Crosstalk between Mast Cells and Pancreatic Cancer Cells Contributes to Pancreatic Tumor Progression

Matthew J. Strouch[1], Eric C. Cheon[1], Mohammad R. Salabat[1], Seth B. Krantz[1], Elias Gounaris[2], Laleh G. Melstrom[1], Surabhi Dangi-Garimella[2], Edward Wang[1], Hidayatullah G. Munshi[2], Khashayarsha Khazaie[2], and David J. Bentrem[1,3]

Abstract

Purpose: To assess the clinical and pathologic significance of mast cell infiltration in human pancreatic cancer and evaluate crosstalk between mast cells and cancer cells *in vitro*.

Experimental Design: Immunohistochemistry for tryptase was done on 53 pancreatic cancer specimens. Mast cell counts were correlated with clinical variables and survival. Serum tryptase activity from patients with cancer was compared with patients with benign pancreatic disease. *In vitro*, the effect of pancreatic cancer–conditioned medium on mast cell migration was assessed. The effect of conditioned medium from the human mast cell line, LAD-2, on cancer and normal ductal cell proliferation was assessed by thymidine incorporation. Matrigel invasion assays were used to evaluate the effect of mast cell–conditioned medium on cancer cell invasion in the presence and absence of a matrix metalloproteinase inhibitor, GM6001.

Results: Mast cell infiltration was significantly increased in pancreatic cancer compared with normal pancreatic tissue (11.4 ± 6.7 versus 2.0 ± 1.4, $P < 0.001$). Increased infiltrating mast cells correlated with higher grade tumors ($P < 0.0001$) and worse survival. Patients with pancreatic cancer had elevated serum tryptase activity ($P < 0.05$). *In vitro*, AsPC1 and PANC-1 cells induced mast cell migration. Mast cell–conditioned medium induced pancreatic cancer cell migration, proliferation, and invasion but had no effect on normal ductal cells. Furthermore, the effect of mast cells on cancer cell invasion was, in large part, matrix metalloproteinase–dependent.

Conclusions: Tumor-infiltrating mast cells are associated with worse prognosis in pancreatic cancer. *In vitro*, the interaction between mast cells and pancreatic cancer cells promotes tumor growth and invasion. *Clin Cancer Res; 16(8); 2257–65. ©2010 AACR.*

Pancreatic cancer is currently the fourth leading cause of cancer death in the United States with an overall 5-year survival rate of less than 5% (1). Chronic inflammation is a major risk factor for the development of this disease and epidemiologic evidence suggests that a history of pancreatitis increases the risk of cancer 14- to 18-fold compared with the general population (2). Even when inflammation is not recognized prior to the development of pancreatic cancer, there is a marked inflammatory desmoplastic reaction that occurs once the cancer develops,

which is considered a hallmark of the disease (3). Nevertheless, the inflammatory microenvironment of pancreatic cancer remains poorly understood.

Mast cells, which have been extensively studied for their orchestration of allergic reactions and autoimmunity, are increasingly recognized as critical components of the tumor stromal microenvironment in a number of human malignancies (4–16). In many cancers, increased mast cell infiltration has been associated with a worse prognosis (6, 8, 11–14), although this correlation has been tumor type–dependent (4, 7, 10). In pancreatic cancer, one study examined the association of mast cells with angiogenesis. The authors reported that mast cell infiltration in pancreatic cancer was associated with an angiogenic phenotype, however, they did not find a correlation with survival and they did not assess the correlation between mast cell infiltration and other pathologic variables such as tumor stage and grade (9).

To date, the majority of studies on mast cell infiltration in human cancers remain correlative, with minimal investigation of how mast cells induce or inhibit cancer progression. In the present study, we evaluate the clinical and pathologic significance of mast cell infiltration in human

Authors' Affiliations: Departments of [1]Surgery and [2]Medicine, The Robert H. Lurie Comprehensive Cancer Center, Feinberg School of Medicine, Northwestern University, and [3]Jesse Brown VA Medical Center, Chicago, Illinois

Corresponding Author: David J. Bentrem, Department of Surgery, Northwestern University, Lurie Building 3-250, 303 East Superior Street, Chicago, IL 60611. Phone: 312-695-4113; Fax: 312-695-1462; E-mail: dbentrem@nmff.org.

Translational Relevance

Mast cells, which have been extensively studied for their orchestration of allergic reactions and autoimmunity, are being increasingly recognized as critical components of the tumor stromal microenvironment in a number of human malignancies. In the present study, we evaluated the clinical and pathologic significance of mast cell infiltration in human pancreatic cancer and established that high numbers of tumor-infiltrating mast cells are associated with higher grade tumors and decreased survival. Furthermore, our examination of the cellular crosstalk between mast cells and pancreatic cancer cells *in vitro* showed that cancer cells induce mast cell migration and that mast cells subsequently promote pancreatic cancer cell growth and invasion. The effect of mast cells on pancreatic cancer cell invasion is, in large part, matrix metalloproteinase–dependent. Our study highlights the significance of mast cell infiltration in human pancreatic adenocarcinoma and suggests a new target for therapeutic intervention in an otherwise treatment-resistant malignancy.

pancreatic cancers and establish that high numbers of tumor-infiltrating mast cells are associated with higher grade tumors and decreased survival. Furthermore, we examine the crosstalk between pancreatic cancer cells and mast cells *in vitro*. Ultimately, we provide evidence that pancreatic cancer cells recruit mast cells to the tumor microenvironment where they induce cancer cell growth and invasion.

