Brief communication

E1AF expression levels are not associated with prognosis in human breast cancer

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Summary

E1AF is a transcription factor involved in regulation of several metastasis-associated genes, and is associated with overexpression of HER2/neu. We were unable to find a clear prognostic value of E1AF expression in human breast cancer. Furthermore, no association of E1AF levels with HER2/neu mRNA levels, hormone receptor status, histological grade, tumor size, or lymph node involvement was found.

Introduction

E1AF (ets-variant 4 or ETV4), a member of the PEA3 group of Ets transcription factors, is involved in the transcriptional regulation of several metastasis-associated genes involved in extracellular matrix degradation and adhesion [1, 2]. Transfection of non-invasive breast cancer cells with E1AF induces an invasive phenotype accompanied by MMP-9 expression [1]. Furthermore, E1AF expression is associated with overexpression of the HER2/neu oncogene in human breast cancer [3], and transfection with a dominant negative E1AF transgene delays mammary oncogenesis in MMTV-neu transgenic mice [4], suggesting an association of E1AF with HER2/neu induced tumorigenesis. Thus, E1AF could be a strong marker of poor prognosis in human breast cancer. On the other hand, recent reports suggest that E1AF suppresses HER2/neu expression, inhibiting breast cancer tumorigenesis and metastasis [5]. E1AF administration is now even considered as a therapeutic agent in breast cancer [6]. Considering these findings we felt it was relevant to assess the prognostic value of E1AF expression levels in breast cancer patients.

Patients, material and methods

Clinical data from 121 patients with unilateral, invasive, operable breast cancer who underwent resection of their primary tumor between October 1989 and December 1995 were retrospectively collected. Patients, with a median age of 57 years (range 31–88 years), had no previous diagnosis of carcinoma, no distant metastases at time of diagnosis, and no evidence of disease within 1 month after primary surgery. Furthermore, patients receiving neo-adjuvant therapy were excluded. Patients underwent modified radical mastectomy (n = 96) or a breast saving procedure (n = 25) followed by radiotherapy in 79 patients. A resection was considered complete when there were no tumor cells in the inked border of the histological section. In case the margin was not free, a re-resection or breast ablation was performed whenever possible or additional radiotherapy was given. Lymph-node involvement was recognized in 67 patients. Subsequent systemic adjuvant therapy (31 endocrine therapy, 9 chemotherapy, 13 both) was given based on established clinical evaluation. Patients were seen (history, physical examination,
Hazard ratios were calculated for RFS at different cut-off levels (straight line with 95% CI). These did not differ significantly ($P$-values = dotted lines) from 1, indicating that E1AF expression has no prognostic value for RFS in breast cancer. The arrow indicates the median value of E1AF expression.

routine laboratory investigations) once every 3 months during the first 2 years, once every 6 months for 5 years and once a year after that. Once a year an X-ray mammography, and in case of suspicion also a mamma-MRI, was made. The median follow-up time was 63 months (range 1.2–164 months). During follow-up, 43 patients had a recurrence (4 local, 2 regional and 37 distant metastases) and 32 patients died (27 confirmed breast cancer-related, 5 unknown). Contralateral breast cancer or second malignancies were not considered as recurrent disease.

Quantitative RT-PCR was carried out using Taqman (E1AF) or Sybr Green (HER2/neu) Universal PCR master mix (PE Applied Biosystems) with 500 nM of each primer and 100 nM of probe in a final volume of 25 µl. The E1AF forward primer was 5’-gac ttc gcc tac gac tca gat gt-3’, the reverse primer 5’-age cca tgg ccc cg-3’, and the probe was 5’-FAM-ccg ggt gcg cat caa tgt acc tc-TAMRA-3’. The HER2/neu forward primer was 5’-ctg gtg aca cag ctt atg ccc t-3’ and the reverse primer was 5’-atc ccc tgg gca atc tgc a-3’. β-Actin was amplified using the Pre-Developed Assay Reagents Taqman RT-PCR assay from Perkin-Elmer (PE Applied Biosystems). Amplifications, with denaturation at 95°C for 10 min, and 40 cycles of 15 s at 95°C (melting) and 60 s at 58°C (annealing and elongation), were performed on a ABI-Prism 7700 sequence detection system (PE Applied Biosystems).

Identity and purity of the E1AF PCR products were confirmed by sequencing of column purified PCR products from eight breast tumor samples on an ABI-Prism 3700 DNA analyzer (PE Applied Biosystems).

Statistical analyses were carried out using SPSS 10.0.5 software (SPSS Benelux BV, Gorinchem, The Netherlands). Relapse-free survival (RFS) time (defined as the time from primary surgery until the diagnosis of recurrent disease) and overall survival (OS) time (defined as the time between date of primary surgery and death by any cause) were used as follow-up parameters. Equality of survival distributions was tested using log-rank testing. Hazard ratios with 95% confidence intervals (95% CI) were obtained by Cox regression analysis. Cases with >72 months of follow-up were censored at 72 months, because of the rapidly declining number of patients thereafter.

Results

E1AF expression levels (E1AF/β-actin ratios) ranged from undetectable in three tumors to 1.6, with a median of 0.02. No association was found between