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Clinical significance of sCIP2A levels in breast cancer

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Abstract. – OBJECTIVE: It has previously found that human oncoprotein cancerous inhibitor of protein phosphatase 2A (CIP2A) was overexpressed in breast cancer, and was positively correlated with lymph node metastasis of the patients. This study aimed to investigate the association between serum CIP2A and prognosis of breast cancer. Then, we investigated whether CIP2A could be as a therapeutic target in breast cancer treatment.

PATIENTS AND METHODS: Preoperative CIP2A levels of 240 patients with breast cancer and 480 cases of controls were measured by ELISA method. The association of CIP2A levels with clinicopathological outcomes was investigated by univariate and multivariate analyses. The effect of CIP2A on breast cancer MDA-MB-231 cells was evaluated by CIP2A siRNA-mediated depletion of the CIP2A protein followed by an analysis of cell proliferation, invasion, colony growth, and xenograft growth and metastasis.

RESULTS: The serum CIP2A levels in patients with breast cancer were (79.0±74.2) ng/mL, which was significantly higher than that in those controls (25.6±21.4) ng/mL for male and (24.8±20.6) ng/mL for female control. Higher preoperative CIP2A levels were significantly associated with the American Joint Committee on Cancer (AJCC) stage, histological grade and lymph node metastasis. Patients with elevated CIP2A levels showed worse survival. In multivariate analysis, elevated preoperative CIP2A levels were independent prognostic factors. Patients with high CIP2A levels had significantly shorter overall survival (OS) and disease-free survival (DFS) times. Knockdown of CIP2A by stable CIP2A siRNA transfection inhibited MDA-MB-231 cell proliferation, invasion, colony growth *in vitro*, and xenograft growth and metastasis *in vivo*.

CONCLUSIONS: Our results suggest that serum CIP2A is significantly higher in patients with breast cancer, which is a potential biomarker to make a distinction between breast cancer patients and healthy controls. Higher serum CIP2A levels positively associated with the aggressive phenotype of breast cancer, and forecasts poor prognosis for patients with breast cancer. Knockdown of CIP2A may be a novel target for prevention and treatment of breast cancer.

Key Words:
CIP2A, Breast cancer, Rognosis, Survival, siRNA.

Introduction

Breast cancer is the most common diagnosed cancer and accounts for the majority of cancer-related deaths in women worldwide[1,2], suggesting that early diagnosis and prevention for this disease is urgently needed. Among the traditional prognostic factors such as tumor grade, tumor size and lymph node status, the prognostic value of serum tumor markers has been widly investigated in breast cancer[3-7].

To date, carbohydrate antigen 15-3 (CA 15-3) is the most widely applied serum marker. However, the lack of sensitivity impeded its clinical use in early stage disease[8]. Other serum markers such as carcinoembryonic antigen (CEA) and tissue polypeptide specific antigen (TPS) are even less sensitive than CA 15-3[9,10]. At present, CA 15-3 and CEA are mainly utilized to scan therapy in metastatic breast cancer in combination with imaging, history and physical examination[10,11]. However, it should be left out that spurious rises of these markers may occur after the start of treatment without any clinical correlation[11-13].

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Cancerous inhibitor of protein phosphatase 2A (CIP2A), which is a cellular protein phosphatase 2A (PP2A) inhibitor, promotes the stability of c-Myc protein by inducing c-Myc serine 62 (S62) phosphorylation and inhibiting its degradation mediated by PP2A[14]. As an experimentally identified oncoprotein, CIP2A contributes to immortalization and malignant transformation of human cells[14]. Importantly, recent studies have found that CIP2A is overexpressed in various human malignancies, such as head and neck squamous cell carcinoma[14], oral squamous cell carcinoma[15], oesophageal squamous cell carcinoma[16], colon[14], gastric[17], prostate[18], acute myeloid leukaemia[19] and breast[20]. CIP2A has also been found to be a prognostic factor for patients with gastric cancer[21], cutaneous malignant melanoma[22], non-small cell lung cancer[23] and breast[24].

CIP2A has found to be overexpressed in human breast cancer tissues[24], and CIP2A allow the diagnosis and prognosis of breast cancer[24], and thus is useful as a diagnostic marker for breast cancer. The remarkable induction of CIP2A in breast cancer tissues may be used as a blood diagnostic marker for breast cancer. Blood is a relatively easily obtainable specimen. It has found that healthy persons and patients exhibit different plasma levels of CIP2A through detailed analysis of it[20]. We, therefore, suggested that serum CIP2A level might be used as a diagnostic marker for breast cancer.

To date, the association between prognosis and CIP2A levels in patients with breast cancer remains unclear. In the present study, we detected serum CIP2A levels in patients with breast cancer and explored its potential effects of CIP2A, and further investigated its potential effect on cell proliferation, migration and xenograft growth.

