[Cancer Biology & Therapy 6:2, e1-e10, EPUB Ahead of Print: http://www.landesbioscience.com/journals/cbt/abstract.php?id = 3623; February 2007]; ©2007 Landes Bioscience

Research Paper

Expression of Extracellular Matrix Metalloproteinase Inducer (EMMPRIN/CD147) in Pancreatic Neoplasm and Pancreatic Stellate Cells

Weiwei Zhang
Mert Erkan
Ivane Abiatari
Nathalia A. Giese
Klaus Felix
Hany Kayed
Markus W. Büchler
Helmut Friess
Jörg Kleeff*

Department of General Surgery; University of Heidelberg; Heidelberg, Germany

*Correspondence to: Jörg Kleeff; Department of General Surgery; University of Heidelberg; Im Neuenheimer Feld 110; Heidelberg 69120 Germany; Tel.: +49.6221.56.4860; Fax: +49.6221.56.6903; Email: joerg_kleeff@med.uni-heidelberg.de

Original manuscript submitted: 11/25/06
Manuscript accepted: 11/25/06

This manuscript has been published online, prior to printing for Cancer Biology & Therapy, Volume 6, Issue 2. Definitive page numbers have not been assigned. The current citation is: Cancer Biol Ther 2007; 6(2):
http://www.landesbioscience.com/journals/cc/abstract.php?id = 3623
Once the issue is complete and page numbers have been assigned, the citation will change accordingly.

KEY WORDS

EMMPRIN, CD147, pancreatic cancer, invasion, vascular endothelial growth factor, matrix metalloproteinase, microenvironment

ABSTRACT

EMMPRIN (extracellular matrix metalloproteinase inducer, CD147) participates in the progression of various malignancies by stimulating the synthesis of specific matrix metalloproteinases (MMP) from peritumoral fibroblasts. In the present study, the expression and functional role of EMMPRIN was investigated in pancreatic neoplasm. QRT-PCR, immunohistochemistry, immunoblot, and ELISA analyses were used to analyze the expression, localization, and release of EMMRPIN. Silencing of EMMPRIN was performed using siRNA oligonucleotides, and functional consequences were assessed using growth assays, invasion assays, as well as MMP1/MMP2 and VEGF ELISA. EMMPRIN mRNA levels were 2.2-fold increased in pancreatic cancer (n = 52) and 2.0-3.5-fold increased in other pancreatic neoplasm (n = 105), but unchanged in chronic pancreatitis (n = 10) compared to normal pancreatic tissues (n = 9). Strong and predominantly membranous immunostaining was observed in the cancer cells and surrounding stromal cells. EMMPRIN serum levels were also significantly increased in pancreatic cancer patients (n = 44) (4.13 ± 0.28 ng/ml) with an AUC of 0.97 compared to healthy volunteers (n = 29) (0.95 ± 0.16 ng/ml; $p < 0.0001$) and with an AUC of 0.74 compared to chronic pancreatitis patients (n = 20) (2.98 ± 0.5 ng/ml; $p = 0.0021$). EMMPRIN silencing did not significantly affect anchorage-dependent or -independent growth of pancreatic cancer cells. In contrast, EMMPRIN silencing in pancreatic stellate cells slightly repressed VEGF and MMP2 levels but strongly increased pro-MMP1 expression under coculture conditions. In conclusion, Increased EMMPRIN expression is present in different pancreatic neoplasm, likely representing a tumor-specific reaction with the potential to modulate the tumor microenvironment rather than a mere reflection of an activated stroma.

INTRODUCTION

EMMPRIN/CD147 (Extracellular matrix metalloproteinase inducer) was originally purified from the plasma membrane of cancer cells as a glycoprotein with a molecular weight of 58 kDa. After biosythesis in the Golgi complex, the core protein of EMMPRIN (non-glycosylated form of EMMPRIN) is modified into the less glycosylated (LG)-EMMPRIN. LG-EMMPRIN can mature into highly glycosylated (HG) EMMPRIN by further glycosylation. The highly glycosylated (HG) form of EMMPRIN is more active in the induction of matrix metalloproteinases (MMPs).[1,2] Specifically, during glycosylation of EMMPRIN in the Golgi complex, the LG-form can associate with caveolin-1 which escorts it to the cell surface. This surface bound less glycosylated form of EMMPRIN (immature form) does not self-aggregate leading to impairment of EMMPIRN glycosylation and MMP induction.[3,4] In the absence of negative regulators such as caveolin-1, EMMPRIN is further glycosylated in the Golgi complex by addition of polylactosamine-type sugars as it matures to the HG-form of EMMPRIN (mature form). The HG-form has a tendency to self-aggregate and is active in the stimulation of MMP production by neighboring cells.[4,5]

Due to its ability to stimulate the synthesis of collagenase-1 (MMP1) in fibroblasts,[6,7] EMMPRIN was designated as tumor collagenase stimulating factor. EMMPRIN contains two extracellular domains, a transmembrane domain and a cytoplasmic domain.[1,3,8-12] Only the distal extracellular domain and its glycosylated form contribute to EMMPRIN's function. Current evidence indicates that the N-terminal Ig domain of EMMPRIN is required for stimulation of MMP production.[13] Thus, inhibition of this interaction interferes with MMP production and MMP-dependent invasion of tumor cells.

EMMPRIN expression is increased in tumors such as bladder carcinoma,[14] lung carcinoma,[15] glioma,[16] melanoma,[17] lymphoma[18] and pancreatic cancer,[19,20] and correlates

©2006 LANDES BIOSCIENCE. DO NOT DISTRIBUTE.

with tumor size, stage, and prognosis in primary breast and ovarian cancer.[21,22] In addition, EMMPRIN also plays a role in tumor invasion,[17,23-25] angiogenesis,[24,26] apoptosis[27] and chemoresistance,[28] as well as in nonmalignant diseases such as rheumatoid arthritis,[29-31] chronic liver disease,[32,33] heart failure[34,35] and atherosclerosis.[35] In the present study, the expression of EMMPRIN was analyzed in the normal pancreas, chronic pancreatitis (CP), and in various pancreatic tumors, and its functional role was specifically assessed in pancreatic cancer.

MATERIALS AND METHODS

Tissue specimens and cell cultures. Tissue specimens were obtained from patients in whom pancreatic resections were carried out. The patients underwent operation for a range of pancreatic diseases: pancreatic ductal adenocarcinoma (PDAC; n = 52), CP (n = 10), acinar cell carcinoma (n=9), mucinous cystic neoplasm (MCN; n = 8), serous cystic neoplasm (SCN; n = 18), intraductal papillary mucinous adenoma (IPMA; n = 22), intraductal papillary mucinous carcinoma (IPMC; n = 19), benign neuroendocrine tumors (n = 8), and neuroendocrine carcinomas (n = 21). Normal human pancreatic tissue samples (n = 9) were obtained through an organ donor program from previously healthy individuals, when no recipient was available. These tissues were then processed identical to the other pancreatic tissues. The study was approved by the ethics committee of the University of Heidelberg, Germany.

Freshly removed tissues were fixed in paraformaldehyde solution for 12–24 h and then paraffin embedded for histological analysis. In addition, a portion of the tissue samples was preserved in RNAlater (Ambion Europe Ltd., Huntington, Ambridgeshire, UK), or snap-frozen in liquid nitrogen immediately upon surgical removal and maintained at -80°C until use. Pancreatic cancer cell lines were grown in DMEM medium (Panc-1, Aspc-1, BxPc-3, Capan-1, Colo-357, SU86.86, MiaPaCa-2, and T3M4), supplemented with 10% fetal calf serum (FCS) and 100 U/ml penicillin and streptomycin (complete medium), and incubated in a 5% CO_2 humidified atmosphere. Pancreatic stellate cells (PSCs) were cultured in low glucose (1000 mg/l) DMEM supplemented with an equal amount of F12 in the presence of 20% FCS and antibiotics (penicillin and streptomycin). Isolation of primary PSC was made with the outgrowth method, as described by Bachem et al.[36]

Real-time quantitative RT-PCR. All reagents and equipment for mRNA and cDNA preparation were purchased from Roche (Roche Applied Science, Mannheim, Germany). RNA was reverse transcribed into cDNA using the cDNA synthesis kit for reverse transcription polymerase chain reaction (PCR) (AMV) according to the manufacturer's instructions. Real-time quantitative PCR (QRT-PCR) was performed with the Light Cycler Fast Start DNA SYBR Green kit. The number of specific transcripts was normalized to the levels of two housekeeping genes (cyclophilin B and hypoxanthine guanine phosphoribosyltransferase). The EMMPRIN forward primer was: 5' TTC AGC CTC TGG GTC TGA GT 3' and the reverse primer: 5' GCC AAG AGG TCA GAG TCG TC 3'.

Immunohistochemistry. Immunohistochemistry was performed using the Dako Envision System (Dako Cytomation GmbH, Hamburg, Germany). Consecutive paraffin-embedded tissue sections (3–5 μm thick) were deparaffinized and rehydrated using routine methods.[37] Antigen retrieval was performed by pretreatment of the slides in citrate buffer (pH 6.0) in a microwave oven for 10 minutes. Endogenous peroxidase activity was quenched by incubation in deionized water containing 3% hydrogen peroxide at room temperature for 10 minutes. After blocking of nonspecific reactivity with diluted normal goat serum, sections were incubated with rabbit anti-human EMMPRIN polyclonal antibodies (10 μg/ml) (Zymed, South San Francisco, CA) at 4°C overnight and then incubated with horseradish peroxidase-linked goat anti-rabbit antibodies, followed by reaction with diaminobenzidine and counterstaining with Mayer's hemotoxylin. In addition, to confirm the specificity of the primary antibodies, tissue sections were incubated in the absence of the primary antibodies and with negative control rabbit IgG. Under these conditions, no specific immunostaining was detected.

