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RUNX1T1: A Novel Predictor of Liver Metastasis in Primary Pancreatic Endocrine Neoplasms

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Abstract

Objectives—Using gene expression profiling on frozen primary pancreatic endocrine tumors (PETs), we discovered RUNX1T1 as a leading candidate progression gene. This study was designed (1) to validate the differential expression of RUNX1T1 protein on independent test sets of metastatic and nonmetastatic PETs and (2) to determine if RUNX1T1 underexpression in primary tumors was predictive of liver metastases.

Methods—Immunohistochemical expression of RUNX1T1 protein was quantified using Allred scores on archival metastatic (n = 13) and non-metastatic (n = 24) primary adult PET tissues using

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custom-designed tissue microarrays. Wilcoxon rank sum/Fisher exact tests and receiver operating characteristic curves were used in the data analysis.

Results—Median RUNX1T1 scores were 2 (2–7) and 6 (3–8) in metastatic versus nonmetastatic primaries ($P < 0.0001$). Eleven of 13 metastatic and 1 of 24 nonmetastatic primaries exhibited RUNX1T1-scores of 4 or less ($P < 0.0001$). Low RUNX1T1 expression was highly associated with hepatic metastases ($P < 0.0001$), whereas conventional histological criteria (Ki-67 index, mitotic rate, necrosis) were weakly associated with metastases ($P = 0.08$ – 0.15). Considering RUNX1T1 expression (Allred) score of 4 or less to be predictive, the sensitivity to predict hepatic metastases was 85%, with a specificity of 96%.

Conclusions—RUNX1T1 protein is underexpressed in well-differentiated metastatic primary PETs relative to nonmetastatic primaries and emerges as a promising novel biomarker for prediction of liver metastases.

Keywords

RUNX1T1; immunohistochemistry; predictor of metastasis; progression marker; pancreas; endocrine neoplasm

Pancreatic endocrine tumors (PETs) are clinically challenging neoplasms. The incidence of these tumors in the United States is estimated to be 4 cases per million persons per year, with a 5-year survival of 44% to 71%.^{1–3} Based on the presence or absence of metastases and/or gross invasion of adjacent organs, they are classified as pancreatic endocrine carcinomas (PECAs) or tumors PETs (World Health Organization, 2004).⁴ Clinical behavior of these neoplasms ranges from benign to highly aggressive. In the presence of negative metastatic workup at the time of first tissue diagnosis, it is difficult to predict which of these tumors will become metastatic (“malignant”) or remain localized to the pancreas (“benign”). Conventional prognostic criteria include the presence of a functional tumor, larger tumor size, presence of metastases, higher mitotic rate, tumor necrosis, vascular invasion, perineural invasion, and higher proliferation rate, conventionally assessed as Ki-67 index.^{4–6} To further refine prognostic evaluation of these neoplasms, a number of histological grading and staging schemes have been proposed.^{5,7–8} However, it has been difficult to establish reliable prognostic models for these neoplasms, due mainly to their rarity and complex biology.

From a practical standpoint, histological tumor characteristics such as cytological atypia and angiovascular and perineural invasion are either not evident or, if present, fail to distinguish between indolent and aggressive tumors. Other frequently evaluated pathological criteria, including tumor size, mitotic count/histological grade, and Ki-67 index, often fail to predict malignant behavior.⁹ Because patients who develop distant metastases from pancreatic endocrine neoplasms are rarely cured and 5-year survival rates diminish significantly,¹⁰ identification of molecular markers of prognosis is vitally important to quantify the risk of (1) future metastases in patients with clinically localized pancreatic endocrine neoplasms and (2) metastatic recurrence in patients with resected pancreatic endocrine primaries. Such molecular markers may therefore identify patients who could benefit from adjuvant

therapies and also provide objective criteria to determine the need for postoperative surveillance.

