Original Research

Tumor-derived intercellular adhesion molecule-1 mediates tumor-associated leukocyte infiltration in orthotopic pancreatic xenografts

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Abstract

Tumor infiltration of immune cells (polymorphonuclear cells [PMNs] and macrophages) was initially thought to be an attempt by the host organism to combat malignancy. It appears, however, that certain subsets of chronically activated immune cells likely promote tumor growth, facilitate tumor cell survival and aid in metastasis. The association between tumor cells and tumor-associated PMNs has been demonstrated in several types of cancer, but the presence of tumor-associated PMNs in pancreatic cancer has not been well studied *in vivo*. Intercellular adhesion molecule-1 (ICAM-1) functions in cell–cell and cell–extracellular matrix adhesion and has a physiological role in PMN tight adhesion of leukocytes via interaction with the ligands LFA-1 and Mac-1. Increased ICAM-1 expression correlates with poor prognosis in pancreatic cancer. Therefore, the aim of this study was to investigate the function of ICAM-1 and tumor-associated PMNs in pancreatic cancer progression using ICAM-1-null (ICAM-1$^{-/-}$) mice. We hypothesize that ICAM-1 null mice have decreased pancreatic cancer progression. Surprisingly, there is no significant difference in pancreatic cancer progression in wild-type versus ICAM-1 null mice. Interestingly, we found that tumor-derived ICAM-1 co-localizes with host PMNs at the leading edge of the tumor in ICAM-1 null mice. These results suggest that tumor-derived ICAM-1 is a sufficient ligand for tumor-associated PMNs and may play a role in subsequent tumor growth and metastasis.

Keywords: neutrophils, ICAM-1, pancreatic cancer

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Introduction

Solid malignancies consist of a diverse population of cells, including tumor cells, fibroblasts, endothelial cells and immune cells.[1,2] While the infiltration of immune cells was initially thought to be an attempt by the host to combat the malignancy, it now appears that certain subsets of chronically activated immune cells actually promote tumor growth and facilitate tumor cell survival, as well as metastasis.[3] Metastatic tumor cells and immune cells have many similar properties including the ability to attach to endothelium as well as cause degradation of the basement membrane, suggesting a role of tumor cell exploitation of leukocyte function in order to enhance metastatic efficiency.[4–7] The association between tumor cells and polymorphonuclear cells (PMNs) has been demonstrated in several types of cancer.[2,6,7] Electron micrographs of circulating PMNs demonstrate close association between Lewis lung carcinoma and B16 melanoma cells during the process of tumor cell arrest and extravasation.[8] *In vitro* studies have demonstrated increased adhesion of human pancreatic carcinoma cells to microvascular endothelium in the presence of reactive oxygen species released from PMNs.[9] These data suggest a role for PMNs in tumor adhesion and metastasis. However, the role of PMNs in pancreatic cancer progression has not been well studied *in vivo*.

Intercellular adhesion molecule-1 (ICAM-1, CD54) functions in cell–cell and cell–extracellular matrix (ECM)

adhesion, including physiological PMN tight adhesion and transendothelial migration via the leukocyte integrins LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18). Additionally, ICAM-1 has been shown to facilitate PMN protease release,[10,11] which could be potentially involved in metastasis. Interestingly, increased ICAM-1 expression correlates with poor prognosis in malignancies such as melanoma, gastric cancer and pancreatic cancer.[12–14] Normal human pancreas expresses low levels of ICAM-1.[15,16] It is postulated that growth factors such as tumor necrosis factor (TNF)-α, interleukin-1 and interferon-γ released by tumor cells increase tumor ICAM-1 expression on endothelial and tumor cells.[17,18] A study of surgical specimens from patients with pancreatic cancer demonstrated a five-fold increase in ICAM-1 expression compared with healthy controls and this increased level of ICAM-1 correlates with increased nodal metastasis and advanced tumor stage,[14] indicating that ICAM-1 may be important in pancreatic cancer progression and metastasis.