Ethics Statement

This study was approved by the Ethics Committee of Yuhuangding Hospital, Yantai, Shandong, China, according to the principles in the Declaration of Helsinki[25]. In addition, written informed consent was obtained from the patients or their next of kin in this study.

Patients and Methods

From 2005 and 2010, we investigated serum CIP2A concentration levels from a total of 240 patients who were treated for stage I-III invasive breast cancer and 480 control subjects (240 males and 240 females) at Yuhuangding Hospital Breast Care Center and Center of Physical Examination. TNM stage was based on the sixth American Joint Committee on Cancer (AJCC) criteria. All the patients with breast cancer had breast surgery. We excluded patients with stage IV disease at diagnosis, carcinoma *in situ*, unknown TNM stage and receiving neoadjuvant chemotherapy. All data including serum CIP2A levels at the time of diagnosis were obtained from the Yuhuangding Hospital breast care center, which is a prospectively maintained database that includes clinical and pathological information, treatment modality, and details of outcomes including disease recurrence and death. Retrospectively to confirm the significance of preoperative serum CIP2A levels in this longer follow-up study, including the survival outcomes, then evaluated the relationship between the level of CIP2A and clinicopathological characteristics. We detect local or distant relapse as the Cheung et al report[26]. Briefly, clinical follow-up was carried out every 6 to 12 months, which included recording patient's history, physical examination, laboratory tests of CIP2A, complete blood counts, and liver function test, chest radiography, mammography, breast and abdominopelvic ultrasonography, and bone scans. In addition, a computed tomography (CT) scan or a fluorine-18 fluorodeoxyglucose positron emission tomography (FDG PET)/CT scan was carried out if necessary. Pathology data, including tumor size, grade, lymph node involvement, and immunohistochemistry results on hormone receptors expression, were reviewed. A cut-off value of 1% or more positively stained nuclei in the high-power fields was used to define estrogen receptor (ER) and progesterone receptor (PR) positivity. Patients were recommended to undergo adjuvant therapy and surveillance according to the NCCN guidelines.

ELISA

Serum samples were shock frozen in liquid N2 and stored at -80°C until analysis. CIP2A ELISA assays (Shanghai, China) were performed according to the manufacturer's protocol. In brief, 100 ml assay diluent was added to the microplate wells. The wells were then precoated with the monoclonal anti-CIP2A antibody followed by adding the patient's serum. The wells were incubated for 2 h at 25°C, aspirated and washed. An-

ti-CIP2A antibody conjugated to horseradish peroxidase (HRP: 200 ml) was added for another 2 h incubation and three washes followed. Then, 200 ml substrate solution (chromogen, hydrogen peroxide, and tetramethyl benzidine) was added to each well. After 30 min incubation, the reaction was stopped adding 50 ml 1 M sulfuric acid. The optical density was measured at 450 nm using an automated microplate reader (Thermo Fisher Scientific, Waltham, MA, USA).

Cell Culture

MDA-MB-231 cell line was purchased from the American Tissue Culture Collection (ATCC, Shanghai, China). The cells were cultured in DMEM (Life Technologies, Inc., Hangzhou, China) supplemented with 10% FCS, 2 mM glutamine, and 1% nonessential amino acids (complete medium).

siRNA Transfection

siRNA (h) targeting CIP2A: 5′-CUGUGGUUGUGUUUGCACUTT-3′, siRNA (h) targeting scrambled: 5′-UAACAAUGAGAGCACGGCTT-3′ was acquired from Shenggong, Shanghai, China. Using oligofectamine reagent (Invitrogen, Beijing, China), MDA-MB-231 cells were transfected with CIP2A siRNA (50 nM) or scrambled siRNA (50 nM) for 24 h according to the manufacturer's instructions and analyzed the expression of CIP2A in the MDA-MB-231 cells by Western blot method.

Stably expressed clones were selected using medium containing G418 (400 μg/ml) for 14-21 days. The stable transfectants were named MDA-MB-231/CIP2A siRNA and MDA-MB-231/control siRNA respectively.

Western Blot Analysis

siRNA stably transfected MDA-MB-231 cells were harvested, pelleted by centrifugation, then washed with ice-cold PBS and lysed with RIPA buffer supplemented with protease inhibitor. Proteins were separated by 8-12% SDS-polyacrylamide gel electrophoresis and transferred onto a nitrocellulose membrane (Life Technologies, Gaithersburg, MD, USA). After extensive washing, the membranes were incubated with horseradish peroxidase (HRP)-coupled secondary antibody (1:2000, Zhongshan Biotech Company, China) at 25 °C for 1 h. Blots were then incubated in fresh blocking solution with an appropriate dilution of primary CIP2A or GAPDH antibody (Santa Cruz Biotech, Shanghai, China) at 4°C for 24 h. Blots were analysed by using ECL Plus Western blotting detection reagents according to the manufacturer's instructions.

