Immunohistochemical Evaluation of Apoptotic Marker PDCD4 in Malignant and Benign Breast Tissues

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ABSTRACT
Breast cancer is one of the most common cancers in Egypt, early diagnosis and treatment save the life of many patients. In this study we aimed to investigate the PDCD4 expression in breast cancer and its role in disease progression. Fifty breast tissue samples were collected from patients who underwent surgical resection; 25 invasive breast carcinoma and 25 benign noncancerous lesions (20 fibroadenomas, 5 fibrocystic diseases). Immunohistochemical analysis for PDCD4 in tissue samples was done with the anti-PDCD4 antibody. The immunohistochemical evaluation showed moderate and strong positive staining in breast cancer patients with grade II invasive duct carcinoma while invasive duct carcinoma grade III and IV patients showed strong positive staining. Patients with fibrocystic disease showed equal staining levels or mild staining. In conclusion; our results showed that PDCD4 could be used as diagnostic and prognostic marker for this disease.

Keywords: Breast cancer, Immunohistochemical analysis, PDCD4.

INTRODUCTION
Breast cancer is the most common cancer in Egypt, recommended screening guidelines and follow-up of abnormal results of mammography; prompt diagnosis, also offering optimal treatment is important to improve breast cancer outcomes. It is also critical that patients at high risk of breast cancer are identified and given high quality follow-up and also early treatment is crucial for them. Cell death is controlled by process of apoptosis that plays a crucial role in normal development of multicellular organisms. Apoptosis abnormalities leads to multiple diseases including cancer. Tumor formation include several steps of genetic transformations which is divided into initiation, promotion and progression. Also, inactivation of tumor suppressors and activation of protooncogenes are crucial events in malignant cell development and progression. Programmed cell death 4 controls cell cycle proliferation and progression; it encodes a protein with tumor suppressor functions that inhibit tumor promoter-induced neoplastic transformation, PDCD4 dysregulation occurs through different mechanism in case of cancer formation. PDCD4 is a tumor suppressor highly conserved protein composed of 469 amino acids, it inhibits both cancer promotion and cancer progression. Suppression of tumorigenesis by PDCD4 occurs through binding to eIF4A, thus inhibiting translation of a set of mRNAs that require eIF4A activity, thus studying the role of PDCD4 as a prognostic marker and its use as target for antineoplastic therapy is of great interest. The aim of this work was to investigate the expression of PDCD4 in human breast carcinoma and its role in disease progression.

Patients and methods
The study was conducted on 50 female patients who underwent surgical resection for the treatment of breast neoplasm from the National Cancer Institute, Cairo University, Egypt. Their age ranged from 27-62 years, twenty five patients had invasive duct carcinoma; by histologic grading: 6 cases with grade II, 10 cases with grade III and 9 cases grade IV. Clinical staging was expressed according to the TNM classification system based on evaluation of findings of physical examination, routine laboratory tests, radiological reports (chest X-ray, liver echography, bone scan and computed tomography) and pathological records, the other twenty five females had benign noncancerous lesions (20 fibroadenomas, 5 fibrocystic diseases) their age ranged from 31-60 years. Informed consents were obtained from all study participants and the study was approved by the local ethics committee.

Sampling
Serum samples and breast tissue samples were collected from female patients

Methods
-Immunohistochemical analysis for PDCD4 in tissue samples; Paraffin sections (5um) were stained with anti-PDCD4 antibody by incubating overnight at 4°C. Secondary staining with biotin-conjugated anti-rabbit IgG and tertiary staining with HRP-conjugated streptavidin, were performed by an ABC kit (VECTASTAIN, Vector

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Figure 1: Strong +3 positive PDCD 4 immunohistochemical cytoplasmic reaction on paraffin embedded breast cancer tissue, invasive duct carcinoma grade III (X 100).

Figure 2: Moderate +2 positive PDCD 4 immunohistochemical cytoplasmic reaction on paraffin embedded breast cancer tissue invasive duct carcinoma grade II (X 200).

Figure 3: Strong +3 positive PDCD 4 immunohistochemical cytoplasmic reaction on paraffin embedded breast cancer tissue, invasive duct carcinoma grade IV (X 400).
Figure 4: Strong +3 positive PDCD 4 immunohistochemical cytoplasmic reaction on paraffin embedded breast cancer tissue, invasive duct carcinoma grade III (X 400).

Figure 5: Positive PDCD 4 immunohistochemical cytoplasmic reaction on paraffin embedded breast cancer tissue, normal control, fibrocystic disease, duct carcinoma and invasive duct carcinoma (X 100).

Figure 6: Different positive PDCD 4 immunohistochemical cytoplasmic reaction on paraffin embedded breast cancer tissue, normal control, fibroadenoma, and duct carcinoma (X100).
or invasive duct breast cancer cells of carcinoma tissues in
progression and development and showed equal staining levels or mild staining.

Table 2 showed invasive duct carcinoma grade II while patient
positive staining in breast cancer patients with invasive
immunohistochemical evaluation with PDCD4 antibody (figures 1,2,3,4) with
positive area as compared with negative controls stained
as negative internal control, but more or less contained
comparison to adjacent noncancerous normal breast tissue.
expressed in breast cancer cells, and the protein amount was significantly
PDCD4 protein was localized in the cytoplasm of breast
cancer cells, and the protein expression was negative in benign tissues in comparison to
the corresponding cancerous breast cells. The results revealed that the level of PDCD4 expression was
positively and directly correlated to the differentiation of higher grades of malignant invasive duct cells. These
results were in agreement with Goke et al who stated that
overexpression of programmed cell death 4 resulted in
inhibition of cancer cell progression and proliferation; so, increased expression is an attempt of body immunity to
decrease tumor size and attack tumor cells. The anti-
proliferative effect of PDCD4 was demonstrated through the
repression of transcription of the mitosis-promoting factor cyclin-dependent kinase (CDK1/cdc2) via
upregulation of p21Waf1/Cip1 in human carcinoid cells.
Tumor suppressor PDCD4 interacts with eIF4A, and
preferentially inhibits the translation of mRNAs with
highly structured 5' UTRs. Increased protein synthesis
is observed in many cancers, including breast cancer, and
frequently arises as a consequence of elevated eIF4F
activity. Deregulation of eIF4F activity results in increased
translation of mRNAs that code for proteins involved in
cellular growth and proliferation, survival, and migration,
and consequently contributes to tumor development and progression.