The aim of this study was to investigate the role of ICAM-1 in pancreatic cancer progression as well as ICAM-1–PMN interactions at the tumor invasion front. To examine the role of ICAM-1, we utilized ICAM-1-null (ICAM-$1^{-/-}$) mice. We hypothesize that ICAM-1 is necessary for pancreatic cancer progression and metastasis.

Material and methods

Animals

Animals were maintained according to the Institutional Animal Care and Use Committee at UT Southwestern and all procedures were performed following the Public Health Service policy on Humane Care of Laboratory Animals. Experiments were performed in C57BL/6 (6–8 weeks old, Wakeland colony, UTSW) and C57BL/6 ICAM-$1^{-/-}$ mice, which express no alternatively spliced ICAM-1 isoforms[19].

Cell culture

The murine pancreatic adenocarcinoma cell line, Pan02, was obtained from Developmental Therapeutics Program, NCI (Frederick, MD, USA). Pan02-luc cells were a generous gift from Dr J Shay, University of Texas Southwestern Medical Center, Dallas, TX. The murine brain endothelial cell line (bEnd.3) was obtained from ATCC (Manassas, VA, USA) and cells were stimulated with TNF-α[20] (10 ng/mL; recombinant human TNF-α) at 37°C for 18 h. All cell lines were otherwise maintained at 37°C in a mixture of 5% CO_2 and 95% air in Dulbecco's minimal essential medium (Mediatech, Inc, Herndon, VA, USA) supplemented with 10% fetal calf serum (Gemini Bio-products, Woodland, CA, USA). For ICC, 2.5×10^4 Pan02 cells were plated on chamber slides and grown in media as described above.

Orthotopic tumor model

Tumor cells were injected as described.[21,22] Briefly, animals were anesthetized using inhaled isoflurane. A small laparotomy was performed exposing the inferior pole of the spleen and tail of the pancreas, which were externalized through the wound. A total of 2.5×10^5 Pan02 cells were injected underneath the capsule of the pancreas using a 30G needle. The skin and abdominal musculature were closed with a non-absorbable suture. Mice were monitored and weighed twice weekly.

Animals were sacrificed at four weeks. At necropsy, liver, nodal, splenic, gastrointestinal and peritoneal metastases were evaluated by visual inspection. Tumors weights were calculated in conjunction with residual pancreas. Tissues were snap frozen in liquid nitrogen.

Intrasplenic tumor model

Animals were anesthetized using inhaled isoflurane. Laparotomy exposed the inferior pole of the spleen and tail of the pancreas, which was externalized through the wound. A total of 2.5×10^5 Pan02-luc cells were injected into the inferior pole of the spleen using a 30G needle. The skin and abdominal musculature were closed with a non-absorbable suture. Body luminescence imaging of mice was performed at 4 h, 24 h, 72 h, 7 days, 14 days and 21 days post-tumor cell injection using the Xenogen IVIS bioluminescence imaging as described.[23] Animals were sacrificed eight weeks after tumor cell injection. At necropsy, liver, lymph node, splenic, gastrointestinal and peritoneal metastases were evaluated by visual inspection.

Protein extraction and Western blot analysis

Total protein from bEnd.3 stimulated with TNF-α and Pan02 cells was extracted with 1 mL Radio ImmunoPrecipitation Assay (RIPA) lysis buffer[24] (150 mmol/L NaCl, 1% NP-40, 0.5% deoxycholic acid, 0.1% SDS, 50 mmol/L Tris pH 8.0) per 100-mm dish. Adherent cells were dislodged with a sterile scraper and incubated at 4°C × 20 min. The cell lysate was stored at −80°C. An equal concentration of thawed protein extract and loading buffer were heated to 90°C × 5 min and separated by a 10% polyacrylamide gel, transferred to a polyvinylidene fluoride membrane, and probed with rabbit anti-mouse ICAM-1 antibodies (Proteintech Group, Inc, Chicago, IL, USA) at 1:500 dilution. Signal was detected with horseradish peroxidase (HRP)-conjugated donkey anti-rabbit IgG (1:50,000 dilution; Jackson ImmunoResearch, West Grove, PA, USA) antibody and visualized by enhanced chemiluminescence (Pierce Biotechnology, Inc, Rockford, IL, USA).