ELISA. The sera from patients and controls were collected, processed, and stored in an identical manner to ensure the validity of the results. There was a short time interval between diagnosis of patients and referral for surgery and serum collection (1–2 weeks). Preoperative blood was collected from 44 pancreatic cancer patients (median age 57 years) and 20 chronic pancreatitis patients (median age 49 years) undergoing pancreatic resection. The diagnosis was confirmed in all cases by histopathological examination. The 29 control blood samples were collected from healthy volunteers (median age 31 years). All sera were obtained according to a standardized sampling and coding protocol. Briefly, after sample collection, the 7.5 ml monovettes (Sarstedt, Nümbrecht, Germany) were incubated at 22°C for 30 min and centrifuged at 2,500 × g for 10 min. Serum was collected, aliquoted into 200 μl portions and stored at -80°C until further processing. The study was approved by the ethics committee of the University of Heidelberg and written informed consent was obtained from all individuals from whom serum samples were collected.

For EMMPRIN ELISA,[38] 96-well Nunc Immuno plates (Nunc, Roskilde, Denmark) were coated overnight at 4°C with 50 μl (5 μg/ml) of anti-EMMPRIN antibodies (R&D systems) in PBS (pH 7.0). PBS with 0.05% Tween20 was used as the wash solution. Nonspecific binding sites were blocked with 100 μl of blocking buffer (3% bovine serum albumin in PBS) for 1 h at 37°C. Either recombinant human EMMPRIN (R&D systems, diluted in blocking buffer) or serum (50 μl per well) were added and incubated for 2 h at 37°C. Fifty μl of 1:5000 diluted biotin-conjugated anti-EMMPRIN antibodies (RDI-CD147, Research Diagnostics, Concord, MA) were added into each well and incubated for 2 h at 37°C. Fifty μl of horseradish peroxidase-conjugated streptavidin 1:1,000 diluted in PBS were added to each well and incubated for 45 min at 37°C. After three washes with PBS-T, 50 μl of TMB substrate reagent A (BD Biosciences, San Diego, CA) were added for 10 min at 37°C to quench the nonspecific color reaction. Subsequently, 50 μl of developing buffer (TMB substrate reagent A and reagent B 1:1 mixed) were added to each well. Colorimetric reactions were stopped by adding 50 μl of 2N H_2SO_4, and analyzed by microplate reader at 450 nm and 570 nm for correction. MMP1/2 and VEGF ELISAs were carried out according to the manufacturer's instructions (Quantikine, R&D systems, Wiesbaden-Nordenstadt, Germany).

Immunoblot analysis and densitometry. Cultured pancreatic cancer and primary pancreatic stellate cells were lysed in ice-cold buffer containing 20 mM Tris-HCl (pH = 7.4),150 mM NaCl, 1% TritonX, 2.5 mM sodium pyrophosphate and 1 tablet EDTA-free protease inhibitor cocktail (Roche, Germany) for 30 minutes.

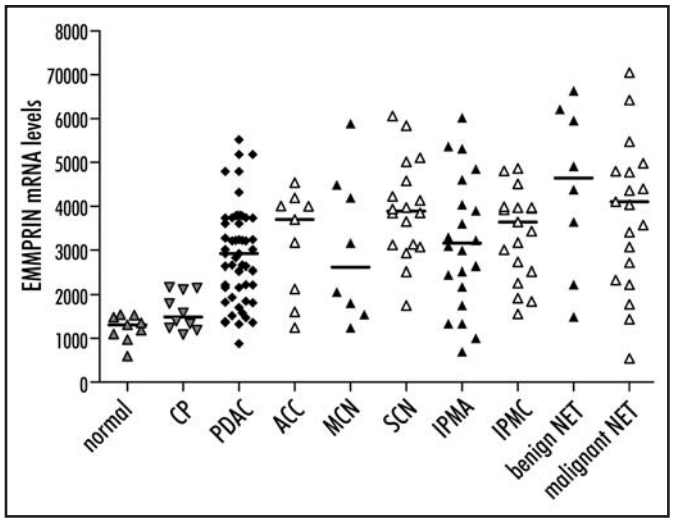

Figure 1. EMMPRIN mRNA expression in pancreatic tissues. Real-time quantitative RT-PCR analysis of EMMPRIN mRNA levels in various pancreatic tumors, chronic pancreatitis tissues, and normal tissues was carried out as described in the Material and Methods section. RNA input was normalized to the average expression of the two housekeeping genes HPRT and cyclophilin B, and is presented as copy number/µl cDNA. Horizontal lines represent the median. CP, chronic pancreatitis; PDAC, pancreatic ductal adenocarcinoma; ACC, acinar cell carcinoma; MCN, mucinous cystic neoplasm; SCN, serous cystic neoplasm; IPMA, intraductal papillary mucinous adenoma; IPMC, intraductal papillary mucinous carcinoma; benign NET, benign neuroendocrine tumor; malignant NET: malignant neuroendocrine tumor.

Cell lysates were then collected after centrifugation at 1,350 rpm for 10 minutes at 4°C. 12.6 µg of the total protein were loaded on 10% polyacrylamide gels and transferred to PVDF membranes. Membranes were blocked in 20 ml T-TBS, 5% skim milk and 0.05% Tween-20 for 1h and incubated with rabbit anti-EMMPRIN polyclonal antibodies (1:100) (Zymed, South San Francisco, CA) or anti-erk2 (1:2500) (Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4°C. Membranes were washed 3 times with 0.05% Tween-20-TBS and incubated with horseradish peroxidase (HRP)-conjugated anti-rabbit antibody (1:2500) for 1h at room temperature. Signals were detected using the enhanced chemiluminescence system (ECL, Amersham Life Science Ltd., Bucks, UK). Films were scanned (ScanoScan 9900F), and densitometric analyses was carried out using the ImageJ program (NIH; http://rsb.info.nih.gov/ij/).

Deglycosylation. For N-glycosidase F (Deglycosylation Kit, Roche, Germany), 5 µl of total cell lysates (20 µg) and 5 µl of reduced denaturation buffer were mixed and incubated for 3 min at 95°C. Then, 10 µl of reaction buffer and 10 µl of reconstituted N-glycosidase F were added and incubated for 1 hour at 37°C. For Endoglycosidase H (Roche, Germany), 5 µl of total cell lysates (20 µg), 1 µl of protease inhibitor and 2 µl (10 mU) of Endoglycosidase H were mixed and incubated overnight at 37°C in a water bath.

siRNA transfections. Synthetic siRNA oligonucleotides for EMMPRIN were purchased from Qiagen (Hilden, Germany) and prepared and stored according to the manufacturer's instructions. Human EMMPRIN RNAi (sense: GGU CAG AGC UAC ACA UUG A; antisense: UCA AUG UGU AGC UCU GAC C) were used. The negative control siRNA (Qiagen) had the target sequence of AAT TCT CCG AAC GTG TCA CGT.

Cells were grown until 50–80% confluence. siRNA transfections were carried out according to the manufacturer's instructions. HiPerFect (Qiagen, Hilden, Germany) transfection reagent was used. The final concentration of both the control and specific oligonucleotides was 5 nM. The efficacy of the siRNA transfection was ascertained by immunoblot analysis after 48 h of transfection.

Invasion assay. To assess cell migration in vitro, transwell migration chambers with 8-µm pore size (BD Biosciences) were used and reconstituted with 600 µl serum-free DMEM medium in both top and bottom chambers for 2–4 h. Cells were trypsinized and seeded in the top chamber at a density of 2.5 x 10^4 cells per well in 600 µl DMEM containing 10% fetal calf serum. After incubation at 37°C for 20 h, cells remaining attached to the upper surface of the filters were carefully removed with cotton swabs, while cells that reached the underside of the chamber were stained with H&E and counted under a microscope in five random fields at a magnification of 200×.

Anchorage-dependent growth assay. Cell growth was determined using the 3-(4,5-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimeric growth assay. Cells were seeded at a density of 2000 cells/well in 96-well plates for up to five days. Each day cell growth was determined by adding MTT solution (50 µg/well) for 4 h. Then, the solution was removed by aspiration and the crystals were solubilized in acidic isopropanol (100 µl/well). The optical density was measured at 570 nm. All assays were performed in triplicate, and repeated three times.

Soft agar assay. Four **x 10^3** cells were suspended in 0.35% low-melting agarose (Hoefer Scientific Instruments, San Francisco, CA), dissolved in 1.5 ml of complete medium, and plated on the top of 1.5 ml of 0.6% agarose in the same medium in 12-well culture plates. Every five days, 0.5 ml of complete medium containing 0.35% agarose were added. After two weeks of incubation, colonies were visualized by MTT staining (300 µg/well) for 24 hours. All assays were performed in triplicate.

