Abnormal p53 immunoreactivity and prognosis in node-negative breast carcinomas with long-term follow-up

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Summary. The expression of the p53 gene product was investigated immunocytochemically in a retrospective series of 164 formalin-fixed paraffin-embedded invasive breast carcinomas with pathologically proven negative lymph nodes. Overall, 78 tumors (48%) showed a variable degree of p53 immunoreactivity. Among these, 38 cases were low expressors (1–10% p53 immunoreactive tumor cells), 21 moderate expressors (10–50% immunoreactive cells) and 19 high expressors (>50% immunoreactive cells). Abnormal p53 expression correlated significantly with tumor size, histological and nuclear grade, DNA ploidy, mitotic rate and proliferation index, and with the lack of estrogen receptors. Disease-free and adjusted survival analysis of the 124 node-negative patients with long term (more than 10 years) follow-up, however, did not reveal an independent prognostic role for p53 expression. These data suggest that the evaluation of p53 immunoreactivity may only play a role in a multi-parametric prognostic assessment of node-negative breast carcinoma.

Key words: p53 gene – Breast carcinoma – Prognosis – Immunocytochemistry

Introduction

The p53 gene, coding for a 375-amino-acid nuclear phosphoprotein, has been extensively investigated in the past decade (for review see Lane and Benchimol 1990). It was formerly considered to be a dominant oncogene, capable of inducing cell transformation, either alone or in association with an activated ras gene (Eliyahu et al. 1984; Jenkins et al. 1984; Parada et al. 1984). Recent experimental studies, however, have demonstrated that wild-type p53 can actually suppress cell transformation, whereas mutated p53 genes play a role in tumorigenesis (Finlay et al. 1989; Friedman et al. 1990). Thus, accumulating evidence has clearly established p53 as a tumor suppressor gene, functionally comparable to the Rb susceptibility gene. Allelic losses of chromosome 17p, where the p53 gene has been mapped (Isobe et al. 1986), and genetic alterations of this gene, including mutations and/or deletions, are frequently detected in many common human malignancies (Fearon et al. 1987; Yokota et al. 1987; Mackay et al. 1988; Nigro et al. 1989). Wild-type p53 protein has a short half-life (6–30 min) and it is expressed at very low levels in normal tissues (Rogel et al. 1985). Conversely, mutant p53 proteins are more stable and have a prolonged half-life (Finlay et al. 1988). Accordingly, the intranuclear accumulation of p53 protein, conveniently investigated by immunocytochemistry, is thought to reflect the occurrence of a mutation of the p53 coding sequence (Rogel et al. 1985; Iggo et al. 1990; Bartek et al. 1991).

p53 abnormalities, as shown by molecular biology techniques or by immunocytochemistry, occur frequently in breast carcinomas (Cattoretti et al. 1988; Bartek et al. 1990; Thompson et al. 1990; Chang et al. 1991; Davidoff et al. 1991 a; Varley et al. 1991). Alterations of p53 have been found in “in situ” carcinomas (Davidoff et al. 1991 b; Walker et al. 1991), are maintained throughout cancer progression (Davidoff et al. 1991 b), and have been related to unfavorable prognostic variables such as stage, metastatic involvement, lack of steroid hormone receptors, aneuploidy, and high proliferative activity (Cattoretti et al. 1988; Davidoff et al. 1991 c). Despite the rapid accumulation of data on p53 alteration in human tumors, particularly in breast carcinoma, its prognostic role in well-defined series of patients still needs to be assessed.

The current investigation was aimed to evaluate the prevalence of abnormal p53 immunoreactivity in a series of breast carcinomas and to define its relationships with several prognostic variables. Furthermore, we have investigated whether p53 immunoreactivity could be an independent prognostic indicator in node-negative breast cancer patients. Two series of previously charac-


Materials and methods

The study population included two separate series of patients with invasive breast carcinoma treated surgically at the Lahey Clinic Medical Center, Burlington, Massachusetts, USA. The first series comprises 124 patients treated with modified radical mastectomy between 1973 and 1982. All patients had histologically proven negative axillary nodes, were without clinical evidence of metastases and did not receive postoperative adjuvant therapy. Patient survival and clinical status were obtained from clinic records, from contact with the patients' physicians, or both. The patients were followed up for at least 10 years. The second series comprises 40 patients with negative nodes treated between 1988 and 1990. Overall, 31 patients were premenopausal whereas 133 patients were postmenopausal. The prognostic role of p53 expression was evaluated only in the first series of patients.

