**Advances in Brief**

**Relative Expression of E-Cadherin and Type IV Collagenase Genes Predicts Disease Outcome in Patients with Resectable Pancreatic Carcinoma**

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**Abstract**

We examined the expression level of several genes that regulate distinct steps of metastasis in formalin-fixed, paraffin-embedded, archival specimens of primary human pancreatic carcinomas from patients undergoing curative surgery. The expression of epidermal growth factor receptor, E-cadherin, type IV collagenase [matrix metalloproteinase (MMP) 2 and MMP-9], basic fibroblast growth factor, vascular endothelial growth factor/vascular permeability factor, and interleukin 8 was examined by a colorimetric in situ mRNA hybridization technique. Down-regulation of E-cadherin and up-regulation of type IV collagenase (MMP-9 and MMP-2) at the periphery of the neoplasms had significant prognostic value. The ratio of type IV collagenase expression (mean of the expression of MMP-2 and MMP-9) to E-cadherin expression (MMP: E-cadherin ratio) at the periphery of the tumors was significantly higher in patients with recurrent disease (4.7 ± 2.1) than in patients who were disease free (2.3 ± 1.7; \( P = 0.0008 \)). Death from pancreatic cancer was significantly associated with a high MMP: E-cadherin ratio (>3.0) by overall survival analysis (\( P < 0.0002 \)), whereas a low MMP: E-cadherin ratio (<3.0) was found in seven of eight patients alive 28–64 months after surgery. Multivariate analysis of overall survival showed that the MMP: E-cadherin ratio was a significant independent prognostic factor, whereas stage, nodal metastasis, and histological type were not. These data show that multiparametric analysis for several metastasis-related genes may allow physicians to assess the metastatic potential and hence predict the clinical outcome of individual patients with resectable pancreatic carcinoma.

**Introduction**

Pancreatic cancer is the fifth leading cause of adult cancer mortality (1, 2). Patients undergoing surgical resection for pancreatic adenocarcinoma at its most common site, the pancreatic head (3), have a 5-year survival rate of only 13–25% (4–6) and a median survival of only 12–24 months (4–7). The disease recurs locally in 80% of patients (8), liver metastasis appears in 50–70% of patients (8, 9), and abdominal carcinomatosis appears in 50% of patients (8, 9). At the University of Texas M. D. Anderson Cancer Center, the current management of patients with pancreatic cancer involves selective laparotomy based on accurate radiographic imaging techniques and reliable, minimally invasive techniques for biliary decompression, a standardized surgical procedure and preoperative patient management, and multimodality therapy in all patients with localized, potentially resectable disease (10, 11). This approach has maximized the overall survival of patients with localized pancreatic carcinoma and allows patients with advanced disease to avoid the morbidity of surgery (12).

The prognosis and choice of therapy for any human neoplasm, especially an aggressive disease such as pancreatic carcinoma, are based on the stage of the disease and its metastatic potential. Traditionally, these parameters have been determined by microscopic examination of tissue sections from the primary neoplasm. Unfortunately, routine histopathological examination of the primary tumor cannot always identify the patients at highest risk for recurrence (13). Recently, however, advances in molecular biology and in our understanding of the pathogenesis of cancer metastasis have provided new tools with which to predict the metastatic potential of human cancers, including pancreatic carcinoma.

Molecular analysis of human pancreatic adenocarcinomas has revealed several abnormalities of oncogenes or tumor suppressor genes, including the K-ras gene (14–16), p53 gene (16–19), p16 gene (20), and DPC4 gene (21). These studies have improved our understanding of pancreatic tumorigenesis, but not necessarily that of the metastatic potential of an individual tumor.

The metastatic potential of solid tumors has been shown to correlate directly with the expression level of several independent genes that regulate: growth (EGF-R), angiogenesis (bFGF, VEGF/vascular permeability factor, and IL-8), invasion (type IV collagenase genes), and multidrug resistance (mdr-1;Refs.