Materials and Methods

Patients. Fifty-three patients with pancreatic adenocarcinoma and 10 patients with benign pancreatic pathology (6 intraductal papillary mucinous neoplasms, 3 mucinous cysts, and 1 ampullary adenoma) underwent pancreatic resection at Northwestern Memorial Hospital between 2002 and 2008. Written informed consent was obtained for inclusion of patients into the Institutional Review Board–approved human pancreatic tumor tissue bank and database. Of the 53 patients with pancreatic adenocarcinoma, 34 underwent a standard Whipple procedure, 8 underwent pylorus-preserving pancreaticoduodenectomy, 10 underwent left pancreatectomy with splenectomy, and 1 underwent total pancreatectomy. Of the 10 patients with benign disease, 7 underwent a pylorus-preserving Whipple and 3 underwent distal pancreatectomy. Patients with adenocarcinoma were staged according to the American Joint Committee on Cancer tumor-node-metastasis staging system and were followed for a median of 18 mo. There were 31 male and 22 female patients (59% and 41%, respectively) with a median age of 65.0 y. Twenty-two patients were stage ≤2A, whereas 31 patients were stage ≥2B. Twenty-

nine patients had grade 3 tumors, 23 patients had grade 2 tumors, and 1 patient had a grade 1 tumor (see Supplementary Table). Thirty-six serum samples from patients with pancreatic cancer and 10 serum samples from patients with benign pancreatic disease were obtained from the Northwestern University Pathology Core Facility.

Immunohistochemistry. Pancreatic tissue specimens were fixed in 10% formalin, embedded in paraffin, and sectioned at 4 μm for routine histologic evaluation. Following deparaffinization, the slides were heated in a microwave in Dako antigen retrieval solution and then washed in Dako wash buffer. Sections were blocked with 1% bovine serum albumin in PBS for 30 min followed by Dako peroxidase block for 30 min. Primary mouse anti-human tryptase antibody (Neomarkers) was used at a concentration of 1:5,000 for 1 h at room temperature. Sections were then treated with Dako secondary antibody (anti-mouse IgG) horseradish peroxidase–linked (Dako Envision Kit K4008) for 30 min at room temperature and developed with 3,3′-diaminobenzidine reagent for 2 min. Counterstaining was done with Gills-2 hematoxylin. The number of mast cells was counted in 10 random fields per sample at 400× magnification.

Serum tryptase activity. A Chemicon quantitative spectrophotometric mast cell degranulation assay kit that measures tryptase activity was purchased from Millipore and performed according to the instructions of the manufacturer. Thirty-six serum samples from patients with pancreatic cancer and 10 serum samples from patients with benign pancreatic pathology were assessed for tryptase activity.

Cell culture. Human pancreatic ductal adenocarcinoma cell lines, PANC-1 and AsPC1, were purchased from American Type Tissue Culture Collection. Cells were grown in DMEM (Sigma Chemicals), supplemented with 10% heat-inactivated fetal bovine serum (Hyclone), and penicillin and streptomycin (100 μg/mL; Life Technologies-Invitrogen). The human pancreatic ductal epithelial (HPDE) cell line was generously provided by Dr. Ming-Sound Tsao (Ontario Cancer Institute, Toronto, Ontario, Canada) and cultured in keratinocyte medium with bovine pituitary extract and epidermal growth factor (Life Technologies-Invitrogen; ref. 17). The human mast cell line, LAD-2, was a generous gift from Dr. Dean Metcalfe at the NIH (Bethesda, MD; ref. 18). LAD-2 cells were cultured in StemPro-34 serum-free medium (Invitrogen) supplemented with penicillin and streptomycin (100 μg/mL) and 100 ng/mL of recombinant human stem cell factor (SCF; Invitrogen).

Preparation of conditioned medium. For the production of conditioned medium, cells were grown to 100% confluency in standard medium. This medium was removed and replaced with serum-free medium for 48 h (pancreatic cancer cells and HPDE cells) or 1 wk (LAD-2 cells). The conditioned medium was removed, filtered, and used for medium transfer experiments.

Mast cell migration assay. The migration was done as detailed previously (19, 20). Briefly, the upper chamber of an

8-μm uncoated 12-well Boyden chamber (BD Biosciences) was seeded with 100,000 LAD-2 cells in 500 μL of serum-free medium. The lower chamber was filled with 750 μL of either nonconditioned DMEM (control), HPDE-conditioned DMEM, PANC-1–conditioned DMEM, or AsPC1-conditioned DMEM. After 6 h, the filter inserts were removed and the number of mast cells that migrated was counted using Guava Technologies' ViaCount Assay (Guava Technologies, Inc.). Migration assays were repeated using AsPC1 and PANC-1 conditioned medium with and without SCF neutralizing antibody (Pepro-Tech) at a concentration of 10 μg/mL. All migration assays were done in triplicate and repeated a minimum of three times.

Proliferation assay. PANC-1, AsPC1, and HPDE cells were grown to 50% confluency. The medium was removed and replaced with either mast cell–conditioned medium or nonconditioned control medium. The cells were then grown for 24, 48, and 72 h. DNA synthesis was assayed by adding 0.5 μCi of methyl-[^{3}H]thymidine/well and incubating cells for 6 h. The cells were washed with PBS, fixed with 10% trichloroacetic acid, and solubilized by adding 250 μL of 0.4 N NaOH to each well. Radioactivity, indicating the incorporation of methyl [^{3}H]thymidine (Amersham) into DNA, was measured by adding scintillation cocktail and counting on a scintillation counter (LKB RackBeta; Wallac). The experiment was done in triplicate and repeated thrice.

