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Quantitative analysis of telomerase activity and telomerase reverse transcriptase expression in renal cell carcinoma

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Abstract To investigate the relationship between the telomerase activity levels and clinicopathological features of tumors, we quantified the telomerase activities of 23 renal cell carcinomas (RCCs) and four non-cancerous tissues, using a modified telomeric repeat amplification protocol assay, and assessed the *hTERT* mRNA levels of these samples by reverse transcription-polymerase chain reaction analysis. Elevated levels of telomerase activity had correlation with tumor stages as well as the degree of nuclear grades. Our findings suggested that telomerase activity is a useful indicator for tumor aggressiveness in RCCs. However, *hTERT* mRNA levels in RCCs had no correlation with nuclear grades and tumor stages. The telomerase activities and the *hTERT* mRNA levels in cancer cells were not always in parallel. These results suggested that telomerase activity is regulated in a posttranscriptional manner as well as a post-translational manner in tumor cells.

Key words Clinical parameter · Posttranscriptional regulation · Renal cell carcinomas · Telomerase · Telomeric repeat amplification protocol · Telomerase reverse transcriptase

Introduction

Telomerase is a specialized type of reverse transcriptase that catalyzes the addition of hexameric TTAGGG repeats to telomeres, the ends of chromosomal DNA [14]. The enzyme consists of three major components: telomerase reverse transcriptase (*hTERT*), telomerase-associated protein (TEP1), and telomerase RNA (TERC) [3, 12, 13]. Telomerase activity has been detected in fetal tissues, adult testes, and adult ovarian follicles. The enzyme seems to be inactivated or repressed in most of the adult tissues, whereas high telomerase activity levels have been detected in many malignant tumors [8, 10]. Telomerase activation appears to be essential for maintaining the telomere length and it is required for cellular immortality. However, the precise role of telomerase in cancer development and the mechanism responsible for telomerase activation are still unclear [19]. *hTERT* is thought to be a rate-limiting determinant of telomerase activity, and its expression has been demonstrated to correlate with telomerase activity in hepatocellular carcinomas [20].

In this study, we improved the current method for assaying telomerase activity, the telomeric repeat amplification protocol (TRAP), to evaluate the levels of this enzyme in different renal cell carcinomas (RCCs). We analyzed the relationship between telomerase activity levels and clinicopathological features of tumors. In addition, as a step towards elucidating the mechanism responsible for telomerase activation in RCCs, we also evaluated *hTERT* mRNA levels by reverse transcription-polymerase chain reaction (RT-PCR) analysis and compared them with the corresponding telomerase activities.

Materials and methods

Tissue samples

Twenty-three samples of RCCs, three adjacent normal kidney tissues, and one renal cyst were obtained during surgery in the Section of Urology of Yamaguchi University Hospital. The histopathological diagnosis of each sample was determined by examining sections of the same specimens as those used for TRAP assay and RT-PCR analysis. All the samples were frozen in liquid nitrogen as quickly as possible after excision and stored at −80 °C until required for use. The TNM stages of the tumors were evaluated

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according to the criteria of UICC (1992), and the nuclear grades were determined according to Skinner's grading [18].

Cell culture

T24 and PC-3 cells derived from human bladder and prostate carcinomas, respectively, were grown in RPMI 1640 (ICN Biomedicals, Ohio, USA) supplemented with 10% fetal bovine serum (FBS). All the cultures were performed in the presence of 5% CO_2 at 37 °C. Cells at 80–90% confluency were harvested and analyzed for telomerase activity and the expression of *hTERT* mRNA.

