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Original contribution

Stromal hyaluronan accumulation is associated with low tumor grade and nodal metastases in pancreatic ductal adenocarcinoma☆

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Summary Pancreatic ductal adenocarcinoma is an aggressive malignancy characterized by abundant desmoplastic stroma. Hyaluronan is a prominent stromal component of pancreatic ductal adenocarcinoma and is associated with unique clinical-pathological profiles in other tumor types. The current study aimed to delineate clinical and pathological features associated with hyaluronan accumulation in pancreatic ductal adenocarcinoma using a novel hyaluronan-binding assay currently being used in a clinical trial targeting hyaluronan. Sixty-four formalin-fixed, paraffin-embedded samples of pancreatic ductal adenocarcinomas from 49 patients treated at a single tertiary care hospital were stained. Fifty-two percent of tumors had high levels of hyaluronan. High levels were associated with low tumor grade and lymph node metastases, novel associations not previously seen in pancreatic ductal adenocarcinoma. This study has elucidated a novel clinical-pathological profile in pancreatic ductal adenocarcinomas using a new assay, suggesting hyaluronan may act as a biomarker for a subset of pancreatic tumors that could be targeted by hyaluronan-degrading agents.

1. Introduction

Pancreatic ductal adenocarcinoma (PDA) is an aggressive malignancy with an 8.5% five-year survival rate and few available chemotherapeutic regimens of limited utility [1]. PDA is characterized by abundant desmoplastic stroma, with the stromal elements frequently far outgrowing the volume of the malignant glandular component. The extracellular matrix of this fibrosis is composed of numerous components, including hyaluronan (HA).

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HA is a glycosaminoglycan composed of repeating glucuronic acid and *N*-acetylglucosamine subunits. It is produced by HA synthases (HAS1, HAS2, and HAS3). HAS2 largely produces intracellular HA, while HAS3 predominantly produces HA that is then extruded from the cell to be assimilated into the extracellular matrix, and has been associated with more aggressive PDAs [2]. As part of the extracellular matrix, HA then interacts with cell surface receptors, most notably CD44 and CD168, which then activate transmembrane receptor tyrosine kinases ultimately involved in numerous cellular processes, including adhesion/motility and survival [2]. HA is then degraded by several hyaluronidase enzymes (HYAL1, HYAL2, HYAL3, KIAA1199, and PH20) [3,4].

Stromal HA accumulation is associated with an aggressive course in several malignancies, including prostate, ovarian, and breast primary carcinomas [5-7]. PDA has both the highest incidence and greatest amount of stromal HA deposition [8]. Extracellular HA accumulation contributes to increased interstitial fluid pressure and decreased vascular permeability in PDA [8,9], and its accumulation has been associated with adverse prognosis in PDA [10]. Administration of polyethylene glycol-conjugated (pegylated) recombinant human hyaluronidase PH20 (PEGPH20) has been shown in animal models to deplete intratumoral HA, increase intratumoral blood vessel patency and permeability, and increase survival when given in combination with gemcitabine [2,8].

PDAs with increased HA accumulation have been shown in preclinical animal models to differentially respond to PEGPH20 combined with gemcitabine and nab-paclitaxel compared to PDAs with reduced HA accumulation, increasing antitumor efficacy of the chemotherapeutic regimen by 25% and increasing median survival time by more than 31% [11]. Initial studies in humans have shown increased survival with PEGPH20 and gemcitabine treatment in tumors with greater HA accumulation compared to tumors with less HA accumulation [12]. A phase 3 randomized controlled study comparing PEGPH20 in combination with gemcitabine and nab-paclitaxel versus placebo with gemcitabine and nab-paclitaxel in patients with metastatic PDA is currently underway.

Different assays predominantly derived from animal cartilage have been used for HA detection for research purposes for decades [13-15]. Phase 2 clinical trial data using a novel histochemical HA binding assay showed patients with HA-high tumors responded better to PEGPH20 in combination with gemcitabine and nab-paclitaxel than those with HA-low tumors, with significantly longer median survival of 11.5 months versus 8.5 months [16]. Now, with enzymatic (PEGPH20) degradation of HA in a phase 3 clinical trial, a precise, reliable method of evaluating HA status could be used as a predictive test to select patients more likely to respond to HA-targeted therapy (HA-high patients).