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3 The abbreviations used are: EGF-R, epidermal growth factor receptor; bFGF, basic fibroblast growth factor; IL, interleukin; MMP, matrix metalloproteinase; ISH, in situ hybridization; VEGF, vascular endothelial growth factor.
22–27). In several reports, the expression of E-cadherin, which is directly related to cell-to-cell cohesion, is inversely correlated with tumor progression and metastasis (22). Most of these correlative studies suggest that the expression of a given gene is necessary but not sufficient to account for the multistep process of metastasis (23). Because each of the discrete steps in the pathogenesis of metastasis is regulated by one or several independent genes, the identification of cells with metastatic potential in heterogeneous primary human tumors requires a multiparametric/multivariate analysis of gene expression (23).

We have previously examined the expression of these metastasis-related genes in colorectal carcinomas (22, 24, 25), gastric carcinomas (26), and prostate carcinomas (27). One factor consistently down-regulated in metastatic tumors is E-cadherin, a transmembrane glycoprotein responsible for homotypic binding and morphogenesis of epithelial tissues (28, 29). In epithelial neoplasms, reduced or absent expression of E-cadherin is associated with a decrease in cellular and tissue differentiation and a higher metastatic potential (30–37). Furthermore, transfection of E-cadherin cDNA into carcinoma cells inhibits motility and invasiveness (38, 39). Another factor altered in metastatic tumors is type IV collagenase, a member of the matrix-degrading proteinase family, the MMPs (40). Type IV collagen is a major component of blood vessel basement membrane, and its degradation is essential in tumor cell invasion of the vasculature. Type IV collagenase consists of two forms of MMPs: (a) a Mr 92,000 type IV collagenase (MMP-9, gelatinase B); and (b) a Mr 72,000 type IV collagenase (MMP-2, gelatinase A), both of which demonstrate a similar catalytic profile (41, 42). The expression of both types of MMPs directly correlates with invasion and metastasis in human carcinomas (43, 44). Because down-regulation of E-cadherin coupled with up-regulation of collagenase type IV favors invasion and metastasis, the ratio of collagenase type IV expression to E-cadherin expression was demonstrated to be associated with metastasis and recurrence of colon carcinoma (22) and distant lymph node metastasis in gastric carcinoma (26).

In this study, we extended this research to adenocarcinoma of the pancreas. We used our recently developed rapid calorimetric ISH technique to detect mRNA in frozen tissues (45) and formalin-fixed, paraffin-embedded tissues (46). We used ISH to study the expression level of several genes that regulate different steps in the metastatic cascade and conclude that the expression level of metastasis-related genes correlates with the clinical outcome of resectable human pancreatic carcinoma.

Materials and Methods

Surgical Specimens. Twenty-two formalin-fixed, paraffin-embedded, archival surgical specimens of primary pancreatic adenocarcinomas from patients treated at the University of Texas M. D. Anderson Cancer Center were chosen at random. Twenty-one patients underwent pancreaticoduodenectomy (Whipple operation), and one patient underwent distal pancreatectomy. Sixteen patients were postoperatively treated with adjuvant chemotherapy and/or radiation therapy. The clinicopathological data of these patients are detailed in Table 1. Patients were followed on a prospective basis, and the information collected was entered in a database. None of the patients received preoperative chemotherapy. The specimens were classified by stages according to the American Joint Committee on Cancer (47) and by histological types according to Solcia et al. (9). Only specimens with intact mRNA as determined by positive reaction with the poly(dT)20 probe (25, 45) were evaluated for expression of the metastasis-related genes.

Oligonucleotide Probes. Specific antisense oligonucleotide DNA probes were designed complementary to the mRNA transcripts of seven metastasis-related genes (Table 2), based on published reports of the cDNA sequences (40, 46, 48–55). The specificity of the oligonucleotide sequences was initially determined by a GenBank/European Molecular Biology Laboratory database search using the Genetics Computer Group sequence analysis program (Genetics Computer Group, Madison, WI) based on the FastA algorithm (56) that showed 100% homology with the target gene and minimal homology with nonspecific mammalian gene sequences. The specificity of each sequence was also confirmed by Northern blot analysis. A poly(dT)30 oligonucleotide was used to verify the integrity of mRNA in each sample. All DNA probes were synthesized with six biotin molecules (hybri-D-biotinylated) at the 3′ end via direct coupling using standard phosphorimidate chemistry (Research Genetics, Huntsville, AL; Refs. 57 and 58). The lyophilized probes were reconstituted to a 1 μg/μl stock solution in 10 mmol/liter Tris-HCl (pH 7.6) and 1 mmol/liter EDTA. The stock solution was diluted with Probe Diluent (Research Genetics) immediately before use. The working dilutions of each probe are shown in Table 2.

