PDAC need more frequent surveillance is also unknown.

Conclusions

Clarification of the classification of pancreatic IPMNs, whether the mixed type IPMN should be defined radiographically or histologically and whether this category is necessary are issues that need to be resolved. Preoperative distinction of premalignant mucinous cysts from nonmucinous cysts should be further pursued and the role and safety of cystic fluid analysis remain to be evaluated in this context. With regard to the diagnosis of malignancy in branch duct IPMNs, criteria with greater specificity are required to reduce the false-positive rate of the 2006 international Sendai guidelines because approximately 80% of branch duct IPMNs that are resected are benign. Preoperative determination of histological subtype could be of interest and value. Surveillance of branch duct IPMNs is of the utmost importance to detect malignant transformation, the development of distinct PDACs and disease recurrence after resection. The best modality for surveillance is not yet known, but the interval for surveillance should not be longer than 6 months.

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MOLECULAR CANCER

RESEARCH Open Access

MicroRNA, *hsa-miR-200c*, is an independent prognostic factor in pancreatic cancer and its upregulation inhibits pancreatic cancer invasion but increases cell proliferation

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Abstract

Background: Recently, the microRNA-200 family was reported to affect cancer biology by regulating epithelial to mesenchymal transition (EMT). Especially, the expression of *miR-200c* has been shown to be associated with upregulating the expression of *E-cadherin*, a gene known to be involved in pancreatic cancer behavior. However, the significance of *miR-200c* in pancreatic cancer is unknown.

Methods: In the present study, we investigated the relationship between *E-cadherin* and *miR-200c* expression in a panel of 14 pancreatic cancer cell lines and in macro-dissected formalin-fixed paraffin-embedded (FFPE) tissue samples obtained from 99 patients who underwent pancreatectomy for pancreatic cancer. We also investigated the effects of *miR-200c* on the proliferation and invasion of pancreatic cancer cells.

Results: We found that patients with high levels of *miR-200c* expression had significantly better survival rates than those with low levels of *miR-200c* expression. We also found a remarkably strong correlation between the levels of *miR-200c* and *E-cadherin* expression.

Conclusions: These data indicate that *miR-200c* may play a role in the pancreatic cancer biology and may be a novel marker for the prognosis of pancreatic cancer.

Introduction

Pancreatic cancer is the fifth leading cause of cancer death and has the lowest survival rate of any solid cancer in the industrialized countries [1,2]. In the past 20 years, 6942 Japanese patients with pancreatic cancer who underwent pancreatectomy showed a very poor prognosis with an overall median survival time (MST) of 11.7 months and a 5-year survival rate of 13.4% [1]. Extensive molecular analysis of pancreatic cancer has led to discoveries of genetic, epigenetic and, more recently, microRNA alterations [3-6].

MicroRNAs (miRNAs) are endogenous, small non-coding RNAs of 14-24 nucleotides that can negatively regulate protein expression at the post-transcriptional level by translational inhibition and/or mRNA degradation, mostly through base pairing with the 3'-UTR of their target mRNAs [7]. Recently, the abnormal expression of miRNAs was shown to be correlated with cancer. The first evidence suggesting a direct link between miRNAs and human cancer was the localization of *miR-15a* and *miR-16-1* within a 30 kb region of minimal loss on chromosome 13 that is deleted in chronic lymphocytic leukemia (CLL) and that both genes are often deleted or down-regulated in CLL [8]. Other miRNAs, such as *miR-143* and *miR-145* have reduced levels of expression in adenomatous and cancerous stages of colorectal neoplasia [9], while *let-7* expression is reduced in lung tumor [10]. The first oncogenic miRNAs (oncomiR-1), the miR-

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17-92 cluster, named from the research on human B cell lymphomas [11], were described as tumor suppressors or oncogenes and brought a novel area of investigation to cancer research [12,13].

Recently, it has been reported that *miR-200c* is a marker of aggressiveness and chemoresistance in female reproductive cancers, that *miR-200c* suppresses invasiveness and restores sensitivity to microtubule-targeting chemotherautic agents in breast and ovrian cancer cells, and that downregulation of *miR-200c* links breast cancer stem cells with normal stem cells [14-16]. Meanwhile, Hurteau *et al.* revealed that over-expression of *miR-200c* leads to reduced expression of *transcription factor 8* (*TCF8*; also termed *ZEB1*) and increased expression of *E-cadherin* in breast cancer cells [17,18]. Also, Park *et al.* reported that *miR-200c* regulates epithelial to mesenchymal transition (EMT) and restores expression of *E-cadherin* in breast and ovarian cancer cells [18-20]. EMT is a central process in the progression of primary tumors toward metastasis (a switch from the polarized, epithelial phenotype to a highly motile fibroblastoid or mesenchymal phenotype). Furthermore, expression of *E-cadherin* can predict disease outcome in patients with resectable pancreatic carcinoma, and the therapeutic restoration of *E-cadherin* was proposed as a strategy to suppress cancer metastasis [21-24].

In the present study, to identify novel relationship between *E-cadherin* and *miR-200c* in pancreatic cancer, we quantified *miR-200c* expression in a panel of 14 pancreatic cancer cell lines and in 99 samples of macro-dissected formalin-fixed paraffin-embedded (FFPE) pancreatic tissues. We also investigated the *in vitro* effects of *miR-200c* upregulation on the proliferation and invasion of pancreatic cancer cells. We found that patients with high levels of *miR-200c* expression had significantly better survival rates compared to those with low levels of *miR-200c* expression. We also found striking correlation between with the levels of *miR-200c* and *E-cadherin* expression. These data suggest that *miR-200c* may be a novel marker for the prognosis of pancreatic cancer.

Materials and methods

Cultured cells

The following 15 pancreatic cancer cell lines were studied: AsPC-1, KP-1N, KP-2, KP-3, PANC-1, BxPC-3 and SUIT-2 (provided by Dr. H. Iguchi, National Shikoku Cancer Center, Matsuyama, Japan); MIA PaCa-2 (Japanese Cancer Resource Bank, Tokyo, Japan); NOR-P1 (established in our laboratory by Dr. Sato); CAPAN-1, CAPAN-2, CFPAC-1, H48N, HS766T and SW1990 (American Type Culture Collection, Manassas, VA, USA). In addition, a human pancreatic ductal epithelial cell line (HPDE, provided by Dr. Ming-Sound Tsao, University of Toronto, Toronto, Ontario, Canada) was studied. The cells were maintained as described previously [25].

Pancreatic tissues

Our study consisted of 99 patients who underwent pancreatic resection for pancreas cancer at the Department of Surgery and Oncology, Kyushu University Hospital (Fukuoka, Japan) from 1992 to 2007. The patients comprised 64 men and 35 women with a median age of 66 years (range, 36-86 years). Survival was measured from the time of pancreatic resection and death was the endpoint. Prognosis was examined in October 2008. The median observation time for overall survival was 15 months and it ranged from 1 to 101 months. Sixty four patients died during follow-up and the other patients were alive and censored.

All resected specimens were fixed in formalin and embedded in paraffin (FFPE) for pathological diagnosis. All tissues adjacent to the specimens were evaluated histologically according to the criteria of the World Health Organization. For all cases, two pathologists were in agreement with regard to pathological features and both confirmed the diagnoses. The stage of tumors was assessed according to the Union Internationale Contre le Cancer (UICC) classification. The clinicopathological characteristics of the tumor collection are described in Table 1. Written informed consent was obtained from all patients, and the study was approved by the Ethics Committee of Kyushu University and conducted according to the Ethical Guidelines for Human Genome/Gene Research enacted by the Japanese Government and the Helsinki Declaration.

miRNA isolation

miRNAs were extracted from cultured cells using a *mir*Vana™ miRNA Isolation Kit (Ambion, Austin, TX, USA) and from macro-dissected FFPE pancreatic tissues using an RNeasy® FFPE Kit (Qiagen, Tokyo, Japan), following the manufacturer's instructions. Considering the influence of genomic DNA contamination, especially from the FFPE materials, Qiagen provides a special gDNA Eliminator spin column to rapidly remove genomic DNA, and we also performed a DNase digestion step. The extracted RNA was quantified by absorbance at 260 nm and its purity was evaluated by the absorbance ratio at 260/280 nm with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Rockland, DE, USA).

Quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR)

The expression of *miR-200c* and *RNU6B* (*U6* snRNA, a reference gene) was measured by qRT-PCR using a TaqMan® MicroRNA Reverse Transcription Kit and TaqMan®

Table 1: Clinicopathological Characteristics of 99 Patients with Pancreatic Cancer

Median age	**65.7 years (range, 36-86 years)**
Sex (Male/Female)	**62 (62.6%)/37 (37.4%)**
Histological diagnosis	
Adenocarcinoma	97 (98.0%)
Adenosquamous carcinoma	2 (2.0%)
pT category	
pT1	6 (6.1%)
pT2	3 (3.0%)
pT3	57 (57.6%)
pT4	33 (33.3%)
pN category	
pN0	33 (33.3%)
pN1	66 (66.7%)
UICC stage	
IA & IB	5 (5.1%) & 4 (4.0%)
IIA & IIB	21 (21.2%) & 64 (64.7%)
III	1 (1.0%)
IV	4 (4.0%)
Histological grade	
G1	20 (20.2%)
G2	43 (43.4%)
G3	36 (36.4%)
Residual tumor category	
R0	60 (61.2%)
R1	38 (38.7%)
Vessel invasion	
Positive	61 (62.2%)
Negative	37 (37.7%)
Neural invasion	
Positive	84 (84.8%)
Negative	15 (15.2%)

Universal PCR Master Mix (No AmpErase® UNG; Applied Biosystems, Tokyo, Japan) and a Chromo4™ System (Bio-Rad, Hercules, CA, USA). We followed the manufacturer's protocols to perform two-step real-time RT-PCR for the measurement of *miR-200c* and *RNU6B* expression. Each sample was run in triplicate. The level of *miR-200c* expression was calculated from a standard curve constructed with small RNAs from the CAPAN-1 pancreatic cancer cell line. The expression levels of *miR-200c* were normalized against the corresponding expression levels of *RNU6B*.

The levels of *E-cadherin* mRNA and *18S* rRNA were measured by qRT-PCR using a QuantiTect SYBR Green RT-PCR Kit (Qiagen, Tokyo, Japan) and a Chromo4™ System, following the manufacturer's protocols [26]. Each sample was run in triplicate. We designed specific primers for *E-cadherin* (forward, 5'-tcagcgtgtgtgactgtgaa-3'; reverse, 5'-aggctgtgccttcctacaga-3'), and *18S* rRNA (forward, 5'-ctttcgaggccctgtaattg-3'; reverse, 5'-cctccaatggatcctcgtta-3') using Primer 3 software and performed BLAST searches to ensure the specificity of the primers. The PCR products amplified using these primers are small (*18S rRNA*, 63 bp; *E-cadherin*, 53 bp), which allowed accurate and sensitive qRT-PCR despite the fragmented RNA extracted from FFPE tissue specimens [27,28]. We also included controls without reverse transcriptase to confirm that there was no influence from genomic DNA contamination. The level of *E-cadherin* mRNA was calculated from a standard curve constructed with total RNA from CAPAN-1 cells and normalized against levels of *18S* rRNA. Accuracy and integrity of PCR products were confirmed with an Agilent 1000 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA).

Cell transfection with miRNA precursors

Upregulation of *miR-200c* expression was achieved by transfection with the *hsa-miR-200c* precursor (Pre-miR™ miRNA Precursor; Applied Biosystems). To verify the specificity of the transfection effect, we used a Pre-miR™ miRNA Precursor Negative Control (Applied Biosystems). Transfections were performed by electroporation using a Nucleofector system (Amaxa Biosystems, Köln, Germany) according to the manufacturer's instructions. PANC-1, SUIT-2 and KP-2 cells (1-2 × 10^6) were transfected with 100 pmol of the indicated precursor or negative control. The degree of mature *miR-200c* upregulation 48 h after transfection was verified by quantifying the expression level of mature *miR-200c*. Cells harvested 48 h after transfection were also used for cell proliferation or invasion assays.

Propidium iodide (PI) assay

Cell proliferation was evaluated using a multiwell fluorescence plate reader and a previously described method [29] with modifications [30,31]. Briefly, cancer cells were seeded at 2 × 10^4 cells/well in Falcon flat-bottom 24-well plates (Becton Dickinson, Franklin Lakes, NJ, USA). 30 μM PI (Wako Ltd., Osaka, Japan) and 600 μM digitonin (Wako Ltd.) were then added to each well. After incubation for 90 min of at 37°C, the fluorescence intensities of labeled nuclei were measured using a CYTO Fluor™ II fluorescence multiwell plate reader (PerSeptive Biosystems, Framingham, MA, USA) to determine total cell numbers.

In vitro Matrigel invasion assay

Invasion of pancreatic cancer cells was evaluated by the numbers of cells invading Matrigel-coated transwell inserts (Becton Dickinson) as reported previously [25,32]. Briefly, transwell inserts with 8 μm pores were coated with Matrigel (20 μg/well; Becton Dickinson). Cancer cells were seeded in the upper chamber at a density of 1.0 × 10^5 cells/cm^2 in 250 μl of Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). After incubation at 37°C, cells that had invaded to the lower surface of the Matrigel-coated membranes were fixed with 70% ethanol, stained with hematoxylin and eosin (H & E) and counted in five randomly selected fields under a light microscope.

Statistical analysis

The *in vitro*data are presented as mean values with error bars representing the minimum and maximum or with the standard deviation (SD). The significance level was $p < 0.05$. *MiR-200c* expression in macro-dissected FFPE samples was split into high and low expression groups using a recursive descent partition analysis. Categorical variables were compared with the chi-square test (Fisher's exact probability test). Survival curves were constructed with the Kaplan-Meier product-limit method and compared by log-rank tests. To evaluate independent prognostic factors associated with survival, a multivariate Cox proportional hazards regression analysis was used, with *miR-200c* expression, age, sex pathological tumor (pT) status, pathological node (pN) status, UICC stage, residual tumor (R) status, histological grade (G) and vessel invasion as covariates (Table 2). Statistical significance was defined as $p < 0.05$. The statistical analyses in the macro-dissected FFPE samples were performed with JMP 7.01 software (SAS Institute, Cary, NC, USA).

Results

Quantitative analysis of *miR-200c* expression in pancreatic cancer cell lines

We investigated *miR-200c* expression in 15 pancreatic cancer cell lines and in a non-neoplastic ductal epithelial cell line (HPDE) by quantitative real-time RT-PCR. As shown in Figure 1a, 4 pancreatic cancer cell lines, CAPAN-1, SW1990, CFPAC-1, and H48N, expressed higher levels of *miR-200c* than HPDE. Two pancreatic cancer cell lines, AsPC-1 and CAPAN-2, expressed similar levels of *miR-200c* to HPDE and 9 pancreatic cancer cell lines, BxPC-3, NOR-P1, KP-1N, KP-2, KP-3, Hs766T, SUIT-2, PANC-1 and MIA PaCa-2, expressed lower levels of *miR-200c* than HPDE.

High levels of *miR-200c* expression correlated with low invasion ability

Having determined the levels of *miR-200c* expression in the 15 pancreatic cancer cell lines, we investigated the invasion ability of the cell lines that expressed high levels of *miR-200c* (CAPAN-1, CFPAC-1, and H48N) and of the cell lines that expressed low levels of *miR-200c* (KP-3, SUIT-2, and PANC-1) using the Matrigel invasion assay. We seeded 7.5 × 10^4 cells per Matrigel-coated well and counted the cells that had invaded the Matrigel 50 h after seeding. As shown in Figure 1b, all cell lines that expressed high levels of high *miR-200c* (CAPAN-1, CFPAC-1, and H48N) showed fewer numbers of invading cells compared to the cell lines that expressed low levels of low *miR-200c* (KP-3, SUIT-2, and PANC-1).

Table 2: Univariate and Multivariate Survival Analyses

	Univariate analysis			Multivariate analysis		
Characteristics	**Hazard Ratio (HR)**	**95% confidence interval**	***P* value**	**Hazard Ratio (HR)**	**95% confidence interval**	***P* value**
Age (< 65)	0.9	0.5 - 1.4	0.62	0.8	0.5 - 1.4	0.50
Sex(Female)	0.9	0.5 - 1.5	0.63	0.9	0.5 - 1.6	0.69
pT (pT1/2)	2.2	1.4 - 3.6	< 0.001	1.8	0.5 - 5.4	0.35
pN (negative)	0.4	0.2 - 0.7	< 0.001	0.5	0.3 - 1.0	0.06
UICC stage	-	-	0.003	-	-	0.01
Histological grade (G3)	1.7	0.9 - 2.8	0.07	0.8	0.6 - 2.6	0.8
Residual tumor (positive)	3.0	1.8 - 5.0	< 0.001	3.2	1.8 - 5.8	< 0.001
Vessel invasion (positive)	2.3	1.4 - 4.1	0.001	1.9	1.0 - 3.6	0.03
Low *miR-200c*	1.8	1.0 - 3.5	0.03	2.2	1.1 - 4.6	0.02

Relative risk of UICC stage was not shown because of 2 parameters.

