immunohistochemistry (IHC) and characterized the genomic status of *IGF1R* using next-generation sequencing (NGS) in a large, multi-institutional population of patients with resected PDAC. We evaluated IGF1R expression as a predictor of disease recurrence and mortality and explored associations between IGF1R status, clinicopathologic characteristics, and genomic features that may impact IGF1R signaling.

MATERIALS AND METHODS

Study population

We evaluated 365 patients with resected PDAC who were treated at three U.S. cancer centers: 129 at Dana-Farber/Brigham and Women's Cancer Center (BWCC; Boston, MA) between September 15th, 2000 and May 21st, 2012; 90 at the University of Rochester Medical Center (URMC; Rochester, NY) between March 1st, 2006 and November 1st, 2013; and 146 at Stanford Cancer Institute (SCI; Stanford, CA) between September 26th, 1995 and May 22nd, 2013. Institutional review boards at each institution granted approval for this study.

Assessment of covariates

From medical records, we ascertained age at surgery, sex, racial background, preoperative body mass index (BMI, kg/m^2), history of diabetes mellitus, tumor location, tumor size, pT and pN stage based on the American Joint Committee on Cancer (AJCC, 8th edition) staging

system, tumor differentiation, presence of lymphovascular or perineural invasion, resection margin status, and history of perioperative chemotherapy and radiation.

Immunohistochemistry for IGF1R

IHC for IGF1R protein was performed on 4-μm sections of formalin-fixed paraffin-embedded (FFPE) cancer resection specimens. After deparaffinization and rehydration, antigen retrieval was performed using pH 9 EDTA-buffered antigen retrieval solution (eBioscience, San Diego, California, USA) in a pressure cooker with microwave heating at 100% power for 17 minutes. After blocking, sections were incubated for 16 hours at 4°C with a rabbit monoclonal anti-IGF1R antibody (Clone D4O6W, Cell Signaling, San Diego, California, USA; dilution, 1:50). An HRP-labeled anti-rabbit secondary antibody (EnVision HRP-labeled polymer Anti-Rabbit, Agilent, Santa Clara, CA, USA) was then applied for 30 minutes, followed by visualization with 3,3-diaminobenzidine and counterstaining with hematoxylin. An isotype-matched control for the primary IGF1R antibody (clone DAK-GO1; mouse IgG1 kappa; dilution, 1:6000; Agilent, Santa Clara, CA, USA) was substituted for the primary anti-IGF1R antibody to aid in confirmation of primary antibody specificity. Pancreatic islet cells served as internal positive controls for IGF1R expression, while smooth muscle cells within the walls of small-caliber vessels served as a negative control. Sections without appropriate expression in positive and negative control cell populations were excluded from analysis. All immunohistochemistry was performed in a single laboratory over a two-month time period to minimize analytic variability.

Evaluation of IGF1R expression

IGF1R expression was evaluated by a single pathologist blinded to clinicopathologic and molecular data (Figure 1). To assess interobserver variability, approximately 40% of the total cases (N=146) were evaluated by a second blinded pathologist. IGF1R expression was evaluated separately in both tumor epithelium and within stromal cells surrounding tumor epithelium. For scoring tumor epithelium, only membranous IGF1R expression was evaluated. Although faint, granular cytoplasmic IGF1R staining in tumor epithelium was occasionally observed, cytoplasmic staining alone was not considered positive. For invasive adenocarcinoma, the broadly accepted histologic score (“H-score”) method(24) was employed. Staining intensity of the predominant expression pattern for each tumor was scored on a four-point scale (0: negative, 1: mild, 2: moderate, 3: intense) along with the percentage of tumor cells that exhibited the staining pattern. The product of the staining intensity and the percentage of positive cells was calculated to provide a predominant H-score for each tumor. The degree of staining intensity and percentage of positive tumor cells for the second most common staining pattern (if present) was recorded and a secondary H-score was obtained, with a combined H-score generated by adding the predominant and secondary H-scores. To evaluate stromal IGF1R evaluation, scoring was limited to stromal cells located within an approximately 250 μm-wide region (one-half of the diameter of a 40x field-of-view) directly surrounding each tumor gland or cell. IGF1R expression in this region was classified as positive (any degree of intensity) or negative (complete absence of expression) (Supplementary Figure S1).

DNA sequencing

Tumor and normal DNA were extracted from FFPE sections of resected PDAC specimens. Massively-parallel sequencing was performed using a customized, hybrid-capture-based platform that targets either 422 (version 1) or 428 (version 2) PDAC-associated genes for detection of mutations, copy number alterations and selected structural variants, as previously described.(25) *IGF1R* copy number status was calculated according to the formula: copy number = (2 * (AGCR-1)/P)+2) where AGCR is the average gene copy ratio after normalization and P represents the tumor purity fraction.

