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PICT1 regulates TP53 via RPL11 and is involved in gastric cancer progression

R Uchi[1,2,7], R Kogo[1,2,7], K Kawahara[3], T Sudo[1], T Yokobori[4], H Eguchi[1], K Sugimachi[1], T Maehama[5], M Mori[6], A Suzuki[3], S Komune[2] and K Mimori*,[1]

[1]Department of Surgery, Kyushu University Beppu Hospital, 4546 Tsurumihara, Beppu 874-0838, Japan; [2]Department of Otorhinolaryngology, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan; [3]Division of Cancer Genetics, Medical Institute of Bioregulation, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan; [4]Department of General Surgical Science, Graduate School of Medicine, Gunma University, 3-39-22 Showa-machi, Maebashi 371-8511, Japan; [5]Department of Biochemistry and Cell Biology, Japan National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan and [6]Department of Gastroenterological Surgery, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, Suita 565-0871, Japan

Background: The TP53 pathway is frequently inactivated in human cancers. PICT1 (also known as GLTSCR2) is a novel regulator of the MDM2-TP53 pathway via its interaction with the ribosomal protein RPL11 in the nucleolus. However, the clinical significance of PICT1 in gastric cancer remains unknown.

Methods: To evaluate PICT1 function, we used shRNA to inhibit *PICT1* expression in gastric cancer cells that expressed wild-type *TP53*. *PICT1* expression and *TP53* mutation status were quantified in 110 cases of primary gastric cancer to explore the impact of *PICT1* expression levels on gastric cancer.

Results: Deficiency of *PICT1* significantly impaired cell proliferation and colony formation via TP53-mediated cell cycle arrest. Following induction of *PICT1* deficiency, RPL11 translocated out of the nucleolus. Of the 110 gastric cancer samples tested, 70 (63.6%) and 40 (36.4%) tumours expressed wild-type and mutant *TP53*, respectively. In gastric cancer patients with wild-type *TP53* tumours, patients with relatively low *PICT1* expression levels had a better prognosis compared with high expression level patients ($P = 0.046$).

Conclusion: The findings suggest that PICT1 has a crucial role in gastric cancer progression by regulating the MDM2-TP53 pathway through RPL11. Clinically, *PICT1* expression is a novel prognostic parameter in gastric cancer patients with wild-type *TP53* tumours.

Gastric cancer is one of the most common malignant tumours in Japan, and it is strongly associated with *Helicobacter pylori* infection. *H. pylori* infection inhibits TP53 function by the activation of MDM2, which promotes TP53 degradation (Wei *et al*, 2010). TP53 is a major tumour suppressor and transcription factor that induces cell cycle arrest, apoptosis and senescence. More than 30% of gastric cancer patients harbour *TP53* mutations, and TP53 inactivation has an important role in tumorigenesis and tumour progression (Fenoglio-Preiser *et al*, 2003). The expression of *TP53* is induced by DNA damage, and TP53 is degraded by MDM2 – an E3 ubiquitin (Ub) ligase (Haupt *et al*, 1997; Kubbutat *et al*, 1997). Therefore, the activation of MDM2 leads to a reduction in TP53 levels and results in tumour progression (Toledo and Wahl, 2006). The MDM2-TP53 pathway is also regulated by certain ribosomal proteins such as RPL5, RPL11, RPL23, and RPS7, which, in response to the nucleolar stress, can

*Correspondence: Professor K Mimori; E-mail: kmimori@beppu.kyushu-u.ac.jp
[7]These authors contributed equally to this work.

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bind to MDM2 in the nucleoplasm and inhibit its function (Zhu *et al*, 2009).

PICT1 (also known as GLTSCR2) was considered to be a tumour suppressor because of its localisation at 19q13 (a region that is frequently deleted in human malignant tumours) and its ability to stabilise PTEN (Okahara *et al*, 2004; Okahara *et al*, 2006; Yim *et al*, 2007). However, using tetracycline-induced *Pict1*-deficient ES cells, we recently showed that Pict1 bound to ribosomal protein Rpl11 in the nucleolus. Loss of Pict1 led to the release of Rpl11 from the nucleolus, whereupon it translocated to the nucleoplasm to inhibit Mdm2, which resulted in stabilisation and accumulation of the p53 protein (Sasaki *et al*, 2011). Moreover, in colorectal cancer (CRC) and esophageal squamous cell carcinoma (ESCC) patients with wild-type *TP53* tumours, patients in the *PICT1* low-expression group had a significantly better prognosis. Thus, *PICT1* appears to act as an oncogene in cells by inactivation of the TP53 pathway. However, little is known about regulation of PICT1 of the MDM2-TP53 pathway in gastric cancer cells and its clinical significance in human gastric cancer cases.

Therefore, we examined the controversial ability of PICT1 to regulate the MDM2-TP53 pathway, especially in gastric cancer cells. In addition, we characterised the clinical significance of *PICT1* expression levels in 110 gastric cancer cases, establishing *PICT1* status as a *bona fide* prognostic marker in gastric cancer.

