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Immunohistochemical Expression of Stem Cell Marker CD24 In Benign and Malignant Breast Lesions Of Iraqi Women

KEYWORDS: CD24, Breast cancer, Immunohistochemistry

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ABSTRACT *CD24 is a small cell surface protein molecule anchored by glycosyl-phosphotidyl-inositol in a wide variety of cancer cell.CD24 is involved in cell adhesion and metastasis. We aimed to evaluate CD24 expression in Fibroadenoma breast lesion and Invasive breast ductal carcinoma and to investigate the relationship between CD24 expression and clinicopathological parameters. The study included 72 Invasive ductal carcinoma (IDC) and 34 Fibroadenoma breast lesion (FA). All cases were reevaluated histopathologically and immunohistochemically was performed with monoclonal CD24 antibody. The results demonstrated that CD24 expression was significantly higher in IDC than FA breast lesion. CD24 staining was detected predominantly in IDC and was significantly increased in high grade IDC. In conclusion, our results suggest that higher CD24 expression may be associated with malignant transformation and progression in breast cancer biology.*

INTRODUCTION

Breast cancer is the most common malignancy affecting women with more than one million cases occurring worldwide annually {1}. Affluent societies carry the greatest risk, with incidence rates of greater than 80 per 100,000 population per year {2}. In Iraq, cancer of the breast is the commonest cancer in females, in 2010 constituted 19.15% of all other malignancy in women. Cancer stem cells (CSCs) can be defined as a population of cells present in tumors, which can undergo self renewal and differentiation. Similar to normal stem cell, CSCs can also give rise to all cancer cells in a tumor and hence termed cancer stem cells {3}. CD24 is a small, heavily glycosylated mucin-like glycosylphosphatidyl-inositol-linked cell surface protein that is expressed in a wide variety of human malignancies, e.g. B-cell , lymphoma renal cell carcinoma, small cell and Non-small cell lung carcinoma, Nasopharyngeal carcinoma, hepatocellular carcinoma, bladder carcinoma, glioma, epithelial ovarian cancer and breast cancer {4,5}. Functionally, CD24 expression might enhance the metastatic potential of tumor cells, because CD24 has been identified as alternative ligand of P-selectin, and adhesion receptor on activated endothelial cells and platelets {6,7}.

MATERIALS AND METHODES

Patients

Our study included 106 patients (72 of Invasive ductal carcinoma –Not otherwise specified- and 34 Fibroadenoma breast lesion) who were diagnosed at the institute of pathology, Baghdad Teaching Hospital, between 2010 and 2014.

Immunohistochemistry staining

Expression of CD24 was analyzed using mouse monoclonal CD24 antibody (abcam, Cambridge, UK) by immunohistochemistry method as described previously {8}. Briefly, 4 µm formalin-fixed paraffin embedded tissue sections were deparaffinised with xylene and then rehydrated in descending concentrations of alcohol. Endogenous peroxidase activity was blocked. The tissues were treated with antigen retrieval by autoclaving for 10 minutes. The slides were then incubated with CD24 antibody (1:50) for 45 min at room temperature. After washing. The staining was visualized using Expose mouse and rabbit specific HRP/DAB(abcam, Cambridge, UK) and haematoxylin. Finally, all sections dehydrated in alcohol, cleared in xylene and mounted for examination. Tonsil tissue was used as positive control to confirm the specificity of staining and negative control consisted of replacement of the primary antibody by PBS.

Evaluation of the Immunohistochemical staining

Semi-quantitative scoring system which relies on the subjective assessment of multiple independent observers was used in this study. The staining of the tissue sections was evaluated by two investigators on two separate occasions after the series were examined on a double-headed microscope blinded to patient's outcome and other clinical findings. The controversy cases were reviewed by the third investigator to achieve a final consensus.CD24 was detecting mainly in the cytoplasm and the scoring was as follows : 0,0% positive cells; 1,1% to 10% positive cells; 2,11% to 50% positive cells; 3, 51% to 75 positive cells; and 4, 76% to 100% positive cells as previously described by Gabriella et al {9}.

Statistical Analysis

All the data of the present study were compiled into a computerized data files, then the frequency and statistical description were calculated using the SPSS software version 16 (Chicago, IL). To analyze statistically significant relationships between the distributions of the categorical value, two-tailed t-test was used to test the variable means between subgroups. Whereas chi square distribution test and ANOVA test, were used when proportion are compared all at (P) values of < 0.05, were considered statistically significant.

RESUTLS

Study of population

Of 106 breast lesion sample which included in the present study, 72 (67.9%) of cases were invasive ductal carcinoma (IDC) (Not otherwise specified) and 34 (32.1%) of cases were fibroadenoma breast lesion. IDC were graded according to modified Bloom-Richardson grading system, 29 (40.3%)

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were grade 3, 31 (43.1%) were grade 2, and only 12 (16.6%) cases were grade 1. Tumor size (for IDC) was categorized in three groups based on TNM classification of breast human cancer: group one tumors were 2.0cm or less in the greatest dimension (T1) comprising 6 (8.3%) tumors, group 2 tumors were more than 2.0cm up to 5cm in the largest dimension (T2) including 41 (56.9%) tumors and group 3 tumors were more than 5cm in greatest dimension (T3) comprising 25 (38.8%) tumors. Of the patients with known lymph node status, 47 (65.3%) tumors were lymph node positive, whereas 25 (34.7%) tumors were node negative.

patients of IDC ranged in age from 28 to 69 years old (mean of 48.5 years), 9 (12.5%) patients were younger than 35 year, 47 (65.3%) patients were 35-50 year of age and 16 (22.2%) patients were over 50 year of age. CD24 expression and clinicopathological parameters are summarized in table 1.

