BJR

Received: 9 May 2016 | Revised: 6 March 2017 | Accepted: 21 March 2017

https://doi.org/10.1259/bjr.20160403

Cite this article as:
Choi SH, Kim HJ, Kim KW, An S, Hong S-M, Kim SC, et al. *DPC4* gene expression in primary pancreatic ductal adenocarcinoma: relationship with CT characteristics. *Br J Radiol* 2017; **90**: 20160403.

FULL PAPER

DPC4 gene expression in primary pancreatic ductal adenocarcinoma: relationship with CT characteristics

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Objective: To investigate the relationship between CT imaging findings and *DPC4* gene expression and to determine the prognostic value of *DPC4* gene expression to predict overall survival in patients with pancreatic ductal adenocarcinoma.

Methods: Between January and December 2011, we retrospectively analyzed 163 pancreatic ductal adenocarcinomas in 163 patients who had undergone surgical resection (mean age = 61.8 years; range = 35–81 years). We divided the study patients into two groups according to *DPC4* gene expression: *DPC4*-expression or *DPC4*-non-expression group. The CT findings were analyzed by two reviewers. The associations between the CT imaging findings and *DPC4* gene expression were evaluated using univariate analysis and multivariate logistic regression analysis. Overall survival was compared according to the *DPC4* gene expression (*DPC4*-expression *vs* *DPC4*-non-expression) using Kaplan–Meier analysis and log-rank testing. To avoid bias, subgroup analyses of CT findings in T3 tumour and overall survival in patients with T3 tumour and R0 resection were performed.

Results: Between *DPC4*-expression group ($n = 75$) and *DPC4*-non-expression group ($n = 88$), three CT findings (*i.e.*, tumour margin, peripancreatic infiltration, and the presence of background intraductal pancreatic mucinous neoplasm) were significantly different in univariate analysis. Of these, a well-defined tumour margin was significantly associated with *DPC4*-expression tumour (adjusted odds ratio = 2.06; $p = 0.032$) in multivariate analysis. Of the total 163 patients, the mean overall survival of the *DPC4*-expression group was significantly longer than that of the *DPC4*-non-expression group (30.0 *vs* 22.0 months; $p = 0.049$). Of the 150 T3 tumours, the presence of well-defined tumour margins was also a significant CT finding (adjusted odd ratio = 2.00; $p = 0.044$) in multivariate analysis. However, of 131 patients with T3 tumour and R0 resection, the overall survival period of the *DPC4*-expression group was not significantly different from that of the *DPC4*-non-expression group (24.0 *vs* 22.0 months; $p = 0.240$).

Conclusion: The presence of well-defined tumour margins on CT was significantly linked with *DPC4*-expression tumour.

Advances in knowledge: A well-defined tumour margin is an independent CT finding associated with *DPC4*-expression pancreatic ductal adenocarcinoma.

INTRODUCTION

Pancreatic ductal adenocarcinoma has a poor prognosis, typically showing aggressive local invasion and early metastasis.[1,2] Although surgical resection is the known curative treatment for pancreatic cancer, the post-resection 5-year survival rate is reportedly only 9–21%.[3] Adjuvant systemic chemotherapy and/or locoregional radiation therapy may provide survival benefits to some select patients; however, those therapies are generally highly toxic and may cause complications. Thus, administering tailored treatment strategies based on the patient's tumour biology may be helpful for managing patients with pancreatic ductal adenocarcinoma. Exploring the genetic and molecular biology of these tumours is an important starting point for planning such individualized treatment strategies.

Recent advances in pancreatic ductal adenocarcinoma biology have led to the discovery of recurrent genetic mutations in *K-ras*, *p53* and *DPC4* and the identification of the core signalling pathways for this disease. Several studies have attempted to correlate the genetic alterations with the clinical features of this cancer,[3–9] which reportedly show

that the loss of *DPC4* (a tumour suppressor gene) and *K-ras* mutation (an oncogene) predict overall survival.[3] Notably, Iacobuzio-Donahue et al[9] recently reported that *DPC4* gene expression can be a good predictive biomarker, in that the *DPC4* non-expression is highly associated with widespread metastasis, whereas *DPC4* expression is associated with locally destructive tumours. Therefore, the *DPC4* gene expression may be an important factor for stratifying pancreatic ductal adenocarcinoma patients to treatment strategies such as systemic chemotherapy or local control. However, the Iacobuzio-Donahue et al[9] study was conducted based on post-mortem analysis rather than in the clinic. In clinical practice, imaging studies such as CT or MRI are the best methods for evaluating tumour growth patterns. So far, there have been no studies on the association between *DPC4* gene expression and the tumour growth patterns of pancreatic ductal adenocarcinoma on imaging modalities.

