Usefulness of aberrant methylation of *tissue factor pathway inhibitor 2 (TFPI-2)* in pure pancreatic juice in diagnosis for pancreatic carcinoma

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Tissue factor pathway inhibitor 2 (TFPI-2) is a Kunitz-type serine proteinase inhibitor and is thought to protect the matrix from degradation therefore counteracting tumor invasion and metastasis. Recently, aberrant methylation of *TFPI-2* was detected frequently in pancreatic carcinoma (PCa) tissues and not in normal pancreatic tissues. We analyzed the aberrant methyaltion of *TFPI-2* in the pure pancreatic juice (PPJ) from patients with various pancreatic diseases and evaluated its clinical usefulness in the diagnosis of PCa.

We evaluated the hypermethylation of *TFPI-2* in PCa cell lines and PPJ aspirated endoscopically from patients with PCa, benign and malignant intraductal papillary mucinous neoplasm of the pancreas (IPMN), chronic pancreatitis (CP) by methylation-specific PCR (MSP) and real-time quantitative MSP (Q-MSP).

The incidence of the aberrant methylation of *TFPI-2* using MSP was 7 (77.8%) of 9 PCa cell lines by Q-MSP and the expression of *TFPI-2* mRNA by quantitative RT-PCR showed inverse correlation to the aberrant methylation of *TFPI-2*. Moreover, the incidence of the aberrant methylation of *TFPI-2* in the PPJ was 21 (58.3%) of 36 PCa patients, 2 (20%) of 10 malignant IPMN and 1 (14.3%) of 7 benign IPMN and 1 (4.8%) of 21 CP by MSP assay. The incidence of the aberrant methylation of *TFPI-2* in the PPJ with PCa was significantly higher than that with IPMN ($p< 0.05$) or CP ($p< 0.001$) as shown in Table. Using Q-MSP analysis, the ratio of hypermethylation *TFPI-2/MyoD1* in the PPJ from patients with PCa, IPMN and CP was 6.62 ± 7.20, 0.58 ± 1.02 and 1.28 ± 1.73, respectively. The ratio of hypermethylation*TFPI-2/MyoD1* in the PPJ with PCa was significantly higher than that with IPMN ($p< 0.01$) or CP ($p< 0.05$). Using the suitable cut-off value set as 2.5 according to the ROC curve, the incidence of the aberrant methylation of *TFPI-2* in the PPJ by real-time MSP was 18 (62.1%) of 29 PCa patients, 1 (5.9%) of 17 IPMN and 3 (14.3%) of 21 CP, respectively. The incidence of quantitative *TFPI-2* hypermethylation in the PPJ with PCa was significantly higher than that with IPMN ($p< 0.001$) or CP ($p< 0.001$).

These results suggest that promoter methylation of *TFPI-2* in the PPJ may be specific to PCa and a useful marker in the diagnosis of PCa.

Table The incidence of aberrant methylation of *TFPI-2* in the pure pancreatic juice from patients with various pancreatic diseases by methylation-specific PCR

Pancreatic disease	Incidence of aberrant methylation of *TFPI-2*	
Pancreatic carcinoma	58.3% (21/36)	
Malignant IPMN	20.0% (2/10)	*
Benign IPMN	14.2% (1/7)	*
Chronic pancreatitis	4.8% (1/21)	**

IPMN: intraductal papillary mucinous neoplasm, * : $p< 0.05$, ** : $p< 0.001$.