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Clinical implications of CIP2A protein expression in breast cancer

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Abstract   The aim of this work is to study the expression and regulatory effects of CIP2A protein in breast cancer and the correlation between CIP2A protein expression and the prognosis of breast cancer. The CIP2A protein level was detected by immunohistochemistry staining. The relationship between CIP2A protein and clinicopathological parameters of breast cancer was determined. It was observed that 448 (35.00 %) of the 1,280 cases positively expressed CIP2A protein. Univariate analysis indicated that CIP2A expression was related to histological grade, lymph node metastasis, distant metastasis, and triple-negative breast cancer ($P = 0.001$, 0.001, 0.001, and 0.001, respectively). Spearman correlation analysis showed that CIP2A expression has line correlation with histological grade, lymph node metastasis, distant metastasis, triple-negative breast cancer, and TNM stage ($P = 0.03$, 0.001, 0.008, 0.001, and 0.001, respectively). After multivariate analysis, tumor size, histological grade, lymph node metastasis, triple-negative breast cancer, distant metastasis, and TNM stage were related to CIP2A expression ($P = 0.035$, 0.001, 0.028, 0.001, 0.001, and 0.001, respectively). CIP2A expression also significantly related to chemotherapeutic sensitivity of breast cancer in the neoadjuvant chemotherapy. In the Cox regression test, histological grade, lymph node metastasis, triple-negative breast cancer, and TNM stage were detected as the independent prognostic factors ($P = 0.001$, 0.006, 0.01, 0.011, and 0.001, respectively). CIP2A expression may be a potential biomarker for chemotherapeutic sensitivity and prognosis of breast cancer.

Keywords   Breast cancer · CIP2A protein · Chemotherapeutic sensitivity · Prognosis

Introduction

Breast carcinoma is the most common malignancy in women and is the second leading cause of cancer deaths in women [1]. Over the last 30 years, deaths from breast cancer have approximately tripled in Japan, which historically has had low incidence of breast cancer [2]. According to the World Health Organization, more than 1.2 million people will be diagnosed with breast cancer each year worldwide [2]. Although many identified molecules play roles in the way breast cancers progress and metastasize, the mechanisms of breast cancer are far from clear [3, 4]. At this point, few molecules exhibit high efficiency in predicting postoperative distant metastasis for breast cancer.

Protein CIP2A also known as cancerous inhibitor of PP2A (CIP2A) is a protein that in humans is encoded by the KIAA1524 gene [5, 6]. PP2A is a cellular tumor suppressor which inhibits cell proliferation and transformation of normal cells to malignant cancer cells. Inhibition of PP2A activity is a prerequisite for human cell transformation. However, the in vivo mechanisms by which PP2A activity is inhibited in spontaneously transformed human cancer cells have been unclear. More specifically, CIP2A was demonstrated to inhibit PP2A activity toward

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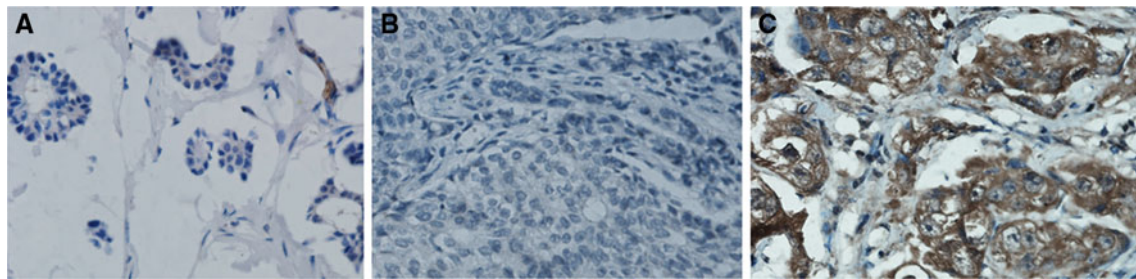e-mail: jinyingyustar@163.com

Fig. 1 The expression of CIP2A protein in breast tumor tissues. **a** in paracancerous tissue (×400); **b** in non-tripe-negative breast cancer (×400); **c** in tripe-negative breast cancer (×400)

oncogenic transcription factor c-Myc and thereby prevent c-Myc proteolytic degradation [7].