Current clinical trials are using a novel HA binding protein to measure tumor HA content, different from previous assays. Tumor necrosis factor-stimulated gene-6 (TSG-6) interacts with HA to remodel the extracellular matrix and contains a HA-binding link module. This link module was fused to the Fc region of IgG1 with an inactivated heparin-binding domain (ΔHep-Fc) and biotinylated to facilitate detection in formalin-fixed, paraffin-embedded tissue samples [17]. Using this novel biotin-TSG-6-ΔHep-Fc assay, the current study aims to determine the clinical and pathological features associated with HA-high and HA-low PDAs.

2. Materials and methods

Clinic records from the gastrointestinal malignancies program at a single tertiary care medical center were queried for patients with PDA that had received gemcitabine and nab-paclitaxel during their clinical courses. Forty-nine patients were identified with a total of 64 formalin-fixed, paraffin-embedded tissue samples available for HA staining. Thirty-seven available samples were obtained prior to gemcitabine and nab-paclitaxel treatment, and 27 tissue samples were procured after initiation of this treatment. None of the patients had received PEGPH20 prior to tissue acquisition. Samples included primary (head, n = 29; uncinate, n = 3; neck, n = 2; body, n = 3; and tail, n = 6) and metastatic (liver, n = 14; lung, n = 3; stomach, n = 1; peritoneum, n = 1; retroperitoneum, n = 1; and porta hepatis lymph nodes, n = 1) PDA. Both biopsy (n = 33) and excision/resection (n = 31) samples were included.

All samples were stained for HA using the biotin-TSG-6-ΔHep-Fc assay, as previously described [17]. Non-neoplastic hepatocytes and PC3 tumor xenograft tissue were included in staining runs as negative and positive controls, respectively. Using the same algorithm as the phase 2 clinical trial by Hingorani and colleagues, the tumor extracellular matrix staining for HA as a proportion of the total tumor surface area was recorded. Tumor stromal staining at any intensity above background stromal staining was recorded as positive. Cases with ≥50% staining were scored as HA-high, and cases with <50% staining were scored as HA-low (the HA status) [16]. All samples were scored by a single pathologist with experience in biotin-TSG-6-ΔHep-Fc stain interpretation.

Clinical and pathological variables were recorded for each sample from clinical notes, radiology reports, and pathology reports. Data included sex, race, CA 19-9 level at presentation, family history of cancer, best response to gemcitabine and nab-paclitaxel, CA 19-9 reduction with treatment, primary tumor location, presence or absence of lymph-vascular invasion, tumor size, histological grade, presence or absence of lymph nodal metastasis, and American Joint Committee on Cancer (AJCC) 7th edition pathologic TNM stage. For the purposes of statistical analysis, well- and moderately differentiated tumors were grouped together as low grade,

and poorly-differentiated and undifferentiated tumors were grouped together as high grade. All variables were analyzed via a univariate generalized mixed effect model.

3. Results

3.1. Clinical and pathological findings

The clinical and pathological features of the patients are summarized in Table 1. Twenty males and 29 females were represented. Thirty-two patients were Caucasian, 9 were Hispanic, 3 were African American, 3 were Asian American, and 2 had no race recorded. Forty-eight patients had information regarding their family history available for review, including 19 with no family history of cancer and 29 with a history of cancer in their family. Primary tumor location was recorded for 48 patients, including 30 in the head of the pancreas, 2 in the neck, 5 in the body, 6 in the tail and 5 in the uncinate process (note: these are the locations of primary tumors as defined by imaging reports, and not all of these primary tumors were sampled for the current study).