ISH. ISH was performed as described previously (45, 46) with a minor modification. ISH was carried out using the Microprobe manual staining system (Fisher Scientific, Pittsburgh, PA; Ref. 59). Tissue sections (4 μm) of formalin-fixed, paraffin-embedded specimens were mounted on Silane-coated ProbeOn slides (Fisher Scientific). The slides were placed in the Microprobe slide holder, dewaxed, and dehydrated with Autodewaxer and Autoolacohol (Research Genetics), followed by enzymatic digestion with pepsin (57). Hybridization of the probe was carried out for 60 min at 45°C, and the samples were then washed three times with 2× SSC for 2 min at 45°C. The samples were incubated for 30 min in alkaline phosphatase-labeled avidin at 45°C, briefly rinsed in 50 mm Tris buffer (pH 7.6), rinsed for 1 min with alkaline phosphatase enhancer (Biomed Corp., Foster City, CA), and incubated for 30 min with the chromogen substrate FastRed (Research Genetics) at 45°C. A positive reaction in this assay stained red. Control for endogenous alkaline phosphatase included treatment of the samples in the absence of the biotiniyalted probe and use of chromogen in the absence of any oligonucleotide probes. To check the specificity of the hybridization signal, the following controls were used: (a) RNase pretreatment of tissue sections; (b) a biotin-labeled sense probe; and (c) a competition assay with unlabeled antisense probe. A markedly decreased or absent signal was obtained after all of these treatments.

Image Analysis to Quantify Intensity of Color Reaction. Stained sections were examined in a Zeiss photomicroscope (Carl Zeiss, Inc., Thomwood, NY) equipped with a three-chip charged-coupled device color camera (model DXC-960 MD; Sony Corp., Tokyo, Japan). The images were analyzed using the Optimas image analysis software (version 5.2; Bothell, WA).
The slides were prescreened by one of the investigators to determine the range in staining intensity of the slides to be analyzed. Images covering the range of staining intensities were captured electronically, a color bar (montage) was created, and a threshold value was set in the red, green, and blue mode of the color camera. All subsequent images were quantified based on this threshold. The integrated absorbance of the selected fields was determined based on its equivalence to the mean log inverse gray scale value multiplied by the area of the field. The samples were not counterstained, so the absorbance was due solely to the product of the ISH reaction. For each section, we determined the absorbance in several 2x2-mm zones located at the center and periphery of the tumors or at the foci of perineural invasion. Three to five different fields in each 2x2-mm zone were quantified to derive an average value. The intensity of staining was standardized to that of the integrated absorbance of poly(dT)20 and determined by comparison with the integrated absorbance of nonpathological pancreatic duct epithelium, which was set at 100.

**Statistical Analysis.** The patients were stratified according to stage (47), histological type (9), invasion status at the periphery and center of the tumor, perineural invasion, amount of fibrous stroma, and hyperplastic changes in the pancreatic duct mucosa adjacent to the tumor. The level of gene expression among the groups was compared by the Mann-Whitney U test (60). Survival analysis was computed by the Kaplan-Meier method.
Expression of Metastasis-related Genes in Pancreatic Cancer

Expression of Metastasis-related Genes at the Periphery of the Lesions. All further analyses therefore examined the expression of metastasis-related genes at the periphery of the lesions. Moreover, the ratio between E-cadherin and collagenase type IV expression predicted disease-free survival. In 12 dead patients with a mean survival time of 25 ± 21 months, the mean ratio was 4.7 ± 2.1, whereas in 10 living patients with a mean survival time of 42 ± 17 months, the mean ratio was 2.3 ± 1.7. The differences in MMP:E-cadherin ratios were highly significant (P = 0.0008, Mann-Whitney U test), as were the differences in overall disease-free survival time (P = 0.0062, Fisher’s exact test) and mean survival time (P = 0.0029, Mann-Whitney U test). Moreover, 11 of 14 patients (79%) with high MMP:E-cadherin ratios (>3.0) died from recurrent disease, whereas 7 of 8 patients (88%) with low MMP:E-cadherin ratios (<3.0) were alive 28–64 months after surgery without disease.