Preparation of proteins and RNA

For preparation of proteins, cultured cells (10^5 cells) or frozen tissues (20–150 mg) were resuspended in 200-μl lysis buffer [10 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid (pH 8.0), 3 mM KCl, 1 mM $MgCl_2$, 1 mM dithiothreitol, 100 μM phenylmethyl-sulfonyl fluoride, 1 μg/ml leupeptin, 2 μg/ml pepstatin, 0.5% n-nonanoyl-N-methylglucamide (Nacalai Tesque, Kyoto, Japan), 10 U/ml RNase inhibitor, and placed on ice for 30 min. The lysate was centrifuged at 16,000 ×*g*, and the supernatant was stored at −80 °C until required for use. The protein concentration of each extract was measured by the Bradford method using bovine serum albumin (BSA) as the standard [2]. Total cellular RNA was isolated from cultured cells or frozen tissues using ISOGEN (Nippon Gene, Tokyo, Japan) according to the manufacturer's instruction.

Conventional TRAP assay

The assay was performed using a 50-μl reaction mixture containing 10 mM Tris-HCl (pH 8.3), 1.5 mM deoxyribonucleoside triphosphates (dNTPs), 2.5 μCi of [α-32P] dCTP (3000 Ci/mmol) (NEN, Massachusetts, U.S.A), 0.5 U EX Taq DNA polymerase (Takara, Kyoto, Japan), the required amount of cell extract (1- or 12-μg protein), 50 pmol TS primer (5′-AAT CCG TCG AGC AGA GTT-3′), 50 pmol ACX primer (5′-GCG CGG CTT ACC CTT ACC CTT ACC CTA ACC-3′), 50 pmol NT primer (5′-ATC GCT TCT CGG CCT TTT-3′), and 100 amol PCR control template (5′-AAT CCG TCG AGC AGA GTT AAA AGG CCG AGA AGC GAT-3′) [7]. The reaction mixture was incubated at 30 °C for 60 min and then subjected to 31 PCR cycles, each comprising 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s. The PCR product was electropheresed on a 10% polyacrylamide gel.

Modified TRAP assay

The assay was performed using a 40-μl reaction mixture containing 20 mM Tris-HCl (pH 8.0), 1 mM dNTPs, 1 mM $MgCl_2$, 2 mM ethylene glycol-bis (*β*-aminoethyl ether)-N, N, N′, N′-tetraacetic acid, 100 μg/ml BSA, 10 pmol TS primer and 2- or 20-μg aliquots of proteins from T24 cell extracts or tissue samples. Each mixture was incubated at 30 °C for 60 min to achieve telomerase-mediated extension of TS primer, then heated at 95 °C for 10 min, and the DNA was precipitated with two vol.of ethanol. In order to amplify the precipitated DNA, the PCR was performed as described above. The PCR product was electropheresed on a 10% polyacrylamide gel. To estimate telomerase activity, we performed the signal of TRAP assay-generated DNA ladder using the Bioimage analyzer (BAS 2000; Fuji, Tokyo, Japan).

RT-PCR

The primers used to amplify the transcripts of *hTERT* and the glyceraldehyde-3-phosphate dehydrogenase gene (*GAPDH*) were designed according to the published sequences as follows: for *hTERT*, forward primer 5′-CCT ATT CCC CTG GTG CGG CCT GCT GC-3′, reverse primer 5′-CGA CAT CCC TGC GTT CTT GGC TTT CA-3′ [17]; for *GAPDH*, forward primer 5′-AAT CCC ATC ACC ATC TTC CA-3′, reverse primer 5′-GCC ATC ACG CCA CAG TTT CC-3′ [23]. cDNAs were synthesized from 1 μg RNA using an RNA PCR kit Ver. 2.1 (Takara) and the reverse primer for *hTERT* mRNA or a random 9-mer primer for *GAPDH* mRNA. PCR amplification of *hTERT* cDNA was performed using a 50-μl reaction mixture containing 10 mM Tris-HCl (pH 8.3), 1.5 mM dNTPs, 2.5 μCi of [α-^{32}P] dCTP, 0.25 U Ampli-Taq Gold (Perkin Elmer/Cetus, CA, USA), 50 pmol forward primer and 50 pmol reverse primer. First, the reaction mixture was heated at 95 °C for 9 min and then subjected to 27 PCR cycles, each comprising denaturation at 94 °C for 45 s, annealing at 68 °C for 45 s and extension at 72 °C for 60 s.

