RESEARCH ARTICLE

Clinical Implications of p57KIP2 Expression in Breast Cancer

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Abstract

Objective: To study the relationship between expression of p57KIP2 and prognosis and other clinicopathological parameters in invasive breast cancers. Methods: We assessed the expression of p57KIP2 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 p57KIP2 protein in the invasive breast cancers and surrounding normal tissue were 30.3% (27/89) and 65% (13/20), respectively. Cases with no p57KIP2 expression exhibited a significantly higher post-operative distant metastasis rate than those with p57KIP2 expression (37.9% vs. 14.8%; P = 0.01). DFS analysis showed that p57KIP2-/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 p57KIP2-/p53+ tumors (64.3% vs. 22.7%; P = 0.001). Survival analysis revealed that p57KIP2 was associated with breast cancer-specific survival overall (P = 0.045, log-rank test). Subgroup analysis demonstrated that individuals with p57KIP2-/C-erbB-2+tumors experienced significantly worse post-operative survival than those with p57KIP2- /C-erbB-2- or other tumors (P = 0.006, log-rank test). p57KIP2-/p53+ tumors were associated with significantly worse post-operative survival than p57KIP2-/p53- or other tumors (P = 0.001, log-rank test). Cox regression analysis showed that p57KIP2 was a non-independent prognostic factor for breast cancer (P = 0.303). Conclusions: p57KIP2 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 p57KIP2/p53 and p57KIP2/C-erbB-2 may provide biomarkers for breast cancer.

Keywords: Breast cancer - P57KIP2 - 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 p21CIP1, p27KIP1, and P57KIP2. 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).

P57KIP2 is the most recently found and cloned CDKI member. It was cloned by Masuoka (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 P57KIP2 gene mutations; for example, Guran reported that there was a P57KIP2 gene mutation in chronic myelogenous leukemia (Guran et al., 1998). However, in most tumors, there is no P57KIP2 gene mutation (Tokino et al., 1996; Karnik et al., 1998). There are reports that decreased P57KIP2 expression has an important effect in ovarian cancer, gastric cancer,

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colorectal cancer, and lymphoma (Kavanagh et al., 2011) . Regarding breast cancer, to our knowledge, there is no previous report about the relationship between $P57^{KIP2}$ expression and prognosis. Thus, we studied the relationship between $P57^{KIP2}$ expression and prognosis and other clinicopathological parameters in invasive breast cancer, assessing the expression of $P57^{KIP2}$ in 89 cases of invasive breast cancer and 20 cases of normal breast tissue.

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 $P57^{KIP2}$ 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 (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

$P57^{KIP2}$ expression was classified semi-quantitatively according to the following criteria: 0 if < 1% of neoplastic cells discretely expressed $P57^{KIP2}$ in their nuclei, 1+ if ≥ 1% and < 10% of neoplastic cells discretely expressed $P57^{KIP2}$ in their nuclei, and 2+ if ≥ 10% of morphologically unequivocally neoplastic cells discretely expressed $P57^{KIP2}$ 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 extract 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 $P57^{KIP2}$, 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.

$P57^{KIP2}$ expression in breast cancer and the relationship between $P57^{KIP2}$ and clinicopathological characteristics

Immunohistochemical examination showed that

Table 1. The Relationship Between the Expression of $p57^{KIP2}$ and Clinicopathological Characteristics

Variables	n	$P57^{KIP2}$-	$P57^{KIP2}$+	X^2 value	P value
Tumor size(cm)					
≤2	36	29	7	6.508	0.039
2--5	41	28	13		
>5	12	5	7		
Metastatic nodes					
0	42	29	13	0.138	0.933
1--3	22	16	6		
≥4	25	17	8		
ER					
negative	52	34	18	1.083	0.298
positive	37	28	19		
PR					
negative	44	31	13	0.026	0.872
positive	45	31	14		
C-erbB-2					
negative	70	53	17	5.682	0.017
positive	19	9	10		
P53					
negative	62	50	12	11.663	0.001
positive	27	12	15		

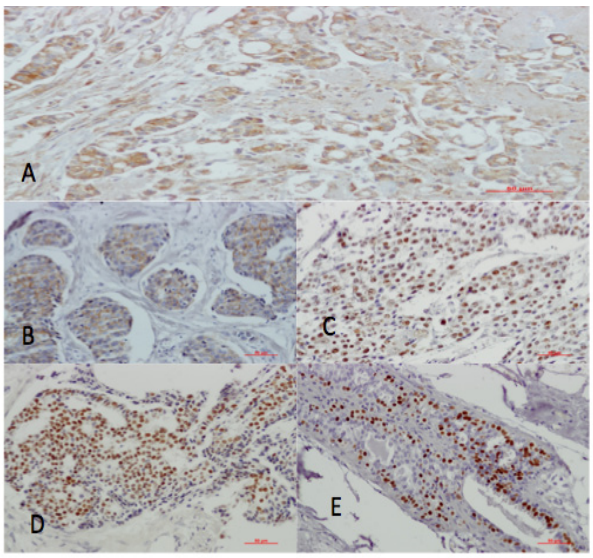

Figure 1. (A) p57KIP2 was Located in the Cell Nucleus in Breast Cancer and (B, C, D, E) Other Tumor-related Proteins, Including Her-2/neu, ER, PR, and P53, which were Found at High or Moderate Levels in Breast Cancer Tissues

