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Cancer Detection and Prevention 30 (2006) 111–117

Cancer Detection and Prevention

www.elsevier.com/locate/cdp

Study of the topographic distribution of ets-1 protein expression in invasive breast carcinomas in relation to tumor phenotype

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Accepted 9 March 2006

Abstract

Background: Ets-1 is a transcription factor, implicated in the regulation of expression of various genes'. The aim of the present study was to investigate the expression of ets-1 protein in invasive breast carcinomas and its correlation with classic clinicopathological parameters, patients' survival and various biological markers. *Methods*: Immunohistochemistry was performed in paraffin-embedded tissue specimens from 149 invasive breast carcinomas to detect the proteins ets-1, p53, topoisomerase IIα, matrix metalloproteinase-7 (MMP-7) and urokinase-type plasminogen activator receptor (uPAR). Results were subjected to univariate and multivariate statistic analysis. *Results*: Ets-1 protein was detected in the 77.9% of the cases in the cytoplasm, in the 46.3% in the nucleus of the malignant cells, and in stromal fibroblasts as well. Cytoplasmic ets-1 was inversely correlated with nuclear and histologic grade of the tumor ($p = 0.004$ and 0.033, respectively) and topoisomerase IIα ($p = 0.057$), while nuclear ets-1 showed a positive association with p53 ($p = 0.002$). Stromal ets-1 revealed a negative correlation with estrogen receptors (ER) ($p = 0.003$) and a positive one with stromal uPAR and MMP-7 as well ($p = 0.048$ and 0.066, respectively). The univariate statistic analysis showed nuclear ets-1 to be related to a shortened overall survival of the postmenopausal patients ($p = 0.032$). *Conclusions*: Ets-1 seems to be related to a different tumor phenotype according to its topographic distribution, with nuclear localization being associated with decreased apoptotic potential of the malignant cells through its relation to the mutant p53 protein, cytoplasmic being related to a favorable tumor phenotype and stromal ets-1 being related to tumor invasion.

Keywords: Ets-1; Breast cancer; Immunohistochemistry; p53; Topoisomerase IIα; uPAR; MMP-7; Estrogen receptors; Tumor phenotype; Patient survival; Disease-free survival; Menopausal status; Stage; Histologic type; Histologic grade; Nuclear grade; Lymph nodes; Prognosticators

1. Introduction

Ets-1 was originally characterized as a v-ets retroviral gene, one of the two oncogenes (v-myb and v-ets) of the avian transforming retrovirus E26 that induces both erythroblastic and myeloblastic leukemias in chickens [1,2]. Thereafter, many cellular homologues were isolated. So far, approximately 30 members of the family have been identified in mammals that are shown to encode nuclear transcription factors to regulate gene expression. The characteristic feature of this family is that they share a conserved ets domain of about 85 amino acids that mediates binding [3,4]. Binding sites for ets proteins lie within the promoters of several genes encoding proteases such as matrix metalloproteinases-1, -2, -9, -3, -7 (MMP-1, -2, -9, -3, -7) as well as the urokinase-type plasminogen activator (uPA) and its receptor (uPAR). All of these proteases are necessary for matrix degradation that is required during tumor invasion [5–8].

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0361-090X/$30.00
doi:10.1016/j.cdp.2006.03.006

Upregulation of expression of the ets-1 gene has been documented in many types of human tumors [9–13] where it usually correlates with the grade of invasiveness and metastasis [10,14–16].

In breast cancer the overexpression of several ets genes, including ets-1 has been reported [17–20] and has been correlated with various metalloproteinases expression [17–18]. Based on these findings, the purpose of the present study was to elucidate the effect of ets-1 on the biological behavior, including invasion, and the prognosis of breast carcinomas. Therefore, we evaluated the ets-1 protein immunoexpression in association with the well-known clinicopathological parameters, various markers such as estrogen and progesterone receptors (ER/PR), p53, topoisomerase IIα (topoIIα), MMP-7, uPAR and patients' survival.

2. Materials and methods

2.1. Tumors studied

Paraffin-embedded breast tissue specimens were retrieved from the archival material of the Department of Pathology, Medical School, University of Athens. Specimens were obtained from 149 female patients operated for primary infiltrating breast cancer. The age of patients ranged from 25 to 86 years (mean age: 56.3 years). From the patient population, 51 women were promenopausal and 98 were postmenopausal. The menopausal status was defined according to the serum levels of patients' hormones. None of them had received radiation or chemotherapy preoperatively.