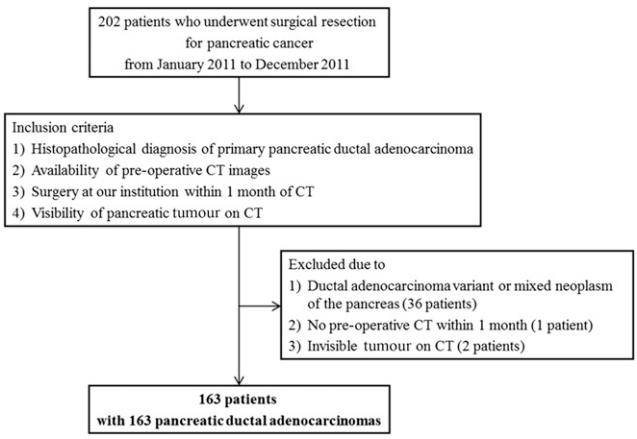
Based on the results of prior studies,[3,8,9] we hypothesized that imaging analysis in clinical practice would show different tumour growth patterns according to the *DPC4* gene expression and that *DPC4* gene expression would predict overall survival in a pancreatic ductal adenocarcinoma cohort. To test our hypothesis, we conducted our present study using a retrospective cohort at a single institution.

METHODS AND MATERIALS

Study population

Asan Medical Center institutional review board approved this retrospective study and waived the requirement for informed consent. We performed a systematic computerized search of Asan Medical Center's database for the terms "pancreas ductal adenocarcinoma" (as the pathologic diagnosis) and "pancreatectomy" (as the procedure code). The patient selection process is presented in Figure 1. Using this search strategy, we identified 202 consecutive patients with pancreatic adenocarcinoma who underwent surgical resection at our hospital between January and December 2011. The inclusion criteria were as follows: (a) histopathological diagnosis of pancreatic ductal adenocarcinoma; (b) pre-operative CT; (c) surgery at our institution within 1 month of CT; and (d) the presence of visible tumours on CT. Among these 202 patients, we excluded 36 cases with the ductal adenocarcinoma variants or mixed neoplasms of the pancreas, 1 patient who did not undergo pre-operative CT within 1 month and 2 patients with an invisible pancreatic mass on CT. Therefore, 163 patients with pancreatic ductal adenocarcinoma were included in this study.

Figure 1. Diagram for selecting the study population.



Review of the medical records

The medical records of the study patients were reviewed to identify each patient's age, sex, initial presentation, surgical history, tumour location, tumour stage, tumour grade and residual tumour. The initial presentation was categorized as "symptomatic" or "incidental". Tumours were classified as symptomatic if the patient presented with the signs and symptoms related to the tumour, *e.g.* abdominal pain or jaundice etc. All other tumours were categorized as incidental. The location of the tumour was categorized as the pancreas head or body/tail based on the pathological reports. The stage of the tumour and presence of a residual tumour (R0, negative resection margins; R1, microscopically positive margins; and R2, macroscopically positive margins) were recorded according to the TNM staging criteria of the American Joint Committee on Cancer.[10]

Immunohistochemical staining for *DPC4*

Immunohistochemical staining was performed at the immunohistochemical laboratory of the Department of Pathology, Asan Medical Center. Whole, representative cancer sections from the available paraffin blocks were labelled with *DPC4*. Briefly, 4-μm-thick tissue sections were deparaffinized and hydrated in xylene and then in serially diluted ethanol solutions, respectively. Endogenous peroxidase was blocked by incubation in 3% hydrogen peroxide for 10 min, and then heat-induced antigen retrieval was performed. The primary antibody for *DPC4*/SMAD4 (SC7966, clone B-8, mouse monoclonal, 1 : 500 dilution; Santa Cruz Biotechnology, Inc., Dallas, TX) was used to stain the section using the Ventana Autostainer (Ventana Medical Systems, Tucson, AZ) according to the manufacturer's protocol. The primary antibody was incubated for 32 min at room temperature, and the sections were counterstained with haematoxylin, dehydrated in ethanol and cleared in xylene. The *DPC4*-labelled area was scored as 0–3 if $<$10%, 10–33%, 34–66% or $>$67% of the area was stained, respectively; a score of 0 indicated total *DPC4* non-expression, whereas Scores 1–3 were considered to indicate *DPC4*-expression labelling.[11,12]

Image analysis

All CT examinations were performed using a Sensation 16 (Siemens Medical Systems, Erlangen, Germany), Somatom® Definition scanner (Siemens Medical Systems), LightSpeed® 16 (GE Healthcare, Milwaukee, WI) or LightSpeed VCT® scanner (GE Healthcare). Non-enhanced, arterial and portal venous-phase images were obtained in 150 patients. Non-enhanced and portal venous-phase images were obtained in 13 patients. For contrast-enhanced CT, 100–120 ml of iopromide (Ultravist® 370 or Ultravist 300; Bayer Schering Pharma, Berlin, Germany) was intravenously administered at a rate of 3 ml s^{-1} using an automatic power injector. The scan parameters, reconstruction thickness and the delay time for the arterial phase of each CT scanner are summarized in Table 1.

