A diagnostic evaluation of serum human telomerase reverse transcriptase mRNA as a novel tumor marker for gynecologic malignancies

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Abstract. Human telomerase reverse transcriptase (hTERT) and epidermal growth factor receptor (EGFR) play an important role in many cancers including gynecological cancers. We previously reported the usefulness of a quantitative highly sensitive detection method for hTERT mRNA in the serum of cancer patients. By this method, we attempted to elucidate the diagnostic evaluation of serum hTERT mRNA for gynecologic malignancies. In 174 female patients with gynecological lesions (47 with ovarian lesions, 63 with uterine lesions, 2 with malignancies in other gynecological lesions, and 62 benign lesions) and 20 healthy individuals, we measured serum hTERT mRNA and EGFR mRNA by using the newly developed real-time quantitative RT-PCR. We examined their sensitivity and specificity in cancer diagnosis, clinical significance in comparison with conventional tumor markers, and their correlations with the clinical parameters by using multivariate analyses. Serum hTERT mRNA showed higher values in patients with gynecologic cancers than in those with benign diseases and healthy individuals. The hTERT mRNA level independently correlated with the presence of cancers (P=0.004 for both ovarian and uterine cancer) and clinical stage (P<0.001). The sensitivity and specificity of hTERT mRNA in cancer diagnosis was 74.4% and 74.1%, respectively. The hTERT mRNA level showed a significant correlation with CA125 by Pearson's relative test (P=0.035) and with histological findings in ovarian cancer by the Friedman test (P<0.004). EGFR mRNA did not display any differences between the diseases. hTERT mRNA is useful for diagnosing gynecologic cancer and is superior to conventional tumor markers. Therefore, serum hTERT mRNA is a novel and available biomarker for gynecologic malignancies.

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Abbreviations: hTERT, human telomerase reverse transcriptase; SCC, squamous cell carcinoma antigen; SqCC, squamous cell carcinoma; HPV, human papilloma virus

Key words: hTERT, real-time RT-PCR, cancer diagnosis, ovarian tumor, tumor marker

Introduction

Ovarian cancer is the fifth most common cancer in women. Despite the fact that it is highly curable if diagnosed early, ovarian cancer kills more women each year than all other gynecologic malignancies (1). There are no proven methods of prevention, and it is often a rapidly progressive and fatal disease. The only validated marker for ovarian cancer is CA125, which is detectable in the serum of >80% of women with ovarian cancer. In contrast, cervical cancer is the third leading cause of cancer death in women. Over half a million new cases are diagnosed every year worldwide. The most common histological type of cervical cancer is squamous cell carcinoma which accounts for >80% of all cervical cancers. An increasing number of reports indicate that other factors are involved such as the human papilloma virus (HPV) to induce cervical carcinogenesis. A routinely used biomarker for advanced cervical cancer is SCC, which is detectable in the serum of <50% of women with cervical cancer. CA125 and SCC are reliable only in monitoring the response to treatment or recurrence, but not as a diagnostic or prognostic marker (2). Thus, there is considerable interest in identifying molecular diagnostic and prognostic indicators to guide treatment decisions.

It is well known that one of the carcinogenic biomarkers, human telomerase reverse transcriptase (hTERT) (3,4), is not

only expressed in mild dysplastic lesions in cervices, but is also often expressed in gynecological malignancies (5). It has been recently shown that hTERT is an important factor during tumourigenesis and that its level is elevated during an early stage of tumourigenesis (6).

In the 1970s, the presence of free DNA was identified in plasma in patients with cancer and in ensuing studies, the neoplastic characteristics of plasma DNA and RNA in cancer patients were founded, for example, the mutation of p53 or the Ras gene, or microsatellite alteration (3,4). Consequently, the free DNA or RNA in plasma may be another important tumor marker. According to recently published reports, manifold nucleic acid tumor markers can be found in plasma, such as total DNA, abnormally expressed genes, tumor suppressor genes of aberrant promoter hypermethylation, altered microsatellites, mutated oncogenes and tumor suppressor genes (7-10). hTERT mRNA in serum was detected in breast cancer but not in benign diseases, suggesting that hTERT is available for cancer diagnosis (8). We have reported the clinical significance of qualitative hTERT mRNA detection in the serum of HCC patients (11) and subsequently proved that combined quantification of hTERT mRNA with EGFR mRNA is useful in the diagnosis of lung cancer, especially adenocarcinoma (12) (unpublished data). Thus, since mRNA in serum may be a clinically useful biomarker like hTERT mRNA, it can be easily applied in the clinical field. Because the expression of hTERT mRNA rises significantly even in cancer cells or tissues from patients with gynecologic malignancies (13-18) and predominantly expresses in ovarian and cervical cancers but only rarely in benign tumors and normal tissue, we attempted to examine whether hTERT mRNA in serum is useful when compared to conventional tumor markers (14,19). From an epigenetic point of view, hTERT methylation is associated with a poorer outcome, irrespective of hTERT expression, indicating that the quantification of hTERT mRNA expression at the pre-translational step should be useful for early detection of malignancies. The purpose of this study is to evaluate the clinical significance of serum hTERT mRNA and EGFR mRNA as tumor markers in patients with ovarian and uterine cancers.