Tumor histology, immunohistochemistry and immunocytochemistry

Antibodies for immunohistochemical analysis were as follows: rabbit anti-mouse ICAM-1 (Proteintech Group Inc, Chicago, IL, USA) at 10 μg/mL, rat anti-mouse ICAM-1 (YN.1; Biolegend, San Diego, CA, USA) at 10 μg/mL, rat anti-mouse neutrophils (7/4; AbDSerotec, Oxford, UK) at 7.5 μg/mL, rabbit anti-myeloperoxidase (MPO, Hycult Biotechnology, The Netherlands) at 1:200, Meca-32 (DSHB;

Table 1 Primer sequences for two-way polymerase chain reaction (PCR) for wild-type (WT) and ICAM-1$^{-/-}$ mice*

Molecule	Sense	Anti-sense
Inull/Geno	ATC-GCT-GCT-TCA-CTA-GTG-C	TGA-CTA-CTG-CCT-AGT-CCC-CTG-C
Puro (wild type)	AGG-CAT-GCT-GGG-GAT-GCG-GT	

*To verify the ICAM-1$^{-/-}$ status in C57BL/6 mice a two-way PCR strategy was employed. DNA was purified from the tail of breeding mice and evaluated using PCR. The sequences of the primer sets are listed in Table 1. A primer from within the selectable marker PURO was used with a primer outside the ICAM-1 locus to amplify the 320 bp mutant allele or an ICAM-1 null genomic primer outside the deletion pairs with a primer from the deleted region to create a 270 bp band

University of Iowa) at 10 μg/mL, MAC157 (ECACC, Cambridge, UK). Frozen tumors were sectioned (10 μm) and dried for 20 min. Chamber slides and tumor sections were fixed with acetone for 1 min, blocked with 20% Aquablock (East Coast Biologics Inc., North Berwick, ME) for 30 min and incubated with primary antibody for 1 h. Slides were subsequently washed with TBS containing 0.2% Tween-20 (TBS-t) and incubated with the appropriate fluorescently labeled secondary antibody (Jackson ImmunoResearch, West Grove, PA, USA) for 1 h. Negative controls were incubated with secondary antibody only. Sections were coated with mounting medium containing 1 μg/mL 4′,6-diamidino-2-phenylindole to stain nuclear DNA. Sections were examined on a Nikon E600 microscope and images captured and analyzed with NIS Elements software.

Polymerase chain reaction

To verify the ICAM-1$^{-/-}$ status in C57BL/6 mice, a two-way polymerase chain reaction (PCR) strategy was employed. DNA was purified from the tail of breeding mice and evaluated using PCR. The PCR reactions (25 μL reactions) were set up as follows: 11.6 μL water, 2.5 μL 10× PCR buffer, 2.5 μL 50 mmol/L $MgCl_2$, 0.4 μL primer Inull/Geno1Sense (20 pm/μL), 0.4 μL primer Inull/Geno2 Anti-sense (20 pm/μL), 0.4 μL primer PURO (20 pm/μL), 6 μL tail DNA, 1 μL of 10 mmol/L dNTP and 0.2 μL Taq polymerase. The sequences of the primer sets are listed in Table 1. A primer from within the selectable marker PURO was used with a primer outside the ICAM-1 locus to amplify the 320 bp mutant allele or a ICAM-1 null genomic primer outside the deletion pairs with a primer from the deleted region to create a 270 bp band. PCR products of heterozygotes show additional bands, likely representing a heteroduplex between wild-type and ICAM-1 strands, which migrate slower than the 320 bp band.[25] PCR conditions were as follows: denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C × 3 min, annealing at 61°C × 45 s and elongation at 72°C × 45 s. Products were kept at 72°C × 3 min and then cooled to 4°C. Products were separated on a 1.5% agarose gel and visualized by ethidium bromide staining.