The original pathological material was reviewed, and conventional histological variables were determined, including tumor size, histological grade, nuclear grade, mitotic rate, and peritumoral lymphatic and blood vessel invasion (Bloom and Richardson 1957; Elston 1987; Lee et al. 1990).

The tumors studied included 148 carcinomas of duct cell type (not otherwise specified), 11 lobular carcinomas, 4 colloid carcinomas and 1 metaplastic carcinoma.

For all tumors, one block was selected for flow cytometry based on abundance of tumor cells and good morphological preservation. Tissues were prepared according to the technique of Hedley et al. (1983). Briefly, 50 μm-thick sections were dewaxed, rehydrated, and mechanically and enzymatically dissociated to yield nuclear suspensions. Nuclei were stained with propidium iodide and counted on a Becton-Dickinson (San Jose, Calif., USA) flow cytometer. At least 10,000 events were measured in each specimen.

One paraffin block was selected for immunocytochemistry in each case, based on good morphological preservation. For the immunolocalization of p53 protein, sections were stained with the monoclonal antibody PAb 1801 (Oncogene Science, Manhasset, N.Y., USA) according to Roncalli et al. (1992). Briefly, dewaxed sections were rehydrated and treated with 0.05% saponin in distilled water for 30 min at room temperature (r.t.) and with 5% normal horse serum for 20 min at r.t. before being subsequently incubated with: (a) PAb1801 mAb diluted 1:4000 in phosphate-buffered saline containing 5% normal horse serum, overnight at 4°C; (b) biotinylated horse anti-mouse immunoglobulin serum (Vector, Burlingame, Calif., USA) diluted 1:200, for 30 min at r.t.; and (c) alkaline phosphatase-labeled streptavidin (Dakopatts, Glostrup, Denmark) diluted 1:100, for 30 min at r.t. Alkaline phosphatase activity was developed with the McGeady reagent (nitro blue tetrazolium and bromo-chloro-indolyl phosphate) containing 1 mM levamisole, for 1 h at r.t. The stained slides were evaluated independently by two of the authors; in the few cases in which the evaluation provided different results, a consensus interpretation was reached after re-examination. Only nuclear staining was considered positive; tumors showing exclusively cytoplasmic staining were regarded as negative. The same authors assessed the percentage of tumor cell nuclei with definite p53 immunoreactivity and the tumors were classified into four groups as follows: negative tumors, 0–1% immunoreactive neoplastic cells; low expressors, 1–10% immunoreactive cells; moderate expressors, 10–50% immunoreactive cells; high expressors, >50% immunoreactive cells.

Statistical differences between variables were analyzed using unpaired t-tests or Wilcoxon rank sum analysis as appropriate. Contingency tables were analyzed with Miettinen's modification of the Fisher exact test. Disease-free and adjusted survival distributions were calculated by the product-limit method of Kaplan and Meier. The statistical significance of differences between distribu-

Results

Overall, of the 164 tumors evaluated, 78 (48%) demonstrated variable levels of p53 immunoreactivity whereas 86 (52%) did not express any immunocytochemically detectable p53 protein. The prevalence of p53-immunoreactive tumors was very similar in the two series of patients. Staining was observed exclusively in the neoplastic tissues (Fig. 1), with the benign breast tissue surrounding the tumors being consistently negative (Fig. 2). In p53-immunoreactive tumors, the intraductal in situ component, when present, was frequently stained, particularly in the “comedo carcinomas” (Fig. 3). Among the 78 p53-immunoreactive tumors, 38 cases (49%) of

[Fig. 1. Invasive breast carcinoma with diffuse staining for p53 protein (high expressor). × 250]

[Fig. 2. Immunoreactivity for p53 is present in neoplastic cells but not in the adjacent benign breast tissue. × 250]