Patients and methods

Patients and sample collection. Consecutive patients, 176 in total, comprising 47 patients with ovarian cancer, 63 with uterine cancer, 2 with other gynecologic cancer, 2 with border lesions, and 62 with benign diseases, that were admitted to Tottori University Hospital between December 2003 and January 2005, were enrolled in this study. The patients with benign diseases were composed of 41 with benign ovarian diseases including 11 with an ovarian dermoid cyst, 4 with endometriosis in the ovary, and 13 with an ovarian cyst, and 21 with benign uterine diseases including 1 patient with invasive hydatidiformmole, 4 with adenomyosis of the uterus, 14 with uterine myoma, and 2 with uterine prolapse. Twenty healthy patients served as controls. The mean age of patients was 55 ranging from 18 to 85. CA125 (ChemilumiACS-CA125II, Bayer Japan, Tokyo) and SCC (SCC RIAbeads, SRL, Tokyo, Japan) in the serum were measured in a routine clinic. Human papilloma virus (HPV) was not examined for uterine lesions. The patients were diagnosed by chief complaints, ultrasonography, computed tomography (CT), CA125 or SCC, cytology or biopsy under internal examination, histological examination after laparoscopy or surgical therapy. The clinicopathological findings (gender, age, etiology, histological findings, CA125 for ovarian disease, SCC for uterine disease, tumor size, clinical staging, and presence of recurrence) were evaluated. Informed consent was obtained from each patient and study protocols followed the ethical guidelines of the 1975 Declaration of Helsinki and were approved by the human research committee of Tottori University (approval no. 138, no. 138-1, 2001). Centrifugation of collected blood and harvesting of serum samples were performed by using three steps of centrifugation to keep the contamination of lymphocytes to a minimum as previously described (9).

To study whether hTERT mRNA in serum is originated and released from original cancer tissues, we harvested the surgically resected cancer tissues and sera in 12 patients. Furthermore, all the patients were stratified into three categories depending on the time of drawing blood (before, during, and 7 days after therapy). The therapeutic effect such as anti-tumor chemicals or surgical treatment was estimated by a t-test.

RNA extraction and real-time quantitative RT-PCR. RNA was extracted with DNase treatment from serum as reported previously (4,9). RNA from 200 μl of serum was dissolved in 200 μl of H_2O. Quantitative RT-PCR was performed by using 1 μl of RNA extract and 2 μl of SYBR-Green I (Roche, Basel, Switzerland) in a one step RT-PCR kit (Qiagen, Tokyo, Japan). RNA was extracted from HCC tissues by using the same volume of serum and dried to a 20-fold concentration. RNAs from HCC tissues were extracted using TRIzol Reagent according to the manufacturer's instructions (Invitrogen Corp., Carlsbad, CA, USA). The primers used in the experiment were used as reported previously for hTERT mRNA (the primers cannot target some splice variants) (9,12) and for EGFR mRNA (12). RT-PCR consisted of an initial incubation at 50˚C for 30 min followed by a 12-min incubation at 95˚C, then 50 cycles at 95˚C (0 sec), 55˚C (10 sec), and 72˚C (15 sec), and a 20-sec melting at 40˚C. The dynamic ranges of RT-PCR analysis for hTERT mRNA and EGFR mRNA were more than approximately 5 copies in this assay and we were able to exclude the possibility of false negativity in serum samples from patients and controls. Control hTERT mRNA for standardization was generated using T7 RNA polymerase in pLIXN-hTERT cDNA kindly provided by Dr H. Tahara (Hiroshima University, Japan) and another control EGFR mRNA was similarly generated using pCRII-TOPO-EGFR (Invitrogen Japan K.K, Tokyo, Japan) retrofitted from pME18SFL3-EGFR purchased as a commercial FLJ cDNA clone (Toyobo, Tokyo, Japan). The PCR yielded products of 131 and 114 bp for hTERT and EGFR, respectively (data not shown). The RT-PCR assay was repeated twice and the quantification was confirmed by using a LightCycler (Roche, Basel, Switzerland) with reproducibility.

