

Original Article

Serum p53 antibodies as a prognostic marker in patients with pancreatic ductal adenocarcinoma (PDAC)

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Received January 15, 2018; Accepted June 10, 2018; Epub August 15, 2018; Published August 30, 2018

Abstract: Objective: p53 alterations play a pivotal role in pancreatic ductal adenocarcinoma (PDAC) progression and may induce a humoral response. PDAC progression is impacted by mutant p53. In the present study, we evaluated whether serum p53 antibodies (p53-Abs) could act as a prognostic marker in PDAC patients. Methods: The levels of serum p53-Abs were determined in PDAC patients using ELISA and their association with clinicopathological features and patient survival was examined. Results: The level of p53-Abs was significantly associated with advanced tumor stage ($P=0.0029$) and tumor metastasis ($P<0.0001$). Patients with low expression for p53-Abs showed better overall survival than those with high expression ($P=0.0028$). Specifically, a negative relationship was observed between serum p53-Abs and survival in patients with an advanced tumor stage ($P=0.027$). However, no significant correlation was observed in patients at an early stage. Conclusions: These results suggest that serum p53-Abs may serve as a biomarker for overall survival in PDAC patients.

Keywords: Pancreatic ductal adenocarcinoma, p53 antibody, biomarker, serum

Introduction

Changes in tumor antigens can lead to the development of autoantibodies, which result from over-expression, mutation, or altered degradation [1]. Tumor antigen-specific autoantibodies have been identified in the sera of patients with various types of solid tumors [2-5]. The long half-life and *in vitro* stability of these antibodies make them potential biomarkers for detection and/or prognosis of cancer. p53, a well-established tumor suppressor protein, plays a key role in the regulation of genes involved in cell cycle, DNA repair or apoptosis, thereby acting as a “gatekeeper” to maintain genomic stability [6]. In response to cellular or genotoxic stress, p53 can induce several responses, including cell cycle arrest, senescence, differentiation, DNA repair and apoptosis [6, 7]. These functions are carried out, in part, by transactivating a series of genes involved in cell cycle control. Mutations in the p53 gene represent the most common genetic alterations in human cancers [8]. Most known p53 alterations are missense mutations occurring in the evolutionarily conserved exons 4-8.

These mutational events impair the function of p53 in the maintenance of genomic stability. Consequently, tumors lacking wild-type p53 are more prone to the accumulation of deleterious mutated p53 and are therefore expected to be more clinically aggressive.

p53 mutations can lead to production of p53 antibodies (p53-Abs), which can be detected in the sera of patients with various malignancy types [9, 10]. Such p53-Abs result from a self-immunization process in response to the accumulation of mutated p53 in tumor cells [11]. Many studies have shown that the serum p53-Abs could be used as a molecular marker in several cancer types, including breast cancer and non-small cell lung cancer (NSCLC) [12, 13].

Pancreatic ductal adenocarcinoma (PDAC) is a type of cancer, in which disease progression is impacted by mutant p53. p53 is found to be mutated in approximately 75% of metastatic PDAC, which has a poor prognosis because of late-stage detection, presence of metastases, and ineffective treatments [14]. In the present

Table 1. Correlation between serum p53-Abs and clinicopathological factors

Variables	p53-Abs			P value
	N	Low expression (n)	High expression (n)	
Age (years)				
≤65	35	15	20	0.4204
>65	82	43	39	
Gleason score				
≤6	48	28	20	0.8489
>6	69	30	39	
Tumor size (cm)				
≤3	40	24	16	0.2419
>3	77	34	43	
Tumor stage				
T1&T2	48	31	17	0.0029
T3&T4	69	27	42	
Metastasis				
M0	43	30	13	<0.0001
M1	74	28	46	

logical type, and staging were recorded. Staging was defined according to the international TNM classification proposed by the American Joint Committee on Cancer (AJCC).

ELISA

p53 autoantibodies from the serum of each patient were quantified using the p53 ELISA-PLUS (auto-antibody) kit (Oncogene Research Products, Cambridge MA, USA). The kit was designed to measure circulating p53-Abs in human serum samples. The results are expressed in O.D. units and are categorized as high/low expression based on the mean value of serum samples from the participants.

Immunohistochemistry (IHC)

The paraffin-embedded sections were subjected to antigen retrieval by heating the slides in a microwave at 100°C for 10 min in 0.1 M citric acid buffer (pH=6.0), and then incubated with p53 antibody (Cell Signaling) at 4°C overnight. After secondary antibody incubation at room temperature for 1 h, the slides were developed in 0.05% diaminobenzidine containing 0.01% hydrogen peroxidase. For negative controls, specific antibodies were replaced with normal goat serum by co-incubation at 4°C overnight preceding the IHC staining procedure. For each section, the percentage of tumor cells that stained positive for p53 was determined: - for no staining (the p53 staining percentage of 0%); + for weak staining intensity (the p53 staining percentage of 1-20%); ++ for moderately strong staining (the p53 staining percentage of 20-40%); +++ for strong staining (the p53 staining percentage of >40%).