MATERIALS AND METHODS

Gastric cancer cell *in vitro* analysis. AGS cells were obtained from the American Type Culture Collection (ATCC). These cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco-BRL, Grand Island, NY, USA) containing 10% heat-inactivated fetal bovine serum (FBS), 100 units ml^{-1} penicillin, and 100 μg ml^{-1} streptomycin in a humidified incubator at 37 °C under 5% CO_2.

Quantitative real-time PCR. *PICT1*, *TP53, MDM2,* and *CDKN1A* mRNA levels were quantified using LightCycler 480 Probes Master kit (Roche Applied Science, Mannheim, Germany) according to the manufacturer's protocol with specific primers shown in the Supplementary Table 1. Gene-expression levels were normalised with respect to *GAPDH*.

Immunoblotting analysis of TP53, CDKN1A, BAX, and PICT1 protein expression. We carried out immunoblotting using a standard protocol and primary antibodies against PICT1, TP53 (Dako, A/S, Glostrup, Denmark), CDKN1A, and BAX (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The proteins were detected using HRP-conjugated secondary antibodies (GE Healthcare Bioscience, Tokyo, Japan). The PICT1 antibody was affinity-purified from antisera as previously described (Okahara *et al*, 2005). β-actin levels (Santa Cruz Biotechnology) were used as a loading control.

Ubiquitin ligase activity assays. We assayed Ub ligase activity as described previously (Furukawa *et al*, 2000; Feng *et al*, 2005) with minor modifications. AGS cells in 9-cm diameter dishes were infected for 3 days with lentiviral *PICT1*-shRNA as described (Inoue-Narita *et al*, 2008). Infected cells were cotransfected with pCAG-HA-Ub (3.5 μg), pcDNA3.1-Myc-TP53 (3.5 μg), and using FuGENE HD (Roche Applied Science). The total amount of plasmid DNA in each transfection was adjusted to 10.5 μg using empty pcDNA3.1 vector as needed. Two days post transfection, cells were treated with 20 μM proteasome inhibitor MG132 (Sigma, St Louis, MO, USA) for 6 h. Cells were boiled for 10 min in 100 μl SDS lysis buffer (50 mM Tris-HCl, pH 7.5, 0.5 mM EDTA, 1% SDS, and 1 mM dithiothreitol), and lysates were diluted in 1 ml 0.5% Nonidet P40 buffer. Ubiquitinated protein was immunoprecipitated (IP) from cell lysates using 2 μg HA-specific antibody (Ab) (Roche Applied Science), resolved by SDS-PAGE, and immunoblotted (IB) with ubiquitinated TP53-specific Ab (Santa Cruz Biotechnology).

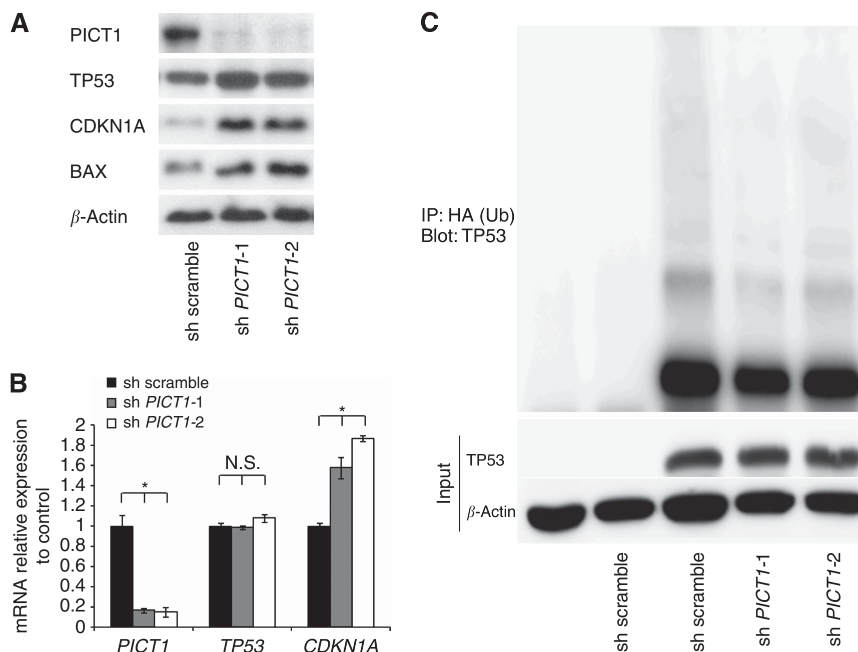

Figure 1. *PICT1* deficiency resulted in TP53 protein accumulation and prevented TP53 ubiquitination. (**A**) Western blot analysis of PICT1, TP53, CDKN1A, and BAX in AGS cells infected with lentivirus expressing scrambled shRNA or *PICT1*-shRNA-1 or -2. β-actin levels were used as a loading control. (**B**) Quantitative real-time PCR of *PICT1*, *TP53*, and *CDKN1A* in AGS cells infected with lentiviral *PICT1*-shRNA. Gene-expression levels were normalised with respect to *GAPDH*. Error bar: mean ± s.d. *$P<0.05$, significant difference from controls. (**C**) Immunoblot of AGS cells transfected with the indicated plasmids and treated with MG132 (20 μM). Lysates were immunoprecipitated and immunoblotted with antibodies to HA (ubiquitin, Ub) and ubiquitinated TP53.

