Abnormalities of Tumor Suppressor Gene *p16* in Pancreatic Carcinoma : Immunohistochemical and Genetic Findings Compared with Clinicopathological Parameters

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Abnormalities of the tumor suppressor gene *p16* have been reported in a variety of human tumors, but are rare in pancreatic carcinoma (PC) except for cancer cell lines and xenografts. Moreover, their clinicopathological significance remains unknown. The purpose of this study is to examine immunohistochemical and genetic alterations of *p16* in primary PC tissues, and to investigate the relationship between abnormalities of *p16* and clinicopathological parameters in order to elucidate their clinicopathological significance.

We investigated p16 expression in 60 PC cases by immunohistochemistry using a monoclonal antibody clone G175-405. In addition, we analyzed genetic alterations of the *p16* gene using DNA extracted from microdissected tissue of PC, by PCR-SSCP, DNA sequencing, and hypermethylation analyses using restriction enzymes. We compared the abnormalities of *p16* alterations with clinicopathological parameters in order to elucidate their significance.

On immunohistochemical study, staining for the p16 protein was strongly positive in 22 (37%) of 60 PC cases, weakly positive in 24 (40%), and negative in 14 (23%). In contrast, *p16* mutations were recognized in 9 (15%) of the 60 PC cases. The incidence of *p16* mutations was 2 (9%) in 22 cases of PC with strongly positive staining, 4 (17%) in 24 with weakly positive staining, and 3 (21%) in 14 with negative staining. Hypermethylation of *p16* was detected in the 2 PC cases with weakly positive staining, although homozygous deletions were not found in any cases. There was no significant correlation between the expression of p16 protein and any of the clinicopathological parameters. In contrast, for PC with *p16* mutation or hypermethylation, the tumor was significantly larger and the survival period significantly shorter than for PC with an intact *p16* gene ($p < 0.05$) (Table).

These findings suggest that *p16* alterations may participate in the aggressiveness of PC.

Table Correlation between clinicopathological parameters and alterations of the *p16* gene in pancreatic carcinomas.

Parameters	Number	*p16* alterations (%)	p
Tumor size			
TS1 & TS2	38	4 (11)	0.049
TS3 & TS4	19	6 (32)	
Tumor location			
Head	40	6 (15)	0.30
Body & tail	19	5 (26)	
Pathological findings			
Pap & well	22	3 (14)	0.54
Mod & por	35	7 (20)	
Clinical stage			
I & II	22	2 (9)	0.15
III & IV	37	9 (24)	
Survival (months)			
>6	25	2 (8)	0.022
$\leqq 6$	23	8 (35)	
LN metastasis			
absent	14	1 (7)	0.067
present	24	8 (33)	
distant metastasis			
absent	35	8 (23)	0.85
present	10	2 (20)	

[a]pap, papillary adenocarcinoma; well, well-differentiated adenocarcinoma; mod, moderately differentiated adenocarcinoma; por, poorly differentiated adenocarcinoma.