

Clinical significance of serum p53 antigen in patients with pancreatic carcinomas

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Abstract

Background—Alterations in the p53 gene are often found in pancreatic cancer, and accumulation of the p53 protein has been noted in tumour cells.

Aims—To investigate whether serum p53 protein concentrations could be used as markers for p53 gene mutations in neoplasms of the pancreas.

Methods—Serum p53 protein concentrations were determined by an enzyme linked immunosorbent assay (ELISA) in 104 cases of pancreatic adenocarcinoma, and 61 matched formalin fixed tissue sections were also stained by an anti-p53 DO-7 monoclonal antibody.

Results—The mean serum concentration of p53 protein in the adenocarcinoma patients was 0.27 (SEM 0.02) ng/ml, and was significantly higher than in 35 healthy blood donors (0.15 (0.02) ng/ml, SD=0.11) or in 15 cases of chronic pancreatitis (0.15 (0.02) ng/ml). Adopting an arbitrary cut off value for the serum p53 protein concentration of 0.37 ng/ml, which corresponded to a value 2 SD above the mean value from the healthy blood donors, positive serum p53 protein concentrations were found in 23 out of 104 (22.1%) patients with adenocarcinomas examined, 16 out of 47 (34.0%) patients with carcinomas with distant metastases, but only seven of 57 patients (12.3%) with carcinomas without metastases ($p < 0.05$). In 11 patients with pancreatic adenocarcinomas, the mean serum p53 protein concentration after tumour resection was 0.21 (0.05) ng/ml, and had decreased compared with the preoperative concentrations (0.25 (0.05) ng/ml) ($p < 0.05$). There were no significant associations between the serum concentrations of p53 protein and serum concentrations of markers such as CA19-9 or CEA; however, serum concentrations of p53 protein demonstrated a potential role as an additional tumour marker. Immunohistochemical studies disclosed that the p53 protein was expressed in 28 out of 61 pancreatic adenocarcinomas (45.9%). Serum p53 protein concentrations in the positively immunostained cases were significantly higher than in the negatively immunostained cases (0.35 (0.05) ng/ml *v* 0.15 (0.01) ng/ml; $p < 0.005$). Furthermore, positive immunostaining for p53 protein was found in eight out of 10 (80%) serum positive p53 protein cases with adenocarcinomas.

Conclusion—An increase in serum p53 protein concentrations appears during the

progression of pancreatic adenocarcinoma and correlates with the accumulation of p53 protein as a result of a mutation of the p53 gene. An analysis of p53 antigen concentrations can detect p53 gene alterations, which could be useful for the selection of treatment regimens.

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The p53 gene is a tumour suppressor gene located on the short arm of chromosome 17. It encodes a 53 kDa nuclear phosphoprotein (p53 protein) which is thought to inhibit cellular proliferation and transformation.¹ Mutations of the p53 gene are the most common genetic alterations found in human malignancies.² Most mutations of the p53 gene lead to an accumulation of p53 protein due to an increased half life. As a result, the accumulation of p53 protein reaches concentrations detectable by immunohistochemistry. By contrast, the product of the wild type p53 gene is undetectable because of its short half life.³ Thus there seems to be a good correlation between the overexpression of p53 protein and p53 gene mutations.⁴

The accumulation of mutant p53 protein in tumour cells can be released into the extracellular environment, such as into the serum,⁵ and can thus be examined by enzyme linked immunosorbent assay (ELISA). Recently, p53 protein has been measured by ELISA in serum samples from patients with carcinomas of the colon and lung,⁵⁻⁷ and were significantly raised compared with negative controls. The p53 protein concentrations correlated with the percentage of p53 gene alteration.⁵⁻⁷ In 40%-60% of pancreatic carcinomas, mutations of the p53 gene and the increased accumulation of p53 protein have been shown by both direct sequencing and by immunohistochemistry.⁸⁻¹² Several investigators have also reported that p53 gene alterations were correlated with a more advanced clinical stage and with decreased survival in patients with pancreatic cancer,^{13 14} but there are conflicting reports regarding the prognostic value of p53 gene expression.^{15 16}

As mentioned above, there have been two main methods for detecting p53 gene mutations in human malignancies: direct sequencing and immunohistochemistry. However, both methods require tissue specimens. In addition, direct sequencing of the p53 gene is time consuming and difficult to perform as a routine test in clinical laboratories. It is also

