Clinical Implications of p57^{KIP2} Expression in Breast Cancer

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Abstract

Objective: To study the relationship between expression of p57^{KIP2} and prognosis and other clinicopathological parameters in invasive breast cancers. Methods: We assessed the expression of p57^{KIP2} in 89 cases of invasive breast cancer and 20 cases of normal breast tissue by immunohistochemical methods and analyzed the results with SPSS software (ver. 16.0). Result: The positive expression rates of p57^{KIP2} protein in the invasive breast cancers and surrounding normal tissue were 30.3% (27/89) and 65% (13/20), respectively. Cases with no p57^{KIP2} expression exhibited a significantly higher post-operative distant metastasis rate than those with p57^{KIP2} expression (37.9% vs. 14.8%; P = 0.01). DFS analysis showed that p57^{KIP2}/C-erbB-2+ tumors also exhibited a significantly higher post-operative distant metastasis rate than the other groups (66.7% vs. 29.2%; P = 0.007), as did p57^{KIP2}/p53+ tumors (64.3% vs. 22.7%; P = 0.001). Survival analysis revealed that p57^{KIP2} was associated with breast cancer-specific survival overall (P = 0.045, log-rank test). Subgroup analysis demonstrated that individuals with p57^{KIP2}/C-erbB-2+ tumors experienced significantly worse post-operative survival than those with p57^{KIP2}/C-erbB-2- or other tumors (P = 0.006, log-rank test). p57^{KIP2}/p53+ tumors were associated with significantly worse post-operative survival than p57^{KIP2}/p53- or other tumors (P = 0.001, log-rank test). Cox regression analysis showed that p57^{KIP2} was a non-independent prognostic factor for breast cancer (P = 0.303). Conclusions: p57^{KIP2} is expressed at low levels in invasive breast cancer and is associated with better overall survival rate and disease-free survival in breast cancer patients, but it was a non-independent prognostic factor for breast cancer. Thus, the connection between p57^{KIP2}/p53 and p57^{KIP2}/C-erbB-2 may provide biomarkers for breast cancer.

Keywords: Breast cancer - P57^{KIP2} - biomarker

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Introduction

Since 2001, when the winner of the Nobel Prize for physiology and medicine found crucial moderators in cyclin-dependent kinase (CDK) and cyclin (Koepp et al., 2001; Duman-Scheel et al., 2002), the study of the cell cycle and associated moderators has become a hot area in tumor research. Factors associated with cell cycle control can be divided into three groups: cyclins, CDK, and cyclin-dependent kinase inhibitor (CDKI). CDK is found at the core of cell cycle control.

Cyclins take positive actions towards CDK and CDKI takes negative actions; together, they form the cell cyclin controlling system. CDKI, as an “anti-oncogene” can arrest cell cycle progress and induce cells to enter apoptosis by arresting cells at checkpoints in the cell cycle (Eddy, 1996).

CDKIs can be divided into two families: the inhibitors of CDK4 (INK4) family and the CDK-interacting protein kinase inhibition protein (CIP/KIP) family, also called the p16 and p21 families, respectively. The INK4 family includes p15, p16, p18, and p19; they are all the specific inhibitors of CDK4/CDK6. The CIP/KIP family includes p21^{CIP1}, p27^{KIP1}, and P57^{KIP2}. They all share a similar domain for inhibition function; they also inhibit the cyclin-CDK complex and cyclin-B-CDK1 complex in the G1 period by combining with cyclin D, E, A, B, and the CDK complex to arrest the cell cycle at the G1 stage (Matsuoka et al., 1995; Buiting et al., 2001).

P57^{KIP2} is the most recently found and cloned CDKI member. It was cloned by Matsuoka (Matsuoka et al., 1995). The human gene is located at chromosome 11p15.5, with an open reading frame of 948 bp, encoding 316 amino acids, in three functional domains. It has maternal expression and paternal inheritance, and a molecular weight of 57 kDa. About 35-40% of breast cancer patients have an allelic imbalance and loss of heterozygosity (Gudmundsson et al., 1995; Karnik et al., 1998; Lichy et al., 1998).