Cell Invasion Assay

Cell invasion assays were carried out in chemotaxis chambers. Briefly, siRNA stably transfected MDA-MB-231cells (MDA-MB-231/CIP2A siRNA and MDA-MB-231/control siRNA) (6×10^4) were suspended in 50 μL serum-free medium, and added to the upper chamber. The lower chamber was full of complete medium. Polycarbonate membrane (8 μm) covered with culture medium containing 20 μL/mL Matrigel was put between the two chambers. Cells were invaded at 37°C for 36 hours. The membrane was fixed in methanol and stained with Giemsa after removing non-invaded cells. Invaded cells were photographed and quantified in 10 random fields per membrane. Each sample was analyzed in triplicate by Student's *t*-test, $p < 0.05$ indicated statistical significance.

Migration Assay

siRNA stably transfected MDA-MB-231 cells (MDA-MB-231/CIP2A siRNA and MDA-MB-231/control siRNA) (6×10^4) were cultivated on a monolayer in a 24-well plate for 24 h. Then, a wound (2 mm width) was scratched into confluent cultures of MDA-MB-231/CIP2A siRNA and MDA-MB-231/control siRNA cells. Randomly marked wound regions with an identical width were studied. Then, the wound closure (the percentage of closed scratch area) was measured (magnification, ×40).

MTT Assay

siRNA stably transfected MDA-MB-231 cells were seeded in 96-well plates at 5×10^3 per well for different times (24 h, 48 h and 72 h), the cell densities were determined by the MTT assay. The results were plotted as means ± SD of 3 separate experiments.

Soft-agar Colony Assay

siRNA stably transfected MDA-MB-231 cells were plated (5×10^4-1×10^5 per well) in a six-well plate and incubated overnight at 37°C. The colonies in the soft agar were counted in all wells after 12 days as the manufecture's instruction.

Tumor Growth and Metastases in vivo

Immunodeficient female mice (4-6 weeks old) were purchased from Shanghai Animal Center.

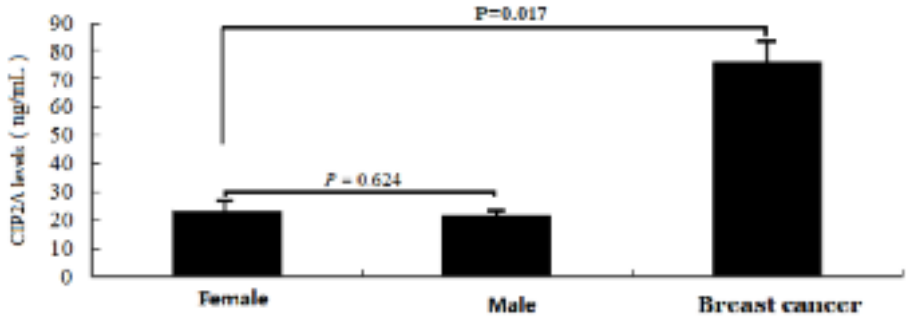

Figure 1. Serum levels of CIP2A in normal female and male controls, breast cancers. The normal controls and breast cancer patients are the average values of three measurements.

Mouse body weight was measured every 3-4 days. For metastasis observation, mice were injected in the abdominal cavity with siRNA stably transfected MDA-MB-231 cells (6×10^6). The experiments were terminated for 21 days. Animals were sacrificed by CO_2 inhalation. The autopsy was carried out for assessment of metastases. The number of the seeded tumor in the abdominal cavity is used to assess metastases. For growth assessment, xenograft tumors were established by s.c. Injection of siRNA stably transfected MDA-MB-231 cells (1×10^8) into the flanks of the female nude mice (n = 6 per group). The experiments stop for 21 days. Tumor growth was monitored thrice a week by calipers to calculate tumor volumes according to the formula [length×width^2]/2.

Immunohistochemistry

Immunohistochemical studies were done on 4-μm-thick sections, which were derived from zinc-fixed, paraffin wax-embedded tumor tissue blocks. These tumors were fetched at the end of the experiments (after 21 days). Sections were subsequently dewaxed, rehydrated, and had endogenous peroxidase activity quenched before CIP2A specific immunohistochemical staining. After specific staining, the sections were dehydrated in alcohol and xylene and subsequently mounted.

Statistical Analysis

The difference between CIP2A and clinicopathological characteristics was analyzed by the *t*-test. Disease-free survival (DFS) was to be from the time of surgery to the locoregional recurrence, distant metastasis, or/and death before recurrence. Overall survival (OS) was to be from the time of surgery to death from any cause. DFS and OS were analyzed using the Kaplan-Meier method and the group differences in survival time were tested using the log-rank test. Multivariate Cox's proportional hazard analysis was carried out to compare and identify independent prognostic factors for DFS and OS and to calculate hazard ratios. All significant parameters in the univariate analysis were entered into a multivariate model.