The expected size of the PCR product for TERT was 388 base pairs. The sequence of the PCR product was confirmed by DNA sequence method. PCR amplification of *GAPDH* was performed using RNA PCR kit Ver. 2.1 according to the manufacturer's instruction. Each reaction mixture was heated at 94 °C for 5 min and then subjected to 17 PCR cycles, each comprising denaturation at 94 °C for 45 s, annealing at 60 °C for 45 s and extension at 72 °C for 60 s. The PCR product was electropheresed on a 5% polyacrylamide gel and estimated using the Bioimage analyzer (BAS 2000; Fuji, Tokyo, Japan).

Detection of alternative spliced *hTERT* transcripts

To detect alternative splicing of *hTERT* transcripts, the cDNA synthesized with the random 9 mer was amplified using the forward primer 5′-GCC TGA GCT GTA CTT TGT CAA-3′ and reverse primer 5′-CGC AAA CAG CTT GTT CTC CAT GTC-3′ by initial heating at 94 °C for 5 min, followed by 27 PCR cycles, each comprising 94 °C for 30 s, 68 °C for 30 s and 72 °C for 60 s [24].

Statistical analysis

To evaluate a relationship between telomerase activities and clinicopathological parameters, we divided tumor samples into two groups: tumors with less than 25% telomerase activity of T24 cells (positive control) and tumors with 25% that of T24 cells or more. To compare these groups, we analyzed clinical data by univariate analysis. All probability (*P*) values refer to either a chi-squared test with Yates correction or Fisher's exact test for tables. Correlation between *hTERT* mRNA levels and telomerase activity levels was assessed by calculating Pearson's correlation coefficient (r). Other statistical analyses were performed using unpaired t-test. The probability (*P*) of < 0.05 was considered to be significant.

Results

Quantitative analysis of telomerase activity

The conventional TRAP assay was designed to enable two reactions, telomere extension, and the PCR to be performed in one tube [5]. However, as the amounts of tissue samples used for the first step of the reaction increased, significant inhibition of the PCR was often observed [19]. The Taq polymerase reaction is inhibited by components derived from cellular extracts that remain after the telomere extension reaction (Fig. 1). Therefore, we attempted to remove the inhibitors of the PCR by inserting an ethanol-precipitation step between telomere extension and the PCR. Figure 1 shows the results obtained with the modified TRAP assay. After ethanol-precipitation, the extension of telomere ladder was clearly observed to be

concentration-dependent. The results indicated that inhibitors in the PC-3 cellular extracts had been removed by the ethanol-precipitation step.

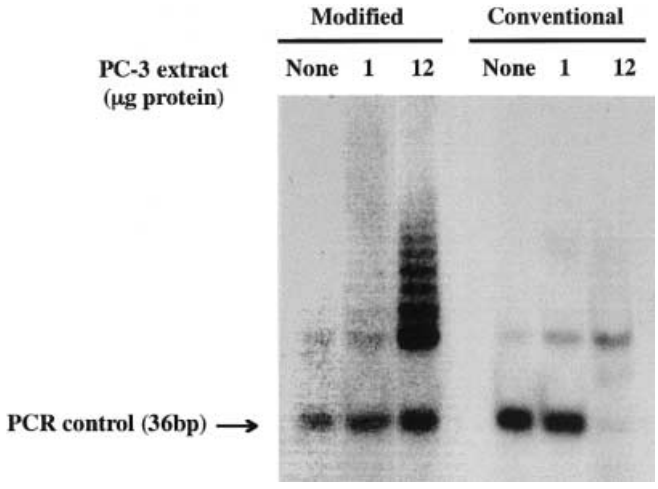

Fig. 1 TRAP assay of PC-3 cell extracts. Inhibitors of the PCR reaction present in the PC-3 cellular extracts were removed by ethanol precipitation. The TRAP assay was performed, as described in "Materials and methods", using 0, 1 and 12 μg protein from PC-3 cell extracts. The results of our modified telomerase activity assay and the conventional TRAP assay are shown in the left and right panels, respectively