In some tumors, there are P57^{KIP2} gene mutations; for example, Guran reported that there was a P57^{KIP2} gene mutation in chronic myelogenous leukemia (Guran et al., 1998). However, in most tumors, there is no P57^{KIP2} gene mutation (Tokino et al., 1996; Karnik et al., 1998). There are reports that decreased P57^{KIP2} expression has an important effect in ovarian cancer, gastric cancer, p21^{CIP1}, and P57^{KIP2}.
Materials and Methods

Patients and tissue specimens
We selected 20 patients with histologically confirmed normal breast tissue and 89 patients who had histologically confirmed breast cancer and underwent radical operations at the First Affiliated Hospital of China Medical University between August 2001 and September 2003. The study protocol was approved by the Ethics Committee of China Medical University. Inclusion criteria for breast cancer patients were: (a) curative operations were performed, (b) resected specimens were examined pathologically, (c) more than 15 lymph nodes were examined pathologically after the operation, (d) no chemotherapy or radiotherapy before the operation, and (e) no distant metastasis before the operation.

Materials
A monoclonal mouse anti-human P57KIP2 antibody (dilution 1:150) was obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Rabbit anti-ER monoclonal antibody (dilution 1:100), monoclonal mouse anti-human PR antibody (dilution 1:100), and monoclonal mouse anti-C-erbB-2 antibody (dilution 1:100), polyclonal rat anti-human p53 antibody (dilution 1:100), and the Polymer Detection System for immunohistological staining (PV9000) were obtained from Zhongshanjinqiao Biotechnology Inc. (China).

Experiment procedures
Thin slices of breast tumor tissues and non-neoplastic breast tissues received in our histopathology unit were fixed in 4% formaldehyde solution (pH 7.0) for periods not exceeding 24 h. Tissues were processed routinely for paraffin embedding, and 4 μm sections were cut and placed on glass slides coated with 3-aminopropyl triethoxysilane for immunohistochemistry. Tissue samples were stained with hematoxylin and eosin to determine the histological type and grade of tumors.

Immunohistochemical analysis
P57KIP2 expression was classified semi-quantitatively according to the following criteria: 0 if < 1% of neoplastic cells discretely expressed P57KIP2 in their nuclei, 1+ if ≥ 1% and < 10% of neoplastic cells discretely expressed P57KIP2 in their nuclei, and 2+ if ≥ 10% of morphologically unequivocally neoplastic cells discretely expressed P57KIP2 in their nuclei. We considered samples scored as 1+ or 2+ to be positive. Nuclear staining for ER, PR, and p53 was graded 1+ if < 10% of the cells were stained, 2+ if 10-50% of the cells were stained, and 3+ if > 50% of the cells were stained. We considered grades 2+ and 3+ to be positive, while the absence of staining and 1+ staining were deemed to be negative. Similar standards were used for staining intensity in HER-2/neu, except that here, only grade 3+ (high intensity) was considered positive.

Statistical analyses
All data were analyzed using the SPSS software (ver. 16.0; SPSS, Chicago, IL, USA). Relationships between tumor markers and other parameters were assessed using the chi-squared test and Fisher’s exact test or the independent t-test. Disease-specific survival was analyzed using the Kaplan-Meier method. The log-rank test was used to analyze differences in survival. Multivariate analysis was performed using Cox regression. P values < 0.05 were considered to indicate statistical significance.

Results
Patient characteristics
The mean age of the 89 patients was 48.3 (range, 32-80) years. Within the total sample, 42 (47.2%) patients had no lymph node metastasis, 22 (24.7%) patients had 1-3 lymph node metastases, and 25 (28.1%) patients had ≥ 4 lymph node metastases. Of the patients, 26 (29.2%) had post-operative distant metastases. Positive expression of P57KIP2, ER, PR, C-erbB-2, and p53 was observed in 27 (30.3%), 37 (41.6%), 45 (50.6%), 19 (21.4%), and 27 (30.3%) of the breast cancer cases, respectively. Tumor sizes of ≤ 2 cm, > 2 cm and ≤ 5 cm, and > 5 cm were seen in 35 (39.3%), 42 (47.2%), and 12 (13.5%) cases, respectively. To exclude any influence of confounding factors, we compared tumor size and adjuvant treatment between patients with distant metastasis and those without; no difference was observed between them.