P57^{KIP2} was located in the cell nucleus in breast cancer (Figure 1A). P57^{KIP2} 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^{KIP2} 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^{KIP2} expression showed a significantly higher post-operative distant metastasis rate than those with P57^{KIP2} expression (37.9% vs. 14.8%; P = 0.049). Subgroup analysis showed that the P57^{KIP2}-/C-erbB-2+ group also showed a significantly higher post-operative distant metastasis rate than P57^{KIP2}-/C-erbB-2- and other groups (62.5% vs. 25.9%; P = 0.007). Interestingly, the P57^{KIP2}- / p53+ group showed a significantly higher post-operative distant metastasis rate than the P57^{KIP2}-/p53+ group and other groups (64.3% vs. 22.7%; P = 0.001).

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

This study showed that the expression of P57^{KIP2} was associated with tumor size (P = 0.039, chi-squared test).

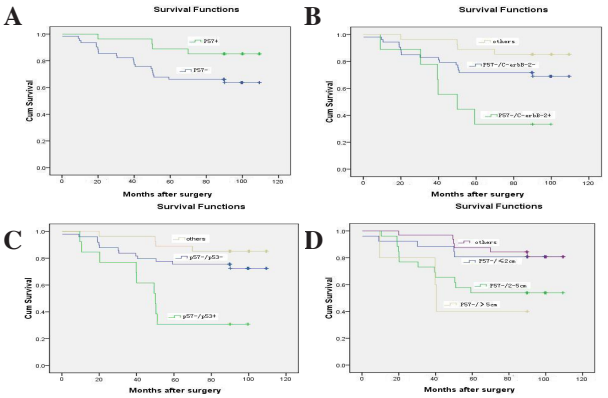

Figure 2. (A) P57^{KIP2} was Associated with Breast Cancer-specific Survival (P = 0.045, log-rank test); (B) P57^{KIP2}-/C-erbB-2+ Group Showed Significantly Worse Post-operative Survival than the P57^{KIP2}-/C-erbB-2- Group and Other Groups (P = 0.06, log-rank test); (C) P57^{KIP2}-/p53+ Group Showed Significantly Worse Post-operative Survival than the P57^{KIP2}-/p53- Group and Other Groups (P = 0.001, log-rank test); (D) the P57^{KIP2}- and Tumor Size > 5 cm Group Showed Significantly Worse Post-operative Survival than the P57^{KIP2}-/Tumor size of 2-5 cm Group, the P57^{KIP2}-/Tumor size < 2 cm Group, and Other Groups (P = 0.014, log-rank test)

Also, the P57^{KIP2}- and tumor size > 5 cm group showed significantly worse post-operative survival than the P57^{KIP2}- / tumor size of 2-5 cm group, the P57^{KIP2}- / tumor size < 2 cm group, and other groups (P = 0.014, log-rank test, Figure 2D).

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^{KIP2} 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^{KIP2} makes cells undifferentiate and overexpression can lead to tumors. The role of P57^{KIP2} 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^{KIP2} 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^{KIP2} promoting the development of tumors. At the same time, P57^{KIP2} has been used as a predictive factor,

to differentiate periods in many kinds of tumors. There have been reports that $P57^{KIP2}$ 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 $P57^{KIP2}$ protein in breast cancer and its relationship to the biological behavior of breast cancer remains unclear. Furthermore, few studies have addressed $P57^{KIP2}$ expression in breast cancer and the relationship between it and prognosis of breast cancer. In our study, we found that $P57^{KIP2}$ was expressed at significantly lower levels in breast cancer than in normal breast tissue (26% vs. 65%; P = 0.031). $P57^{KIP2}$ is recognized as a suppressor gene in breast cancer; loss or functional inhibition of $P57^{KIP2}$ gene makes the incidence of breast cancer higher than with normal $P57^{KIP2}$ expression. At the same time, low expression of $P57^{KIP2}$ 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 $P57^{KIP2}$ was significantly related to the expression of both. Interestingly, breast cancer-specific survival was not only related to $P57^{KIP2}$ independently, but also to $P57^{KIP2}$ expressed together with C-erbB-2+ and/or p53. Thus, $P57^{KIP2}$ may be a non-independent prognostic factor for breast cancer progression.