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difficult to obtain fresh surgical tissue specimens from pancreatic adenocarcinomas before the operation. Needle biopsy of the pancreas also has a relatively high risk of complications. The ELISA assay does not require a tissue specimen and is easy to perform. Vojtesek *et al*¹⁷ reported a significant correlation between p53 protein immunostaining and the quantification of p53 protein concentrations by ELISA in tumour cytosol from breast cancer cells. Therefore, if the serum concentrations of p53 antigen are also correlated with alterations of the p53 gene in pancreatic carcinomas, then an analysis of serum p53 antigen concentrations by ELISA would be expected to be a useful screening procedure for detecting the mutant p53 gene in this neoplasm. In the present study, we investigated preoperative serum p53 antigen concentrations by ELISA in 104 samples of pancreatic adenocarcinomas, and analysed the expression of p53 protein in 61 matched sections by immunohistochemical staining. We also studied the association between serum concentrations of p53 protein and tumour markers such as CA19-9 or CEA.

Methods

PATIENTS

Serum samples were obtained preoperatively from 104 patients with adenocarcinomas of the pancreas, including 11 cases of cystadenocarcinoma, and from 30 patients with non-cancerous pancreatic disease consisting of six benign tumours (five cystadenomas, one solid and cystic tumour), nine islet cell tumours, and 15 cases of chronic pancreatitis. In 11 of the 104 patients with pancreatic cancer, serum samples after resection of the tumour were also available. Patients who had previously received radiotherapy or chemotherapy were omitted from this study. We also obtained serum samples from 35 healthy volunteers as negative controls. All of the samples were stored at -70°C until analysis. Written informed consent was obtained from those patients with pancreatic disease who had received a pancreatectomy or intraoperative biopsy at the Kyoto University Hospital. The 104 cases of adenocarcinoma consisted of 62 male and 42 female patients, with a mean age of 62 (range 39–85) years. Patients were staged using the TNM classification: 12 cases were determined as stage I, two cases as stage II, 24 cases as stage III, and 66 cases as stage IV. Forty seven out of 104 (45.2%) patients showed distant metastases such as liver metastasis or peritoneal dissemination. The histological diagnosis was determined by the microscopical examination of haematoxylin and eosin stained, paraffin wax embedded sections according to the World Health Organisation (WHO) classification,¹⁸ with minor modifications.

DETERMINATION OF p53 ANTIGEN CONCENTRATIONS BY ELISA

Serum concentrations of the p53 antigen were analysed by a p53 mutant selective ELISA

assay kit (Oncogene Science, Cambridge, UK) according to the manufacturer's protocol. Briefly, microtitre wells were precoated with PAb 240, a mouse monoclonal antibody specific for most native mammalian mutant p53 proteins.¹⁹ A 100 μl aliquot of the serum was added to each well, and was incubated at 4°C overnight. After washing the wells, 100 μl rabbit polyclonal reporter antibody, which also recognises mutant mammalian p53 proteins, was added to each well and was incubated at room temperature for two hours. After washing the wells, 100 μl horseradish peroxidase conjugated goat antirabbit IgG was added to each well, and was incubated at room temperature for one hour. After washing the wells again, the colourless solution was converted into a blue/green solution by incubating each well with 100 μl chromogenic substrate 2,2'-azino-di-[3-ethyl-benzthiazoline sulphonate] at room temperature for 30 minutes. The coloured reaction product was quantified by examining its absorbance at 405 nm using a spectrophotometer. The concentration of mutant p53 protein in the serum samples was then determined by comparison against a standard curve generated from a known amount of mutant p53 protein (0, 0.25, 0.5, 1, 2, and 4 ng/ml). The minimum concentration detectable was 0.05 ng/ml according to the manufacturer. In all 104 cases with pancreatic cancer, both CA19-9 and CEA serum concentrations were also analysed by conventional methods.

IMMUNOHISTOCHEMISTRY

Matched samples for immunostaining were available in 61 cases of adenocarcinoma, six cases of benign tumours, six cases of islet cell tumours, and in four cases of chronic pancreatitis. The 61 adenocarcinoma sections consisted of 43 primary tumours and 18 metastatic tumours. They were fixed in 10% (v/v) formalin, and then paraffin wax embedded sections were cut at 4 μm thickness.