Recently, we and others^{11–17} have carried out gene expression profiling in PETs. To identify candidate progression genes in pancreatic endocrine neoplasms, we used Affymetrix 2.0 gene chip on fresh-frozen metastatic primary PECAs (MP PECAs) that already had metastasized to the liver at the time of resection of the pancreatic primary and a set of frozen non-MP PETs that remained free of clinically detectable metastases until the last patient follow-up. All of the pancreatic endocrine primaries used in this experiment were nonfunctional. We applied rigorous criteria, taking into consideration the *P* values and fold changes in the differentially expressed genes, including gene function/class and pathway analysis using Metacore from GeneGo, to select our leading “candidate progression genes” that were differentially expressed between the 2 sets of pancreatic primaries (metastatic and nonmetastatic). Among these, CD24 antigen, insulin receptor, TMPRSS6, SERPINA1, SMURF1, RNF43, and AKR1C2 were notably up-regulated, whereas RUNX1T1, protocadherin 9, RASSF5, RERG, ST14, glucagon, and PDGFRL were notably down-regulated. The down-regulation of RUNX1T1, represented by 2 different probe sets, is shown in metastatic as compared with nonmetastatic pancreatic endocrine primaries from our original Affymetrix 2.0 gene chip analysis on RNA extracted from frozen tumor tissues (Fig. 1). From our “candidate progression gene list,” we validated underexpression of several leading candidate progression genes including RUNX1T1 and overexpression of TMPRSS6, SERPINA1, and others on an independent test set of archival PECAs (with liver metastases) using quantitative real-time polymerase chain reaction.

In this study, we have further validated the differential expression of RUNX1T1 in pancreatic endocrine neoplasms with and without synchronous liver metastases at the protein level. Furthermore, we found RUNX1T1 protein expression in the primary tumor tissue to be more informative than other conventional pathological criteria as predictors of liver metastases. This is an important finding with potential clinical and therapeutic implications that merits further evaluation in larger-scale clinical validation studies.

MATERIALS AND METHODS

Patients and Specimens

This validation study included 37 consecutive patients who met the inclusion criteria among a larger patient cohort who had undergone resection of their well-differentiated, primary PETs (27 nonfunctional, 4 gastrinomas, 2 insulinomas, 1 glucagonoma, 1 VIPoma (vasoactive intestinal polypeptide tumor), and 2 tumors with unknown functional status) at the Moffitt Cancer Center between 1996 and 2008. The study protocol was approved by the institutional review board at the University of South Florida, Tampa, Fla. Patients were identified by searching the Anatomic Pathology Database, the personal consultation files of neuroendocrine oncologists (J.S., L.K.K.) and the Moffitt Cancer Center Cancer Registry. Patients were included in the study if (1) metastases to the liver were present at the time of surgery as documented by preoperative imaging and biopsy, operative findings, and/or the surgical pathology report (metastatic primaries); or (2) there was no evidence of hepatic metastases at the time of surgery and during postoperative follow-up, which varied from 1 to

12 years (nonmetastatic primaries). Poorly differentiated neuroendocrine carcinomas and any tumors less than 0.5 cm were excluded from the study.

Histopathologic Analysis, Grading, and Staging

All archival slides were reviewed by 2 surgical pathologists (E.B.H.-J., N.A.N) in consultation with an experienced neuroendocrine pathologist (A.N.) to select the most optimal slides for (1) confirmation of the original pathological diagnosis, (2) selection of most representative archival tumor block for tissue microarray (TMA), and (3) assessment of number of mitoses per 10 high-power fields (HPFs), Ki-67 proliferation index (%) by counting up to 2000 neoplastic cells in viable tumor areas with the highest nuclear labeling, and the presence or absence of focal or geographic (fields of) tumor necrosis. The tumor size, presence or absence of regional lymph node metastases, and gross invasion of adjacent organs were determined from surgical pathology reports. Criteria established by the World Health Organization (DeLellis et al, WHO Blue Book 2004) were used to classify primary pancreatic endocrine neoplasms as either well-differentiated PECAs or nonmetastatic, clinically localized PETs, based on the presence or absence of synchronous liver metastases, respectively.