Table 1. Scan parameters, reconstruction thickness and delay time for the arterial-phase images obtained using each CT scanner

Variable	Somatom® Sensation 16 (Siemens Medical Systems, Erlangen, Germany)	Somatom Definition (Siemens Medical Systems)	LightSpeed® 16 (GE Healthcare, Milwaukee, WI)	LightSpeed VCT® (GE Healthcare)
Beam collimation (mm)	16 × 0.75	64 × 0.6	16 × 1.25	64 × 0.625
Beam pitch	1	1	0.984	0.984
Gantry rotation time (s)	0.5	0.5	0.5	0.5
kV/mAs[a]	120/200	120/200	120/200	120/200
Reconstruction thickness				
Axial pre/arterial/portal (mm)	5/3/3	5/3/3	5/2.5/2.5	5/2.5/2.5
Coronal arterial/portal (mm)	5/5	5/5	5/5	5/5
Delay time for the arterial phase (s)[b]	10	15	10	15

[a]Automated dose modulation using the maximum allowable tube current.
[b]After attenuation of the aorta at the thoracolumbar junction had reached 100 HU.

The qualitative CT findings were reviewed by the consensus of two radiologists (14 and 7 years of experience in abdominal radiology, respectively). These reviewers knew that the patients in the study population had pancreatic ductal adenocarcinoma, but they were blinded to the initial presentations, operative findings, pathological reports, *DPC4* gene expression and radiological reports. The reviewers evaluated tumour size, tumour location, homogeneity, tumour margins, intratumoural calcification, organ invasion, pancreatic duct dilatation, upstream pancreatic atrophy, bile duct dilatation, arterial invasion, venous invasion, peripancreatic infiltration, the presence of intraductal pancreatic mucinous neoplasm (IPMN) in the background parenchyma, lymph node enlargement and tumour density on the arterial and portal venous phases.

The longest diameter of the tumour on the axial images was measured by a radiologist. The tumour location was categorized as either the pancreas head or body/tail. A tumour located to the right of the left edge of the portal–superior mesenteric vein confluence was considered to be a tumour on the pancreas head, and a tumour located to the left of the left edge of the portal–superior mesenteric vein confluence was considered a tumour on the pancreas body/tail.[13] The homogeneity of the tumour was categorized as homogeneous or heterogeneous. A tumour with mixed-density necrosis or haemorrhage in >70% of the lesion was considered heterogeneous; otherwise, the tumour was considered homogeneous. The tumour margins were categorized as well or ill defined. In order to evaluate the tumour margin, we used the axial image that showed the longest diameter of the tumour. On this axial image, we assessed for the presence of spiculation/infiltration at the tumour margin. If the spiculated or infiltrative involvement of tumour margins was >70% (252°/360°), the tumour was considered as ill defined; otherwise, the tumour was considered well defined. The tumour margins were evaluated in only 150 of the pancreatic ductal adenocarcinomas in our series because we could not evaluate the margins in 13 adenocarcinomas that arose from IPMNs in the background parenchyma. A tumour arising from an IPMN in the background parenchyma was determined when a soft-tissue-enhancing mass was present in the background pancreatic parenchyma and demonstrated a diffuse pattern of pancreatic ductal dilatation or a segmented cystic appearance.[14,15] The presence of calcification within the tumour was evaluated on non-enhanced CT. We considered intratumoural calcification when high attenuating foci in the tumour (visually opaque as bone or >200 HU) was noted.

Pancreatic duct dilatation was defined as the main pancreatic duct that measured ≥4 mm in diameter. Upstream atrophy was defined as the presence of decreased pancreatic parenchymal volume distal to the tumour. Bile duct dilatation was defined as the dilatation of both the extrahepatic bile duct (>8 mm) and intrahepatic bile duct (>2 mm). Peripancreatic infiltration was defined as peritumoural fatty stranding, and adjacent organ invasion was defined as encasement or infiltration into the adjacent organs such as the duodenum, stomach, kidneys or spleen.[16] To evaluate vascular invasion, we used the following criteria: tumour thrombus, vessel occlusion, stenosis, contour deformity and more than half of the perimeter in contact with the tumour.[17] We evaluated all possible adjacent vessels, including the celiac trunk, common hepatic artery, superior mesenteric artery, gastroduodenal artery, pancreaticoduodenal artery, splenic artery, portal vein, superior mesenteric vein, splenic vein, gastroepiploic vein and gastrocolic trunk, because our aim was to evaluate the tumour characteristics and not surgical resectability. Lymph node enlargement was defined by a short axis measuring >1 cm, abnormal round morphology or central necrosis.[13]

The pancreatic tumours were compared in terms of density within the pancreatic parenchyma on visual assessment and

classified as hypodense, isodense or hyperdense on arterial-phase and portal venous-phase images. Also, the arterial and portal enhancement ratio was determined using the Hounsfield unit (HU) values on the contrast-enhanced arterial-phase and portal venous-phase images by manually drawing a region of interest within the tumour and the downstream parenchyma. If there was insufficient pancreatic parenchyma downstream of the tumour, the region of interest was placed on the pancreatic parenchyma upstream of the tumour.[18] The arterial enhancement ratio was defined as the HU value of the tumour divided by the HU value of the pancreatic parenchyma as measured on arterial-phase imaging. The portal enhancement ratio was defined as the HU value of the tumour divided by the HU value of the pancreatic parenchyma as measured on the portal-phase images. Because arterial-phase images were only available for 150 patients, the arterial-phase density and arterial enhancement ratios were evaluated in these cases.