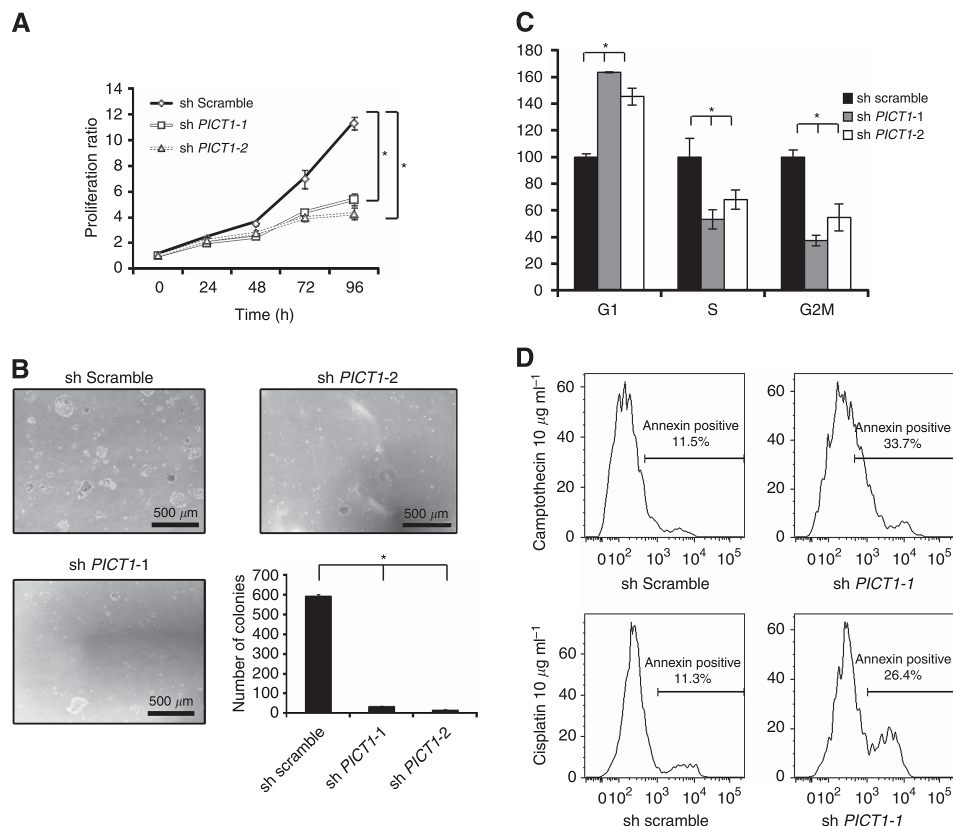

Figure 2. *PICT1* deficiency inhibited proliferation of gastric cancer cells expressing wild-type *TP53*. (**A**) Cell proliferation was evaluated using MTT assay. Error bar: mean ± s.d. *$P<0.05$, significant difference from controls. (**B**) Tumour transformation assay. The number of colonies were counted under an inverted microscope. Representative images of AGS colonies 14 days post transfection with shRNA-targeting *PICT1* or scrambled (control) are shown. Graph shows the number of colonies in each sample. Error bar: mean ± s.d. *$P<0.05$, significant difference from controls. (**C**) Cell cycle assay. Graph shows flow cytometric profiles of the percentage of cells in each cell cycle phase. Error bar: mean ± s.d. *$P<0.05$, significant difference from controls. (**A–C**) Results represent three trials. (**D**) *PICT1* deficiency increased apoptosis in gastric cancer cells treated with anticancer drugs in gastric cancer cells. Forty-eight hours after lentiviral *PICT1*-shRNA infection, AGS cells were treated with camptothecin and cisplatin (each 10 μg ml^{-1}) for 12 h and were stained with annexin V-APC (BD Biosciences).

Cellular proliferation assays. AGS cells were seeded on 96-well plates at a density of 1×10^3 cells per well. After 24 h, cells were infected with lentiviral *PICT1*-shRNA. Cell proliferation was evaluated with the MTT assay using Cell Proliferation Kit I (Roche Applied Science) following the manufacturer's protocol.

Tumour transformation assay. Forty-eight hours after lentiviral *PICT1*-shRNA infection, AGS cells were trypsinised and well mixed with prewarmed RPMI1640 medium containing 10% FBS and 0.33% agar (Difco Noble Agar, BD Biosciences, San Jose, CA, USA). Two milliliter of this mixture from each group was seeded into a 60-mm plate precoated with 3 ml RPMI1640 medium containing 0.5% agar. The plates were incubated at 37 °C and 5% CO_2 for 14 days. The number of colonies was counted under an inverted microscope.