Statistics

Data were analyzed with GraphPad software (GraphPad Prism version 4.00 for Windows; GraphPad Software, San Diego, CA) (www.graphpad.com) and means were analyzed using *t*-tests. Non-parametric tests were employed and significance was considered for $P < 0.05$.

Results

Loss of ICAM-1 does not affect tumor growth or rate of metastasis

To evaluate the contribution of ICAM-1 in pancreatic cancer progression and metastasis, C57BL/6 wild type (ICAM-1$^{+/+}$) and ICAM-1 null (ICAM-1$^{-/-}$) mice were injected with 2.5 × 10^5 Pan02 cells into the tail of the pancreas (Figure 1). Tumors were established for four weeks. Animals were sacrificed and pancreas/tumor size and metastatic burden was evaluated by visual inspection. There was no difference in final combined pancreas/tumor weights (Figure 2), metastatic incidence or the number of metastatic events (Table 2) between the two groups. Taken together, these data indicate that loss of host-derived ICAM-1 does not affect pancreatic cancer progression or metastasis.

Pan02 cells express ICAM-1

Increased tumor expression of ICAM-1 is known to correlate with a worse prognosis in pancreatic cancer.[14] Therefore, following our *in vivo* experiments in which we established that loss of host ICAM-1 did not alter pancreatic cancer progression or metastasis, we measured tumor-derived ICAM-1 status of the Pan02 cells via Western blot analysis and immunocytochemistry (Figure 3). bEnd.3 cells stimulated with TNF-α are well known to express ICAM-1[20] and were used as a positive control. Western blot analysis (Figure 3a) with anti-mouse ICAM-1 shows identical 66 kDa bands expressed by bEnd.3 and unstimulated Pan02 cells. This was confirmed with PCR analysis (data

Figure 1 PCR of wild-type (ICAM-1$^{+/+}$) and ICAM-1$^{-/-}$ mice. DNA from wild-type and ICAM-1$^{-/-}$ mice was amplified using PCR to verify ICAM-1$^{-/-}$ status. Two-way PCR was employed and products were separated on a 1.5% agarose gel. The wild-type band is 270 bp and the mutant, ICAM-1$^{-/-}$ band is 320 bp. Heterozygote mice express an additional band, likely representing a heteroduplex between wild-type and ICAM-1 strands, which migrate slower than the 320 bp band.[25] PCR, polymerase chain reaction; ICAM, intercellular adhesion molecule

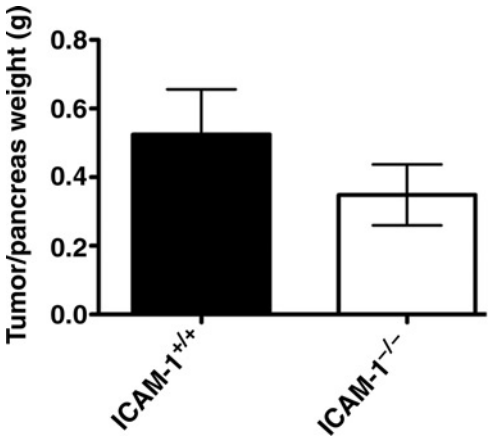

Figure 2 Combined tumor/pancreas weight in ICAM-$1^{+/+}$ ($n = 9$) and ICAM-$1^{-/-}$ mice ($n = 10$). A total of 2.5×10^5 Pan02 cells were injected into the tail of the pancreas of C57BL/6 ICAM-$1^{+/+}$ and ICAM-$1^{-/-}$ mice. Four weeks after tumor cell injection, mice were sacrificed and at necropsy, tumor/pancreas weight was compared between the two groups. There was no significant difference in the mean tumor/pancreas weight in ICAM-$1^{+/+}$ versus ICAM-$1^{-/-}$ (ICAM-$1^{+/+}$: 0.52 g and ICAM-$1^{-/-}$: 0.35 g; P = NS). ICAM, intercellular adhesion molecule