Statistical analysis

Statistical analysis was carried out with the SAS software system (SAS Institute, Cary, NO,

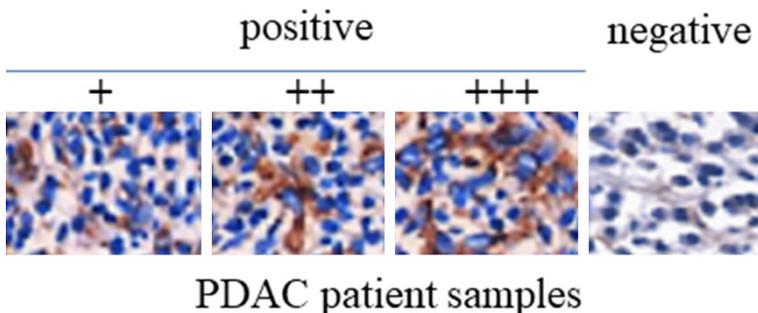


Figure 1. Representative immunohistochemistry (IHC) staining of p53 in pancreatic ductal adenocarcinoma (PDAC) patients, positive (left) or negative (right) for p53.

study, we evaluated the potential prognostic value of serum p53-Ab level and its predictive significance in PDAC.

Patients and methods

Patients

The 117 subjects in this study were recruited from Tianjin Nankai Hospital. The primary PDACs were newly diagnosed and confirmed by pathology and had not received any therapy including radiotherapy, chemotherapy, and surgical resection. Patients had donated a blood sample for routine clinical examination prior to any treatment and excess sera were kept frozen at -80°C and were used for the present analysis. For each patient, the age, histopatho-

Table 2. ELISA result of p53-Abs expression in serum and IHC result of p53 level in tumor tissues

Serum	Tissue			
	-	+	++	+++
High	0	32	7	11
Low	0	6	1	0

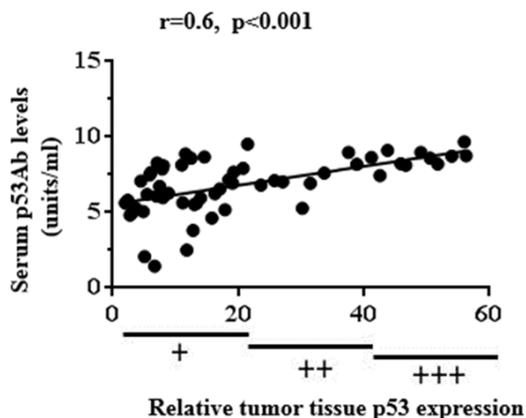


Figure 2. Correlation between serum p53-abs content and tissue p53 expression.

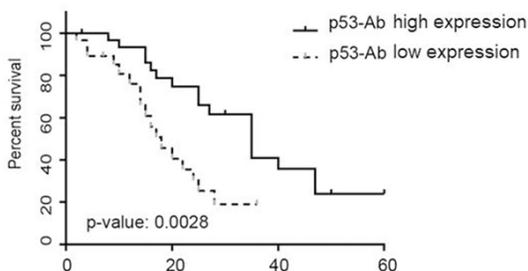


Figure 3. Kaplan Meier analysis of overall survival (OS) of pancreatic ductal adenocarcinoma (PDAC) patients based on the presence of anti-p53 serum antibodies. Patients with high expression for p53 antibodies (p53 Abs) (n=59) had a significantly shorter survival time than those who had low expression (n=58).

USA) and differences were considered significant at $P \leq 0.05$. The association between serum p53 and various clinical pathological characteristics were analyzed by Chi-square test. Pearson's correlation analysis was used for the correlation between serum level and tumor tissue content level of P-53Abs. Survival curves without recurrent disease were constructed using the Kaplan-Meier method. The statistical significance between curves was analyzed by the log-rank test.

Results

Association between the presence of p53-Abs and clinicopathological features

All 117 cases of 60 male and 57 female were analyzed for p53-Abs and clinicopathological features. There were 35 patients with an age ≤ 65 years old and 82 patients with an age > 65 years old. There were 40 patients with tumor size ≤ 3 cm and 77 patients with the tumor size > 3 cm. We also tested the serum p53 with ELISA and evaluated the relationship between the p53-Abs and various clinicopathological characteristics. As shown in **Table 1**, p53-Abs were significantly associated with tumor stage ($P=0.0029$) and metastasis ($P<0.0001$). However, there was no significant association between p53-Abs and age, Gleason score, or tumor size ($P=0.4204$, 0.8489 , 0.2419 , respectively).