A mouse monoclonal antibody (mAb) against the p53 protein, DO-7, was used in this study. As DO-7 recognises the amino terminus of the p53 protein, it can react with any variant form of p53 so long as it is translated.²⁰ In our previous study¹¹ using DO-7 in 10 formalin fixed samples of human pancreatic adenocarcinoma cell lines, we identified a correlation between p53 gene mutations and p53 protein overexpression; positive immunostaining was found in the nuclei of all three p53 gene missense mutations and in two of the four other p53 gene alterations. No positive immunostaining was found in any of the three wild type alleles of the p53 gene (Table I).¹¹ Non-immunised mouse IgG was used as a negative control for the immunostaining.

The tissue sections were deparaffinised and treated with citrate buffer (pH 6.0) for 10 minutes at 95°C , and then endogenous peroxidase activity was quenched with 0.3% hydrogen peroxide in methanol for 30 minutes. The sections were washed in phosphate buffered saline (PBS) and processed with normal horse serum for 30 minutes at room

TABLE I Correlation between p53 gene alterations and p53 protein immunoreactivity in human pancreatic adenocarcinoma cell lines

p53 gene	p53 protein immunoreactivity		Total
	Negative	Positive	
Missense mutation	0	3	3
Other alterations*	2	2	4
Wild type	3	0	3
Total	5	5	10

*Two cases of splicing mutations, and one case each of gross DNA deletion through exon 2 to exon 9, and a 1 bp deletion.

TABLE II Serum concentrations of p53 protein in the 169 samples

Histological type	n	Serum p53 protein (mean (SE) (ng/ml))
Adenocarcinoma	104	0.27 (0.02)
Islet cell tumour	9	0.18 (0.05)
Benign tumour	6	0.16 (0.03)
Chronic pancreatitis	15	0.15 (0.02)
Healthy blood donors	35	0.15 (0.02)

Serum concentrations of p53 protein in the 104 cases of adenocarcinoma were significantly higher than in the 35 healthy blood donors ($p < 0.005$) or in the 15 cases of chronic pancreatitis ($p < 0.05$) (Mann-Whitney U test).

temperature to block non-specific staining. The tissue sections were then incubated with the primary antibody overnight at 4°C. After several washes with PBS, the sections were incubated with biotinylated antimouse IgG (Vector, Burlingame, USA) for two hours at 4°C. After several washes with PBS, the sections were incubated with horseradish-avidin D (Vector) for one hour at 4°C. The histochemical visualisation reaction for the peroxidase was then performed with 3,3-diaminobenzidine and hydrogen peroxidase. The immunostaining was evaluated by the relative ratio of p53 protein positive cells in the tumour tissues, and was classified into two

groups: tumours with less than 10% positive cells were defined as negative, whereas those with greater than 10% positive cells were defined as positive.

STATISTICAL ANALYSIS

Statistical analysis for both the serum p53 protein concentrations and the immuno-histochemical staining for p53 protein were performed by the Mann-Whitney U test. Comparisons between preoperative and post-operative serum p53 protein concentrations were made using the paired *t* test. Correlations between serum p53 protein concentrations and p53 protein immunostaining and between serum p53 protein concentrations and distant metastases were analysed by χ^2 test. A *p* value < 0.05 was considered to be significant.

Results

SERUM p53 CONCENTRATIONS

Table II and Fig 1 list the serum p53 protein concentrations assayed by ELISA. In adenocarcinomas of the pancreas, the serum concentrations of p53 protein ranged from 0.062 to 1.29 ng/ml, and averaged 0.27 (SEM) 0.02 ng/ml. These concentrations were significantly higher than those in healthy blood donors (0.15 (0.02) ng/ml) ($SD = 0.11$, $p < 0.005$), and were also higher than in patients with chronic pancreatitis (0.15 (0.02) ng/ml) ($p < 0.05$). Serum p53 concentrations in patients with chronic pancreatitis, benign tumours (0.16 (0.03) ng/ml), and islet cell tumours (0.18 (0.05) ng/ml) did not differ significantly from those of healthy blood donors. Thus patients with benign tumours, chronic pancreatitis, and healthy volunteers could be defined collectively as the non-malignant group. Serum p53 protein concentrations in the adenocarcinoma group were significantly higher than in the non-malignant group (0.15 (0.01) ng/ml) ($p < 0.001$) (data not shown).