Pathology reports of biopsies and excisions/resections were reviewed for pathological findings. Twenty-four patients had documentation of the presence or absence of lymph-vascular invasion, including 13 with lymph-vascular invasion, 8 without, and 3 patients with findings indeterminate for lymph-vascular invasion. Twenty-four patients had gross and/or microscopic measurements of the primary tumors, ranging from 1.0 to 6.2 cm in greatest dimension, with a median size of 3.0 cm. Twenty-nine patients had information regarding histological grade. For the purposes of this analysis, histological grades 1 and 2 were grouped as low grade, and grades 3 and 4 were grouped as high grade: 19 patients had low grade tumors, and 10 patients had high grade tumors. Twenty-seven patients had information available to determine the pathological T stage according to the AJCC 7th edition staging manual. One patient had a pT1 tumor, 2 patients had pT2 tumors, 23 patients had pT3 tumors, and one patient had a pT4 tumor. Twenty-seven patients had information reported regarding lymph nodal assessment. Eight patients had negative lymph nodes (pN0), and 19 had at least one positive lymph node (pN1), with the number of positive lymph nodes ranging from 1 to 37 (median: 3). Twenty-five patients had information available for assignment into AJCC stage groupings, including 3 patients with stage I disease (one stage IA and 2 stage IB), 20 patients with stage II disease (4 stage IIA and 16 stage IIB), and 2 patients with stage IV disease.

CA 19-9 levels were available for 40 patients at diagnosis, with values ranging from <1 to >10,000 U/mL and a median of 227.5 U/mL. In addition, 37 patients had subsequent CA 19-9 levels drawn after therapy and could be assessed for a biochemical response, defined for the purposes of this study as a >50% reduction in CA19-9 level from the initial level. Nineteen patients had a biochemical response after treatment, and 21 patients had no biochemical response. Forty-four patients had subsequent imaging available to assess for treatment response by RECIST criteria, including 5 with partial response, 30 with stable disease, and 9 patients with progression [18].

Table 1 Clinical and pathological features of patients

Features	
Sex	N = 49 patients
Male/Female	20:29
Race	N = 47 patients
Caucasian	32
Hispanic	9
African American	3
Asian American	3
Primary tumor location in pancreas	N = 48 patients
Head	30
Neck	2
Body	5
Tail	6
Uncinate	5
CA19-9 (U/mL)	N = 40 patients
Range	<1-10000
Median	227.5
pT (Tumor) stage	N = 38 patients
pT1	1
pT2	2
pT3	34
pT4	1
pN (Node) stage	N = 26 patients
pN0	7
pN1	19
Grade	N = 29 patients
Low	19
High	10
Lymph-vascular invasion	N = 24 patients
Present	13
Not identified	8
Indeterminate	3
Pathologic stage (AJCC 7th Edition)	N = 25 patients
IA	1
IB	2
IIA	4
IIB	16
IV	2

3.2. Correlation of HA staining with pathological findings

Overall, 33 of 64 (52%) of samples, independent of treatment status, were determined to be HA-high. Twelve of 25 (48%) males and 21 of 39 (54%) females had samples that were HA-high. Twenty-two (52%) Caucasian, 4 (33%) Hispanic, 3 (75%) African American, and 2 (50%) Asian

American patients had HA-high samples, respectively. Of 19 patients with no family history of cancer, 13 had HA-high samples and 8 patients had HA-low samples, including 2 patients with both HA-high and -low samples. No significant difference was found in HA status by sex, race, or family cancer history.

Seventeen patients with primary tumors in the head of the pancreas had HA-high samples and 16 had HA-low samples, including 3 patients that had both HA-high and -low samples. Of the patients with uncinate tumors, 3 patients had HA-high samples and 2 had HA-low samples. Both of the patients with neck tumors had HA-low tissue samples. Of the patients with body tumors, 2 had HA-high tissue samples and 3 had HA-low samples. Four patients with tail tumors had HA-high samples, and 3 patients had HA-low tumors, including one patient with both HA-high and -low samples. No significant association between HA status and the location of primary tumors was identified.

In 13 patients with lymph-vascular invasion, 8 patients had HA-high tissue samples and 6 patients had HA-low samples, including one patient with both HA-high and -low samples. In 8 patients without lymph-vascular invasion, 5 had HA-high tissue samples and 3 had HA-low samples. Of the 3 patients with findings indeterminate for lymph-vascular invasion, 2 patients had HA-high samples and 2 patients had HA-low samples, including one patient with both HA-high and -low samples. No significant association between lymph-vascular invasion and HA status was identified.

Of 10 patients with tumors measuring <3.0 cm (median size for the study set), 6 had HA-high samples and 5 had HA-low samples, including one patient with both HA-high and -low samples. Of 14 patients with tumors measuring ≥3.0 cm, 9 patients had HA-high samples and 6 patients had HA-low samples, including one patient with both HA-high and -low samples. No significant association between tumor size and HA status was identified.

