Abstract

Background The purpose of this study was to evaluate telomerase activity in peripheral whole blood from head and neck squamous cell carcinoma (HNSCC) patients as a biomarker for diagnosis of HNSCC or detection of recurrence during follow-up.

Materials and methods Telomerase activity was measured from peripheral whole blood extracts by telomerase repeat amplification protocol (TRAP) in HNSCC patients before and after surgery and in a control group. Sixty-two HNSCC patients and 42 control subjects were included.

Results Telomerase activity was found in 41 out of 62 (66.1%) HNSCC patients before surgery and in 8 out of 42 (19.0%) controls (p<0.001). Among 41 HNSCC patients who showed positive telomerase activity before surgery, 32 (78.1%) showed a conversion of telomerase activity to negative after surgery. In follow-up, 6 out of 8 (75%) showed conversion of telomerase activity from negative to positive after recurrence. Telomerase activity was changed to negative in 4 out of 6 (66%) recurred patients with positive telomerase activity after second surgery.

Conclusion The telomerase activity in peripheral whole blood extracts of HNSCC patients might be a useful biomarker for detecting recurrence after treatment. Further study with larger sample size using a more sensitive detection method of telomerase activity is necessary to verify these results.

Keywords Telomerase · Head and neck cancer
also detected in the circulating epithelial cells of peripheral blood in hepatic cancer patients [21–26].

In this study, we analysed telomerase activity in peripheral whole blood extracts before and after surgery in HNSCC patients and in the control group subjects to evaluate its role as a useful biomarker for diagnosis or detection of recurrence during follow-up in HNSCC.

Materials and methods

Patients and sample collection

In this study, we included 62 patients with histologically confirmed HNSCC after curative surgery at Hanyang University Medical Center during the period from June 2002 until February 2006. We only included cases that were first diagnosed and had received no prior treatment including chemotherapy, radiotherapy or surgery. There were 54 men and 8 women, and the mean age was 63.2 years old (range, 41–90 years). The primary sites of HNSCC were 29 larynx cancer, 19 oral cancer, 6 oropharynx cancer, 5 hypopharynx cancer, 2 paranasal sinus cancer and 1 unknown primary metastatic cancer. The TNM stage distribution (AJCC sixth edition) was as follows: 21, Stage I; 10, Stage II; 9, Stage III; and 23, Stage IV. 38 (61.3%) of the HNSCC patients received postoperative radiotherapy and one patient received postoperative concurrent chemoradiotherapy. The recurrence was noted in 23 (37.1%) out of 62 HNSCC patients during the mean follow-up time of 33±18 months. Of 23 recurred patients, 12 patients underwent the second surgery with curative intention.

The control group included 42 patients, ages 32–66 years (mean age, 43 years), who underwent surgery for chronic paranasal sinusitis, chronic otitis media, chronic tonsillitis or vocal nodule; 35 (87.5%) were men and 5 (12.5%) were women. We excluded patients from the control group if they had a history of malignancy.

Informed consent was obtained from each patient and the institutional review board of Hanyang University Hospital approved the study protocol. We obtained 10 ml of peripheral blood 1 day before and about 7 days after the first or second surgery from HNSCC patients and 1 day before surgery from the control group. We stored the blood at –70°C prior to the experiment.

Telomerase assay

To assay telomerase activity in the samples, we used the TRAPEZE® Gel-Based Telomerase Detection Kit (Chemicon® International, Temecula, CA, USA), which is based on a telomeric repeat amplification protocol assay (TRAP).

A total of 330 µl of peripheral whole blood was suspended in 200 µl of 1X CHAPS lysis buffer. After incubating for 30 min at 4°C, the suspension was centrifuged at 12,000×g for 20 min at 4°C. The protein concentration of supernatants was measured; 1 µg of lysates was used in each assay. In those cases with weak or negative signal, the assay was repeated with increased amount of lysate up to 3 µg of protein. The TRAP reaction mixture included 5 µl of 10× TRAP reaction buffer, 1 µl of 50× dNTPs mix, 1 µl of TS primer, 1 µl of TRAP primer mix, 0.4 µl of Taq polymerase and 1 µg of lysates, and dH2O was added up to the total amount of 50 µl. The mixture was incubated at 30°C for 30 min and amplified in a thermal cycler (Perkin Elmer, Gene Amp PCR system 20400; Norwalk, CT, USA) with a 3-step polymerase chain reactions (PCR) of 94°C/30 seconds, 59°C/30 seconds and 72°C/1 minute for 30 cycles. A total of 5 µl of loading dye (0.25% bromophenol blue, 0.25% xylene cyanol, 50% glycerol, 50 mM EDTA) was placed into each reaction tube; 25 µl of the solution was taken and electrophoresis was conducted in a 12.5% non-denaturing polyacrylamide gel with 0.5× tris-borate EDTA (TBE) buffer. After the electrophoresis, the gel was stained with ethidium bromide for 30 min and destained for 20 min in deionised water at room temperature. As a positive control, a positive telomerase cell host provided in the TRAPEZE® Kit was used. The peripheral blood sample was treated with heat at 85°C to deactivate telomerase and was used as the negative control. Telomerase activity is considered positive if the telomeric repeat ladder appears with the 36 bp internal control band. If the 36 bp internal control band appears without the telomeric repeat ladder, telomerase activity is considered negative. In this study, the telomerase activity in the peripheral whole blood extracts was weaker than in the positive control provided with the TRAPEZE® Kit (Fig. 1).

Statistical analysis

Pearson’s chi-square test was used to compare telomerase activity between the HNSCC patient group and the control group and to evaluate the relationship between telomerase activity and clinicopathologic parameters in HNSCC patients. The relationship between telomerase activity and survival rate was tested using the Kaplan–Meier method and log-rank test. All statistical data were analysed with SPSS version 15.0 (SPSS, Chicago, IL, USA). Differences were considered statistically significant at p<0.05.

Results

Telomerase activity in peripheral whole blood extract before surgery

Telomerase activity was detected in 41 out of 62 patients (66.1%) in the HNSCC group before surgery. Telomerase activity was detected in 8 out of 42 (19.0%) control group subjects. This difference represents a significantly higher telomerase activity in the HNSCC than in the control groups (p<0.001) (Table 1).