Anoikis assay. Cells were seeded at a density of 1 x 10^5 cells per well in 12-well plates coated with 2 ml of a 20 mg/ml solution of polyhydroxyethylmethacrylate.[27,39] After incubation for three days under standard culture conditions, the cell suspension was collected and centrifuged at 1,350 rpm for 10 min and dissolved in 1:4 diluted binding buffer (1 ml binding buffer and 3 ml distilled water). 5 µl Annexin V-FITC (human AnnexinV-FITC kit, Bender MedSystems, Burlingame, CA) were added, vortexed for a few seconds, and incubated for 10 min at room temperature. Thereafter, apoptotic cells were detected by FACS after adding 10 µl of the 20 µg/ml propidium iodide stock solution (final concentration 1 µg/ml).

Statistical analysis. Data are expressed as the mean ± standard error of the mean (SEM) for in vitro assays and median and individual data points for QRT-PCR and ELISA results unless indicated otherwise. The Mann-Whitney test, and the Kruskal-Wallis test were utilized and groups were compared using Dunn's multiple comparison test. **A p value <0.05 was taken as the level of significance.** The mean difference between groups was estimated with a 95% confidence interval (CI). The receiver operating characteristic curve (ROC) program (ACOMED Statistik, Leipzig, Germany) was used to assess the sensitivity and specificity of serum EMMPRIN levels for differentiation of pancreatic cancer patients from healthy controls and chronic pancreatitis patients. The area under the curve (AUC) was calculated by the trapez-method.[40,41] SE and CI of AUC were

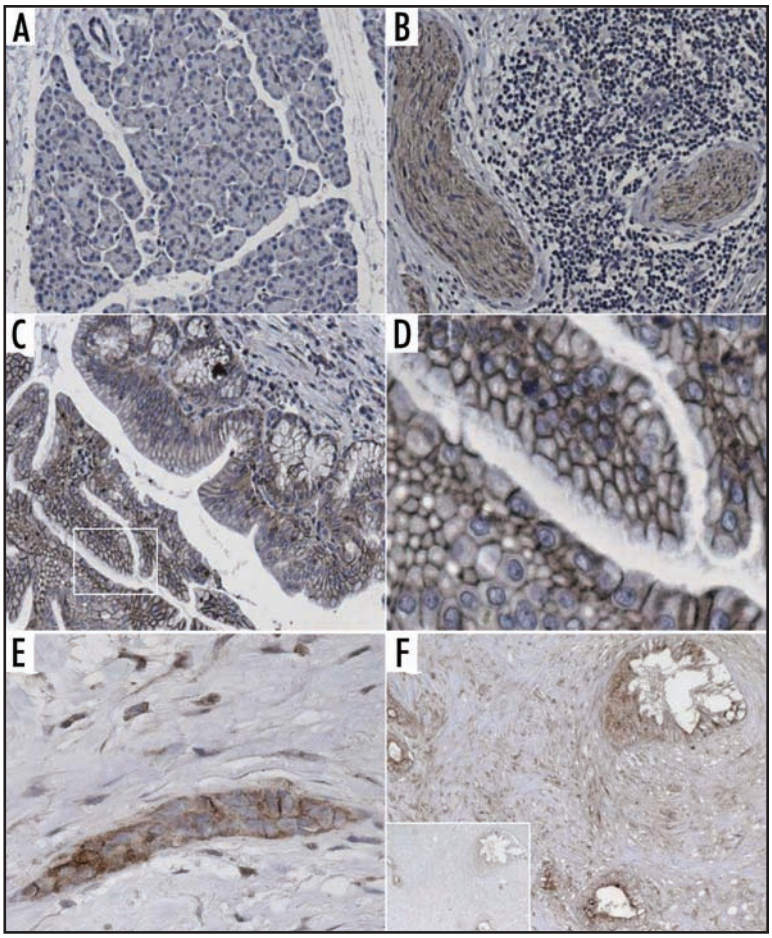

Figure 2. Localization of EMMPRIN in pancreatic tissues. Immunohistochemistry using a specific EMMPRIN antibody was carried out as described in the Material and Methods section. EMMPRIN in the normal pancreas (A), chronic pancreatitis (B; depicting inflammatory cells and nerves) and pancreatic ductal adenocarcinoma (C–F). (D) is a 400× magnification of the focus region in (C), F-insert: negative control using isotype-matched IgG.

calculated according to Hanley et al.[40] The confidence-interval of the curve was calculated by the exact local binomial CI of sensitivity and specificity. It is a conservative estimator for the CI.

RESULTS

EMMPRIN expression and localization in pancreatic tissues. To quantify the mRNA levels of EMMPRIN, QRT-PCR was performed using specific primers for EMMPRIN in different types of pancreatic tissues, including 52 cases of pancreatic ductal adenocarcinoma (PDAC), ten cases of chronic pancreatitis (CP), and nine normal pancreatic tissues. This analysis revealed a 2.2-fold increase of median EMMPRIN mRNA levels in pancreatic cancer tissues (2920 arbitrary units) in comparison with normal tissues (1308 arbitrary units; $p < 0.001$). A large number of other pancreatic tumors, including cystic and neuroendocrine tumors, also displayed increased EMMPRIN mRNA levels, ranging from 2.0-fold to 3.5-fold compared to normal pancreatic tissues ($p < 0.01$) (Fig. 1). Interestingly, EMMPRIN mRNA levels were not significantly different in CP tissues (1486 arbitrary units) compared to the normal pancreas (1308 arbitrary units) (Fig. 1). However, the mean difference between normal and CP tissues was 374 arbitrary units with a 95% confidence interval of 13-735 arbitrary units suggesting that a larger patient cohort would be required to confirm the absence of a significant difference between normal and CP tissues.

Immunostaining was performed next in 20 cases of PDAC, 10 cases of chronic pancreatitis and nine normal pancreatic tissues to determine the localization of EMMPRIN (Fig. 2). EMMPRIN was moderately to weakly present in ductal and acinar cells in chronic pancreatitis and the normal pancreas (Fig. 2A). There was also moderate staining in the nerves in both pancreatic cancer and chronic pancreatitis tissues (Fig. 2B). EMMPRIN was strongly expressed on the membrane and partly in the cytoplasm of pancreatic cancer cells (Fig. 2C and D). In addition, there was strong expression in the stromal cells adjacent to the tumor mass in pancreatic cancer tissues (Fig. 2E) but not in stromal cells in chronic pancreatitis tissues.

EMMPRIN serum levels in patients and healthy controls. To analyze whether increased tissue levels of EMMPRIN would result in increased serum levels, EMMPRIN protein

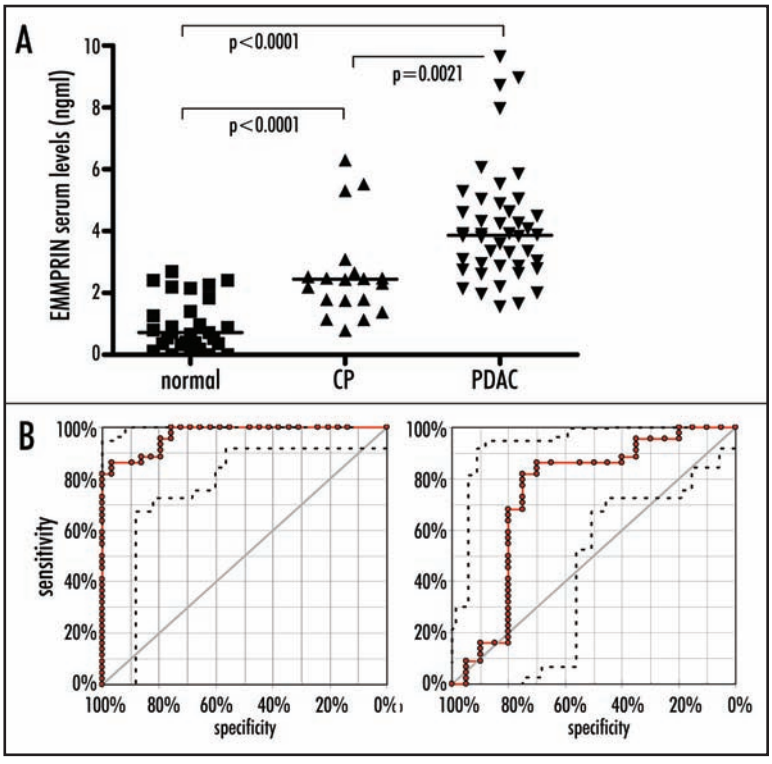

Figure 3. EMMPRIN serum levels in patients and healthy controls. (A) EMMPRIN ELISA was performed as described in Materials and Methods. Soluble EMMPRIN was detected in the sera of pancreatic cancer (n = 44) and chronic pancreatitis (n = 20) patients and healthy volunteers (n = 29). Horizontal lines represent the median values. (B) Specificity and sensitivity of soluble EMMPRIN were analyzed for pancreatic cancer patients vs. normal volunteers (left) and for pancreatic cancer patients vs. chronic pancreatitis patients (right) using ROC analysis (red line). The dashed lines represent the 95% confidence intervals.