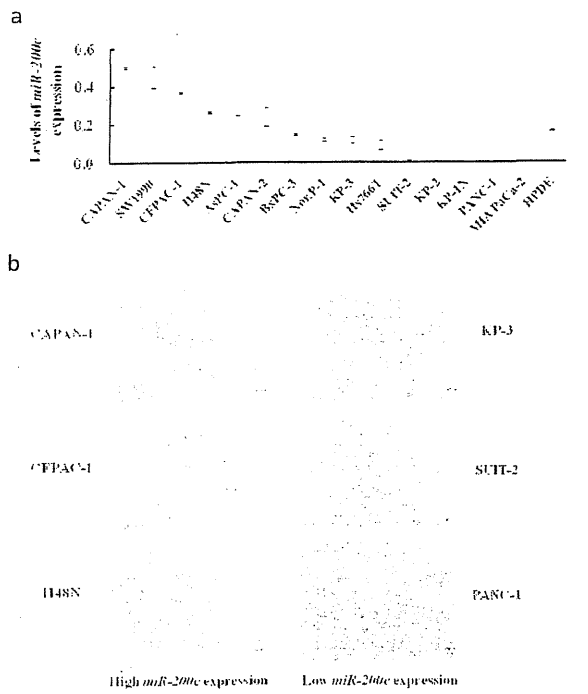

Figure 1 The levels of *miR-200c* expression in cell lines and the correlation of *miR-200c* expression level and the invasion ability of pancreatic cancer cell lines. a. The levels of *miR-200c* expression in 15 pancreatic cancer cell lines and in an HPDE cell line. b. Pictures of invading cells from cell lines expressing high levels of *miR-200c* (CAPAN-1, CFPAC-1 and H48N) and from cell lines expressing low levels of *miR-200c* (KP-3, SUIT-2 and PANC-1). H & E staining. Original magnification, 10 ×. Each sample was run in triplicate. Error bars represent SD.

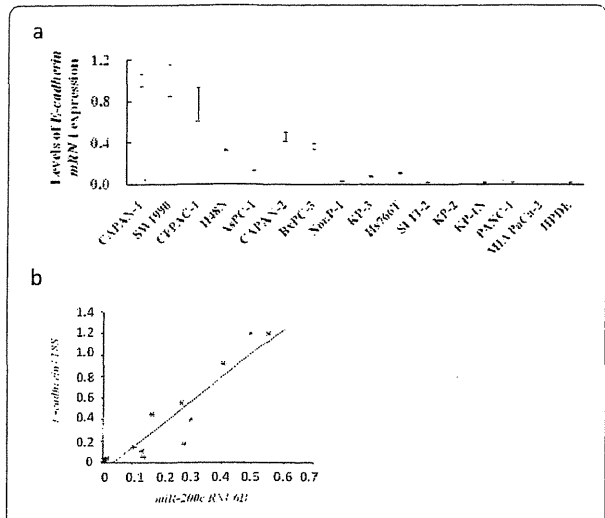

Figure 2 *E-cadherin* expression in cell lines and the correlation between *E-cadherin* and *miR-200c* expression. a. The levels of *miR-200c* expression in 15 pancreatic cancer cell lines and in an HPDE cell line. b. The correlation between *miR-200c* and *E-cadherin* mRNA levels in all cell lines ($p < 0.0001$). Each sample was run in triplicate. Error bars represent SD.

Quantitative analysis of *E-cadherin* mRNA levels in cell lines and significant correlations between *miR-200c* and *E-cadherin* mRNA levels

We investigated *E-cadherin* mRNA levels by qRT-PCR in the 15 pancreatic cancer cell lines and in the HPDE cell line. Similar to the results of *miR-200c* expression, there were high or low *E-cadherin* mRNA levels in these cell lines (Figure 2a), and we found there were significant correlations between *miR-200c* and *E-cadherin* mRNA levels in all cell lines (Pearson's test $p < 0.0001$, Figure 2b)

Upregulation of *miR-200c* in pancreatic cancer cell lines

To upregulate the expression of mature *miR-200c*, we transfected the pancreatic cancer cell lines that expressed *miR-200c* at low levels with the *miR-200c* precursor. 24 h after transfection, we isolated total RNA (including small RNAs) and investigated the levels of *miR-200c* expression. As shown in Figure 3a, SUIT-2 cells transfected with the hsa-*miR-200c* precursor (precursor group) showed a 38-fold increase in mature *miR-200c* expression compared with cells transfected with the miRNA Precursor Negative Control (control group). Similar increases of *miR-200c* expression were seen in KP-3 and PANC-1 cell lines (data not shown).

Upregulation of *miR-200c* enhanced the levels of *E-cadherin* mRNA in pancreatic cancer cells

We also investigated the levels of *E-cadherin* mRNA in the precursor and control groups. As shown in Figure 3b, the SUIT-2 precursor group, which expressed *miR-200c* at levels 38-fold higher than the control group, showed 3.9-fold higher *E-cadherin* mRNA levels compared to the control group 24 h after transfection.

Upregulation of *miR-200c* stimulated proliferation in cancer cells

After confirmation of the upregulation of *miR-200c* in pancreatic cancer cells, we monitored changes in cell proliferation in PANC-1, SUIT-2, and KP-3 cell lines. As shown in Figure 4a, the upregulation of mature *miR-200c* expression in the precursor group enhanced cell proliferation in an upregulation rate-dependent manner for 96 h after transfection in SUIT-2 cells (upper), and for 120 h in KP-3 (middle) and PANC-1 cells (bottom).

Upregulation of *miR-200c* inhibited invasion of cancer cells

Next, we investigated the effect of upregulation of mature *miR-200c* expression on the invasive potential of pancreatic cancer cells. Representative microphotographs of cells invading through Matrigel-coated membranes 36 h after transfection are shown for the control and *miR-200c* precursor cells in the left and right panels of Figure 4b, respectively. The numbers of invading PANC-1 cells were

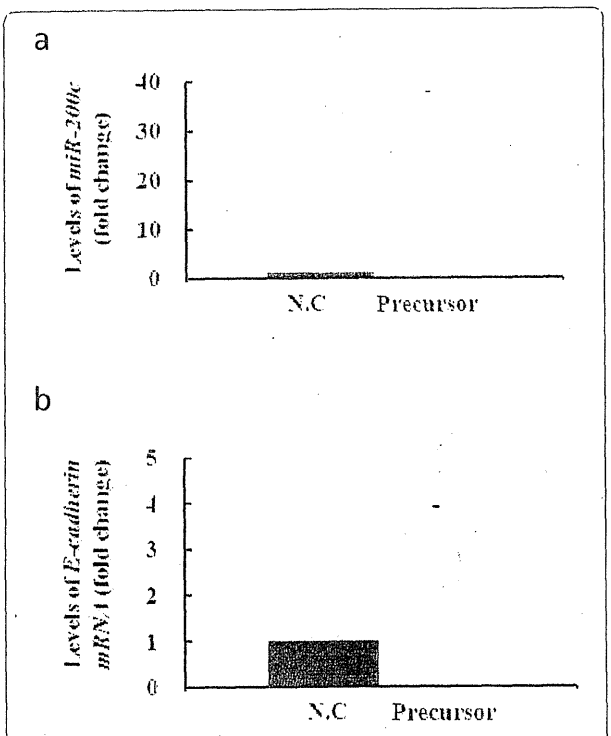

Figure 3 Upregulation of *miR-200c* in pancreatic cancer cell lines and enhanced expression of *E-cadherin*. a. SUIT-2 cells were transfected with *hsa-miR-200c* precursor and showed a 38-fold increase in mature *miR-200c* expression compared with the control 24 h after transfection. b. Upregulation of *miR-200c* enhanced expression of *E-cadherin* 3.9-fold relative to the control group. Each sample was run in triplicate. Error bars represent SD.

significantly inhibited in an upregulation rate-dependent manner when cells were transfected with the *miR-200c* precursor ($p < 0.001$), and the number of cells invading in the precursor group was approximately 75% less than the number of cells invading in the control group (Figure 4c). Similar to the inhibition rate of the PANC-1 precursor group, the KP-3 precursor group also showed a significant inhibition of invasion compared to the control group, with the control group invasion rate inhibited in the precursor group by approximately 75% (Figure 4d, e).