Invasion assay. The upper chamber of an 8-μm Matrigel-coated 12-well Boyden chamber (BD Biosciences) was seeded with 100,000 PANC-1, AsPC1, or HPDE cells. The lower chamber was filled with 750 μL of either mast cell–conditioned medium or nonconditioned DMEM. At 48 h, the membrane inserts were removed, fixed, and stained with Diff-Quick (IMEB, Inc.). The number of invading cells was manually counted at 200× magnification in three random areas of the membrane (20). For the invasion assay with the matrix metalloproteinase (MMP) inhibitor GM6001 (Chemicon), 200,000 AsPC1 cells were seeded in the upper chamber of the Boyden chamber and the lower chamber was filled with either nonconditioned control medium, mast cell–conditioned medium, or mast cell–conditioned medium with 10 μm of GM6001. All invasion assays were done in triplicate and repeated thrice.

Statistical analysis. Data from cell culture experiments were analyzed by ANOVA, followed by Dunnett's multiple comparison test as appropriate for post hoc testing and paired *t* tests where appropriate. The analyses were done with the Prism software package (GraphPad). Data were expressed by mean ± SEM.

Statistical analysis for the patient data analysis was done with SAS Software (SAS, Inc.). The relationship between mast cell counts and patient characteristics was assessed with Student's *t* test. Sensitivity analysis was done to identify mast cell count thresholds for patient clustering. Patients were divided into three groups, low (0-8; $n = 16$), medium (8-13; $n = 19$), and high (>13; $n = 18$), and dif-

ferences in clinical and pathologic variables among these three groups were evaluated with a χ^2 test. The Kaplan-Meier method was used to estimate the disease-specific survival and recurrence-free survival for the three groups (low, medium, and high mast cell counts). These were compared using a log rank test. For all tests, $P < 0.05$ values were considered statistically significant. The project was approved by the Institutional Review Board at Northwestern University.

Results

Pancreatic cancer and mast cell infiltration. Because mast cells were identified in the tumor microenvironment of various human malignancies (4–16), we first sought to confirm that mast cells infiltrate human pancreatic adenocarcinoma. A subset of 12 pancreatic cancer patient specimens were immunohistochemically stained with
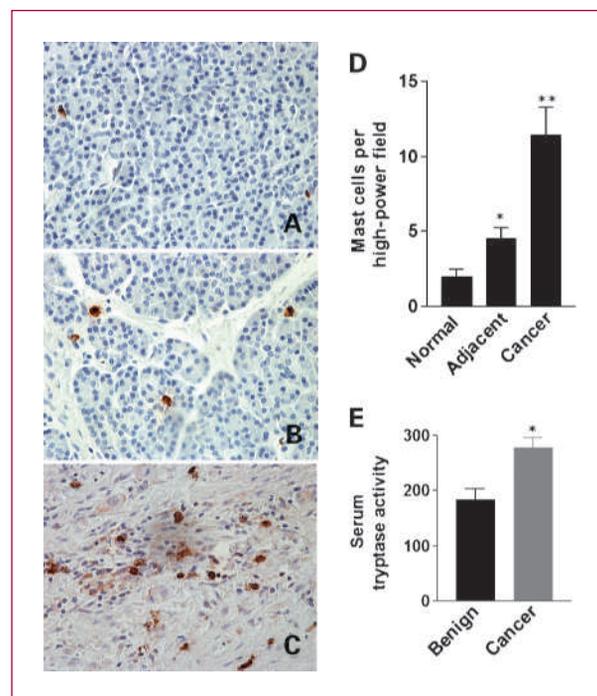
Fig. 1. Mast cells infiltrate human pancreatic adenocarcinoma. Immunohistochemistry (magnification, ×400) for mast cell–specific tryptase (seen with dark red staining) in normal pancreas from a patient with a benign cystic neoplasm (A), an adjacent histologically normal area of pancreas from a patient with pancreatic adenocarcinoma (B), and pancreatic adenocarcinoma (C). D, tryptase-positive mast cells were counted at 400× magnification in 12 pancreatic cancer specimens and compared with the adjacent normal pancreas and 10 normal areas of pancreas from patients with benign disease. Mast cell infiltration was greater in cancer versus adjacent normal ($P < 0.01$) and in cancer versus benign normal ($P < 0.001$). Mast cell infiltration was greater in adjacent normal versus benign normal ($P < 0.05$). E, serum tryptase activity was measured by a quantitative spectrophotometric assay in 36 patients with pancreatic cancer and compared with 10 patients with benign pancreatic disease (*, $P < 0.05$; **, $P < 0.001$).

Table 1. Pancreatic cancer patient demographics and clinical characteristics by mast cell count category

	n	Mast cell count category			P*
		Low (<8)	Medium (8-13)	High (>13)	
Age (y)					0.4309
<65	30	8 (27%)	13 (43%)	9 (30%)	
≥65	23	8 (35%)	6 (26%)	9 (39%)	
Gender					0.1076
Male	31	7 (23%)	10 (32%)	14 (45%)	
Female	22	9 (41%)	9 (41%)	4 (18%)	
Chronic pancreatitis					0.1346
Yes	13	2 (18%)	4 (30%)	7 (52%)	
No	40	14 (35%)	16 (40%)	10 (25%)	
Stage					0.8084
≤IIA	22	6 (27%)	9 (41%)	7 (32%)	
≥IIB	31	10 (32%)	10 (32%)	11 (35%)	
Lymph node					0.6712
Positive	31	9 (29%)	10 (32%)	12 (39%)	
Negative	22	7 (32%)	9 (41%)	6 (27%)	
T stage					0.0827
≤2	9	0 (0%)	4 (44%)	3 (56%)	
≥3	44	16 (36%)	15 (34%)	13 (30%)	
Histology grade					0.0002
G_2	24	10 (42%)	13 (54%)	1 (4%)	
G_3	29	6 (21%)	6 (21%)	17 (58%)	

*χ^2 test.