Of 19 patients with low grade tumors, 13 patients had HA-high tissue samples and 7 had HA-low samples, including one patient with both HA-high and -low samples. Among the 10 patients that had high grade tumors, 3 patients had HA-high samples and 8 had HA-low samples, including one patient with both HA-high and -low samples. Low tumor grade was associated with HA-high status ($P < .001$, Table 2).

Regarding tumor staging, one patient had a pT1 tumor and a HA-low sample. Two patients had pT2 tumors with HA-high tissue samples, including one patient with both HA-high and -low samples. Twenty-three patients had pT3 tumors, with 13 having HA-high samples and 11 having HA-low samples, including one with both HA-high and -low samples. One patient had a pT4 tumor with HA-low samples. No significant association between pathological T stage and HA status was identified.

Of the 8 patients with negative lymph nodes, 3 had HA-high samples and 6 had HA-low samples, including one patient with both HA-high and -low samples. Among the patients with positive lymph nodes, 13 had HA-high samples and 7 had HA-low samples, including one patient with both HA-high and -low samples. HA-high status was significantly correlated with positive lymph nodes (pN1 status).

Of the 3 patients with AJCC 7th edition stage I disease, 2 had HA-high samples and 2 HA-low samples, including one patient with both HA-high and -low samples. Of 20 patients with stage II disease, 12 had HA-high samples and 9 had HA-low samples, with one patient having both HA-high and -low samples. Of the 2 patients with stage IV disease, one patient had HA-high samples and one had HA-low samples. No significant correlation was identified between HA status and AJCC stage grouping.

Table 2 Hyaluronan (HA) status of select variables

	N = 39		N = 41	
	pN0	pN1	Low grade	High grade
HA-high	4	19	17	5
HA-low	8	8	10	9
P	$P < .001$		$P < .001$	

3.3. Correlation of HA staining with clinical findings

Regarding CA19-9, 10 patients with initial CA 19-9 levels above the median for the study group (227.5 U/mL) had HA-high samples and 11 had HA-low samples, including one patient that had both HA-high and -low samples. Twelve patients with CA 19-9 levels below the median had HA-high samples and 11 patients had HA-low samples, including 3 patients with both HA-high and -low samples. Ten patients that had a biochemical response during treatment with gemcitabine and nab-paclitaxel had HA-high samples and 9 had HA-low samples, including 2 patients with both HA-high and -low samples. Nine non-responders had HA-high samples and 13 had HA-low samples, including 2 patients with both HA-high and -low samples. No significant association of HA status was identified with initial CA 19-9 levels or with CA 19-9 biochemical response to treatment.

Of 5 patients with a partial treatment response by RECIST criteria, 4 patients had HA-high tissue samples and 3 had HA-low samples, including 2 with both HA-high and -low samples. The 9 patients that experienced disease progression had similarly heterogeneous findings, with 4 patients having HA-high samples and 5 patients having HA-low samples. Among the 30 patients with stable disease, 15 patients had HA-high samples and 17 had HA-low samples, including 2 patients with both HA-high and -low samples. No significant association between HA status and treatment response to gemcitabine and nab-paclitaxel was identified.

3.4. HA staining in patients with multiple samples

A small number of patients had multiple samples stained, allowing for a limited comparison of HA staining in different

situations. Nine patients had tissue samples from both primary and metastatic PDAs. Eight patients had one sample each from primary and metastatic tumors, and one patient had samples from the primary tumor, a metastasis, and a pancreatic recurrence. Three patients had concordantly HA-high primary and metastatic samples, and 3 had concordantly HA-low samples. Three patients had discordant HA statuses, with all 3 having HA-high primary tumors and HA-low metastases.

Eight patients had tissue obtained by both biopsy and excision/resection. Six patients had concordant HA statuses between biopsy and excision/resection samples, including 4 patients that had HA-high samples and 2 patients that had HA-low samples. Two patients had discordant HA-statuses between biopsy and excision/resection samples: both patients had HA-high biopsy samples and HA-low excision/resection samples.