Telomerase activity levels in renal lesions

Telomerase activity was determined in four non-cancerous tissues and in 23 RCCs (Table 1, Fig. 2a). The means of telomerase activity levels in non-cancerous tissues and RCCs were 1.4% (range, 0.0–2.8%) that of T24 cells and 18.2% (range, 0.0–66.0%), respectively. To analyze clinicopathological parameters of RCCs, we divided tumor samples into two groups: tumors with less than 25% telomerase activity levels of T24 cells and tumors with 25% that of T24 cells or more. There was a correlation between elevated telomerase activity levels and tumor stages ($P = 0.03$, Table 2). The mean of telomerase activity level in G1- and G2-RCCs was 11.2% (range, 0.0–37.0%) and that of G3-RCCs was 34.1% (range, 5.2–66.0%). The telomerase activity level in G3-RCCs was significantly higher than that in G1-RCCs and G2-RCCs ($P = 0.04$). Thus telomerase activity correlated with nuclear grades of tumors in RCCs.

Quantitative analysis of *hTERT* mRNA in renal lesions

Next, we carried out RT-PCR analysis to determine *hTERT* mRNA levels of 18 RCCs and three adjacent normal tissues (Table 1, Fig. 2b). Six samples (five RCCs and one renal cyst) were excluded from this analysis because the amounts of total RNA obtained

Table 1 Clinical findings, telomerase activities and hTERT mRNA levels in renal cell carcinomas and adjacent normal tissue (*M* Male, *F* Female)

Case	Age (years)/ Gender	Histopathology	Nuclear grade	TNM staging	Telomerase activity[a]	*hTERT* mRNA[b]
1	36/F	Clear cell subtype	G1	pT2N0M0	0.5	ND[c]
2	74/F	Clear cell subtype	G2	pT2N0M0	12.0	3.2
3	76/M	Clear cell subtype	G1	pT1N0M0	8.8	3.8
4	60/M	Clear cell subtype	G2	pT3aN0M1	6.2	0.2
5	68/M	Mixed subtype	G3	pT3aN0M0	25.0	0.7
6	68/M	Clear cell subtype	G3	pT2N0M0	58.0	2.2
7	66/M	Clear cell subtype	G2	pT3aN0M0	9.2	ND[c]
8	74/M	Clear cell subtype	G3	pT3aN0M0	66.0	ND[c]
9	61/F	Clear cell subtype	G3	pT2N0M0	5.2	0.2
10	41/F	Clear cell subtype	G2	pT2N0M0	0.0	0.5
11	46/M	Clear cell subtype	G2	pT2N0M0	3.5	ND[c]
12	43/F	Clear cell subtype	G2	pT2N0M0	20.0	0.8
13	78/F	Mixed subtype	G2	pT3bN0M0	5.4	3.7
14	42/M	Mixed subtype	G3	pT2N0M0	12.0	2.1
15	67/M	Mixed subtype	G3	pT3aN3M0	28.0	1.1
16	40/M	Clear cell subtype	G2	pT2N0M0	37.0	0.8
17	55/M	Clear cell subtype	G3	pT3aN0M0	45.0	0.5
18	42/F	Mixed subtype	G2	pT2N0M0	10.0	ND[c]
19	75/M	Clear cell subtype	G2	pT2N0M0	9.0	0.8
20	61/M	Clear cell subtype	G2	pT2N0M0	0.0	0.4
21	77/F	Clear cell subtype	G2	pT2N0M0	22.0	0.2
22	45/M	Clear cell subtype	G2	pT3aN3M0	26.0	0.7
23	84/M	Clear cell subtype	G2	pT2N0M0	9.5	0.9
24	67/M	Renal cyst			2.8	ND[c]
1N[d]	36/F	Adjacent normal tissue			0.0	0.0
2N[d]	74/F	Adjacent normal tissue			1.0	0.0
3N[d]	76/M	Adjacent normal tissue			1.6	0.6