Assessment of *KRAS*, *CDKN2A*, *SMAD4, and TP53*

We evaluated the status of the key PDAC driver genes *KRAS*, *CDKN2A, SMAD4*, and *TP53* using an integrated sequencing and IHC approach, as previously described.(25) Briefly, we performed targeted pyrosequencing for *KRAS* hotspot alterations and next-generation sequencing using our customized panel to determine the molecular status of all four driver genes. Additionally, IHC for CDKN2A (p16), SMAD4, and TP53 was performed on whole slide sections of each tumor. *KRAS* status was classified as mutant or wild-type based on NGS (or pyrosequencing if the predefined NGS coverage goals were not reached); the status of *CDKN2A* and *SMAD4* was classified as intact or lost based on IHC results; and for *TP53*, NGS and IHC data were integrated to yield a final classification of wild-type or altered.

Outcome measures and eligibility for survival analyses

We defined disease-free survival (DFS) as time between surgery and disease recurrence, and overall survival (OS) as time between surgery and all-cause mortality. Follow-up continued through June 28th, 2016 for DFCI/BWCC patients, March 17th, 2016 for URMC patients, and March 11th, 2016 for SCI patients. Metastatic disease (liver metastases, peritoneal implants) was found intraoperatively in nine patients who underwent pancreatic resection, and these were excluded from outcome analyses; similarly, patients with 30-day or in-hospital mortality (N=11) and those who received neoadjuvant therapy (N=24) were also excluded. The final study population for primary analyses of DFS and OS was 317 and 321 patients, respectively (Supplementary Figure S2). Sensitivity analyses incorporating patients who received neoadjuvant treatment included 341 and 345 patients for DFS and OS analyses, respectively.

Statistical analyses

Interobserver reliability of predominant H-scores was assessed using the intraclass correlation coefficient.(26) For statistical analyses, we categorized IGF1R H-scores into tertiles to allow for comparisons between extremes of expression. We evaluated associations between driver gene status and IGF1R expression using Fisher's exact test. For survival analyses, we analyzed associations of IGF1R predominant H-score tertiles with DFS and OS using multivariable-adjusted Cox proportional hazards regression, calculating hazard ratios (HR) and 95% confidence intervals (95% CI); we also generated Kaplan-Meier curves, from which we calculated median survival times. Multivariable-adjusted models were built using stepwise selection with Cox proportional hazards regression using entry and keep thresholds

of P=0.15 and P=0.05, respectively. Age at surgery (continuous variable) and sex were entered into the model *a priori* and the following covariates were considered for stepwise selection: racial background, body mass index (<25, ≥25, unknown); history of diabetes mellitus; tumor location; AJCC 8th ed. pT stage; AJCC 8th edition pN stage; degree of tumor differentiation; lymphovascular invasion; perineural invasion; *KRAS* status; *CDKN2A* status, *SMAD4* status, and *TP53* status; perioperative systemic treatment, perioperative radiation treatment; resection margin status; and cancer center. The variables selected for the final multivariable-adjusted model using stepwise selection were age at surgery, sex, AJCC 8th edition N stage, tumor differentiation, perineural invasion, resection margin status, *CDKN2A* status, perioperative systemic treatment, and perioperative radiation treatment. We confirmed the validity of the proportionality of hazards assumption by evaluating a time-dependent variable resulting from the cross-product of the exposures of interest (predominant and combined IGF1R H-scores) and time (DFS and OS).

We conducted linear trend tests across tertiles of IGF1R predominant H-score by assigning each subject the median H-score value for their corresponding tertile and modeling it as a continuous variable. We performed secondary outcome analyses based on tertiles of IGF1R combined H-score. Moreover, we conducted sensitivity analyses including patients who received neoadjuvant therapy. Since patient demographic and clinical characteristics may influence their metabolic status and IGF signaling pathways, we conducted tests of interaction by strata of potential effect modifiers (sex, age, BMI, and diabetes mellitus) by creating an interaction term as the cross-product of IGF1R predominant H-score (continuous variable) and the covariate of interest (as a binary variable), and entering it into the multivariable models. Last, we evaluated the association between *IGF1R* gene copy number and IGF1R predominant H-score using the Pearson correlation coefficient; we also compared the IGF1R predominant H-scores based on IGF1R staining in the stromal component using the Kruskal-Wallis test. All hypothesis tests were two-sided and statistical significance was set at P<0.05; statistical analyses were performed using SAS software (version 9.4; SAS Institute, Cary, N.C.).

RESULTS

In our patient cohort, 13% of cases showed complete absence of IGF1R expression, with 317 (87%) cases showing variable degrees of membranous IGF1R expression. Heterogeneity of expression was noted within tumors, with 30% of tumors showing at least two different expression intensities and many tumors containing areas of complete negativity. The interclass correlation coefficient was 0.67 for the predominant H-score, indicating adequate reliability between two pathologists. Baseline patient and tumor characteristics of 365 subjects with resected PDAC by tertiles of IGF1R predominant H-scores are presented in Table 1 and representative IHC images from each tertile are provided in Figure 1. The median H-score was 99 and H-scores were not associated with clinicopathological features. Higher H-scores were associated with the presence of *KRAS* mutations (Fisher's exact test P=0.019) without specificity for a distinct allele, but not with alterations in *CDKN2A*, *SMAD4*, or *TP53* (Table 2).