Statistical analysis

To determine any relationship between the demographic and clinical characteristics and *DPC4* gene expression, the demographic and clinical characteristics of the *DPC4*-expression patients and *DPC4*-non-expression patients were compared using the Fisher exact or χ^2 tests (for categorical variables) or the Student's *t*-test (for continuous variables). The associations between *DPC4* gene expression and the CT findings of the pancreatic ductal adenocarcinomas were analyzed using univariate analysis with the Fisher exact or χ^2 tests (for categorical variables) or the Student's *t*-test (for continuous variables). Subsequently, among the CT findings that demonstrated potential significance on univariate analysis, multivariate logistic regression analysis was performed to determine any independently significant CT findings that predict *DPC4*-expression tumour.

To evaluate the relationship between overall patient survival and the *DPC4* gene expression, the overall survival rates at 1, 2 and 3 years were analyzed and compared between *DPC4*-expression and *DPC4*-non-expression patients using the Fisher exact test. In addition, we evaluated the relationship between overall patient survival and the independent CT finding for predicting *DPC4*-expression tumour. According to the *DPC4* gene expression and the presence of independent CT finding, the survival curves were drawn using the Kaplan–Meier method, and univariate comparisons were performed using the log-rank test. Overall survival was calculated from the date of pancreatic surgery to the date of death. The last date of data collection was 15 April 2015.

To minimize bias, we performed subgroup analysis of CT findings in T3 tumours using univariate analysis and multivariate logistic regression analysis. We calculated the sensitivity, specificity, positive-predictive value and negative-predictive value of this significant CT finding for predicting *DPC4*-expression tumour. In addition, subgroup analysis of overall survival in patients with T3 tumours and R0 resection was performed. These statistical analyses were performed using SPSS® v. 21.0 statistical software (IBM Corp., New York, NY; formerly SPSS Inc., Chicago, IL). In this study, $p < 0.05$ was considered statistically significant.

RESULTS

Patient characteristics

The demographic and pathologic characteristics of the 163 included patients are summarized in Table 2. There were 92 males (mean age = 61.7 years; range = 41–81 years) and 71 females (mean = 62.0 years; range = 35–78 years), with a mean age of 61.8 years (range = 35–81 years). Most tumours were symptomatically detected (68.1%; 111 of 163 patients) and diagnosed as Stage II (93.3%; 152 of 163 patients). Among the 163 patients with pancreatic ductal adenocarcinoma, 75 patients (46.0%) demonstrated *DPC4* expression and 88 patients (54.0%) demonstrated *DPC4* non-expression on the immunohistochemical analysis. There were no significant differences in any basic demographic/clinical characteristics between the *DPC4*-expression and *DPC4*-non-expression groups ($p \geq 0.05$).

CT findings associated with *DPC4* gene expression in overall subjects

Of the 163 tumours, there were 8 T1 tumours, 5 T2 tumours and 150 T3 tumours. There was no T4 tumour. The univariate analyses showed that three CT findings—tumour margin, background IPMN and peripancreatic infiltration—were significantly different between the *DPC4*-expression patients and *DPC4*-non-expression patients (Table 3). Notably, *DPC4*-expression tumours tended to be well defined (55.4%) in comparison with *DPC4*-non-expression tumours (37.6%) ($p = 0.031$ according to the Fisher exact test) (Figures 2 and 3). The presence of the background IPMN was more frequently observed in *DPC4*-expression tumours than *DPC4*-non-expression tumours (13.3% *vs* 3.4%; $p = 0.020$). Peripancreatic infiltration was observed more frequently in *DPC4*-non-expression tumours than *DPC4*-expression tumours (94.3% *vs* 81.3%; $p = 0.010$).

According to the multivariate logistic regression analysis using backward elimination with tumour margins, peripancreatic infiltration and IPMN background as covariates, the presence of well-defined tumour margins was the single, independently significant CT finding for predicting a *DPC4*-expression tumour (adjusted odds ratio = 2.06; 95% confidence interval = 1.07–3.97; $p = 0.032$). The other covariates were eliminated from the multivariate model.

Relationship between the *DPC4* gene expression and overall survival in overall subjects

The median follow-up period for all 163 study patients was 24.0 months (95% confidential interval = 20.3–27.7 months). *DPC4*-non-expression significantly affected overall survival ($p = 0.049$; Figure 4). The median overall survival period of the *DPC4*-expression group was 30.0 months and that of the *DPC4*-non-expression group was 22.0 months. The estimated 1-, 2- and 3-year overall survival rates of the *DPC4*-expression group (76.0%, 57.3%, and 37.3%, respectively) were better than those of the *DPC4*-non-expression group (71.6%, 38.6%, 27.3%, respectively). However, the *p*-values for the differences ($p = 0.449$, 0.001, and 0.058, respectively) were only statistically significant for the 2-year survival rate. Based on the tumour margin, the median overall survival period of the well-defined

Table 2. Baseline demographic and pathological tumour characteristics of the 163 patients with primary pancreatic ductal adenocarcinoma