mast cell–specific tryptase and compared with adjacent normal pancreas as well as 10 histologically normal areas of pancreas from patients that had resection for benign disease (representative immunohistochemistry shown in Fig. 1A-C). Mast cells were identified in the stroma of all cancers. There was a statistically significant increase in mast cell infiltration in the stroma of pancreatic cancer compared with adjacent normal pancreatic tissue (11.4 ± 6.7 versus 4.5 ± 2.5, $P < 0.01$) and normal pancreatic tissue from patients with benign disease (11.4 ± 6.7 versus 2.0 ± 1.4, $P < 0.001$). Furthermore, there was a statistically significant increase in mast cell number in the adjacent normal pancreas compared with normal pancreas from patients with benign disease (4.5 ± 2.5 versus 2.0 + 1.4, $P < 0.05$; Fig. 1D).
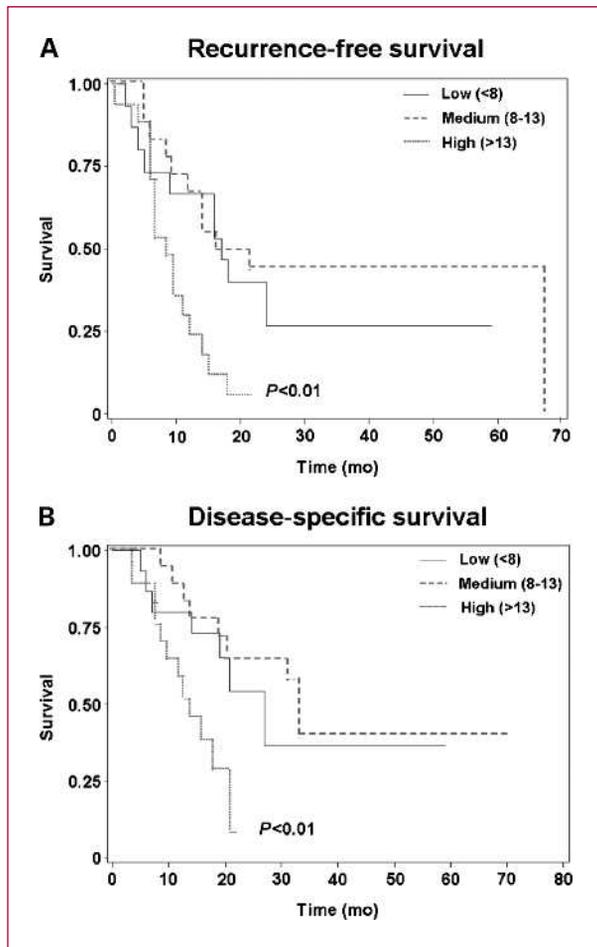
We next evaluated whether there was a correlation between the number of infiltrating mast cells and various clinical and pathologic factors. Examination of 53 tumors stained with mast cell–specific tryptase showed that there was a statistically significant increase in mast cell number in patients with advanced grade tumors (14.31 ± 5.67 for grade 3 tumors versus 8.97 ± 2.75 for grade 2 tumors, $P < 0.001$; Table 1). There was also a trend towards higher mast cell counts in males versus females ($P = 0.10$). Age, T stage, presence of lymph node metastases, presence of chronic pancreatitis, and overall stage did not correlate with mast cell number.

Because patients with pancreatic cancer have high numbers of tryptase-positive tumor-infiltrating mast cells, we hypothesized that patients with pancreatic cancer might have an elevated serum tryptase level. Serum tryptase activity was elevated in 36 serum samples of patients with pancreatic cancer compared with 10 serum samples from patients who underwent pancreatic resection for benign disease ($P < 0.05$; Fig. 1E), demonstrating that the tumor inflammatory microenvironment may be reflected in the patient's serum.
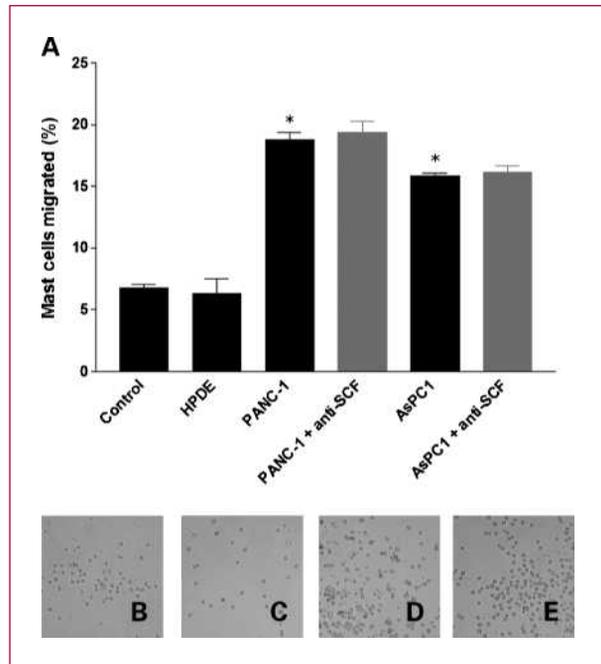
Mast cell count and prognosis. After establishing that the number of tumor-infiltrating mast cells correlated with tumor grade, we sought to evaluate whether mast cell infiltration correlated with patient survival. Sensitivity analyses for mast cell number and survival resulted in three roughly equal groups of low (<8, $n = 16$), medium (8-13, $n = 19$), and high (>13, $n = 18$) mast cell counts. Kaplan-Meier analysis for recurrence-free survival and disease-specific survival was done comparing patients with low, medium, and high tumor mast cell counts. Median recurrence-free survival was significantly worse in patients with a high mast cell count compared with those with a low mast cell count (8 versus 16 months, $P < 0.05$; Fig. 2A). Similarly, median disease-specific survival was also significantly worse in patients with a high mast cell count compared with those with a low mast cell count (12 versus 19 months, $P < 0.05$; Fig. 2B). In both survival analyses,

there were no statistically significant differences between the low and medium cell count groups.