[a] The telomerase activitiy levels in each sample were normalized according to the activity of T24 cells (control. 100%) and presented as relative ratios
[b] *hTERT* in each sample was calculated by dividing the radioactivity associated with *hTERT* products by that of the *GAPDH* products (internal control)
[c] ND: Not determined
[d] 1–3N mean adjacent normal tissues of case numbers 1–3, respectively

from these samples were inadequate for repeated performance of the RT-PCR experiment. *hTERT* mRNA levels in each sample was calculated by dividing the radioactivity associated with *hTERT* products by that of the *GAPDH* products (internal control). There was no significant correlation of *hTERT* mRNA level with nuclear grades and tumor stages ($P = 0.76$ and $P = 0.80$, respectively). In addition, RT-PCR analysis showed no correlation between the telomerase activities and *hTERT* mRNA levels in RCCs ($r = -0.05$, $P = 0.86$, Fig. 3).

Detection of alternative splicing of *hTERT* transcripts

Two alternative splicing patterns have been reported [21]. One occurs at the α-splicing site resulting in a 36-bp deletion, and the other at the β-splicing site causes a 182-bp deletion resulting in a nonsense mutation. The PCR using the primer set described in "Materials and methods" should yield four PCR products from alternatively spliced RNAs: 457-bp, 421-bp, 275-bp and 239-bp bands.

We detected alternatively spliced RNAs not only in adult normal kidney but also in RCCs. In our samples, the 257-bp band was the major transcript and the 421-bp band was not detected (Fig. 4). The 275-bp and 239-bp bands with the splicing event at the β-splicing site direct

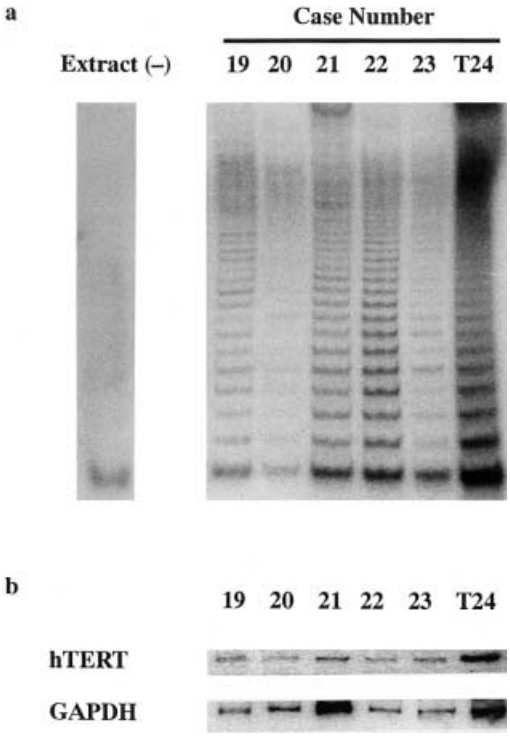

Fig. 2 TRAP assay and RT-PCR analysis of *hTERT* mRNA in clinical samples. The case numbers are the same as those listed in Table 1. **a** Telomerase activity in each RCC sample determined using the modified TRAP assay. Buffer containing no sample extract was used as a negative control. **b** RT-PCR products of *hTERT* and *GAPDH* (control) with corresponding samples

Table 2 Relationship between telomerase activity levels and tumor stages showing the number of cases in the corresponding categories

Telomerase activity level	Tumor stages	
	T1 + 2[a]	T3[a]
< 25% level of T24 cells	13	3
≥25% level of T24 cells	2	5
		$P = 0.03$[b]