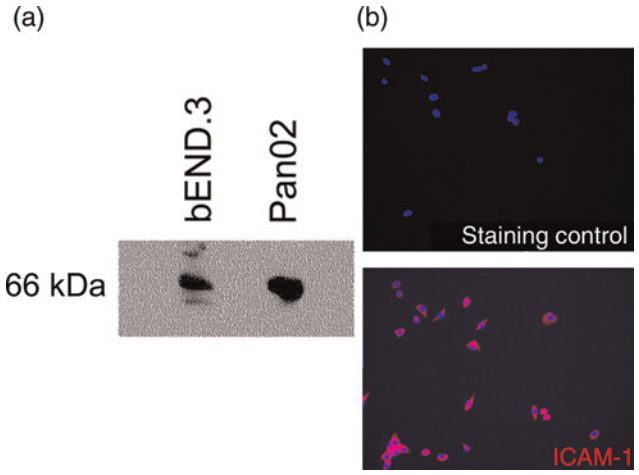

Figure 3 Pan02 cells express ICAM-1. (a) Cell lysates from TNF-α stimulated bEnd.3 cells or Pan02 cells were separated by 10% SDS-PAGE gel, transferred to a PVDF membrane and probed for ICAM-1. ICAM-1 is expressed by stimulated bEND.3 cells and unstimulated Pan02 cells. (b) Pan02 cells were plated onto chamber slides and stained with anti-ICAM-1 (red) and 4′,6-diamidino-2-phenylindole for nuclear stain (blue). Total magnification was $\times 200$. Images were overlayed using Elements software. ICAM, intercellular adhesion molecule; TNF, tumor necrosis factor; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PVDF, polyvinylidene fluoride

not shown). Immunocytochemistry of Pan02 cells (Figure 3b) also demonstrated ICAM-1 expression in unstimulated Pan02 cells.

ICAM-1 and PMNs at the tumor invasion front

Although PMNs can be cytotoxic to tumor cells, they have been shown under certain circumstances to promote tumor adhesion, transendothelial migration and facilitate in the activation of angiogenesis.[7,26] Studies in melanoma using light and electron microscopy have shown circulating PMNs in close association with metastatic tumor cells.[8] However, the co-localization of PMNs and ICAM-1 on tumor cells has not previously been demonstrated in pancreatic cancer *in vivo*.

To further investigate the interaction of PMNs and tumor cells via ICAM-1 expression, co-localization of ICAM-1 and PMNs was performed on frozen sections of tumors from ICAM-$1^{+/+}$ and ICAM-$1^{-/-}$ animals (Figure 4, Suppl. Figure 1). In tumors from ICAM-$1^{+/+}$ animals, ICAM-1 is expressed in areas of tumor as well as normal pancreas (Figure 4a). However, in tumors from ICAM-$1^{-/-}$ mice, ICAM-1 expression is seen only in the areas of tumor and along infiltrating tumor cells (arrows), not in the uninvolved pancreas (Figure 4d). PMN infiltration is seen in tumors from ICAM-$1^{+/+}$ and ICAM-$1^{-/-}$ mice at the tumor invasion front (Figures 4b and e), where it is co-localized with MPO (Figures 4c and f). Further, we found an increase in the number of PMNs in tumors from ICAM-$1^{-/-}$ mice compared with tumors from control animals (Figure 5). Taken together, these data indicate that host-derived ICAM-1 is not necessary for PMN infiltration at the zone of tumor invasion. As angiogenesis is required for solid tumor growth beyond 1–2 mm[3,27] it is possible that infiltrating PMNs may play a role in activating angiogenesis.[26] Having found an increase in PMN infiltration in the tumors from ICAM-$1^{-/-}$ mice, we hypothesized that there would be an increase in microvessel density (MVD) in the tumors from ICAM-$1^{-/-}$ mice compared with control. Frozen tumor sections from ICAM-$1^{+/+}$ and ICAM-$1^{-/-}$ mice were stained with Meca-32, an endothelial cell marker. There was a significant increase in MVD in tumors from ICAM-$1^{-/-}$ mice compared with control (Figure 6).

