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RESEARCH

The Predictive Role of p16 Deletion, p53 Deletion, and Polysomy 9 and 17 in Pancreatic Ductal Adenocarcinoma

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Abstract In this study, we investigated p53 and p16 deletions, and chromosome 9 and 17 amplifications in pancreatic ductal adenocarcinoma (PDAC), and further analyzed their associations with clinical characteristics and prognosis of PDAC. A total of 32 PDAC and 23 peritumoral tissues were collected. Molecular abnormalities of CEP9/p16 and CEP17/p53 were detected using Fluorescence in situ hybridization (FISH). Deletions of p16 and p53 were detected in 50 % and 65.7 % of PDAC, respectively. Polysomy 9 and 17 were identified in 75 % and 71.8 % of PDAC, respectively. No p16 and p53 deletion, polysomy 9 and 17 were identified in peritumoral tissues. We also observed significant correlations of p16 deletion, polysomy 9 and 17 with shorter survival of PDAC. P16 deletion, polysomy 9 and 17 are predictive markers for poor prognosis of PDAC patients, but p53 deletion is not associated with the clinical characteristics and prognosis of PDAC.

Keywords Pancreatic ductal adenocarcinoma · Prognosis · p16 · p53 · Deletion · Polysomy

Introduction

Pancreatic cancer is the 13th in incidence, but the 8th leading cause of cancer related deaths worldwide [1]. Despite the progress in tumor therapy during the past several decades, the overall survival rate of pancreatic cancer is still less than 5 % [2]. Besides the highly aggressive nature of pancreatic cancer, the lack of specific symptoms leads to about 80 % to 90 % of patients being diagnosed at advanced stages of cancer and is responsible for poor prognosis of pancreatic cancer [3]. Only 15–20 % of patients are able to undergo radical excision of the tumor [4]. Even for these patients with resectable tumors, the 5-year survival rates are only 16 % [5]. This may indicate an urgent need for a prognostic factor of pancreatic cancer

Recent studies have demonstrated that abnormality in tumor-suppressor genes is involved in the development and prognosis of pancreatic cancer [6]. The p53 gene is a crucial tumor-suppressor gene on chromosome 17p and a gatekeeper to guard cell growth against cancer in almost all cell types. Abnormal p53 function is mostly caused by mutations and deletions of the gene [7]. P53 is somatically mutated in 50 % to 75 % of the PDAC patients [8, 9]. However, the prognostic role of p53 mutation in pancreatic cancer remains controversial [10, 11]. In contrast, deletions of the p53 gene that are also responsible for abnormal p53 function have not been extensively investigated in PDAC.

P16 is another tumor suppressor gene located on chromosome 9p21, which regulates cell-cycle progression through inhibiting cyclin D/CDK4/6 functions [6]. Inactivation of p16 function occurs in 80 % to 95 % cases of sporadic PDAC through mechanisms involved in mutation, deletion, or promoter hypermethylation [12]. Among these genetic abnormalities, homozygous deletions accounted for 40 %, and 38 % contained point mutations and loss of the wild-type allele [12, 13]. However, the predictive role of p16 mutations is conflicting. One study has linked p16 mutations to significantly shorter survival of PDAC patients, whereas another study did not find any significant correlation between p16 mutations and the survival of PDAC [14, 15]. Particularly, the prognostic role of p16 deletion has not been investigated in pancreatic cancer.

In contrast to deletion, chromosome amplification has also been thought to be an early event in the carcinogenetic

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process [16]. A previous study demonstrated that polysomy 17 is a predictive marker for PDAC [17]. However, the relationships of p16 deletions and chromosome 9 amplifications, p53 deletions and chromosome 17 amplifications, as well as their associations with the clinicopathological characteristics and prognosis of PDAC have not been investigated. Currently, FISH is the most popular method used to identify homozygous and hemizygous deletions [18, 19], particular, to identify deletions and chromosomal amplifications at the same time, which can permit an analysis for the effect of chromosome amplification on locus deletion. Moreover, FISH assay is available in many clinical laboratories, and can be performed on archival paraffin-embedded tissues and is relatively less expensive than PCR-based assay. In addition, FISH assay is quite sensitive because phenotypic and anatomic changes must occur after DNA changes [19]. In this study, we performed FISH assay in 32 PDACs and further analyzed the correlations of p53 and p16 deletions as well as polysomy 9 and 17 with the clinical characteristics and prognosis of PDAC.