Pancreatic cancer induces mast cell migration. Mast cells are produced in the bone marrow and migrate to peripheral tissues where they exert their effect (21). Observing that mast cells accumulate in the pancreatic cancer tumor stroma but not in normal pancreatic tissue, we hypothesized that pancreatic cancer epithelial cells secrete mediators to orchestrate mast cell migration. To test this, a phenotypically normal human ductal epithelial cell line, HPDE, a primary pancreatic cancer cell line, PANC-1, and a metastatic pancreatic cancer cell line, AsPC1, were used to produce conditioned serum-free medium. Using an 8-μm uncoated Boyden chamber, conditioned medium from HPDE, PANC-1, and AsPC1 cells was compared with control nonconditioned medium and we showed that the

Fig. 3. Mast cell migration assay. LAD-2 cells (100,000) were plated in the upper chamber of an 8-μm uncoated Boyden chamber. The bottom chamber was filled with 750 μL of either control, HPDE-conditioned medium, PANC-1–conditioned medium, AsPC1-conditioned medium, PANC-1–condition medium with SCF neutralizing antibody at 10 μg/mL, or AsPC1-conditioned medium with SCF neutralizing antibody at 10 μg/mL. After 6 h, the number of LAD-2 cells that migrated was counted using Guava Technologies' ViaCount Assay (Guava Technologies) and was reported as the percentage of mast cells. LAD-2 cells migrated in response to (B) nonconditioned control medium, (C) HPDE-conditioned, (D) PANC-1–conditioned, and (E) AsPC1-conditioned medium (*, $P < 0.01$).

Fig. 2. Mast cell count and survival. Sensitivity analysis was done to identify mast cell count thresholds for patient clustering. Patients were divided into three groups of low (0-8; $n = 16$), medium (8-13; $n = 19$), and high (>13; $n = 18$). Statistical analysis for the patient data analysis was done with SAS Software (SAS). The Kaplan-Meier method was used to compare the disease-specific survival and recurrence-free survival among the three mast cell count groups (low, medium, and high) using log rank test.
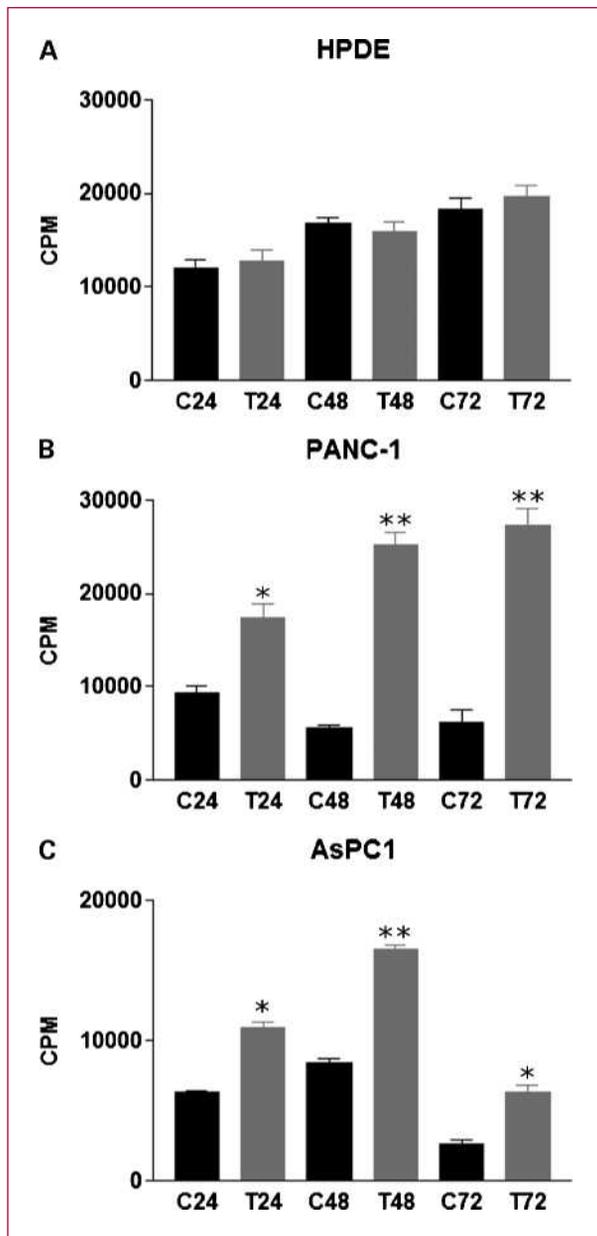
PANC-1 and AsPC1 conditioned medium induced migration of LAD-2 cells whereas the conditioned medium from HPDE cells did not induce LAD-2 migration ($P < 0.01$; Fig. 3).

We next sought to identify whether a specific pancreatic cancer cell product was responsible for mast cell migration. Huang et al. showed that SCF is an important factor in mast cell migration to the tumor microenvironment (22). Therefore, we did mast cell migration assays using conditioned medium from AsPC1 and PANC-1 cells in the presence or absence of 10 μg/mL of anti-SCF neutralizing antibody. We found that inhibition of SCF did not result in a decrease in LAD-2 migration (Fig. 3A).

Mast cells induce pancreatic cancer cell proliferation and invasion. After establishing that mast cells accumulate in pancreatic cancer in response to secreted cancer cell signals, we evaluated the effect that mast cells had on pancreatic cancer cell proliferation and invasion. Using conditioned medium from LAD-2 cells, we assessed the proliferative effect of conditioned medium compared with nonconditioned medium on HPDE, PANC-1, and AsPC1 cells. There was no statistically significant change in

Fig. 4. Mast cells induce pancreatic cancer cell proliferation. One human pancreatic ductal epithelial cell line, (A) HPDE and two pancreatic cancer cell lines, (B) PANC-1 and (C) AsPC1, were grown in either control medium (C) or mast cell conditioned medium (T) and proliferation was measured by thymidine incorporation at 24, 48, and 72 h (*, *P* < 0.01; **, *P* < 0.001).