Table 1. The Relationship Between the Expression of p57KIP2 and Clinicopathological Characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>n</th>
<th>P57KIP2</th>
<th>P57KIP2+</th>
<th>X² value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor size(cm)</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>≤2</td>
<td>36</td>
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<td>7</td>
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<td>41</td>
<td>28</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;5</td>
<td>12</td>
<td>5</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metastatic nodes</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>0</td>
<td>42</td>
<td>29</td>
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<td>0.138</td>
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<tr>
<td>1--3</td>
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<td>≥4</td>
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<td>17</td>
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<td>ER</td>
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<td>52</td>
<td>34</td>
<td>18</td>
<td>1.083</td>
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<tr>
<td>positive</td>
<td>37</td>
<td>28</td>
<td>19</td>
<td></td>
<td></td>
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<tr>
<td>PR</td>
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<td>31</td>
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<td></td>
<td></td>
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<tr>
<td>C-erbB-2</td>
<td></td>
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<td>70</td>
<td>53</td>
<td>17</td>
<td>5.682</td>
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<tr>
<td>positive</td>
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<td>9</td>
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<td></td>
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<tr>
<td>p53</td>
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<tr>
<td>negative</td>
<td>62</td>
<td>50</td>
<td>12</td>
<td>11.663</td>
<td>0.001</td>
</tr>
<tr>
<td>positive</td>
<td>27</td>
<td>12</td>
<td>15</td>
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</table>
P57<sup>KIP2</sup> was located in the cell nucleus in breast cancer (Figure 1A). P57<sup>KIP2</sup> was expressed significantly higher in breast cancer than in normal breast tissue (26% vs. 65%, P = 0.031). We also found that expression of P57<sup>KIP2</sup> was associated with the tumor size, and the expression of C-erbB-2 (Figure 1B) and p53 (Figure 1C; P = 0.039, 0.017, and 0.001, respectively), but not with the number of lymph nodes with metastases, or the expression of ER (Figure 1D) or PR (Figure 1E; P = 0.933, 0.298, and 0.872, respectively; Table 1).

**Prognostic analysis**

We investigated post-operative distant metastasis rates and overall survival rates among the different groups. Cases without P57<sup>KIP2</sup> expression showed a significantly higher post-operative distant metastasis rate than those with P57<sup>KIP2</sup> expression (37.9% vs. 14.8%; P = 0.049). Subgroup analysis showed that the P57<sup>KIP2</sup> -/C-erbB-2+ group also showed a significantly higher post-operative distant metastasis rate than P57<sup>KIP2</sup> -/C-erbB-2+ and other groups (62.5% vs. 25.9%; P = 0.007). Interestingly, the P57<sup>KIP2</sup> -/p53+ group showed a significantly higher post-operative distant metastasis rate than the P57<sup>KIP2</sup> -/p53+ and other groups (64.3% vs. 22.7%; P = 0.001).

Survival analysis revealed that P57<sup>KIP2</sup> was associated with breast cancer-specific survival (P = 0.045, log-rank test; Figure 2A). Subgroup analysis demonstrated that the P57<sup>KIP2</sup> -/C-erbB-2+ group showed significantly worse post-operative survival than the P57<sup>KIP2</sup> -/C-erbB-2+ and other groups (P = 0.06, log-rank test; Figure 2B). Moreover, the P57<sup>KIP2</sup> -/p53+ group showed significantly worse post-operative survival than the P57<sup>KIP2</sup> -/p53- group and other groups (P = 0.001, log-rank test; Figure 2C). From a Cox regression analysis, P57<sup>KIP2</sup> was shown to be a non-independent prognostic factor for breast cancer (P = 0.303).

This study showed that the expression of P57<sup>KIP2</sup> was associated with tumor size (P = 0.039, chi-squared test).

**Discussion**

Breast cancer is the most common malignant tumor in females. There were 230,480 people with newly confirmed breast cancer in the United States of America in 2011, representing 30% of all newly confirmed malignant tumors in females. In China, breast cancer is also the most common malignant tumor in females and the disease incidence is increasing rapidly, according to statistics from the cities of Shanghai and Tianjin. Thus, research on breast cancer is receiving increased attention in China.