Custom Pancreatic Endocrine Tissue Microarray

A PET TMA was constructed. Each of the 37 tumors was represented by five 1-mm cores of viable tumor tissue and, where available in the selected tumor block, five to ten 1-mm cores of “adjacent nonneoplastic pancreatic tissue containing histologically normal islets” (“paratumorous islets”) were also included.

RUNX1T1 Immunohistochemistry

We used commercially available rabbit anti-human RUNX1T1 (CBFA2T1/myeloid translocation gene 8 [MTG8]) antibody (Sigma, St Louis, Mich) in 1:200 dilution to stain the PET TMA. Four-micron-thick TMA sections were immunostained for RUNX1T1 protein using the immunohistochemical (IHC) staining protocol optimized in the Tissue Core Laboratory at our institute (A.N.). The IHC staining was carried out using a Ventana Discovery XT automated system (Ventana Medical Systems, Tucson, Ariz) per manufacturer's protocol using proprietary reagents. Briefly, slides were deparaffinized on the automated system with EZ Prep solution (Ventana). Enzymatic retrieval was used with protease 1 solution (Ventana). The detection system used was the Ventana Omni UltraMap kit, and slides were then counterstained with hematoxylin. Slides were dehydrated and cover-slipped per standard tissue core laboratory protocol.

IHC Controls

RUNX1T1 antibody was optimized using human cerebellar tissue as positive control to show RUNX1T1 protein expression, where it resulted in crisp brown staining of the nuclei of cells of the granular and molecular layers. The primary antibody was replaced by normal rabbit serum in the reagent negative control slides of human cerebellar tissue, resulting in the absence of brown nuclear staining for RUNX1T1 protein.

Interpretation and Scoring of RUNX1T1 Immunostaining

A semiquantitative measure of RUNX1T1 expression, the Allred score¹⁸ (RUNX1T1 score), was determined independently by the study pathologists who were blinded to the metastatic status of each primary tumor tissue in the TMA. Any discrepancy in RUNX1T1 scores assigned by the pathologists was settled by joint review on multihead microscope. The RUNX1T1 score for each primary tumor was based on overall evaluation of all 5 tumor cores in the TMA. Essentially, the RUNX1T1 score for each specimen (metastatic or non-MP or its matched paratumorous islets) was calculated as the sum of scores for the intensity of immunostain and the percentage of cells stained. The intensity of immunostain was scored 0 to 3, where 0 = absence of staining, 1 = mild but unequivocal staining, 2 = intermediate intensity of staining, and 3 = most intense staining. The proportion of stained cells was scored 0 to 5 as follows: no cells stained = 0, less than 1% of cells stained = 1, 1% to 10% = 2, 11% to 33% = 3, 34% to 66% = 4, and greater than 66% stained = 5. The possible range of RUNX1T1 score for each tumor or its matched sample of multiple paratumorous islets was 0 to 8. In the case of variation in RUNX1T1 expression among multiple cores for a given tumor or its paratumorous islets, the average intensity and percent staining scores for all the interpretable cores were used to calculate the overall RUNX1T1 score.

Statistical Analysis

The Wilcoxon rank sum test was used to test for differences in medians of 2 independent populations. Associations between variables were assessed by Fisher's exact test. Receiver operating characteristic (ROC) analysis was used to choose the cut point in RUNX1T1 Allred score (high vs low expression) to optimize the sensitivity and specificity of RUNX1T1 protein expression for predicting the presence of liver metastases.