Francisco et al found that cancer cells had lower
susceptibility to apoptosis induced by reducing specific
cell receptors and ligands, also alteration of genetic
episodes leads to decreased cell death and escape
immunosurveillance process; this causes tumor
progression, invasion and new angiogenesis formation
leading to metastases. As the tumor progress the rate of
apoptosis may increase due to presence dysfunctional cells
leading to increased expression of PCDC4 in an attempt to
lower rate of carcinogenesis and tumor formation.

Apoptosis stimuli leads to activation of multiple proteases
like Caspase 8 and Caspase 9 which stimulate other
caspases such as Caspase 3 that indicated the final step of
apoptotic signal. Some data revealed that caspase 3 is
an indicator of apoptotic activity in precancerous tissues.
Some studies demonstrated that increased apoptotic
Discussion
During tumorigenesis, the rate of cell death is changed
according to progression of disease. Programmed cell
death 4 (PDCD4) which is a protein expressed during
apoptosis and has a role in programmed cell death was
found to be an important inhibitor of neoplastic transformation. Overexpression of PDCD4 can cause
suppression of tumor cells through inhibition of
transcription of mitosis promoting factors. The results of our study on human breast invasive duct
carcinoma revealed that level of PDCD4 protein
expression was negative in benign tissues in comparison to
the corresponding cancerous breast cells. The results
revealed that the level of PDCD4 expression was
positively and directly correlated to the differentiation of
higher grades of malignant invasive duct cells. These
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Table 1: clinicopathological Features of studied groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Cancer patients</th>
<th>Benign patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numbers</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Age (years)</td>
<td>27-62</td>
<td>32-60</td>
</tr>
<tr>
<td>TNM classification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T stage</td>
<td>T1,T2</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>T3,T4</td>
<td>19</td>
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<tr>
<td>- LN metastasis</td>
<td>-ve</td>
<td>3</td>
</tr>
<tr>
<td>+ve</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Distant metastasis</td>
<td>-ve</td>
<td>16</td>
</tr>
<tr>
<td>+ve</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>CEA ng/ml</td>
<td>T1, T2</td>
<td>2.3</td>
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<tr>
<td></td>
<td>T3, T4</td>
<td>5.9</td>
</tr>
<tr>
<td>CA15.3 U/ml</td>
<td>T1, T2</td>
<td>24.4</td>
</tr>
<tr>
<td></td>
<td>T3, T4</td>
<td>39.6</td>
</tr>
</tbody>
</table>

Labs, Burlingame, CA). Specific stainings were visualized by 3,3'-diaminobenzidine (DAB) staining. The slides were then counterstained with hematoxylin.

-Serum CA15.3 and CEA were assayed by enzyme-linked immunosorbent assay (ELISA) using Quantikine Human R&D Systems, Minneapolis, MN according to the manufacturer’s instructions.

RESULTS

Table (1) showed clinicopathological features of studied groups. The studied samples were subjected to
immunohistochemical study; immunohistochemical analysis with the anti-PDCD4 antibody showed that
PDCD4 protein was localized in the cytoplasm of breast cancer cells, and the protein amount was significantly
expressed in breast cancer cells of carcinoma tissues in comparison to adjacent noncancerous normal breast tissue
as negative internal control, but more or less contained
positive area as compared with negative controls stained
with PDCD4 antibody (figures 1,2,3,4) while figures 5 and
6 showed invasive duct carcinoma in comparison to
normal control and fibrocystic disease. The
immunohistochemical evaluation showed moderate
positive staining in breast cancer patients with invasive
duct carcinoma grade II while patients with invasive duct
carcinoma grade III and IV showed strong positive
staining. Our results denote that the staining increases with
the progression of disease. Patients with fibrocystic disease
showed equal staining levels or mild staining. (Table 2).

Table 2: Immunohistochemical evaluation of studied groups

<table>
<thead>
<tr>
<th>Samples numbers</th>
<th>Staining</th>
<th>Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>23 fibrocystic</td>
<td>-</td>
<td>(Equal staining levels with antibody in cell)</td>
</tr>
<tr>
<td>disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 fibrocystic</td>
<td>+</td>
<td>(Tissues with less than 30% positive area)</td>
</tr>
<tr>
<td>disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 grade II cancer</td>
<td>++</td>
<td>(Tissues with more than 30% -50% positive area)</td>
</tr>
<tr>
<td>21 grade III, IV</td>
<td>+++</td>
<td>(Tissues with more than 50% of positive area)</td>
</tr>
<tr>
<td>cancer</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
activity is linked with increased proliferation in cancer tissue\textsuperscript{17,18}. This phenomenon, which could be due to abnormal cell replication followed by programmed cell death in more rapidly growing tissue, could explain our results, which suggest that decreased apoptotic activity is an indicator of high-risk normal tissue only in the presence of low proliferative activity\textsuperscript{19,20}.

In conclusion our results showed that PDCD4 expression is increased in patients with breast cancer, so it could be used as diagnostic and prognostic marker for this disease. Further studies are needed to determine role of apoptotic markers in cancer progression and metastases.

REFERENCES