Materials and Methods

Case Selection

This study was done ethically with pre-approval from the Ethics Committee for Human Studies at Shanghai Jiaotong University. A total of 32 PDAC samples and 23 peritumoral tissues were collected. PDAC specimens were collected from patients who underwent partial pancreatoduodenectomy and radical lymphadenectomy, but received no neoadjuvant or adjuvant chemoradiotherapy therapy from January 2005 to July 2010 at First People's Hospital, Shanghai Jiaotong University. Diagnosis of carcinomas was based on morphological criteria, immunohistochemical staining, and clinical findings according to the seventh edition of the tumor-node-metastasis (TNM) classification of the International Union against Cancer [20]. The follow-up ended on April 30, 2011. Survival information was obtained through letters and phone calls. Among the patients, 12 cases survived over 1 year and 20 cases survived less than 1 year. The average survival time was calculated from patients with positive or negative expression of the marker or markers.

Fluorescence in situ Hybridization (FISH)

The PathVision single-color or dual-color fluorescence in situ hybridization kit and probes were purchased from Vysis Inc. (Downers Grove, IL). The probes included centromere (CEP9)/p16 dual color probe (Cat#: 32-190078), p53 single color probe (Cat#: 08 L64-020), and CEP17 single color probe (05 J03-017). FISH was performed according to the manufacture's protocol. All slides were viewed using an Olympus BX51 epi-fluorescence microscope equipped with a set of the appropriate filters (Vysis Inc.). Samples showing sufficient FISH efficiency (>90 % nuclei with readable signals) were assessed. The hybridization results were evaluated by two independent researchers who were unaware of clinical outcome. Hemizygous deletions of the p16 and p53 locus were defined as >50 % nuclei containing a single locus probe signal but two CEP9 or CEP17 signals. Homozygous deletions of p16 and p53 were identified by the simultaneous lack of both loci signals and by the presence of CEP9 or CEP17 signals in more than 30 % of cells. Polysomy 9 and 17 (chromosomal gains) was defined as >10 % of nuclei containing three or more CEP 9 and CEP 17 signals [21].

Statistical Analysis

All analyses were performed using SPSS v13.0 (SPSS Inc., Chicago, IL, USA). Categorical variables were assessed using the Chi-square test and Fischer's exact test. Univariate analyses of overall survival and survival curves were performed following the Kaplan–Meier method, and the Cox proportional hazards model was used for multivariate analysis. All probabilities are two-tailed. A $p<0.05$ was considered statistically significant.

Results

P16 and p53 Deletion, Polysomy 9 and 17 were Observed in PDAC Tumors But Not in Peritumoral Tissues

FISH was used to measure genetic alterations in 32 resected PDAC tumors. 65.7 % of cases had p53 deletion (21 out of 32), 50 % had p16 deletion (16 out of 32), polysomy 9 was identified in 75 % of PDAC and polysomy 17 was identified in 71.8 % of PDAC (Fig. 1, Table 1). No p53 and p16 deletion, polysomy 9 and 17 were observed in peritumoral

