Utility of Different Immunohistochemical Stain for Diagnosis of Invasive Breast Cancer

Dujuan Wang\(^1\), Li Chen\(^1\), Lihua Zeng\(^2\), Shuzhen Han\(^3\)

\(^{1,2,3}\) Department of Clinical Pathology, Affiliated Dongguan Houjie Hospital, Guangdong Medical College, Dongguan, Guangdong 523945, China

Abstract:
Breast cancer is a malignant tumor that seriously affects females’ physical health, which is the leading cause of cancer death among Chinese female. Estimating early diagnostic and prognostic markers are helpful to conduct treatment for patients with breast cancer. Accumulating investigations focused on the role of Jab1 and S100A8 proteins in the development and metastasis. In our study, we performed the immunohistochemical stain for Jab1 and S100A8 in breast carcinoma and para-carcinoma samples. We have found that the positive rate of Jab1 and S100A8 in breast cancer was higher than that in para-carcinoma tissues. The expression level of Jab1 and S100A8 in breast cancer might have a close relationship with the histologic grade and lymphatic metastasis. The two proteins might be promising supplementary targets for the treatment and prognosis of breast cancers in clinical pathology.

Keywords: Breast cancer; Jab1; S100A8; Immunohistochemical stain

Introduction
Breast cancer (BC) is one of the most common cancers in women and is a major cause of death worldwide with nearly 1.7 million new cases diagnosed each year. WHO (World Health Organization) estimated that worldwide over 521,000 women died in 2012 due to breast cancer [1]. Breast cancer in China has characteristics of advance morbidity peak compared with western countries, and the average occurrence age is 10 years earlier than that in foreign countries[2]. There are many risk factors that might cause breast cancer, including genetic factors, environmental factors and mental factors[3]. Recent therapeutic advances have improved survival for many patients with breast cancer. These advances have been most impressive for targeted therapies, such as those targeting the estrogen receptor (tamoxifen) and the human epidermal growth factor receptor (EGFR) 2 (Her2) [4,5]. However the clinical benefit is limited because of intrinsic and acquired drug resistance[6,7]. Identification of key signaling molecules relevant to those patients who have no specific target medicine is therefore an important step toward the goal of improving breast cancer therapy [8-10]. Based on the limited medical resources in China, we must take positive researches to explore more diagnostic and prognostic markers, which can use widely in clinical pathology.

\(c\)-Jun activation domain-binding protein-1 (Jab1) is a multifunctional signaling protein that can mediate many of its biological effects, including tumor apoptosis, cell cycle regulation and promotion of cell survival [10]. Jab1 has found to be a master regulator of the ‘wound response’ and it also interacts with many components of known cell signaling pathways such as NF-\(\kappa\)B, Smad4 and P27 in breast cancer [11,12]. Additional evidence showed that Jab1 is a key gene in breast cancer progression comes from the recent finding that it is a downstream target for Her2 [13]. Recent studies have showed that abnormal expression of S100 protein is often related to tumor, such as thyroid cancer, colorectal cancer, prostatic cancer, bladder cancer, lung cancer, breast cancer and so on[14-20]. It is known that abnormal expression of multiple S100 proteins is associated with breast cancer, including S100A2, S100A4, S100A6, S100A7, S100A8, S100A9, and S100A11[21]. Increasing studies have reported S100A8 is overexpression in lung cancer and breast cancer[22,23], and it is associated with the progress, metastasis and chemo-resistance in breast cancer[24-26]. In this study, we will use immunohistochemical stain to detect the different expression of Jab1 and S100A8 in invasive breast carcinoma and para-carcinoma tissues and show the correlationship of these two markers and clinical characteristics.

Materials and methods

Tissue specimens
The breast cancer tissues were collected between 2014 and 2016 at Affiliated Dongguan Houjie Hospital of Guangdong Medical College. The diagnostic criterion was followed Breast tumours, Pathology and Genetics (WHO2012) . The study samples consisted of 30 invasive breast cancers with non-specific patients and 30 para-carcinoma tissues. All the samples were selected for this study based on the availability of archived paraffin-embedded BC and tissue blocks for immunohistochemical analysis. Each case were reviewed for tumor histologic grade, tumor size, lymph node status.

Immunohistochemical Analysis
A total of 60 formalin-fixed, paraffin-embedded human specimens (30 primary BC specimens and 30 para-carcinoma specimens) were analyzed. Immunohistochemical (IHC) analyses of Jab1 and S100A8 were performed using the Bond-Max system (Leica Biosystems, Wetzlar, Germany). Antigens were retrieved according to the Bond Max ER1 antigen retrieval protocol. Antibodies used in this study: Jab1 (mouse anti-human, 1/1000, Santa Cruz Biotechnology, Santa Cruz, CA) and S100A8 (mouse anti-human antibody, 1/800, Lifespan Bioscience, Seattle, USA).

The percentage of tumor cells exhibiting intense staining for Jab1 and S100A8 were evaluated in 10 high-power microscopy fields. Cases were considered positive when more than 10% of tumor cells were stained or negative when 10% or less were stained[27]. For each specimen, protein staining in the nucleus were scored separately.

**Statistical analysis**

Statistical analyses were performed using the GraphPad Prism 6 for Windows. Pearson chisquare test (or Fisher exact test when appropriate) was used to compare the Jab1 and S100A8 expression between groups. Paired t-tests were performed to determine whether there were significant differences between the mean percentage of them in different groups. Data were considered statistically significant when the p-value was less than 0.050.