The MMP:E-cadherin expression ratio at the periphery of the resected pancreatic cancers also correlated with the overall survival analyzed by the Kaplan-Meier method. These data, shown in Fig. 2, differed significantly between the two groups of patients (P < 0.001, log-rank test). Patient survival also correlated with the expression of E-cadherin and MMP-9 (P = 0.0008 and 0.0249, respectively, Mann-Whitney U test). Neither tumor stage (I, II, or III) nor histological classification (state of differentiation) correlated with the expression of metastasis-related genes, the ratio of MMP:E-cadherin, or overall disease-free survival.

The prognostic significance of the clinicopathological parameters (Tables 1 and 4) and the expression of metastasis-related genes were analyzed by univariate and multivariate analysis using the Cox proportional hazards model and the log normal model. The data summarized in Table 5 demonstrate that

#### Table 3 Intratumoral heterogeneity of the expression of E-cadherin, MMP-9, and MMP-2

<table>
<thead>
<tr>
<th>Site</th>
<th>No. of cases</th>
<th>E-Cadherin Mean ± SD</th>
<th>MMP-9 Mean ± SD</th>
<th>MMP-2 Mean ± SD</th>
<th>MMP:E-cadherin ratio Mean ± SD</th>
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<tbody>
<tr>
<td>Center</td>
<td>22</td>
<td>77 ± 24f</td>
<td>163 ± 56e</td>
<td>149 ± 74d</td>
<td>2.2 ± 1.3f</td>
</tr>
<tr>
<td>Periphery</td>
<td>22</td>
<td>63 ± 21f</td>
<td>200 ± 59f</td>
<td>186 ± 79e</td>
<td>3.6 ± 2.1g</td>
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<tr>
<td>Perineural invasion</td>
<td>9</td>
<td>70 ± 12</td>
<td>166 ± 31</td>
<td>147 ± 54</td>
<td>2.3 ± 0.6h</td>
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</table>

* Expressions at the periphery of the lesions
dMMP:E-cadherin was calculated as an average of the MMP-2:E-cadherin expression ratio and the MMP-9:E-cadherin expression ratio.

Results

**Intratumoral Heterogeneity for the Expression of E-Cadherin and Type IV Collagenase Genes in Pancreatic Carcinomas.** The integrity of mRNA in each sample was first verified by using a poly(dT)20 probe (25, 45). All samples had an intense histochemical reaction, indicating that the mRNA was well preserved. Previous reports analyzing the expression of metastasis-related genes in human colon carcinomas (22) and human gastric carcinomas (26) concluded that the expression level of E-cadherin and collagenase type IV varied between the center and periphery of the lesions. Moreover, the ratio between E-cadherin and collagenase type IV expression predicted disease outcome. For this reason, we examined the expression level of E-cadherin, MMP-9, and MMP-2 at the periphery (invasive edge), the center of the tumor, and in the adjacent nonpathological duct epithelium. The expression level of these metastasis-related genes differed at the periphery and center of the tumors (Table 3; Fig. 1). In 20 of 22 cases (91%), the expression of E-cadherin was significantly lower at the periphery than in the center (P = 0.0167). In contrast, the expression level of type IV collagenase was higher at the periphery as compared with the center of the tumors. In fact, up-regulation of MMP-9 and MMP-2 at the periphery was found in 19 of 22 (86%) and 20 of 22 (91%) cases, respectively. The mean expression of MMP-9 and MMP-2 was significantly higher at the periphery than at the center (P = 0.0102 and 0.0347, respectively). Because down-regulation of E-cadherin and up-regulation of type IV collagenases were found at the periphery of the tumors, the MMP:E-cadherin expression level ratio was highest at the periphery of all tumors, differing significantly from that found in the center (P = 0.0039; Mann-Whitney U test). These data agree with the previous reports (22, 26) suggesting that analysis of the metastatic potential of human neoplasms should focus on the tumor’s peripheral zone. All further analyses therefore examined the expression of metastasis-related genes at the periphery of the tumors (Table 4).