[a] T1 + 2 means T1 and T2 tumors; T3, T3a and T3b tumors
[b] *P* expresses the Fisher's exact probability

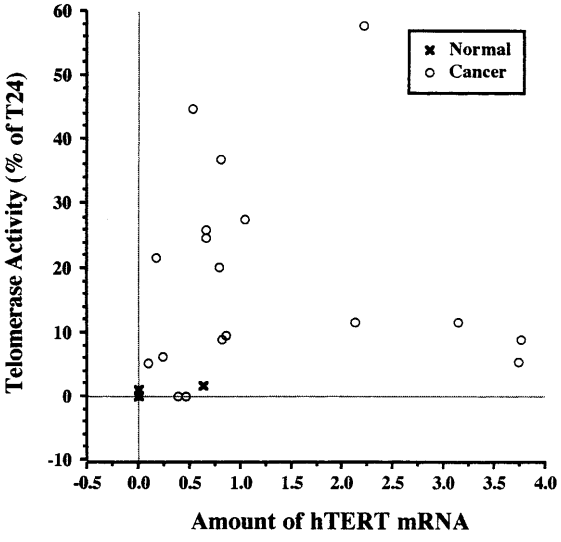

Fig. 3 Telomerase activity versus *hTERT* mRNA level. There was no correlation between the telomerase activity levels and *hTERT* mRNA levels in RCCs ($r = -0.05$, $P = 0.86$)

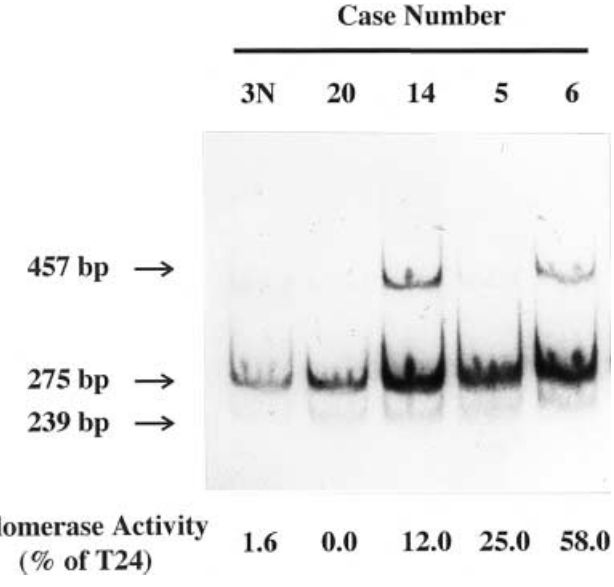

Fig. 4 RT-PCR analysis of alternatively spliced transcripts of *hTERT* mRNA. The PCR yielded three PCR products: 457-bp, 275-bp and 239-bp bands. The case numbers are the same as those in Table 1. Telomerase activity of each sample is shown at the bottom

an inactive form of the enzyme. The 457-bp band is only expected to code for an active reverse transcriptase. The sum of the densities of all alternative spliced transcripts corresponded to mRNA values presented in Table 1. However, the densities of the 457-bp bands of the five samples did not correlate with telomerase activity. Thus, the discrepancy between the telomerase activities and *hTERT* mRNA levels found in RCCs cannot be accounted for by alternative splicing of *hTERT* transcripts.

Discussion

RCC is a well-known tumor showing unpredictable behavior. Various parameters such as age, nuclear grade, clinical stage, and morphological feature are helpful for predicting clinical outcome [16]. However, no single parameter has given a reliable prediction of the survival outcome in the individual patients. Therefore, additional parameters in RCCs are necessary to improve the assessment of tumor aggressiveness.

