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Short Report

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Telomerase mRNA Expression in Ductal Carcinomas of the Breast

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Recently, telomerase expression has been shown to be closely associated with cellular immortality and cancer; the majority of malignant tumours from all cancer types tested express human telomerase RNA (hTR), but most normal somatic tissues do not express hTR significantly (1).

hTR expression has been shown to correlate with poor clinical outcome in breast tumours (2). However, some studies revealed no correlation between hTR activity and disease outcome (3). The aim of this study was to determine hTR activity in breast carcinomas with a nonisotopic in situ hybridization technique.

Telomerase activity in the archival paraffin blocks of 18 cases of invasive ductal carcinoma of the breast was retrospectively examined. The slides were reviewed and tumours classified according to the Scarff-Bloom-Richardson grading system. Staging was performed according to the American Joint Committee (AJCC) classification (1999). Then 5 µm paraffin sections from the representative paraffin blocks were recut for in situ hybridization.

Nonisotopic in situ hybridization (NISH) was performed with a commercially available telomerase mRNA probe (Biogenex HK864-2K). Signal screening was performed by 5-bromo-4-chloro-3indoyl phosphate/nitro blue tetrazolium (BCIP/NBT).

Normal adult testis and normal breast tissues were used as controls. In the study group, the percentage of positive cells was determined by two authors (HK and EK) on a blind basis, and discrepancies were resolved after joint observations using a dual-headed microscope.

Statistical analysis was based on two-sided chi- square test. The computations were performed using GraphPad InStat version 2.04 (GraphPad Software USA). The Satistical difference was considered significant if the p value was less than 0.05.

The cases included in this study had a minimum follow-up of 2 years. The patients' age at diagnosis ranged from 28 to 70 years with a median of 53 years. Telomerase activity was very high in adult testicular tubules, but negative in breast tissues. The intensity of telomerase activity in the study group was similar in each case and of a moderate degree compared to adult testicular tissue. The presence of hTR expression was observed in the cytoplasm of the carcinoma cells. The Table summarizes the age and follow-up results, and the histological grade, stage and percentage of hTR positive cells.

We found telomerase activity in 44% (8/18) of cases in the study group (Figure). We did not find any significant statistical correlation between telomerase activity and tumour grade or stage or the follow-up ($p > 0.05$). The most significant expression (60%) was observed in the patient with distant metastases (stage: T4N2M1), who was not included in the study group due to her death 2 months after diagnosis.

The Telomeric Repeat Amplification Protocol (TRAP) assay has been recently developed to measure telomerase activity using total tissue extraction (3). Although the most sensitive method, the TRAP assay uses tissue extracts, and the exact cell type expressing telomerase

Table. Clinical features and morphologic findings of the cases.

Case	Age	Grade	Stage	Follow-up	Telomerase Activity (%)
1	54	2	T2N1	3 y, NED	30
2	53	1	T3N1	4 y, NED	30
3	64	2	T2N0	3 y, NED	10
4	50	2	T2N0	4 y, NED	5
5	46	2	T2N1	4 y, NED	5
6	58	2	T2N0	3 y, NED	< 5
7	64	1	T2N0	3 y, NED	< 5
8	59	2	T2N0	3 y, NED	< 5
9	50	1	T2N1	3 y, NED	0
10	39	1	T3N1	4 y, NED	0
11	58	3	T2N1	6 months later relapsed	0
12	45	2	T2N1	3 y, NED	0
13	42	2	T2N0	4 y, NED	0
14	55	2	T2N1	4 y, NED	0
15	36	2	T2N1	3 y, NED	0
16	54	2	T2N0	3 y, NED	0
17	70	2	T2N1	4 y, NED	0
18	28	2	T3N1	3 y, NED	0

NED: no evidence of disease

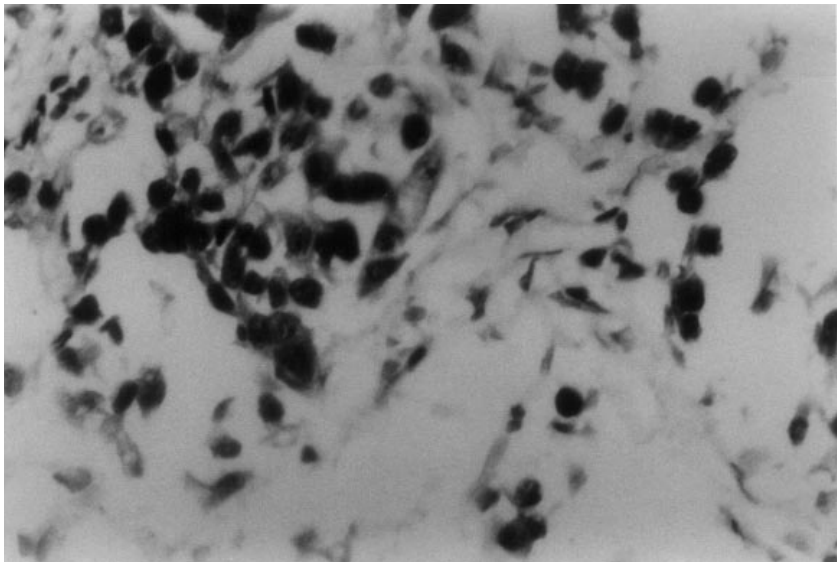

Figure. Intracytoplasmic telomerase activity in invasive ductal carcinoma cells (Telomerase mRNA Probe using BCIP/NBTchromogen X 40).

activity could not be identified. On the other hand, in situ hybridization (ISH) allows precise detection of signals in normal epithelium, premalignant lesions and carcinoma within a single specimen section. It has also been reported that there are good correlations between hTR expression detected by ISH and telomerase activity detected by TRAP (4).

In the current study, telomerase activity was investigated in ductal carcinomas of the breast by ISH. hTR expression was present in 44% of cases. This incidence is in accordance with previously published studies on breast carcinomas using the TRAP assay (2,5). The high levels of telomerase activity detected by the TRAP assay may be attributable to the false positive

results due to contamination of tumour tissues by inflammatory cells or lymphoid nodules.

Telomerase activity has been reported to be either absent or low in normal breast tissues (2,6). In our study, no telomerase activity was detected in either normal control breast tissues or residing in the vicinity of the carcinoma areas of the study group. Telomerase activity was entirely intracytoplasmic in the ductal carcinoma areas, similar to transitional cell carcinomas of the upper urinary tract (6).

The hTR level is up-regulated in the early preneoplastic stages, increases further during progression and telomerase activity is detected only in late stage tumours (2,6,7). Higher levels of activity are associated with poorer clinical outcome (3,8). In our study, the only case with distant metastases expressed the highest hTR. This finding correlates with the suggestion that tumours expressing this enzyme may have a more aggressive phenotype (2,5). Very recently it has been reported that a gene or genes on the normal human chromosome 3p efficiently represses the telomerase in human breast cancer cells and this repression is associated with a permanent growth arrest of tumour cells (9). Telomerase may be important in cancer diagnostics as a target for cancer therapy.

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