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Prognostic implication of CDC25A and cyclin E expression on primary breast cancer patients

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Abstract

Defect in cell cycle control is a hallmark character of cancer. We have investigated the association of Ki67 labeling index, cyclin E and CDC25A expressions with clinical follow-up data in breast carcinomas. Flow cytometry was used to detect gene amplification of cyclins in 44 tumor tissue with invasive breast carcinomas. Multivariate Cox proportional hazard ratio test was used to show the correlations. Cyclin E or CDC25A were upregulated in 34% of the tumors. Among the whole total material, expression of cyclin E and of CDC25A were found upregulated in 31.9% and 39.4% of cells, respectively. Both CDC25A and cyclin E protein expression levels were correlated with Ki67 expression level ($p < 0.001$). In addition, the expression of CDC25A was associated significantly with poor survival ($P = 0.028$), whereas no correlation was found with cyclin E. These findings suggest a possible prognostic value for CDC25A as a cell cycle marker and may imply in characteristic of high risk breast cancer patients.

Keywords: Breast cancer; Ki 67 labeling index; Cyclin E; CDC25A; Survival

1. Introduction

Cell cycle checkpoints are critical episodes in controlling cell proliferation paradigm (Tandis et al., 1998; Qu et al., 2003). Indeed, cell cycle is regulated by the multiple actions of cyclins, cyclin-dependent kinase (CDK) and CDK inhibitors (Bortner and Rosenberg, 1997). Many tumors, such as colon, breast and gastric carcinomas appear to deregulate or amplify cyclin expression, especially cyclin E. This crucially exerts its action during the transition of G1 into S phase of the cell cycle. Cyclin E activity is also needed for the initiation of DNA replication and regulating genes essential for proliferation and progression through S phase (Ohtsubo et al., 1995; Ekholm-Reed et al., 2004; Kawamura et al., 2004; Berglund and Landberg, 2006). Amplification of the cyclin E gene in human breast carcinoma probably plays an important role in tumorigenesis and progression of the carcinoma (Han et al., 2003).

Cell division cycle 25A phosphatase (CDC25A) acts by dephosphorylating threonine 14, tyrosine 15(or both) on CDKs, and activating cyclin/CDK complexes to stimulate cell proliferation (Galaktionov et al., 1995). Previous study suggests that the expression of CDC25A is a frequent event in primary breast tumors (Wu et al., 1998), due to its oncogenic peculiarities. Cyclin E has been implicated as an activator of CDC25A phosphatase, which could account for the greater activation of overexpressed CDC25A (Sandhu et al., 2000). Recent studies have suggested that higher levels of CDC25A and cyclin E expression are associated with poor characteristics of human neoplasms, such as breast carcinoma. However,

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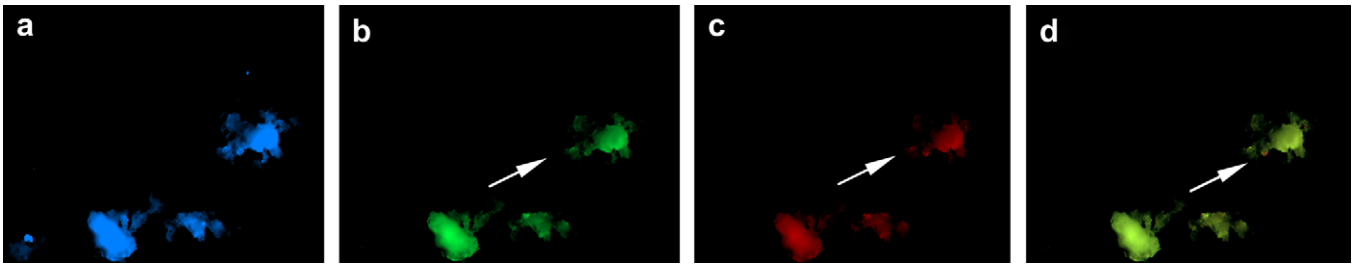

Fig. 1. Expression and co-expression of Ki67 and CDC25A proteins by immunofluorescence. b–d show expression of three investigated proteins at cellular level. a, tumor breast cells with DAPI filter, b, expression of Ki67 (isotype IgG1/conjugated with FITC), c, expression of CDC25A, isotype IgG2a/conjugated with Pe-Cy5, d, co-expression of Ki67 (FITC) and CDC25A (Pe-Cy5). Arrows typically show different range of expressions in a group of cells.

the data were not obtained by flow cytometry and immunofluorescence (Nielsen et al., 1996; Cangi et al., 2000; Lindahl et al., 2004; Husdal et al., 2006; Bonin et al., 2006). Despite a few reports on cyclin E and CDC25A, their intercorrelation with Ki67 expression as the proliferative activity of carcinoma cell has not being denoted (Han et al., 2003; Konigsberg et al., 2008). Hence, we explored the possible relation between its expression status and important clinical outcomes in breast cancer patients. The investigations looked at protein expression status of cyclin E and CDC25A in breast carcinoma, and a potential association between the expression status of either cyclin E or CDC25A and clinical outcomes.