Immunohistochemistry. For the immunohistochemical analyses, 12 patients [patients with ovarian cancer (OC), 3 patients with squamous cell carcinoma (SqCC), 1 with endometrioid

Table I. Statistical analysis of comparison of tumor markers with clinicopathological findings.

Clinical parameters	No. of patient	Multivariate analyses and Friedrich test (p-value)			
		hTERT mRNA	EGFR mRNA	SCC	CA125
Age mean: 55 years (range 18 to 85)		<0.001	N.S.	N.S.	0.028
Disease					
Malignant	112	0.004	N.S.		
Border	2				
Benign	62				
Organ					
Uterus	63	N.S.	N.S.	N.S.	N.S.
Cervical	37				
Body	15				
Benign	21				
Ovary	47	0.045	N.S.	N.S.	N.S.
Benign	41				
Other	2				
Histological findings					
Uterus		N.S.	N.S.	0.021	N.S.
Squamous cell carcinoma	29				
Endometrioid	15				
Other	40				
Ovary		0.004	N.S.	N.S.	<0.001
Serous	24				
Mucinous	9				
Other	55				
Tumor size		0.044	N.S.	N.S.	N.S.
Tumor marker					
SCC (ng/μl)		N.S.	N.S.		N.S.
CA125 (mAU/ml)		0.035	N.S	N.S.	
Stage					
1	25	<0.001	N.S.	0.033	0.028
2		8			
3		12			
4		2			
Before therapy	80	N.S.	N.S.	N.S.	0.043
During therapy	6				
After therapy	26				
Recurrence					
Yes	43	N.S.	N.S.	N.S.	N.S.
No	69				

N.S., not significant.

carcinoma (EC) and 1 with cervical dysplasia] with positive mRNAs or tumor markers, who underwent surgical treatment, were chosen. Resected lung tissue specimens fixed in 4% paraformaldehyde were processed for paraffin embedding. Five-micron-thick sections of the tissue specimens were deparaffinized through a series of xylene baths, and then the