The cut off value for the serum p53 protein concentration in the pancreatic cancer cases was arbitrarily defined as 0.37 ng/ml, which was 2 SD above the mean of the healthy blood donors. Therefore, serum p53 protein concentrations above 0.37 were defined as positive, and those below 0.37 were designated as negative. Positive serum p53 protein samples were found most often in cases of pancreatic carcinoma, which showed raised concentrations in 23 out of 104 patients examined (22.1%). By contrast, raised concentrations were seen in only one out of nine patients with islet cell tumours (11.1%), in two out of 35 healthy volunteers (5.7%), in none of the six patients with benign tumours, and in none of the 15 patients with chronic pancreatitis. Pancreatic carcinomas with distant metastases also exhibited significantly higher serum p53 protein concentrations compared with those without metastases (0.31 (0.04) ng/ml *v* 0.21 (0.02) ng/ml, $p < 0.05$). Positive serum p53 protein concentrations were found in 16 out of 47 (34.0%) metastatic cases, but in only seven

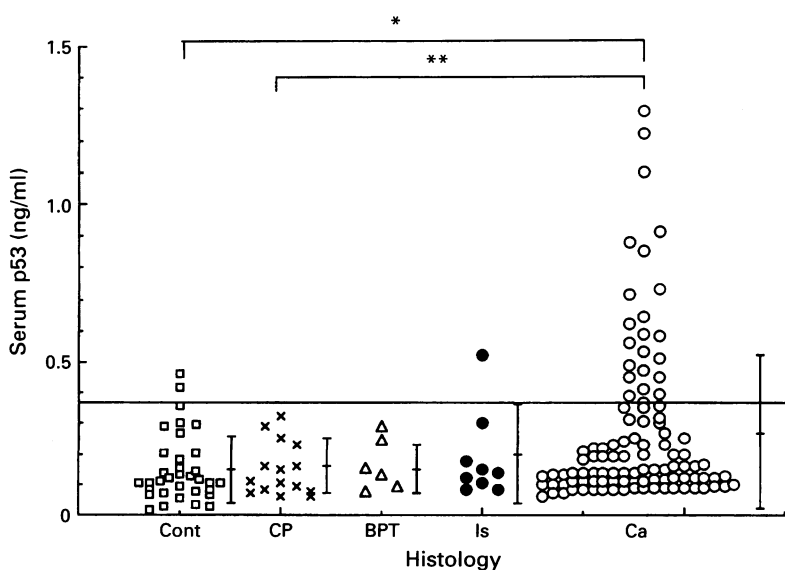


Figure 1: Differences in serum p53 protein concentrations between the different histological types. The horizontal line indicates the cut off value (0.37 ng/ml). Cont=controls (healthy blood donors); CP=chronic pancreatitis; BPT=benign pancreatic tumour; Is=islet cell tumour; Ca=adenocarcinoma. The mean serum concentration of p53 protein in the cases of adenocarcinoma was significantly higher than in the healthy blood donors and patients with chronic pancreatitis (* $p < 0.005$; ** $p < 0.05$, Mann-Whitney U test).

TABLE III Correlation between serum p53 protein concentrations and distant metastasis in 104 patients with pancreatic adenocarcinomas

Serum p53 protein	Distant metastases		Total
	Negative	Positive	
Negative	50	31	81
Positive	7	16	23
Total	57	47	104

Distant metastases=liver metastasis or peritoneal dissemination. Negative=serum p53 protein <0.37 ng/ml; positive=serum p53 protein \geq 0.37 ng/ml. Positive serum p53 protein concentrations were found in 16 out of 47 (34.0%) patients with carcinomas with distant metastases, but in only seven out of 57 (12.3%) patients with carcinomas without metastases (χ^2 test, $p < 0.05$).

TABLE IV Correlation between serum p53 protein concentrations and the tumour markers CA19-9 and CEA in 104 patients with pancreatic adenocarcinomas

Serum p53 protein	CA19-9 (n (%))		CEA (n (%))		Total
	Negative	Positive	Negative	Positive	
Negative	20 (19.2)	61 (58.6)	40 (38.5)	41 (39.4)	81
Positive	9 (8.7)	14 (13.5)	11 (10.6)	12 (11.5)	23
Total	29 (27.9)	75 (72.1)	51 (49.1)	53 (50.9)	104

Negative=serum p53 protein <0.37 ng/ml; positive=serum p53 protein \geq 0.37 ng/ml. The cut off values were 37 U/ml for CA19-9 and 2.5 ng/ μ l for CEA.