Quantitative analysis of *miR-200c* and *E-cadherin* mRNA levels in macro-dissected FFPE pancreatic cancer tissues

We measured *miR-200c* versus *E-cadherin* mRNA levels in macro-dissected FFPE samples from 99 patients who underwent pancreatic resection for pancreatic cancer at our institution from 1992 to 2007. The median *miR-200c* expression level in the macro-dissected pancreatic cancer samples was 0.30, and the median *E-cadherin* expression level was 4.41. Similar to the results from cultured cells, we also found that there was a significant correlation between *miR-200c* and *E-cadherin* mRNA levels in all macro-dissected pancreatic cancer tissues (Pearson's test $p < 0.0001$, Figure 5a).

Univariate and multivariate analyses of *miR-200c* expression for survival time of patients with pancreatic cancer after curative resection

We classified the patients into two groups of high versus low *miR-200c* expression (cut-off value: 0.64; the partition was constructed by the overall survival time). The high and low *miR-200c* expression groups were composed of 21 and 78 cases, respectively. In univariate survival analyses based on the Cox proportional hazard model, the *miR-200c* levels and conventional prognostic factors, such as pT status (pT3 and pT4 versus pT1 and pT2), pN status (pN1 versus pN0), UICC stage (IV, III and IIB versus IIA, and versus IA and IB), tumor grade (G3 versus G1 and G2), R factor (R1 versus R0) and vessel invasion (positive versus negative), were investigated for overall survival (Table 2). We found that pT status, pN status, UICC stage, R factor and vessel invasion were significantly associated with a shorter overall survival ($p < 0.001$, $p < 0.001$, $p = 0.002$, $p < 0.001$, and $p = 0.001$, respectively). We also found that high *miR-200c* expression was associated with a longer overall survival ($p = 0.03$). The median survival time (MST) and 5-year survival rate were 42 months and 33.5% in the high *miR-200c* expression group, and 19 months and 11.2% in the low *miR-200c* expression group, respectively (Figure 5b). In multivariate survival analyses, we found that the overall survival time was significantly dependent on UICC stage ($p = 0.01$), R factor ($p < 0.001$), vessel invasion ($p = 0.03$) and high *miR-200c* expression ($p = 0.02$).

Discussion

The present study shows, for the first time, the involvement of *miR-200c* in pancreatic cancer progression and prognosis. We have found that high *miR-200c* expression was related to low invasion ability, and that upregulation of *miR-200c* expression inhibited cell invasion and stimulated cell proliferation in pancreatic cancer cell lines. We also have found a significant correlation between *miR-200c* and *E-cadherin* expression, and that upregulation of *miR-200c* expression correlated with increased expression of *E-cadherin* in pancreatic cancer cell lines. This finding is consistent with previous reports investigating other cancers [14-19]. On the other hand, reduced expression of E-cadherin is regarded as a main molecular event in the dysfunction of the cell-cell adhesion system, triggering cancer invasion and metastasis [33,34]. Recently, Liu *et al.* revealed that E-cadherin stimulated cell proliferation at intermediate seeding densities, and Mees *et al.* revealed that metastasis suppressor gene EP300 was regulated by *miR-200c* in ductal adenocarcinomas of the pancreas [35,36]. These studies indicate that

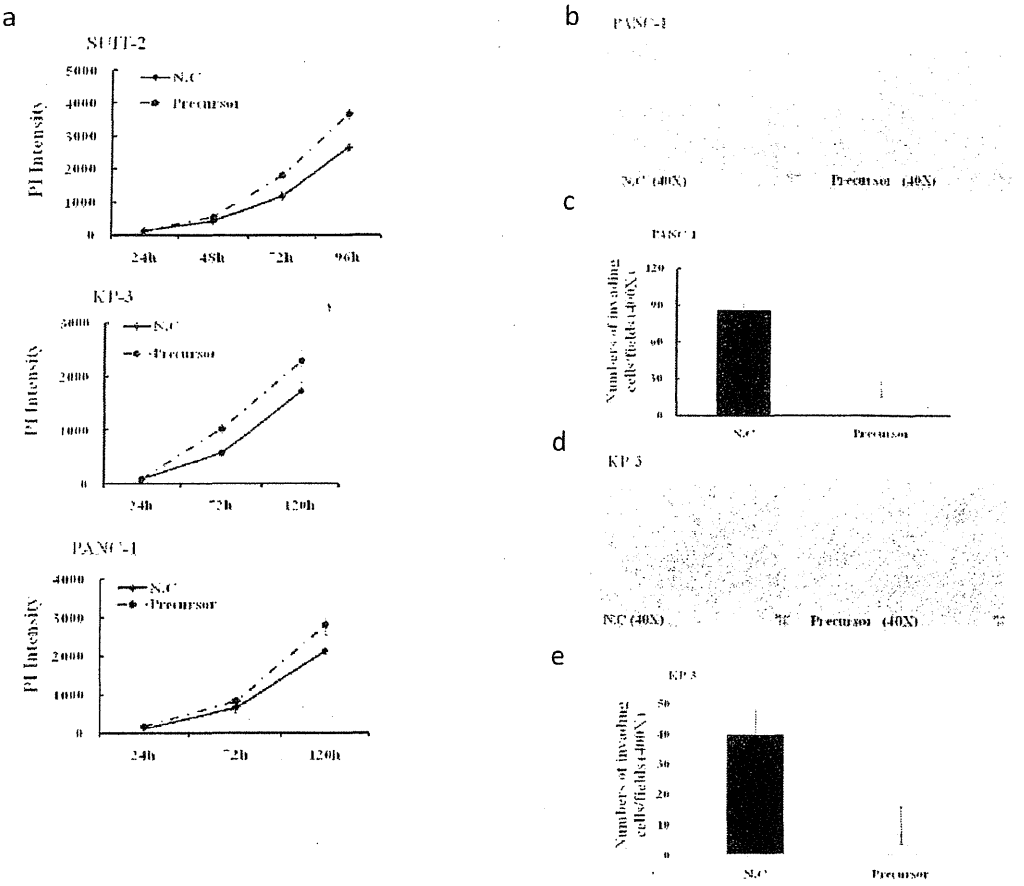

Figure 4 Upregulation of *miR-200c* stimulated proliferation and inhibited invasion. a. the upregulation of mature *miR-200c* expression enhanced the cell proliferation in an upregulation rate-dependent manner in SUIT-2 (upper), KP-3 (middle), and PANC-1 (bottom) cells. b and d. Pictures of invading cells (PANC-1 and KP-3, respectively). Original magnification, 10 ×. c and e. The number of invading cells (PANC-1 and KP-3, respectively) in five randomly selected fields observed under a light microscope (H&E staining, magnification, 400 ×). Error bars represent SD.

miR-200c plays a key role in the enhancement of proliferation and inhibition of invasion in pancreatic cancer via regulation of E-cadherin. Such inconsistent function is similar to gamma-interferon, which can inhibit tumor growth and enhance metastasis in a TS/A mammary adenocarcinoma model [37].

Furthermore, univariate and multivariate analyses of 99 macro-dissected FFPE pancreatic cancer samples revealed that high *miR-200c* expression was associated with a better prognosis. *E-cadherin* is considered as a prognosis factor in some cancers [38], and we also found that high *E-cadherin* expression was associated with a better prognosis in univariate analyses of macro-dissected FFPE pancreatic cancer samples but not in multivariate analyses (data not shown). These findings indicate that E-cadherin can be used as a prognosis factor by immunohistochemistry to detect E-cadherin protein or by qRT-PCR to measure *E-cadherin* mRNA levels. However, it is difficult to generate a highly specific E-cadherin protein antibody or specific *E-cadherin* mRNA primers, especially when using fragmented RNA from FFPE samples. miRNAs are small RNAs of 14 -24 nucleotides and are more stable than mRNA from FFPE samples [39] and the technologies of miRNA extraction and of qRT-PCR can be controlled more easily than those for mRNAs. Taken together, these findings suggest that *miR-200c* can be a better independent prognosis factor than *E-cadherin* mRNA in univariate or multivariate analyses, while the latter can be used as a prognosis factor in univariate analyses only. Furthermore, Mitchell, *et al.* reported that circulating miRNAs are stable blood-based markers for cancer detection [40], suggesting that quantifying the levels of *miR-200c* from patients' pancreatic juice or blood may provide an important marker for indicating the suitability for surgery.

