

Highly sensitive RT-PCR using the QIAGEN[®] OneStep RT-PCR Kit detects telomerase RNA in breast cancer patients*

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Use of the QIAGEN[®] OneStep RT-PCR Kit resulted in the development of a sensitive and accurate assay for the detection of telomerase RNA subunits in the serum of breast cancer patients. This finding may prove useful in cancer diagnosis and followup.

Breast cancer is the leading cause of death of women aged 35–54 in western populations. Early detection is the single most important factor in surviving breast cancer. Several studies indicate that mammographic screening can reduce breast cancer mortality, but such screening has a high rate of both false positive and false negative results. A sensitive molecular diagnostic test using genetic markers in blood could assist accurate detection of breast cancer.

Telomerase is a ribonucleoprotein enzyme that adds telomeric repeats to the ends of eukaryotic chromosomes after cell division.

Normal somatic cells have low or undetectable levels of telomerase, whereas the vast majority of cancer biopsies, including those from breast cancer patients, have detectable telomerase activity. Telomerase consists of two subunits, telomerase RNA template (hTR) and telomerase reverse transcriptase protein (hTERT). We report here the use of the QIAGEN[®] OneStep RT-PCR Kit for detection of hTR and hTERT in both tumor and serum samples taken from breast cancer patients. ►

Table 1. Primer and template concentrations, and primer sequences for RT-PCR

Target	Template concentration	Primer concentration	Primer sequences
<i>hTR</i>	1 ng (from tumor samples), or 1–5 µl / 100 µl extract obtained from 100 µl serum	0.15 µM of each primer	P1: 5'-GAA GGG CGT AGG CGC CGT GCT TTT GC-3' P2: 5'-GTT TGC TCT AGA ATG AAC GGT GGA AGG-3'
<i>hTERT</i>	1 ng (from tumor samples), or 1–5 µl / 100 µl extract obtained from 100 µl serum	0.3 µM of each primer	P3: 5'-TGA CAC CTC ACC TCA CCC AC-3' P4: 5'-CAC TGT CTT CCG CAA GTT CAC-3'
<i>GAPDH</i>	1 ng (from tumor samples), or 1–5 µl / 100 µl extract obtained from 100 µl serum	0.075 µM of each primer	P5: 5'-CGG AGT CAA CGG ATT TGG TCG TAT-3' P6: 5'-AGC CTT CTC CAT GGT GGT GAA GAC-3'

* Data excerpted from Chen, X. q., Bonnefoi, H., Pelte, M.-F., Lyautey, J., Lederrey, C., Movarekhi, S., Schaeffer, P., Mulcahy, H.E., Meyer, P., Stroun, M., and Anker, P. (2000) Telomerase RNA as a detection marker in the serum of breast cancer patients. *Clin. Cancer Res.* **6**, 3823 (reference 1). Published with permission of the American Association for Cancer Research.

Materials and methods

Blood was collected with informed consent from 18 females undergoing surgery for breast cancer and from 2 females undergoing surgery for benign breast disease. RNA was isolated using standard procedures. RT-PCR of *hTR*, *hTERT*, and *GAPDH* from tumor and serum samples was carried out using the QIAGEN OneStep RT-PCR Kit. Reactions were set up according to the manufacturer's instructions. Details of primer and template concentrations are given in Table 1. RT-PCR

conditions for all templates were: initial incubation 50°C, 30 minutes; second incubation 95°C, 15 minutes (activation of HotStarTaq™ DNA Polymerase), then 50 cycles of 94°C, 30 seconds; 65°C, 1 minute; 72°C, 1 minute, followed by 72°C, 10 minutes (final extension).

PCR products were run on agarose gels (Elchrom Scientific Precast ClearoseBG S50), stained with SYBR®gold (Molecular Probes) for 45 minutes, and destained twice in a darkroom with deionized water for 30 minutes. All experiments were performed at least twice, and only samples clearly positive twice were scored as positive.

Results

RT-PCR was carried out using RNA isolated from tumor samples (from 18 patients undergoing surgery) and from serum samples (from all 20 patients). Results are shown in Figure 1 and Table 2. Expression of *GAPDH* mRNA (positive control) was detected in all patient samples and also in the serum of healthy control individuals.