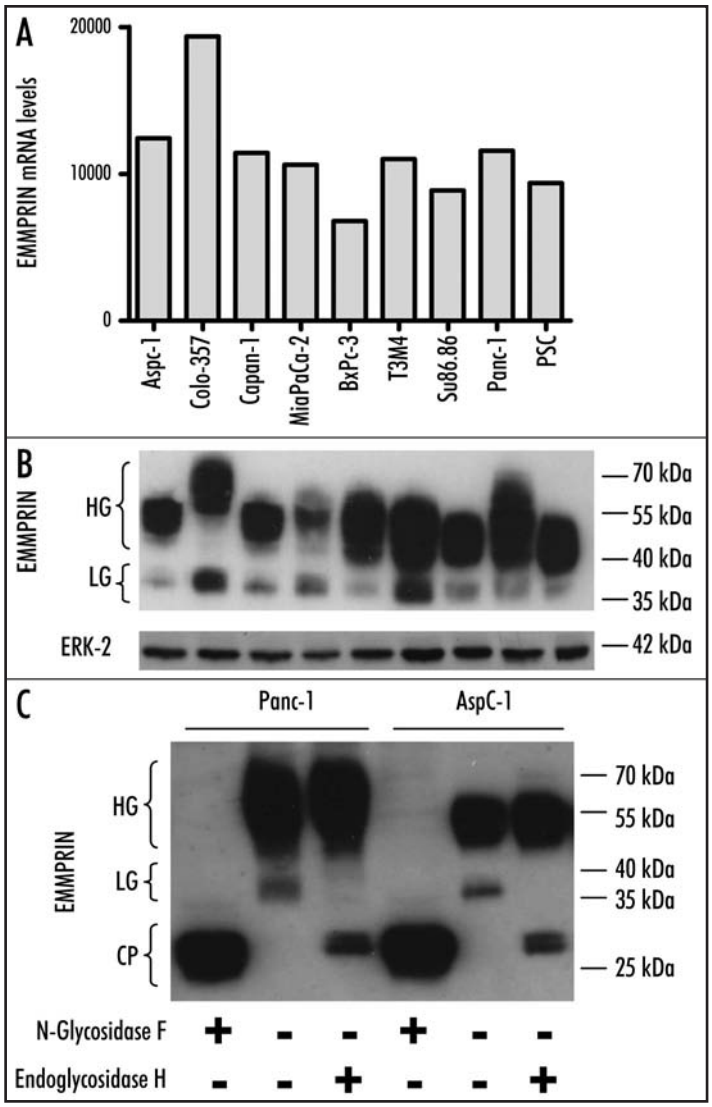

Figure 4. Differential expression and glycosylation patterns of EMMPRIN in pancreatic ductal adenocarcinoma cell lines. (A) Real-time quantitative RT-PCR analysis of EMMPRIN mRNA levels in 8 pancreatic cancer cell lines as well as in PSC, as described in Material and Methods. RNA input was normalized to the average expression of the two housekeeping genes HPRT and cylophilin B, and is presented as copy number/µl cDNA. (B) Immunoblot analysis was carried out to detect expression of EMMPRIN in eight pancreatic cancer cell lines and PSC. Equal loading of the protein samples was confirmed using an ERK-2 antibody. (C) Deglycosylation analysis was performed to confirm the highly glycosylated form and less glycosylated form by using N-glycosidase F and endoglycosidase H in Panc-1 and Aspc-1 cells, as described in Material and Methods. HG (45–70 kDa) is the highly glycosylated EMMPRIN form, LG (35–40 kDa) is the less glycosylated form of EMMPRIN, and CP (20–30 kDa) is the core protein.

concentration was determined by ELISA in the serum of healthy volunteers, chronic pancreatitis and pancreatic cancer patients. As shown in Figure 3A, mean EMMPRIN levels in the serum of healthy volunteers (n = 29) were low (0.95 ± 0.16 ng/ml), but increased 3.1-fold and 4.3-fold in the sera of chronic pancreatitis patients (n = 20) (2.98 ± 0.5 ng/ml; $p < 0.0001$) and pancreatic cancer patients (n = 44) (4.13 ± 0.28 ng/ml; $p < 0.0001$) in accordance with the mRNA data. ROC curve analysis (Fig. 3B) was performed to assess the effectiveness of serum EMMPRIN levels to discriminate between pancreatic cancer patients, chronic pancreatitis patients and healthy volunteers. A cut-off level of 2.7 ng/ml EMMPRIN resulted in a sensitivity of 81.8% to discriminate between pancreatic cancer patients and normal controls, and between cancer and chronic pancreatitis patients. At the same cut-off level, the specificity was 100% in cancer patients versus normal controls, and 75% in cancer versus chronic pancreatitis patients. The AUC for the discrimination of cancer versus control was 0.97 with a SE of 0.019 and a 95% CI of 0.90-0.99. The AUC for the discrimination of cancer versus CP was 0.74 with a SE of 0.062 and a 95% CI of 0.62-0.84. Although there was a difference in median age between the three groups, this difference is unlikely to have contributed to the observed differences in EMMPRIN levels inasmuch as there was no correlation between age and EMMPRIN levels within the cohort (data not shown). There was also no correlation between EMMPRIN serum levels and the stage (TNM) of the disease for pancreatic cancer patients. There was no obvious difference of the 3 CP and the four cancer patients that exhibited the highest EMMPRIN serum levels (Fig. 3A). Specifically, the 3 CP patients had no signs of malignancy (initially, and also during follow-up), and the four cancer cases were not more advanced in comparison to the other 40 cases.

Expression of EMMPRIN in pancreatic cancer cell lines. To further investigate the expression of EMMPRIN in pancreatic cancer, we quantified and compared its mRNA levels and protein levels in eight human pancreatic cancer cell lines (Panc-1, Aspc-1, BxPc-3, Capan-1, Colo-357, SU86.86, MiaPaCa-2 and T3M4) as well as in primary PSC using QRT-PCR and immunoblot analysis. As shown in Figure 4A, EMMPRIN mRNA was highly expressed in all pancreatic cancer cell lines and PSC.

Cell lysates of all cell lines demonstrated that EMMPRIN existed in pancreatic cancer cell lines and PSC in the highly glycosylated form (HG) migrating at ~75–45 kDa, with significant differences in the amount and size of the mature form in different cell lines. In addition, a less glycosylated (LG) form migrating at ~40–35 kDa could also be observed, the size being almost equal, while the amount was significantly different in different cell lines (Fig. 4B).

Next, to confirm the specificity of the observed EMMPRIN variants, deglycosylation of different parts of the oligosaccharide chains of EMMPRIN using N-glycosidase F or endoglycosidase H was performed. In Panc-1 and Aspc-1 cells lysates, treatment with N-glycosidase F resulted in a shift of both the HG form, with 45–70 kDa, and the LG form, with 35–40 kDa, to a single band of approximately 20–30 kDa representing the core protein. As expected, after treatment with endoglycosidase H, the LG form shifted to a lower band of approximately 20–30 kDa, while the HG form did not change (Fig. 4C). This is in accordance with the mechanism that N-glycosidase F targets and deglycosylates both highly and less glycosylated forms to the nonglycosylated form, whereas endoglycosidase H only targets and deglycosylates the less glycosylated form to the nonglycosylated form.

Effects of EMMPRIN silencing on proliferation and invasion of pancreatic cancer cells. Panc-1 and Aspc-1 pancreatic cancer cell lines, which express different levels of HG EMMPRIN, were chosen for further experiments. Transfection of Panc-1 cells with EMMPRIN siRNA for 24 h, 48 h and 72 h reduced EMMPRIN expression significantly to 30.5%, 16.8% and 12.1%, respectively (by densitometric analysis), compared with control samples (Fig. 5A). Transfection of Aspc-1 and PSC cells with EMMPRIN siRNA for the indicated times significantly reduced EMMPRIN expression compared with the control group as well. The efficacy of silencing is summarized in Table 1. To investigate the effects of EMMPRIN

on invasion, Panc-1 and Aspc-1 cells were treated with EMMPRIN siRNA for 48h. Although there was no significant change in Panc-1 invasiveness, a 63% inhibitory effect was observed in Aspc-1 cells (Fig. 5B).

The effect of EMMPRIN silencing on the growth of Panc-1 and Aspc-1 cells was assessed in order to exclude the possibility of growth inhibition appearing as reduction of invasiveness. Although there was a slight reduction of cell growth in both cell lines at longer time points, there was no difference at 48 hours (Fig. 5C). Anchorage-independent growth using soft agar assay also did not reveal any significant differences in the colony formation capacity between these two groups (Fig. 6A). Since Aspc-1 cells do not grow in soft agar, we performed an anoikis assay using Annexin V-FITC labeling for FACS analysis to assess apoptosis. This analysis revealed that silencing of EMMPRIN did not alter apoptosis of Aspc-1 cells (Figs 6B and C).

Table 1 **Densitometric analysis of EMMPRIN levels in Panc-1, Aspc-1 and PSC following siRNA silencing**

	Total (HG + LG)	HG	LG
Panc-1	17.4 ± 0.4%	22.9 ± 3.4%	19.0 ± 6.3%
Aspc-1	31.8 ± 1.2%	32.8 ± 12.9%	1.8 ± 1.3%
PSC	43.8 ± 6.6%	46.6 ± 7.5%	18.1 ± 9.3%

Indicated cells were subjected to immunoblot analysis after transfection with control RNAi and EMMPRIN RNAi for 48 hours. Films were scanned, and densitometric analysis was carried out as described in the Methods section. Data are presented as mean percent ± SEM optical density of EMMRPIN RNAi transfected versus control RNAi transfected cells of two independent experiments. HG is the highly glycosylated EMMPRIN form; LG is the less glycosylated form of EMMPRIN.

