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ORIGINAL ARTICLE

CD24 expression in ductal carcinoma in situ and invasive ductal carcinoma of breast: An immunohistochemistry-based pilot study

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Abstract

CD24 is a small, heavily glycosylated cell surface protein, that is expressed in a large variety of solid tumors. It is considered to play an important role in tumor progression and metastasis. We aimed to evaluate CD24 expression in invasive ductal carcinomas (IDCa), ductal carcinoma *in situ* (DCIS) and non-tumorous breast tissues, and to investigate the relationship between histopathological parameters, estrogen and progesterone receptors, and c-erbB2 expressions. The study included 34 IDCa, 25 DCIS, and 13 non-tumorous breast tissues. All cases were reevaluated histopathologically, and immunohistochemistry was performed with monoclonal CD24 antibody. The results clearly demonstrated that CD24 expression, including membranous and cytoplasmic staining, was significantly higher in DCIS and IDCa than in the non-tumorous breast ($p = 0.001$, $p = 0.000$, and $p = 0.035$, $p = 0.000$, respectively). Cytoplasmic staining was detected predominantly in neoplastic tissues and was significantly increased in high grade DCIS ($p = 0.013$). In invasive carcinomas, although the level of membranous staining was significantly positively correlated with tumor grade ($p = 0.040$), there was no such an association with the cytoplasmic level. However, it showed a trend towards pT ($p = 0.089$). In conclusion, our results suggest that higher CD24 expression may be associated with malignant transformation and progression in breast cancer biology. Furthermore, higher membranous expression and, in particular, cytoplasmic staining seem to predict malignant transformation, and different patterns of CD24 expression may be associated with different pathological features in breast tumors.

Keywords: CD24; Invasive ductal carcinoma; Ductal carcinoma in situ; Breast

Introduction

CD24 is a small cell surface protein that contains a heavily glycosylated protein core comprising 27 amino acids, and is attached to cell membrane by a phosphatidylinositol anchor [18,21]. Some studies have identified CD24 as an alternative ligand for P-selectin [2,3,18]. Functionally, it is considered to play a critical role in the metastasis of tumor cells through P-selectin [18,21]. Many tumor cell lines can bind to platelets via P-selectin, and CD24 expression might enhance the metastatic potential of tumor cells [2,3,18]. The ability of tumor cells to bind to platelets in the blood is considered important, because stabilized platelets-tumor thrombi can protect tumor cells from destruction and can promote tumor extravasation and tissue penetration [2,18]. The major ligand of P-selectin is a cell surface mucin P-selectin glycoprotein ligand-1 (PSGL-1), and certain PSGL-1-negative tumor cell lines can bind P-selectin through surface mucin CD24. CD24 can be substituted for PSGL-1 despite the absence of PSGL-1,

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and it has been found to contribute not only to the rolling of tumor cells where P-selectin exists but also to the attachment of tumor cells to activated platelet [2,3].

It has originally been described as a B marker, and its expression has been reported in the developing brain and the pancreas, regenerating muscles, keratinocytes, renal tubules, and various adult rat tissues, such as the gastrointestinal tract, the respiratory system, the salivary gland, and the prostate [4,16,21]. CD24 expression has been found in B cell lineage neoplasia, such as acute lymphoblastic leukemia (ALL) [10,22], in chronic lymphocytic leukemia (CLL) [23], and in a variety of non-Hodgkin lymphomas [1,14]. The reduction or absence of CD24 expression on ALL cells predicts a better prognosis [20]. Recently, several studies have investigated CD24 expression in various epithelial tumors such as lung cancer, gallbladder carcinoma, hepatocellular carcinoma, gastrointestinal carcinoma, choriocarcinoma, nasopharyngeal carcinoma, cholangiocarcinoma, glioma, prostatic cancers, pancreatic cancer, renal cell carcinoma, ovarian and breast cancers [4,7,8,12,15,17–19,21,25,28]. These studies showed that higher CD24 expression was significantly associated with shorter patient survival and/or a significant prognostic marker especially in non-small cell lung cancer, in intrahepatic cholangiocarcinoma, and in epithelial ovarian and breast cancers [7,15,17,18,21,28]. CD24 positivity was significantly related to younger patient age, higher pT stages, and higher PSA relapse rate in prostatic adenocarcinomas [19]. Its expression was significantly correlated with higher tumor grade in pancreatic cancer [18]. In hepatocellular carcinomas, CD24 expression was also correlated with serum levels of HBs-Ag, elevated serum AFP levels, and p53 mutations [12]. Droz et al. [8] noted strong CD24 staining in renal cell carcinoma irrespective of the histological tumor type. Lim and Oh [21] showed that the positivity of CD24 expression was increased with positive nodal status in gastric and colonic adenocarcinomas.