HPDE proliferation with LAD-2–conditioned medium versus nonconditioned medium at 24, 48, or 72 hours (Fig. 4A). In both PANC-1 and AsPC1 cells following 24, 48, and 72 hours of treatment with LAD-2–conditioned medium, there was a statistically significant increase in proliferation at all three time points (*P* < 0.01, *P* < 0.001, and *P* < 0.001, and *P* < 0.001, *P* < 0.001, *P* < 0.01, respectively, for PANC-1 and AsPC1 cells at 24, 48, and

72 hours; Fig. 4B and C). In the AsPC1 proliferation assay, we noted a decrease in thymidine incorporation at 72 hours compared with 48 hours, which may be attributed to contact inhibition.

Because we noted the greatest effect on proliferation in both cancer cell lines at 48 hours, we chose this time point to perform Boyden chamber Matrigel invasion assays. LAD-2–conditioned medium induced a statistically significant increase in Matrigel invasion in both PANC-1 (13.0 ± 1.0 versus 7.3 ± 0.6 cells per 200× high-power field, *P* < 0.05) and AsPC1 (88.3 ± 11.0 versus 19.7 ± 0.6 cells per 200× high-power field, *P* < 0.01) cancer cells (Fig. 5). Of note, the HPDE cells showed no Matrigel invasion at 48 hours with both nonconditioned and LAD-2–conditioned medium.

To evaluate whether the effect of mast cell–conditioned medium on pancreatic cancer cell invasion was MMP-dependent, AsPC1 cells were treated with either control medium, mast cell–conditioned medium, or mast cell–conditioned medium with a broad-spectrum MMP inhibitor, GM6001. We showed that the addition of GM6001 to the mast cell–conditioned medium led to a statistically significant reduction in invasion compared with conditioned medium treatment (51.6 ± 0.8 versus 174.3 ± 22.2 cells per 200× high-power field, *P* < 0.01; Fig. 5G). Zymography of the mast cell–conditioned medium did not show the presence of MMP-2 or MMP-9 (data not shown).

Discussion

Pancreatic cancer remains one of the deadliest cancers with a roughly equivalent incidence of disease and mortality (1). One of the universal features of this disease is the dense desmoplastic stromal reaction comprised of epithelial cancer cells, fibroblasts, endothelial cells, extracellular matrix, and inflammatory cells (3, 23). Further defining the interaction of pancreatic cancer epithelial cells and stromal cells will provide insight into understanding pancreatic cancer tumorigenesis and progression. However, few studies have examined this crosstalk in pancreatic cancer with the majority of work focusing on pancreatic stellate cells (24). Nevertheless, inflammatory cells are increasingly recognized to play a key role in the tumor microenvironment in many human cancers (25). Specifically, mast cells have been found to infiltrate a number of human cancers and have been associated with angiogenesis and disease progression (26). The clinical significance of mast cell infiltration in pancreatic cancer and the crosstalk between mast cells and pancreatic cancer cells was the emphasis of the current study.

Previously, Esposito et al. evaluated patients with pancreatic cancer and showed that mast cell infiltration correlated with increased tumor microvessel density and lymph node metastases (9). The authors did not find a correlation with mast cell number and patient survival in their cohort, which included patients with metastatic disease. In contrast, although we did not see a correlation between

mast cell count and lymph node status, we found a strong positive correlation between increased mast cell count and tumor grade. In addition, we found that a higher mast cell count was associated with decreased recurrence-free and disease-specific survival. Interestingly, there was a strong trend towards increased mast cells in male pancreatic cancer patients compared with female patients. Although the reasons for this finding are unclear, mast cells have been shown to express estrogen receptor and *in vitro* treatment of mast cells with estrogen resulted in the inhibition of tumor necrosis factor-α release, a major mast cell–derived cytokine and autocrine growth factor (27, 28). This, in part, may account for the varying levels of mast cell infiltration seen in men and women. In a recent evaluation of the prognostic influence of tumor-infiltrating mast cells in follicular lymphoma, there was also a trend towards higher mast cell counts in males (29). Given these find-

ings, the association of gender and mast cell count warrants further investigation. Another interesting finding of the present study is that patients with pancreatic cancer had significantly higher serum tryptase activity compared with patients with benign pancreatic pathology. The serum activity assay might be a reflection of the tumor microenvironment and the measurement of serum tryptase activity levels may serve as a means to differentiate benign and malignant pancreatic lesions. However, further studies are needed to assess the sensitivity and specificity of serum tryptase levels for the detection of malignancy.

Because the majority of studies involving mast cells in human cancer remain correlative, we sought to evaluate the mechanisms by which mast cells contribute to a worse prognosis in patients with pancreatic cancer. Our evaluation of human pancreatic cancer specimens showed that mast cells are located in the tumor stroma but not in direct