Variable	*DPC4*-expression ($n = 75$)	*DPC4*-non-expression ($n = 88$)	*p*-value
Age (years)	61.9 ± 9.5	61.8 ± 9.1	0.941
Sex (M : F)	43 : 32	49 : 39	0.832
Tumour location			
Head	48 (64.0%)	64 (72.7%)	0.231
Body/tail	27 (36.0%)	24 (27.3%)	
Initial presentation			
Symptomatic[a]	48 (64.0%)[a]	63 (71.6%)[a]	0.300
Incidental	27 (36.0%)	25 (28.4%)	
Type of operation			
Total pancreatectomy	11 (14.7%)	13 (14.8%)	0.985
Pancreaticoduodenectomy	46 (61.3%)	51 (58.0%)	0.661
Distal pancreatectomy	18 (24.0%)	24 (27.3%)	0.634
Tumour grade			
Grade 1	11 (14.7%)	9 (10.2%)	0.569
Grade 2	50 (66.7%)	65 (73.9%)	
Grade 3	14 (18.7%)	14 (15.9%)	
T stage			
T1	5 (6.7%)	3 (3.4%)	0.179
T2	4 (5.3%)	1 (1.1%)	
T3	66 (88.0%)	84 (95.5%)	
Tumour stage (TNM)			
I	8 (10.7%)	3 (3.4%)	0.066
II	67 (89.3%)	85 (96.6%)	
Residual tumour			
R0	67 (89.3%)	75 (85.2%)	0.435
R1	8 (10.7%)	13 (14.8%)	
R2	0 (0.0%)	0 (0.0%)	

F, female; M, male.
Data are represented as mean ± standard deviation or number (percentage).
[a]111 patients with initial symptoms, including 74 patients with pain, 30 patients with jaundice, 5 patients with indigestion and 2 patients with nausea and vomiting.

tumour group was not significantly different from that of the ill-defined tumour group (27.0 *vs* 22.0 months; $p = 0.070$).

Subgroup analysis of CT findings in T3 tumours and overall survival in patients with T3 tumour and R0 resection

Of the 150 T3 tumours, there were 66 *DPC4*-expression tumours (44.0%) and 84 *DPC4*-non-expression tumours (56.0%). *DPC4*-expression tumours showed well-defined tumour margin (54.1% *vs* 37.0%; $p = 0.043$) more frequently than *DPC4*-non-expression tumours, and *DPC4*-non-expression tumour showed peripancreatic infiltration (97.6% *vs* 87.9%; $p = 0.018$) more frequently than *DPC4*-expression tumour. The other CT findings did not show any significant difference between the two groups (Table 4). According to the multivariate logistic regression analysis, the presence of well-defined tumour margins was the independently significant CT finding for predicting a *DPC4*-expression tumour (adjusted odds ratio = 2.00; 95% confidence interval = 1.02–3.94; $p = 0.044$). Using this CT finding, the sensitivity, specificity, positive-predictive value and negative-predictive value for predicting *DPC4*-expression tumour were 54.1%, 63.0%, 52.4% and 64.6%, respectively.

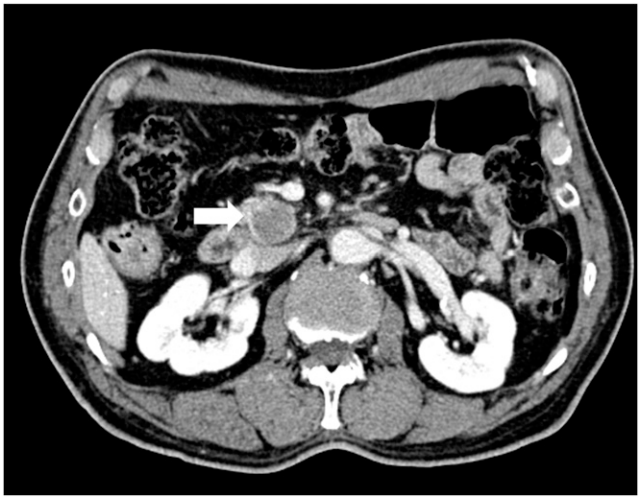
Regarding the relationship between *DPC4* gene expression and overall survival, of the 131 patients with T3 tumour and R0

Table 3. Univariate analysis of the CT features and *DPC4* gene expression in the total 163 patients with pancreatic ductal adenocarcinoma