Five patients had a tissue sample obtained before receiving chemotherapy or radiation treatment and a separate sample obtained after treatment. One patient had an initial HA-high tumor sample and a later HA-low sample obtained after treatment with gemcitabine and nab-paclitaxel. Another patient had an initial HA-high sample and a subsequent HA-low sample after treatment with gemcitabine and nab-paclitaxel plus combined capecitabine/radiation therapy. A third patient had an initial HA-low tumor sample and an additional HA-low sample obtained after folinic acid/fluorouracil/irinotecan/oxaliplatin (FOLFIRINOX) therapy with subsequent fluorouracil/radiation combination therapy. The fourth patient had an initial HA-high tumor sample and a HA-high sample obtained after gemcitabine/nab-paclitaxel and FOLFIRINOX regimens. The fifth patient had an initial HA-high sample and a second HA-high sample obtained after adjuvant gemcitabine/nab-paclitaxel treatment for the initial tumor resection and also neoadjuvant FOLFIRINOX in preparation for resection of a pancreatic recurrence.

4. Discussion

4.1. Staining and interpretation of HA

The biotin-TSG-6-ΔHep-Fc assay produced brown staining of extracellular HA. As discussed in the Materials and Methods section, interpretation of the assay requires peritumoral stroma as well as background stroma for comparison, as the HA status is a relative measure of HA deposition in peritumoral stroma compared to background stroma. Heterogeneity of staining within samples was seen as expected, and as accounted for by the HA status [16].

4.2. Review of previous studies

A first glance at the literature reveals several investigations of HA accumulation in PDA. However, closer examination reveals considerable heterogeneity in the experimental models, staining assays, grading schema, and, therefore, clinical-pathological associations. In order to understand the novelty of the findings in the current study, an in-depth discussion of the previous studies is required.

Jacobetz and coauthors used a mouse model of PDA to demonstrate a difference of survival with combined PEGPH20 and gemcitabine treatment (28.5 days) versus gemcitabine alone (15.0 days). In this study, HA was assessed using a commercially-available histochemical biotinylated HA binding protein from Merck KGaA (Darmstadt, Germany). HA staining was scored semiquantitatively as 3+, 2+, 1+, or 0, using a ratio of both strength and area of positive staining [8]. This study convincingly demonstrated the use of PEGPH20 in combination with chemotherapy, though no correlation with quantity or quality of HA staining was commented on. The HA grading system used also relied on pixel analysis using digital imaging software, which, while precise for research purposes, is not practically applicable for routine clinical practice.

Cheng and colleagues demonstrated that high tumor HA accumulation was a biomarker of poor prognosis after resection of PDA in humans, comparable to AJCC stage in multivariate analysis [10]. This study used a polyclonal immunohistochemical antibody to HA from Abcam (Cambridge, MA) and a weighted HA scoring system similar to that of Jacobetz and co-authors based on both intensity (scored as 0-3) and quantity (0%-100%) of tumor staining for HA, multiplied together (for a total score of 0 to 300). HA staining was classified into 2 broad categories: weak (score of 1-150) and strong (151-300) expression, with poor prognostic implications for the strong subgroup.

Whatcott and colleagues showed high accumulation of HA in the extracellular matrix of both primary and metastatic PDA using archival human tumor tissue. Patients with tumors with high HA accumulation had significantly shorter median survival of 9.3 months versus 24.3 months for patients with tumors with low HA accumulation [19]. This study used the immunohistochemical antibody to HA from Abcam and a scoring system combining intensity and quantity of tumor staining similar to previous studies. However, analysis of staining was performed using digital slide scanning and automated pixel counts. Also, this study used a slightly different scoring system, defining low HA as a score of 0-112 and high HA as a score of 113-300.

Gebauer and colleagues directly compared the utility of the polyclonal HA immunostain from Abcam to the biotinylated HA binding protein from Merck. Their study utilized a semiquantitative staining evaluation, with intensity graded as 0, 1+, 2+, and 3+ on tissue microarrays, with 2+ and 3+ considered positive. They demonstrated significantly different staining patterns of extracellular HA between the 2 modalities, with anti-HA predigestion eliminating staining with the HA binding protein, but not eliminating staining with the immunostain, and thus suggesting greater specificity for detection of extracellular

HA using the HA binding protein [20]. Therefore, Gebauer and co-authors validated the use of an HA binding protein for HA detection similar to that used in the current study, though they used a different scoring regimen, and using this similar assay, they found no association with stromal HA accumulation and prognosis in PDA.