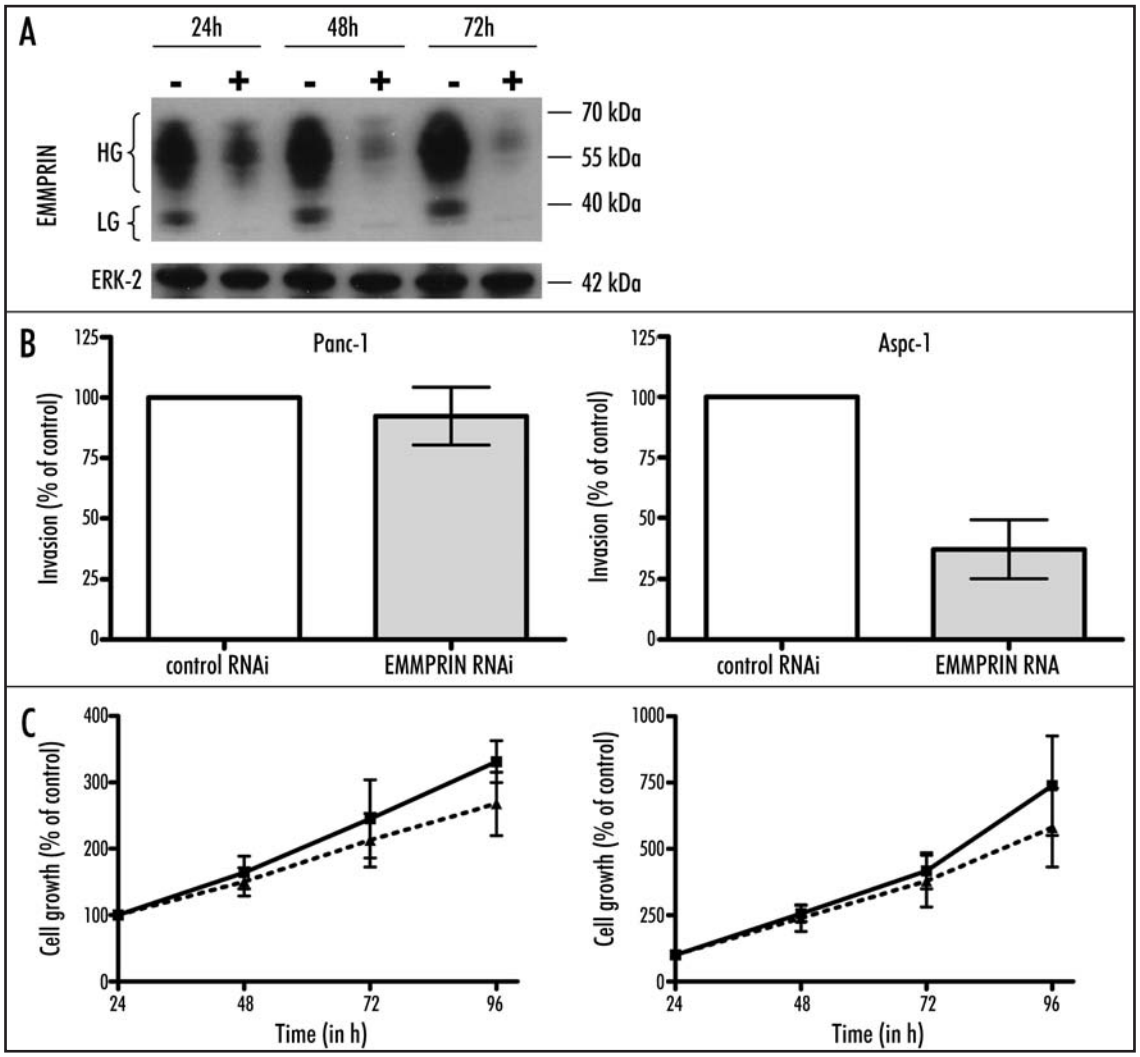

Figure 5. Silencing of EMMPRIN expression by siRNA and its effects on invasion and anchorage-dependent growth in Panc-1 and Aspc-1 cells in vitro. (A) Expression of EMMPRIN after transfection with EMMPRIN siRNA (+) and siRNA control (-) at different time points in Panc-1 cells. Equal loading of the protein samples was confirmed using an ERK-2 antibody. (B) An in vitro cell invasion assay was performed to evaluate the effect of EMMPRIN silencing on invasiveness in Panc-1 and Aspc-1 cell lines. Cells were transfected with EMMPRIN siRNA or control siRNA for 48 h as described. The values shown are the mean ± SEM obtained from three independent experiments. (C) Anchorage-dependent growth was tested in Panc-1 and Aspc-1 cell lines after transfection with EMMPRIN siRNA (dash line) or control siRNA (straight line) as described in Materials and Methods. Data are expressed as mean ± SEM obtained from three independent experiments.

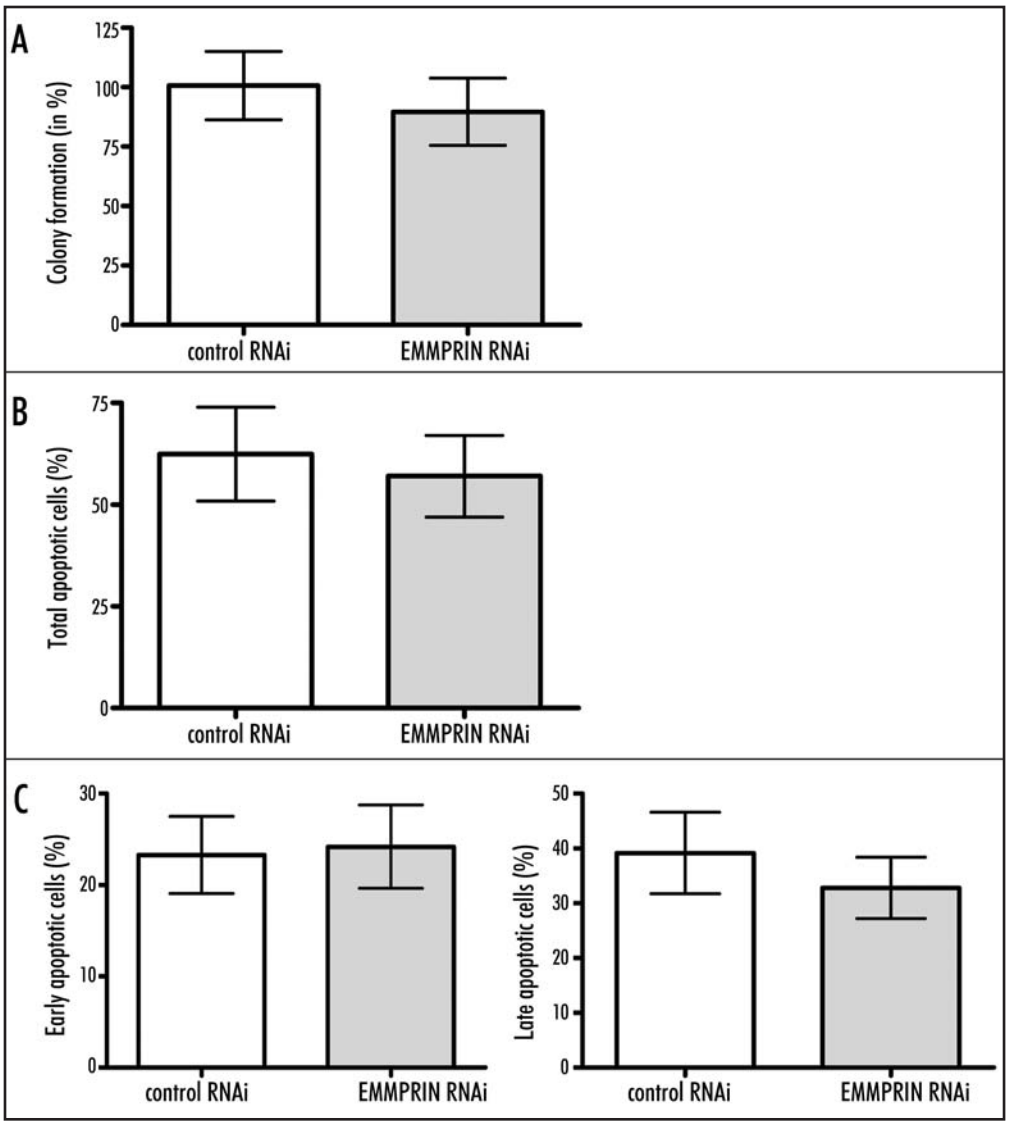

Figure 6. Effect of EMMPRIN silencing by siRNA on anchorage-independent growth in Panc-1 and Aspc-1 cells. (A) Anchorage-independent cell growth for individual clones was measured in Panc-1 cells by soft agar assay as described in Material and Methods. Data are presented for control and EMMPRIN siRNA transfected as indicated. Data are presented as mean ± SEM obtained from three independent experiments. (B and C) Anchorage-independent cell growth was measured in Aspc-1 by anoikis assay as described in Material and Methods. Total apoptotic cells, early apoptotic cells and late apoptotic cells were detected using FACS and Annexin V-FITC labeling. Data are presented as mean ± SEM obtained from three independent experiments.