Foci of perineural invasion, a characteristic of human pancreatic adenocarcinoma (9, 64), were found and examined in nine tumors. Although most of the foci of perineural invasion were found at the periphery of the tumor, the expression of E-cadherin and type IV collagenase was similar to that found in the center of the tumor (Table 3).
the most significant independent prognostic parameter for survival was the MMP:E-cadherin ratio, followed by the expression level of E-cadherin.

Discussion

In archival surgical specimens of primary human pancreatic carcinomas, we examined the expression level of several genes that regulate steps of pancreatic cancer metastasis. The present results show that the expression of metastasis-related genes, especially the ratio between the expression of MMP-2/MMP-9 and E-cadherin, closely correlated with disease recurrence and patient survival. In contrast, nodal metastasis, advanced pathological stage, and stage of differentiation were not predictive of decreased patient survival, results that may be due to the relatively small sample size (4, 65–67). We have reported previously that the metastatic potential of human colon cancers (22) and human gastric cancers (26) can be identified by a multiparametric analysis for the expression of genes that encode for EGF-R (growth), bFGF and IL-8 (angiogenesis), E-cadherin (cohesion), and MMP-2 and MMP-9 (invasion). Specifically, the down-regulation of the cell-to-cell cohesion molecule E-cadherin (28, 29) concurrent with the up-regulation of the proteolytic enzymes MMP-2 and MMP-9 accurately predicted metastasis and disease recurrence (22, 26). Similar findings were also reported for human prostate cancer cells with low and high metastatic potential (27).

Like other neoplasms, human pancreatic adenocarcinomas consist of multiple cell types, including tumor cells and host fibroblasts, epithelial, endothelial, and infiltrating cells (68). Because metastases can be produced by a small subpopulation of tumor cells (<1.0% of the tumor; Refs. 68–70), detecting the expression of metastasis-related genes in the minority of tumor cells requires a sensitive technique. We chose ISH because it identifies the cellular source of the mRNA as well as intratumor
neoplasms directly correlate with invasion and metastasis (43, 76–79), and specific inhibitors of MMPs have been shown to inhibit tumor cell invasion (78–81). Thus, a decrease in the expression of E-cadherin and an increase in collagenase type IV activity should enhance tumor cell invasion and metastasis. Regardless of the pathological stage of the primary tumor, a high MMP:E-cadherin ratio of ≥3.0 directly correlated with disease recurrence and inversely correlated with survival. Our data conclude that this MMP:E-cadherin expression ratio is at the periphery of patients undergoing curative pancreatectomy.

Perineural invasion is characteristic of human pancreatic cancer (4, 9, 64, 82). The MMP:E-cadherin ratio in cancer cells in the foci of perineural invasion was <3.0. Thus, these cells did not share the phenotype of cancer cells at the periphery of the lesions. Perineural invasion may therefore relate to the affinity of tumor cells to migrate to peripheral neural tissue expressing neural cell adhesion molecule (83).

In summary, we used the ISH technique described here to examine the concurrent expression of metastasis-related genes in formalin-fixed, paraffin-embedded specimens of resected human pancreatic adenocarcinomas from patients undergoing curative surgery. Using quantification of gene expression by col-

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of cases</th>
<th>Survival period (mo)</th>
<th>Expressiona</th>
<th>MMP:E-cadherin ratioa</th>
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<td>133 ± 30d</td>
<td>187 ± 35</td>
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<td>II</td>
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<td>25 ± 23d</td>
<td>123 ± 21</td>
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<td>III</td>
<td>11</td>
<td>25 ± 17</td>
<td>119 ± 32</td>
<td>198 ± 67</td>
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<td>Histologyf</td>
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<td>WDA</td>
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<td>25 ± 1</td>
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<td>MDA</td>
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<td>PDA</td>
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<td>MMP:E-cadherin ratio</td>
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<td>≥3.0</td>
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| * The intensity of cytoplasmic staining quantitated by an image analyzer was compared with poly(dT)20 and then standardized by the expression of nonneoplastic pancreas duct epithelium set at 100. This normalization was repeated for each stained specimen.