Expression of *hTR* mRNA was detected in 17 of 18 tumors studied and in 5 of 18 serum samples. No expression was detected in serum samples from 21 healthy control individuals or in the 2 patients with benign breast disease.

Expression of *hTERT* mRNA was detected in 17 of 18 tumors studied and in 4 of 16 serum samples (2 samples were not available for analysis). No expression was detected in serum samples from 21 healthy controls or in the 2 patients with benign breast disease. Three of the serum samples positive for *hTERT* were negative for *hTR*, so at least one telomerase subunit was detected in serum from 8 of 18 breast cancer patients.

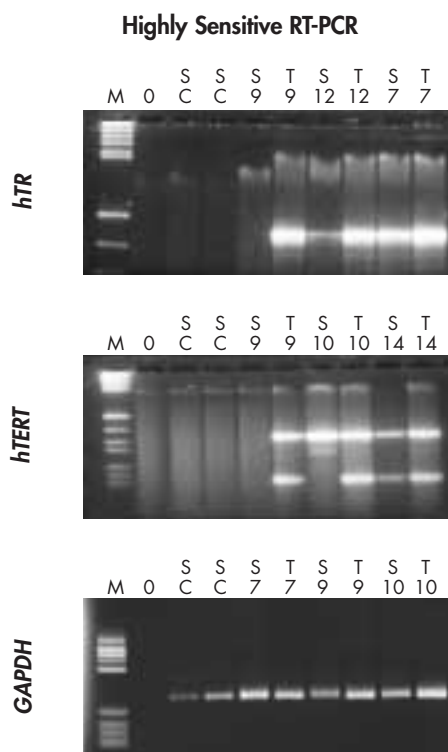


Figure 1 Agarose gel electrophoresis of RT-PCR products from GAPDH, *hTR*, and *hTERT* templates. The smaller product amplified from *hTERT* is probably a primer-dimer. **M:** markers; **S:** serum; **T:** tumor; **C:** healthy control; **7, 9, 10, 12, 14:** patient numbers.

Discussion

The RT-PCR assay used in this study detected expression of at least one telomerase subunit in 44% of the serum samples from breast cancer patients. No expression was detected in any samples from healthy control individuals. Tumor DNA has previously been found in serum samples from breast cancer patients using a range of molecular markers (for example, reference 2). However, this is the first report of detection of tumor-related RNA in serum from breast cancer patients. No single DNA marker previously used detected such a high percentage of positive cases as we report here.

Telomerase activity is not easily observed in serum as its RNA components tend to degrade. However, this RT-PCR assay successfully detected the individual telomerase components.

It is unclear why the expression of *hTR* and *hTERT* were discordant in some samples. These subunits are independently expressed in vivo, and little is known about their mechanism of assembly.

In the future, the use of real-time RT-PCR might increase the sensitivity of this assay and therefore increase the number of positive cases detected. As telomerase activity is commonly found in neoplastic disease, an RT-PCR assay for detection of telomerase in serum might be useful in the detection of a wide range of cancers. ■

References

1. Chen, X. q. et al. (2000) Telomerase RNA as a detection marker in the serum of breast cancer patients. *Clin. Cancer Res.* **6**, 3823
2. Chen, X. q., Bonnefoi, H., Diebold-Berger, S., Lyautey, J., Lederrey, C., Faltin-Traub, E., Stroun, M., and Anker, P. (1999) Detecting tumor-related alterations in plasma or serum DNA of patients diagnosed with breast cancer. *Clin. Cancer Res.* **5**, 2297

Table 2. Expression of *hTR* and *hTERT* in 20 breast cancer patients

Patient No.	<i>hTR</i>		<i>hTERT</i>	
	Tumor	Serum	Tumor	Serum
1	+	-	+	NA
2	-	-	+	+
3	+	-	+	-
4	+	+	+	NA
5	+	+	+	-
6	+	+	+	-
7	+	+	+	+
8	+	-	+	-
9	+	-	+	-
10	+	-	+	+
11	+	-	-	-
12	+	+	+	-
13	+	-	+	-
14	+	-	+	+
15	+	-	+	-
16	+	-	+	-
17*	NA	-	NA	-
18	+	-	+	-
19	+	-	+	-
20*	NA	-	NA	-

+: product detected by RT-PCR; -: no product detected by RT-PCR; NA: not available. *: Patients with benign breast disease