Table 2 Metastatic incidence and number of metastatic events*

Metastatic incidence						
Treatment	***n* =**	**Lymph**	**GI**	**Liver**	**Spleen**	**Total**
WT	9	44%	33%	44%	33%	78%
ICAM$^{-/-}$	10	20%	20%	40%	10%	60%

Metastatic events							
Treatment	***n* =**	**Lymph**	**GI**	**Liver**	**Spleen**	**Other**	**Total**
WT	9	5	6	7	3	0	21
ICAM$^{-/-}$	10	8	6	6	3	2	25

*At necropsy, lymph, gastrointestinal (GI), liver, splenic, GI and other metastases were evaluated by visual inspection

Tumor cell engraftment is reduced after blocking tumor-derived ICAM-1

Having established that loss of host-derived ICAM-1 does not affect pancreatic cancer progression, we blocked tumor-derived ICAM-1 with the anti-ICAM-1 antibody, YN.1. Pan02-luc cells were incubated with a blocking dose (5 μg/mL) of YN.1 or an isotype-matched control antibody for 1 h and 2.5×10^5 cells were injected into the inferior pole of the spleen of ICAM-$1^{-/-}$ mice. Animals were imaged using bioluminescence imaging at various time points following tumor cell injection for luciferase activity (Figure 7). Animals injected with tumor cells treated with