Effects of EMMPRIN on VEGF, MMP1 and MMP2 production. In the next set of experiments we analyzed the effects of EMMPRIN silencing in pancreatic cancer and stellate cells on VEGF, MMP1, and MMP2 secretion. Both Aspc-1 and PSC can produce VEGF. Interestingly, after coculture, VEGF production increased compared to either cell line alone (calculated as VEGF levels normalized to the cell number; Fig. 7A). Although silencing of EMMPRIN expression in PSC resulted in slightly reduced VEGF secretion in coculture experiments, silencing EMMPRIN in Aspc-1 had no effect on VEGF production (Fig. 8A). Aspc-1 cells did not produce MMP-1 at detectable levels. In contrast, PSC cells released significant amounts of MMP-1 into the cell culture supernatant (Fig. 7B). Interestingly, coculture of Aspc-1 and PSC resulted in suppression of MMP-1 production. EMMPRIN silencing in PSC cells but not Aspc-1 cells led to marked up-regulation of MMP-1 (Fig. 8B). Aspc-1 cells also did not produce MMP2 at detectable levels (Fig. 7C). As with MMP1, PSC cells released significant amounts of MMP-2 into the supernatant, and this was not inhibited by coculturing. In contrast to MMP1, EMMPRIN silencing in PSC but not in Aspc-1 cells resulted in a slight suppression of MMP2 levels (Fig. 8C).

DISCUSSION

In the present study we demonstrated increased expression of EMMPRIN in human pancreatic cancer tissues, mainly localized on the membranes of pancreatic cancer cells and in tumor-associated fibroblasts. However, increased expression of EMMPRIN is not specific for pancreatic cancer, since it has also been observed in a number of other human tumors, such as breast cancer,[42] glioma[43] and laryngeal cancer.[44]

As is hinted by its name, one can expect EMMPRIN (extracellular matrix metalloproteinase inducer) expression to be proportional to the amount of ECM, as it is involved in its turn-over. Although CP tissues exhibit a strong desmoplastic reaction which is comparable to that of PDAC tissues, EMMPRIN levels were not significantly increased compared to normal pancreatic tissues. In contrast, all examined pancreatic neoplasms (serous cystic neoplasm, mucinous cystic neoplasm, intraductal papillary mucinous neoplasm, neuroendocrine tumors, and acinar cell carcinoma), regardless of whether they were desmoplastic, displayed significantly increased EMMPRIN expression. These findings suggest that increased EMMPRIN levels are more specific for neoplastic (versus inflammatory) changes in the pancreas, but not directly related to an activated stroma.

Moreover, in accordance with our QRT-PCR and immunohistochemistry data, EMMPRIN serum levels clearly distinguished pancreatic cancer patients from healthy volunteers. However, this specificity decreased when pancreatic cancer patients were compared to chronic pancreatitis patients. Nevertheless, our findings suggest that EMMPRIN could have a potential role as a diagnostic and/or as a follow-up marker for pancreatic cancer, although large patient cohorts are required to validate this point.

Besides its ability to stimulate MMP production, the second best characterized function of EMMPRIN is adhesion.[45] EMMPRIN had a moderate effect on cancer cell invasion in one of the two investigated pancreatic cancer cell lines. Similar effects have been

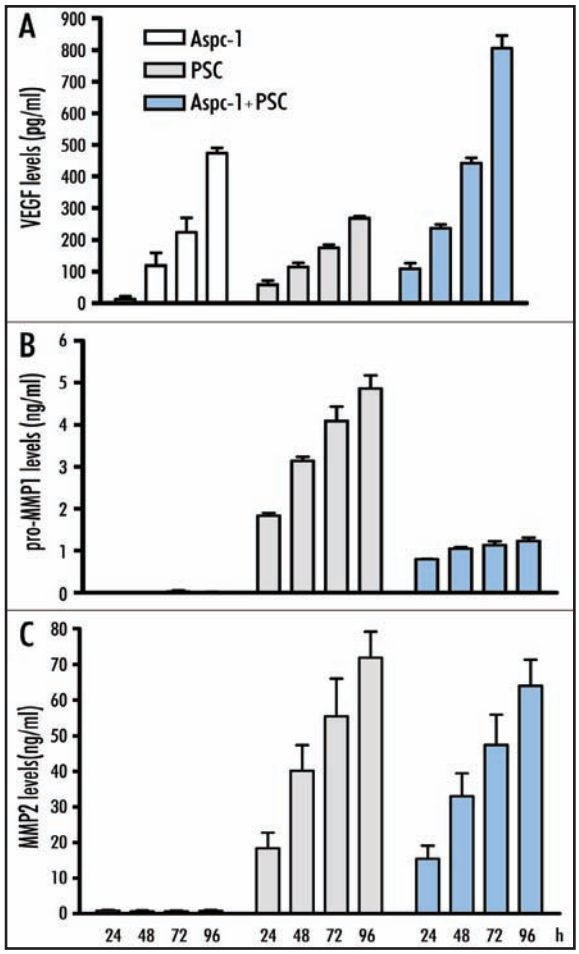

Figure 7. Detection of VEGF, pro-MMP1 and MMP2 production in single-cultured or cocultured Aspc-1 and PSC cells. After the indicated time, supernatants were collected and submitted to VEGF (A), pro-MMP1 (B) or MMP2 (C) ELISA. Data are presented as mean ± SEM obtained from three independent experiments. The amount of secreted protein was normalized to 10^5 cells.

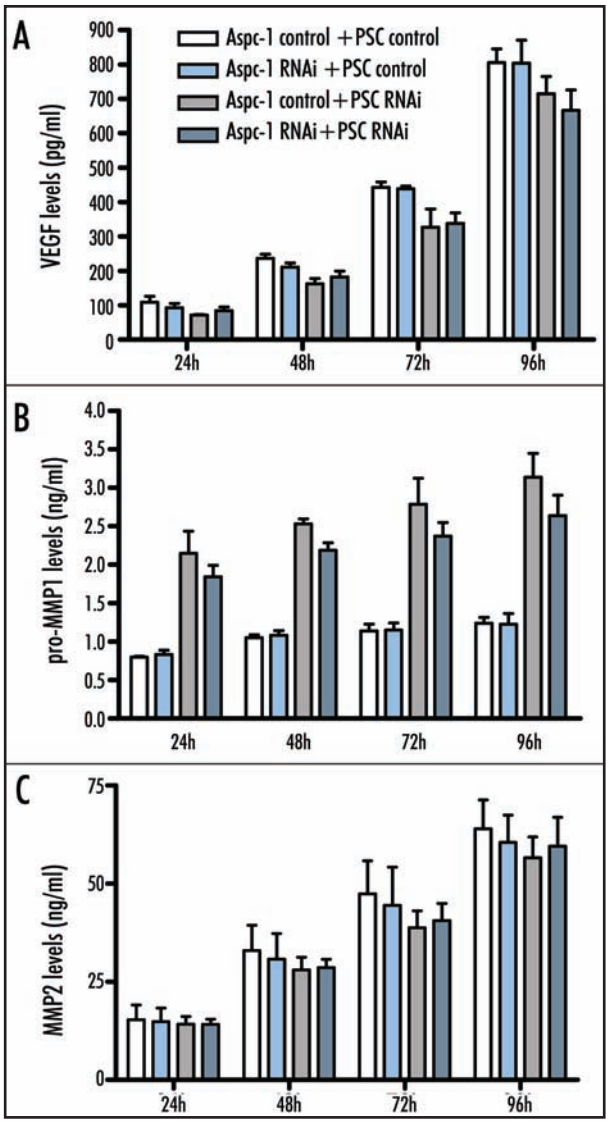

Figure 8. Effect of EMMPRIN silencing on VEGF, pro-MMP1 and MMP2 production in cocultured PSC and Aspc-1 cells. Aspc-1 cells transfected with either control siRNA or EMMPRIN siRNA were cocultured with PSC (also transfected with either control siRNA or EMMPRIN siRNA). After the indicated time, the supernatants were collected and submitted to VEGF (A), pro-MMP1 (B) or MMP2 (C) ELISA. Data are presented as mean ± SEM obtained from three independent experiments. The amount of secreted protein was normalized to 10^5 cells.

observed in prostate,[23] ovarian[46] and other cancer cells.[17,24,25] Thus, EMMPRIN might have direct effects on the invasion of human pancreatic cancer cells, although more cell lines have to be analyzed to confirm this assumption. The effects of adhesion on cell growth are also a well known phenomenon. In the present study, silencing of endogenous EMMPRIN did not significantly affect pancreatic cancer cell growth. These findings are in agreement with other studies that could not find a direct effect of EMMPRIN on proliferation of cells.[47] In contrast, it has recently been shown that EMMPRIN confers resistance to anoikis in breast cancer cells.[27] However, silencing of EMMPRIN in pancreatic cancer cells did also not affect anchorage-independent growth or anoikis.

In addition to having direct effects on cancer cell growth, EMMPRIN also has the potential to indirectly stimulate tumor growth.[47] One of the main determinants of tumor growth is angiogenesis, which is a function of stromal tissue around the cancer cells.[48] It is widely accepted that the EMMPRIN-MMP-VEGF system plays a very important role in tumor progression as a result of stroma-cancer interactions. For example, in breast cancer, increased EMMPRIN expression results in immediate stimulation of VEGF and MMP production in tumor and stromal cells, and tumor-derived MMP can increase soluble VEGF or release biologically active angiogenic growth factors from matrix-bound complexes to modulate VEGF secretion in an MMP-dependent fashion in vivo.[26,49-51] Recent findings also illustrate that EMMPRIN stimulates fibroblast-mediated tumor growth in head and neck squamous cell carcinoma in vivo,[52] and revealed that fibroblasts have a more profound influence on the development and progression of carcinomas.