In conclusions, $P57^{KIP2}$ was expressed at low levels in invasive breast cancer. Expression of $P57^{KIP2}$ 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 $P57^{KIP2}$/p53 and $P57^{KIP2}$/C-erbB-2 may be useful as biomarkers for breast cancer. The underlying genetic mechanism of action of $P57^{KIP2}$ as expressed in breast cancer, however, remains unclear. The relationships between $P57^{KIP2}$ and C-erbB-2 and p53 gene expression and the biological behavior of breast cancer need further investigation.

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References

Buiting K, Kanber D, Horsthemke B, et al (2010). Imprinting of RB1(the new kid on the block). *Brief Funct Genomics*, **9**, 347-53.

De P, Smith BR, Leyland-Jones B (2010). Human epidermal growth factor receptor 2 testing: where are we? *J Clin Oncol*, **28**, 4289-92

Duman-Scheel M, Weng L, Xin SJ, et al (2002). Hedgehog regulates cell growth and proliferation by inducing cyclin D and cyclin E. *Nature*, **417**, 299-304.

Eddy AA (1996). Expression of genes that promote renal interstitial fibrosis in rats with proteinuria. *Kidney Int*, **54**, S49-54.

Gudmundsson J, Barkardottir RB, Eiriksdottir G, et al (1995). Loss of heterozygosity at chromosome 11 in breast cancer: association of prognostic factors with genetic alterations. *Brit J Cancer*, **72**, 696-701.

Güran S, Bahçe M, Beyan C, Korkmaz K, Yalçin A (1998). P53, p15INK4B, p16INK4A and p57KIP2 mutations during the progression of chronic myeloid leukemia. *Haematologia (Budap)*, **29**, 181-93.

Hatada I, Inazawa J, Nakayama M, et al (1996). Genomic imprinting of human P57-KIP2 and its reduced expression in Wilms' tumors. *Hum Mol Genet*, **5**, 783-8.

Karnik P, Paris M, Williams BRG, T, et al (1998). wo distinct tumor suppressor loci within chromosome 11p15 implicated in breast cancer progression and metastatsis. *Hum Mol Genet*, **7**, 895-903.

Kavanagh E, Joseph B (2011). The hallmarks of CDKN1C (p57, KIP2) in cancer. *Biochim Biophys Acta*, **1816**, 50-6.

Koepp DM, Schaefer LK, Ye X, et al (2001). Phosphorylation-dependent ubiquitination of cyclin E by the SCFFbw 7 ubiquitin ligase. *Science*, **294**, 173-7.

Kondo M, Matsuoka S, Uchida K, et al (1996). Selective maternal-allele loss in human lung cancers of the maternally expressed P57-KIP2 gene at 11p15.5. *Oncogene*, **12**, 1365-8.

Lichy JH, Zavar M, Tsai MM, et al (1998). Loss of heterozygosity on chromosome 11p15 during histological progression in microdissected ductal carcinoma of the breast. *Am J Pathol*, **153**, 271-8.

Li Y, Millikan RC, Newman B, et al (1999). P57(KIP2) polymorphisms and breast cancer risk. *Hum Genet*, **104**, 83-8.

Matsuoka S, Edwards MC, Bai C, et al (1995). $P57^{KIP2}$, a structurally distinct member of the p21CIP Cdk inhibitor family is a candidate tumor suppressor gene. *Gene Dev*, **9**, 650-62.

Matsuoka S, Thompson J, Edwards M, et al (1996). Imprinting of the gene encoding a human cyclin-dependent kinase inhibitor,P57-KIP2, on chromosome 11p15. *P Natl Acad Sci USA*, **93**, 3026-30.

Procter M, Suter TM, de Azambuja E, et al (2010). Longer-term assessment of trastuzumab-related cardiac adverse events in the Herceptin Adjuvant (HERA) trial. *J Clin Oncol*, **28**, 3422-8.

Seizinger B, Deimling A (1991). Report of the committee on chromosome and gene loss in human neoplasia. *Cytogenet Cell Genet*, **58**, 1080-96.

Tokino T, Urano T, Furuhata T, et al (1996). Characterization of the human $P57^{KIP2}$ gene:alternative splicing, insertion/deletion polymorphisms in VNTR sequences in the coding region, and mutational analysis. *Hum Genet*, **97**, 625-31.

Zhang P, Liegeois N, Wong C, et al (1997). Altered cell differentiation and proliferation in mice lacking P57-KIP2 indicates a role in Beckwith-Wiedemann syndrome. *Nature*, **387**, 151-63.