out of 57 (12.3%) non-metastatic cases (χ^2 $p < 0.05$; Table III). In 11 patients with pancreatic adenocarcinomas, which could be resected, the mean postoperative serum concentration of p53 protein was 0.21 (0.05) ng/ml. This was decreased compared with the preoperative p53 protein concentrations (0.25 (0.05) ng/ml) (paired *t* test, $p < 0.05$). The postoperative p53 protein concentrations in 11 cases of pancreatic cancer were also higher than in healthy blood donors, although this difference was not significant. There were no significant associations between the serum p53 protein concentration and other clinicopathological findings such as age or sex (data not shown).

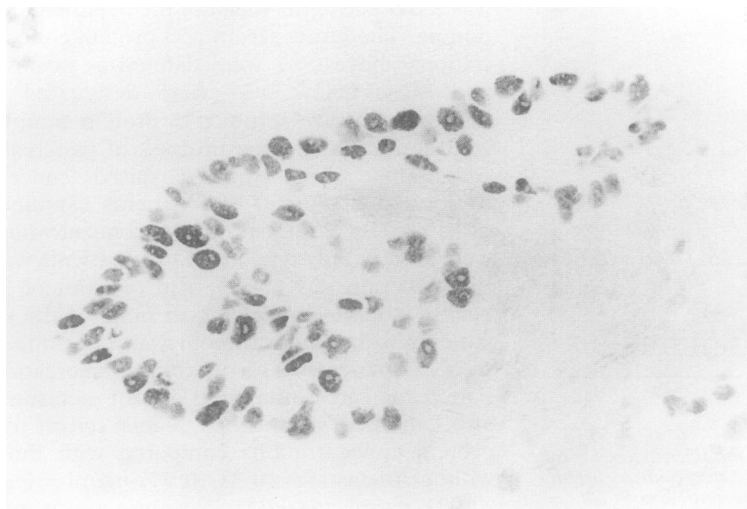


Figure 2: Photomicrograph showing intense p53 protein immunoreactivity in the nuclei of pancreatic carcinoma cells (original magnification: $\times 400$).

CORRELATION BETWEEN SERUM CONCENTRATIONS OF p53, AND CA19-9 OR CEA IN PANCREATIC ADENOCARCINOMAS

Of the 104 patients with pancreatic adenocarcinomas, positive serum CA19-9 concentrations (>37 U/ml) were found in 75 (72.1%) cases, and positive serum CEA concentrations (>2.5 ng/ml) were noted in 53 (50.9%) cases (Table IV). The serum concentrations of p53 protein did not correlate significantly with either CA19-9 or CEA concentrations (Table IV). Instead, positive serum p53 protein concentrations were found more often in patients negative for CA19-9 than patients positive for CA19-9 (Table IV). Of the 22 patients showing both negative CA19-9 and CEA serum concentrations, six patients had positive concentrations of serum p53 protein (data not shown).

p53 PROTEIN IMMUNOHISTOCHEMISTRY

Most of the p53 protein immunoreactivity was seen in the nuclei of carcinoma cells, and was found homogeneously in the carcinoma tissues (Fig 2). An increasing relative ratio of p53 positive cells was found in sections that tended to show strong immunoreactivity. The localisation of p53 protein to the nucleus was interpreted as being immunopositive. In total, positive p53 protein staining was found in 28 out of 61 (45.9%) cases of adenocarcinoma. Positive p53 protein staining was found in 10 out of 18 (55.6%) metastatic cases versus 18 out of 43 (41.9%) primary cases; this difference was not significant. Non-malignant tissue adjacent to the carcinoma tissue on each slide did not show any positive staining. Furthermore, there was no detectable p53 protein immunoreactivity in the sections from the six islet cell tumours, the six benign tumours, or from the four cases of chronic pancreatitis (data not shown). There were no significant associations between p53 protein immunoreactivity and other clinicopathological findings such as age or sex (data not shown).