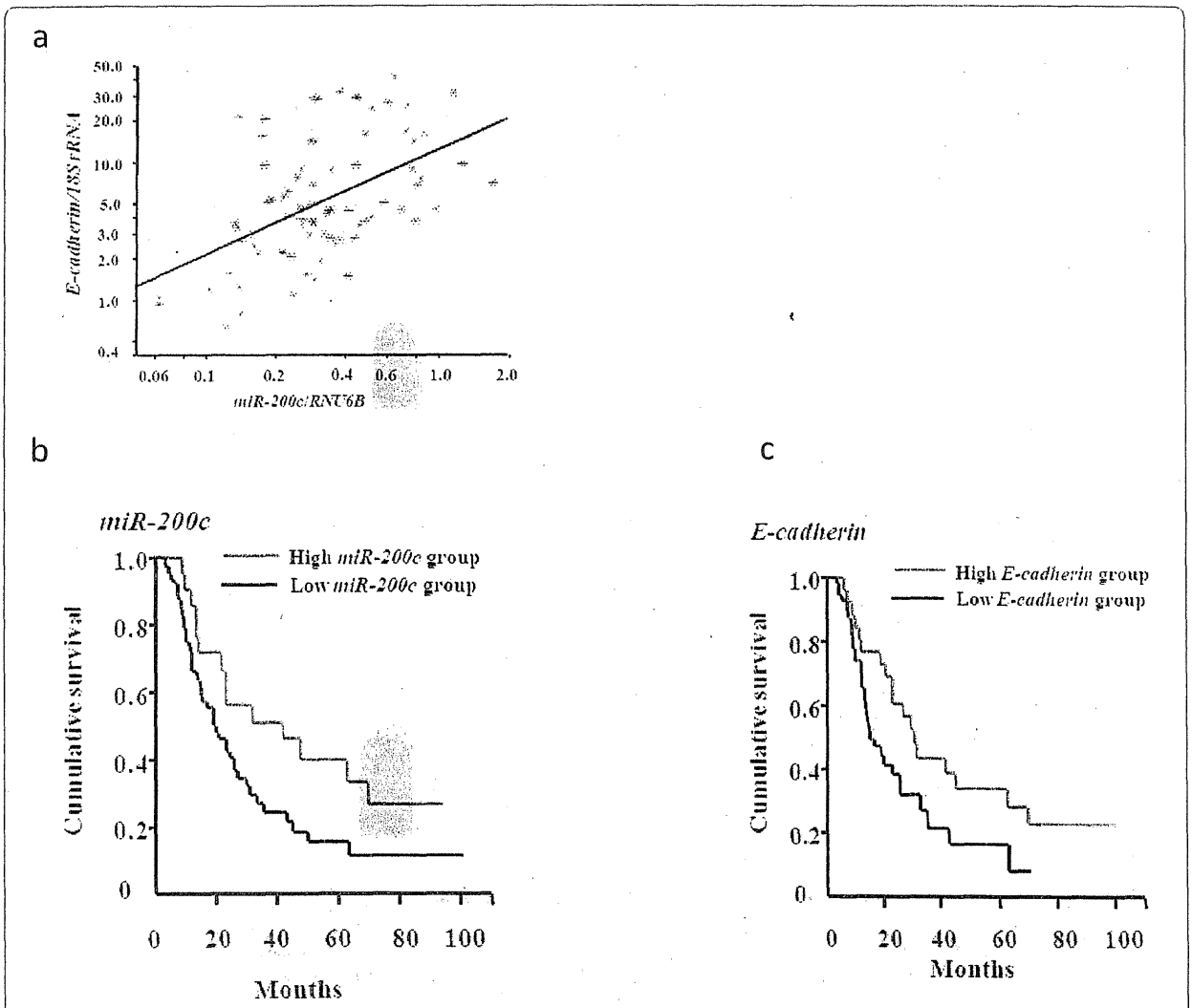

Figure 5 The correlation between *miR-200c* and *E-cadherin* expression in macro-dissected FFPE pancreatic cancer tissues. a. There was a significant correlation between *miR-200c* and *E-cadherin* mRNA levels in all macro-dissected pancreatic cancer tissues (Pearson's test $p < 0.0001$). b and c. Overall survival time after resection of pancreatic cancer with high *miR-200c* expression levels versus *low miR-200c* expression levels and with high *E-cadherin* mRNA expression levels versus low *E-cadherin* expression levels. Each sample was run in triplicate.

In conclusion, our results have revealed that high levels of *miR-200c* expression inhibit cancer invasion and stimulate cancer cell proliferation, possibly via up-regulation of *E-cadherin*, and that high levels of *miR-200c* expression correlate with better survival of patients with curative resection of pancreatic cancer. We believe that research into *miR-200c* may bring about new opportunities for the development of drugs and therapeutic strategies for the treatment of pancreatic cancer. On the other hand, the *miR-200* family members, like *miR-200a/b/c*, *miR-141*, and *miR-429*, have similar, but not identical functions ([19,20] and [36]). We believe that it is necessary to investigate the other family members to complete the picture regarding the *miR-200* family and pancreatic cancer.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
Conception and design: JY, KO, KM, and MT; analysis and interpretation: JY, TK, and KN; data collection: HF; writing the article: JY; critical revision of the article: KO, KM, NS, and MT; final approval of the article: JY and MT; statistical analysis: JY, KO, and KN; overall responsibility: KO and KM. All authors read and approved the final manuscript.

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Significance of Renal Rim Grade on Computed Tomography in Severity Evaluation of Acute Pancreatitis

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Objectives: Multifactor scoring systems, such as the Acute Physiology and Chronic Health Evaluation (APACHE) II, are useful for predicting the severity of acute pancreatitis (AP); however, they are rather complicated. The aim of this study was to introduce renal rim grade (RRG) as a severity assessment measure for AP.

Methods: One hundred twenty-two eligible AP patients who underwent abdominal computed tomography (CT) on admission were evaluated for RRG (grades 1–3). The end points were the severity of illness and hospital mortality. Furthermore, RRG was compared with the Balthazar score, the CT severity index, the Ranson score, and the Acute Physiology and Chronic Health Evaluation (APACHE) II score, using a receiver operating characteristic analysis.

Results: The exacerbation rates into severe disease were 3% (grade 1), 48% (grade 2), and 89% (grade 3). The mortality rates were 3% (grade 1), 8% (grade 2), and 31% (grade 3). The area under the receiver operating characteristic curves to predict the severe disease and mortality using the RRG system were comparable with other scoring systems.

Conclusions: Renal rim grade is useful for the evaluation of the severity of AP.

Key Words: acute pancreatitis, computed tomography (CT), Gerota fascia, renal rim grade, renal rim sign, loss of renal rim sign

(*Pancreas* 2010;39: 41–46)

Acute pancreatitis (AP) is reported to affect 5.4 to 79.8 per 100,000 population per year,[1] and 15% to 25% of them demonstrate a severe disease course.[2] The patients with the severe disease develop a systemic inflammatory response syndrome, often leading to a fulminating course with pancreatic necrosis and/or multiple organ dysfunction syndrome (MODS).[3] Therefore, the prognosis of AP greatly depends on the measures taken to prevent multiple organ dysfunction syndrome (MODS). An early prediction of the prognosis is one of the most important determinations in cases of AP about the intensity of care and the use of advanced treatments.[4,5]

Several scoring systems based on clinical and laboratory data or those based on imaging grading were reported to be useful for the prediction of severe disease and mortality in AP. The Ranson score[6] and the Acute Physiology and Chronic Health Evaluation (APACHE) II score[7] have been adopted in many guidelines and consensus conferences for AP.[4,8–12] The Balthazar score[13] and the computed tomography severity index (CTSI)[14] consisting of the degree of pancreatic and peripancreatic inflammation and that of pancreatic necrosis is widely accepted with an excellent predictive power.[15–18] However, these clinical, laboratory, and imaging evaluation systems need physicians to assess many aspects. The physicians cannot evaluate the severity of patients with AP in a short time, as is done using the C-reactive protein at 48 hours[19] and the 4-variable BALI (BUN, age, LDH, and IL-6) model.[20]

In AP, diffuse peripancreatic inflammation is usually provoked. The extension of the peripancreatic inflammation is once blocked by the Gerota fascia, which is the boundary between the pararenal space (pancreatic side of the fascia) and the perirenal space (renal side of the fascia; Fig. 1A). In addition, only when peripancreatic inflammation spreads more extensively and becomes more severe do the inflammatory changes extend beyond the Gerota fascia to the perirenal spaces (Fig. 1B). The Gerota fascia seems to work as a barrier against inflammatory extension. We call the noninflamed perirenal fat tissue, which is surrounded by inflamed pararenal fat tissue with the blockade of inflammatory extension by the Gerota fascia, as *renal rim*. We call the appearance of renal rim as *renal rim sign*. In addition, if the inflammatory changes extend to the perirenal space as Figure 1B, the demarcation line consisting the Gerota fascia between the pararenal and the perirenal spaces would disappear on CT. We call the appearance of the renal rim as *renal rim sign* and the disappearance of the renal rim as *loss of renal rim sign*. Such inflammatory changes of fat tissue can be visualized on abdominal CT. In this study, we analyzed the significance of assessment for pararenal and perirenal inflammation (renal rim grade [RRG]) in AP.

