Original Article

Nonselective β-adrenergic blockade impacts pancreatic cancer tumor biology, decreases perineural invasion and improves patient survival

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Background: Beta (β) adrenergic signaling mediates progression and invasion in many cancer subtypes. β-blockade, often prescribed for a chronic medical illness such as hypertension, is associated with improved outcomes in breast and prostate cancer. While hypertension is a frequent comorbid condition found in patients diagnosed with pancreatic ductal adenocarcinoma (PDAC), the impact of β-blockade in this disease remains unknown.

Methods: Clinical data were prospectively collected for 1,933 patients undergoing pancreatectomy between 2000 and 2016 at a high-volume pancreatic surgery center of excellence. β-blocker use was utilized to stratify patients into independent cohorts and histopathologic and oncologic outcomes data were investigated. Differences between these two cohorts prompted investigations in patient-derived models of PDAC. A proposed mechanism of action of β-blockade on tumor biology is explored.

Results: In total, 457 of 1,933 patients taken to the operating room for a diagnosis of PDAC were prescribed β-blockade by their primary care physician or cardiologist. Three-hundred and ninety-seven patients were taking β1-selective-blockers and 60 received non-selective β-blockers. When stratified by selectivity, non-selective β-blockade is associated with decreased perineural invasion (PNI) compared to β1-selective or no β-blockade (Noβ) (non-selective 68.3%, β1-selective 84.9%, Noβ 85.9%, P<0.001). Non-selective β-blockade is also associated with longer overall survival (OS) (median: non-selective 26.1 months, β1 18.5 months, Noβ 18.8 months, P<0.01). With these data associating β-blockade and PNI, a direct local mechanism involving noradrenergic hormones was hypothesized. *In vitro* human derived PDAC cell line demonstrated increased cell growth and migration with norepinephrine administration; however, co-administration of propranolol successfully abrogated norepinephrine-induced growth and migration.

Conclusions: Non-selective blockade is independently associated with decreased PNI and improved OS in resected PDAC. *In vitro*, norepinephrine stimulates cell growth and migration. While propranolol does not impact cell growth in isolation, co-administration with norepinephrine abrogates the increased growth and migration seen with norepinephrine administration alone. This work highlights the impact of commonly used antihypertensive medications on tumor biology and may elucidate a rationale for the use of common antihypertensive medications as therapeutic adjuncts in PDAC.

Keywords: β blocker; perineural invasion (PNI); pancreatic ductal adenocarcinoma (PDAC); norepinephrine

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a deadly disease with high rates of disease-specific mortality. Surgical resection offers the only potential for cure, however, even in these selected populations, median survival remains poor with the overwhelming majority ultimately succumbing to disease (1-3). Aggressive tumor biology, a complex tumor microenvironment (TME) and broad resistance to existing chemotherapeutics are prominent drivers for these poor outcomes (1,4,5). The signaling mechanisms that mediate these processes remains poorly understood, but offers the potential for highly leveraged targets for therapeutic intervention.

The dense network of peripheral neurons that reside in the TME and interact closely with cancer cells is a component of particular interest in PDAC. Distinct neoplastic invasion into or around peritumoral nerves, or perineural invasion (PNI), is a common histologic feature of PDAC independently associated with inferior survival (6,7). Adrenergic signaling from peripheral sympathetic neurons has been implicated in the regulation of growth, progression and invasion of many cancer subtypes (8-10). Notable work investigating β-adrenergic signaling and its mechanistic relationship to the TME have been performed in the field of prostate cancer (11,12). Magnon *et al.* reported that the deletion of β2 and β3 adrenergic receptors prevented early phases of tumor development (11). While Zahalka *et al.* utilized mouse models of prostate cancer to show endothelial β2-adrenergic receptor signaling in the TME helped to activate angiogenesis exponentially fueling tumor growth (12). Thus, adrenergic neuron to cancer cell signaling may be involved in both the process of PNI as well as a general dictation of tumor invasiveness and biologic tumor behavior. Both epidemiologic and preclinical studies have suggested inhibition of this signaling via β-adrenergic blockade may reduce rates of tumor progression and improve survival outcomes for melanoma, breast, prostate and colon cancer (8,12-16). Similar associations of incidental β-blockade and improved survival have also been suggested in PDAC patients (17,18). β-blockers are widely available, safe and inexpensive, thus their use as a potential therapeutic adjunct for cancer treatment is immensely appealing.

In this study, we leverage a large, single institution's experience of patients with resected PDAC to investigate the association between β-blockade and markers of tumor biology, including PNI and overall survival (OS). Furthermore, human-derived PDAC models are used to study this relationship *in vitro* and a proposed mechanism of tumor biology is derived.

Methods

Patient cohort

Clinicopathologic data for patients undergoing pancreatic resection at Johns Hopkins Hospital between 2000 and 2016 were collected in a prospectively maintained database and included in this study. Inclusion criteria included a confirmed diagnosis of PDAC upon final histopathologic analysis. Subsequently, cohorts were generated based on use of β-blockers in the perioperative period as determined by review of prescribed "home" medications at the time of resection. The specificity of prescribed β-blockade was identified: β1 selective (Atenolol, Bisoprolol, Metoprolol and Nebivolol) and Nonselective blockade (Propranolol, Carvedilol, Labetalol, Sotalol and Timolol). The primary outcomes of clinical interest were PNI and OS. Follow-up data through May 2018 were retrieved from the Institutional database. The date of death was obtained from medical records, local obituary, or the Social Security Death Index. OS was calculated from the date of surgery to the date of death from any cause, or censored at the date of last follow-up. This study was approved by the Institutional Review Board of Johns Hopkins Hospital (No. 00074221), conducted in accordance with the Declaration of Helsinki. Because of the retrospective nature of the research, the requirement for informed consent was waived.