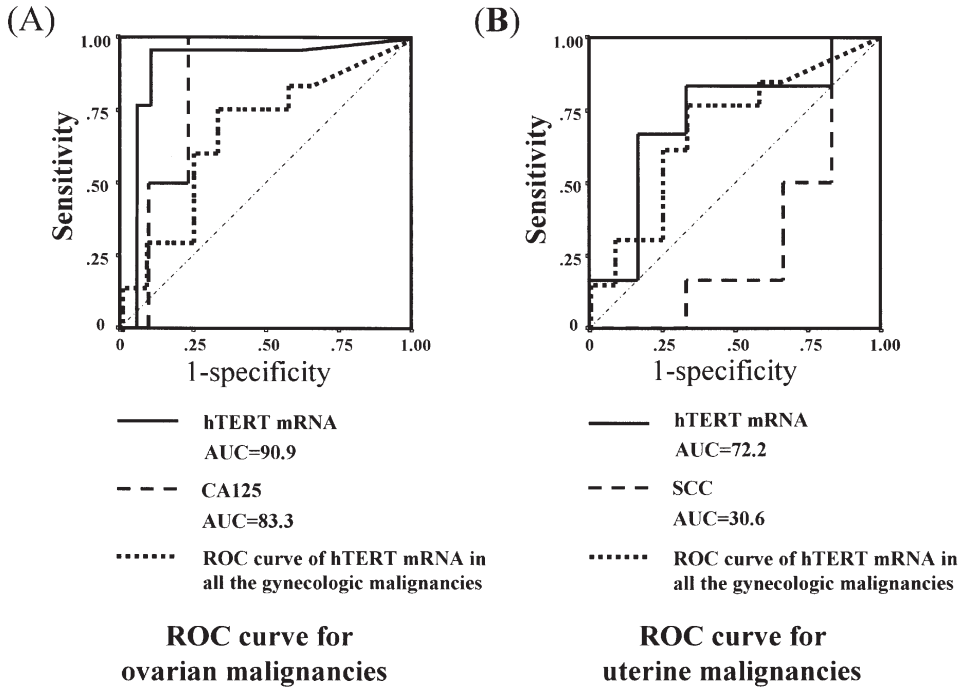


Figure 1. Receiver operator characteristic (ROC) curve analysis of hTERT mRNA and EGFR mRNA expression. The curves shown were obtained by processing quantified raw data by SPSS13.0 software and the sensitivity/specificity values were predicted from the area under the curves and the calculated data. (A) For ovarian malignancies, the bold solid line and dotted line correspond to hTERT mRNA and CA125 level, respectively. AUC for hTERT mRNA is 90.9 and 83.3 for CA125. (B) For uterine malignancies, the bold solid line and dotted line correspond to hTERT mRNA and SCC level, respectively. Fine dotted lines in both (A) and (B) demonstrate the ROC curve for all the gynecologic malignancies. AUC for hTERT mRNA is 72.2 and 30.6 for SCC.

samples were rehydrated in graded alcohols. The sections were incubated with the following monoclonal antibodies: anti-hTERT (Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-EGFR, anti-CA125 (Sigma Chemical, MO, USA), and anti-SCC (Sigma Chemical). For the staining with hTERT, EGFR, CA125, and SCC, the sections were pretreated at 121˚C with 10-mmol/l citrate buffer at pH 6.0 for 10 min by an autoclave. Immunoreactivity was visualized with fluorochrome-conjugated secondary antibodies (20). Finally, the sections were processed with standard avidin-biotin immunochemistry according to the manufacturer's recommendations (Vector Laboratories, Burlingham, CA, USA). The final chromogen used was diaminobenzidine, and the nuclear counterstain used was hematoxylin. As a negative control, the staining procedure was performed with the primary antibody omitted. The degree of expression was estimated for hTERT, EGFR, CA125, and SCC by a Percentage Score (PS), Intensity score (IS) and Overall Score (OAS). PS was classified as follows: 0, <10%; 1, 10-25%; 2, 26-50%; 3, 51-75%; 4, >76%. IS was classified as follows: 0, no staining; 1, light brown; 2, brown; 3, dark brown. OAS was PS multiplied by IS.

Statistical analysis. To examine significant clinicopathological findings affecting hTERT and other markers, a multivariate analysis was performed using SPSS13.0 (SPSS Corp., Tokyo, Japan). Stratified categories in each clinical parameter were evaluated by the Friedman test. To examine correlations among hTERT mRNA, EGFR mRNA, CA125 or SCC, we conducted Pearson's relative test. Probability values < 0.05 were considered to be statistically significant. To assess the accuracy of diagnostic tests, the matched data sets regarding CA125, SCC, EGFR mRNA, and hTERT mRNA were analyzed by using receiver operator characteristic (ROC) curve analysis in SPSS13.0. This assay demonstrated a strong linear relation between copy number and PCR cycles using RNA controls ($r^2 \geq 0.99$). The correlation of hTERT mRNA between HCC tissue and serum was analyzed by using both Paired t-test and Spearman's test. Sensitivity was estimated as the mean of confidence interval (CI) of area under the curve (AUC), on the basis of which the corresponding specificity was led from the output table of SPSS. Predictive cut-off value was evaluated as the nearest point on the ROC curve from the upper left shoulder of the graph.

Results

mRNA quantification and clinical parameters. In each quantitative assay, a strong linear relation was demonstrated between copy number and PCR cycles using RNA controls. The copy numbers of hTERT mRNA were significantly higher in the gynecologic cancer patients than in the healthy individuals (P<0.01, each). Clinicopathological findings showed significant relations to hTERT mRNA in the serum (Table I). By multivariate analysis, the hTERT mRNA level was significantly correlated with age, the presence of cancer, ovarian disease in organs, ovarian malignancies, tumor size, and CA125 (P<0.001, P=0.004, P=0.045, P=0.004, P=0.044, P=0.035, and P<0.001, respectively). On the other hand, EGFR mRNA did not show any significant correlation with any parameters or other markers. SCC was significantly associated with uterine malignancies (P=0.021). By the Friedman test,