At present, CIP2A is reported to be overexpressed in prostate cancer, lung cancer, oral squamous cell carcinoma, and gastric cancer [8, 9]. Furthermore, the expression of CIP2A correlates with breast cancer aggressivity [10]. However, there is still no clinical study addressing the clinical implications of CIP2A expression in breast cancer patients. Therefore, we examined 1,280 mastectomy specimens obtained from patients with breast cancers to investigate the expression of CIP2A in relation to clinicopathological features, immunohistochemical markers, and amplification of key genes in breast cancer and to determine the prognostic impact of CIP2A expression.

Materials and methods

Patients and tissue specimens

One thousand two hundred and eighty patients who were histologically confirmed breast cancer and underwent radical operations in the Harbin Medical University between January 2002 and January 2008. The mean age of the enrolled patients was 51.34 ± 8.02 (mean $\pm$ SD). The inclusion criteria were as follows: (a) curative operations were performed; (b) resected specimens were pathologically examined; (c) more than 10 lymph nodes were pathologically examined after operation. The Ethics Committee of Harbin Medical University approved this study's protocol.

Thin slices of tumor tissue of all cases received in our histopathology unit were fixed in 4 % formaldehyde solution (pH 7.0) for periods not exceeding 24 h. The tissues were processed routinely for paraffin embedding, and 4-μm-thick 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 histological type and grade of tumors.

Immunohistochemical analysis

Briefly, immunohistochemical staining was performed using the standard streptavidin-peroxidase (SP) method with the UltraSensitive TM S–P Kit (Maixin-Bio, China) according to the manufacturer's instructions, and signals were visualized using the DAB substrate, which stains the target protein yellow. Briefly, one paraffin-embedded block of the tissue was cut at 4 μm and placed on poly-L-lysine-coated slides. The slides were deparaffinized in xylene, rehydrated in a gradient of ethanol solutions, and then immersed in 10 mM sodium citrate buffer (pH 6.0),

Table 1 Correlations between CIP2A expression and clinicopathological features ($n = 1,280$)

Variables	n	CIP2A$^-$	CIP2A$^+$	χ^2 value	P value
Age				2.682	0.110
<40 (years)	205	123	82		
>40 (years)	1,075	709	366		
Tumor size				3.701	0.296
T1	249	174	75		
T2	926	588	338		
T3	94	63	31		
T4	11	7	4		
Histological grade				27.057	0.001
I	112	84	28		
II	817	558	259		
III	351	190	161		
Lymph node metastasis				63.711	0.001
Negative	683	376	307		
Positive	597	456	141		
Distant metastasis				78.125	0.001
Negative	922	667	255		
Positive	358	165	193		
Triple-negative breast cancer				33.886	0.001
Yes	379	201	178		
No	901	631	270		

Table 2 Multivariate analysis of the factors related to CIP2A expression

Varies	OR	95 % CI for OR	P value
Age	1.060	0.897–1.252	0.479
Tumor size	1.387	1.023–1.881	0.035
Histological grade	2.324	1.908–2.831	0.001
Lymph node metastasis	1.923	1.406–2.632	0.028
Triple-negative breast cancer	1.969	1.076–3.610	0.001
Distant metastasis	6.451	3.511–9.082	0.001
TNM stage	4.695	2.513–8.850	0.001

CI confidence interval

pretreated in a microwave oven for 10 min, followed by a 10-min rinse with phosphate-buffered saline (PBS). The sections were incubated with 3 % hydrogen peroxide for 10 min to block endogenous peroxidase activity at room temperature. Nonspecific reactions were blocked by incubating the sections in a solution containing normal serum. Then, the slides were incubated in a humid chamber at 4 °C overnight with primary antibody. Following washings with PBS, sections were incubated for 30 min in the secondary biotinylated antibody (Multilink Swine anti-goat/mouse/rabbit immunoglobulin; Dako, Inc.). Following washings, Avidin Biotin Complex (1:1,000 dilution, Vector Laboratories, Ltd.) was applied to the sections for 30–60 min at room temperature. The immunoreactive products were visualized by the catalysis of 3,3′-diaminobenzidine (DAB) by horseradish peroxidase in the presence of H_2O_2. Sections were then counterstained in Gill's hematoxylin and dehydrated in ascending grades of methanol before clearing in xylene, then mounting under a coverslip.