Demographics and clinicopathological characteristics

All patient data were collected from the prospectively maintained institutional database. Patient demographics included age, sex, race, history of hypertension (HTN), pain on presentation and β-blocker use. Neoadjuvant chemotherapy and/or radiation was noted as was the type of surgery: pancreaticoduodenectomy, distal or total pancreatectomy. Tumor pathology characteristics including tumor size, stage, grade of differentiation, PNI, lymphovascular invasion, presence of nodal disease and margin status were extracted from final pathology reports. Resections were considered "R0" when malignant cells were greater than 1 mm from the surgical margin.

Cell lines, growth and invasion assays

The human Panc10.05 pancreatic tumor cell line was

obtained and established in accordance with IRB approved protocols. These cells were cultured in RPMI-1640 based medium supplemented with 10% FBS, 1% penicillin/streptomycin as previously described (19,20). Tumor cells were grown in 2D culture at 37 ℃. Ten μM of norepinephrine and/or 1 μM of propranolol, atenolol and losartan potassium were added to culture media for growth and invasion assessments based on previous literature (21). In short, growth curves were analyzed by the addition of 1.5×10^4 cells resuspended equally in medium with or without supplemental norepinephrine and/or propranolol, atenolol and losartan potassium. At 0, 24, 48, 72, 96, and 120 hours, cells were counted and total number compared. Assays were run in biological quadruplicate and technical replicates were performed twice.

Invasion was estimated by scratch cell migration assay. Panc10.05 cells were grown in culture medium with or without supplemental norepinephrine and/or propranolol, atenolol and losartan potassium. Following 100% confluence, a scratch was performed, and closure percentage estimated at 0, 8, 24 and 32 hours was assessed to measure tumor cell migration. The area of the wound was obtained by the measurement of at least 6 random points per group. The area of closure was measured at each time point as:

$$\%\,Wound\ Healing = \left(A_{\text{original wound}} - A_{\text{healing wound}}\right) / A_{\text{original wound}} \times 100\% \quad [1]$$

Statistical analysis

Statistical analysis was performed using Stata/MP 12.1 (Stata Corp, College Station, TX). Categorical variables were expressed as percentages and compared using χ^2 or Fisher exact test. Šidák correction was utilized for multiple comparisons. Continuous variables were presented as median and interquartile range (IQR) and compared using Kruskal-Wallis test. Kaplan-Meier curves were used to estimate median survival with the log-rank test utilized for subgroup comparison. A multivariate regression model was used with PNI as the outcome of interest. A Cox proportional-hazards regression model for OS was also used. For both models, a stepwise backward approach was used selecting all covariates with a P value <0.10 on univariate analysis used as the cutoff for inclusion. A 2-sided P value of <0.05 was considered statistically significant.

Results

A total of 1,933 patients underwent pancreatic resection for PDAC at Johns Hopkins Hospital from 2000 to 2016 and were included in the study. Approximately half (51.9%) were male with a median age of 67 (IQR, 59–75) at the time of operation. A history of hypertension was recorded in 40.4% of patients with a prescribed β-blocker documented in 23.4%. Of these, 392 (20.3%) were prescribed β1 selective, and 60 (3.1%) non-selective β-blockers. Additional detailed demographic and clinicopathologic characteristics for all included patients are summarized in *Table 1*.

When compared to patients without a listed β-blocker prescription, patients taking β-blockers in the perioperative period were more likely to be older (71 *vs.* 66, P<0.001) and have a history of hypertension (62.1% *vs.* 33.7%, P<0.001). Tumor characteristics were not statistically significant when comparing these two groups: including similar tumor size, T stage, nodal status, grade, resection margin status and lymphovascular invasion. A non-significant trend was appreciated with fewer patients prescribed β-blockers had associated findings of PNI on histopathologic analysis (82.7% *vs.* 85.8%, P=0.101). This trend reached statistical significance when analyzed according to selectivity of β blockade. Non-selective β-blocker use was associated with significantly less PNI (nonselective β-blocker: 68.3%, β1 selective: 84.9%, No β-blocker: 85.9%, P=0.001). Additional detailed findings are summarized in *Table 1*.

PNI

Further analyses were performed with the presence of PNI set as the primary outcome to investigate the likelihood of a type I statistical error. On multivariate analysis a significantly lower rate of PNI was associated with neoadjuvant treatment (OR: 0.33; 95% CI: 0.23–0.48, P<0.001) and nonselective β-blocker use (OR: 0.36; 95% CI: 0.17–0.77, P=0.008). A higher T stage (OR: 2.39; 95% CI: 1.47–3.90, P<0.001), positive nodal status (OR: 1.86; 95% CI: 1.31–2.64, P=0.001), positive lymphovascular invasion (OR: 3.22; 95% CI: 2.22–4.68, P<0.001) and high grade (OR: 2.38; 95% CI: 1.26–4.48, P=0.007) were independently associated with significantly higher rates of PNI (*Table 2*).