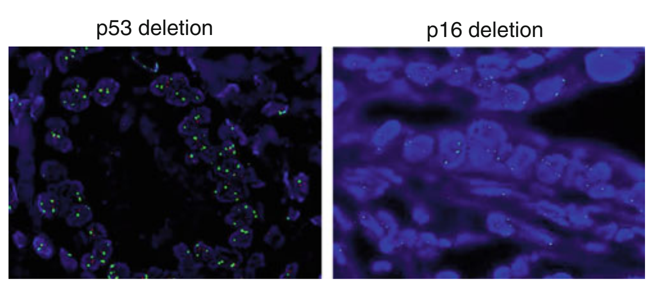

Fig. 1 Molecular abnormalities in patients with PDAC. Molecular abnormalities detected by FISH. Original magnification x1000. Representative of p16 and p53 deletions

Table 1 Comparison of p53 and p16 deletion, polysomy 9 and polysomy 17 in PDAC with peritumor tissues

Variable	Tumor tissue		Peritumor tissue	
	Neg No. (%)	Pos No. (%)	Neg No. (%)	Pos No. (%)
P53 deletion	11 (34.3)	21 (65.7)	23 (100)	0
P16 deletion	16 (50.0)	16 (50.0)	23 (100)	0
Polysomy 9	8 (25.0)	24 (75.0)	23 (100)	0
Polysomy 17	9 (28.2)	23 (71.8)	23 (100)	0

tissues. All p53 deletions are hemizygous deletions, while all p16 deletions are homozygous deletions.

P53 and p16 Deletion, and Polysomy 9 and 17 Were Not Associated with Clinicopathological Characteristics of PDAC

We analyzed the associations of p53 and p16 deletion, polysomy 9 and 17 with the clinicopathological characteristics of PDAC. No markers exhibited significant associations with age, gender, tumor location, TMN stages, lymph node metastasis, and tumor grade (Table 2). Also, no correlation between any two markers among p53 deletion, p16 deletion, polusomy 9, and polysomy 17 was observed.

Polysomy 9 and 17 Correlated with Survival in Patients with PDAC

Kaplan-Meier survival analysis revealed that p53 deletion had no association with average survival time in PDACs. In contrast, the p16 deletion and p16/p53 deletions exhibited significant correlations with shorter average survival times (p=0.002). Average survival time in patients with polysomy 9 or polysomy 17 was significantly shorter than those without polysomy 9 and 17 (p=0.019, p=0.004, respectively) (Table 3, Fig. 2). This suggested that p16 deletion, polysomy 9 and 17 are predictive markers for poor prognosis of PDAC. Cox multivariate analysis further showed that polysomy 17 and p16 deletion negatively correlated with survival (Table 3), suggesting that polysomy 17 and p16 deletion are an independent risk factor.

Discussion

Mutation of p53 was observed in 50 % to 75 % of the PDAC patients, whereas p53 deletion in PDAC is rarely reported. The inactivation of p16 gene by mutation, deletion and methylation is observed in up to 95 % of sporadic PDAC cases. In this study, we investigated the occurrence of p16 and p53 deletions and chromosome 9 and 17 amplifications by FISH. Polysomy 9 and 17 were observed in 75 % and 71.8 % of PDAC patients, respectively, while hemizygous

Table 2 Association of p53 deletion, p16deletion, polysomy9 and polysomy17 with the clinicopathological characteristics of PDAC

Variables examined	P53		Pol17		P16		Pol9	
	Positive No. (%)	p	Positive No. (%)	p	Positive No. (%)	p	Positive No. (%)	p
Age								
≤60	6 (66.7)	1.000	7 (77.8)	0.667	5(50.0)	1.000	7(70.0)	1.000
>60	13(72.2)		11(61.1)		8(44.4)		12(66.7)	
Gender								
Male	13(72.2)	1.000	13(72.2)	0.423	8(44.4)	1.000	13(72.2)	0.677
Female	6(66.7)		5 (55.6)		5(50.0)		6(60.0)	
Location								
Head	17(73.9)	0.558	16(69.6)	0.582	11(45.8)	1.000	16(66.7)	1.000
Body and tail	2 (50.0)		2 (50.0)		2 (50.0)		3 (75.0)	
TNM								
I+IIa	9(69.2)	1.000	8(61.5)	0.673	6(42.9)	0.695	8(57.1)	0.429
IIb+III+IV	9(75.0)		9(75.0)		7(58.3)		9(75.0)	
Lymph node								
Negative	11(73.3)	1.000	10(66.7)	1.000	7(43.8)	0.688	10(62.5)	1.000
Positive	7(70.0)		7(70.0)		6(60.0)		7(70.0)	
Tumor grade								
G1	2(66.7)	1.000	1(33.3)	0.250	1(33.3)	1.000	1(33.3)	0.234
G2+G3	17(70.8)		17(70.8)		12(48.0)		18(72.0)	