Fig. 5. Mast cell–conditioned medium induces pancreatic cancer invasion through a MMP-dependent mechanism. Using an 8 μm Matrigel-coated Boyden chamber, PANC-1 and AsPC1 cancer cells were plated in the upper chamber whereas either mast cell–conditioned medium or unconditioned control medium was placed in the lower chamber. At 48 h, the number of invading cells per 200× field in response to either conditioned medium (C.M.) or nonconditioned (Control) medium were tabulated for (A-C) PANC-1 and (D-F) AsPC1 cells (*, $P < 0.05$; **, $P < 0.01$). G, the invasion assay was repeated with AsPC1 cells in the presence or absence of a broad-spectrum MMP inhibitor, GM6001 (**, $P < 0.01$ for C.M. + GM6001 versus C.M.).
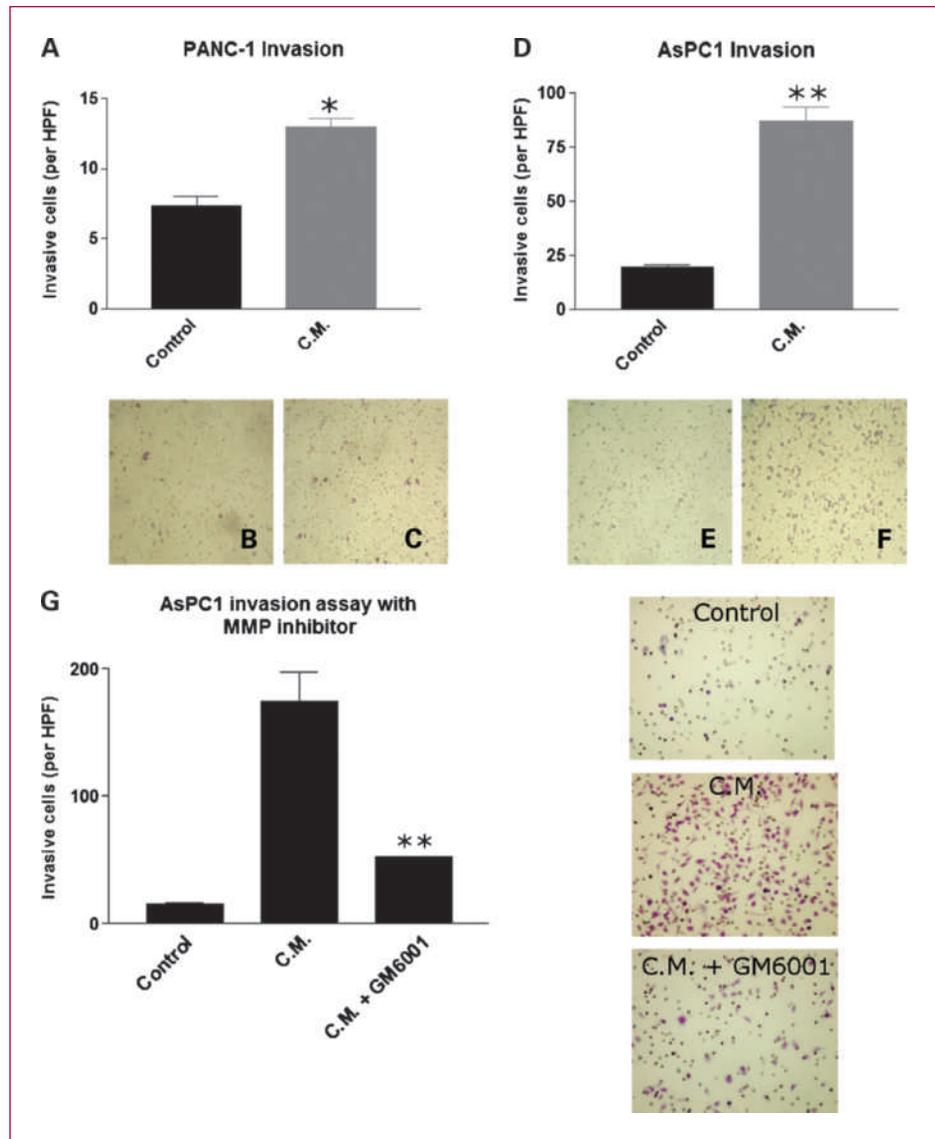
contact with pancreatic cancer epithelial cells. Thus, we hypothesized that mast cells affect cancer cells via secreted signals rather than contact-dependent mechanisms. Our *in vitro* assays on human mast cells and pancreatic cancer cells were designed to evaluate cellular crosstalk via secreted mediators. We first showed that conditioned medium from two pancreatic cancer cell lines induced mast cell migration. On the contrary, conditioned medium from normal HPDE cells did not induce mast cell migration, indicating that this is an acquired property of the cancer cells. These *in vitro* findings were consistent with our human data, in which very few mast cells were found in normal pancreatic tissue whereas a dense mast cell infiltration was seen in pancreatic cancer. Our study implicates pancreatic cancer epithelial cells as mediators of mast cell infiltration. Furthermore, unlike a prior report on tumor-infiltrating mast cells (22), we found that mast cell migration was not SCF-dependent.

We next evaluated the effect of mast cells on pancreatic cancer cells and showed that treatment with mast cell–conditioned medium resulted in increased proliferation of both AsPC1 and PANC-1 cells. However, treatment with conditioned medium did not increase HPDE cell proliferation. Furthermore, we found that mast cell-conditioned medium promoted invasion through Matrigel of both pancreatic cancer cell lines. Through the use of a broad-spectrum MMP inhibitor, GM6001, we showed that the effect of mast cells on pancreatic cancer cell invasion is, in large part, an MMP-dependent process. Because mast cells have been reported to produce MMPs, we did gel zymography on the mast cell–conditioned medium but did not show the presence of MMP2 or MMP9. Because

GM6001 is a broad-spectrum MMP inhibitor, it is possible that it blocked the effects of other MMPs not detected by gel zymography. Alternatively, the mast cell–conditioned medium might induce pancreatic cancer cell MMP production which would also be inhibited by the addition of GM6001.