OS

Cox proportional-hazards regression model was performed to assess factors and characteristics independently associated with OS in resected PDAC patients with detail found in *Table 3*. As anticipated, patients with more advanced features and aggressive histopathologic findings had inferior survival. Notably poor prognostic indicators included increasing

Table 1 Clinicopathologic characteristics of pancreatic cancer patients receiving resection between 2000 and 2016

Variable	Entire cohort (n=1,933)	Patients without β-blockers (n=1,476)	Patients on β-blockers (n=457)	P value*
Age (years), median (IQR)	67 (59 to 75)	66 (58 to 74)	71 (64 to 77)	<0.001
Male, n (%)	1,003 (51.9)	752 (51.0)	251 (54.9)	0.137
Race	1,633 (87.2)			0.986
Caucasian	121 (6.5)	1,246 (87.2)	387 (87.2)	
Non-Caucasian	119 (6.4)	183 (12.8)	57 (12.8)	
History of HTN, n (%)				<0.001
No	1,130 (59.6)	961 (66.3)	169 (37.9)	
Yes	765 (40.4)	488 (33.7)	277 (62.1)	
Pain on presentation, n (%)				0.909
No	1,272 (68.6)	970 (68.5)	302 (68.8)	
Yes	583 (31.4)	446 (31.5)	137 (31.2)	
Neoadjuvant treatment, n (%)	329 (17.6)	242 (17.0)	87 (19.6)	0.373
Surgery type				0.22
Pancreaticoduodenectomy	1,548 (80.1)	1,187 (80.4)	361 (79.0)	
Distal pancreatectomy	294 (15.2)	215 (14.6)	79 (17.3)	
Total pancreatectomy	91 (4.7)	74 (5.0)	17 (3.7)	
Tumor size (cm), median (IQR)	3.1 (2.2–4.0)	3.0 (2.2–4.0)	3.0 (2.3–3.6)	0.906
T stage, n (%)				0.312
T1	201 (10.6)	146 (10.1)	55 (12.4)	
T2	428 (22.6)	321 (22.1)	107 (24.0)	
T3	1,210 (63.8)	938 (64.6)	272 (61.1)	
T4	58 (3.1)	47 (3.2)	11 (2.5)	
Nodal status, n (%)				0.524
N0	555 (28.8)	419 (28.4)	136 (30.0)	
N1	1,374 (71.2)	1,056 (71.6)	277 (70.0)	
Grade, n (%)				0.938
Well differentiated	80 (4.3)	62 (4.3)	18 (4.1)	
Moderately differentiated	1,023 (54.4)	780 (54.2)	243 (55.1)	
Poorly differentiated	777 (41.3)	597 (41.5)	180 (40.8)	
Resection margin, n (%)				0.595
R0	1,294 (67.6)	985 (67.3)	309 (68.7)	
R1	619 (32.4)	478 (32.7)	141 (31.3)	
PNI, n (%)				0.101
No	286 (14.9)	208 (14.2)	78 (17.3)	
Yes	1,630 (85.1)	1,258 (85.8)	372 (82.7)	
Lymphovascular invasion, n (%)				0.821
No	894 (47.7)	685 (47.6)	209 (48.1)	
Yes	981 (52.3)	755 (52.4)	226 (51.9)	

*, comparison of Patients without β-blockers and patients on β-blockers.

Table 2 Univariate and multivariate logistic regression analysis of characteristics associated with PNI in patients with pancreatic cancer resection

Variable	PNI present (n=1,630)	PNI absent (n=286)	Univariate P value	OR	95% CI	Multivariate P value
Age, years						
<65	593 (36.4)	117 (40.9)	ref.			
>65	1,037 (63.6)	169 (59.1)	0.144			
Male	843 (51.7)	150 (52.5)	0.82			
Race						
Caucasian	1,385 (87.7)	233 (84.4)	ref.			
Other	195 (12.3)	43 (15.6)	0.138			
History of HTN, n (%)						
No	940 (58.6)	179 (65.6)	ref.			
Yes	665 (41.4)	94 (34.4)	0.029	1.41	0.99–1.98	0.052
Pain on presentation, n (%)						
No	1069 (68.2)	191 (70.7)	ref.			
Yes	499 (31.8)	79 (29.3)	0.402			
Neoadjuvant treatment, n (%)	215 (13.6)	108 (40.6)	<0.001	0.33	0.23–0.48	<0.001
Current Beta-blocker use						
None	1,264 (77.5)	208 (72.7)	ref.			
β1 selective	326 (20.0)	59 (20.6)	0.265	0.8	0.54–1.19	0.265
β1/β2 non-selective	41 (2.5)	19 (6.6)	<0.001	0.36	0.17–0.77	0.008
Surgery type						
Pancreaticoduodenectomy	1,336 (82.0)	198 (69.2)	ref.			
Distal pancreatectomy	219 (13.4)	73 (25.5)	<0.001	0.77	0.51–1.18	0.227
Total pancreatectomy	75 (4.6)	15 (5.2)	0.306	0.82	0.39–1.76	0.615
Tumor size						
<3 cm	696 (42.7)	164 (57.3)	ref.			
>3 cm	934 (57.3)	122 (42.7)	<0.001	1.11	0.78–1.59	0.551
T stage, n (%)						
T1	115 (7.1)	85 (31.5)	ref.			
T2	355 (22.0)	71 (26.3)	<0.001	2.09	1.26–3.47	0.004
T3	1,098 (67.9)	105 (38.9)	<0.001	2.39	1.47–3.90	<0.001
T4	49 (3.0)	9 (3.3)	<0.001	1.32	0.52–3.37	0.557
Nodal status, n (%)						
N0	379 (23.3)	172 (60.4)	ref.			
N1	1,250 (76.7)	113 (39.6)	<0.001	1.86	1.31–2.64	0.001
Grade, n (%)						
Well differentiated	57 (3.6)	23 (9.0)	ref.			
Moderately differentiated	862 (53.5)	156 (60.7)	0.002	1.82	1.00–3.32	0.05
Poorly differentiated	691 (42.9)	78 (30.4)	<0.001	2.38	1.26–4.48	0.007
Resection margin, n (%)						
R0	1,069 (66.0)	219 (77.9)	ref.			
R1	552 (34.0)	62 (22.1)	<0.001	1.13	0.77–1.66	0.529
Lymphovascular invasion, n (%)						
No	666 (41.8)	228 (80.9)	ref.			
Yes	927 (58.2)	54 (19.2)	<0.001	3.22	2.22–4.68	<0.001