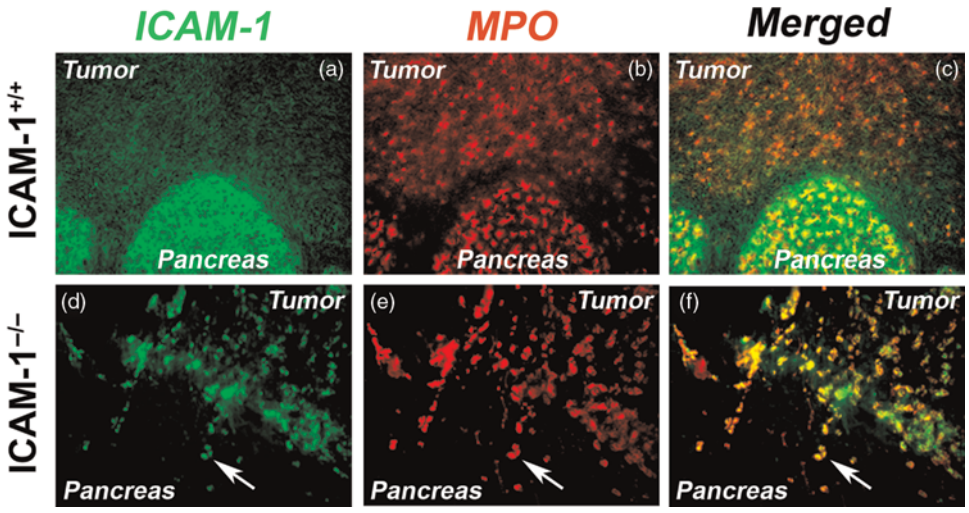

Figure 4 Neutrophils at the leading edge: Frozen sections of tumor from ICAM-$1^{+/+}$ and ICAM-$1^{-/-}$ mice were co-localized with ICAM-1 and MPO antibodies and FITC (ICAM-1) or Cy3 (MPO) conjugated secondary antibodies and photographed at ×400 total magnification using FITC and Cy3 filters. Images were overlayed using Elements software. There was ICAM-1 expression in the ICAM-$1^{+/+}$ normal pancreas and tumor (a); however, the only ICAM-1 expression in the ICAM-$1^{-/-}$ was in the areas of tumor (d) and along infiltrating tumor cells (arrow). Similarly, PMN infiltration was seen in the same areas (b, e). In the overlayed images (c, f), there are areas of co-localization between the ICAM-1 and MPO, suggesting a role for tumor-derived ICAM-1 in PMN infiltration. ICAM, intercellular adhesion molecule; MPO, myeloperoxidase; PMN, polymorphonuclear cell; FITC, fluorescein isothiocyanate

YN.1 had a significant decrease in luciferase activity seven days following tumor cell injection, which represents a decrease in tumor cells engraftment following treatment with the anti-ICAM-1 antibody. These data suggest a role of tumor-derived ICAM-1 in tumor cell implantation.

Discussion

Pancreatic cancer is the fourth leading cause of cancer-related death, with a five-year survival of <5% and a median survival of six months after diagnosis.[28] Although the molecular basis of metastasis and recurrence has been investigated in recent decades, major contributing factors are still poorly understood. Enhanced cell–cell or cell–ECM adhesion in the tumor as well as enhanced extravasation are thought to be important steps in metastasis and local recurrence.[7,12]

In this paper, we have attempted to better delineate the importance of tumor-derived ICAM-1 and PMN interaction and its role in pancreatic cancer progression. We have shown using an ICAM-$1^{-/-}$ mouse model that loss of host-derived ICAM-1 does not affect pancreatic cancer growth or metastasis. We have also demonstrated the PMN–ICAM-1 co-localization at the tumor invasion front, suggesting a role of PMNs in pancreatic tumor growth and metastasis. Furthermore, blocking tumor-derived ICAM-1 prior to tumor cell injection resulted in decreased

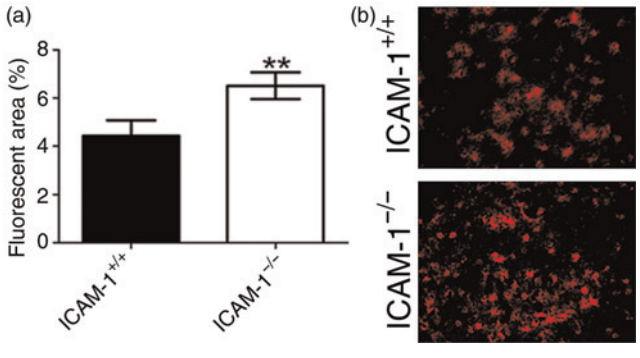

Figure 5 Increased PMNs in tumors from ICAM-$1^{-/-}$ mice. Frozen sections of tumor from ICAM-$1^{+/+}$ and ICAM-$1^{-/-}$ mice were stained with the mouse neutrophil antibody 7/4 and Cy3-conjugated secondary antibodies and photographed at ×400 total magnification using Cy3 filters. The total area fraction of $7/4^{+}$ cells was measured using Elements software and values graphed using GraphPad Prism software. Data are displayed as mean ± SEM and represents five images per tumor and three tumors per group. There were significantly more PMNs in the tumors from ICAM-$1^{-/-}$ compared with ICAM-$1^{+/+}$ (0.065 versus 0.044 % area fraction; $P = 0.007$). PMN, polymorphonuclear cell; ICAM, intercellular adhesion molecule