Telomerase activity is thought to be required for cellular immortality during cancer development. Recently it was reported that insufficient levels of telomerase lead to telomere attrition [1]. It was also demonstrated that the telomere attrition in aging telomerase-deficient p53 mutant mice promotes the development of epithelial cancers by a process of fusion-bridge breakage that leads to the formation of complex non-reciprocal translocations [1]. However, the precise role of telomerase in cancer development is still unclear. In this study, with the improved TRAP method, we found that telomerase activity levels increase along with nuclear grade. We also found that there was a positive correlation between elevated telomerase activity levels and tumor stages, although previous reports have demonstrated no correlation between telomerase activity and clinical parameters in RCCs [4, 9, 14, 17, 22]. The results indicated that telomerase activity of RCCs may be a useful indicator for tumor progression.

It has been suggested that *hTERT* is a rate-limiting determinant of telomerase activity [14]. Recently, several groups reported that telomerase activity is positively regulated at the transcription level of *hTERT* gene by Myc [18, 23]. However, we found that the telomerase activity levels and *hTERT* mRNA contents of RCCs were not always in parallel (Fig. 3). There are two possible explanations for this. Firstly, alternative splicing in *hTERT* transcripts was also demonstrated to affect the telomerase activity during the development of human embryos [21]. However, in our cases, *hTERT* transcripts in RCCs did not correlate with the levels of telomerase activity (Fig. 4). Secondly, the involvement of post-transcriptional and post-translational mechanism in regulation of telomerase activity was suggested. We observed that some RCCs had low *hTERT* mRNA levels despite having high telomerase activities. Our findings indicated that telomerase protein complex is more stable than *hTERT* m RNA in vivo and that the telomerase activity is regulated at multiple steps. Similar findings were reported by Lin et al., who found that *TERT* mRNA is expressed at very low levels during activation of human lymphocytes, regardless of high telomerase activity [11]. In addition, Li et al. reported that *hTERT* is a phosphoprotein, and its phosphorylation is an essential step in the generation of a functional telomerase complex in the initiation and maintenance of telomerase activity in breast cancer cells [9].

Our findings indicated that it is important for evaluation of cancer stage and grade of RCCs to analyze the telomerase activity of tumors quantitatively, but not simply to examine the presence of *hTERT* mRNA, since telomerase activity is regulated not only at de novo expression but also at post-translational modification.

In order to evaluate the tumor progression, further investigation of the regulatory mechanism for telomerase activity, including stability, phosphorylation, and proteolysis of TERT protein, remains to be determined.

References

1. Artandi SE, Chang S, Lee S-L, Alson S, Gottlleb GJ, Chin L, DePinho RA (2000) Telomerase dysfunction promotes non-reciprocal translocations and epithelial cancers in mice. Nature 406: 641
2. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248
3. Counter CM, Meyerson M, Eaton EN, Ellison LW, Caddle SD, Haber DA, Weiberg RA (1998) Telomerase activity is restored in human cells by ectopic expression of hTERT (hEST2), the catalytic subunit of telomerase. Oncogene 16: 1217
4. Feng J, Funk WD, Wang SS, Weinrich SL, Avilion AA, Chiu C, Adams RR, Chang E, Allsopp RC, Yu J, Le S, West MD, Harley CB, Andrews WH, Greinder CW, Villeponteau B (1995) The RNA component of human telomerase. Science 269: 1236
5. Kanaya T, Kyo S, Takakura M, Ito H, Namiki M, Inoue M (1998) hTERT is a critical determinant of telomerase activity in renal-cell carcinoma. Int J Cancer 78: 539
6. Kim NW, Wu F (1997) Advances in quantification and characterization of telomerase activity by the telomeric repeat amplification protocol (TRAP). Nucleic Acids Res 25: 2595
7. Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PLC, Coviello GM, Wright WE, Weinrich SL, Shay JW (1994) Specific association of human telomerase activity with immortal cells and cancer. Science 266: 2011
8. Kinoshita H, Ogawa O, Mitsumori K, Kakehi Y, Terachi T, Yoshida O (1998) Low frequency of positive telomerase activity in a chromophobe subtype of renal cell carcinoma. J Urol 159: 245
9. Li H, Zhao L, Yang Z, Funder JW, Liu J-P (1998) Telomerase is controlled by protein kinase Cα in human breast cancer cells. J Biol Chem 273: 33436
10. Lin Y, Miyamoto H, Fujinami K, Uemura H, Hosaka M, Iwasaki Y, Kubota Y (1996) Telomerase activity in human bladder cancer. Clin Cancer Res 2: 929
11. Liu K, Schoonmaker MM, Levine BL, June CH, Hodes RJ (1999) Constitutive and regulated expression of telomerase reverse transcriptase (hTERT) in human lymphocytes. Proc Natl Acad Sci USA 96: 5147
12. Mehle C, Piatyszek MA, Ljungberg B, Shay JW, Roos G (1996) Telomerase activity in human renal cell carcinoma. Oncogene 13: 161