Hingorani and co-investigators used the same biotin-TSG-6-ΔHep-Fc HA binding assay and the same definitions for HA-high and HA-low as used in the current study [16]. Their data was arguably the most clinically relevant, as it came from clinical trials of human patients and demonstrated significantly greater response to combined chemotherapeutic and HA-targeted therapy in patients with HA-high tumors. Significantly, the HA scoring system used was also relatively simple to apply without the need for computational aids.

While these previous studies have suggested relationships between HA accumulation in PDA and clinical and pathological relationships, details of the staining and interpretation render application of most of these schema for routine clinical practice impractical. Mouse xenografts are an important first investigation, but translation of the findings to human tissue is frequently discordant. While digital pixel counts may provide precise measurements, they require technology that is impractical for everyday usage. Significantly, the findings by Gebauer and colleagues strongly suggest that immunohistochemical staining is insufficiently specific for the detection of HA in clinical samples. With these details in mind, the current study was undertaken using an assay and grading scheme similar to that used by Hingorani and colleagues, most likely to have clinical utility in routine patient care.

4.3. Novel findings and implications of the current study

Approximately half of all PDAs tested in the current study were HA-high, slightly higher than the 34% identified in Hingorani's study [16]. This suggests that although most PDAs are characterized by extensive stromal acquisition, the amount of HA in the stroma is heterogeneous from tumor to tumor. The finding that HA-high status is associated with low tumor grade and metastasis to regional lymph nodes (N1 status) are both novel discoveries in the current study.

Histological tumor grade is based on the amount of the tumor that forms glands reminiscent of normal pancreatic ducts. In brief, well-differentiated tumors are composed of a mixture of well-formed and irregular glandular structures, and poorly-differentiated tumors are composed of a mixture of irregularly-shaped glands, infiltrative single cells, and sheets of non-gland-forming structures. Moderately-differentiated tumors are comprised of an intermediate amount of glandular structures [21]. Multiple studies have shown the prognostic utility of grouping well- and moderately-differentiated tumors into a low grade category, distinct from the poorly differentiated, or high grade category. High tumor grade accounts for 28.1-