Cell cycle assays. Seventy-two hours after lentiviral *PICT1*-shRNA infection, AGS cells were fixed in 70% ethanol at −20 °C and resuspended in PBS containing five μg ml^{-1} propidium iodide (PI) and 0.25 mg ml^{-1} RNase A. Data were collected on a FACSVantage flow cytometer (BD Biosciences) and analysed with FlowJo v9.3.3 (TreeStar, Ashland, OR, USA).

Confocal microscopy. Lentiviral *PICT1*-shRNA and *RPL11*-DsRed expression plasmids were cotransfected into AGS cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Seventy-two hours after lentiviral *PICT1*-shRNA infection, cells were fixed in 4% formaldehyde and dehydrated with cold methanol-acetone (1 : 1). Fixed cells were immunostained with anti-NPM antibody (Sigma), followed by incubation with Alexa350-labelled secondary antibodies (Invitrogen). Cells were then examined using confocal microscopy (LSM5, Carl Zeiss, Jena, Germany).

Apoptosis assays with anticancer drugs. Forty-eight hours after lentiviral *PICT1*-shRNA infection, AGS cells were treated with camptothecin and cisplatin (both at 10 μg ml^{-1}) for 24 h and were stained with annexin V-APC (BD Biosciences). Annexin-V-positive cells were measured using a FACSVantage flow cytometer (BD Biosciences) and analysed using FlowJo v 9.3.3 (TreeStar).

Gene-set-enrichment analysis of gastric cancer cell lines with *PICT1* expression. Gene-expression profiles and *TP53* status of 34 gastric cancer cell lines were obtained from the Cancer Cell Line Encyclopedia (CCLE) (Barretina *et al*, 2012). Of the 34 gastric cancer cell lines, 11 (32.4%) and 23 (67.6%) tumours expressed wild-type and mutant *TP53*, respectively. We used gene-set-enrichment analysis (GSEA) to clarify the correlation between *PICT1* expression and gene signatures for each *TP53* status group (Subramanian *et al*, 2005). A GSEA algorithm was applied to identify the enrichment of specific functions in the list of genes preranked according to their *P*-value for the test of differences in expression between cell lines. The statistical significance of the enrichment score was calculated by permuting the genes 1000 times as implemented in the GSEA software. To collapse each probe set on the array to a single gene, the probe with the highest

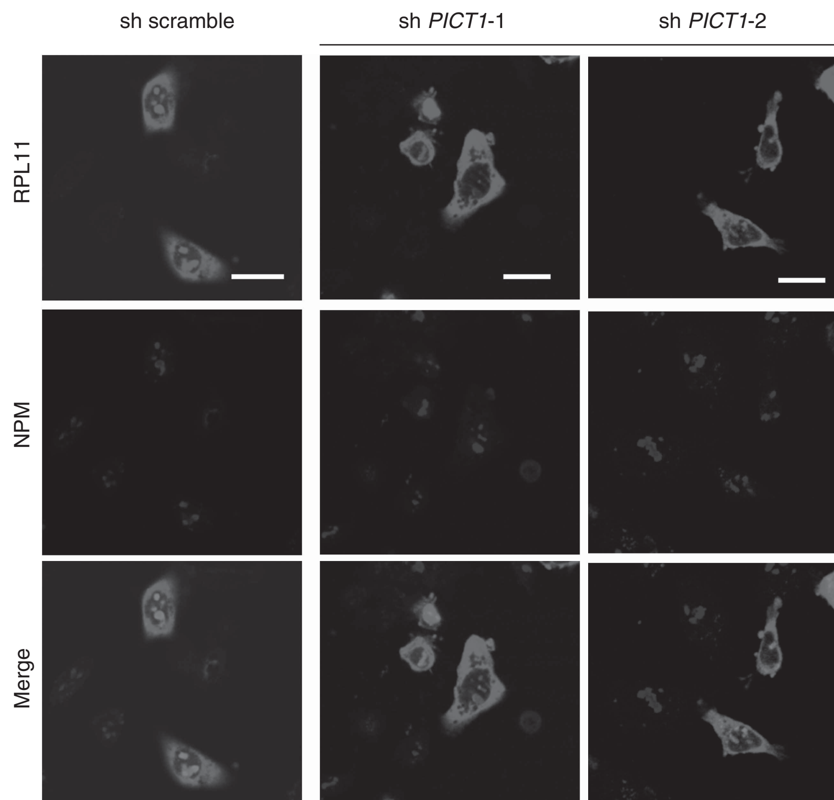

Figure 3. PICT1 deficiency induced translocation of RPL11 out of the nucleolus. Confocal microscopy of AGS cells cotransfected with lentiviral *PICT1*-shRNA and RPL11-DsRed expression plasmid. Scale bars, 5 μm. Confocal microscopy of AGS cells cotransfected with lentiviral *PICT1*-shRNA and *RPL11*-DsRed expression plasmids. Endogenous nucleophosmin (NPM) was detected using antibody to NPM (blue). Cell fluorescence at 72 h is shown. Scale bars, 5 μm.

variance among multiple probes that corresponded to the same gene was selected. Gene sets were derived from the REACTOME pathway database (Croft *et al*, 2011).