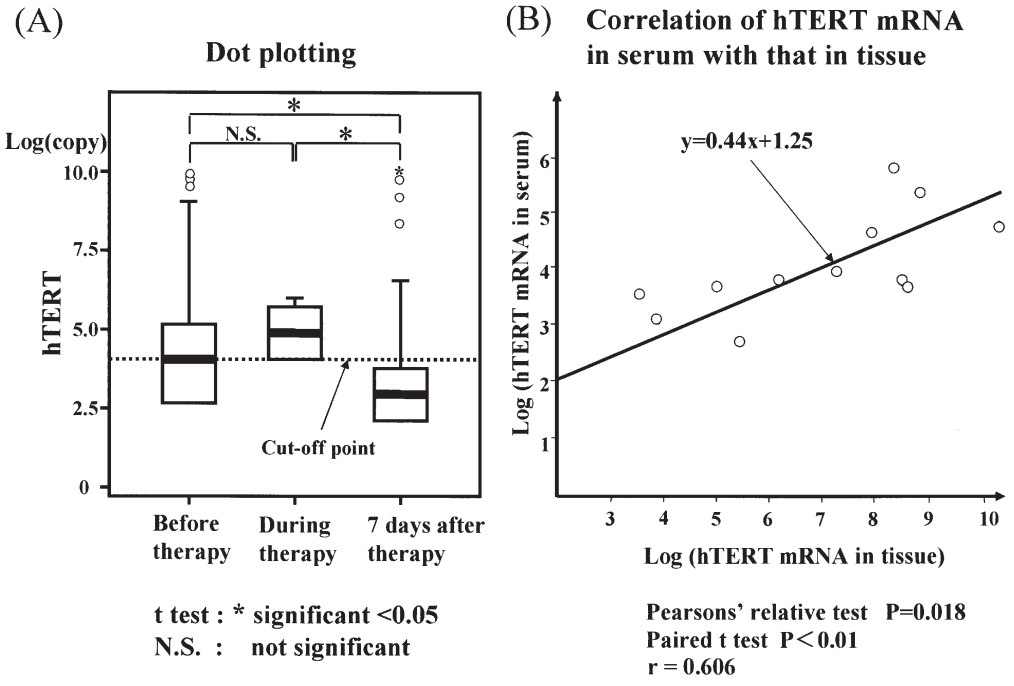


Figure 2. The quantification of both mRNAs in the serum before, during, and 7 days after any treatment including chemotherapy or surgical treatment was stratified into three groups and shown in (A). The data were evaluated by a logarithm of quantification. hTERT mRNA expression among the three groups was evaluated by a t-test (*P<0.05). N.S. indicates not significant. (B) A dot plot representing the significant correlation of hTERT mRNA level in serum with that in gynecologic cancer tissues in 12 patients. Only a minority of the cases that were positive for mRNA in the tissue specimens (n=12 for hTERT) were included in this analysis. Positive is defined as 'being above the predictive cut-off values for both mRNAs obtained from this study in 89 lung tumors and 27 healthy individuals'. These data for hTERT mRNA were analyzed by the Spearman's relative test and the Paired t-test (P<0.018 and P<0.01, respectively). The data were evaluated by a logarithm of quantification.

hTERT mRNA, SCC, and CA125 were significantly correlated with the clinical stage (P<0.001, P=0.033, and P=0.028, respectively). Pearson's relative test between clinical parameters denoted that the hTERT mRNA level was significantly associated with the presence of cancer (P=0.009) and, in the ovary, with CA125 and age (P=0.049, and P=0.045, respectively).

To examine the sensitivity and specificity of tumor markers for diagnosis of gynecologic malignancies, ROC curve analysis showed the sensitivity/specificity of hTERT mRNA for gynecologic malignancies to be 74.4%/74.1% (data not shown). For ovarian malignancies, the mean of confidence interval (CI) of area under the curve (AUC) of hTERT mRNA and CA125 was 90.9% and 83.3%, respectively, and the sensitivity/specificity of hTERT mRNA in ovarian cancer was 90.9%/90.0% (Fig. 1A). The sensitivity/specificity of hTERT mRNA and SCC in uterine cancer was 70.1%/81.5% and 50.0%/67.5%. On the other hand, the sensitivity/ specificity of hTERT mRNA and CA125 in ovarian cancer was 100%/76.5% and 100%/75.5%. For uterine malignancies, AUC of hTERT mRNA and SCC was 72.2% and 30.6% (Fig. 1B), respectively. The optimal cut-off values for hTERT mRNA and EGFR mRNA were calculated as $10^{4.1}$ and $10^{2.99}$ copies/0.2 ml, respectively.