The studies investigating CD24 expression in breast are limited in number, and these have usually been performed in breast cancer lines [2,3,5,11,16,21,24,29]. Fogel et al. [11] were the first to report CD24 expression in breast cancer tissue using immunohistochemistry on frozen tissue, and they proposed that CD24 might be a useful marker for human breast cancer. Kristiansen et al. [16] described CD24 expression as a prognostic marker of disease-free survival and overall survival. However, these studies have usually investigated invasive breast carcinomas; non-invasive breast tumors are limited in number. Therefore, we aimed to investigate CD24 in non-tumorous breast, ductal carcinoma *in situ* (DCIS), and invasive ductal carcinomas (IDCa) in an immunohistochemistry-based pilot study, and to identify its possible role during malignant transformation. In addition, we planned to examine the relationship between CD24 expression and histopathological parameters, estrogen receptor (ER), progesterone receptor (PR), and c-erbB2 in invasive carcinomas.

Materials and methods

This study included 34 cases of IDCa and 25 cases of DCIS, involving 9 pure DCIS and 16 cases found around the invasive component. Thirteen cases of non-tumorous breast tissue were also used as a control group. All cases were randomly selected from archival material of the Department of Pathology, including excisional biopsies or mastectomies. All hematoxylin/eosin (HE)-stained sections were re-evaluated histopathologically. IDCa were graded according to the modified Nottingham classification of Bloom and Richardson [9], and the cases of DCIS were also graded as 1, 2, and 3 according to the Van Nuys classification system [26]. The pathological stage (pT) of invasive carcinomas was assessed according to the American Joint Committee on Cancer [6]. Data regarding ER, PR, and c-erbB2 status were taken from the archival reports.

Immunohistochemistry was performed on selected slides using the streptavidin-biotin-peroxidase technique for mouse monoclonal antibody CD24 (Neomarkers, Clone 24C02, 1:100 dilution, Fremont, CA, USA). The sections with a thickness of 4 μm were cut, deparaffinized in xylene, and dehydrated in descending dilutions of ethanol. Labvision otostainer 360 was used for immunohistochemistry. Background staining was minimized by incubation with goat serum (UltraVision HRP kit, LabVision, USA). Sections were incubated with a primary antibody followed by testing with a streptavidin-biotin-peroxidase kit (UltraVision Large Volume Detection System Anti-Polyvalent, HRP, LabVision, USA). The sections were visualized with diaminobenzydine (DAB). Finally, they were counterstained with Mayer's hematoxylin and mounted with mounting medium. Tonsil tissue was used as a positive control. Negative cases had to definitely show no CD24 immunoreactivity in any part of the tissue. All of the other cases with weak but unequivocal staining were defined as positive. The pattern of CD24 expression was separately evaluated as membranous (apical or circumferential) or cytoplasmic staining pattern in the tumoral cells and non-tumorous breast tissue. The level of immunopositivity was scored semiquantitatively according to the following criteria: 1+, minimal (<10%) positivity of cells; 2+, moderate (10–50%) positivity of cells; and 3+, diffuse/marked (>50%) positivity of cells.

Statistical analysis was planned according to overall staining and different CD24 immunostaining patterns.