RESULTS

We determined RUNX1T1 protein expression by immunohistochemistry in primary pancreatic endocrine neoplasms resected from 37 patients, 18 of whom were male and 19 female, with a median age of 54 years (range, 27–79 years). All tumors were well-differentiated PETs or carcinomas (World Health Organization Classification).⁴ Twenty-five primary tumors (67%) were found in the tail of the pancreas, 11 (30%) were in the pancreatic head, and 1 (3%) was in the body. Based on documented clinical data and/or pancreatic hormone elevations, 27 (73%) were nonfunctional, 8 (22%) were functional (4 gastrinomas, 2 insulinomas, 1 glucagonoma, and 1 VIPoma), whereas functional status was unknown in 2 (5%) of the PETs. Table 1 shows the European Neuroendocrine Tumor Society (ENETS) stage and TNM classification⁶ for the pancreatic endocrine neoplasms studied. Sixteen tumors were confined to the pancreas and no larger than 4 cm (stage I–IIA, 43%), 8 tumors were larger than 4 cm or had local invasion or regional node metastasis (stage IIB–IIIB, 22%), and 13 had liver metastases (stage IV, 35%).

Clinicopathologic Characteristics and Liver Metastases

Patients with liver metastases presented at a younger median age of 48 years (range, 28–79 years) than those without metastases, who had a median age of 57 years (range 27–78

years), but the difference was not statistically significant ($P = 0.25$). Median primary tumor size of 3.9 cm (range, 1.5–11.5 cm) in patients with hepatic metastases was greater than the median tumor size of 2.5 cm (range, 0.9–10 cm) in those without ($P = 0.12$). The median mitotic rate of 2.5 mitoses/10 HPFs (range, 1–12 mitoses/10 HPFs) in primary tumors with hepatic metastases was greater than the median rate of 1 mitosis/10 HPFs (range, 1–11 mitoses/10 HPFs) in primaries without metastases ($P = 0.05$). The median Ki-67 proliferative index of 5% (range, 1%–12%) in tumors from patients with liver metastases was greater than the median Ki-67 index of 1% (range, 1%–12%) in the absence of metastases ($P < 0.05$).

Prediction of Liver Metastases by Pathological Characteristics

Pathological characteristics that have been regarded as markers of malignant behavior in well-differentiated neuroendocrine tumors are more than 2 mitoses/10 HPFs, Ki-67 index greater than 2%, and the presence of tumor necrosis (Table 2). In this analysis, these 3 criteria of malignancy had only borderline association with the presence of hepatic metastases. Neither the primary tumor size greater than 2 cm nor the presence of regional lymph node metastases was significantly associated with hepatic metastases.

RUNX1T1 Expression in Primary Pancreatic Endocrine Neoplasms and Matched Pancreatic Islets

Immunohistochemical staining for RUNX1T1 protein was nuclear both in the tumor cells (Fig. 2B) and also in the paratumorous islet cells (Figs. 2E, F). Overall, RUNX1T1 expression was decreased in MP PECA tissues (Figs. 2C, D) relative to non-MP PETs (Figs. 2A, B). Although all pancreatic endocrine neoplasms showed at least some degree of nuclear RUNX1T1 protein, this expression was most often decreased in the metastatic primaries. Eleven (85%) of 13 primary PECAs with hepatic metastases and only 1 (4%) of 24 nonmetastatic primaries had a RUNX1T1 score of 4 or less ($P < 0.0001$) (Fig. 3). Median RUNX1T1 score was 2 (range, 2–7) in the primaries with hepatic metastases, compared with a median score of 6 (range, 3–8) in the primaries without liver metastases ($P < 0.0001$). Similar to pancreatic endocrine neoplasms, nuclear RUNX1T1 protein expression was also found to be decreased in the matched paratumorous pancreatic islets from 7 metastatic primaries (median RUNX1T1 score, 3; range, 3–7) than those from 13 nonmetastatic primaries (median RUNX1T1 score, 6; range, 3–8) ($P = 0.06$).M

Prediction of Liver Metastases by RUNX1T1 Expression

Figure 4 shows the ROC curve that expresses the relationship between sensitivity and specificity of RUNX1T1 as a diagnostic test for the presence of hepatic metastases. Diagnostic tests that discriminate well have an area under the ROC curve that approaches 1, whereas those tests that perform poorly will have a curve that falls close to the diagonal dashed line and an area under the curve closer to 0.5. The area under the ROC curve of 0.9022 (95% confidence interval, 0.7818–1.0000) indicates that RUNX1T1 expression in the primary tumor tissue is a good test for predicting the presence of hepatic metastases. Furthermore, low RUNX1T1 expression in the primary tumor tissue may also be useful in predicting the degree of risk (high vs low) of future development of distant metastases.