Evaluation of serum hTERT quantification as a tumor biomarker. All patients were stratified into three categories regarding the timing of blood drawing (before, during, and after therapy) and the therapeutic effect such as anti-tumor chemicals or surgical treatment was estimated by a t-test. Although any correlations between before and during the drawing of blood were not observed, hTERT mRNA significantly decreased after therapy, compared with before and during the therapy (P<0.05, each) (Fig. 2A), suggesting that the measurement of hTERT may be useful for the evaluation of a therapeutic effect. Moreover, in the case of uterine malignancies, hTERT mRNA and SCC were significantly useful biomarkers to evaluate a therapeutic effect (P=0.001 and P=0.026, respectively). In ovarian malignancies, hTERT mRNA and CA125 were significantly useful for the diagnosis of cancer (P=0.001 and P=0.043, respectively).

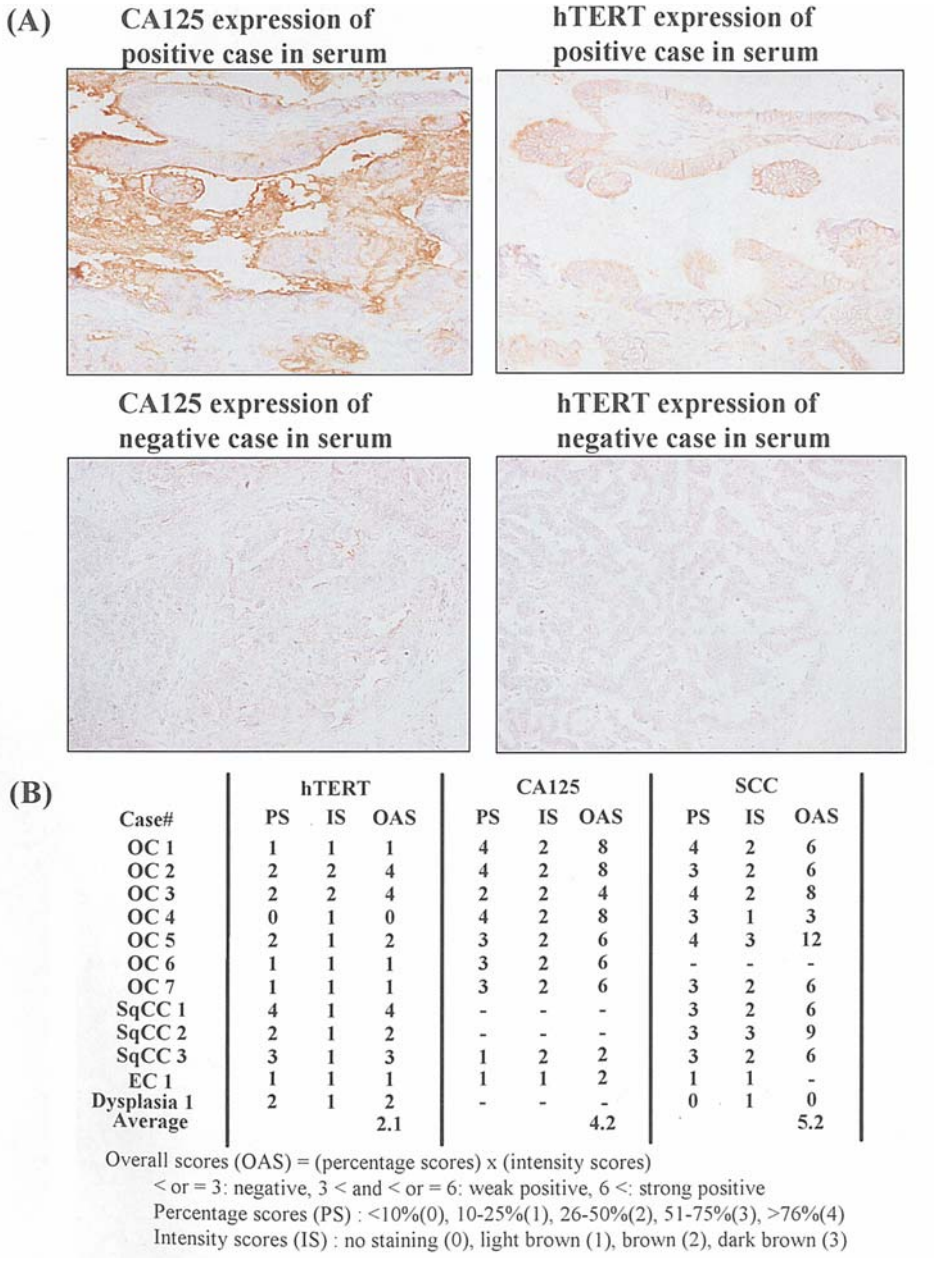
Correlation of hTERT mRNA and EGFR mRNA detection in paired serum and tumor tissue samples. The copy number of hTERT mRNA in serum was significantly correlated with that in cancer tissue (P=0.018 in Pearson's relative test, P<0.01 in the Paired t-test , and r=0.606) (Fig. 2B).

