Telomerase Activity in Benign and Malignant Breast Lesions: a Pilot Prospective Study on Fine-Needle Aspirates

Raffaella Villa, Nadia Zaffaroni, Marco Folini, Gabriele Martelli, Giuseppe De Palo, Maria Grazia Daidone, Rosella Silvestrini*

The enzyme telomerase contributes to the maintenance of telomere stability (1). Although telomerase reactivation in somatic cells is not sufficient per se for the cells to proliferate indefinitely, telomerase expression and telomere stabilization appear to occur concomitantly with the attainment of immortality in cancer cells (1–3) and may contribute to tumorigenesis and neoplastic progression. It is not yet known at what stage of cancer development telomerase is reactivated, but scientific interest has recently been focused on defining the utility of assaying for telomerase as a diagnostic and prognostic tool (4). In particular, investigators have focused on breast cancer for its high incidence and relevance. These investigations have been favored by the existence of ongoing screening programs and the possibility of obtaining samples for pathologic and biologic characterization through the use of the relatively noninvasive method of fine-needle aspiration. Only two studies (5,6) of which we are aware have provided results on the diagnostic potential of measuring telomerase activity in fine-needle aspirates (FNAs) of breast lesions. However, the actual diagnostic predictivity of results obtained with the telomeric repeat amplification protocol (TRAP) assay has not been conclusively defined, owing to the lack of histologic confirmation for some of the cytologically diagnosed lesions and the small number of cases investigated. In this study, we prospectively assessed the diagnostic accuracy of findings of telomerase activity in a consecutive series of FNAs from 116 solid breast lesions that were subsequently submitted for histologic examination—i.e., in a series that is much larger than those previously examined (5,6). Telomerase activity was measured by use of the polymerase chain reaction-based TRAP assay described by Kim et al. (2). The protein content of each sample was quantified, and the TRAP assay was performed at two protein concentrations (0.6 and 6.0 μg) per sample, as suggested by Sugino et al. (6). The reliability of the TRAP assay results was supported by the concordance of data obtained from the FNAs and their corresponding surgical specimens (Fig. 1, A) and by the demonstration of a lack of TRAP assay inhibitors in the telomerase-negative samples (Fig. 1, B). The TRAP assay results were independently scored by two of the investigators (R.Villa and M. Folini) without knowledge of the histologic diagnosis.

Women entering the study had a median age of 42 years (range, 18–86 years), had palpable solid breast lumps (median size, 2.2 cm; range, 0.5–4.5 cm), and were examined at the Division of Diagnostic Oncology and the Outpatient Clinic of the Istituto Nazionale per lo Studio e la Cura dei Tumori of Milan, Italy during the period from November 1996 through April 1997. All of the lesions were subsequently subjected to surgical removal and histologic examination. Eighty lesions were diagnosed as being benign (59 fibroadenomas and 21 proliferative and nonproliferative dysplasias), and 36 were diagnosed as being malignant.

The TRAP assay results and the histologic findings were not strictly congruent. In fact, the telomerase signals for some benign tumors were as strong as those observed for some carcinomas. In particular, among the 75 assessable benign lesions, 49% (27 fibroadenomas and 10 dysplasias) showed a positive or faintly positive telomerase signal at least at one of the two protein concentrations tested (Table 1). However, TRAP assay positivity dropped to 16% (nine fibroadenomas and three dysplasias) when positivity at both protein concentrations was considered and to 17% (nine fibroadenomas and four dysplasias) when positivity only at the lowest protein concentration was considered. Such data suggest the prevalence of weak telomerase activity in positive benign lesions, as previously reported by other investigators (5). Patients with telomerase-negative benign lesions were comparable to patients with telomerase-positive benign lesions with respect to age, tumor size, and tumor histologic features.

Among the 36 histologically diagnosed cancers, 27 (75%) showed a strong telomerase signal at least at one of the two protein concentrations tested, with an accuracy that seemed to be independent of the specific protein concentration used in the TRAP assay (Table 1). Requiring positive TRAP signals at both protein concentrations did not improve the identification of cancerous lesions, but limited TRAP assay positivity to only 39%. Most of the nine carcinomas not showing telomerase activity were from women who were more than 65 years of age; these tumors were small and of a pure or mixed-lobular histologic type. No association was observed between the number of morphologically identified putative tumor cells and the TRAP assay results.

