Abstract. To study the activity of telomerase and the relationship between telomerase and other prognostic variables in cases of invasive ductal breast carcinomas, fifty fine-needle aspiration biopsies (FNABs) obtained from the same number of female patients, diagnosed cytologically and confirmed histologically after surgery, were examined. The same cases were studied immunocytochemically using monoclonal antibodies to telomerase, estradiol receptors (ER) and HER-2 (CB11) and a standard alkaline phosphatase (APAAP) method. Telomerase activity was found in 72% of the carcinomas studied. An association was found between telomerase activity, ER receptors and HER-2 expression (p<0.005). A relationship between telomerase activity, histological grade and lymph node status (LNS) was found as well (p<0.005). The above results seem to be significant prognostic factors and should be taken into consideration in the follow-up of patients after appropriate treatment for breast cancer.

The incidence of breast carcinoma is gradually increasing but early correct diagnosis has improved the outcome in the past decade. Fine-needle aspiration biopsy (FNAB) proved to be a powerful diagnostic tool for the early detection of breast malignancy with well-documented accuracy, cost effectiveness and minimal risk of side-effects (1, 2).

The most important clinicopathological prognostic parameters so far identified are the absence or presence of lymph node metastasis and the grade of the tumor. A promising emerging molecular marker is telomerase, a ribonucleoprotein enzyme complex, which, when (re)activated or up-regulated, allows tumor cells to escape cellular senescence and proliferate indefinitely (3).

Human telomerase is a large ribonucleoprotein complex composed of at least three components: the RNA subunit hTR, which provides the template sequence for the synthesis of telomerase repeats (4), the catalytic subunit hTER, which possesses conserved reverse transcriptase motifs (5), and the telomerase-associated protein hTEP1, which may play a role in coordinating the telomerase holoenzyme structure and/or recruiting telomerase regulatory factors (6). Telomerase activity has been found in breast carcinoma tissues, whereas it was not detected in most benign tumors except for some large fibroadenomas (7, 8).

HER-2/neu is a prognostic factor for breast cancer and overall disease-specific survival (DSS). According to sentinel lymph node status and the estradiol receptors (ER) status, patients with elevated HER-2 expression, LN-positive and ER-negative tumors have a significantly shorter DSS.

The present study examined, on FNABs, the association between telomerase activation and other clinicopathological variables such as tumor grade, LNS, HER-2/neu and ER, in invasive ductal breast carcinomas.

Materials and Methods

Samples from 50 FNABs (one from each female patient included in the study) were studied.

In all cases, some samples in superfrost plus slides were fixed in 96% ethyl alcohol and used for Pap staining, while some others were air-dried and used for Giemsa staining and immunocytochemistry.

All cases were diagnosed cytologically as invasive ductal breast carcinomas and confirmed by histological examination after surgery. As verified by histological study, in this series there were eight grade I carcinomas, eighteen grade II carcinomas and twenty-four grade III carcinomas. In twenty-nine cases, positive axillary lymph nodes were found, while the remaining twenty-one cases were negative for metastases to axillary lymph nodes.
Figure 1. Telomerase activity on FNAB of invasive ductal breast carcinoma. (x250)

Figure 2. ER expression on FNAB of invasive ductal breast carcinoma. (x250)

Figure 3. Her-2/neu expression on FNAB of invasive ductal breast carcinoma. (x250)
**Immunocytochemistry.** Monoclonal antibodies to telomerase, Her-2/neu (DAKO) and ER (DAKO) were used at a 1:250, 1:50 and 1:50 dilution, respectively, with an optimum incubation time of one hour. Immunostaining was performed using the alkaline phosphatase (APAAP) method. A step of microwave heating in a sodium citrate solution was performed prior to incubation. A very light hematoxylin counter stain was also performed.

**Statistical analysis.** The $\chi^2$-test (Chi-square) was used. A $p$ value $<$0.05 was considered as significant.

**Evaluation of immunostaining.** The slides were examined using a $\times$ 40 objective lens. Nuclear immunostaining was observed for telomerase and ER (Figures 1+2) and transmembrane staining for Her-2/neu (Figure 3).

**Results**

The results are summarized in Table I and Table II. In all samples of invasive ductal breast carcinomas, neoplastic cells were seen either as small or large aggregates or separately. Telomerase immunostaining was distinct in the nucleus or rarely in the cytoplasm (Figure 1) of neoplastic cells in two cases of grade I (25%), in twelve cases of grade II (66.6%) and in twenty-two cases of grade III (90%) (Table I).

Telomerase immunostaining was observed in twenty-seven cases of lymph node-positive status (LNS+) (93%) and in nine cases of lymph node-negative status (LNS-) (43%) $p<0.005$ (Table I). Telomerase activity was found in thirty cases of positive Her-2/neu (30%), in five ER-positive cases (27.5%) and in thirty-one cases of the ER-negative cases (72.5%) (Table II).

The above results were found to be statistically significant $p<0.005$ (Tables I and II). A statistically strong overexpression between telomerase, grade, LNS and Her-2/neu expression was observed, while a statistically significant inverse association was found between telomerase activity and ER status.

**Discussion**

Knowledge of the mechanisms by which human telomerase is regulated might be important for a better understanding of the pathogenesis of breast carcinoma. To gain insight into the mechanisms controlling telomerase activity in invasive ductal carcinoma of the breast on FNABs, we examined the catalytic activity of telomerase and we correlated that activity to other prognostic variables.

Telomerase activity was detected in 27% of the carcinomas, in accordance with a recent study from the Breast Cancer Center of London, UK (4). In other recent studies, the telomerase activation varied from 67% to 88% using the TRAP assay method (1, 2, 9, 10). A significant association between telomerase activity, grade and nodal status was found according to some recent studies (1, 4, 11, 12), but other studies failed to detect such an association (13, 14).

In our series of invasive breast cancers, telomerase activity seemed to be inversely associated with ER content, in agreement with another recent study (10), but in partial disagreement with previous data in which such a relationship was not observed (15). ER-negative, telomerase-positive tumors did not differ significantly from ER-positive, telomerase-negative tumors as regards other characteristics such as histological grade or LNS of the disease. Progesterone seems to induce the down-regulation of telomerase activity and is mainly related to the accumulation of cells in the 60/61-phase of the cycle (16). A strong association was found between telomerase activation and Her-2/neu expression ($p<0.005$), but there are no data from other studies.

In summary, our findings suggest that the determination of telomerase activity as a prognostic marker in breast cancer should be included in future validation studies. The detection of telomerase activity by the APAAP method has been shown to be useful in FNABs from ductal breast
carcinomas and may prove to be useful in the detection and confirmation of malignancies on non surgical specimens, the majority of which currently are obtained on an outpatient basis. In the future, the study of telomerase activity may prove useful in many other malignancies as an important marker for the outcome of the diseases.

References


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