Table 3 Univariate and multivariate cox proportional-hazards regression analysis of characteristics associated with OS in patients with pancreatic cancer resection

Variable	Univariate P value	HR	95% CI	Multivariate P value
Age	<0.001	1.01	1.01–1.02	<0.001
Male	0.753			
Race				
Caucasian	ref.			
Other	0.029	0.9	0.76–1.07	0.251
History of HTN, n (%)				
No	ref.			
Yes	0.008	1.08	0.96–1.20	0.207
Pain on presentation, n (%)				
No	ref.			
Yes	0.295			
Neoadjuvant treatment, n (%)	0.8			
Current Beta-blocker use				
None	ref.			
β1 selective	0.107	1.06	0.93–1.21	0.4
β1/β2 non-selective	0.01	0.62	0.44–0.88	0.007
Surgery type				
Pancreaticoduodenectomy	ref.			
Distal pancreatectomy	0.01	1.03	0.87–1.22	0.733
Total pancreatectomy	0.775	1.14	0.89–1.45	0.298
Tumor size	<0.001	1.12	1.08–1.16	<0.001
T stage, n (%)				
T1	ref.			
T2	<0.001	1.01	0.78–1.30	0.938
T3	<0.001	1.32	1.04–1.67	0.021
T4	<0.001	1.5	1.03–2.17	0.032
Nodal status, n (%)				
N0	ref.			
N1	<0.001	1.39	1.21–1.59	<0.001
Grade, n (%)				
Well differentiated	ref.			
Moderately differentiated	0.002	1.57	1.18–2.08	0.002
Poorly differentiated	<0.001	2.04	1.53–2.71	<0.001
Resection margin, n (%)				
R0	ref.			
R1	<0.001	1.44	1.28–1.62	<0.001
PNI, n (%)				
No	ref.			
Yes	<0.001	1.23	1.02–1.47	0.028
Lymphovascular invasion, n (%)				
No	ref.			
Yes	<0.001	1.12	0.99–1.25	0.06

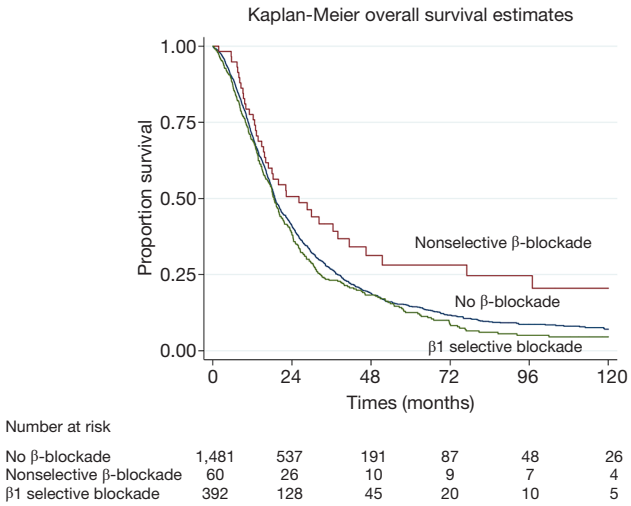

Figure 1 OS estimates of resected PDAC patients from the time of surgery subgrouped by β-blocker use.

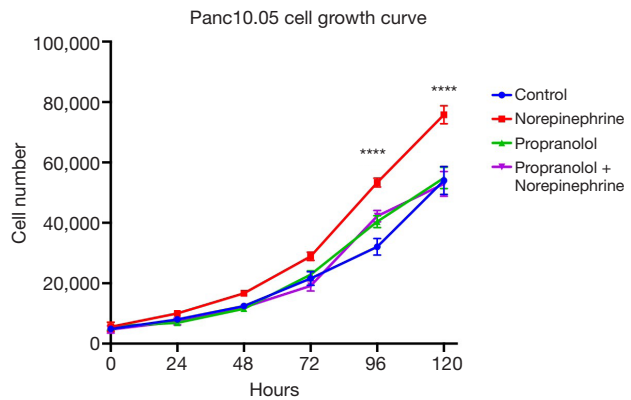

Figure 2 The effects of 10 μM norepinephrine and/or 1 μM propranolol on the growth rate in total cell number of human derived pancreatic adenocarcinoma. Data is representative of mean ± SEM in quadruplicate measurements (Šidák multiple comparisons test). ****, P<0.001.