In vitro and *in vivo*, data presented are means of the three or more independent experiments ± *SE*. Statistically significant differences were determined by X^2 and Student's *t*-test. p values < 0.05 were considered significant. *SPSS* 11.0 was used for all statistical analyses (SPSS Inc., Chicago, IL, USA.

Results

Detection of Serum CIP2A Levels in Control Persons and Breast Cancer Patients

The serum CIP2A levels in different control groups were analyzed by ELISA method. The measurements were performed on the serum of 480 normal persons. The mean value of serum CIP2A levels was at 25.6±21.4 ng/mL for male controls and 24.8±20.6 ng/mL for female controls. They showed no statistical significance between the two groups (Figure 1; p = 0.624). The serum CIP2A level in patients with breast cancer was 79.0±74.2 ng/mL, which was significantly higher than that in those controls (Table I; p=0.017).

Serum CIP2A Levels and Clinically Relevant Parameters in Breast Cancer Patients

Within the patients with lymph node metastasis, the serum CIP2A levels were 135.6±104.5 ng/ml,

which was significantly higher than that in those patients without lymph node metastasis (56.0 ±53.5 ng/ml; p=0.027; Table I). The patients with N3 lymph node status had significantly higher serum CIP2A levels when compared with those with N0 (p=0.02) and N1 (p=0.04; data not shown). In patients with high AJCC stage and high histological grade, the serum levels of CIP2A were also significantly higher (Table I). No correlation was observed between serum CIP2A levels and patient age, menopausal status, tumor size, ER, PR, and HER2 expression (Table I).

Serum CIP2A levels, OS and DFS

To evaluate the relation of serum CIP2A levels with survival, patients were dichotomized according to their median serum CIP2A levels. The median serum CIP2A levels in breast cancer patients was 79 ng/ml, and was selected as a cut-off, because it classified half of the breast cancer patients into high and low CIP2A levels groups. At a median follow-up of 5.8 years, 40 of 240 (15%) of patients had died (23 patients with low CIP2A (≤79 ng/ml) and 17 with high (>79 ng/ml). Age, tumor size, nodal status, tumor grade, and log CIP2A serum levels were significantly associated with breast cancer-specific survival in univariate analysis as well (Table II). The independent effect of CIP2A levels on OS was assessed by multivariate Cox proportional hazard models adjusted for age, tumor size, nodal status, and tumor grade. In these multivariate analyses, log CIP2A serum concentrations remained significantly associated with OS (Table

Table I. Serum CIP2A levels and clinically relevant parameters in breast cancer patients.

Variables	Number	CIP2A levels (ng/ml) Mean	p-value
Age			0.536
<50 years	112	83.0±74.5	
≥50 years	128	81.0 ±72.4	
Menopausal status			0.48
No	138	76.6±71.4	
Yes	102	79.0±69.5	
Size			0.079
<2 cm	99	72.4±67.3	
≥2 cm	141	93.7±88.5	
AJCC stage			0.02
I+II	130	65.3±61.7	
III	110	112.0±98.5	
Histological grade			0.037
1+2	174	68.5±63.4	
3	66	106.8±93.4	
Lymph node metastasis			0.027
No	92	56.0±53.5	
Yes	148	135.6±104.5	
ER expression			0.092
Negative	98	67.4±64.9	
Positive	142	98.3±87.3	
PR expression			0.156
Negative	110	67.4±66.4	
Positive	130	87±74.8	
HER2 expression			0.073
Negative	102	64.6±61.3	
Positive	138	92.4±83.5	

Table II. Uni- and multivariate Cox proportional hazard models for overall survival (OS).

	Univariate			Multivariate		
Variable	HR for overall survival	95% CI	*p* value	HR for overall survival	95% CI	*p*-value
Age	1.06	1.01-1.10	0.016	1.04	1.02-1.08	0.02
Tumor size	1.04	1.02-1.08	0.01	1.03	1.01-1.06	0.01
nodal status	2.17	1.12-3.97	0.03	1.34	0.56-2.15	0.36
tumor grade	2.32	1.42-3.25	0.015	2.33	1.34-3.15	0.004
Log. CIP2A	3.86	1.84-10.3	0.003	3.21	1.29-8.94	0.02

CIP2A siRNA inhibits CIP2A expression

II). Kaplan-Meier estimates for OS demonstrated a significantly poorer outcome if CIP2A levels were higher than the median CIP2A levels of 79 ng/ml in our population investigated (p=0.02, log-rank test; Figure 2A). Survival curves of Figure 2B showed elevated CIP2A levels were clearly associated with poor DFS. Patients with <79 ng/ml levels of CIP2A levels showed better DFS than those with elevated CIP2A levels (p=0.014).