In conclusion, the current study provides evidence that mast cell infiltration is associated with higher grade tumors and worse survival in patients with pancreatic adenocarcinoma. Our *in vitro* investigation of the crosstalk between mast cells and pancreatic cancer epithelial cells showed that mast cells could be recruited to the tumor microenvironment by pancreatic cancer cell signaling. Mast cells could subsequently contribute to tumor cell proliferation and invasion in an MMP-dependent manner. Further evaluation of specific mast cell functions that promote the invasiveness of pancreatic cancer are likely to open new possibilities for therapeutic intervention.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

References

1. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2008. CA Cancer J Clin 2008;58:71–96.

2. McKay CJ, Glen P, McMillan DC. Chronic inflammation and pancreatic cancer. Best Pract Res Clin Gastroenterol 2008;22:65–73.

3. Mahadevan D, Von Hoff DD. Tumor-stroma interactions in pancreatic ductal adenocarcinoma. Mol Cancer Ther 2007;6:1186–97.

4. Ali G, Boldrini L, Lucchi M, Mussi A, Corsi V, Fontanini G. Tryptase mast cells in malignant pleural mesothelioma as an independent favorable prognostic factor. J Thorac Oncol 2009;4:348–54.

5. Amini RM, Aaltonen K, Nevanlinna H, et al. Mast cells and eosinophils in invasive breast carcinoma. BMC Cancer 2007;7:165.

6. Beer TW, Ng LB, Murray K. Mast cells have prognostic value in Merkel cell carcinoma. Am J Dermatopathol 2008;30:27–30.

7. Dabiri S, Huntsman D, Makretsov N, et al. The presence of stromal mast cells identifies a subset of invasive breast cancers with a favorable prognosis. Mod Pathol 2004;17:690–5.

8. Elpek GO, Gelen T, Aksoy NH, et al. The prognostic relevance of angiogenesis and mast cells in squamous cell carcinoma of the oesophagus. J Clin Pathol 2001;54:940–4.

9. Esposito I, Menicagli M, Funel N, et al. Inflammatory cells contribute to the generation of an angiogenic phenotype in pancreatic ductal adenocarcinoma. J Clin Pathol 2004;57:630–6.

10. Hedstrom G, Berglund M, Molin D, et al. Mast cell infiltration is a favourable prognostic factor in diffuse large B-cell lymphoma. Br J Haematol 2007;138:68–71.

11. Molin D, Edstrom A, Glimelius I, et al. Mast cell infiltration correlates

with poor prognosis in Hodgkin's lymphoma. Br J Haematol 2002; 119:122–4.

12. Nonomura N, Takayama H, Nishimura K, et al. Decreased number of mast cells infiltrating into needle biopsy specimens leads to a better prognosis of prostate cancer. Br J Cancer 2007;97:952–6.

13. Ribatti D, Ennas MG, Vacca A, et al. Tumor vascularity and tryptase-positive mast cells correlate with a poor prognosis in melanoma. Eur J Clin Invest 2003;33:420–5.

14. Takanami I, Takeuchi K, Naruke M. Mast cell density is associated with angiogenesis and poor prognosis in pulmonary adenocarcinoma. Cancer 2000;88:2686–92.

15. Tuna B, Yorukoglu K, Unlu M, Mungan MU, Kirkali Z. Association of mast cells with microvessel density in renal cell carcinomas. Eur Urol 2006;50:530–4.

16. Yodavudh S, Tangjitgamol S, Puangsa-art S. Prognostic significance of microvessel density and mast cell density for the survival of Thai patients with primary colorectal cancer. J Med Assoc Thai 2008;91: 723–32.

17. Ouyang H, Mou L, Luk C, et al. Immortal human pancreatic duct epithelial cell lines with near normal genotype and phenotype. Am J Pathol 2000;157:1623–31.

18. Kirshenbaum AS, Akin C, Wu Y, et al. Characterization of novel stem cell factor responsive human mast cell lines LAD 1 and 2 established from a patient with mast cell sarcoma/leukemia; activation following aggregation of FcεRI or FcγRI. Leuk Res 2003;27:677–82.

19. Ottaviano AJ, Sun L, Ananthanarayanan V, Munshi HG. Extracellular

matrix-mediated membrane-type 1 matrix metalloproteinase expression in pancreatic ductal cells is regulated by transforming growth factor-β1. Cancer Res 2006;66:7032–40.

20. Munshi HG, Stack MS. Analysis of matrix degradation. Methods Cell Biol 2002;69:195–205.

21. Gurish MF, Boyce JA. Mast cells: ontogeny, homing, and recruitment of a unique innate effector cell. J Allergy Clin Immunol 2006;117:1285–91.

22. Huang B, Lei Z, Zhang GM, et al. SCF-mediated mast cell infiltration and activation exacerbate the inflammation and immunosuppression in tumor microenvironment. Blood 2008;112:1269–79.

23. Farrow B, Albo D, Berger DH. The role of the tumor microenvironment in the progression of pancreatic cancer. J Surg Res 2008;149:319–28.

24. Vonlaufen A, Phillips PA, Xu Z, et al. Pancreatic stellate cells and pancreatic cancer cells: an unholy alliance. Cancer Res 2008;68:7707–10.

25. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature 2008;454:436–44.

26. Conti P, Castellani ML, Kempuraj D, et al. Role of mast cells in tumor growth. Ann Clin Lab Sci 2007;37:315–22.

27. Kim MS, Chae HJ, Shin TY, Kim HM, Kim HR. Estrogen regulates cytokine release in human mast cells. Immunopharmacol Immunotoxicol 2001;23:495–504.

28. Zhao X, Dong Z, Yang Z. [Expression of estradiol receptors in mast cells of the upper airway]. Zhonghua Er Bi Yan Hou Ke Za Zhi 1999; 34:135–7.

29. Taskinen M, Karjalainen-Lindsberg ML, Leppa S. Prognostic influence of tumor-infiltrating mast cells in patients with follicular lymphoma treated with rituximab and CHOP. Blood 2008;111: 4664–7.