Detection of *preproenkephalin (ppENK)* hypermethylation in the pancreatic juice from patients with pancreatic carcinomas:compared with *p53* mutations

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Aberrant methylation of CpG islands is a common mechanism for tumor suppressor genes in a variety of human malignancies. The *preproenkephalin (ppENK)* gene encodes a native opioid peptide, met-enkephalin, which is known to be a potent regulator of development, cell proliferation and angiogenesis. *ppENK* hypermethylation is recognized in 90% in pancreatic carcinoma (PCa), but not normal pancreas tissues. We analyzed the *ppENK* hypermethylation in pure pancreatic juice (PPJ) in patients with PCa and chronic pancreatitis (CP), and elucidated the usefulness for diagnosis of PCa. Furthermore, *p53* mutations were also studied in the same PCa patients.

PPJ was collected endoscopically from 28 patients with PCa and 20 patients with CP. DNA was extracted from PPJ. Methylation specific PCR was performed for hypermethylation analyses with the DNA extracted from these samples. In addition, single-strand conformation polymorphism and sequencing were performed simultaneously for the analyses of *p53* mutations.

The incidence of *ppENK* hypermethylation in PPJ was 50% (14 of 28) in patients with PCa. In contrast, only one of 20 cases presented hypermethylation in patients with CP ($p < 0.002$). *p53* mutations were observed in 12 of 28 PCa cases (42.9%), but not in 20 CP cases. In combination analyses of *ppENK* hypermethylation and *p53* mutations both were positive in 7 and both were negative in 9 of 28 patients. *ppENK* hypermethylation alone was positive in 7 and *p53* mutations alone in 5 patients. *ppENK* hypermethylation or *p53* mutations were observed in 19 of 28 shown in Table, so that positivity improved to 67.9% upon their combination.

These results suggest that *ppENK* hypermethylation in PPJ would be specific for cancer, and the combination assay with *p53* could enhance the genetic diagnosis of PCa.

Table. Comparison of *ppENK* hypermethylation and *p53* mutations from patients with pancreatic cancer

		p53 mutations +	-	Total
ppENK hypermethylation	+	7	7	14
	-	5	9	14
Total		12	16	28