Patients and samples. One hundred ten gastric cancer samples were obtained during surgery and used after obtaining informed consent. All patients underwent resection of the primary tumour at the Kyushu University Beppu Hospital between 1992 and 2000. None of the patients received neoadjuvant chemotherapy or radiotherapy before surgery. Resected cancerous tissues were immediately cut and stored in RNA later (Applied Biosystems, Foster City, CA, USA), frozen in liquid nitrogen, and kept at $-80\,^{\circ}$C for subsequent RNA and DNA extraction. Following isolation of RNA, cDNA was synthesised from 8.0 μg total RNA as described previously (Yokobori *et al*, 2009). Genomic DNA was extracted from cancer tissues using the QIAamp DNA mini kit according to the manufacturer's protocol (QIAGEN, Valencia, CA, USA), followed by direct DNA sequencing.

DNA sequencing of *TP53*. Genomic DNA and RNA were extracted from the 110 gastric cancer tissues and their *TP53* status determined using direct DNA sequencing of *TP53* exons five to eight, the area where most TP53 mutations occur. Exons five to eight of the *TP53* gene were amplified and sequenced using BigDye Terminator v3.1 (Applied Biosystems) as previously described (Yokobori *et al*, 2009).

Statistical analyses. Differences between two groups were estimated with Student's *t*-test and χ^2 test. Overall survival curves were plotted according to the Kaplan–Meier method, with the log-rank test applied for comparison. Survival was measured from the day of the surgery. All differences were statistically significant at the level of $P<0.05$. Statistical analyses were performed using the JMP 5 for Windows software package (SAS Institute).

RESULTS

PICT1 deficiency resulted in TP53 protein accumulation and inhibited proliferation of gastric cancer cells expressing wild-type *TP53*. We performed a detailed analysis of the function of *PICT1* in the gastric cancer cell line AGS that expresses wild-type *TP53*. Following treatment with lentivirus PICT1-shRNA, a marked reduction in PICT1 protein was confirmed (Figure 1A). *PICT1* deficiency in AGS cells induced TP53 protein accumulation and upregulation of CDKN1A and BAX, which are major TP53 transcription targets (Figure 1A) (el-Deiry *et al*, 1993; Miyashita and Reed, 1995). The accumulation of TP53 was not observed in two gastric cancer cell lines, NUGC3 and MKN7, which carry mutant *TP53* (Supplementary Figure 1A). The data demonstrated that there was no significant change in *TP53* mRNA levels or an increase in *CDKN1A* mRNA levels (Supplementary Figure 1B). In ES cells, Pict1 deficiency prevented p53 degradation by decreasing Mdm2-mediated ubiquitination (Sasaki *et al*, 2011). Thus, we next asked whether reducing PICT1 expression by cancer cells affected TP53 degradation by ubiquitination. We transfected AGS cells with plasmids expressing hemagglutinin-tagged Ub (HA-Ub) and TP53, and we carried out immunoprecipitation and immunoblotting to detect ubiquitinated TP53 (Ub-TP53). As anticipated, when we infected TP53-expressing AGS cells with a lentivirus expressing PICT1-shRNA, the laddering was less intense (Figure 1C).

Next, to analyse the phenotype induced by *PICT1* deficiency, we performed a cell-proliferation assay. PICT1 deficiency inhibited

