Quantitative measurement of *hTERT* mRNA in sera of the patients with pancreatic carcinoma by real-time RT-PCR

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Human telomerase reverse transcriptase (hTERT) is regarded as a catalytic component of telomerase and a rate-limiting determinant of the enzymatic activity of human telomerase. Its expression reflects the telomerase activity. Usually, telomerase is inactivated or its activity repressed in the majority of normal somatic cells but is activated in germ cells, embryonal cells, proliferating cells of renewable tissues, and activated lymphocytes as well as in most malignant tumor cells. We examined whether the detection of *hTERT* mRNA in sera by real-time RT-PCR is useful or not for the diagnosis of pancreatic carcinoma (PCa).

The serum samples were obtained from 16 patients with PCa, 4 with chronic pancreatitis (CP), and 23 healthy subjects and the RNA was extracted from the serum immediately and stored at –80℃ until the assay. The RNA samples were reverse-transcribed after DNase I treatment, and measured by the real-time RT-PCR.

The *hTERT* mRNA in sera was detected in 13 (57%) of the 23 control subjects and 16 (100%) of 16 PCa patients by the real-time RT-PCR and the agarose gel electrophoresis. Based upon the standard curve using *hTERT* cDNA as a positive control, a linear relationship between PCR products and cycle number of *hTERT* cDNA was confirmed in the range from 0.03pg to 30ng. A linear relationship about 18S rRNA was also confirmed by real-time RT-PCR. The mean ±SD of the ratio of *hTERT* mRNA X 10^{-2}/18S in sera from the control subjects, patients with PCa, and patients with CP was 0.07±0.18, 0.13±0.25, and 0.002±0.05, respectively. With a standard value of 0.07 as the mean value of normal subjects, 3 (13%) of 23 normal subjects, 6 (38%) of 16 patients with PCa, and none (0%) of 4 with CP showed elevated values. The PCa patients with the high values of *hTERT* mRNA/18S in sera showed advanced clinical stages such as liver metastasis and peritoneal dissemination, whereas some of the PCa patients with its low value less than 0.07 in sera underwent surgical resection and obtained longer survival. However, the values of *hTERT* mRNA/18S in sera were elevated in a part of normal subjects as well as PCa patients. Moreover, the values of *hTERT* mRNA/18S with PCa patients considerably overlapped with those with normal subjects even in the high range. It is probably because the *hTERT* mRNA derived from proliferating cells of renewal tissues or activated lymphocytes may be included in the serum. Therefore, assay of *hTERT* mRNA in the serum may be troublesome and not useful for the clinical application from the results of our study although *hTERT* mRNA has been reportedly supposed to be a useful tumor marker.