IGF1R expression and risk of disease recurrence and survival

Median follow-up time among patients who were alive at the end of the study (*N*=77, 23%) was 33.7 months. Median DFS and OS in our study population were 13.3 and 21.0 months, respectively. Higher IGF1R predominant H-scores were significantly associated with worse DFS (adjusted P_{trend}=0.002) (Table 3, Figure 2); patients whose tumors demonstrated H-scores in the highest tertile had an adjusted HR for DFS of 1.73 (95% CI 1.24–2.44) compared with those in the lowest tertile, with median DFS of 10.8 and 16.1 months, respectively. The association between higher IGF1R H-scores and OS was similar (P_{trend}=0.046); patients with H-scores in the highest and lowest tertiles had a median OS of 17.4 and 25.8 months, respectively (HR 1.39, 95% CI 1.00–1.92) (Table 3).

Outcome analyses based on tertiles of IGF1R combined H-scores, identified by summing the predominant and secondary H-scores to examine the utility of more granular assessment of intratumoral IGF1R expression, demonstrated similar results to those found using the predominant H-scores (Table 3). Compared with the lowest tertile, IGF1R combined H-scores in the highest tertile were associated with a HR for DFS of 1.77 (95% CI 1.26–2.51, P_{trend}=0.001) and a HR for OS of 1.47 (95% CI 1.06–2.03, P_{trend}=0.021). Sensitivity analyses including patients who received neoadjuvant therapy revealed similar results (Supplementary Table S1).

Stratified analyses

We conducted subgroup analyses across strata of several potential effect-modifying variables that may influence IGF signaling (Table 4). The association between higher IGF1R predominant H-score and DFS was significantly modified by preoperative BMI ($P_{interaction}$=0.032). Among patients with overweight or obesity (define as BMI ≥25 kg/m^2), H-scores in the highest tertile were associated with a HR for DFS of 4.27 (95% CI 2.03–8.96), while IGF1R predominant H-scores were not associated with DFS in patients with BMI <25 kg/m^2 (highest vs. lowest tertiles, HR 0.72, 95% CI 0.24–2.21). We found no significant interactions by patient sex, age, or diagnosis of diabetes mellitus (Table 4).

SNV and CNV analyses

Mutations in *IGF1R* were uncommon, with no tumors harboring likely inactivating alterations such as frameshift or nonsense mutations, and only two tumors harboring missense mutations (c.3086G>A [p.R1029K] and c.3852G>T [p.E1284D]). Neither missense mutation occurred at a mutational hotspot nor had been previously reported in pancreatic adenocarcinoma or other tumor types based on publicly available sequencing databases (COSMIC, cBioPortal). We conducted CNV analyses in tumor tissue to evaluate associations between *IGF1R* gene copy number and IGF1R predominant H-scores. Overall, the median estimated *IGF1R* gene copy number was 2.06 (range 0.61–4.69). Higher H-scores were modestly associated with higher estimated gene copy number (Pearson correlation coefficient=0.26, *P*<0.001), although the median *IGF1R* gene copy number in tumors in the highest and lowest tertiles of IGF1R predominant H-scores were 2.09 (range 1.29–4.69) and 2.04 (range 1.09–3.33), respectively.

IGF1R protein expression in tumor stroma

We assessed IGF1R expression in peritumoral stromal cells in relation to IGF1R predominant H-scores in tumor cells (Supplementary Figure S1). Positive IGF1R stromal expression was observed in 76 (21%) cases and was associated with lower H-scores in tumor cells ($P<0.001$); the median H-score among cases with positive stromal expression was 55, compared to 100 in cases with negative stromal expression. Positive IGF1R stromal expression was not associated with DFS (HR 1.03, 95% CI 0.75–1.42) or OS (HR 0.98, 95% CI 0.71–1.35). On *post-hoc* analyses, we found no significant difference in preoperative BMI comparing cases with positive and negative IGF1R stromal expression (median BMI, 26.5 vs. 23.7; $P=0.15$).

DISCUSSION

In a large, multi-institutional population of patients with resected PDAC, higher IGF1R tumor expression was associated with increased risk of disease recurrence independent from potential confounders. This association was influenced by preoperative BMI. Among patients with BMI ≥25 kg/m^2, higher IGF1R expression was associated with a 4-fold increased risk of recurrence; in contrast, we found no association between IGF1R expression and survival outcomes among patients with BMI <25 kg/m^2. We also found that *KRAS*-mutant tumors have higher IGF1R expression, supporting the notion that *KRAS* wild-type pancreatic cancer is molecularly distinct from *KRAS* mutant pancreatic cancer at a level beyond *KRAS* itself.(27) Finally, we identified expression of IGF1R in stromal cells, where it was inversely proportional to IGF1R expression in tumor cells.