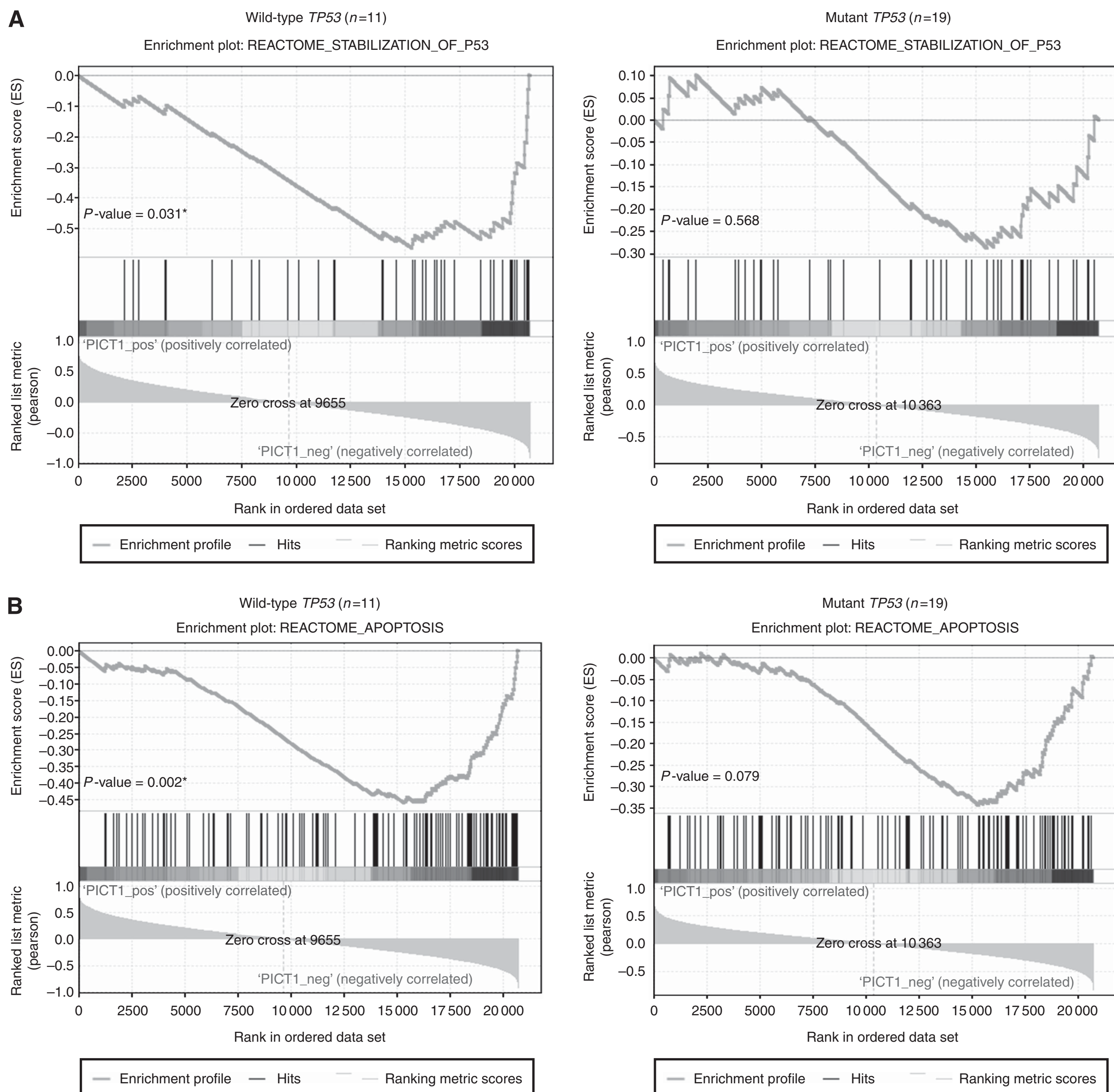

Figure 4. GSEA in gastric cancer cell lines revealed correlations between *PICT1* expression and TP53 stabilisation. Enrichment plots of expression signatures of REACTOME_STABILIZATION_OF_P53 and REACTOME_APOPTOSIS. The barcode plot indicates the position of the genes in each gene set; red and blue colours represent positive and negative Pearson's correlation with *PICT1* expression, respectively. These gene-expression signatures were obtained from the REACTOME database (Croft *et al*, 2011). (**A**) GSEA for the gene signature of the REACTOME_STABILIZATION_OF_P53. (**B**) GSEA for the signature of REACTOME_APOPTOSIS. *$P<0.05$, significant difference was predicted by statistic analysis.

anchorage-dependent growth (Figures 2A and B) and also decreased the proportion of cells in the S and G2/M phases, as measured by a cell cycle assay, which indicated that *PICT1* depletion induced G1 arrest (Figure 2C) in AGS. In contrast, *PICT1* deficiency in NUGC3 and MKN7, which carry mutant *TP53,* did not inhibit cell growth (Supplementary Figure 1B). Moreover, *PICT1* deficiency increased the number of apoptotic cells after treatment with antigastric cancer drugs, such as cisplatin and camptothecin (Figure 2D). Thus, inhibition of *PICT1* expression induced TP53 accumulation and inhibited transformation in not only ES cells but also human gastric cancer cells.

PICT1 deficiency induced translocation of RPL11 out of the nucleolus. In ES cells, Pict1 binds to Rpl11 in the nucleolus, whereas in the absence of Pict1, Rpl11 is released from the nucleolus to the nucleoplasm (Sasaki *et al*, 2011). The released Rpl11 binds to Mdm2, which inhibits Mdm2-mediated ubiquitination of p53. Translocation of Rpl11 out of the nucleolus is one of the most important events leading to p53 accumulation in Pict1-deficient ES cells (Sasaki *et al*, 2011). Thus, we next explored whether PICT1 depletion in cancer cells affected RPL11 localisation by cotransfecting gastric cancer cells with lentiviral *PICT1*-shRNA and *RPL11*-DsRed expression vectors. RPL11 appeared in both the nucleolus and the cytoplasm in cells transfected with scrambled shRNA but translocated out of the nucleolus in cells transfected with *PICT1*-shRNA (Figure 3). Thus, PICT1 appears to be essential for the nucleolar localisation of RPL11 in gastric cancer cells.