tumor size (OR: 1.12; 95% CI: 1.08–1.16, P<0.001), higher stage (OR: 1.50; 95% CI: 1.03–2.17, P=0.032), higher grade (OR: 2.04; 95% CI: 1.53–2.71, P<0.001), positive nodal metastases (OR: 1.39; 95% CI: 1.21–1.59, P<0.001), PNI (OR: 1.23; 95% CI: 1.02–1.47, P=0.028) and positive resection margin (OR: 1.44; 95% CI: 1.28–1.62, P<0.001). Interestingly, the receipt of neoadjuvant treatment was not significantly associated with OS in univariate analysis of this cohort. Nonselective β-blocker use was independently associated with significantly improved OS in this cohort (OR: 0.62; 95% CI: 0.44–0.88, P=0.007).

A median OS of 18.6 months was found for all patients. The administration of β1 selective blockade was not associated with an increase in OS (18.8 *vs.* 18.5 months, P=0.105), however, patients with nonselective β-blocker had significantly longer median OS compared to no β-blocker use (26.1 *vs.* 18.5 months, P=0.0016) (*Figure 1*).

Growth and invasion assays

The significant associations with nonselective β-blocker use with PNI and OS prompted additional investigation of tumor biology in an *in vitro* setting. Panc10.05 growth was assessed with or without supplemental norepinephrine and propranolol. PDAC tumor growth was enhanced with a statistically significant increase of cell numbers at 96 (P<0.001) and 120 hours (P<0.001) with the addition of norepinephrine. However, norepinephrine did not significantly increase growth in the presence of propranolol. While propranolol alone did not decrease growth compared to untreated controls, the growth enhancement by norepinephrine was mitigated with nonselective β-blockade (*Figure 2*). These results were consistent in an additional human derived PDAC cell line: AsPC-1 (*Figure S1*). To further investigate the importance of non-selective β-blockade, the impact of norepinephrine on the growth of Panc10.05 was assessed in the presence of the β1-selective atenolol and an alternative antihypertensive, losartan.

Cell migration scratch assay similarly revealed significantly enhanced invasiveness as represented by tumor cell motility at 24 hours (P<0.01) and 32 hours (P<0.05) in the cells supplemented with norepinephrine as compared to control treated Panc10.05 cells (*Figure 3*). This was again mitigated with the addition of propranolol with no significant difference in tumor cell motility comparing propranolol and the combination propranolol and norepinephrine (*Figure 3*). Notably, neither atenolol nor losartan recapitulated the mitigating effects of propranolol on norepinephrine with a significant increase in both growth and motility comparing treated cells (atenolol or losartan) to the combination of atenolol or losartan and norepinephrine (*Figures S2,S3*).

Discussion

Perioperative non-selective β-blocker use was independently associated with decreased PNI and improved OS in patients undergoing resection for PDAC. *In vitro* PDAC modeling