MATERIALS AND METHODS

Among the 157 consecutive patients with AP who were admitted to our department from 1992 through 2005, 122 patients (78%) in whom abdominal CT study was performed within 24 hours after admission were eligible for this study. The mean interval between the disease onset and the day of undergoing CT was 1.6 days (95% CI, 1.3–1.9 days; range, 0–7 days) in these patients. The diagnosis of AP was based on the clinical, the laboratory, and the radiographic findings according to the criteria of the Research Committee of Intractable Disease of the Pancreas of the Japanese Ministry of Health, Labor and Welfare.[21] Severe AP was diagnosed according to the 1992 Atlanta criteria of severity.[12] All the patients initially received conservative management using proteinase inhibitor and prophylactic antibiotics. Mechanical ventilation for respiratory failure, catecholamine administration for hypotension, and continuous hemodiafiltration for renal failure were initiated, if necessary. The intrapancreatic arterial administration of proteinase inhibitor and prophylactic antibiotics were provided to the patients experiencing necrotizing pancreatitis and for patients in stage 3 or 4 in the Japanese

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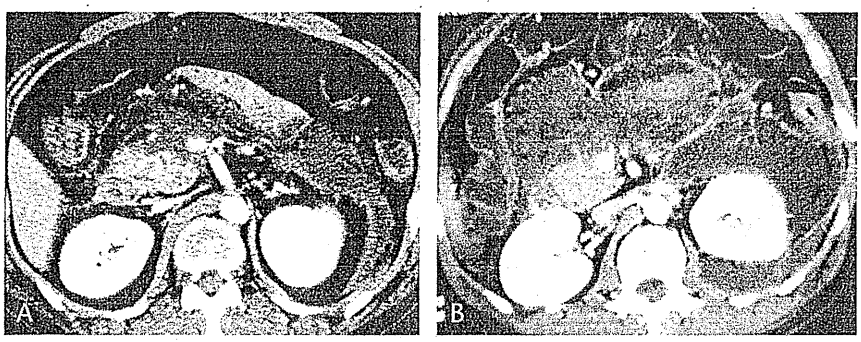

FIGURE 1. Computed tomographic images of a patient whose RRG progressed from grade B to C after 2 weeks. A, Computed tomographic image at admission (grade B, renal rim sign is positive). B, Computed tomographic image 2 weeks after admission (grade C, loss of renal rim sign is positive).

severity scoring system based on 17 clinical signs and 11 laboratory data.[21–23] The patients diagnosed with infected pancreatic or intestinal necrosis underwent operative therapy or radiological intervention.

The Ranson score at 48 hours after admission and the APACHE II score on admission were calculated for all patients enrolled in this study. All 122 patients underwent contrast-enhanced CT within 24 hours after admission and were evaluated for RRG (Table 1). Patients who were not subjected to CT study within 24 hours after admission were excluded from this study. If there is no apparent inflammatory extension to the pararenal space, it is assessed as grade 1 (Figs. 2A, B). If the inflammation extended to the pararenal space along the pancreatic side of the Gerota fascia and the CT attenuation of this space increased, it is assessed as grade 2 (Figs. 2C–E). If the inflammation extended to the retroperitoneal perirenal tissue beyond the Gerota fascia and the CT attenuation around the kidney (renal side of the Gerota fascia) increased, then it is assessed as grade 3 (Figs. 2F–I). Peripancreatic inflammation extends to the perirenal space along with the bridging septa. When the perirenal bridging septa is thickened, the RRG would be assessed as grade 3 (Fig. 2I).

One board-certified radiologist (K.A., with 22 years of experience), 1 pancreatic surgeon (M.H., with 25 years of experience), and 2 junior surgeons (with more than 6 and 7 years of experience) independently performed evaluations and judgment of RRG in each patient. They were blinded to the severity and mortality of the AP but were cognizant of the patient age, sex, and clinical symptoms. All the images were reviewed in random order on films with window level (40–50 H) and window width (450 H).

The evaluation end points were the development of severe disease as defined by the 1992 Atlanta classification of severity[12] and the hospital mortality. The correlation between the RRG system and the other grading systems (the Balthazar score, the CTSI, the Ranson score, and the APACHE II score) was analyzed using the Spearman rank correlation test. The receiver operating characteristic (ROC) curve was constructed for performance to predict severe disease and mortality of AP, and the area under the ROC curve (AUC) was calculated. The sensitivity, the specificity, the overall correctness of prediction, the positive and the negative predictive values, the false-positive and the negative rates, and the likelihood ratio of a positive test were calculated, and the cutoff point giving the best Youden index was determined.[24] This cutoff point was also used to calculate the predicted and the observed severity and mortality of AP. The differences in the AUCs were assessed as previously described.[25] To assess the degree of observer agreement for the quality of depiction of inflammation, we used the Cohen κ coefficient. The scale for the κ coefficients for interobserver agreement was $\kappa < 0.20$, poor; 0.21 to 0.40, fair; 0.41 to 0.60, moderate; 0.61 to 0.80, substantial; and 0.81 to 1.00, almost perfect.[26,27] Differences were considered to be significant if the $P < 0.05$.

RESULTS

The 122 eligible patients (85 men and 37 women) of the total 157 patients were enrolled in this study. Their ages ranged from 13 to 81 years (mean, 53 years). Alcohol use was suspected as the etiology in 52 patients (42%): biliary stones in 28 (23%), idiopathic in 24 (20%), and postoperative in 6 (5%). Sixty-six patients (54%) were graded as mild, and the other 56 patients (46%) were graded as severe disease according to the 1992 Atlanta criteria of severity. Sixteen patients (13%) died of AP during their hospital stay. Because our institution is a tertiary referred center, the severest patients are not rare.

The rates for developing severe disease in each RRG were as follows: 3% (1/37) in grade 1, 48% (24/50) in grade 2, and 89% (31/35) in grade 3. The mortality rates in each RRG were 3% (1/37) in grade 1, 8% (4/50) in grade 2, and 31% (11/35) in grade 3 (Table 2). The grade can progress from lower to higher grade. Figure 1 shows an example of such case.

The correlation between RRG and the other severity evaluation systems was statistically significant (Table 3). Using the ROC curves, all the 5 scores were found to be reliable in predicting severe disease and mortality. Tables 4 and 5 show the performances of the various scoring systems calculated at the cutoff point giving the best Youden index. The AUCs to predict severe disease and mortality for RRG were 0.86 and 0.76, respectively (Fig. 3, Tables 4, 5). According to the AUCs in the ROC analysis, the predicting power of RRG was comparable to

TABLE 1. Renal Rim Grade

RRG	Definition
1	No increase in the attenuation of the anterior pararenal and the perirenal spaces
2	Increase in the attenuation of the pararenal space (pancreatic side of the Gerota fascia); renal rim sign (+)
3	Increase in the attenuation of both the pararenal and the perirenal spaces (renal side of the Gerota fascia); loss of renal rim sign (+). When the perirenal bridging septa is thickened, RRG would be assessed as grade 3.