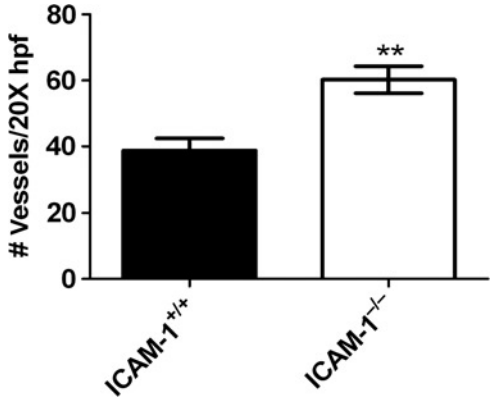

Figure 6 Increased MVD in ICAM-$1^{-/-}$ tumors. Frozen sections from ICAM-$1^{+/+}$ and ICAM-$1^{-/-}$ tumors were stained with the endothelial cell marker, Meca-32 and Cy3-conjugated secondary antibody. Sections were photographed at ×200 total magnification using Cy3 filters. Data are displayed as mean ± SEM and represent five images per tumor and three tumors per group. There was a significant increase in the MVD in tumors from ICAM-$1^{-/-}$ mice compared with ICAM-$1^{+/+}$ tumors (38.8 versus 60.3 vessels/20× hpf, respectively; $P = 0.003$). MVD, microvessel density; ICAM, intercellular adhesion molecule

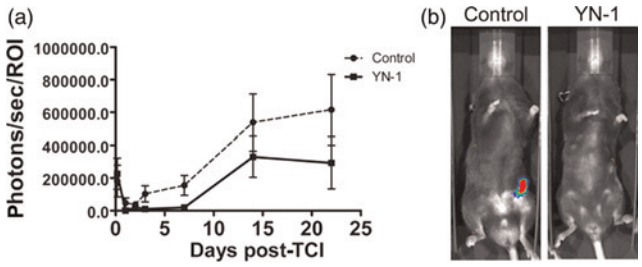

Figure 7 Blocking tumor-derived ICAM-1 decreases tumor cell engraftment. Pan02-luc cells were preincubated with anti-ICAM-1 blocking antibody or control and injected into the inferior pole of the spleen of ICAM-1$^{-/-}$ mice. (a) BLI was performed at various time points post-tumor cell injection (TCI). There was significantly less luciferase activity in the animals receiving YN.1-treated cells seven days post-TCI. (b) Representative images of BLI. These data represent decreased tumor cell engraftment after blocking tumor-derived ICAM-1, suggesting a possible role of tumor-derived ICAM-1 in tumor cell implantation. ICAM, intercellular adhesion molecule; BLI, bioluminescence imaging

tumor cell engraftment. However, these data could also represent antibody-dependent cellular cytotoxicity of tumor cells by host antigen-presenting cells.[29,30]

Tumors consist of a heterogeneous population of cells, including tumor cells, endothelial cells, fibroblasts and immune cells, such as PMNs and macrophages. Recent evidence suggests that immune cell infiltration can either antagonize tumor growth via immune surveillance or promote tumor growth and metastasis via immune enhancement.[31] The cancer-promoting effect of tumor-associated macrophages has been established,[31] but the role of PMNs in cancer progression has been less well studied. Hanahan and colleagues[26] have shown that infiltrating neutrophils are responsible for the initial 'angiogenic switch' in mouse models of pancreatic cancer. PMNs secrete a wide variety of factors upon stimulation, including matrix metalloproteinase (MMP)-9. MMP-9 is known to act as a regulator of vascular endothelial growth factor (VEGF) levels by mobilizing VEGF from an extracellular reservoir,[32] which may contribute to the increase in angiogenesis seen in ICAM-1$^{-/-}$ mice.

The role of the PMN–ICAM-1 interaction in pancreatic cancer has not been studied *in vivo*. The evidence we have presented contributes to a body of work demonstrating the importance of PMNs and ICAM-1 in pancreatic cancer progression and metastasis. Our data allow us to hypothesize that tumor-derived ICAM-1 serves as an important docking point for PMNs that functionally promote tumor cell metastasis.

Author Contributions: All authors participated in the design, interpretation of the studies and the analysis of the data as well as review of the manuscript. CLR, SPD, JET, JGC, RAB and CCB performed the experiments, including murine work and *in vitro* assays. CWS provided the transgenic (ICAM-1) null mice. CLR, CWS, RAB and CCB wrote the manuscript.

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