Immunohistochemistry. The expression of hTERT, CA125, and SCC was also examined at the protein level (Fig. 3A). hTERT protein was not so highly expressed, however, it was diffusely stained in the cytoplasm of cancer cells and its expression was stronger in tumorous lesions than in normal adjacent lesions. It tended to be expressed more strongly in squamous cell carcinoma. hTERT was expressed more strongly in cervical squamous cell carcinoma than in ovarian cancer and overall areas (OAS) tended to correlate with serum mRNA level in each patient (Fig. 3B). However, the relationship between mRNA expression and the pathological type of cancer was not statistically significant.

Table II. Mean ± SE of measured values in gynecologic malignancies and benign tumors.

	hTERTmRNA	EGFR mRNA	SCC	CA125
All malignancies	5.12±1.94	5.03±4.79	11.78±4.50	569.67±262.99
Uterine cancer	4.19±0.55	4.03±0.44	16.15±8.33	148.43±57.24
Uterine benign diseases	3.19±3.05	N.E.	N.E.	N.E.
Ovarian cancer	5.32±2.37	4.08±3.81	N.E.	777.64±368.36
Ovarian benign diseases	2.49±0.54	N.E.	N.E.	165.60±122.91
Benign tumor	3.27±3.10	3.28±3.17	N.E.	992.58±340.72

N.E., not examined.



Case#	hTERT PS	hTERT IS	hTERT OAS	CA125 PS	CA125 IS	CA125 OAS	SCC PS	SCC IS	SCC OAS
OC 1	1	1	1	4	2	8	4	2	6
OC 2	2	2	4	4	2	8	3	2	6
OC 3	2	2	4	2	2	4	4	2	8
OC 4	0	1	0	4	2	8	3	1	3
OC 5	2	1	2	3	2	6	4	3	12
OC 6	1	1	1	3	2	6	-	-	-
OC 7	1	1	1	3	2	6	3	2	6
SqCC 1	4	1	4	-	-	-	3	2	6
SqCC 2	2	1	2	-	-	-	3	3	9
SqCC 3	3	1	3	1	2	2	3	2	6
EC 1	1	1	1	1	1	2	1	1	-
Dysplasia 1	2	1	2	-	-	-	0	1	0
Average			2.1			4.2			5.2

Figure 3. (A) Representative immunohistochemistry in a cancerous portion of the ovary (magnification, x200). Nuclear and cytoplasmic immunoreactivity were observed by using a respective antibody to detect the expression of CA125 level [upper left: CA125-positive case in serum: Case 2 (OC 2) in (B); lower left: CA125-negative case in serum] and hTERT level [upper right: hTERT mRNA-positive case in serum: Case 2 (OC 2) in (B); lower right: hTERT mRNA-negative case in serum]. (B) Immunohistochemical estimation in hTERT mRNA- or tumor marker-positive patients in serum was performed as follows: Overall scores = (percentage scores) x (intensity scores). Percentage scores: <10% (0), 10-25% (1), 26-50% (2), 51-75% (3), and >76% (4). Intensity scores: no staining (0), light brown (1), brown (2), dark brown (3). < or = 3, negative; 3 < and < or = 6, weak positive; >6, strong positive.

The expression of CA125 and SCC was high in gynecologic cancer tissues.

Discussion

Gynecologic malignancies include ovarian cancer, uterine cervical cancer, endometrial cancer, and trophoblastic neoplasms. With ovarian tumors, due to their location within the abdominal cavity, it is difficult to make a preoperative pathological diagnosis of cancer without laparotomy. From this point of view, the tumor markers that have carbohydrate antigens like CA125, as well as diagnostic imaging, are useful in the diagnosis of ovarian cancer. SCC antigen (SCC), a marker for SqCC, is clinically useful in the management of advanced cervical cancer and, in early-stage cervical cancer, determining serum SCC levels allows more refined preoperative estimation of the likelihood for adjuvant radiotherapy than current clinical parameters. At present, there are no useful markers for endometrial cancer that exhibit both high sensitivity and specificity, although CA125 is often used in clinical practice for the detection of disease as well as in the monitoring of patients. Therefore, the role of preoperative serum SCC in the management of patients with early-stage cervical cancer deserves further investigation. Women with endometriosis have an increased risk of some malignancies, especially ovarian cancer (21). For the early detection of ovary cancer, considerable effort has been expended in the last few years in identifying potential markers that might substitute or complement CA125, ultimately in the design of screening strategies, for which we are developing a more feasible method exclusively by blood drawing (22,23).