To use the detection of telomerase activity prospectively for a differential diagnosis of breast lesions, we evaluated the specificity, the sensitivity, and the predictive value of the TRAP assay results on our case series. Overall, the area under the receiver-operating-characteristic curve calculated by considering the two threshold levels of protein concentration was 0.683. The specificity (defined as the frequency of samples correctly identified as benign by the TRAP assay among the total number of histo-
logicidally diagnosed informative benign lesions) ranged from 51% (95% confidence interval [CI] = 42%–60%) to 85% (95% CI = 78%–92%) on the basis of the threshold level of protein used to define a positive assay (i.e., 6.0 and 0.6 μg protein, respectively); the predictive value ranged from 75% to 80%. The sensitivity of the TRAP assay (i.e., the frequency of samples correctly identified as malignant) ranged from 39% (95% CI = 30%–48%) to 75% (95% CI = 62%–88%), with the predictive value ranging from 41% to 60%. Moreover, when a positive signal was detected at least at one of the two protein concentrations tested, the level of sensitivity of the TRAP assay was similar (75%) to that reported by other investigators (6), thus providing indirect support for the reproducibility of the assay. For the 81 cases with available cytoclogic information, the sensitivity, the specificity, and the predictive accuracy of the cytoclogic diagnoses were higher than the corresponding measures estimated by use of the TRAP assay.
Table 1. Telomerase activity in fine-needle aspirates (FNAs) of breast lesions as a function of histologic diagnosis

<table>
<thead>
<tr>
<th>Diagnosis based on surgical specimens</th>
<th>No. of specimens with TRAP* assay results</th>
<th>Telomerase activity in FNAs: No. of positive samples†‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Assessable</td>
<td>Unassessable</td>
</tr>
<tr>
<td>Benign lesion</td>
<td>75</td>
<td>5</td>
</tr>
<tr>
<td>Fibroadenoma</td>
<td>56</td>
<td>3</td>
</tr>
<tr>
<td>Dysplasia</td>
<td>19</td>
<td>2</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>36</td>
<td>—</td>
</tr>
</tbody>
</table>

*TRAP = telomeric repeat amplification protocol.
†A = telomerase activity detected at least at one of the two protein concentrations tested (0.6 and 6.0 μg); B = telomerase activity detected at the lowest protein concentration tested; C = telomerase activity detected at the highest protein concentration tested; and D = telomerase activity detected at both protein concentrations tested.
‡Numbers in parentheses show the frequency of positive cases among the total number of informative cases.
§Fraction of positive samples for telomerase activity, detected at least at one of the two protein concentrations tested, was significantly higher for carcinomas than for benign lesions (continuity adjusted chi-squared value = 4.975; two-sided \(P = .026\) for one degree of freedom).

In conclusion, our results on FNAs from solid breast lumps confirm that differences exist in telomerase activity between neoplastic and benign lesions (6–8). However, the low specificity of the TRAP assay suggests caution with respect to the application of measuring telomerase activity, at least as a single prospective diagnostic tool. In fact, the high incidence of telomerase-positive samples among benign lesions and the poor predictive values for both malignant and benign lesions are far from acceptable for routine diagnostic application and indicate the need for assay improvements to increase predictivity. Moreover, false-positive cases should be followed to monitor the evolution of the lesions, although the occurrence of a positive TRAP signal in fibroadenomas and dysplasia is in agreement with previous observations of a substantial fraction of S-phase cells in benign breast lesions (9) and may reflect the presence of a proliferative disease, mainly in young patients. Such positive signals could also be due to the presence of normal breast stem cells, which can differentiate into either epithelial cells or myoepithelial cells (6), or of normal breast tissue under hormonal stimulation (10). These findings should be taken into account, since immortality or an active proliferative state may be acquired independently of malignant transformation (11).

References


Notes

Supported by grants from the Associazione Italiana per la Ricerca sul Cancro and the Italian National Research Council (grant no. 97.00490. CT04).

We thank B. Canova and B. Johnston for editorial assistance.