13. Morin GB (1989) The human telomere terminal transferase enzyme is a ribonucleoprotein that synthesizes TTAGGG repeats. Cell 59: 521
14. Müller M, Heicappell R, Krause H, Sachsinger J, Porsche C, Miller K (1999) Telomerase activity in malignant and benign renal tumors. Eur Urol 35: 249
15. Nakamura TM, Morin GB, Chapman KB, Weinrich SL, Andrews WH, Lingner J, Harley CB, Cech TR (1997) Telomerase catalytic subunit homologs from fission yeast and human. Science 277: 955
16. Nakayama J, Saito M, Nakamura H, Matsuura A, Ishikawa F (1997) TLP 1: A gene encoding a protein component of mammalian telomerase is a novel member of WD repeats family. Cell 88: 875
17. Nakayama J, Hidetoshi T, Tahara E, Saito M, Ito K, Nakamura H, Nakanishi T, Tahara E, Ide T, Ishikawa F (1998) Telomerase activation by hTRT in human normal fibroblasts and hepatocellular carcinomas. Nat Genet 18: 65
18. Skinner DG, Colvin RB, Vermillion CD, Pfister RC, Leadbetter WF (1971) Diagnosis and management of renal cell carcinoma. Cancer 28: 1165
19. Störkel S, Thoenes W, Jacobi GH, Engelmann U, Lippold R (1990) Prognostic parameters of renal cell carcinoma. Eur Urol 18: 36
20. Sugimura K, Yoshida N, Hisatomi H, Nakatani T, Ikemoto S (1999) Telomerase activity in human renal cell carcinoma. BJU Int 83: 693
21. Takamura M, Kyo S, Kanaya T, Hirano H, Takeda J, Yutsudo M, Inoue M (1999) Cloning of human telomerase catalytic subunit (hTERT) gene promoter and identification of proximal core promoter sequences essential for transcriptional activation in immortalized and cancer cells. Cancer Res 59: 551
22. Tatematsu K, Nakayama J, Danbara M, Shinoya S, Sato H, Omine M, Ishikawa F (1996) A novel quantitative 'stretch PCR assay', that detects a dramatic increase in telomerase activity during the progression of myeloid leukemias. Oncogene 13: 2265
23. Tso JY, Sun X-H, Kat T-H, Reece KS, Wu R (1985) Isolation and characterization of rat and human glyceraldehyde-3-phosphate dehydrogenase cDNAs: genomic complexity and molecular evolution of the gene. Nucleic Acids Res 13: 2485
24. Ulaner GA, Hu J, Vu TH, Giudice LC, Hoffmann AR (1998) Telomerase activity in human development is regulated by human telomerase reverse transcriptase (hTERT) transcription and by alternate splicing of hTERT transcripts. Cancer Res 58: 4168
25. Wu KJ, Grandori C, Amacker M, Vermot NS, Polack A, Lingner J, Favera RD (1999) Direction activation of TERT transcription by c-MYC. Nat Genet 21: 220
26. Yoshida K, Kakamoto S, Sumi S, Higashi Y, Kitahara S (1998) Telomerase activity in renal cell carcinoma. Cancer 83: 761