CIP2A expression was classified semiquantitatively according to the following criteria: 0 if <1 % of neoplastic cells discretely expressed CIP2A in their cell cytoplasmic; 1+ if ≥1 and <10 % of morphologically unequivocal neoplastic cells discretely expressed CIP2A in their cell cytoplasmic; and 2+ if ≥10 % of morphologically

Table 3 Spearman correlation analysis between clinicopathological features and CIP2A expression

Clinicopathological features	CIP2A expression (P; Spearman correlation)
Age	0.776 (0.285)
Tumor size	0.877 (0.381)
Histological grade	0.030 (2.175)
Lymph node metastasis	0.001 (3.734)
Triple-negative breast cancer	0.008 (2.647)
TNM stage	0.001 (4.413)
Distant metastasis	0.001 (5.439)

unequivocal neoplastic cells discretely expressed CIP2A in their cell cytoplasmic. Samples scored as 1+ or 2+ were considered positive.

Statistical analysis

All data were analyzed with SPSS statistics software (version 13.0, Chicago, IL, USA). Relationships between tumor markers and other parameters were studied using chi-square test, Fisher's extract test, or independent *t* tests. Disease-specific survival was analyzed using the Kaplan–Meier method. The log-rank test was used to analyze survival differences. Multivariate analysis was performed using the Cox proportional hazards model selected in forward stepwise. A *P* value of <0.05 was considered statistically significant.

Results

CIP2A staining in breast tissues

Immunochemical staining tests showed that CIP2A protein was located in the cytoplasm as yellow-to-brown staining in the breast tumor tissues (Fig. 1). In normal breast tissue, CIP2A was weakly expressed in a cytoplasm. A stronger staining quality was observed in invasive carcinomas. The expression status data of CIP2A in the 1,280 invasive carcinomas are shown in Table 1.

There was no association observed between CIP2A staining intensity and patient age or tumor size ($P = 0.110$ and 0.296, respectively). However, we found that patients with high membranous staining of CIP2A demonstrated high histological grade, more lymph node metastasis and triple-negative breast cancer ($P = 0.001$, 0.001, and 0.001, respectively) (Table 1). Patients with high cytoplasmic staining of CIP2A were prone to have distant metastasis ($P = 0.001$) (Table 1).

Relationship between CIP2A expression and various clinicopathological factors

After logistic regression analysis, the expression of CIP2A had no significant association with patient age ($P = 0.479$). CIP2A expression was significantly related to the tumor

Table 4 Correlations between CIP2A expression and chemotherapeutic resistance in breast cancers ($n = 164$; n(%))

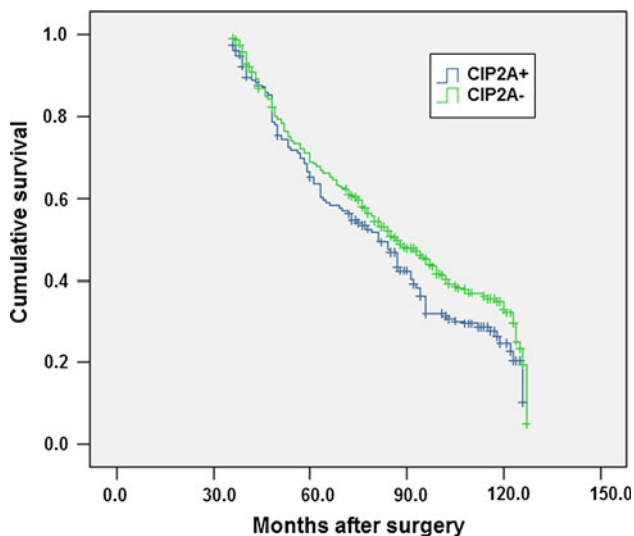|	n	CIP2A+	χ^2 value	P value
Complete response	21	3(14.29)	13.866	0.003
Partial response	76	21(27.63)		
Stable disease	39	18(46.15)		
Progressive disease	28	16(57.14)		

Fig. 2 Patients with high cytoplasmic staining of CIP2A were significantly correlated with shortened disease-free survival ($P = 0.001$)

size, histological grade, lymph node metastasis, triple-negative breast cancer, TNM stage, and distant metastasis ($P = 0.035$, 0.001, 0.028, 0.001, 0.001, and 0.001, respectively) (Table 2). We also performed Spearman correlation analysis to investigate the correlation between CIP2A expression and clinicopathological features. Finally, histological grade, lymph node metastasis, triple-negative breast cancer, TNM stage, and distant metastasis were observed to have significant correlation with CIP2A expression ($P = 0.035$, 0.001, 0.028, 0.001, 0.001, and 0.001, respectively) (Table 3).

