RESEARCH NOTE

# DETECTION OF hTERT mRNA IN GASTROINTESTINAL TRACT CANCER SPECIMENS

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Abstract. Human telomerase consisting of telomerase RNA template (hTR) and telomerase reverse transcriptase (hTERT) provides a mechanism for synthesis of telomere repeats that prolongs life span of cells. Telomerase activity is present in germ-line and malignant tumor cells but not in most normal human somatic cells. This study determined hTERT mRNA level in tissue samples from patients with gastrointestinal tract (GI) cancers. Tissue samples were obtained from 22 GI cancer patients, 3 gastrointestinal stomal tumors (GIST) and 25 corresponding non-cancerous tissues. hTERT expression was determined by real-time reverse transcriptase-polymerase chain reaction (RT-PCR) using Taqman probe. hTERT mRNA was detected in 12 of 22 cancerous tissue samples. Six of 8 tissue samples obtained from patients with hepatocellular carcinoma and cholangiocarcinoma were positive for hTERT. However, hTERT mRNA was not detected in GIST and non-cancerous tissues. These results suggest that hTERT may be an effective target for cancer therapies to treat many type of GI cancers including cholangiocarcinoma and hepatocellular carcinoma.

## INTRODUCTION

Gastrointestinal tract (GI) cancers include cancers of the esophagus, stomach, hepatobiliary system, pancreas, and small and large intestines. Among them, stomach and liver cancers are the most common malignancies worldwide. Thus, GI cancers are a major public health problem (Neal, 2006).

Human telomerase, an enzyme that prevents the loss of telomere repeat, that maintain the stability of cells comprises a telomerase RNA template (hTR) and telomerase transcriptase protein (hTERT) (Kawahara *et al*, 2007). hTR acts as a template for synthesis of telomeric DNA, whereas hTERT catalyzes the synthesis of the telomere ends of linear chromosomes (Yan *et al*, 1999). Activation of telomerase has been implicated in cell immortality and cancer growth. Telomerase activity is undetectable in most normal cells and tissues, but is present in immortalized cells and 85-90% of human cancer specimens (Deng *et al*, 2007). As telomerase is constitutively active in most cancer cells, but not in normal cells, hTERT appears as a good target for cancer treatment. hTERT antisense oligodeoxynucleotide transferred into bladder cancer cells increases tumor necrosis factor-$\alpha$ (TNF-$\alpha$)-induced apoptosis of tumor cells (Gao *et al*, 2007), and over expression of hTERT protects TNF-$\alpha$-

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induced apoptosis in maturation-resistant acute promyelocytic leukemia (APL) cell line (Kondo *et al*, 1998).

To use hTERT as a target for GI cancer treatment, knowledge of the incidence of the expression of hTERT in GI cancer specimens is necessary. We examined hTERT mRNA level in tissue samples of patients with cholangiocarcinoma and hepatocellular carcinoma in comparison with non cancerous tissues using real-time reverse transcriptase polymerase chain reaction (real time RT-PCR).

## MATERIAL AND METHODS

### Patients and sample preparation

Twenty-two tumor tissues, 3 gastrointestinal stomal tumors (GIST) and 25 corresponding non-cancerous tissues from patients undergoing surgery for GI at Rajavithi Hospital, Thailand, between July 2003 and April 2006, were studied. Tumor samples and non-cancerous tissues (as negative control) were collected at the time of surgery and histopathologically characterized to confirm diagnosis. Pathological data, including tumor staging, were also collected. Tissue samples were preserved in RNAlater reagent (QIAGEN) and stored at -80ºC until further analysis.

### RNA extraction

Total RNA from tissue samples was extracted using Trizol (Invitrogen, Carlsbad, CA) and ethanol precipitation, according to the manufacturer's instructions.

### Primers and probes

Primers and probes for hTERT mRNAs were 5′-TGACACCTCACCTCACCCAC-3′, 5′-CACTGTCTTCCGCAAGTTCAC -3′, and TaqMan 5′ (6-FAM)- ACCCTGGTCCGAGG TGTCCCTGAG -(TAMRA-Q) 3′. Primers for porphobilinogen deaminase (PBGD) mRNA were 5′-CGGAAGAAAACAGCCCAAAGA-3′ and were 5′-TGAAGCCAGGAGGAAGCACA GT-3′. Probes and primers were purchased from Operon.

### Analysis of hTERT expression by real time RT-PCR

Quantitative detection of hTERT mRNA was performed with Quantitect Multiplex RT PCR Kit (QIAGEN) using Chromo4 Real-Time PCR Detector (MJ Research) according to the manufacturer's instructions. Reaction was performed in 25 μl solution cantaining 1x Master mix, 0.25 μl of RT mix, 0.4 μM of each primer pair, 0.2 μM of probe and 250 ng of RNA. Reaction mixture was reverse transcribed at 50ºC for 20 minutes, and heated at 95ºC for 15 minutes before being amplified employing 40 cycles of thermocycling, each cycle at 94ºC for 45 seconds and at 60ºC for 1.15 minutes. Samples were normalized on the basis of PBGD mRNA content. Amplification of PBGD was performed with Quantitect Multiplex RT-PCR NR kit (QIAGEN). PCR was performed in 20 μl solution containing 1x SYBR RT Master mix buffer, 0.2 μl of Quantitect RT mix, 0.4 μM of each primer and 200 ng of RNA. PCR reaction was performed following reverse transcription at 50ºC for 20 minutes and heating at 95ºC for 15 minutes by amplification through 35 cycles, each cycle at 94ºC for 15 seconds, 48ºC for 20 seconds and at 72ºC for 30 seconds. Tissue samples with more than 150 copies of PBGD mRNA were used for analysis of hTERT mRNA.