# MMP:E-cadherin was calculated as an average of the MMP-2:E-cadherin expression ratio and the MMP-9:E-cadherin expression ratio.

* Stage was classified according to the American Joint Committee on Cancer Cancer Staging Manual (47). I, no direct extension and no regional nodal involvement; II, direct extension into adjacent tissue but no lymph node involvement; III, regional lymph node involvement with or without direct tumor extension.

* Mean ± SD.

* The significance in expression level was determined by the Mann-Whitney U test.

*NS, not significant (P > 0.05).

* Difference between stage II cases and stage III cases.

* Difference between stage I cases and stage II cases.

* Histology was classified according to Solcia et al. (9). WDA, well-differentiated adenocarcinoma; MDA, moderately differentiated adenocarcinoma; PDA, poorly differentiated adenocarcinoma; MUC, mucinous carcinoma.

* Significantly different; P = 0.0169 by Mann-Whitney U test.

* Difference between dead and living patients.

* Significantly different; P = 0.0062 by Fisher’s exact test.

* Significantly different; P = 0.0029 by Mann-Whitney U test.

* Difference between cases with low (<3.0) and high (≥3.0) MMP:E-cadherin ratios.

moral heterogeneity in expression, whereas Northern blot analysis reveals only the average levels of mRNA of all of the cells in a sample (25). As was the case for human colon carcinomas (22) and human gastric carcinomas (26), the expression of E-cadherin and collagenase type IV differed between the center and periphery of the pancreatic cancers.

E-Cadherin is a cell surface glycoprotein involved in calcium-dependent homotypic cell-to-cell adhesion (71). It is localized at the epithelial junction complex and is responsible for the organization, maintenance, and morphogenesis of epithelial tissues (29, 72). Reduced levels of E-cadherin are associated with a decrease in cellular/tissue differentiation and increased histological grade in different epithelial neoplasms (30–34, 73–75). Transfection of E-cadherin-encoding cDNA into invasive cancer cells has been shown to inhibit their invasiveness (38).

Once cells detach from the primary tumor, they must invade the host stroma if they are to metastasize. Degradation of blood vessel basement components, especially type IV collagen, is one of the necessary steps in metastasis. The levels of $M_c$ 72,000 and $M_c$ 92,000 type IV collagenase in human and rodent neoplasms directly correlate with invasion and metastasis (43, 44, 76–79), and specific inhibitors of MMPs have been shown to inhibit tumor cell invasion (78–81). Thus, a decrease in the expression of E-cadherin and an increase in collagenase type IV activity should enhance tumor cell invasion and metastasis. Regardless of the pathological stage of the primary tumor, a MMP:E-cadherin ratio of ≥3.0 directly correlated with disease recurrence and inversely correlated with survival. Our data conclude that this MMP:E-cadherin expression ratio is at the periphery of patients undergoing curative pancreatectomy.

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In summary, we used the ISH technique described here to examine the concurrent expression of metastasis-related genes in formalin-fixed, paraffin-embedded specimens of resected human pancreatic adenocarcinomas from patients undergoing curative surgery. Using quantification of gene expression by col-

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Table 4 Relationship between expression of metastasis-related genes and clinicopathological features

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<td>NS</td>
</tr>
<tr>
<td>P#</td>
<td>NS</td>
<td>0.0008</td>
<td>0.0249</td>
<td>NS</td>
</tr>
</tbody>
</table>

---

a The intensity of cytoplasmic staining quantitated by an image analyzer was compared with poly(dT)20 and then standardized by the expression of nonneoplastic pancreas duct epithelium set at 100. This normalization was repeated for each stained specimen.

b MMP:E-cadherin was calculated as an average of the MMP-2:E-cadherin expression ratio and the MMP-9:E-cadherin expression ratio.

c Stage was classified according to the American Joint Committee on Cancer Cancer Staging Manual (47). I, no direct extension and no regional nodal involvement; II, direct extension into adjacent tissue but no lymph node involvement; III, regional lymph node involvement with or without direct tumor extension.

d Mean ± SD.

e The significance in expression level was determined by the Mann-Whitney U test.