In the present study, we tried to mimic the tumor-stroma interaction in pancreatic cancer in vitro by coculturing PSC with pancreatic cancer cells. In accordance with the literature, VEGF production increased in coculture of PSC with cancer cells. To provide insight into pancreatic cancer-stellate cell interactions we repeated coculture experiments with various combinations of EMMPRIN silencing. Silencing of EMMPRIN expression in PSC but not in pancreatic cancer cells resulted in reduced VEGF secretion, slightly decreased

MMP2 production, and a marked up-regulation of pro-MMP-1 in coculture experiments. This is in general agreement with studies finding that EMMPRIN in fibroblasts can modulate MMP/VEGF expression.[26] Interestingly, we could show that the effects were predominantly exerted by the stellate cells (fibroblasts) and not by cancer cell-derived EMMPRIN. This is in line with a number of reports regarding the effects of fibroblast-derived EMMPRIN.[53]

Nevertheless, although EMMPRIN's name reflects its ability to induce MMPs, its role in the modulation of various MMPs should not be oversimplified to an on/off function. EMMPRIN silencing had paradoxical effects on MMP-1 (collagenase) and MMP-2 (gelatinase) expression. Interestingly, collagenases are the MMPs that can effectively degrade the fibrillary collagens (type-I, II, III), which are the main components of the ECM.[54] Cleavage by these enzymes makes the collagen molecules unwind to form gelatin, after which they can be degraded by the gelatinases.[55] However, the basement membrane, which is mostly composed of collagen IV and laminin, is a substrate for gelatinases.[56] Therefore, the effect of EMMPRIN may even vary during the course of tumor progression, as breaching of the basement membrane takes place as an early event. This step brings tumor cells into direct contact with fibrillary collagens, which are known to support tumor growth and increase resistance to therapy.[57]

Our observation that EMMRIN has different effects on MMP1 and MMP2 production has not been previously observed in other cell lines. This discrepant observation may be due to the fact that MMPs are regulated at multiple levels, including transcription, activation of the zymogen forms, extracellular inhibitors, location inside or outside the cell, and internalization by endocytosis. Moreover, there are several mechanisms that may inactivate MMPs, such as TIMPS, RECK and alpha2-macroglobulin.[55] Thus, further research is required to elucidate these mechanisms.

Differential modification of EMMPRIN through glycosylation may be cell-type-specific or associated with the malignancy of cells. Variable highly and less glycosylated forms of EMMPRIN were detected in different pancreatic cancer cell lines as well as in PSC. The functional consequences of this different glycosylation pattern in pancreatic cancer cells are not clear. In our experiments, unlike in breast cancer cells,[58] there was no correlation of the glycosylation pattern and the basal invasiveness of pancreatic cancer cells. It is clear, however, that the HG form of EMMPRIN is the form that induces MMP production in fibroblasts and breast cancer cells.[1,13] All cultured pancreatic cancer cells, as well as primary pancreatic stellate cells, expressed the active HG form of EMMPRIN in our study.

In conclusion, EMMPRIN is highly expressed in pancreatic cancer cells as well as in PSC. EMMPRIN does not directly influence pancreatic cancer cell growth and has only moderate effects on pancreatic cancer cell invasion. In contrast, PSC-derived EMMPRIN has the potential to modulate VEGF and MMP production, thereby contributing to a more favorable peritumoral microenvironment.

References

1. Sun J, Hemler ME. Regulation of MMP-1 and MMP-2 production through CD147/extracellular matrix metalloproteinase inducer interactions. Cancer Res 2001; 61:2276-81.
2. Guo H, Zucker S, Gordon MK, Toole BP, Biswas C. Stimulation of matrix metalloproteinase production by recombinant extracellular matrix metalloproteinase inducer from transfected Chinese hamster ovary cells. J Biol Chem 1997; 272:24-7.
3. Tang W, Hemler ME. Caveolin-1 regulates matrix metalloproteinases-1 induction and CD147/EMMPRIN cell surface clustering. J Biol Chem 2004; 279:11112-8.
4. Tang W, Chang SB, Hemler ME. Links between CD147 function, glycosylation, and caveolin-1. Mol Biol Cell 2004; 15:4043-50.
5. Yu XL, Jiang JL, Li L, Feng Q, Xu J, Chen ZN. The glycosylation characteristic of hepatoma-associated antigen HAb18G/CD147 in human hepatoma cells. Int J Biochem Cell Biol 2006; 38:1939-45.
6. Kataoka H, DeCastro R, Zucker S, Biswas C. Tumor cell-derived collagenase-stimulatory factor increases expression of interstitial collagenase, stromelysin, and 72-kDa gelatinase. Cancer Res 1993; 53:3154-8.
7. Biswas C, Zhang Y, DeCastro R, Guo H, Nakamura T, Kataoka H, Nabeshima K. The human tumor cell-derived collagenase stimulatory factor (renamed EMMPRIN) is a member of the immunoglobulin superfamily. Cancer Res 1995; 55:434-9.
8. Yoshida S, Shibata M, Yamamoto S, Hagihara M, Asai N, Takahashi M, Mizutani S, Muramatsu T, Kadomatsu K. Homo-oligomer formation by basigin, an immunoglobulin superfamily member, via its N-terminal immunoglobulin domain. Eur J Biochem 2000; 267:4372-80.
9. Kasinrerk W, Fiebiger E, Stefanova I, Baumruker T, Knapp W, Stockinger H. Human leukocyte activation antigen M6, a member of the Ig superfamily, is the species homologue of rat OX-47, mouse basigin, and chicken HT7 molecule. J Immunol 1992; 149:847-54.
10. Jiang JL, Chan HC, Zhou Q, Yu MK, Yao XY, Lam SY, Zhu H, Ho LS, Leung KM, Chen ZN. HAb18G/CD147-mediated calcium mobilization and hepatoma metastasis require both C-terminal and N-terminal domains. Cell Mol Life Sci 2004; 61:2083-91.
11. Yurchenko V, Pushkarsky T, Li JH, Dai WW, Sherry B, Bukrinsky M. Regulation of CD147 cell surface expression: Involvement of the proline residue in the CD147 transmembrane domain. J Biol Chem 2005; 280:17013-9.
12. Chiampanichayakul S, Peng-in P, Khunkaewla P, Stockinger H, Kasinrerk W. CD147 contains different bioactive epitopes involving the regulation of cell adhesion and lymphocyte activation. Immunobiology 2006; 211:167-78.
13. Intasai N, Mai S, Kasinrerk W, Tayapiwatana C. Binding of multivalent CD147 phage induces apoptosis of U937 cells. Int Immunol 2006; 18:1159-69.
14. Muraoka K, Nabeshima K, Murayama T, Biswas C, Koono M. Enhanced expression of a tumor-cell-derived collagenase-stimulatory factor in urothelial carcinoma: Its usefulness as a tumor marker for bladder cancers. Int J Cancer 1993; 55:19-26.
15. Polette M, Gilles C, Marchand V, Lorenzato M, Toole B, Tournier JM, Zucker S, Birembaut P. Tumor collagenase stimulatory factor (TCSF) expression and localization in human lung and breast cancers. J Histochem Cytochem 1997; 45:703-9.
16. Sameshima T, Nabeshima K, Toole BP, Yokogami K, Okada Y, Goya T, Koono M, Wakisaka S. Expression of emmprin (CD147), a cell surface inducer of matrix metalloproteinases, in normal human brain and gliomas. Int J Cancer 2000; 88:21-7.
17. Kanekura T, Chen X, Kanzaki T. Basigin (CD147) is expressed on melanoma cells and induces tumor cell invasion by stimulating production of matrix metalloproteinases by fibroblasts. Int J Cancer 2002; 99:520-8.
18. Nabeshima K, Suzumiya J, Nagano M, Ohshima K, Toole BP, Tamura K, Iwasaki H, Kikuchi M. Emmprin, a cell surface inducer of matrix metalloproteinases (MMPs), is expressed in T-cell lymphomas. J Pathol 2004; 202:341-51.
19. Li M, Wang H, Li F, Fisher WE, Chen C, Yao Q. Effect of cyclophilin A on gene expression in human pancreatic cancer cells. Am J Surg 2005; 190:739-45.
20. Li M, Zhai Q, Bharadwaj U, Wang H, Li F, Fisher WE, Chen C, Yao Q. Cyclophilin A is overexpressed in human pancreatic cancer cells and stimulates cell proliferation through CD147. Cancer 2006; 106:2284-94.
21. Reimers N, Zafrakas K, Assmann V, Egen C, Riethdorf L, Riethdorf S, Berger J, Ebel S, Janicke F, Sauter G, Pantel K. Expression of extracellular matrix metalloproteases inducer on micrometastatic and primary mammary carcinoma cells. Clin Cancer Res 2004; 10:3422-8.
22. Davidson B, Goldberg I, Berner A, Kristensen GB, Reich R. EMMPRIN (extracellular matrix metalloproteinase inducer) is a novel marker of poor outcome in serous ovarian carcinoma. Clin Exp Metastasis 2003; 20:161-9.
23. Wang L, Wu G, Yu L, Yuan J, Fang F, Zhai Z, Wang F, Wang H. Inhibition of CD147 Expression reduces tumor cell invasion in human prostate cancer cell line via RNA interference. Cancer Biol Ther 2006; 5:608-14.
24. Liang Q, Xiong H, Gao G, Xiong K, Wang X, Zhao Z, Zhang H, Li Y. Inhibition of basigin expression in glioblastoma cell line via antisense RNA reduces tumor cell invasion and angiogenesis. Cancer Biol Ther 2005; 4:759-62.
25. Li Y, Shang P, Qian AR, Wang L, Yang Y, Chen ZN. Inhibitory effects of antisense RNA of HAb18G/CD147 on invasion of hepatocellular carcinoma cells in vitro. World J Gastroenterol 2003; 9:2174-7.
26. Tang Y, Nakada MT, Kesavan P, McCabe F, Millar H, Rafferty P, Bugelski P, Yan L. Extracellular matrix metalloproteinase inducer stimulates tumor angiogenesis by elevating vascular endothelial cell growth factor and matrix metalloproteinases. Cancer Res 2005; 65:3193-9.
27. Yang JM, O'Neill P, Jin W, Foty R, Medina DJ, Xu Z, Lomas M, Arndt GM, Tang Y, Nakada M, Yan L, Hait WN. Extracellular matrix metalloproteinase inducer (CD147) confers resistance of breast cancer cells to Anoikis through inhibition of Bim. J Biol Chem 2006; 281:9719-27.
28. Yang JM, Xu Z, Wu H, Zhu H, Wu X, Hait WN. Overexpression of extracellular matrix metalloproteinase inducer in multidrug resistant cancer cells. Mol Cancer Res 2003; 1:420-7.
29. Tomita T, Nakase T, Kaneko M, Shi K, Takahi K, Ochi T, Yoshikawa H. Expression of extracellular matrix metalloproteinase inducer and enhancement of the production of matrix metalloproteinases in rheumatoid arthritis. Arthritis Rheum 2002; 46:373-8.
30. Zhu P, Ding J, Zhou J, Dong WJ, Fan CM, Chen ZN. Expression of CD147 on monocytes/macrophages in rheumatoid arthritis: Its potential role in monocyte accumulation and matrix metalloproteinase production. Arthritis Res Ther 2005; 7:R1023-33.
31. Zhu P, Lu N, Shi ZG, Zhou J, Wu ZB, Yang Y, Ding J, Chen ZN. CD147 overexpression on synoviocytes in rheumatoid arthritis enhances matrix metalloproteinase production and invasiveness of synoviocytes. Arthritis Res Ther 2006; 8:R44.