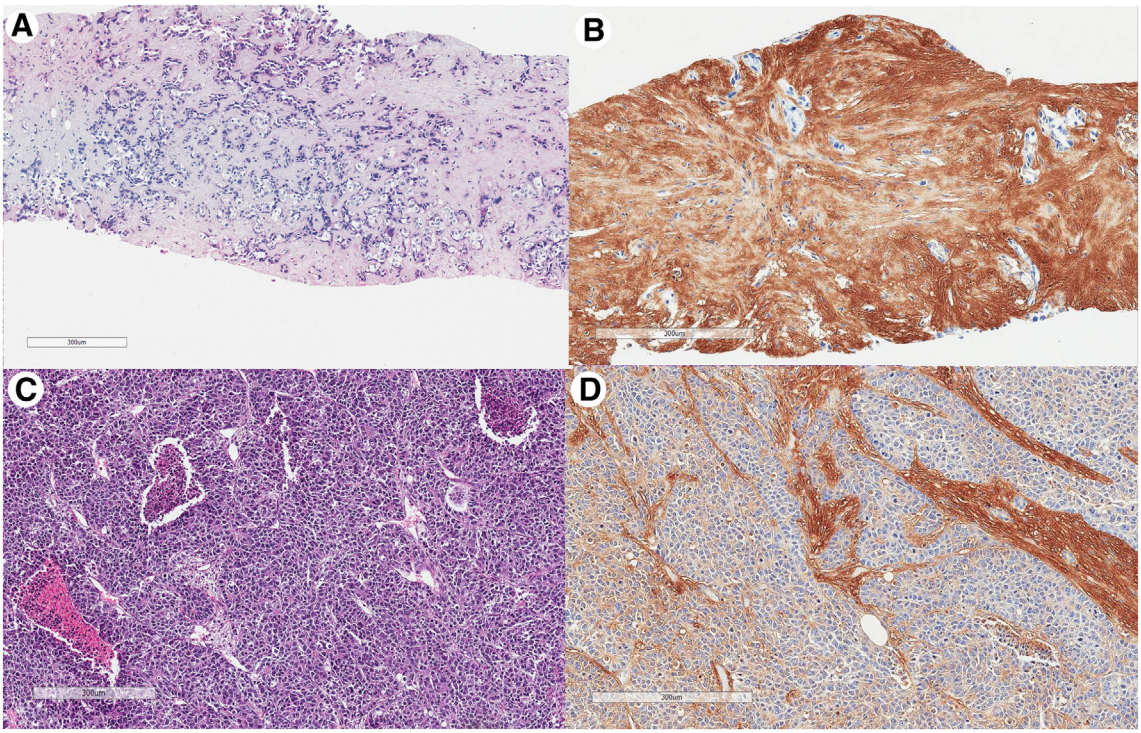

Figure Comparison of hyaluronan (HA) staining in high grade and low grade pancreatic ductal adenocarcinomas (PDAs). A, Low grade PDA with abundant stroma (hematoxylin and eosin). B, HA stain showing the PDA in FigureA is HA-high. C, High-grade PDA with minimal stroma (hematoxylin and eosin). D, HA stain showing the PDA in FigureC is HA-low.

37.0% of PDA and has been associated with poor prognosis independent of stage [22-24].

Strong HA staining has been previously associated with poor prognosis, though independent of tumor grade [10]. In the current study, HA-high status was associated with low tumor grade. The study showing no association between HA status and tumor grade by Cheng and coauthors was performed using a different, immunohistochemical assay to assess for HA and a different quantification scheme for defining HA-high and -low status, as described above. Furthermore, the finding in the current study that high grade tumors were HA-low is predictable from the histopathologic examination, as high grade tumors often have a greater cellular component and lesser stromal component (see Figure).

Similarly, while positive lymph node metastases are a poor prognostic indicator, the current study is the first to find an association between HA-high status and positive lymph nodes in PDA. The reasons for this association can only be speculated about at this point, but could reveal more about HA tumor biology in the future. For instance, this finding suggests that HA accumulation may play a role in lymphatic spread. Studies in colorectal cancer have shown that HA degradation products in the interstitium are associated with lymphatic spread and nodal metastases [25], and stromal HA accumulation is associated with nodal metastases in breast carcinoma [6]. Other data suggest increased HA accumulation and processing contributes to lymph node metastasis in prostate cancer [26,27].

In fact, evolving research indicates that stromal HA alone may not be the driver of an aggressive phenotype in PDA, but specifically the accumulation of low molecular weight HA degradation products after processing by specific hyaluronidases like KIAA1199 [4,28]. This evolving understanding of the role of HA processing may offer further explanation for the association of HA-high tumors with some features, such as N1 status and low tumor grade, and not others in the current study.

4.4. Limitations of the current study and opportunities for further investigation

While the current study highlights several new findings regarding HA in PDA, there are several limitations to the current cohort. The current cohort is relatively small, considering the large amount of variables involved. Variability in primary tumor location, availability of primary and metastatic tumors for testing, and heterogeneity of treatment, all limited evaluation of these variables. The cohort was chosen to comprise patients that had received gemcitabine/nab-paclitaxel during their treatment course. However, given the relatively small sample size and the highly heterogeneous nature of treatment and clinical course in a retrospective series, it was decided to abandon any direct comparison of pre- and post-gemcitabine/nab-paclitaxel treatment samples. Similarly, due to this heterogeneity, the current study did not attempt to analyze true survival statistics, such as overall survival and disease-free survival. While a subset of patients had multiple tissue samples, the small sizes of these sub-cohorts argue against attempting to delineate any differentiation between biopsies and resections, primaries and metastases, and pre- and post-treatment samples. Additional studies will also be required to assess interobserver variability, as samples in the current studied were scored by a single pathologist with experience interpreting this HA stain.

5. Conclusion

While the cohort is relatively small and heterogeneous, the current study is a first investigation into the clinical-pathologic phenotype of HA-high PDAs using a novel, clinically validated biotin-TSG-6-ΔHep-Fc HA binding protein and HA scoring algorithm. HA-high PDAs are associated with lymph nodal metastases and low grade, a unique biological tumor profile. Further investigations into HA's relationships to these tumor parameters is warranted and could shed light on the tumor microenvironment in PDA, the niche of HA in PDA tumor biology, and other potential avenues of targeted PDA therapy in the future.

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