As determined by Western blot analysis, siRNA stably transfected MDA-MB-231 cells displayed significant reduction in the expression levels of CIP2A protein (Figure 3A). Control siRNA did not exhibit any effect on protein levels of CIP2A (Figure 3A). These data confirmed the suppression effect of siRNA, and proved the efficiency of siRNA transfection.

Proliferation Assays

Next, we determined the effect of CIP2A siRNA or control siRNA on proliferation in MDA-MB-231 cells. As shown in Figure 3B, CIP2A siRNA significantly inhibited MDA-MB-231 cells proliferation compared with control siRNA at 24, 48, and 72 hs, respectively.

Colony Formation Assay

MDA-MB-231/CIP2A siRNA or MDA-MB-231/control siRNA cells (8 x10^3/ml) were maintained in 1 ml of 0.3% basal medium Eagle's agar (BMEA) containing serum at 37°C in a humidified incubator for 14 days. Using three different plates, the cell colonies were counted under a microscope. As showed in Figure 3C, compared with MDA-MB-231/control siRNA, MDA-MB-231/CIP2A siRNA cells demonstrated a significant decrease in colony formation. After 14 days culture, the colonies that MDA-MB-231/CIP2A siRNA cells formed was 46.4% of MDA-MB-231/control siRNA.

Effect of CIP2A knockdown on MDA-MB-231Cell Invasion

The result (Figure 3D) from Matrigel invasion assay indicates that CIP2A knockdown with siR-

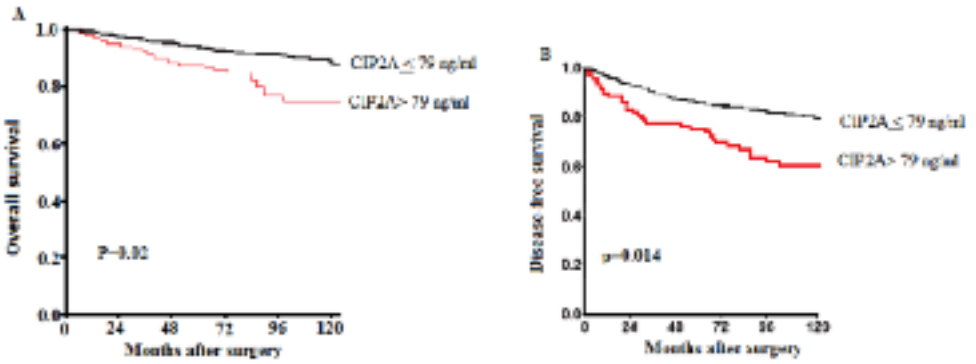

Figure 2. Overall survival (OS) and disease-free survival (DFS) according to preoperative CIP2A levels in breast cancer patients.