hTERT expression has been examined in many kinds of cancers, precancerous lesions and normal tissues (15,24). Although telomerase was initially expected to be a useful tumor marker due to its specificity to cancer, it has only been clinically applied to surgically resected tissues because no significant telomerase expression has been found in blood and urine samples (25). The hTERT mRNA or endogeneous RNA component derived from cancer cells does not appear to be detectable in serum due to its instability in the serum containing RNase. However, since RNAs in serum are stable within 24 h after drawing blood, it has been suggested that they can be detected even in blood only if we overcome technical problems (26,27). In addition, although normal cells and lymphocytes express low levels of hTERT mRNA, the contamination of the cell fraction could be suppressed to a minimal level by introducing three steps of centrifugation of blood samples (28,29). Finally, hTERT mRNA can be detected in serum from breast cancer patients and its maximum sensitivity was at most 40% (30).

We have therefore developed a highly sensitive quantitative assay which specifies new primers capable of detecting a small amount of RNA. We further demonstrated that sensitivity and specificity of hTERT mRNA in serum of patients with HCC is superior to that of other conventional tumor markers (31) in lung cancer as well, and that hTERT mRNA decreased the expression level by half-time (2-3 days) in a large-scale study of HCC (unpublished data). In lung cancer, the induction of a combination of hTERT mRNA and EGFR mRNA into diagnosis and in the follow-up of lung cancer may be of benefit to patients (unpublished data). In addition, we performed the quantification of EGFR mRNA in SqCC and ovarian cancer. An increased EGFR expression was observed in ~70% of ovarian carcinomas (32,33) and it was thought that the measurement of EGFR expression in ovarian cancer was feasible and could give important prognostic information (34). However, EGFR mRNA did not show a significant usefulness for diagnosis (Table I).

We examined the clinical significance of a gynecologic malignancy diagnosis by using our method to detect hTERT mRNA in serum. In the present study, hTERT mRNA was useful for cancer diagnosis in gynecologic organs and maybe even for the early detection of cancer, indicating that hTERT mRNA, unlike EGFR may be a more excellent diagnostic molecule than CA125 for ovarian cancer than SCC for uterine cervical cancer (35). Judging from the half-time in serum, hTERT mRNA could be quantified after 3-4 weeks after anti-tumor therapy such as chemicals or surgery in order to estimate the therapeutic effect.

Some reports say that trichostatin A (TSA) may primarily target for hTERT to drive a proliferative signal or carry out the anti-apoptotic effect of hTERT through a $p21^{waf1}$-dependent and p53-independent pathway even in dysplastic cells (14). Therefore, inhibition of histone deacetylation may selectively regulate the transcriptional repression of hTERT in normal cells compared to several cancers. Thus, a definite link between the chromatin environment of the telomerase gene promoters and transcriptional activity may be important for development of a novel cancer detection modality.

Furthermore, it is speculated that hTERT-positive but morphologically non-malignant cells within particular tissues may be susceptible to the subsequent development of invasive cancer, and the greater frequencies of activation of telomerase in low-malignant-potential (LMP) tumors that border behavior as frank carcinomas were nearly significant compared with normal ovary or normal cystadenomas (36).

The employment of biomarkers, which is expected to contribute to the careful follow-up of patients who are undergoing chemotherapy, may result in an improvement in the prognosis in resected cases. However, we believe that the association with response and survival may be more evident if this higher sensitive mRNA-based detection assay is used rather than current protein-based assays.

In conclusion, hTERT mRNA is useful in the diagnosis for gynecologic cancers as well as hepatoma and is superior to conventional tumor markers, although hTERT mRNA, combined with other biomarkers such as CA125 in order to elevate the specificity for detection, should be used for cancer diagnosis in screening the presence of malignancies or monitoring a clinical condition since hTERT mRNA does not have organ-specificity. Therefore, serum hTERT mRNA is a novel and available biomarker for gynecologic malignancies. A prospective study or a validity test with masked samples to conclude the diagnostic superiority of hTERT mRNA is needed.

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