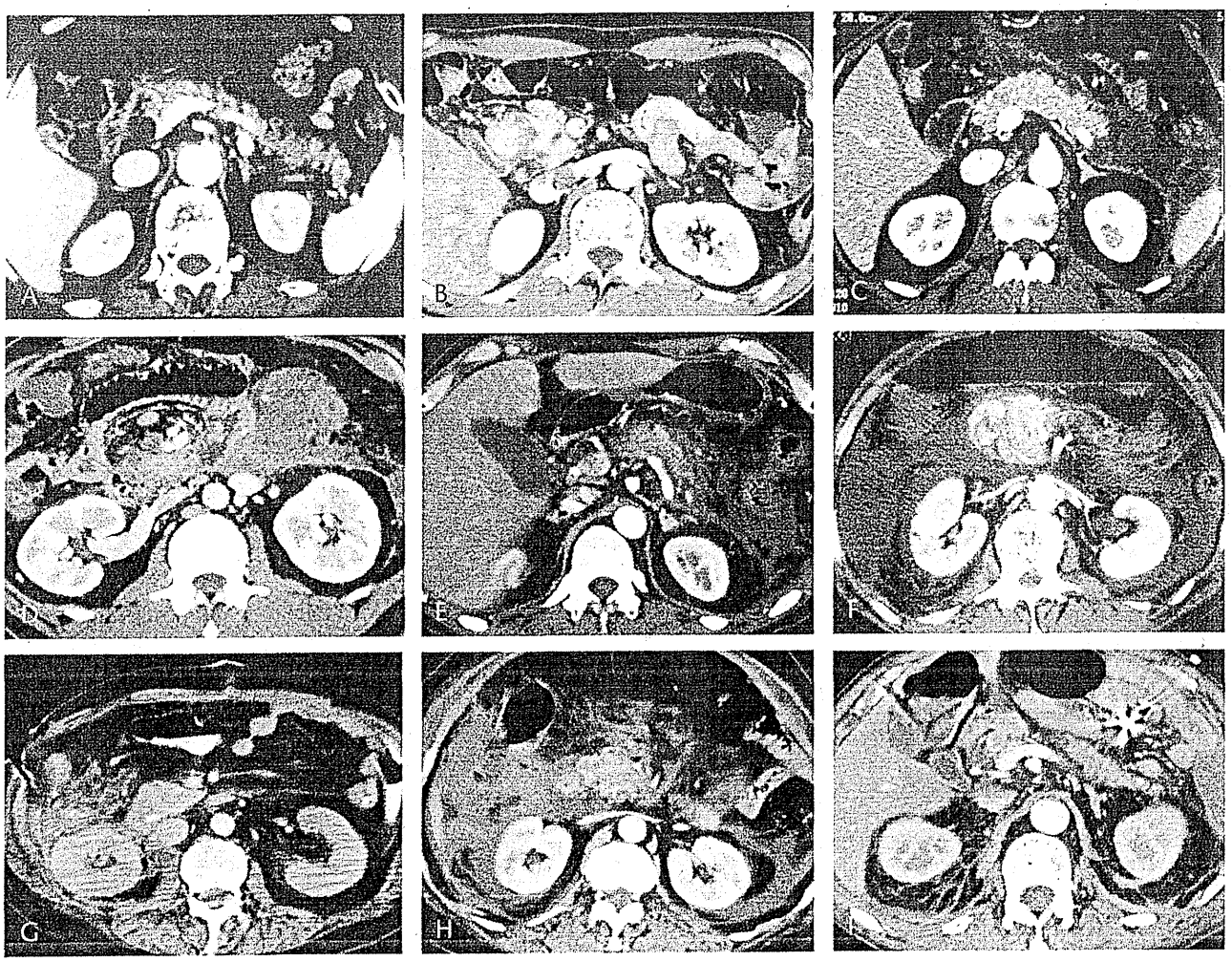

FIGURE 2. Typical CT images in each RRG. A and B, Grade 1; C–E, grade 2; and F–I, grade 3.

other 4 scoring systems (data not shown). The AUC for the Balthazar score in predicting severe disease was significantly smaller than those for the other 4 scores including RRG. The AUC for the APACHE II score in predicting the mortality of AP was significantly larger than those for the other 4 scores including RRG.

The κ coefficients for RRG was 0.81 (95% confidence interval [CI], 0.68–0.95), indicating almost perfect agreement.

DISCUSSION

In this study, we introduced a new CT grading classification (RRG) of AP. The merit of this grading system is the simplicity in evaluation. The peripancreatic inflammation in AP commonly extends to the pararenal spaces. The inflammation further extends to the perirenal space across the Gerota fascia via the bridging septae,[28,29] although, simultaneously, the inflammatory fluid spreads inferiorly to reach the pelvic retroperitoneum and superiorly along the diaphragm.[30] An increase of the pancreatic and the peripancreatic pressures may cause a

TABLE 2. Relationship Between RRG and the Patients' Outcome

RRG	Rate of Severe Disease	Mortality Rate
1	3% (1/37)	3% (1/37)
2	48% (24/50)	8% (4/50)
3	89% (31/35)	31% (11/35)

TABLE 3. Correlation Between RRG and Other Clinical and Radiological Scores

	RRG (n = 122)				
	Grade 1 (n = 37)	Grade 2 (n = 50)	Grade 3 (n = 35)	ρ	P
Balthazar score	B (A–D)	D (A–E)	E (B–E)	0.68	<0.0001
CTSI	1 (0–3)	4 (0–10)	6 (2–10)	0.74	<0.0001
Ranson score	0 (0–8)	3 (0–8)	5 (1–10)	0.66	<0.0001
APACHE II score	3 (0–14)	9 (0–28)	12 (3–34)	0.60	<0.0001

The data are expressed as the median, with the range in parentheses.

TABLE 4. Comparison of the Performance of the Scoring Systems in Predicting Severity of Acute Pancreatitis

Parameter	RRG	Balthazar	CTSI	Ranson	APACHE II
Cutoff point*	>1	>B	>3	>1	>7
Youden index	0.53	0.41	0.51	0.60	0.61
Sensitivity, %	98	42	75	98	91
Specificity, %	55	98	76	62	70
Overall correctness of prediction, %	75	68	75	79	80
Positive predictive value, %	65	97	72	69	72
Negative predictive value, %	97	59	78	98	90
False-positive rate, %	45	2	24	38	30
False-negative rate, %	2	58	25	2	9
LR of positive test	2.16	23.76	3.09	2.59	3.01
AUC	0.86	0.74†	0.84	0.90	0.87
95% CI (AUC)	0.80–0.92	0.66–0.83	0.77–0.91	0.85–0.95	0.81–0.93

*Value giving the best Youden index.
†Significantly smaller than the AUC of RRG ($P = 0.0014$).
LR indicates likelihood ratio.

decrease in the pancreatic tissue blood flow, pancreatic ischemia, and necrosis.[31] Hence, the assessment of the retroperitoneal inflammation may reflect not only the degree of autodigestion but also the resultant pancreatic ischemia. The degree of retroperitoneal inflammatory extension efficiently reflects the severity of AP. The predictive power of RRG was comparable to other scoring systems in predicting severe disease and mortality.

As for the CT classification for AP, the Balthazar score and the CTSI were reported by Balthazar et al in 1985[13] and 1990,[14] respectively. The Balthazar score needs the assessments of both the pancreatic and the peripancreatic changes, and the CTSI needs moreover the degree of pancreatic necrosis. They are already widely accepted and have an excellent predictive power for severe disease.[15–18] However, the classification system needs physicians to assess many aspects, such as the presence of pancreatic enlargement, peripancreatic inflammation, and the degree of fluid collections and pancreatic necrosis. Especially, regarding fluid collection, there are many locations to evaluate, and it is difficult to assess all of them promptly.

The importance of the assessment of peripancreatic changes in AP has been well elucidated. The extrapancreatic (EP) score that was focused on 7 points limited to EP changes at CT was first reported by Schroder et al.[32] Hjelmqvist et al[33] also recommended early CT scan and assessment using the EP score. Although the EP score is useful and correlates with patients' prognoses, relatively many points have to be assessed. In addition, Lankisch et al[34] also suggested that the EP changes especially in pararenal space paralleled the severity of AP. However, they also indicated that the extension of peripancreatic fluid to the splenic area did not correlate with mortality. Recently, Ishikawa et al[29] and De Waele et al[35] also focused on the relationship between retroperitoneal inflammation and AP. Ishikawa et al[29] classified the patients with AP into 5 grades according to the detailed 3-dimensional pathways of inflammatory extension. De Waele et al[35] reported a new scoring system based on the systemic inflammation signs on CT as EP inflammation on CT score. Although these classifications have a good predictive power of the outcomes, physicians need to understand the complicated

TABLE 5. Comparison of the Performance of the Scoring Systems in Predicting Mortality of Acute Pancreatitis

Parameter	RRG	Balthazar	CTSI	Ranson	APACHE II
Cutoff point*	=3	>D	>3	>3	>12
Youden index	0.46	0.36	0.46	0.48	0.77
Sensitivity, %	69	67	88	81	94
Specificity, %	77	69	58	67	83
Overall correctness of prediction, %	76	67	62	69	84
Positive predictive value, %	31	24	24	27	99
Negative predictive value, %	94	93	97	96	84
False positive rate, %	23	33	42	33	17
False negative rate, %	31	31	13	19	6
LR of positive test	3.04	2.14	2.11	2.46	5.52
AUC	0.76	0.70	0.75	0.82	0.92†
95% CI (AUC)	0.65–0.87	0.59–0.82	0.65–0.86	0.72–0.92	0.86–0.98

*Value giving the best Youden index.
†Significantly larger than the AUC of RRG ($P = 0.026$).