Table 3 Univariate and multivariate survival analysis (log-rank test) of molecular features

Variables	Univariate analysis	Multivariate analysis		
	p	p	RR	95 % CI
P53 deletion (Pos vs. Neg)	0.701			
P16 deletion (Pos vs. Neg)	0.002	0.003	7.210	1.929–26.944
Polysomy 9 (Pos vs. Neg)	0.019	0.194		
Polysomy 17 (Pos vs. Neg)	0.004	0.004	7.072	1.857–26.935
P53 del+p16del (Pos vs. Neg)	0.002	0.713		

p53 and homozygous p16 deletions were observed in 65.7 % and 50 % of PDAC patients, respectively. Interestingly, p16 deletion, polysomy 9 and 17 were demonstrated to be prognostic markers in PDAC, whereas, deletions of p53 was not associated with clinical characteristics and prognosis of PDAC.

A previous study revealed that upon to 95 % of the p53 deletions are hemizygous deletion, which are significantly associated with p53 mutation [6]. Moreover, a study in chronic lymphocytic leukemia revealed that hemizygous deletion is responsible for the high expression of nuclear p53 protein [6]. In this study, all p53 deletions were hemizygous, and 65.7 % PDAC patients had p53 deletion. The rate for p53 deletion is similar to the rate of p53 abnormality observed in PDAC (50 % to 75 %) [9]. This suggests that p53 deletion might be the main p53 abnormality in PDAC. However, it lacks of association between p53 deletion and clinical characteristics as well as prognosis of PDAC observed in the current study.

Previous studies demonstrated that most p16 deletions are homozygous [12]. In this study, we found that all p16 deletions are homozygous. The rate for p16 deletion (50 %) is little higher than that in previous reports (40 %) [6]. The prognostic role of p16 loss in pancreatic cancer remains controversial in a previous report. In this study, p16 deletions were significantly associated with prognosis of PDAC. In addition, p16 deletion occurs at the stage of PDAC when malignancy of tumor has already been established [2]. Therefore, it is easy to understand the correlation between p16 deletions and the prognosis of PDAC.

The copy number changes of chromosomes have been thought to be an early event in the carcinogenetic process [16]. Studies in breast cancer demonstrated that up to 93 % of tumors have chromosome 17 copy number changes, which are associated with tumorigenesis of breast cancer [22]. In contrast, polysomy 17 was only identified in about 32 % of pancreatic adenocarcinoma [23, 24]. In this study, polysomy 17 was identified in 71.8 % of PDAC, which significantly correlated with poor survival (p=0.005). Several important tumor-suppressor genes, such as *TP53* and *BRCA1,* and an oncogene, human epidermal-growth-factor receptor 2 (Her-2), are located on chromosome 17 [24, 25]. The high rate of polysomy 17 may be responsible for the overexpression of Her-2 found in pancreatic cancers [26, 27]. In contrast, tumor-suppressor genes *TP53* and *BRCA1* are located in regions that are often deleted and mutated