**Result and discussion**

**Patient Characteristics and Demographics**

Samples from 30 patients with non-specific invasive breast cancer (median age, 48 years old; rang, 29-74 years old) were used in the present study. The cancer occurred in left breast is 16cases and right breast is 14cases. All the characteristics have show in Table 1. As reported, in China the average occurrence age is 47 years old, and the most women patients are from 40-49 years old[2]. Among our patients there is 14 cases (about 47% of total patients) occured among 40-50 years old. The tumor size almost is 2–5cm. Our data is consistent with this report.

**Table 1 The characteristics of Breast cancer patients**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Age(years)</th>
<th>Tumor size (cm)</th>
<th>Histologic grade</th>
<th>Lymphnode status(number)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer</td>
<td>2</td>
<td>22</td>
<td>5</td>
<td>1 17 12</td>
</tr>
<tr>
<td></td>
<td>20–40</td>
<td>40–60 ≥60</td>
<td>≤2 2–5 ≥5</td>
<td>0 1'3 4'9 ≥9</td>
</tr>
</tbody>
</table>

**Jab1 Expression in breast cancer**

Jab1 was initially identified as c-Jun activation domain-binding protein-1 (Jab1) and subsequently discovered to be the fifth component of the constitutive photomorphogenetic-9 (COP9) [28]. This protein regulates a variety of cellular and developmental processes, including signal transduction, cells proliferation, cell cycle, apoptosis, DNA damage response (DDR) and tumorigenesis [29]. Previous studies have reported Jab1 overexpressed in breast cancers [30]. In our study, we used immunohistochemical analysis to reveal that 96% of the BC samples has positive staining for Jab1 (Figure 1). We found that the positive cells in para-carcinomas were almost in normal ductal epithelium cells, especially in gland epithelium cells. The positive rate was higher (96%) than that in the para-carcinomas tissues, which was 63% (P=0.0025<0.01; Table 2). The positive rate of Jab1 between different positive percentage groups has significant difference, but there is no difference between 10–50% group and 50–75% group. Above 75% group the positive rate is up to 83%. However the different positive rate between breast cancer and para-carcinoma is not related to tumor size, lymph node status and histologic grade. This finding suggested that Jab1 might be potential biomarker in BC, and it was consistent with other researches.

**Figure 1 Expression of Jab1 and S100A8 proteins in breast cancer and para-carcinoma (×20)**

**S100A8 Expression in breast cancer**

In breast cancer, glandular epithelial cell gets gene mutation under the action of multiple carcinogenic factors, and the immunophenotype of these disorder cells will be changed. They can interact with the surrounding microenvironment, moreover they will be invasive because the microenvironment factors will squeeze and destroy the surrounding normal tissues and damages the normal tissue structure of the breast[31,32]. S100 protein family is a kind of calcium binding protein with cell and tissue specificity[33], and increasing investigations have showed the overexpression in breast cancer. S100A8 is expressed by breast cancer cells as well as by infiltrating immune and myeloid cells. High breast cancer cell S100A8 protein expression was a significant prognostic factor for OS in 417 patients cohort[34]. Strong
expression and secretion of S100A8 may be associated with the loss of ER in BC, and may be involved in the poor prognosis of Her2+/basal-like subtypes of BC [24]. In our study, the positive rate of S100A8 in invasive breast cancer is 67%, which is higher than that in para-carcinoma(10%, P<0.0001; Figure1, Table 2). The percentage positive cells of 50~75% group is significantly different from above 75% group. As expression of Jab1 positive rate, there have no obvious relationship with tumor size, histologic grade and lymph node status because of the limited cancer sample number (Table 3).

The mechanism about S100A8 overexpression in progression, invasion, metastasis and chemo-resistance of tumor involves the tumor microenvironment. The S100A8 promotes the migration and invasion of human breast cancer cells through actin polymerization and epithelial-mesenchymal transition. Ye Y have demonstrated that during premetastatic phase, an inflammatory response and inflammation-induced vascular hyperpermeability leading to an abnormal pulmonary microenvironment in a TGFβ-dependent manner, resulting an increasing expression of S100A8[22]. It will promote the circulating breast tumor cells to seed the lung. Becker A also reported that S100A8 has been implicated in the induction of tumor associated macrophaged (TAM) or myeloid-derived suppressor cells (MDSC), which support tumor development and spread[35]. Also there are increasing studies showed the signaling pathways of S100A8 in breast cancer, such as p38-MAPK, IL6 –JAK2-STAT3[36] and so on.

**Table 2 The positive expression of Jab1 and S100A8 in different groups**

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Jab1 (%)</th>
<th>S100A8 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>≤10</td>
<td>10-50</td>
</tr>
<tr>
<td>BC</td>
<td>30</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Para-BC</td>
<td>30</td>
<td>11</td>
<td>5</td>
</tr>
</tbody>
</table>

**Table 3 The positive expression of Jab1 and S100A8 with different clinical characteristics**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Jab1</th>
<th>S100A8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BC(n=30)</td>
<td>Para-BC(n=30)</td>
</tr>
<tr>
<td>Tumor size (cm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤2</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>2-5</td>
<td>22</td>
<td>14</td>
</tr>
<tr>
<td>≥5</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Tissue classified</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>II</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>III</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>LN Status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>20</td>
<td>11</td>
</tr>
<tr>
<td>1^3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>4^9</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>≥9</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>

**Conclusion**

Overexpression of Jab1 and S100A8 in breast cancer was testified in our study, which has significant difference between breast cancer and para-carcinoma. Moreover, the positive rate has no difference between different groups with tumor size, age, histologic grade and lymph node metastasis. Limited studies have not clarified the mechanism of Jab1 and S100A8 in breast cancer clearly, however evaluation of Jab1 and S100A8 protein expression may provide additional prognostic information beyond traditional breast cancer prognostic biomarkers in clinical pathology.

**References**