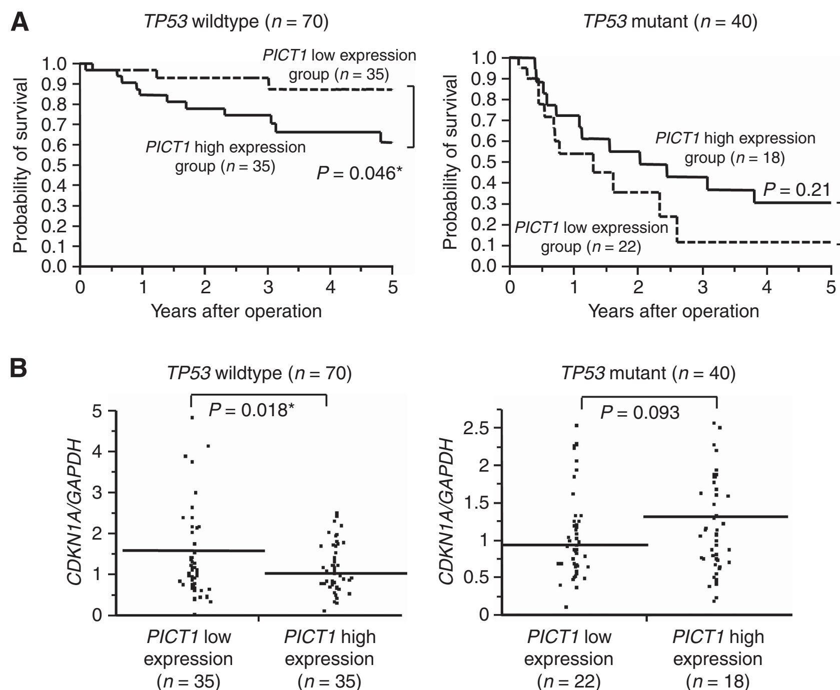

Figure 5. Low *PICT1* expression levels were associated with a better prognosis in gastric cancer patients with wild-type *TP53* tumours. (**A**) Kaplan–Meier overall survival curves for 110 gastric cancer patients according to *PICT1* level. Left, patients with wild-type *TP53* tumours ($n = 70$); Right, patients with mutant *TP53* tumours ($n = 40$). A log-rank test was performed to determine significance. (**B**) Quantitative real-time PCR analysis of *CDKN1A* in 110 gastric cancer tissues and classification based on *PICT1* level (*PICT1/GAPDH* = 1). Horizontal line: borderline of *PICT1* high or low. Left, wild-type *TP53* tumours ($n = 70$); Right, mutant *TP53* tumours ($n = 40$). A Student's *t*-test was performed to determine significance. *$P < 0.05$, significant difference was predicted by statistic analysis.

GSEA revealed correlations between PICT1 and TP53 pathways in *TP53* wild-type gastric cancer cell lines. We used the array data of gastric cancer cell lines from CCLE and asked whether *PICT1* expression was highly correlated with previously curated gene-expression signatures (Croft *et al*, 2011). In these cell lines, 11 lines (including AGS) have wild-type *TP53* and 19 cell lines have mutated *TP53* (Barretina *et al*, 2012). We used GSEA to examine possible correlations between *PICT1* expression and TP53-associated pathways for each *TP53* status groups. Although the gene signature, REACTOME_STABILIZATION_TP53, was not correlated with *PICT1* expression in *TP53*-mutated cell lines ($P = 0.568$), it was significantly negatively correlated with *PICT1* expression in *TP53* wild-type cell lines ($P = 0.031$) (Figure 4A). In addition, the gene pathway, REACTOME_APOPTOSIS, was negatively correlated with *PICT1* expression only in *TP53* wild-type gastric cancer cell lines ($P = 0.002$) (Figure 4B). These results suggested that PICT1 induces TP53 ubiquitination and apoptosis not only in AGS cell lines but also in other *TP53* wild-type gastric cancer cell lines.

Low *PICT1* expression levels were associated with a better prognosis in gastric cancer patients with wild-type *TP53* tumours. We investigated whether *PICT1* also behaved as an oncogene in clinical samples. Genomic DNA and RNA were extracted from 110 gastric cancer tissues and their *TP53* status was determined by direct DNA sequencing of *TP53* exons five to eight. *PICT1* expression levels in these samples were determined using qRT–PCR. Of the 110 gastric cancer samples tested, 70 (63.6%) and 40 (36.4%) tumours had wild-type and mutant *TP53*, respectively. We divided the two groups according to their *PICT1* expression level (*PICT1* high-expression group, *PICT1/GAPDH* > 1; low-expression group, *PICT1/GAPDH* < 1, Supplementary Figure 2A). In gastric cancer cases with wild-type *TP53* tumours ($n = 70$), the *PICT1* high-expression group ($n = 35$) had a poorer prognosis for overall survival as compared with the low-expression group ($n = 35$, $P = 0.046$) (Figure 5A, left). However, for mutant *TP53* ($n = 40$) and total gastric cancer cases ($n = 110$), *PICT1* expression levels did not correlate with overall survival (Figure 5A, right, Supplementary Figure 2B). Next, we investigated whether MDM2 was a prognostic marker in gastric cancer cases. There was no significant difference between the MDM2 high-expression group and the low-expression group in either wild-type *TP53* or mutant *TP53* gastric cancer cases (Supplementary Figure3).