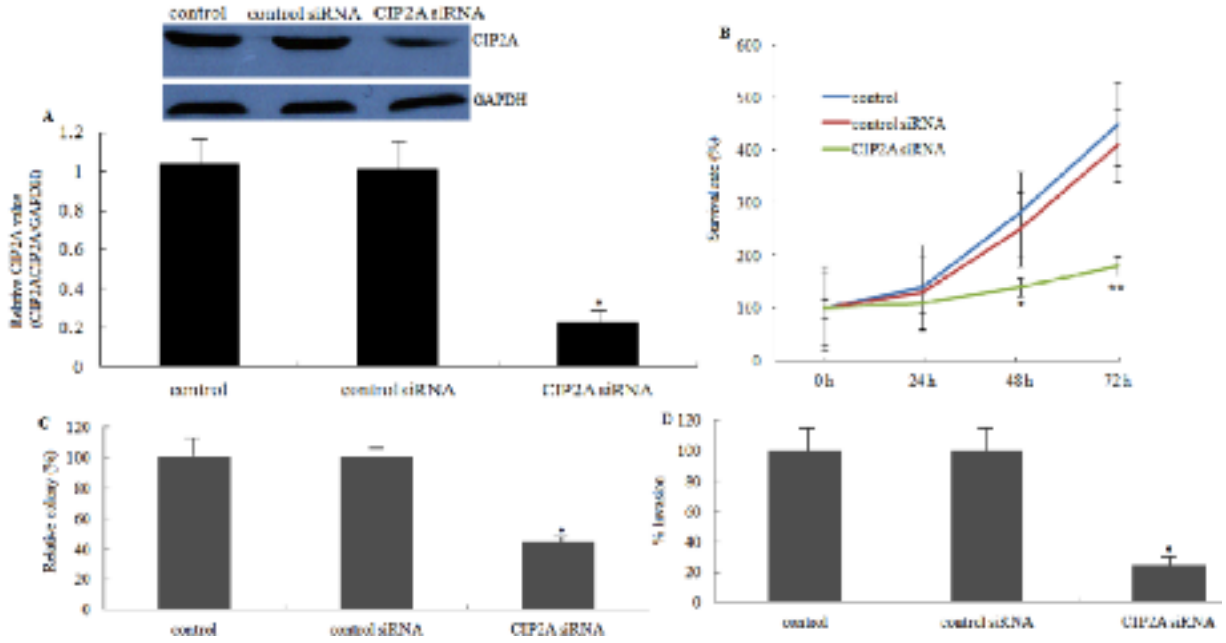

Figure 3. Effect of CIP2A siRNA knocks down on CIP2A expression, proliferation, colony formation and invasion. ***A,*** CIP2A expression in siRNA stably transfected MDA-MB-231 cells by Western blot assay; ***B,*** Effect of CIP2A siRNA knocks down on proliferation at 24, 48, and 72 hs in siRNA stably transfected MDA-MB-231 cells by MTT assay. ***C,*** Effect of CIP2A siRNA knocks down on colony formation at 14 days in siRNA stably transfected MDA-MB-231 cells. ***D,*** Effect of CIP2A siRNA knocks down on invasion of siRNA stably transfected MDA-MB-231 cells. Vs. control/control siRNA, *p<0.05; **p<0.01.

NA significantly inhibited the invasion of MDA-MB-231 cells by 76%, as compared with control siRNA transfected MDA-MB-231 cells.

In vivo Inhibition of Tumor Growth and Metastasis

All mice receiving MDA-MB-231/CIP2A siRNA were alive and exhibited a healthier appearance. As shown in Figure 4A, MDA-MB-231/CIP2A siRNA groups showed significantly decreased tumor volume by (69 ± 8)% (p=0.03) relative to the control siRNA groups. Control siRNA failed to inhibit tumor growth.

Immunohistochemical staining revealed that high levels of CIP2A expression was in the orthotopic tumors of control siRNA groups, and low levels of CIP2A expression was in the orthotopic tumors of CIP2A siRNA groups (Figure 4B).

We then tested the effect of CIP2A siRNA on MDA-MB-231 pseudometastatic models. The mice were injected with stable CIP2A siRNA or control siRNA-transfected MDA-MB-231 cells (1×10^8) at the site of the abdominal cavity on day 0. After 21 days, the animals were sacrificed, and autopsy was used to remove organs. There were many off-white nodus in peritoneum, mesentery, retina, intestinal and gastric wall. Autopsy showed a reduction of seeded tumor nodes in abdominal cavity organs in CIP2A siRNA-transfected groups. The number of seeded tumor was 47.6±9.4 and 165.8±15.6 in MDA-MB-231/CIP2A siRNA groups and control siRNA groups, respectively. Differences of seeded tumor reached statistical significance (Figure 4C, p = 0.02). Immunohistochemical staining revealed high levels of CIP2A expression in the control siRNA groups, and low levels of CIP2A expression in the CIP2A siRNA groups (data not shown).

Discussion

Serum tumor markers are soluble molecules, which are released into the blood stream by cancer cells or other cell types belonging to tumor microenvironment[27]. To measure these molecules is considered an economic and noninvasive diagnostic assay, it is able to give information about the presence or absence of disease as well as its