Variable		*DPC4*-expression ($n = 75$)	*DPC4*-non-expression ($n = 88$)	*p*-value
Tumour size (mm)	Longest axial diameter	29.0 ± 12.2	28.4 ± 9.9	0.738
Tumour location	Head	48 (64.0%)	64 (72.7%)	0.231
	Body/tail	27 (36.0%)	24 (27.3%)	
Tumour homogeneity	Homogeneous	13 (17.3%)	11 (12.5%)	0.385
	Heterogeneous	62 (82.7%)	77 (87.5%)	
Tumour margins[a]	Well defined	36 (55.4%)	32 (37.6%)	0.031
	Ill defined	29 (44.6%)	53 (62.4%)	
IPMN background	Presence	10 (13.3%)	3 (3.4%)	0.020
	Absence	65 (86.7%)	85 (96.6%)	
Intratumoural calcification	Presence	3 (4.0%)	1 (1.1%)	0.239
	Absence	72 (96.0%)	87 (98.9%)	
Pancreatic duct dilatation	Presence	54 (72.0%)	68 (77.3%)	0.439
	Absence	21 (28.0%)	20 (22.7%)	
Upstream atrophy	Presence	19 (25.3%)	26 (29.5%)	0.549
	Absence	56 (74.7%)	62 (70.5%)	
Bile duct dilatation	Presence	36 (48.0%)	47 (53.4%)	0.491
	Absence	39 (52.0%)	41 (46.6%)	
Peripancreatic infiltration	Presence	61 (81.3%)	83 (94.3%)	0.010
	Absence	14 (18.7%)	5 (5.7%)	
Organ invasion	Presence	15 (20.0%)	9 (10.2%)	0.079
	Absence	60 (80.0%)	79 (89.8%)	
Artery invasion	Presence	23 (30.7%)	35 (39.8%)	0.226
	Absence	52 (69.3%)	53 (60.2%)	
Vein invasion	Presence	23 (30.7%)	32 (36.4%)	0.443
	Absence	52 (69.3%)	56 (63.6%)	
Lymph node enlargements	Presence	39 (52.0%)	40 (45.5%)	0.405
	Absence	36 (48.0%)	48 (54.5%)	
Arterial-phase density[b]	Hypodensity	69 (95.8%)	74 (94.9%)	0.780
	Isodensity	3 (4.2%)	4 (5.1%)	
	Hyperdensity	0 (0.0%)	0 (0.0%)	
Portal-phase density	Hypodensity	69 (94.5%)	82 (93.2%)	0.726
	Isodensity	4 (5.5%)	6 (6.8%)	
	Hyperdensity	0 (0.0%)	0 (0.0%)	
Arterial enhancement ratio (%)[b]		0.60 ± 0.16	0.56 ± 0.16	0.225
Portal enhancement ratio (%)		0.64 ± 0.17	0.65 ± 0.17	0.715

IPMN, intraductal papillary mucinous neoplasm.
[a]Tumour margin was evaluated in 150 pancreatic ductal adenocarcinomas because 13 adenocarcinomas with an IPMN in the background parenchyma could not be evaluated.
[b]Arterial-phase density was assessed in 150 pancreatic ductal adenocarcinomas due to the unavailability of arterial-phase images in 13 adenocarcinomas. The arterial enhancement ratio and arterial/portal enhancement ratio were calculated in 150 pancreatic ductal adenocarcinomas.

Figure 2. A 75-year-old male with *DPC4*-expression pancreatic ductal adenocarcinoma. The transverse portal venous-phase CT scan showed a 4.1-cm, well-defined, hypodense tumour (arrow) in the pancreatic head. Pylorus-preserving pancreaticoduodenectomy was performed. The patient was alive throughout the 50-month follow-up period.



resection patients, the overall survival period of the *DPC4*-expression group was not significantly different from that of the *DPC4*-non-expression group (24.0 *vs* 22.0 months; $p = 0.240$). Based on the tumour margin, of the 125 patients with T3 tumour and R0 resection patients who were available for evaluation of the tumour margin, the median overall survival period of the well-defined tumour group was 20.0 months and that of the ill-defined tumour group was 22.0 months. There was no significant difference in overall survival period between the two groups ($p = 0.195$; Figure 5).

Figure 3. A 55-year-old female with *DPC4*-non-expression pancreatic ductal adenocarcinoma. The transverse portal venous-phase CT scan showed a 3.6-cm, ill-defined, hypodense tumour (arrow) in the pancreatic head. The Whipple procedure was performed. This patient died 4 months after surgery.

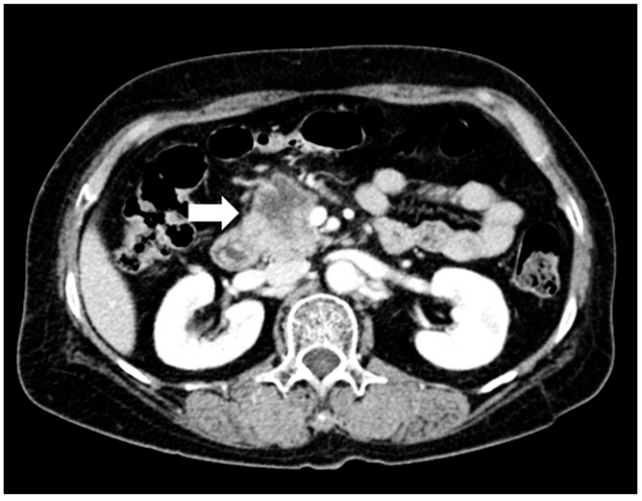
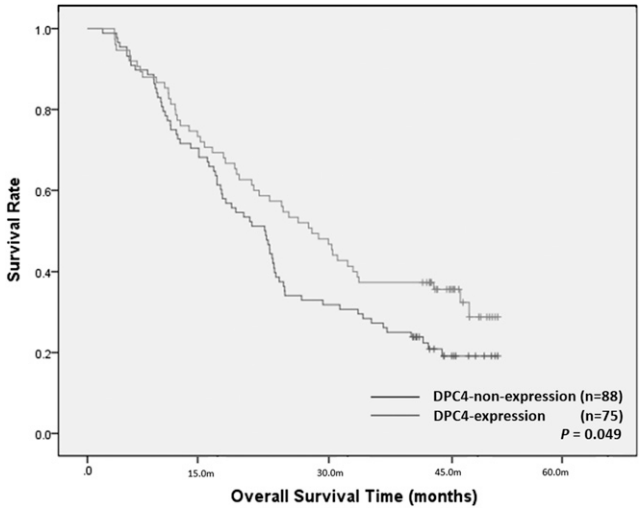