Receiver operating characteristic curve analysis was used to choose the optimal cutoff value for RUNX1T1 score that would achieve maximum sensitivity in identifying hepatic metastases without significant decrease in specificity (increasing false-positive rate). If RUNX1T1 score of 4 or less is considered indicative of hepatic metastases, the sensitivity of this cutoff for predicting hepatic metastases is 85%, with a specificity of 96%.

DISCUSSION

Pancreatic endocrine neoplasms are uncommon tumors with a broad range of biologic behavior. Recently, we used comprehensive genome-wide expression profiling approach and discovered a novel set of candidate progression genes that were differentially expressed in surgically resected MP PECAs relative to non-MP (clinically localized) PETs. Several of these genes, including RUNX1T1,¹⁹ had not been previously identified as progression-associated genes in PETs. We have successfully validated some of these candidate progression genes at the protein level using immunohistochemistry on independent test sets of metastatic and non-MP pancreatic endocrine neoplasms. These data were presented at the recent Meetings of ENETS (2009) and the United States and Canadian Academy of Pathology (2009). We present our data to support validation of decreased expression of RUNX1T1 protein as a predictor of liver metastases in the primary well-differentiated PECAs.

In the present study, there was no significant difference in the primary tumor size between the metastatic and nonmetastatic primaries. Although mitotic count and Ki-67 indices were significantly different between the 2 groups of primaries, these 3 pathological criteria (size, mitotic count, and Ki-67 index) showed only a borderline association with liver metastases. On the other hand, presence of liver metastases at the time of surgery was nearly always associated with low RUNX1T1 protein expression in the primary PECA tissues. Furthermore, tumor mitotic rate and Ki-67 proliferative index were inversely related to RUNX1T1 protein expression. Because IHC expression of RUNX1T1 protein showed a striking difference between the 2 sets of primaries (metastatic and nonmetastatic), we used ROC curve analysis to further elucidate the relationship between sensitivity and specificity of RUNX1T1 as a prognostic test to predict the presence of liver metastases. Using a cutoff of RUNX1T1 score of 4 or less in the primary tumor tissue as a predictor of liver metastases, we found low-RUNX1T1 expression (score = 4) to have a sensitivity of 85% and specificity of 96%. In the present study, we did not have an adequate number of cases to determine association between RUNX1T1 protein expression and lymph node metastases because only 6 (16%) of 37 cases had regional lymph node metastases (Table 1). This may in part be due to the case selection bias because these cases were selected based on the presence or absence of synchronous liver metastases at the time of resection of the primary, regardless of their lymph node status. It will be important to further investigate the association between RUNX1T1 protein expression in the primary PET tissues and lymph node metastases in larger series.

In mammals, including humans, there are 3 Runt homology domain transcription factors (Runx1, Runx2, Runx3) that function as master regulatory genes for the differentiation of distinct tissues.²⁰ Runx proteins also function as cell context-dependent tumor suppressors

or oncogenes. Abnormalities in various Runx genes have been linked to neoplastic transformation, adverse prognosis, and progression in a number of malignancies.^{20–22} In recent years, a large body of literature has emerged on these genes including RUNX1 (also known as AML1) that has recently been shown to be involved in the pathogenesis of pituitary adenomas through the transforming growth factor β (TGF- β) pathway.²³ In cell culture experiments, RUNX1 and RUNX2 genes have also been shown to regulate Galectin 3 (LGALS3) expression in human pituitary tumor cells and thus contribute to pituitary tumor progression. Furthermore, IHC analyses have shown a close correlation between Galectin 3 expression and RUNX1/RUNX2 level in pituitary tumors.²⁴ These studies have contributed to improved understanding of the molecular underpinnings of genesis and progression of pituitary adenomas and may have potential biologic implications in other endocrine neoplasms, including PECAs.