fNS, not significant (P > 0.05).

g Difference between stage II cases and stage III cases.

h Difference between stage I cases and stage II cases.

i Histology was classified according to Solcia et al. (9). WDA, well-differentiated adenocarcinoma; MDA, moderately differentiated adenocarcinoma; PDA, poorly differentiated adenocarcinoma; MUC, mucinous carcinoma.

j Significantly different; P = 0.0169 by Mann-Whitney U test.

k Difference between dead and living patients.

l Significantly different; P = 0.0062 by Fisher’s exact test.

m Significantly different; P = 0.0029 by Mann-Whitney U test.

n Difference between cases with low (<3.0) and high (≥3.0) MMP:E-cadherin ratios.

---
Fig. 2 Correlation of survival with the MMP:E-cadherin expression ratio in resected pancreatic cancer. Overall survival of patients with resected pancreatic carcinomas stratified by the expression ratio between MMP and E-cadherin genes in the surgical specimens was analyzed by the Kaplan-Meier method. The overall survival differed significantly between the group with tumors exhibiting a MMP:E-cadherin ratio of <3.0 and ≥3.0 ($P = 0.001$, log-rank test).

### Table 5 Univariate analysis of overall survival rates

<table>
<thead>
<tr>
<th>Covariates</th>
<th>$P$</th>
<th>Risk ratio</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Univariate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nodal metastasis</td>
<td>0.3379</td>
<td>0.5370</td>
<td>0.150–1.917</td>
</tr>
<tr>
<td>Negative, positive</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>TNM$^a$ stage</td>
<td>0.9794</td>
<td>1.0240</td>
<td>0.169–6.205</td>
</tr>
<tr>
<td>Stages I, II, III</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histological type</td>
<td>0.3425</td>
<td>0.4630</td>
<td>0.094–2.272</td>
</tr>
<tr>
<td>WDA, MDA$^b$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP:E-cadherin ratio $&lt;3.0$</td>
<td>0.0002</td>
<td>0.2600</td>
<td>0.973–1.008</td>
</tr>
<tr>
<td>Multiple regression$^c$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP:E-cadherin</td>
<td>0.0121</td>
<td>0.3220</td>
<td>0.129–0.805</td>
</tr>
<tr>
<td>$&lt;3.0$, ≥3.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E-Cadherin</td>
<td>0.7220</td>
<td>1.0140</td>
<td>0.992–1.036</td>
</tr>
<tr>
<td>MMP-9</td>
<td>0.3270</td>
<td>0.9960</td>
<td>0.990–1.002</td>
</tr>
<tr>
<td>Model 2</td>
<td></td>
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<td></td>
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<tr>
<td>Nodal metastasis</td>
<td>0.3004</td>
<td>0.7700</td>
<td>0.339–1.749</td>
</tr>
<tr>
<td>Histological type</td>
<td>0.0154</td>
<td>1.8990</td>
<td>0.730–4.938</td>
</tr>
<tr>
<td>MMP:E-cadherin ratio $&lt;3.0$</td>
<td>0.0015</td>
<td>0.3690</td>
<td>0.155–0.881</td>
</tr>
</tbody>
</table>

$^a$ $P$ was computed using the Wald statistics by the Cox proportional hazards model.

$^b$ TNM, tumor-node-metastasis.

$^c$ WDA, well-differentiated adenocarcinoma; MDA, moderately differentiated adenocarcinoma.

$^d$ Log normal model.

orimetric scanning and standardization to nonpathological duct mucosa cells allowed an examination of individual specimens. We conclude that the ratio between the expression of MMP-2/ MMP-9 and E-cadherin at the periphery of the neoplasms would accurately predict outcome. These data show that multiparametric analysis for several metastasis-related genes may allow physicians to assess the metastatic potential and hence predict the clinical outcome of individual patients with resectable pancreatic carcinoma.

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### References

Expression of Metastasis-related Genes in Pancreatic Cancer


Relative Expression of E-Cadherin and Type IV Collagenase Genes Predicts Disease Outcome in Patients with Resectable Pancreatic Carcinoma

Hiroki Kuniyasu, Lee M. Ellis, Douglas B. Evans, et al.


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