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ORIGINAL ARTICLE

Adverse outcome and resistance to adjuvant antiestrogen therapy in node-positive postmenopausal breast cancer patients—The role of p53

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Summary The prognostic and predictive relevance of p53 immunoreactivity is used here as a tentative approach for defining more accurately the benefit of adjuvant hormonal therapy in postmenopausal node-positive breast cancer patients. Ninety-seven postmenopausal patients with axillary lymph node metastasis were treated with an antiestrogen for a period of 3 years after primary surgery and radiotherapy. The p53 status of the primary tumor was assessed by immunohistochemistry and 24% of the samples showed positive expression of p53. Within the average follow-up time of 59 months, disease recurrence was diagnosed in 34 patients (35%). Multivariate analysis showed high clinical stage, negative estrogen receptor status and p53 positivity to be independent prognostic factors predicting both shortened disease-free survival and worse overall survival. p53 immunoreactivity was associated with worse clinical outcome irrespective of hormone receptor status. The data suggest that adjuvant therapy with antiestrogens is insufficient in this patient population with p53-positive tumors.

Introduction

p53 is a tumor suppressor gene localized to chromosome 17 encoding a 53-kD nuclear phosphoprotein, and it has a principal role in controlling the

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cell cycle. DNA damage increases the level of p53, resulting in cell cycle arrest, DNA repair or apoptosis. p53 induction can also be seen in hypoxia without any known damage to DNA. Somatic p53 mutation is detected in approximately 26% of breast tumors according to the IARC TP53 database and is often associated with large tumor size and positive nodal status,[1] and a poorer clinical outcome.[1,2] The prognostic value of p53 immunopositivity seems to be limited in node-negative breast carcinoma patients.[3,4] However, recent data suggest that p53 accumulation might provide prognostic information in a metastatic setting; p53-positive patients had significantly shorter survival compared with p53-negative patients after pathologic bone fracture.[5]

The predictive value of mutant p53 has been investigated in several studies with conflicting conclusions regarding the possible resistance to hormonal therapy in metastatic breast cancer,[2,6,12] including in the adjuvant setting,[7–9] and also to anthracycline-based chemotherapy.[10,11] As the issue is of high clinical relevance, in order to further evaluate the prognostic and predictive value of p53 immunopositivity we investigate here the clinical outcome of node-positive postmenopausal breast cancer patients treated with an antiestrogen as an adjuvant therapy in relation to p53 status.

Materials and methods

The series is based on 97 node-positive postmenopausal breast cancer patients treated in Northern Finland during the years 1992–1995, belonging initially to a multicenter randomized trial involving the use of 20 mg tamoxifen or 40 mg toremifene daily for 3 years as an adjuvant therapy after primary surgery and radiotherapy. Patients with positive, unknown or negative estrogen and/or progesterone receptors (PRs) in their primary tumors were included in the early stage of the trial. The average age of the patients was 63 years (range 48–83). Postmenopausal status was verified by measuring follicle-stimulating hormone (FSH >30 IU) in patients for whom less than 1 year has elapsed since menopause, or in cases where hysterectomy had been performed before breast cancer was detected. Eighty-six patients underwent mastectomy and axillary evacuation, whereas segmental resection of the breast with axillary dissection was preferred in 11 cases. Patients receiving adjuvant chemotherapy were excluded. Postoperative irradiation covering the ipsilateral axilla, the supraclavicular region and the chest wall on the side of the tumor was given in 90 cases. Seven patients did not receive radiotherapy for personal reasons or on account of retarded wound healing. Patients with a contraindication for the use of antiestrogens or with primarily detected distant metastases were excluded. The average follow-up time was 59 months (range 7–96). The stage of disease was determined according to the TNM classification of tumors issued by the International Union Against Cancer (UICC) and the WHO classification for the characterization of tumor histopathology. Estrogen and PRs were mostly analyzed by radioimmunoassay, but were assessed by immunohistochemistry in a few cases. p53 status was measured from tumor samples available in Oulu University Hospital.

p53 status was defined immunohistochemically. A mouse monoclonal antibody against the anti-p53 antibody (Do7) was purchased from Novocastra Laboratories (Newcastle upon Tyne, UK). The antibody recognizes abnormal p53 accumulation in a cell, while a wild-type p53 protein is not usually detectable in normal cells, as it decays rapidly in normal conditions. The dilution for the primary antibody for p53 was 1:1000. Before application of the primary antibody, the sections were heated in a microwave oven in 10 mM citrate buffer, pH 6.0, for 10 min. After a 60-min incubation with the primary antibody at room temperature, a biotinylated secondary anti-mouse antibody (Dakopatts; Copenhagen, Denmark) was applied (dilution 1:200), followed by the avidin–biotin–peroxidase complex (Dakopatts). Careful rinses with phosphate-buffered saline (PBS) were done between each step of the procedure. For all the immunostainings, the color was developed by diaminobenzidine, whereafter the sections were lightly counterstained with hematoxylin and mounted with Eukitt (Kindler, Freiburg, Germany). Negative control stainings were carried out by substituting non-immune mouse serum and PBS for the primary antibody. Lung cancer tissue samples known to be p53-mutated were used as positive controls.

Only nuclear positivity of tumor cells was considered significant and the results were evaluated from the whole sample as follows; negative = less than 1% of cells staining, weak positivity = 1–10% of cells staining and moderate positivity = 11–50% of cells staining. In strong positive samples more than 50% of cells showed immunoreactivity. In a statistical analysis all positive cases were analyzed together and compared as a single group with negative cases.

The protocol was approved by the Ethical Committee of the Faculty of Medicine, University of Oulu, and Oulu University Hospital.