RUNX1T1, also known as MTG8 or ETO, is a member of the MTG family of transcriptional corepressors and an important regulator of leukemogenesis.²⁵ In acute myeloid leukemia (M2 subtype), the t(8;21)(q22;q22) translocation produces a chimeric gene made up of the 5' region of the RUNX1 gene fused to the 3' region of RUNX1T1. The chimeric protein is thought to associate with the nuclear corepressor/histone deacetylase complex to block hematopoietic differentiation, thus resulting in leukemic transformation. The role of RUNX1T1, as an oncogene or a tumor suppressor gene, appears to be highly context dependent with regard to the effects demonstrated by the RUNX1-RUNX1T1 fusion gene in acute myeloid leukemia.^{26,27} Leukemias expressing this translocation are associated with a good prognosis compared with other subtypes.²⁸ RUNX1T1 also binds to the fourth zinc finger of Bcl-6, enhances Bcl-6 repression of genes, and forms a complex with Bcl-6 in B-cell lymphoma. Therefore, RUNX1T1 is a corepressor involved in the pathogenesis of acute leukemias and B-cell lymphomas. Furthermore, it has been a focus of novel therapeutic strategies in these malignancies.^{29–32} A genome-wide screen to detect somatic mutations of genes in colorectal cancer identified RUNX1T1 as 1 of 189 new candidate cancer genes, related to TGF- β signaling.¹⁹ Based on our gene expression data and the present validation study of well-characterized PETs with and without synchronous liver metastases, we have identified a novel role for RUNX1T1 as a prognostic indicator in these neoplasms. RUNX1T1 appears to be a promising candidate gene likely involved with the metastatic process in these tumors. The underexpression of this protein appears to be a potential marker of metastasis that may be used for early detection of clinically aggressive subsets of pancreatic endocrine neoplasms.

CONCLUSIONS

Decreased IHC expression of RUNX1T1 protein in the primary tumor tissue appears to be a surrogate end point for prediction of risk of liver metastases in patients with surgically resected primary PETs. Underexpression of this novel prognostic marker may identify patients with resected primary PETs who are at high risk for metastatic recurrence and may be candidates for closer follow-up for early detection of metastatic disease. Furthermore, RUNX1T1 protein expression in the primary tumor tissue emerges as a stronger predictor of liver metastases as compared with the other conventional pathological criteria, such as tumor size, grade, and Ki-67 index.

FUTURE DIRECTIONS

In the future, we would like to carry out further clinical validation of RUNX1T1 as a predictor of liver metastases on larger-scale retrospective and prospective series of resected primary PETs. We are also in the process of evaluating IHC expression of RUNX1T1 protein in combination with other candidate progression proteins to develop a molecular progression score, which will identify patients at high risk for metastatic recurrence. The ultimate goal will be to identify potential patient candidates for adjuvant therapy trials, thus contributing to personalized management of patients with these unpredictable neoplasms. Furthermore, because RUNX1T1 gene has CpG islands, epigenetic regulation may be responsible for down-regulation of this gene in the primary PETs that metastasize. Future investigation into the molecular mechanisms of RUNX1T1 down-regulation, such as the DNA methylation status of this gene, will provide useful insights about its role as a metastasis-associated gene in pancreatic endocrine neoplasms. Finally, the link between RUNX1T1 expression and TGF- β signaling also merits further investigation, as this may open newer opportunities for codevelopment of TGF- β inhibitor-based targeted therapy and its companion diagnostic to successfully treat metastatic progression in these unpredictable malignancies.