Figure 4. Overall survival times after surgical resection in the 163 patients with pancreatic ductal adenocarcinomas. The Kaplan–Meier curves for overall survival based on *DPC4* gene expression demonstrated a higher overall survival rate in the *DPC4*-expression group ($n = 75$) than in the *DPC4*-non-expression group ($n = 88$) ($p = 0.049$).



DISCUSSION

As expected, our current study results showed that the well-defined tumour margins on CT were independently and significantly associated with the *DPC4*-expression tumour in both overall subjects (adjusted odds ratio = 2.06; $p = 0.032$) and T3 tumour subgroup (adjusted odds ratio = 2.00; $p = 0.044$). Although *DPC4* non-expression significantly affected overall survival in the total 163 patients ($p = 0.049$), of the 131 patients with T3 tumour and R0 resection, the overall survival period of the *DPC4*-expression group was not significantly different from that of the *DPC4*-non-expression group (24.0 *vs* 22.0 months; $p = 0.240$).

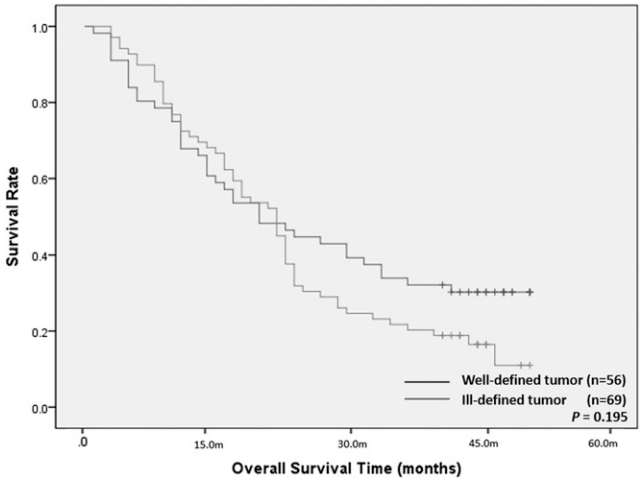
In pancreatic ductal adenocarcinoma, the *DPC4* gene is one of the most important tumour-suppressor genes,[3,19] as it demonstrates a 55–66% inactivation rate.[20,21] In our current study, the frequency of *DPC4* gene non-expression was 54.0%, which is similar to the values reported in previous studies.[3,20,21] According to the results of recent studies, *DPC4*-expression tumours tend to manifest as local disease rather than widespread disease.[3,9,22] Our present study demonstrated that *DPC4*-expression tumours were more likely to be well defined and showed, less frequently, peripancreatic infiltration than *DPC4*-non-expression tumours. We observed that well-defined tumour margins are significantly associated with *DPC4*-expression tumour in patients with pancreatic ductal adenocarcinoma. Considering the fact that the presence of well-defined tumour margins is representative of less infiltrative disease behaviour, this imaging feature could be well correlated with the locally limited disease pattern of *DPC4*-expression tumour. Although our results were modest, *DPC4* gene expression may enable clinicians to stratify patients to receive either chemoradiotherapy when the *DPC4* gene is expressive or only systemic chemotherapy when the *DPC4* gene is non-expressive.[9] However, this

Table 4. Univariate analysis of the CT features and *DPC4* gene expression in 150 patients with T3 tumours