IGF-1 and IGF-2 act through endocrine, paracrine, and autocrine mechanisms.(7,11,16,28) Binding of these ligands to IGF1R leads to downstream activation of the MAPK/RAS-RAF-ERK, the PI3K/AKT, and the JAK/STAT pathways.(12–14) The multifaceted role of IGF signaling in driving multiple mechanisms of tumor survival and progression in PDAC (7–11) has led to investigation into the potential clinical relevance of this pathway. Previous efforts have evaluated the protein expression of IGF1R in PDAC as an indicator of activity of this axis; while our rate of tumor IGF1R positivity of any degree (87%) is somewhat higher than prior reported 41–64% rates of IGF1R positivity in PDAC, this difference is likely due to our use of a more granular scoring method that does not classify tumors with patchy and/or weak IGF1R expression as negative.(19,20,29,30)

We explored associations between IGF1R expression and the main driver gene alterations in PDAC and found that *KRAS*-mutant tumors have higher levels of IGF1R expression than *KRAS* wild-type tumors. Activating *KRAS* mutations are present in more than 90% of PDAC and are a key event in pancreatic carcinogenesis(25,31,32). Recent experimental evidence suggests that PI3K/AKT activation in *KRAS*^{G12D} pancreatic ductal epithelial cells is mediated, in part, by autocrine IGF-2 and IGF1R signaling and is not entirely due to the direct activity of mutant *KRAS*.(9) While further work is needed to shed light on the interplay between KRAS activity and IGF1R signaling, our findings further support an interaction between the IGF pathway and this central molecular driver of pancreatic cancer.(33,34) The lack of association between IGF1R expression and alterations in *CDKN2A*, *SMAD4* and *TP53* suggests that IGF1R signaling is governed by factors that are distinct

from those associated with these driver genes. Finally, in the context of the IGF1R expression heterogeneity, the absence of significant genomic alterations in IGF1R implicates epigenetic, transcriptional, or post-transcriptional mechanisms as the main regulators of IGF1R expression.

PDAC is characterized by a dense, desmoplastic stroma with a rich cellular component (35,36). Recent evidence indicates that local IGF signaling activity in the PDAC microenvironment can be driven by reciprocal tumor-stromal cell crosstalk. *In vitro* and *in vivo* studies have revealed that macrophages and activated fibroblasts within the tumor microenvironment enhance tumor motility, metastatic potential, and chemoresistance through production of IGFs and paracrine signaling through IGF1R on PDAC tumor cells. (15–18,34) However, there are limited data evaluating whether peritumoral stromal cells express IGF1R.(7,19) We observed positive IGF1R expression in peritumoral stromal cells of 21% of cases in our cohort; moreover, positive stromal expression was inversely associated with the degree of IGF1R expression in tumor cells. In a previous study of 105 resected PDAC, Valsecchi *et al*(19) found positive stromal expression of IGF1R in half their study cohort. The reason for this discrepancy is unknown, but it may stem from differences in the stromal regions being assessed,(37) the scoring methodology, and sample size. Together with our data, these results suggest a role for paracrine IGF signaling in PDAC and suggest hypotheses for future experimental testing.

Previous studies have evaluated the prognostic role of tumor expression of IGF1R in patients with resected PDAC.(19,20) Valsecchi *et al*(19) showed that IGF1R overexpression (defined as strong, complete membranous staining in >30% of tumor cells) was associated with worse survival (unadjusted HR, 2.05; 95% CI, 1.25–3.37; P=0.004); however, no association was found upon assessment of expression according to H-scores (comparing H-score ≥200 vs. H-scores 0–199, HR, 0.95; 95% CI, 0.36–2.52). A different study of 122 patients found that tumor expression of IGF1R (classified as negative or positive, where positive expression corresponded to moderate or strong staining in ≥10% of tumor cells by IHC) was associated with higher mortality (unadjusted HR, 1.71; 95% CI, 1.09–2.69; P=0.020).(20) Using a more scoring granular scoring approach in a larger, we show that higher tumor expression of IGF1R is significantly associated with higher risk of disease recurrence and death after resection. Our results are also consistent with a recent proteomic study wherein IGF1R was identified as a dominant regulator of a protein expression signature associated with shortened PDAC survival.(38) We also evaluated IGF1R stromal expression and found that stromal IGF1R positivity is not associated with risk of recurrence and death.

Obesity is a known risk factor for PDAC(21) and previous studies have shown that cancer development in the context of obesity is associated with elevated IGF-1 and an increase in AKT/mTOR signaling activity.(10) Furthermore, obesity is commonly associated with insulin resistance, compensatory hyperinsulinemia, and increased hepatic IGF-1 synthesis. (23) We found that tumor IGF1R expression was not associated with preoperative BMI. However, the prognostic role of IGF1R expression was significantly modified by BMI. Among patients with BMI ≥25 kg/m^2, high IGF1R expression was associated with higher risk of recurrence and mortality, although this association was not present among patients with BMI <25 kg/m^2. We hypothesize that obesity may not directly influence IGF1R

expression by tumor cells, but rather that tumors with high IGF1R expression arising in individuals with obesity may have a more aggressive clinical course. Theoretically, high IGF1R expression may render such tumors more responsive to higher levels of circulating IGF1R ligands. However, mechanistic studies will be needed to more clearly define the role of IGF signaling in obesity-associated PDAC.