CORRELATION BETWEEN SERUM p53 CONCENTRATIONS AND p53 PROTEIN IMMUNOHISTOCHEMICAL STAINING IN PANCREATIC ADENOCARCINOMAS

We analysed the correlation between serum p53 protein concentrations and the immunohistochemical staining for p53 protein in 61 matched cases of pancreatic adenocarcinoma. The average serum p53 protein concentration in the positive immunostaining cases was 0.35 (0.05) ng/ml, and was significantly higher than in the negative staining cases (0.15 (0.01) ng/ml) ($p < 0.005$; Table V). Furthermore, positive immunostaining for p53 protein was found in eight out of 10 (80%) cases positive for p53 protein in serum (Table VI). In addition, serum negative p53 was found in 31 of 33 (93.9%) cases with negative p53 protein immunostaining (Table VI). In the negative staining group, there were no significant differences in the serum p53 protein concentrations between carcinoma, benign pancreatic tumour,

TABLE V Differences in serum p53 protein concentrations versus p53 protein immunoreactivity in 61 patients with pancreatic adenocarcinoma

p53 protein immunoreactivity	n	serum p53 protein (mean (SE) (ng/ml))
Negative	33	0.15 (0.01)*
Positive	28	0.35 (0.05)

* $p < 0.005$, Mann-Whitney U test.

TABLE VI Correlation between serum p53 protein concentrations and p53 protein immunostaining in 61 patients with pancreatic adenocarcinoma

Serum p53 protein	Immunostaining		Total
	Negative	Positive	
Negative	31	20	51
Positive	2	8	10
Total	33	28	61

Negative=serum p53 protein < 0.37 ng/ml; positive=serum p53 protein ≥ 0.37 ng/ml. Positive serum p53 concentrations were found in eight out of 28 (28.6%) positive immunostaining cases, but in only two out of 33 (6.1%) negative immunostaining cases (χ^2 test, $p < 0.05$).

and chronic pancreatitis cases (data not shown).

Discussion

In the present study, we examined the serum concentrations of p53 antigen in cases of pancreatic cancer by ELISA, and analysed whether they correlated with the immunohistochemical staining for p53 protein. To the best of our knowledge, there have been no previous reports on measuring the concentrations of serum p53 antigen in patients with pancreatic cancer. Serum p53 antigen concentrations in the cases of adenocarcinoma were significantly higher than in healthy blood donors or in patients with chronic pancreatitis. Moreover, patients with positive serum p53 antigen concentrations above 0.37 ng/ml were most often found in the adenocarcinoma group (22.1%). These results were similar to those from a recent report investigating serum p53 antigen concentrations by ELISA in colon carcinomas, in which raised concentrations were detected in 20% of the adenoma cases and in 32% of the carcinoma cases.⁵

In the present study, pancreatic adenocarcinomas with distant metastases showed significantly higher serum p53 concentrations than tumours without metastases. In addition, positive serum p53 concentrations were also found more often in patients with distant metastases (34.0%) than in those without metastases (12.3%). This is compatible with a previous study reporting that p53 gene mutations in pancreatic cancer cases may occur more often in metastatic lesions than in primary tumours.³ Fontanini *et al*⁶ also showed that the concentrations of mutant p53 antigen detected in the serum of patients with lung cancer were significantly higher in those patients with lymph node involvement and late stage disease. Furthermore, they also reported that the serum p53 antigen concentrations were associated with the extent of tumour

necrosis.⁶ These results suggest that serum p53 protein concentrations may be raised during the progression of these malignancies. This is a reasonable assumption considering the fact that increases in the serum p53 protein concentration can result from the destruction of tumour cells. Nevertheless, the mechanisms responsible for this still remain unclear. In 11 patients with pancreatic adenocarcinomas which could be resected the postoperative serum concentrations of p53 protein had decreased compared with their preoperative concentrations, but were still higher than in healthy blood donors. This suggests that these tumours could specifically produce the mutant p53 protein. Recently, circulating antibodies against p53 protein have been identified in the serum of patients with various types of cancer.²¹ Several studies have shown that such antibodies are usually associated with the accumulation of mutant p53 protein within the tumour cells.²² Anti-p53 protein antibodies were detected in eight out of 29 patients with pancreatic cancer (28%),²² which is similar to the frequency of serum positive p53 antigen cases in our study (22.1%). The presence of p53 protein antibodies were thought to be an early marker of cancer,^{23, 24} whereas the p53 antigen can be detected during the progressive stage. Thus it remains to be elucidated whether there is a relation between p53 protein antibodies and p53 antigen in the serum of cancer patients. In cases of pancreatic cancer, CA19-9 and CEA tumour markers have been utilised for both diagnosis and monitoring.²⁵⁻²⁷ In this study, the serum p53 protein concentrations did not correlate significantly with either CA19-9 or CEA. Patients with serum positive p53 antigen concentrations were found more often in cases negative for CA19-9 than in positive cases. Of the 22 patients showing negative serum concentrations for both CA19-9 and CEA, six patients had a positive serum p53 protein concentration. Thus the presence of serum p53 protein probably represents a different biological process from CA19-9 or CEA. Serum p53 protein concentrations can thus be regarded as an additional tumour marker to improve the serological sensitivity of CA19-9 and CEA in patients with pancreatic cancer.