Variable		*DPC4*-expression ($n = 66$)	*DPC4*-non-expression ($n = 84$)	*p*-value
Tumour size (mm)	Longest axial diameter	29.7 ± 11.8	28.9 ± 9.8	0.985
Tumour location	Head	41 (62.1%)	61 (72.6%)	0.217
	Body/tail	25 (37.9%)	23 (27.4%)	
Tumour homogeneity	Homogeneous	13 (19.7%)	11 (13.1%)	0.274
	Heterogeneous	53 (80.3%)	73 (86.9%)	
Tumour margins[a]	Well defined	33 (54.1%)	30 (37.0%)	0.043
	Ill defined	28 (45.9%)	51 (63.0%)	
IPMN background	Presence	5 (7.6%)	3 (3.6%)	0.279
	Absence	61 (92.4%)	81 (96.4%)	
Intratumoural calcification	Presence	3 (4.5%)	1 (1.2%)	0.206
	Absence	63 (95.5%)	83 (98.8%)	
Pancreatic duct dilatation	Presence	49 (74.2%)	66 (78.6%)	0.534
	Absence	17 (25.8%)	18 (21.4%)	
Upstream atrophy	Presence	17 (25.8%)	25 (29.8%)	0.588
	Absence	49 (74.2%)	59 (70.2%)	
Bile duct dilatation	Presence	34 (51.5%)	44 (52.4%)	0.916
	Absence	32 (48.5%)	40 (47.6%)	
Peripancreatic infiltration	Presence	58 (87.9%)	82 (97.6%)	0.018
	Absence	8 (12.1%)	2 (2.4%)	
Organ invasion	Presence	14 (21.2%)	9 (10.7%)	0.077
	Absence	52 (78.8%)	75 (89.3%)	
Artery invasion	Presence	23 (34.8%)	35 (41.7%)	0.385
	Absence	43 (65.2%)	49 (58.3%)	
Vein invasion	Presence	23 (34.8%)	32 (38.1%)	0.682
	Absence	43 (65.2%)	52 (61.9%)	
Lymph node enlargements	Presence	36 (54.5%)	39 (46.4%)	0.324
	Absence	30 (45.5%)	45 (53.6%)	
Arterial-phase density[b]	Hypodensity	62 (95.4%)	70 (94.6%)	0.832
	Isodensity	3 (4.6%)	4 (5.4%)	
	Hyperdensity	0 (0.0%)	0 (0.0%)	
Portal-phase density	Hypodensity	62 (93.9%)	79 (94.0%)	0.978
	Isodensity	4 (6.1%)	5 (6.0%)	
	Hyperdensity	0 (0.0%)	0 (0.0%)	
Arterial enhancement ratio (%)[b]		0.60 ± 0.16	0.56 ± 0.16	0.224
Portal enhancement ratio (%)		0.65 ± 0.18	0.65 ± 0.17	0.979

IPMN, intraductal papillary mucinous neoplasm.
[a]Tumour margin was evaluated in 142 pancreatic ductal adenocarcinomas because 8 adenocarcinomas with an IPMN in the background parenchyma could not be evaluated.
[b]Arterial-phase density was assessed in 139 pancreatic ductal adenocarcinomas due to the unavailability of arterial-phase images in 11 adenocarcinomas. The arterial enhancement ratio and arterial/portal enhancement ratio were calculated in 139 pancreatic ductal adenocarcinomas.

Figure 5. Overall survival times after surgical resection in the 125 patients with T3 tumour and R0 resection who were available for evaluation of the tumour margin. There was no significant difference in overall patient survival between the well-defined tumour group ($n = 56$) and the ill-defined tumour group ($n = 69$) ($p = 0.195$).



study could not define patient management implications in patients with pancreatic ductal adenocarcinoma, as we mainly focused on the correlation between imaging findings and tumour gene expression. Further study will be needed to define the clinical implications.

Although the presence of well-defined tumour margins was significantly associated with *DPC4*-expression tumours, the frequency of well-defined tumours in the *DPC4*-expression group was not remarkably higher than that in the *DPC4*-non-expression group (55.4% *vs* 37.6%). Using this CT finding in T3 tumours, the sensitivity, specificity, positive-predictive value and negative-predictive value for predicting *DPC4*-expression tumour were not high (54.1%, 63.0%, 52.4% and 64.6%). In addition, of the 125 T3 tumour with R0 resection patients, there was no significant difference in overall survival between well-defined and ill-defined tumours. This could be explained due to multiple factors. In other words, the tumour margin had significant associations with multiple factors that had no significant relationship with overall survival. Although our study demonstrated that the presence of well-defined tumour margins was significantly associated with *DPC4*-expression tumour, our results were modest, and we should not overestimate the results because tumour margin was the best predictor out of a series of poor predictors.

Our current analysis is the simplest and most explorative form of a radiogenomic study. Radiogenomics—the identification of imaging traits that correspond to different molecular phenotypes with clinical and biological relevance—is one of the most important fields in the development of personalized medicine because it can enable individualized treatment strategies by predicting individual risk stratification, responses and toxicity before definite treatment. To date, there have been no radiogenomic studies on pancreatic cancer. Indeed, the number of clinically relevant gene mutations that have been found for this cancer remains limited (*e.g. p53*, *Kras*, *DPC4*, *erb2* and *TGF-beta*). The Pancreatic Cancer Genome Project is currently ongoing,[23] with the prospect of extensive radiogenomic studies on pancreatic cancer. This initial study provides baseline data and is a step towards further evaluations of the radiogenomic features of pancreatic ductal adenocarcinoma.

Our present study had several limitations of note. First, as we analyzed only patients with resectable and localized pancreatic ductal adenocarcinoma, results might be influenced by selection bias. Second, we did not obtain the interobserver variability of the qualitative image analysis. It was due to the consensus review. However, experienced abdominal radiologists performed the image analyses, and disagreements were uncommon during the consensus review. Third, we used various CT scanners and parameters due to the retrospective study design.

In summary, our present study is the first to analyze the association between *DPC4* gene expression and CT imaging findings in patients with pancreatic ductal adenocarcinoma. We showed that the presence of well-defined tumour margins was significantly linked with *DPC4*-expression tumour. However, there was no significant difference in overall patient survival according to tumour margins on CT. Further investigations and validations are needed to confirm this finding, preferably using a larger, more comprehensive and prospective cohort.

FUNDING

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (No. 2014R1A1A1006823) and a grant from the Asan Institute for Life Sciences, Asan Medical Center, Seoul, Republic of Korea (No. 2015-0636).

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