P57<sup>KIP2</sup> is a member of the family of cyclin-dependent kinases inhibitors (CDKIs). CDKI can combine with cyclin-dependent kinase (CDK) and inhibit its activity in controlling the cell cycle. It plays a double role in the process of adjusting tumor cells: on the one hand, it can affect the invasion and metastasis of tumor cells, while on the other, it can adjust apoptosis and differentiation in tumor cells.

Abnormal expression of P57<sup>KIP2</sup> makes cells undifferentiate and overexpression can lead to tumors. The role of P57<sup>KIP2</sup> in apoptosis in tumor cells may make it useful as a target for therapy (Kavanagh et al., 2011). There is little variation in the expression of P57<sup>KIP2</sup> gene in tumor cells: it controls expression by loss of imprinting, loss of heterozygosity, methylation of promoters, and controlling microRNA, with decreased expression of P57<sup>KIP2</sup> promoting the development of tumors. At the same time, P57<sup>KIP2</sup> has been used as a predictive factor.
to differentiate periods in many kinds of tumors. There have been reports that P57\textsuperscript{KIP2} undergoes frequent loss of heterozygosity in breast, bladder, lung, ovarian, kidney, and testicular carcinomas (Seizinger et al., 1991). The p57 gene is paternally imprinted in humans (Matsuoka et al., 1996), and loss of the maternal (expressed) allele occurs in a variety of pediatric tumors, including Wilms’ tumor (Hatada et al., 1996), and in adult lung tumors (Kondo et al., 1996). Mice lacking p57 exhibit altered cell proliferation and differentiation and manifest a number of developmental defects (Zhang et al., 1997). On the basis of this evidence, p57 has been designated a putative tumor suppressor gene (Li et al., 1999).

Currently, the expression status of P57\textsuperscript{KIP2} protein in breast cancer and its relationship to the biological behavior of breast cancer remains unclear. Furthermore, few studies have addressed P57\textsuperscript{KIP2} expression in breast cancer and the relationship between it and prognosis of breast cancer. In our study, we found that P57\textsuperscript{KIP2} was expressed at significantly lower levels in breast cancer than in normal breast tissue (26% vs. 65%; \textit{P} = 0.031). P57\textsuperscript{KIP2} is recognized as a suppressor gene in breast cancer; loss or functional inhibition of P57\textsuperscript{KIP2} gene makes the incidence of breast cancer higher than with normal P57\textsuperscript{KIP2} expression. At the same time, low expression of P57\textsuperscript{KIP2} is associated with the overall survival rate and disease-free survival in breast cancer patients.

In the past decade, C-erbB-2+ and p53 have become significant molecular markers for breast cancer. At the same time, some C-erbB-2 inhibitors have been approved by FDA for breast cancer therapy and have shown favorable results (De et al., 2010; Procter et al., 2010).

In breast cancer, C-erbB-2+ and p53 status influence the prognosis and probability of response to systemic therapies, while P57\textsuperscript{KIP2} was significantly related to the expression of both. Interestingly, breast cancer-specific survival was not only related to P57\textsuperscript{KIP2} independently, but also to P57\textsuperscript{KIP2} expressed together with C-erbB-2+ and/or p53. Thus, P57\textsuperscript{KIP2} may be a non-independent prognostic factor for breast cancer progression.

In conclusions, P57\textsuperscript{KIP2} was expressed at low levels in invasive breast cancer. Expression of P57\textsuperscript{KIP2} was associated with overall survival and disease-free survival in breast cancer patients, but it was a non-independent prognostic factor in breast cancer. Thus, the connection between P57\textsuperscript{KIP2}/p53 and P57\textsuperscript{KIP2}/C-erbB-2 may be useful as biomarkers for breast cancer. The underlying genetic mechanism of action of P57\textsuperscript{KIP2} as expressed in breast cancer, however, remains unclear. The relationships between P57\textsuperscript{KIP2} and C-erbB-2 and p53 gene expression and the biological behavior of breast cancer need further investigation.

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References