Positive immunostaining by the anti-p53 protein DO-7 antibody was found in 45.9% of cases of pancreatic adenocarcinoma, which is similar to the results from previous reports^{16, 28, 29} which showed p53 gene overexpression in 62 out of 133 (47%), in 16 out of 34 (47%), and in 19 out of 48 (40%) cases of adenocarcinoma, using DO-7, the PAb 1801 monoclonal antibody, and the CM-1 polyclonal antibody respectively. However, our incidence of positive immunoreactivity was slightly lower than in the other reports.^{8-11, 30} This is probably due to differences in the antibody used, the preparation of the sections, and the method of evaluation.

There was more frequent positive p53 antigen immunostaining in the metastatic cases (55.6%) than in the primary cases (41.9%), although this difference was not significant.

This is compatible with the serum p53 protein results, which showed higher p53 concentrations in patients with distant metastases than in those without. We used two different antibodies: PAb 240 for the ELISA and DO-7 for the immunohistochemistry. PAb 240 only recognises the mutant form of the p53 protein,¹⁹ whereas DO-7 recognises both wild type and mutant forms.²⁰ However, the wild type p53 protein is usually undetectable by DO-7 because of its short half life. Therefore, it is probable that the results obtained by both antibodies are compatible.

For the correlation between serum p53 antigen concentrations and p53 protein immunostaining, a high specificity (80%) was shown for the serum p53 protein concentrations, but the sensitivity was not as high. Twenty of the 28 (71.4%) positive immunostaining cases were negative for the serum p53 antigen by ELISA, and were considered to be false negatives. On the other hand, two of the 33 (6.1%) negative immunostaining cases were positive on the ELISA, and were considered to be false positives. The reason for these false-negative cases may be the fact that not all tumours with p53 gene overexpression necessarily release p53 protein into the blood stream,⁵ or that the presence of p53 protein antibodies can impair the detection of p53 protein in the serum.⁷ The false positives could have resulted from an underestimation of the p53 protein immunostaining due to the heterogeneity of the tumour cells. Alternatively, they could have arisen from non-specific cross reactions with other serum proteins that have similar epitopes to p53,⁵ or from a stabilisation of the p53 protein due to binding with other serum proteins via an unknown mechanism. However, there may have been more positive p53 immunostaining cases in the patients with serum positive p53 concentrations in this study than were actually detected, because 13 out of the 23 patients with serum positive p53 concentrations were unresectable cases, and thus their tumour specimens were not available for immunohistochemistry. Therefore, the serum concentrations of p53 protein are expected to be more closely correlated with p53 antigen immunostaining in tumour cells than was actually reported here.

As p53 is the most frequent gene to be mutated in human cancers, it is a popular target for therapy. Recently, antisense oligonucleotides targeting the p53 gene have been reported to have an antiproliferative effect in various cell lines such as acute myeloid leukaemia, chronic myeloid leukaemia, and pancreatic cancers by the suppression of p53 gene expression.³¹⁻³³ Moreover, Fan *et al* showed that the p53 gene status was an important determinant of both radiosensitivity and chemosensitivity in lymphoid cell lines, and that p53 mutations were often associated with a decreased sensitivity to DNA damaging agents.³⁴ Therefore, the investigation of p53 gene alterations in pancreatic cancers is important for the selection of therapeutic treatments, especially in cases of unresectable tumours.

In conclusion, an increase in serum p53 antigen concentrations can represent an accumulation of the p53 protein, which results from mutations of the p53 gene. It would seem that the serum p53 protein concentrations represent different biological processes from tumour markers such as CA19-9 or CEA. In the future, assaying serum p53 protein concentrations may be a procedure for the detection of p53 gene alterations, by which we could select the best therapeutic regimens in patients with pancreatic carcinomas.

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