Table 1 Association between Cytoplasmic expression of CD24 and Clinicopathological parameters of breast carcinoma.

Characteristic	All cases	CD24 Positive	CD24 Negative	P Value
All carcinomas	72(100%)	34(47.3%)	38(52.7%)	
Age at surgery (yr.)				0.321
<35	9(100%)	3(33.3%)	6(66.7%)	
35 – 50	47(100%)	27(57.4%)	20(42.6%)	
>50	16(100%)	8(50%)	8(50%)	
Histological grade				0.031
I	12(100%)	5(41.7%)	7(58.3%)	
II	31(100%)	19(61.3%)	12(38.7%)	
III	29(100%)	14(48.3%)	15(51.7%)	
Tumor size (cm)				0.391
<2 cm	6(100.%)	1(16.7%)	5(83.3%)	
2 – 5 cm	41(100.%)	26(63.4%)	15(36.6%)	
>5 cm	25(100%)	11(44%)	14(56%)	
Lymph node status				0.251
Positive LN	47(100%)	31(65.9%)	16(34.1%)	
Negative LN	25(100%)	7(28%)	18(72%)	

Expression of CD24 in breast lesions

Expression of CD24 in breast lesions was determined by using Immunohistochemistry. Tonsil biopsy tissue which used as positive control shown strongand uniform staining of CD24. For Fibroadenoma breast lesions, 21 out of 34 breast FA (61.8%) were 0 (no staining), whereas 7 (20.6%) cases out of 34 cases were +1 (1% to 10% positive cells), 4 (11.8%) were +2(11% to 50% positive cells), 2 (5.8%) cases out of 34 Fibroadenoma cases were +3 (51% to 75% positive cells) and zero cases for +4 (>75%), figure 1 shows CD24 expression in fibroadenoma. Considering the IDC cases, 38 (52.7%) cases out of 72 were 0 (no staining), 14 (19.4%) cases of IDC were +1, 11 (15.3%) cases were +2, 7 (9.8%) cases were +3 and 2 (2.8%) cases of IDC were score +4 staining, figure 2 shows CD24 expression in Invasive breast carcinoma.

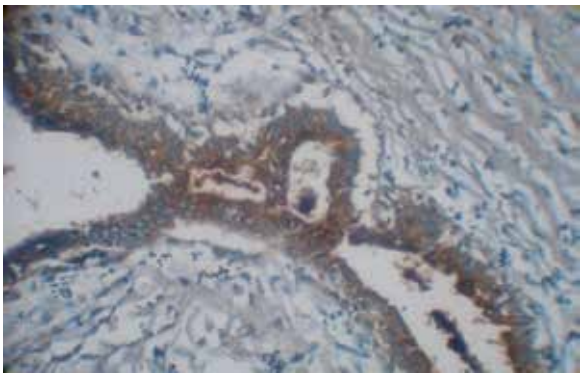

Figure 1 Immunohistochemical Expression of CD24 in Fibroadenoma, score +2.

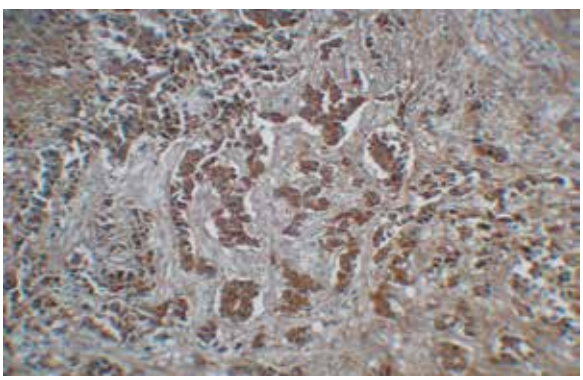

Figure 2 Immunohistochemical Expression of CD24 in Invasive breast carcinoma, Score +4.

Relationship of CD24 expression with Clinicopathological parameters

The results clearly demonstrated that CD24 expression was significantly higher in IDC than Fibroadenoma breast lesion (P=0.019). For IDC, we did not find any significant association of CD24 expression with age of patient, tumor size and lymph node status, only a grade of tumor (P=0.031) was linked to CD24 positivity.

DISCUSSION

CD24 is highly expressed in ovarian, breast, prostate, bladder, renal, no small cell carcinoma and other human cancers {10,11}. In this immunohistochemistry – based study we describe the expression of CD24 protein in breast cancer. Basically, we can confirm the findings of Fogel *et al* {12}, who were the first to describe CD24 expression of breast cancer in an immunohistochemistry study based on frozen sections.

The reported a CD24 immuno-reactivity in benign ducts and an additional cytoplasmic marker as a marker of breast cancer. We used a commercially a viable monoclonal CD24 antibody that Is applicable to paraffinized tumor tissue. The aim of this study was to investigate the expression of stem cell marker CD24 in benign and malignant breast lesions and correlated with Clinicopathological factors in a collection of unselected breast tumor samples from an Iraqi population.

As we see the expression of CD24 was highly significant (P=0.019) in IDC than Fibroadenoma breast lesions. In a series of 72 breast carcinomas, we were demonstrate the significant correlation between CD24 cytoplasmic expres-

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sion and histological grade and this is reflected the association of higher CD24 expression and the malignancy transformation and aggressiveness of breast cancer biology, and this finding is similar to other findings of Fogel *et al* and Bircan S *et al* {10,13} on the other hand we were unable to verify correlation between CD24 expression and lymph node status, tumor size and age of the patients and this is agree also with G. Kristiansen *et al*{10}.

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