The key role for IGF signaling in numerous cancer types has motivated the development of compounds targeting this pathway, including anti-IGF1R monoclonal antibodies like ganitumab (AMG 479).(39–41) Ganitumab showed encouraging results in combination with gemcitabine in a randomized, phase II trial of patients with metastatic pancreatic cancer;(42) however, a subsequent phase III, randomized placebo-controlled trial of ganitumab combined with gemcitabine compared to single-agent gemcitabine failed to show significant survival benefits.(43) Our findings, together with previous experimental and observational evidence, support an important role for the IGF pathway in PDAC biology and suggest that novel therapeutic approaches to targeting this complex signaling pathway may offer clinical utility.(15,18,44)

Despite progressive improvement in the rates of cancer recurrence and survival with the use of multi-drug chemotherapy regimens in the adjuvant setting, outcomes for patients with resected PDAC remain suboptimal and are offset by significant treatment-related toxicities.(2,4–6) Characterizing markers such as IGF1R may help link the biology and behavior of patient's tumors to overall body composition and metabolic state. By elucidating these links, it may be possible to use markers such as IGF1R to help guide the selection of patients with the highest risk of recurrence who may therefore derive the greatest benefit from more aggressive treatment approaches.

Our study has multiple strengths, including a large sample size of patients with resected PDAC drawn from several centers in different geographic regions of the U.S. and highly annotated clinical, pathological, and treatment data, allowing for multivariable adjustment of survival analyses, stratified analyses by potential effect modifiers, and evaluation of relationships with key PDAC driver genes. In addition to evaluating IGF1R protein expression, we also conducted molecular analyses of *IGF1R.* Lastly, our IHC assessment also enabled outcome analyses based on a combined H-score incorporating the predominant and secondary patterns of IGF1R expression and assessment of IGF1R expression in peritumoral stroma. Our study also had limitations. While we included cases from multiple academic and community centers in the U.S. to capture a diversity of patients, most of our patient population was White. Studies with larger proportions of patients from different racial backgrounds are warranted. The results of our survival analyses are applicable to patients with non-metastatic disease amenable to surgical resection with curative intent; whether higher IGF1R tumor expression is associated with disease progression and mortality in patients with metastatic pancreatic cancer still needs to be determined. We identified a single preoperative BMI measurement for stratified analyses. Many patients with PDAC lose weight prior to their cancer diagnosis, such that we may be underestimating the number of patients who were chronically overweight or obese. Moreover, the timing of preoperative BMI assessment was not standardized across institutions; prospective studies with longitudinal assessments are needed. We also recognize that BMI is an imperfect parameter

to assess adiposity and central obesity. Finally, we evaluated archival tissue specimens, but did not conduct functional experiments to interrogate IGF1R signaling.