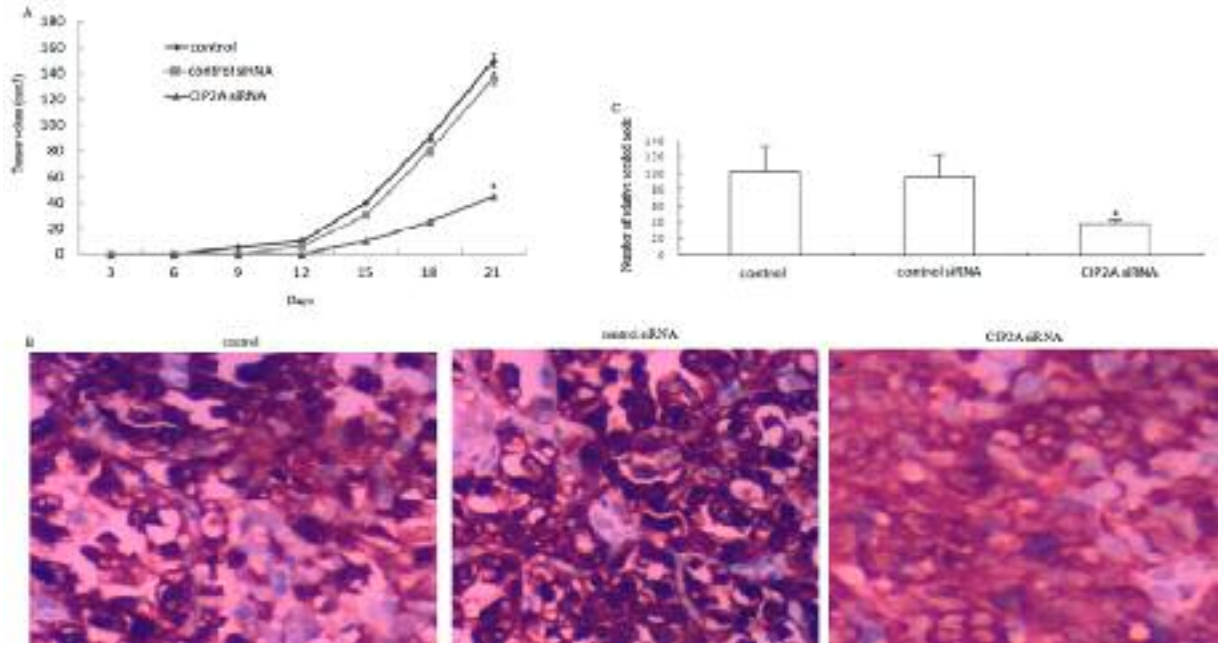

Figure 4. Tumor growth and metastasis inhibition *in vivo* by CIP2A siRNA. ***A,*** Survival curves of pseudometastatic neuroblastoma-bearing mice in response to small interfering RNA (CIP2A siRNA) and control siRNA (n=6). ***B,*** Immunohistochemistry (IHC) staining performed on formalin-fixed and paraffin-embedded tumor sections of CIP2A subcutaneous (x200). ***C,*** Seeded tumor in abdominal cavity organ of each animal was evaluated for the extent of metastatic invasion. Significant differences between the control siRNA and CIP2A siRNA groups are indicated ($p<0.05$). Mean values of 3 independent experiments are shown.

evolution. In case of breast cancer (BC), different serum markers were used, and to date, the most used in clinical practice are carcinoembryonic antigen (CEA), tissue polypeptide specific antigen (TPS), cytokeratin 19 fragment (CIFRA-21-1), the soluble form of MUC-1 protein (CA15-3), circulating cytokeratins such as tissue polypeptide antigen (TPA) and the proteolytically cleaved ectodomain of the human epidermal growth factor receptor 2 (s-HER2). Although all of these markers are routinely used in clinical practice, none is useful for screening programs and/or early diagnosis of BC[27,28].

CIP2A was recently identified as a human oncoprotein, and overexpressed in many cancers, including breast cancer[14-19]. In breast cancer, CIP2A expression has positive correlation with histological grade, lymph node metastasis, distant metastasis, triple-negative breast cancer, and TNM stage[24]. Tumor size, histological grade, lymph node metastasis, triple-negative breast cancer, distant metastasis, and TNM stage were related to CIP2A expression[24]. However, the clinical role of serum CIP2A levels and its association with breast cancer has not yet been established.

The present study provides the first evidence that serum CIP2A was significantly higher in patients with breast cancer. Higher preoperative blood CIP2A levels were significantly associated with AJCC stage, histological grade and lymph node metastasis. Patients with elevated CIP2A levels showed worse survival. In multivariate analysis, elevated preoperative CIP2A levels were independent prognostic factors. Patients with high CIP2A levels had significantly shorter overall survival (OS) and disease-free survival (DFS) times. We, therefore, suggested that CIP2A might be a novel target for prevention and treatment of breast cancer.

In lung cancer cells, silencing CIP2A by siRNA inhibited the proliferation and clonogenic activity *in vitro*[29]. In hepatocellular cancer (HCC), inhibition of CIP2A determines the effects of erlotinib on apoptosis[30]. In breast cancer, CIP2A may be a target for gene treatment *in vitro*[20]. In the present study, we found that CIP2A inhibition significantly suppresses proliferation, invasion, colony growth *in vitro*, and xenograft growth and metastasis *in vivo*. These results demonstrated a critical role of CIP2A in driving disease progression and spread of breast cancer.