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FIGURE 1.

Box plots showing down-regulation of 2 of the probe sets for RUNX1T1 gene in the primary PECAs that had already metastasized to the liver (MP1 group) as compared with those that were nonmetastatic (clinically localized) at the time of their resection (NM1 group). Data from gene expression profiling experiment on frozen tumor tissues from metastatic and nonmetastatic pancreatic endocrine primaries.

FIGURE 2.

A, A primary, well-differentiated PET that had no clinical evidence of liver metastasis (non-MP PET) at the time of resection of the pancreatic primary (hematoxylin-eosin stain, original magnification $\times 200$). B, Same case as in Figure 2A: A non-MP (clinically localized) PET with distinct (2 to 3+) nuclear immunoreactivity for RUNX1T1 protein in the tumor cells (RUNX1T1 protein immunostain, original magnification $\times 200$). C, A primary, well-differentiated PECA that had clinical evidence of synchronous liver metastasis at the time of resection of the pancreatic primary (hematoxylin-eosin stain, original magnification $\times 200$).

D, Same case as in Figure 2C: An MP PECA that shows lack of nuclear expression of RUNX1T1 protein in most of the tumor cells (RUNX1T1 protein immunostain, original magnification $\times 200$). E, Section of paratumorous histologically normal pancreatic tissue featuring a pancreatic islet from an MP PECA. RUNX1T1 expression in the islet cell nuclei is lower (only scattered islet cells show nuclear positivity for RUNX1T1) as compared with the paratumorous islet from a non-MP PET in Figure 2F (RUNX1T1 protein immunostain, original magnification $\times 400$). F, Section of paratumorous histologically normal pancreatic tissue featuring a pancreatic islet from a nonmetastatic PET. RUNX1T1 expression in the islet cell nuclei is higher (most islet cells show nuclear positivity for RUNX1T1) as compared with the paratumorous islet from an MP PECA (Fig. 2E) (RUNX1T1 protein immunostain, original magnification $\times 400$).

FIGURE 3.
RUNX1T1 protein expression scores in 37 primary PETs.

FIGURE 4.

Receiver operating characteristic curve showing the relationship between sensitivity and specificity of RUNX1T1 expression in primary PET tissue as a prognostic test to predict the presence of hepatic metastases.

TABLE 1

Pathological Staging of Primary Pancreatic Endocrine Neoplasms in 37 Patients Who Underwent Surgical Resection of Their Primaries

	n (%)
ENETS stage *	
I: tumor ≤ 2 cm, confined to the pancreas	2 (5)
IIA: tumor 2–4 cm, confined to the pancreas	14 (38)
IIB: tumor >4 cm and confined to the pancreas, or local invasion of duodenum /bile duct	1 (3)
IIIA: local invasion of other adjacent organs, celiac axis, or SMA; no positive nodes	1 (3)
IIIB: regional node metastasis, no distant metastases	6 (16)
IV: distant metastasis	13 (35)

* Based on the pathological staging scheme for PETs proposed by ENETS.⁶

SMA indicates superior mesenteric artery.

TABLE 2

Association of Low RUNX1T1 Expression and Conventional Prognostic Criteria With the Presence of Hepatic Metastases

Prognostic Marker	Marker Value	Hepatic Metastases		Association <i>P</i>
		Present	Absent	
RUNX1T1 Allred score 4	Yes	11	1	<0.0001
	No	2	23	
Ki-67 >2%	Yes	9	9	0.09
	No	4	15	
2 Mitoses/10 HPFs	Yes	8	7	0.08
	No	5	17	
Necrosis	Yes	4	2	0.15
	No	8	20	
Primary >2 cm	Yes	10	19	1.00
	No	3	5	
Regional node metastases	Yes	6	6	0.47
	No	6	11	

Associations between RUNX1T1 expression, various prognostic markers of pancreatic endocrine neoplasms, and the presence of hepatic metastases were analyzed by Fisher exact test.