Routine histological examination was performed with haematoxylin-eosin staining. Conventional histological classification of the World Health Organization was applied [21]. Histologically, 117 out of 149 tumors were categorized as invasive ductal carcinomas and 32 as invasive lobular. The combined histological grade (1, 2 and 3) of invasive ductal carcinomas was obtained according to Elston [22]. Nuclear grade was based on nuclear pleomorphism. Nuclear grading is the cytologic evaluation of tumor nuclei in comparison with the nuclei of normal mammary epithelial cells. The most widely employed system of nuclear grading is usually reported in terms of three categories: cell differentiated (grade 1) with small nuclei, uniform nuclear chromatin and little variation in nuclear size, intermediate (grade 2) with open vesicular nuclei, visible nucleoli and moderate variability in both nuclear size and shape and poorly differentiated (grade 3) with vesicular nuclei, often prominent nucleoli, exhibiting marked variation in nuclear size and shape [23] Tumor staging was performed according to the TNM system of the International Union against Cancer [24]. Tumor size ($\leq$20 mm, 20–50 mm and $\geq$50 mm) and lymph node status were evaluated separately. According to the number of positive nodes, three groups were formed (0, 1–4 and $\geq$4 positive axillary lymph nodes).

Follow-up was available for 144 patients. The mean observation period was 98.9 months (range 5–135 months).

2.2. Immunohistochemistry

Briefly, 4-μm thick sections were cut, dewaxed, rehydrated and incubated with 0.3% hydrogen peroxide (H_2O_2) for 30 min to block endogenous peroxidase activity. To enhance antigen retrieval, sections were microwave-treated in 0.01 M citrate buffer (pH 6.0) at 750 W for 10 min. After rinsing with 0.01 M Tris-buffered saline (TBS), blocking solution (TBS with 5% non-fat dry milk) was applied for 30 min to block non-specific antibody binding. Subsequently, sections were incubated overnight at 4 °C with a rabbit antibody (C-2D) against the C-terminal peptide of the ets-1 (Santa Cruz Biotechnology, Santa Cruz, CA) at a dilution of 1:500. After additional rinsing in TBS, sections were incubated with biotinylated horse-anti-rabbit secondary antibody (Vector Laboratories, Burlingame, CA) for 30 min, at room temperature and then incubated with avidin-biotinylated peroxidase complex (Vectastain Elite ABC kit, Vector Laboratories) for 30 min. The peroxidase reaction was developed with a 0.5 mg/ml solution of 3,3′-diaminobenzidine tetrahydrochloride (Sigmal Chemical Co, St Louis, MO) supplemented with 0.01% H_2O_2. Finally, sections were counterstained with Harris haematoxylin.

The other immunomarkers assessed in the present study in combination with ets-1 had been previously detected [25,26] with the following antibodies: (1) Anti-ER clone 1D5 and anti-PR clone 1A6 (DAKO, Glostrup, Denmark) at dilutions 1:450 and 1:150, respectively. (2) Anti-topoIIα clone JH 2.7 (Biocare Medical, Walnut Creek, CA) at a dilution 1:100 and (3) Anti-p53 clone BP 53.12.1 (Oncogene, Cambridge, MA) at a dilution 1:50. (4) Anti-MMP-7, clone 1D2 (Neomarkers, Fremont, CA, USA) at a dilution 1:130 and (5) Anti-uPAR (catalogue No. 3932) (American Diagnostica Inc) at a dilution 1:100.

2.3. Evaluation of immunostaining

A semiquantitative estimation based on the staining intensity and the percentage of positive cells was performed by two independent pathologists, the same who performed the histological examination according to the same standard criteria. Ets-1 was detected in the cytoplasm and the nucleus of the malignant cells, stromal fibroblasts and the endothelium of the vessels. Cytoplasmic and nuclear ets-1 was scored on a scale of 0–3: 0 = no staining or weak, in less than 10% of tumor cells, 1 = weak to moderate staining in 10–20% of cells, 2 = moderate staining in 21–40% of cells, and 3 = strong staining in more than 40% of tumor cells. The fraction of positively stained tumor cells was scored by cell counts after having examined at least 10, randomly selected high-power fields (×400) in one section from each case. Positive controls included sections from carcinoma with known immunopositivity. Negative controls had the primary