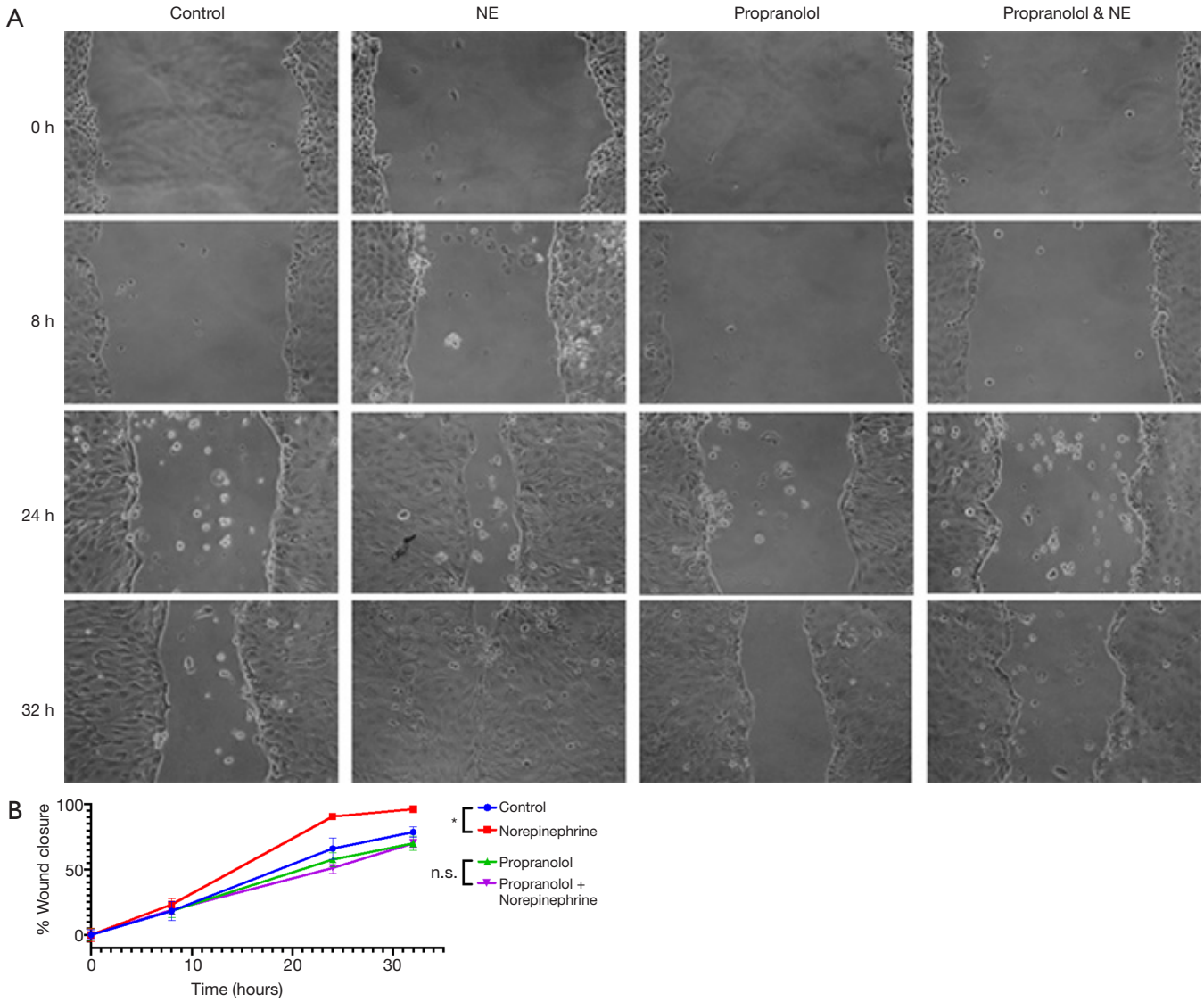

Figure 3 The effects of 10 µM norepinephrine and/or 1 µM propranolol on tumor cell motility as determined by 2-D scratch test of human derived pancreatic adenocarcinoma. (A) 20× magnification light microscopy images of Panc10.05 cell growth following supplementation with propranolol and/or norepinephrine; (B) Graphical representation of the percent wound closure at 0, 8, 24 and 32 hours respectively of Panc10.05 supplemented with propranolol and/or norepinephrine. Data is representative of mean ± SEM of 6 measurements (Šidák multiple comparisons test). *, P<0.05; n.s., non-significant.

of noradrenergic stimulation demonstrates increased cancer tumorgenicity that is abrogated with non-selective β-blockade. Together, these findings suggest a possible mechanism of action whereby enhanced local noradrenergic signaling within the PDAC TME may lead to increased tumorgenicity. This mechanism suggests that selective antagonism of noradrenergic signaling may play a role in mitigating the invasive phenotype of disease, a finding that is manifest in clinical data suggesting decreased PNI with the use of non-selective β- blockade. β-blockers may therefore be an intriguing therapeutic adjunct for PDAC.

PNI is appreciated in multiple tumor subtypes, however it is especially prevalent in PDAC reported in as high as 80–100% of cases (22,23). This histologic characteristic is universally associated with poorer prognosis and as anticipated, an independent predictor of poor survival in

this study (6,7,22,23). The presence of PNI in this study, was significantly associated with general factors of more aggressive tumor biology including poor differentiation, lymphovascular invasion, lymph node metastasis and larger tumor size. Non-selective β-blockers use however, was an independent protective factor for PNI with a remarkable absolute difference of nearly 20%. Interestingly however, there was no correlation with non-selective β-blockers and other clinicopathologic features: patients had tumors of similar size, grade and stage regardless of β-blocker use, only PNI was affected. Renz *et al.* reported a trend of lower PNI rate in patients with pancreatic cancer taking nonspecific β-blockers that perhaps did not reach statistical significance due to a smaller number of patients than this present study (17). This highlights a mechanism of adrenergic signaling, potentially confined to the perineural TME, leading to this histopathologic phenomenon.

The adrenergic signaling pathway's effect on cancer biology has been previously investigated, often motivated by observations of the impact of stress on increased cancer progression (9,24,25). β2-adrenergic signaling in particular, has been shown to have multiple potential downstream molecular effects including stimulating epithelial proliferation, triggering DNA damage, suppressing p53 levels, and increasing matrix metalloproteinase related tissue invasion and tumor cell motility (17,25-27). Previous published work reported β2-dependent PDAC promotion associated with increased pancreatic nerve density and upregulation of neurotrophins, while also identifying an epidemiologic relationship of nonselective β blocker use and improved OS (17). This present study supports these previously published findings, similarly finding longer OS associated with nonspecific β-blockade while utilizing a much larger patient series. The importance of the β2 pathway is further supported by the findings of this study; while individuals on non-selective β-blockers had decreased rates of PNI and improved OS, those with β1 selective blockade had no appreciated advantage.

Interestingly, neoadjuvant therapy was independently associated with less PNI but did not affect OS. Whether decreased PNI following neoadjuvant therapy is an association of the therapy received or of patient selection alone is unknown (i.e., those with aggressive tumors and PNI at diagnosis are more likely to progress on a neoadjuvant approach and fail to reach surgery). This study spans a large time period and treatment philosophies have changed as neoadjuvant therapy for locally advanced and borderline resectable PDAC has proven to be safe, efficacious and lead to encouraging survival (28-30). Previous reports have similarly shown PNI to be decreased following neoadjuvant treatment, although the survival benefit remains to be proven and is challenging to truly compare in retrospective studies (7). The combination of β-blockers and gemcitabine led to a significant survival advantage compared to gemcitabine alone in a preclinical mouse model of pancreatic cancer (17). Whether or not β-blocker use synergizes and augments the effect of modern neoadjuvant therapy and its interaction with PNI warrants future investigation.