In conclusion, we show that higher IGF1R protein expression in PDAC is associated with increased risk of disease recurrence after cancer resection and worse overall survival. Upon stratification by preoperative BMI, the association between IGF1R and patient outcomes was seen predominantly among patients with BMI ≥25 kg/m^2, suggesting that IGF signaling pathways may play an important role in patient outcomes in obesity-associated PDAC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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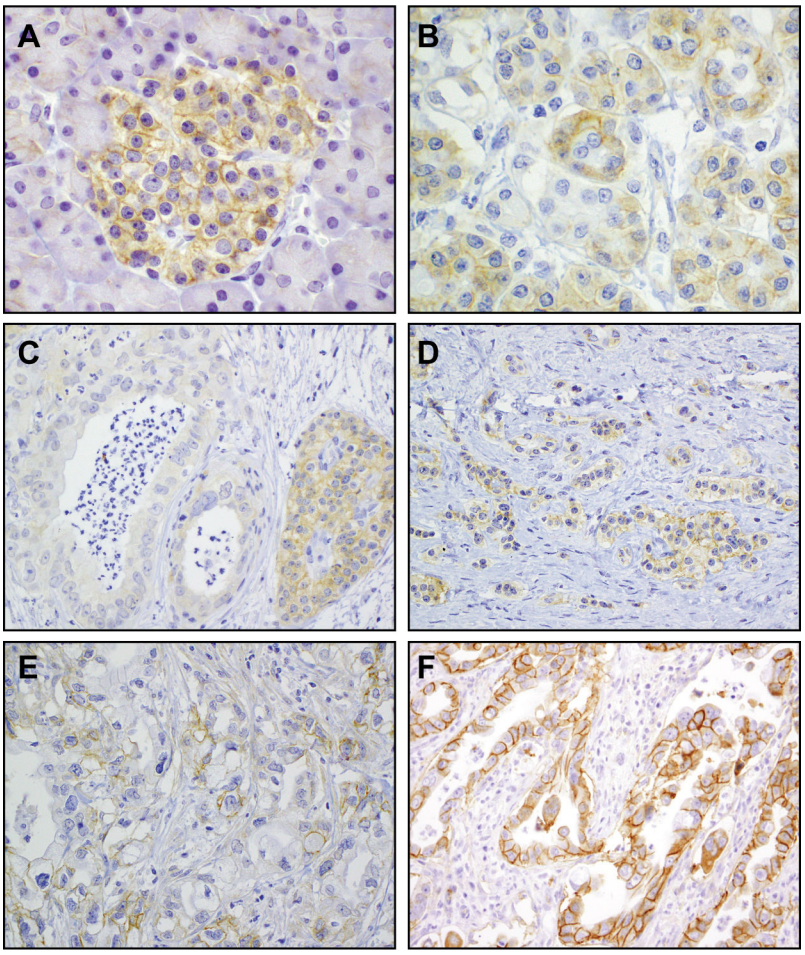
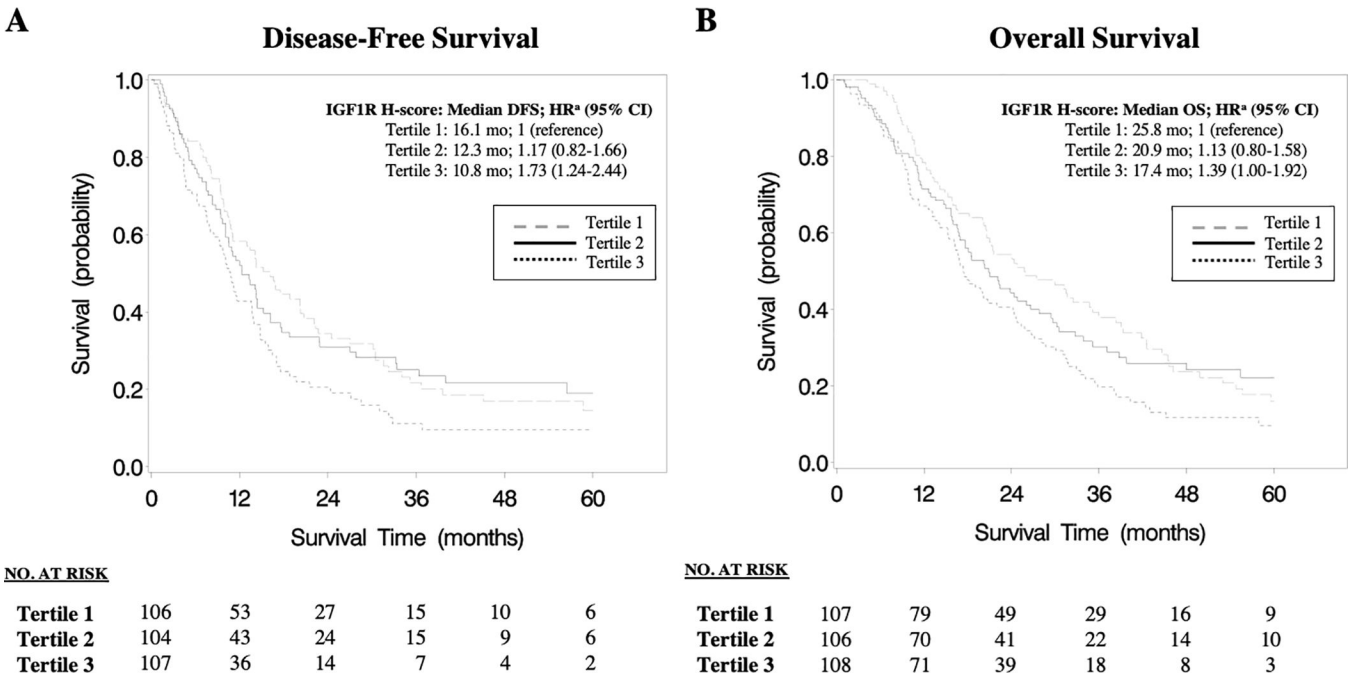


Figure 1.

Expression of IGF1R in normal pancreas and invasive pancreatic ductal adenocarcinoma **A.** Membranous IGF1R expression occurs in a patchy distribution within pancreatic islets. **B.** Pancreatic exocrine cells exhibit faint granular expression within the cytoplasm with only rare and weak membranous IGF1R expression. **C.** Complete absence of IGF1R expression (intensity 0 in 100% of cells, H-score 0) in PDAC with retained expression in an adjacent non-neoplastic islet. **D.** Patchy, weak (intensity 1 in 50% of cells, H-score 50) IGF1R expression in PDAC. **E.** Patchy, moderate (intensity 2 in 50% of cells, H-score 100) IGF1R expression in PDAC. **F.** Diffuse, strong (intensity 3 in 100% of cells, H-score 300) IGF1R expression in PDAC.



NO. AT RISK

Tertile 1	106	53	27	15	10	6
Tertile 2	104	43	24	15	9	6
Tertile 3	107	36	14	7	4	2

NO. AT RISK

Tertile 1	107	79	49	29	16	9
Tertile 2	106	70	41	22	14	10
Tertile 3	108	71	39	18	8	3

Figure 2.

Kaplan-Meier survival curves of patients with resected PDAC by tertiles of IGF1R predominant H-score. **A.** Disease-free survival. **B.** Overall survival. [a]Cox proportional-hazards model adjusted for age at the time of surgery, sex, N stage (American Joint Committee on Cancer 8th edition), tumor grade of differentiation, perineural invasion, resection margin status, *CDKN2A* status, perioperative systemic treatment, and perioperative radiation treatment.