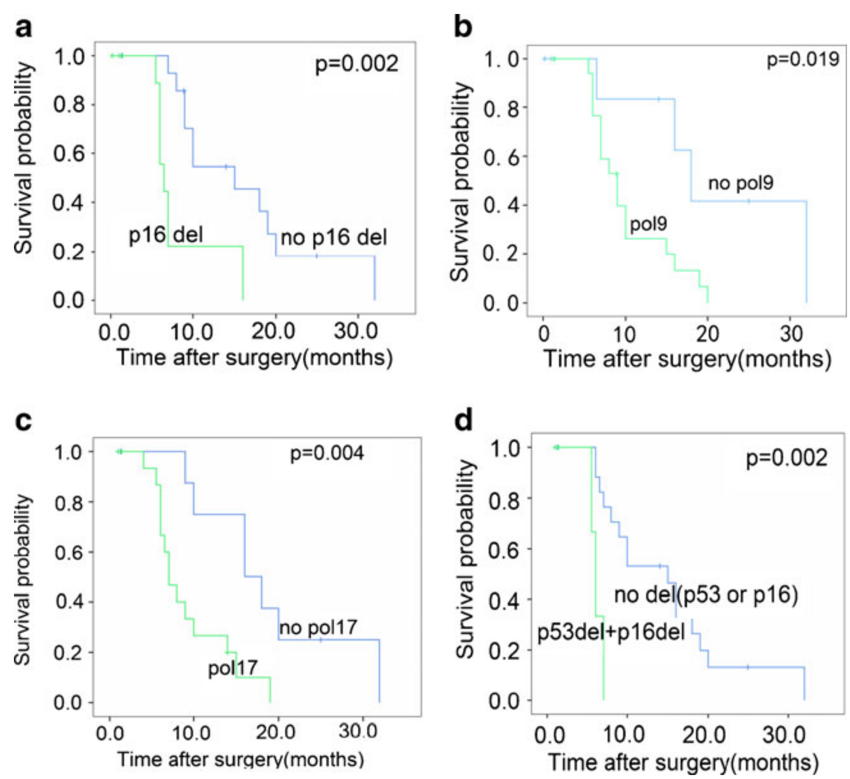

Fig. 2 Survival analysis in patients with PDAC. Kaplan-Meier plots of overall survival in patients with PDAC and with positive and negative p16 deletion **a**, polysomy 9 **b**, polysomy 17 **c** and p16/p53 deletion **d**

[25]. In this study, 65.7 % of *TP53* were deleted in PDAC. Although the rate of p53 mutations in the left allele was not analyzed in this study, previous studies demonstrated that *TP53* mutations can be identified in upon to 75 % of the PDAC patients [8, 9]. Therefore, the positive correlation of polysomy 17 with poor prognosis in PDAC may be associated with oncogene expression, such as Her-2 overexpression, due to increase in copy number of chromosome 17.

Changes in copy number of chromosome 9 have been found in several tumors [16, 28], which was proposed as a biomarker that is responsible for increased risk of developing cancer. Conversely, no report explored the clinical relationship between chromosome 9 and pancreatic cancer. In this study, polysomy 9 was identified in 75 % of PDAC, which significantly correlated with the poor prognosis ($p=0.019$). The tumor suppressor gene p16 is located on the 9p chromosomal region [29]. Therefore, polysomy 9 should lead to p16 gene overexpression and the suppression of tumor growth, which is contrary to findings in this study. This can be explained by the observation of p16 dysfunction in up to 95 % of PDAC due to mutation, deletion, or promoter hypermethylation [12, 13]. In this study, we also found p16 deletion in 75 % of PDAC. The presence of other oncogenes in chromosome 9, such as c-Myc and ABL1 genes [30, 31], may also contribute to the role of polysomy 9 in the poor prognosis of PDAC.

In conclusion, patients with p16 deletion, polysomy 9 and 17 survive a shorter time and these biomarkers could predict tumor prognosis in PDAC patients. In contrast, p53 deletion is not associated with clinical characteristics of PDAC. Our study also implicated that oncogenes in chromosome 9 and 17 may also play a more important role in PDAC prognosis**.**

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