32. Pushkarsky T, Zybarth G, Dubrovsky L, Yurchenko V, Tang H, Guo H, Toole B, Sherry B, Bukrinsky M. CD147 facilitates HIV-1 infection by interacting with virus-associated cyclophilin A. Proc Natl Acad Sci USA 2001; 98:6360-5.
33. Wu E, Trauger SA, Pache L, Mullen TM, von Seggern DJ, Siuzdak G, Nemerow GR. Membrane cofactor protein is a receptor for adenoviruses associated with epidemic keratoconjunctivitis. J Virol 2004; 78:3897-905.
34. Yoon YW, Kwon HM, Hwang KC, Choi EY, Hong BK, Kim D, Kim HS, Cho SH, Song KS, Sangiorgi G. Upstream regulation of matrix metalloproteinase by EMMPRIN; extracellular matrix metalloproteinase inducer in advanced atherosclerotic plaque. Atherosclerosis 2005; 180:37-44.
35. Spinale FG, Coker ML, Heung LJ, Bond BR, Gunasinghe HR, Etoh T, Goldberg AT, Zellner JL, Crumbley AJ. A matrix metalloproteinase induction/activation system exists in the human left ventricular myocardium and is upregulated in heart failure. Circulation 2000; 102:1944-9.
36. Bachem MG, Schneider E, Gross H, Weidenbach H, Schmid RM, Menke A, Siech M, Beger H, Grunert A, Adler G. Identification, culture, and characterization of pancreatic stellate cells in rats and humans. Gastroenterology 1998; 115:421-32.
37. Kayed H, Kleeff J, Kolb A, Ketterer K, Keleg S, Felix K, Giese T, Penzel R, Zentgraf H, Buchler MW, Korc M, Friess H. FXYD3 is overexpressed in pancreatic ductal adenocarcinoma and influences pancreatic cancer cell growth. Int J Cancer 2006; 118:43-54.
38. Tang Y, Kesavan P, Nakada MT, Yan L. Tumor-stroma interaction: Positive feedback regulation of extracellular matrix metalloproteinase inducer (EMMPRIN) expression and matrix metalloproteinase-dependent generation of soluble EMMPRIN. Mol Cancer Res 2004; 2:73-80.
39. Frisch SM, Francis H. Disruption of epithelial cell-matrix interactions induces apoptosis. J Cell Biol 1994; 124:619-26.
40. Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. Radiology 1982; 143:29-36.
41. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: A nonparametric approach. Biometrics 1988; 44:837-45.
42. Caudroy S, Polette M, Tournier JM, Burlet H, Toole B, Zucker S, Birembaut P. Expression of the extracellular matrix metalloproteinase inducer (EMMPRIN) and the matrix metalloproteinase-2 in bronchopulmonary and breast lesions. J Histochem Cytochem 1999; 47:1575-80.
43. Sameshima T, Nabeshima K, Toole BP, Yokogami K, Okada Y, Goya T, Koono M, Wakisaka S. Glioma cell extracellular matrix metalloproteinase inducer (EMMPRIN) (CD147) stimulates production of membrane-type matrix metalloproteinases and activated gelatinase A in cocultures with brain-derived fibroblasts. Cancer Lett 2000; 157:177-84.
44. Suzuki S, Sato M, Senoo H, Ishikawa K. Direct cell-cell interaction enhances pro-MMP-2 production and activation in coculture of laryngeal cancer cells and fibroblasts: Involvement of EMMPRIN and MT1-MMP. Exp Cell Res 2004; 293:259-66.
45. Yurchenko V, Constant S, Bukrinsky M. Dealing with the family: CD147 interactions with cyclophilins. Immunology 2006; 117:301-9.
46. Zou W, Yang H, Hou X, Zhang W, Chen B, Xin X. Inhibition of *CD147* gene expression via RNA interference reduces tumor cell invasion, tumorigenicity and increases chemosensitivity to paclitaxel in HO-8910pm cells. Cancer Lett 2006.
47. Zucker S, Hymowitz M, Rollo EE, Mann R, Conner CE, Cao J, Foda HD, Tompkins DC, Toole BP. Tumorigenic potential of extracellular matrix metalloproteinase inducer. Am J Pathol 2001; 158:1921-8.
48. Korc M. Role of growth factors in pancreatic cancer. Surg Oncol Clin N Am 1998; 7:25-41.
49. Fang J, Shing Y, Wiederschain D, Yan L, Butterfield C, Jackson G, Harper J, Tamvakopoulos G, Moses MA. Matrix metalloproteinase-2 is required for the switch to the angiogenic phenotype in a tumor model. Proc Natl Acad Sci USA 2000; 97:3884-9.
50. Sounni NE, Roghi C, Chabottaux V, Janssen M, Munaut C, Maquoi E, Galvez BG, Gilles C, Frankenne F, Murphy G, Foidart JM, Noel A. Up-regulation of vascular endothelial growth factor-A by active membrane-type 1 matrix metalloproteinase through activation of Src-tyrosine kinases. J Biol Chem 2004; 279:13564-74.
51. Matrix metalloproteinase-9 triggers the angiogenic switch during Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. Nat Cell Biol 2000; 2:737-44.
52. Rosenthal EL, Vidrine DM, Zhang W. Extracellular matrix metalloprotease inducer stimulates fibroblast-mediated tumor growth in vivo. Laryngoscope 2006; 116:1086-92.
53. Rosenthal EL, Zhang W, Talbert M, Raisch KP, Peters GE. Extracellular matrix metalloprotease inducer-expressing head and neck squamous cell carcinoma cells promote fibroblast-mediated type I collagen degradation in vitro. Mol Cancer Res 2005; 3:195-202.
54. Lovejoy B, Welch AR, Carr S, Luong C, Broka C, Hendricks RT, Campbell JA, Walker KA, Martin R, Van Wart H, Browner MF. Crystal structures of MMP-1 and -13 reveal the structural basis for selectivity of collagenase inhibitors. Nat Struct Biol 1999; 6:217-21.
55. Somerville RP, Oblander SA, Apte SS. Matrix metalloproteinases: Old dogs with new tricks. Genome Biol 2003; 4:216.
56. Han YP. Matrix metalloproteinases, the pros and cons, in liver fibrosis. J Gastroenterol Hepatol 2006; 21(Suppl 3):S88-91.
57. Armstrong T, Packham G, Murphy LB, Bateman AC, Conti JA, Fine DR, Johnson CD, Benyon RC, Iredale JP. Type I collagen promotes the malignant phenotype of pancreatic ductal adenocarcinoma. Clin Cancer Res 2004; 10:7427-37.
58. Riethdorf S, Reimers N, Assmann V, Kornfeld JW, Terracciano L, Sauter G, Pantel K. High incidence of EMMPRIN expression in human tumors. Int J Cancer 2006; 119:1800-10.