Prognostic analysis

We further studied the relationship between CIP2A expression and chemotherapeutic sensitivity in 164 neo-adjuvant chemotherapy breast cancers. CIP2A expression was expressed in 14.29, 27.63, 46.15, and 57.14 % in complete response, partial response, stable disease, and progressive disease patients ($P = 0.003$) (Table 4).

Furthermore, CIP2A along with histological grade, lymph node metastasis, triple-negative breast cancer, and TNM stage were shown to be the prognostic factors of breast cancer in Cox model regression analysis ($P = 0.001$, 0.001, 0.006, 0.010, and 0.011, respectively); (Figs. 2, 3); (Table 5).

Discussion

A recently characterized PP2A inhibitor protein, namely cancerous inhibitor of PP2A (CIP2A), has been found to be overexpressed at a high frequency in most of the human cancer types [9, 10]. However, our understanding of gene expression programs regulated by CIP2A is almost absent. Moreover, clinical relevance of the CIP2A-regulated transcriptome has not been addressed thus far [11]. Huang et al. reported that CIP2A protein was abundantly expressed in bladder cancer cell lines but not in non-tumor epithelial cell lines. Furthermore, CIP2A was specifically expressed in transitional cell carcinoma (TCC) of the bladder tumor tissues but not in adjacent non-tumor bladder tissue. They raised that the role of CIP2A in bladder cancer progression and its usefulness for the surveillance of recurrence or progression of human bladder cancer [12]. In another study, Xue et al. investigated its expression pattern, clinical significance, and biological function in urothelial cell carcinoma (UCC) of the bladder. The study indicated that CIP2A expression was significantly associated with tumor stage, histological grade, and lymph node status. CIP2A is an independent predictor of poor prognosis of bladder UCC patients, and inhibition of its expression might be of therapeutic significance [13]. In hepatocellular carcinoma, inhibition of CIP2A determines the effects of erlotinib on apoptosis. Overexpression of CIP2A was reported to up-regulate phospho-Akt and protected Hep3B cells from erlotinib-induced apoptosis. CIP2A may be useful as a therapeutic biomarker for predicting clinical response to erlotinib in HCC treatment [14].

Recently, a study found that CIP2A was a major molecular determinant of the sensitivity of TNBC cells to bortezomib-induced apoptosis through CIP2A-dependent p-Akt down-regulation. It may be used as a target of bortezomib in human triple-negative breast cancer cells [15]. CIP2A signature was shown to cluster with basal-type and human epidermal growth factor receptor (HER) 2-positive (HER2+) breast cancer signatures. Accordingly, CIP2A protein expression was significantly associated with basal-like ($P = 0.0014$) and HER2+ breast cancers. CIP2A expression also associated with MYC gene amplification. However, there is still no clinical study addressing the clinical implications of CIP2A expression in breast cancer patients.