Table 1.

Baseline characteristics of 365 patients with resected PDAC by IGF1R expression.

	Overall (*N* = 365)	IGF1R predominant H-score tertiles			*P* [a]
		1 (*N* = 121)	2 (*N* = 122)	3 (*N* = 122)	
IGF1R predominant H-score, median (range)	99 (0–300)	5 (0–50)	99 (60–140)	200 (150–300)	
Age, median (IQR)	67 (14)	66 (15)	68 (14)	67 (15)	0.83
Women (n, %)	169 (46%)	56 (46%)	59 (48%)	54 (44%)	0.81
Racial background (n, %)					
White	284 (78%)	96 (79%)	96 (79%)	92 (75%)	0.80
Black	5 (1%)	3 (3%)	1 (1%)	1 (1%)	
Asian	33 (9%)	11 (9%)	11 (9%)	11 (9%)	
Other/Unknown	43 (12%)	11 (9%)	14 (11%)	18 (15%)	
Body mass index (kg/m2), median (IQR) [b]	26.2 (7.4)	27.4 (7.3)	25.4 (8.0)	25.3 (7.1)	0.15
Diabetes mellitus (n, %)					
No	238 (71%)	84 (76%)	75 (66%)	79 (71%)	0.56
Yes (NOS)	32 (10%)	9 (8%)	8 (7%)	15 (13%)	
Yes, managed with diet	7 (2%)	4 (3%)	1 (1%)	2 (2%)	
Yes, managed with oral medication	42 (12%)	12 (11%)	19 (17%)	11 (10%)	
Yes, managed with insulin	18 (5%)	2 (2%)	11 (9%)	5 (4%)	
Unknown	28	10	8	10	
Tumor location (n, %)					
Head/Uncinate	267 (73%)	89 (74%)	88 (72%)	90 (74%)	0.63
Body/Tail	89 (24%)	28 (23%)	33 (27%)	28 (23%)	
Overlapping sites	9 (3%)	4 (3%)	1 (1%)	4 (3%)	
Tumor size in cm, median (IQR)	3.1 (1.5)	3.1 (1.6)	3.3 (2.0)	3.1 (1.5)	0.47
N stage (AJCC 8th edition), (n, %)					
N0 (0 positive lymph nodes)	116 (32%)	45 (37%)	41 (34%)	30 (25%)	0.28
N1 (1–3 positive lymph nodes)	132 (36%)	40 (33%)	41 (34%)	51 (42%)	
N2 (≥4 positive lymph nodes)	116 (32%)	36 (30%)	40 (32%)	40 (33%)	
Nx (cannot be assessed)	1	-	-	1	
Tumor differentiation (n, %)					
Well/Moderately differentiated	211 (59%)	72 (61%)	74 (63%)	65 (54%)	0.36
Poorly differentiated/Undifferentiated	145 (41%)	46 (39%)	44 (37%)	55 (46%)	
Unknown	9	3	4	2	
Perineural invasion (n, %)					
Absent	41 (12%)	10 (9%)	18 (16%)	13 (11%)	0.26
Present	302 (88%)	103 (91%)	96 (84%)	103 (89%)	
Unknown	22	8	8	6	
Resection margin status (n, %)					
R0	179 (49%)	60 (50%)	65 (54%)	54 (44%)	0.57
R1	177 (49%)	58 (48%)	53 (44%)	66 (54%)	

	Overall (*N* = 365)	IGF1R predominant H-score tertiles			*P* [a]
		1 (*N* = 121)	2 (*N* = 122)	3 (*N* = 122)	
R2	7 (2%)	3 (2%)	2 (2%)	2 (2%)	
Rx (unknown)	2	-	2	-	
Neoadjuvant treatment (n, %)					
No	341 (93%)	114 (94%)	115 (94%)	112 (92%)	0.68
Yes	24 (7%)	7 (6%)	7 (6%)	10 (8%)	
Adjuvant treatment (n, %)					
No	102 (29%)	33 (28%)	39 (33%)	30 (25%)	0.47
Yes	255 (71%)	86 (72%)	81 (67%)	88 (75%)	
Unknown	8	2	2	4	

Abbreviations: PDAC, pancreatic ductal adenocarcinoma; IGF1R, insulin-like growth factor-1 receptor; IQR, interquartile range; NOS, not otherwise specified; AJCC, American Joint Committee on Cancer.

[a] Chi-square or Fisher's exact test (where appropriate) for categorical variables; Kruskal-Wallis test for continuous variables.

[b] Available for 186/365 subjects

Table 2.

Associations between main driver gene alterations and IGF1R expression.