## RESULTS

Tissues from 22 patients with confirmed diagnosis of GI cancer, consisted of 5 cholangiocarcinoma patients; 4 CA sigmoid patients; 3 each with hepatocellular carcinoma, CA ampulla and CA G.B; 1 each with CA duodenum and P-CCA; and 2 patients from CA colon. Tumors and non-cancerous tissues were extracted for total RNA and hTERT mRNA level was quantitated by real time RT-PCR using Taqman probe. Fig 1 shows the signals in a series of amplification

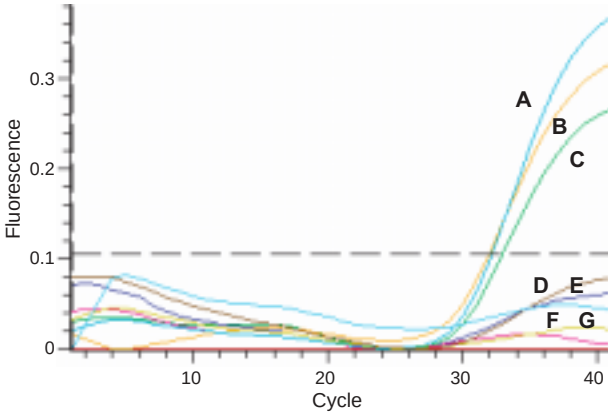


Fig 1–Amplification curve of hTERT cDNA in tumor and corresponding non-cancerous tissue samples. RT-PCR reaction was performed in 25 μl volumes consisting 250 ng of RNA template, 0.4 μM of specific primer pair, 1x Master mix, 0.25 μl of RT mix and 0.2 μM of TaqMan probe. Reverse transcription was carried out at 50ºC for 20 minutes, followed by initial denaturation step at 95ºC for 15 minutes. cDNA was then amplified with 40 cycles at 94ºC for 45 seconds and 60ºC for 1.15 minutes. Fluorescence signal was acquired at the end of each cycle. A and B, signal from tissue of cholangiocarcinoma patients; C, signal from tissue of hepatocellular carcinoma patient; D and E, signal from corresponding non-cancerous tissues; F and G, signal from GIST.

curves of hTERT mRNA from tissue samples demonstrating that hTERT mRNA were significantly higher in tumor tissues than in the corresponding non-cancerous tissues. hTERT mRNA was undetectable in all non-cancerous tissues (including GIST), whereas it was observed in 12 of 22 (55%) of cancerous tissue samples obtained from cancer patients. Surprisingly, 6 of 8 (75%) of tissue samples obtained from hepatocellular carcinoma and cholangiocarcinoma were positive for hTERT.

## DISCUSSION

In the present work, we used the real-time RT-PCR for quantification of hTERT mRNA expression in samples from patients with GI cancer including cholangiocarcinoma and hepatocellular carcinoma. To normalize hTERT mRNA expression for sample-to-sample differences in RNA input, RNA quality, reverse transcriptase efficiency and RT-PCR performance, amplification of mRNA encoding for PBGD was processed in a separate RT-PCR. Over 50% of cancerous tissues exhibited presence of hTERT mRNA and in particular, 75% of hepatocellular carcinoma and cholangiocarcinoma patients showed expression of hTERT gene, whereas hTERT mRNA was not found in all GIST and non-cancerous tissues. In a previous study, hTERT mRNA was detected in 28 of 33 (85%) sera obtained from cholangiocarcinoma patients (Leelawat *et al*, 2006). Recent studies have shown correlation between the expression of hTERT mRNA expression and telomerase activity in biopsy specimens and bile from biliary tract cancers (Itoi *et al*, 2001) and in breast cancer (Hosseini *et al*, 2006). Moreover, hTERT mRNA was also detected in tissues from many types of cancers such as gastric, breast and thyroid cancer (Saji *et al*, 1999; Kirkpatrick *et al*, 2003; Hu *et al*, 2004). These data support that hTERT mRNA is a useful tumor marker for the detection of cancer. The presence of hTERT is unrelated to patients' age, gender, tumor size, tumor location and stage (Saji *et al*, 1999; Kirkpatrick *et al*, 2003; Hu *et al*, 2004, Leelawat *et al*, 2006).

A number of studies have demonstrated that telomerase can be developed as a new tumor-targeting gene therapy by utilizing telomerase promoter mechanism (Song *et al*, 2003; Hong *et al*, 2007). hTERT antisense has been studied in acute promyelocytic leukemia (APL) cell line, in which the overexpression of hTERT protected TNF-α-induced apoptosis in maturation-resistant APL cell line (Kondo *et al*, 1998). Recently, a new tumor-targeting gene therapy strategy based upon the targeting

and replacements of hTERT RNA, using a trans-splicing ribozyme, has been developed (Hong *et al*, 2007).

The high incidence of hTERT expression in GI cancer specimens shown in this study indicate that these cancers should be considered as candidates for future tumor-targeting gene therapy.

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