In our study, 35.00 % of the enrolled cases positively expressed CIP2A protein. Spearman correlation analysis showed that CIP2A expression has line correlation with histological grade, lymph node metastasis, distant metastasis, triple-negative breast cancer, and TNM stage. After multivariate analysis, tumor size, histological grade, lymph node metastasis, triple-negative breast cancer, distant metastasis, and TNM stage were related to CIP2A expression. We also investigated the relationship between CIP2A expression and chemotherapeutic sensitivity in breast cancer. It was found that CIP2A protein expression was related to the chemotherapy resistance in breast
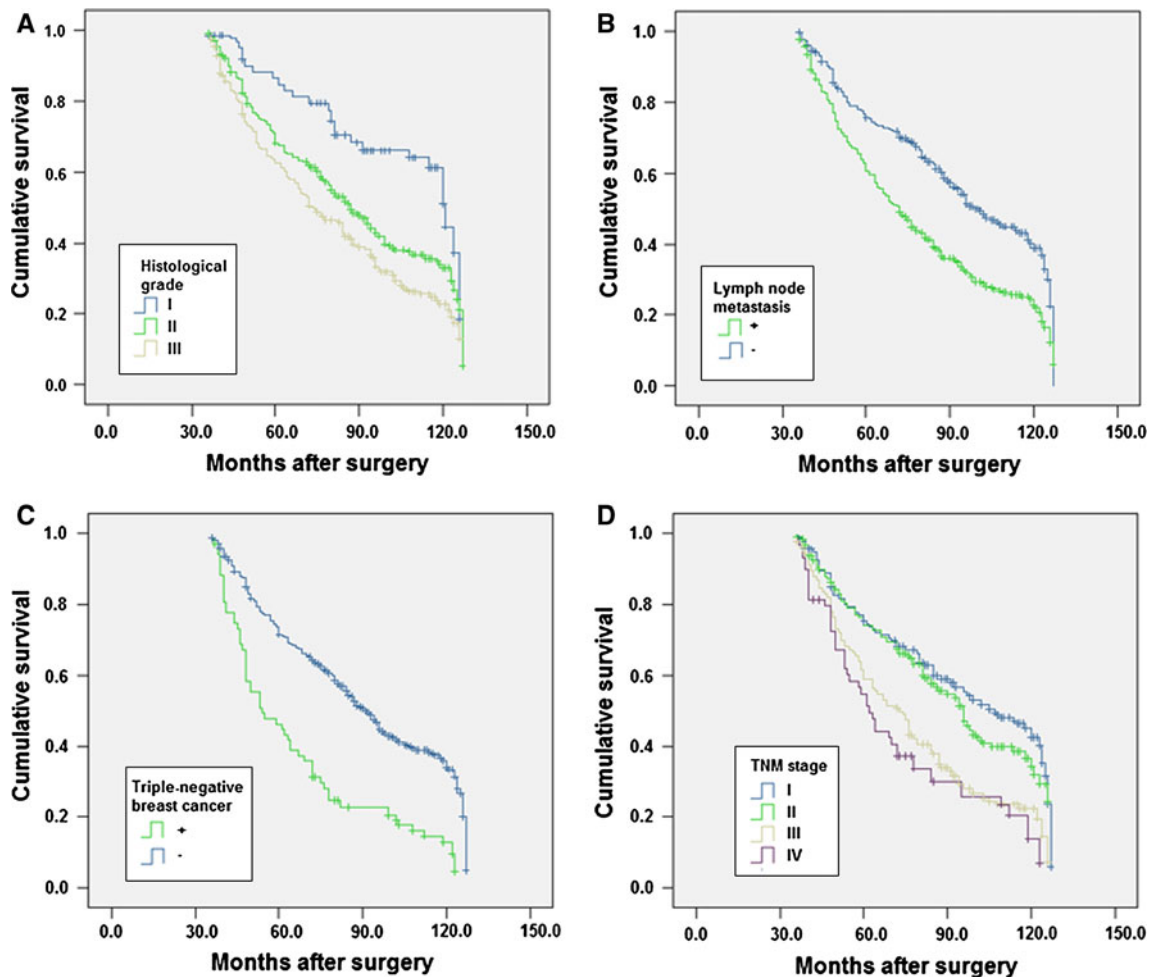
Fig. 3 Histological grade (**a**), lymph node metastasis (**b**), triple-negative breast cancer (**c**), and TNM stage (**d**) were shown to be the prognostic factors of breast cancer ($P = 0.01$, 0.001, 0.001, and 0.001, respectively)

Table 5 Cox model regression analysis of the breast cancer prognostic factors

Varies	OR	95 % CI for OR	P value
Age	0.651	1.457–0.927	0.064
Tumor size	1.047	0.859–1.275	0.651
Histological type	0.232	1.095–1.386	0.001
Lymph node metastasis	1.011	1.215–3.314	0.006
Triple-negative breast cancer	1.315	1.004–1.071	0.010
TNM stage	1.866	1.156–3.011	0.011
CIP2A expression	1.799	1.555–2.079	0.001

CI confidence interval

cancer. Finally, histological grade, lymph node metastasis, triple-negative breast cancer, TNM stage, and distant metastasis were detected as the independent prognostic factors. In conclusions, CIP2A expression may be a potential biomarker for chemotherapeutic sensitivity and prognosis of breast cancer.

Conflict of interest None.

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