	Overall (*N* = 365)	IGF1R predominant H-score tertiles			*P* [a]
		1 (*N* = 121)	2 (*N* = 122)	3 (*N* = 122)	
KRAS (n, %)					
Wild-type	27 (8%)	13 (11%)	11 (9%)	3 (3%)	0.019
Mutant	324 (92%)	101 (89%)	108 (91%)	115 (97%)	
Unknown	14	7	3	4	
KRAS mutation[b] (n, %)					
G12D	130 (40%)	39 (38%)	45 (42%)	46 (40%)	0.94
G12V	106 (33%)	36 (36%)	33 (30%)	37 (32%)	
G12R	49 (15%)	15 (15%)	16 (15%)	18 (16%)	
Codon 61	24 (8%)	5 (5%)	10 (9%)	9 (8%)	
Other mutation[c]	4 (1%)	2 (2%)	-	2 (2%)	
Two mutations	11 (3%)	4 (4%)	4 (4%)	3 (2%)	
CDKN2A (n, %)					
Intact	116 (33%)	36 (32%)	38 (32%)	42 (36%)	0.78
Lost	235 (67%)	78 (68%)	81 (68%)	76 (64%)	
Unknown	14	7	3	4	
SMAD4 (n, %)					
Intact	178 (51%)	61 (54%)	59 (50%)	58 (49%)	0.77
Lost	173 (49%)	53 (46%)	60 (50%)	60 (51%)	
Unknown	14	7	3	4	
TP53 (n, %)					
Wild-type	125 (36%)	45 (39%)	43 (36%)	37 (31%)	0.44
Altered	226 (64%)	69 (61%)	76 (64%)	81 (69%)	
Unknown	14	7	3	4	

Abbreviations: IGF1R, insulin-like growth factor-1 receptor.

[a] Fisher's exact test.

[b] Among 324 patients with *KRAS*-mutant PDAC.

[c] G12C (*N* =2), G13D (*N*=1), A146T (*N*=1).

Table 3.

IGF1R expression, disease-free survival, and overall survival in patients with resected PDAC.

	IGF1R H-score tertiles			P_{trend}[a]
	1	2	3	
Predominant H-score				
Disease-free survival				
No. at risk	106	104	107	
No. events	75	66	77	
Median, months	16.1	12.3	10.8	
Multivariable-adjusted, HR (95% CI)[a]	1 (reference)	1.17 (0.82–1.66)	1.73 (1.24–2.44)	0.002
Overall survival				
No. at risk	107	106	108	
No. events	79	76	89	
Median, months	25.8	20.9	17.4	
Multivariable-adjusted, HR (95% CI)[a]	1 (reference)	1.13 (0.80–1.58)	1.39 (1.00–1.92)	0.046
Combined H-score				
Disease-free survival				
No. at risk	103	110	104	
No. events	73	69	76	
Median, months	16.1	12.3	10.8	
Multivariable-adjusted, HR (95% CI)[a]	1 (reference)	1.20 (0.84–1.70)	1.77 (1.26–2.51)	0.001
Overall survival				
No. at risk	104	112	105	
No. events	78	78	88	
Median, months	25.8	20.9	17.8	
Multivariable-adjusted, HR (95% CI)[a]	1 (reference)	1.08 (0.76–1.52)	1.47 (1.06–2.03)	0.021

Abbreviations: IGF1R, insulin-like growth factor-1 receptor; PDAC, pancreatic ductal adenocarcinoma; HR, hazard ratio; CI, confidence interval.

[a]Adjusted for age at the time of surgery, sex, N stage (American Joint Committee on Cancer 8th edition), tumor grade of differentiation, perineural invasion, resection margin status, *CDKN2A* status, perioperative systemic treatment, and perioperative radiation treatment.

Table 4.

Stratified analyses of IGF1R expression and disease-free survival in patients with resected PDAC.[a]

Subgroup	No. of patients	IGF1R predominant H-score tertiles			$P_{interaction}$
		1	2	3	
		HR (95% CI)	HR (95% CI)	HR (95% CI)	
Sex					
Women	147	1 (reference)	1.34 (0.77–2.32)	1.16 (0.66–2.04)	0.17
Men	170	1 (reference)	0.87 (0.53–1.44)	2.18 (1.37–3.48)	
Age, years					
≤ 67 (median)	163	1 (reference)	0.91 (0.55–1.50)	1.56 (0.99–2.46)	0.61
> 67 (median)	154	1 (reference)	1.60 (0.92–2.78)	2.09 (1.20–3.64)	
BMI, kg/m^2					
< 25	70	1 (reference)	0.96 (0.38–2.42)	0.72 (0.24–2.21)	0.032
≥ 25	97	1 (reference)	1.28 (0.60–2.71)	4.27 (2.03–8.96)	
Diabetes mellitus					
No	203	1 (reference)	1.54 (0.99–2.38)	2.01 (1.29–3.13)	0.24
Yes	89	1 (reference)	0.68 (0.32–1.46)	1.42 (0.64–3.17)	

Abbreviations: IGF1R, insulin-like growth factor-1 receptor; HR, hazard ratio; CI, confidence interval; BMI, body mass index.

[a]Hazard ratios for disease-free survival adjusted for the following covariates, except when a covariate defines a subgroup for stratified analyses: age at the time of surgery, sex, N stage (American Joint Committee on Cancer 8th edition), tumor grade of differentiation, perineural invasion, resection margin status, *CDKN2A* status, perioperative systemic treatment, and perioperative radiation treatment.