These data are suggestive of progress being made in other academic cancer biology laboratories studying the oncologic effects of commonly used pharmaceuticals. Perhaps most striking is the similarity between this work and another antihypertensive class, the angiotensin II receptor blockers (ARBs). Epidemiologic studies have suggested improved survival following resection for PDAC in individuals taking ARBs (31). In advanced pancreatic cancer inhibition of angiotensin signaling has also been associated with improved prognosis, and has led to its use as an adjuvant to accepted therapies in multiple clinical trials (32,33). The effect of ARBs on the TME is an area of intense focus for several laboratories and the clinical effect of these agents is the focus on at least one ongoing clinical trial (NCT 03563248). Nevertheless, the effect of ARBs on the TME appears distinct from the adrenergic pathway as supported by a failure of losartan to abrogate norepinephrine induced growth or motility in this study (*Figures S2*,*S3*).

The findings of this work need to be viewed in the context of several limitations. Firstly, while this series represents nearly 2,000 consecutive patients with resected PDAC, only 60 patients were given nonselective beta blockers in the preoperative period. This rate of 3.1% is similar to previous reports of 2.7% in a series of 631 patients with pancreatic cancer (17). Nevertheless, to the authors' knowledge this represents the largest report in literature. Although a prospective database from a large tertiary referral center was leveraged, the main clinical outcome of interest (survival) was collated and associated in a retrospective fashion, risking associated bias of retrospective work. Inevitably, accurate long-term follow-up for all patients can be challenging as many patients are referred to our tertiary center for resection. These patients typically receive their initial surveillance with the surgical providers for the first year, however are then frequently transitioned to follow-up by home institutions and may be lost to follow-

up, only later to be identified by documented deaths. This follow-up limitation, however, is equally distributed across all groups and time frames. The categorization of β-blocker use was based upon documentation at the time of resection and data reliably determining patient treatment adherence to these home medications are unavailable. Similarly, it remains possible that not all individuals taking β-blockers were captured in the medical record at the time of patient evaluation. Additionally, the duration of β-blocker use was not readily available via our data abstraction methods. In our *in vitro* models, significant effects on tumor growth and motility after simultaneous norepinephrine and nonselective β-blocker treatment were noted after a period of only 1 to 3 days. In the future, it will be interesting to study if the timing or duration of clinical β-blocker treatment impacts the ability to modulate the TME. Next, our co-culture experiments did not include any additional cell types. The TME is complex and β-blockers may impact tumorgenicity through other cell types, such as endothelial cells as suggested in prostate cancer (11). Future studies including additional cell types to our co-culture are being pursued. Lastly, the *in vitro* effects of nonselective β-blockers were investigated without concurrent chemotherapy. The majority of patients in this study did not receive neoadjuvant chemotherapy, thus the observed modulating effects in the absence of chemotherapeutics are largely applicable, however future efforts investigating potential synergistic effects on tumor response of nonselective β-blockers with concomitant chemotherapy are of interest and relevance in the current era of PDAC management.

Conclusions

In conclusion, non-selective β-blocker use in patients with resected PDAC was associated with significantly lower rates of PNI and longer OS. PDAC patients continue to have a dismal survival and thus there is an urgent need for effective therapeutic approaches. β-blockers are readily available, inexpensive, safe and well-understood drugs. This study helps to provide additional rationale for future prospective studies investigating non-selective β-blockers as an adjuvant to existing chemotherapy regimens in PDAC patients.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/apc-19-45). JLC serves as an unpaid editorial board member of *Annals of Pancreatic Cancer* from March 2017 to December 2020. CLW serves as an unpaid editorial board member of *Annals of Pancreatic Cancer* from March 2017 to December 2020. LZ serves as the Editor-in-Chief of *Annals of Pancreatic Cancer* and receives grant support from Bristol-Meyer Squibb, Merck, iTeos, Amgen, NovaRock, Inxmed, and Halozyme. LZ is a paid consultant/Advisory Board Member at Biosion, Alphamab, NovaRock, Akrevia, Datarevive, and Mingruzhiyao. LZ holds shares at Alphamab and Mingruzhiyao. The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was approved by the Institutional Review Board of Johns Hopkins Hospital (No. 00074221), conducted in accordance with the Declaration of Helsinki. Because of the retrospective nature of the research, the requirement for informed consent was waived.

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Supplementary

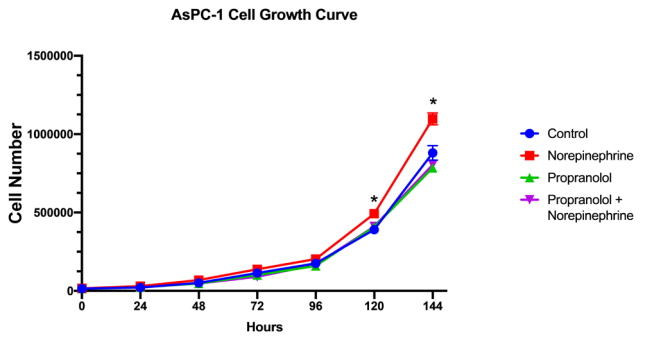

Figure S1 The effects of 10 μM norepinephrine and/or 1 μM propranolol on the growth rate in total cell number of human derived pancreatic adenocarcinoma: AsPC-1. Data is representative of mean ± SEM of 6 measurements (Šidák multiple comparisons test). *, P<0.05.