Clinicopathologic variables of *PICT1* expression in gastric cancer cases. We also analysed the association between *PICT1* expression levels and clinicopathologic factors in gastric cancer patients with wild-type *TP53* tumours and found that *PICT1* expression was significantly associated with tumour depth ($P = 0.03$, Table 1). Moreover, to clarify the relationship between *PICT1* expression and the function of TP53 in gastric cancer cases, we examined the association between *PICT1* and *CDKN1A* expression levels using qRT–PCR. In wild-type *TP53* cases, the *PICT1*-low-expression group ($n = 35$) showed significantly higher *CDKN1A* expression than the high-expression group ($n = 35$) ($P = 0.018$, Figure 5B, left). In contrast, for *TP53*-mutant cases, there were no significant differences between *PICT1* and *CDKN1A* expression levels (Figure 5B, right).

DISCUSSION

We recently reported that *PICT1* is a novel oncogene that regulates the MDM2-TP53 pathway (Sasaki *et al*, 2011). The present study disclosed the function of *PICT1* in gastric cancer, the most common malignant tumour in Japan. As expected, inhibition of *PICT1* in gastric cancer cells resulted in wild-type TP53 accumulation and led to TP53-mediated cell cycle arrest and apoptosis. One of the critical findings of the current study was that

Table 1. *PICT1* expression and clinocopathologic factors in gastric cancer patients with wild-type *TP53*

	***PICT1* low expression (*n*=35)**		***PICT1* high expression (*n*=35)**		
Factors	**Number**	**%**	**Number**	**%**	***P*-value**
Age (mean ± s.d.)	66.5 ± 2.00		64.5 ± 2.00		0.477
Gender					
Male	22	62.9	21	60.0	0.806
Female	13	37.1	14	40.0	
Histological grade					
Well and moderately	17	48.6	18	51.4	0.811
Poorly and Signet	18	51.4	17	48.6	
Tumour depth[a]					
m, sm, mp	21	60.0	12	34.3	0.03*
ss, se, si	14	40.0	22	65.7	
Lymph node metastasis					
Absent	16	45.7	17	48.6	0.811
Present	19	54.3	18	51.4	
Lymphatic invasion					
Absent	13	37.1	9	25.7	0.302
Present	22	62.9	26	74.3	
Venous invasion					
Absent	32	91.4	26	74.3	0.052
Present	3	8.6	9	25.7	
Peritoneal dissemination					
Absent	34	97.1	30	85.7	0.075
Present	1	2.9	5	14.3	
Stage					
Stages I, II	26	74.3	19	54.3	0.081
Stages III, IV	9	25.7	16	45.7	

*$P<0.05$.
[a]Tumour invasion of mucosa (m), submucosa (sm), muscularis propria (mp), subserosa (ss), penetration of serosa (se), and invasion of adjacent strucures (si).

PICT1 deficiency induced RPL11 translocation out of the nucleolus in gastric cancer cells, as we previously demonstrated for *Pict1*-deficient ES cells. Thus, this is the first demonstration that PICT1 regulates the nucleolar localisation of RPL11 in cancer cells and promotes TP53 accumulation, thereby reducing cancer progression.

As for the clinical usefulness of *PICT1* expression in gastric cancer cases, we clarified that high expression of *PICT1* correlated with a poorer prognosis in gastric cancer cases with wild-type *TP53*. We previously reported that *PICT1* high expression levels indicated a poor prognosis in CRC and ESCC cases not only in *TP53* wild-type cases but in all cases. On the other hand, our analysis of gastric cancer cases showed the prognostic significance of *PICT1* expression but only in the *TP53* wild-type group. It should be noted that our study patients with wild-type *TP53* tumours had a significantly better prognosis than those with mutant *TP53*. As such, this *TP53* status-related prognostic difference may explain this discrepancy. About 30% of human gastric cancers harbour *TP53* mutations (Fenoglio-Preiser *et al*, 2003). Therefore, in 70% of gastric cancer cases with wild-type *TP53*, PICT1 expression status might determine the clinical outcome.

Activation of TP53 is induced by many types of cellular stress. PICT1 binds to ribosomal protein RPL11, leading to its localisation in the nucleolus. Reduction in *PICT1* levels in response to nucleolar stress in particular releases RPL11 from the nucleolus followed by its translocation to the nucleoplasm. Serum depletion and some anticancer drugs, such as low-dose actinomycin D, mycophenolic acid (MPA), 5 fluorouracil, and camptothecin, are known to induce nucleolar stress, which is also referred to as ribosomal stress because of the inhibition of ribosomal biogenesis (Sun *et al*, 2008; Boulon *et al*, 2010). In this study, apoptosis was significantly induced in *PICT1*-deficient cells following treatment with camptothecin and cisplatin (Figure 2D). In oligodendroglioma, loss of heterozygosity (LOH) of 19q13 was associated with better disease-free survival after chemotherapy (Cairncross *et al*, 1998; Smith *et al*, 1999; Mariani *et al*, 2006). These results indicate that gastric cancer patients having low *PICT1* expression levels may retain higher chemotherapeutic susceptibility.

In summary, the level of *PICT1* expression regulates the MDM2-TP53 pathway via RPL11 in gastric cancer and may be a novel prognostic factor in gastric cancer patients with wild-type *TP53* tumours. Therefore, inhibition of *PICT1* expression or interfering with the interaction between PICT1 and RPL11 might provide useful strategies for treating gastric cancer.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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