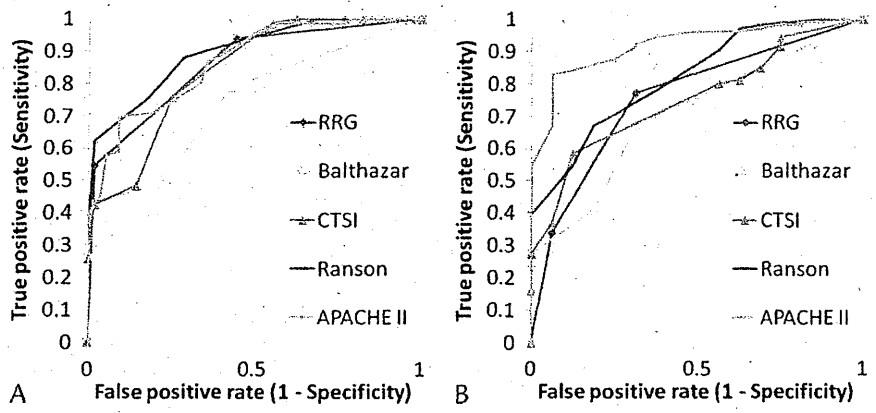

FIGURE 3. Receiver operating characteristic curves for the RRG, the Balthazar score, the CTSI, the Ranson score, and the APACHE II score. A, Receiver operating characteristic curves for predicting severity defined by the 1992 Atlanta classification. B, The ROC curves for predicting hospital mortality.

retroperitoneal anatomy and evaluate many aspects. We also focused on the inflammatory extent to the retroperitoneal perirenal space. Renal rim grade has good power for prediction of severe disease and mortality, and it is easy to use. We consider that easiness to use is important clinically, particularly in emergency setting.

However, our study had 2 major limitations. First, it was a nonrandomized retrospective study. Second, all patients did not undergo CT at admission. Hence, we analyzed only the subgroup of the patients who underwent contrast-enhanced CT within 24 hours after admission. Therefore, we need to assess RRG prospectively in all consecutive patients with AP. Although there are some limitations, RRG is useful and accurate clinically in the severity assessment of AP.

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Original Article

Can the Physiologic Ability and Surgical Stress (E-PASS) Scoring System Predict Operative Morbidity After Distal Pancreatectomy?

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Abstract

Purpose. Mortality rates after pancreatic resection are now lower than 5% in high-volume centers; however, morbidity remains high. This stresses the importance of identifying accurate predictors of operative morbidity after pancreatic resection. The Estimation of Physiologic Ability and Surgical Stress (E-PASS) scoring system was developed for a comparative audit of general surgical patients. Our previous study confirmed its usefulness for predicting morbidity after pancreaticoduodenectomy. In the present study, we evaluated whether the E-PASS scoring system can predict the occurrence of complications after distal pancreatectomy (DP).

Methods. The subjects were 46 patients who underwent DP for pancreatic disease. We studied correlations between the incidence of postoperative complications and the preoperative risk score (PRS), surgical stress score (SSS), and comprehensive risk score (CRS) of the E-PASS scoring system.

Results. A collective total of 20 postoperative complications developed in 13 (28.3%) of the 46 patients. All E-PASS scores, particularly PRS and CRS, were significantly higher in the patients with postoperative complications than in those without complications. The complication rate increased with increasing PRS, SSS, and CRS scores.

Conclusion. The E-PASS scoring system is useful for predicting morbidity after DP.

Key words E-PASS scoring system · Distal pancreatectomy · Complication

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Introduction

Advances in surgical techniques and perioperative management have reduced the operative mortality rate after pancreatic resection to less than 5% in high-volume centers; however, morbidity rates have changed little and range from 30% to 40%.[1–16] The majority of perioperative complications are not life-threatening, but they can prolong the hospital stay, increase costs, necessitate readmission, and delay adjuvant therapy. Thus, it is important to identify the predictive and intraoperative risk factors associated with operative morbidity after distal pancreatectomy (DP).

Haga et al. devised and validated the Estimation of Physiologic Ability and Surgical Stress (E-PASS) scoring system for risk stratification of patients undergoing elective general gastrointestinal (GI) surgery.[17] It has been externally validated in a different geographical setting from where it was originally developed, and is reproducible for accurately predicting outcomes after elective GI surgery.[18] We previously reported its usefulness for predicting morbidity after pancreaticoduodenectomy (PD).[19] This system comprises a preoperative risk score (PRS), a surgical stress score (SSS), and a comprehensive risk score (CRS), calculated from the PRS and SSS. The E-PASS was based on the premise that morbidity and mortality rates are correlated with the patient's physiological risk and anticipated surgical stress. The aim of this study was to evaluate whether the E-PASS scoring system could predict postoperative complications in patients undergoing DP.

Patients and Methods

Patients and Treatments

Between April 2005 and December 2007, 46 consecutive patients underwent DP for malignant or benign pancre-

Table 1. Equations for Estimation of Physiologic Ability and Surgical Stress (E-PASS) scoring system

Preoperative risk score (PRS)
$= -0.0686 + 0.00345X_1 + 0.323X_2 + 0.205X_3 + 0.153X_4 + 0.148X_5 + 0.0666X_6$
Where X_1 is age, X_2 is presence (1) or absence (0) of severe heart-disease[a], X_3 is presence (1) or absence (0) of severe pulmonary disease[b], X_4 is presence (1) or absence (0) of diabetes mellitus, X_5 is performance status index[c] (0–4), X_6 is American Society of Anesthesiologists physiological status classification

Surgical stress score (SSS)
$= -0.342 + 0.0139X_1 + 0.0392X_2 + 0.353X_3$
Where X_1 is blood loss/body weight (ml/kg), X_2 is operation time (h), X_3 is extent of skin incision (0 = minor incision for laparoscopic or thoracoscopic surgery, 1 = laparotomy or thoracotomy alone, 2 = both laparotomy and thoracotomy)

Comprehensive risk score (CRS)
$= -0.328 + 0.936$ (PRS) $+ 0.976$ (SSS)

[a] Severe heart disease was defined as heart failure of New York Heart Association Class III or IV or severe arrhythmia requiring mechanical support
[b] Severe pulmonary disease was defined as a condition with a % vital capacity <60% or a % forced expiratory volume in 1.0 second <50%
[c] Performance status index was based on the definition by the Japanese Society for Cancer Therapy

atic diseases at Kumamoto University Hospital. Written informed consent was obtained from all patients before the treatment. The same three surgeons performed the operations using almost uniform procedures. The pancreas was routinely transected with a scalpel, the pancreatic duct was ligated, and the pancreatic stump was closed with monofilament sutures. A closed-suction drain was placed in the vicinity of the pancreatic stump. D2 lymph node dissection was performed in patients with pancreatic cancer.[20]

E-PASS Scoring System

The equations used in the E-PASS scoring system are shown in Table 1. The PRS is calculated using factors such as age, the presence or absence of severe heart disease, severe lung disease, or diabetes mellitus, American Society of Anesthesiologists (ASA) physiological status classification, and performance status index defined by the Japanese Society for Cancer Therapy,[21] which is the same as that defined by the Eastern Cooperative Oncology Group. The performance status index is defined as follows: grade 0, conditions without symptoms that restrict social activities; grade 1, conditions with mild symptoms that restrict muscular labor but do not restrict walking or mild exertion; grade 2, conditions that require some physical assistance for daily living; grade 3, conditions that require frequent physical assistance for daily living; grade 4, conditions that require constant physical assistance. Patients in grade 2 are not restricted to bed for more than half a day, those in grade 3 are restricted to bed for more than half a day, and those in grade 4 are restricted to bed all day. According to a previous study,[22] the expected in-hospital mortality rate was estimated as $Y = -0.465 + 1.192(\text{CRS}) + 10.91(\text{CRS})^2$.

Postoperative Complications

The postoperative complications, apart from pancreatic fistula (POPF), were assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0 (NCI CTCAE v.3.0).[23,24] In this study, adverse events of grade 2–5 occurring within 30 days after surgery were considered to be postoperative complications. Adverse events corresponding to grade 1 were excluded because medical treatment was not required. Postoperative pancreatic fistula was assessed according to an international study group (ISGPF) definition, that is a as drainage output of any measurable volume of fluid on or after postoperative day (POD) 3 with amylase content greater than three times the serum amylase activity.[25] Three different grades of POPF (grades A, B, C) are defined according to the clinical impact on the patient's hospital course. Grade B and grade C were considered to be postoperative complications in this study. Grade A was excluded because it had no clinical impact. The overall complication rate was defined as the proportion of patients with at least one complication. Operative and hospital mortality was defined as death within 30 days after surgery or during hospitalization, respectively.

Statistical Analysis

We used the chi-squared test, Fisher's exact test and the Mann–Whitney *U*-test for statistical analysis, as appropriate. Receiver operator characteristic (ROC) curves were plotted to assess the extent to which CRS, PRS, and SSS could accurately predict morbidity. The area under the ROC curve (AUC) was used as a measure of overall diagnostic accuracy. Statistical significance was considered at $P < 0.05$.