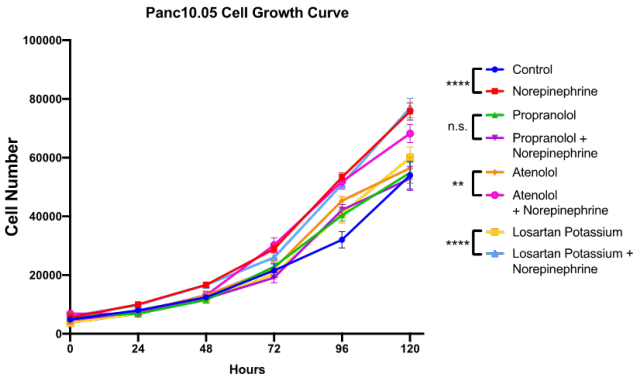

Figure S2 The effects of 10 μM norepinephrine and/or 1 μM propranolol, atenolol or losartan potassium on the growth rate in total cell number of human derived pancreatic adenocarcinoma. Data is representative of mean ± SEM of at least 4 measurements (Šidák multiple comparisons test). **, P<0.01; ****, P<0.001; n.s., non-significant.

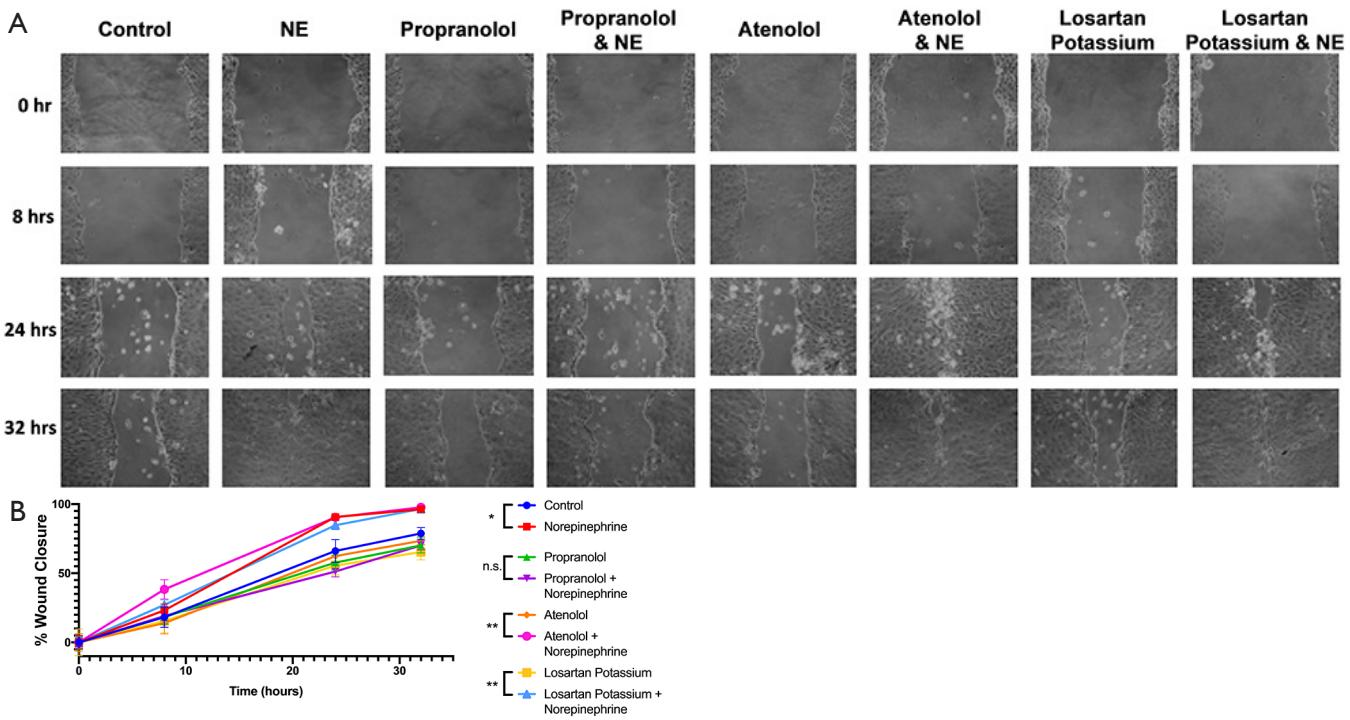

Figure S3 The effects of norepinephrine and/or propranolol, atenolol or losartan potassium on tumor cell motility as determined by 2-D scratch test of human derived pancreatic adenocarcinoma. (A) 20× magnification light microscopy images of Panc10.05 cell growth following supplementation with 10 μM norepinephrine and/or 1μM propranolol, atenolol or losartan; (B) graphical representation of the percent wound closure at 0, 8, 24 and 32 hours respectively of Panc10.05 cells supplemented with norepinephrine and/or propranolol, atenolol or losartan. Data is representative of mean ± SEM of 6 measurements (Šidák multiple comparisons test). *, P<0.05; **, P<0.01; n.s., non-significant.