2. Material and methods

2.1. Study subjects

A total of 60 Iranian patients affected with primary breast cancer, diagnosed between 1993 and 2006, were recruited. Modified radical mastectomy (MRM) was performed in Day hospital, Tehran, Iran. Among this population, 44 patients were eligible for flow cytometric assay because of available complementary information. After confirmation by frozen section, fresh tissue samples were promptly delivered to the laboratory. No patients had a previous malignancy. The demographic records of probands through generations1–4 were obtained by genetic counsellors in order to identify the pedigrees of those affected.

Positive axillary lymph node metastasis (ALNM) was histologically confirmed in 34/60 (56.6%) patients. The ratio of involved ALNM with >20 was detected in 20 (35.1%) patients and 14 (24.6%) patients had ALNM ratio <20. Histological grade (HG) III differentiated carcinoma was determined in 27 (61.47%) patients, while grades II and I consisted of 11 (25%) and 6 (13.6%) cases. In the course of the patients' clinical follow-up, information on family history (FH), age, gender, laterality, metastasis, recurrency and deceasing was upgraded by surgeons and oncologist. All the participants had with primary breast cancer and none had received preoperative therapy. However, they received almost identical postoperative chemotherapy regimens.

2.2. Flow cytometry

2.2.1. Tissue and cell preparation

Fresh breast tumor samples were incubated in mixture of MaCoy RPMI culture media, PH 7.2, (Cat#78-5192, Grand island biological company- USA) and 500 μl trypsin 0.01x (Sigma–Aldrich, St Louis, MO, USA) for 30 min at 37 °C. To inhibit trypsin activity in the reaction mixture, they were washed twice in KCl 0.075 M. Methanol (Merck, Germany) and acetic acid (Merck – Germany) at 6:1 was used to fix cells. The cells were pelleted, resuspended in fresh solution and stored as cell suspensions at −20 °C. The mean numbers of cells per tumor specimen were in the range 446 ± 387.

2.2.2. Staining

Cells were stained by monoclonal antibodies as follows: using monoclonal anti-CDC25A, isotype IgG2a (clone DCS121purified mouse immunoglobulin, Sigma, USA) was added to cells and incubated at 4 °C for 25 min. Cells were washed twice by PBS to remove unbound antibodies. Conjugated, secondary antibody goat anti-mouse IgG2a/PE-Cy5 (0.5 ml, 1:4; GE/Amersham Biosciences) was used. Cells were

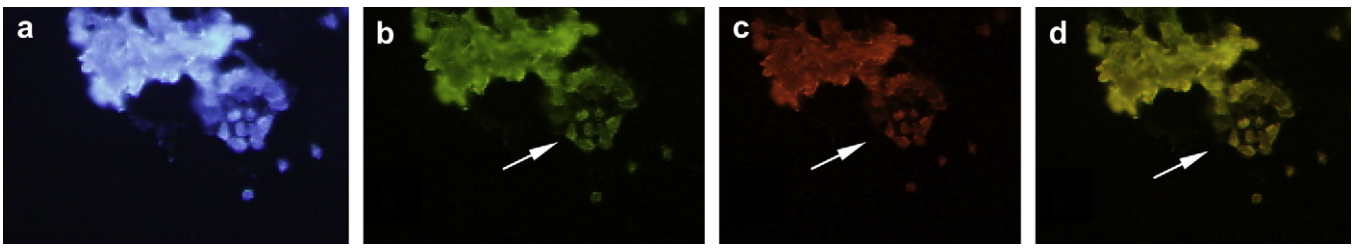

Fig. 2. Expression and co-expression of Ki67 and Cyclin E proteins by immunofluorescence. b–d show expression of three investigated proteins at cellular level. a, tumor breast cells with DAPI filter, b, expression of Ki67, isotype IgG1/conjugated with FITC, c, expression of cyclin E, isotype IgG2b/conjugated with R-PE, d, co-expression of Ki67 (isotype IgG1/FITC) and cyclin E (R-PE). Arrows typically show different range of expressions in a group of cells.