Relationship between the presence of p53-Abs and IHC staining of p53

Tissue samples from a total of 57 (48.7%) of the 117 patients were assayed for p53 accumulation in tumor sections using IHC. Representative IHC staining of positive and negative p53 expression is shown in **Figure 1**. IHC staining of p53 expression in tumor tissues and p53-Abs in the serum are shown in **Table 2**. An association was noted between p53 accumulation in tumors and the presence of p53-Abs in the sera (**Figure 2**, $r=0.6$, $p<0.001$).

Presence of p53-Abs correlates with overall survival in PDAC patients

Figure 3 illustrates the Kaplan-Meier curves for PDAC patients who were high or low expression for serum p53-Abs. Log-rank analysis revealed a significant difference in the overall survival (OS) between the two groups ($P=0.0028$) in which longer survival was observed in the group of patients low for serum p53-Abs. Specifically, overall median survival time was 20 (I-II), 9 (III-IV) months for high expression groups, and 22 (I-II), 18 (III-IV) months for low expression groups, respectively. During the follow-up period, 81% of patients with high expression for p53-Abs had died, whereas 72.4% of patients with low expression for p53-Abs had died. Comparison was also made within the group at early stage (I-II) or advanced stage (III-IV). It was observed that p53-Abs had a significant effect

Table 3. p53 antibodies (p53-Abs) and pancreatic ductal adenocarcinoma (PDAC) patient survival

Variable	High p53-Abs level	Low p53-Abs level
Stage I-II		
No. of cases (n)	17	31
Mean survival time (months)	20±2	22±0.5
Analysis of variance (<i>P</i> value)	0.884	
Stage III-IV		
No. of cases (n)	42	27
Mean survival time (months)	9±0.3	18±0.8
Analysis of variance (<i>P</i> value)	0.027	

on advanced stage patient survival (*P*=0.027), but no effect on early-stage patients (*P*=0.884) (Table 3).

Discussion

As the fourth leading cause of cancer-related death, PDAC has a dismal prognosis, with 5-year survival rates of less than 5% [15]. Its high mortality rate is partially due to the lack of effective early detection and diagnosis strategies. Therefore, there is an urgent need to identify novel biomarkers for early PDAC detection, as well as prognostic biomarkers to help guide clinical practice.

Since its discovery in 1982, by Crawford et al. [16], progressive studies have attempted to demonstrate the clinical value of p53-Abs. In the present study, we measured the serum concentration of p53-Abs by ELISA in PDAC patients and assessed the correlation between serum p53-Abs and clinicopathological characteristics. To the best of our knowledge, there have been few, if any, studies examining serum p53-Abs in PDAC patients. We show that high p53-Abs expression is more frequently observed in patients at advanced stages, compared with those at early stages (60.9% vs 35.4%; *P*=0.0029). Moreover, a higher incidence of high serum p53-Abs was observed in patients with distant metastases than those with primary cancer (62.2% vs 30.2%; *P*< 0.0001). This agrees with previous reports suggesting that p53 mutation occurs in 75% of metastatic PDAC, and that mutant p53 drives pancreatic cancer metastasis [17, 18].

Similar observations have been made for other cancer types. It has been reported that p53-Abs concentrations were significantly higher in the

sera of lung cancer patients with lymph node involvement and late-stage disease [19]. In patients with hepatocellular carcinoma, a higher frequency of positive p53-Abs was found in those with vascular invasion [13]. In addition, the p53-Abs expression was positively associated with p53 IHC staining (*P*<0.0001), indicating that this immune response is triggered by the accumulation of p53 in the tumor. This relationship between p53 over-expression and serum p53-Abs presence has been documented in many other cancer types as well [20-22].

Numerous investigations have been carried out to examine the relationship between the presence of p53-Abs and patient survival. Laudanski et al. showed that there was a significant association between the presence of serum p53-Abs and poor survival in NSCLC patients [23]. In another study led by Bergqvist et al., poor survival was observed in advanced NSCLC adenocarcinoma patients with high serum p53-Abs [24]. However, significantly better survival without recurrence was found to be associated with the presence of serum p53-Abs in patients with hepatocellular carcinoma [13]. Similarly, the presence of serum p53-Abs has been reported to be correlated with improved overall survival in serous ovarian cancer [25]. In the present study, overall PDAC survival rates for p53-Abs low expression patients were significantly longer than those for p53-Abs high expression patients (*P*=0.0028). More specifically, p53-Abs had significant effects on the survival of advanced stage patients (*P*=0.027), but no effects on early stage patients (*P*=0.884). Collectively, these results suggest that serum p53-Abs may be used as a novel biomarker for patient survival in PDAC.

In summary, our study demonstrates that the serum p53-Abs are strongly associated with advance tumor stage and cancer metastasis. The serum p53-Abs are significantly correlated with p53 over-expression in tumor tissues. Patients with low expression for p53-Abs exhibit better survival than those with high level p53-Abs. Therefore, circulating p53-Abs may serve as a novel biomarker for prognosis prediction in PDAC patients.

Disclosure of conflict of interest

None.

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