Conclusions

It was found that serum CIP2A is significantly higher in the patients with breast cancer and plays an important role in breast cancer progress. Higher levels of CIP2A were positively correlated with the aggressive phenotype of breast cancer, and predicted poor outcome of patients. Sliencing CIP2A by stable CIP2A siRNA transfection inhibited proliferation, invasion, colony growth, and xenograft growth and metastasis. Therefore, serum CIP2A level may be as a marker for diagnosis of breast cancer, and as a novel potential target for prevention and treatment of breast cancer.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.
2) Seymour JD, Calle EE, Flagg EW, Coates RJ, Ford ES, Thun MJ. Diet Quality Index as a predictor of short-term mortality in the American Cancer Society Cancer Prevention Study II Nutrition Cohort. Am J Epidemiol 2003; 157: 980-988.
3) Elston CW, Ellis IO, Pinder SE. Pathological prognostic factors in breast cancer. Crit Rev Oncol Hematol 1999; 31: 209-223.
4) Molina R, Auge JM, Farrus B. Prospective evaluation of carcinoembryonic antigen (CEA) and carbohydrate antigen 15.3 (CA 15.3) in patients with primary locoregional breast cancer. Clin Chem 2010; 56: 1148-1157.
5) Sandri MT, Salvatici M, Botteri E. Prognostic role of CA15.3 in 7942 patients with operable breast cancer. Breast Cancer Res Treat 2012; 132: 317-326.
6) Uehara M, Kinoshita T, Hojo T. Long-term prognostic study of carcinoembryonic antigen (CEA) and carbohydrate antigen 15-3 (CA 15-3) in breast cancer. Int J Clin Oncol 2008; 13: 447-451.
7) Maric P, Ozretic P, Levanat S. Tumor markers in breast cancer evaluation of their clinical usefulness. Coll Antropol 2011; 35: 241-247.
8) [No authors listed] Clinical practice guidelines for the use of tumor markers in breast and colorectal cancer. Adopted on May 17, 1996 by the American Society of Clinical Oncology. J Clin Oncol 1996; 14: 2843-2877.
9) Guadagni F, Ferroni P, Carlini S, Mariotti S, Spila A. A reevaluation of carcinoembryonic antigen (CEA) as a serum marker for breast cancer: a prospective longitudinal study. Clin Cancer Res 2010; 7: 2357-2362.
10) Duffy MJ, Evoy D, McDermott EW. CA 15-3: uses and limitation as a biomarker for breast cancer. Clin Chim Acta 2010; 411: 1869-1874.
11) Harris L, Fritsche H, Menel R, Norton L, Ravdin P. American Society of Clinical Oncology 2007 Update of recommendations for the use of tumor markers in breast cancer. J Clin Oncol 2011; 25: 5287-5312.
12) Sturgeon CM, Duffy MJ, Stenman UK, Lilja H, Brunner N, National Academy of Clinical Biochemistry Laboratory Medicine practice guidelines for use of tumor markers in testicular, prostate, colorectal, breast and ovarian cancers. Clin Chem 2008; 54: e11-79.
13) D'Alessandro R, Roselli M, Ferroni P, Mariotti S, Spila A. Serum tissue polypeptide specific antigen (TPS): a complementary tumor marker to CA 15-3 in the management of breast cancer. Breast Cancer Res Treat 2013; 68: 9-19.
14) Junttila MR, Puustinen P, Niemela M, Ahola R, Arnold H, Bottzauw T, Ala-aho R, Nielsen C, Ivaska J, Taya Y, Lu SL, Lin S, Chan EK, Wang XJ, Grenman R, Kast J, Kallunki T, Sears R, Kahari VM, Westermarck J. CIP2A inhibits PP2A in human malignancies. Cell 2007; 130: 51-62.
15) Basile JR, Czerninski R. The role of CIP2A in oral squamous cell carcinoma. Cancer Biol Ther 2010; 10: 700-702.
16) Qu W, Li W, Wei L, Xing L, Wang X, Yu J. CIP2A is overexpressed in esophageal squamous cell carcinoma. Med Oncol 2010; 14: 236-241.
17) Li W, Ge Z, Liu C, Liu Z, Bjorkholm M, Jia J, Xu D. CIP2A is overexpressed in gastric cancer and its depletion leads to impaired clonogenicity, senescence, or differentiation of tumor cells. Clin Cancer Res 2008; 14: 3722-3728.
18) Vaarala MH, Vaisanen MR, Ristimaki A. CIP2A expression is increased in prostate cancer. J Exp Clin Cancer Res 2010; 29: 136-141.
19) Wang J, Li W, Li L, Yu X, Jia J, Chen C. CIP2A is over-expressed in acute myeloid leukaemia and associated with HL60 cells proliferation and differentiation. Int J Lab Hematol 2011; 33: 290-298.
20) Come C, Laine A, Chanrion M, Edgren H, Mattila E, Liu X, Jonkers J, Ivaska J, Isola J, Darbon JM, Kallioniemi O, Thezenas S, Westermarck J. CIP2A is associated with human breast cancer aggressivity. Clin Cancer Res 2009; 15: 5092-5100.
21) Khanna A, Bockelman C, Hemmes A, Junttila MR, Wiksten JP, Lundin M, Junnila S, Murphy DJ, Evan GI, Haglund C, Westermarck J, Ristimaki A. MYC-dependent regulation and prognostic role of CIP2A in gastric cancer. J Natl Cancer Inst 2009; 101: 793-805.
22) Shi F, Ding Y, Ju S, Wu X, Cao S. Expression and prognostic significance of CIP2A in cutaneous malignant melanoma. Biomarkers 2014; 19: 70-6.

23) Dong QZ, Wang Y, Dong XJ, Li ZX, Tang ZP, Cui QZ, Wang EH. CIP2A is overexpressed in non-small cell lung cancer and correlates with poor prognosis. Ann Surg Oncol 2011; 18: 857-865.

24) Yu G, Liu G, Dong J, Jin Y. Clinical implications of CIP2A protein expression in breast cancer. Med Oncol 2013; 30: 524-526.

25) Deutsch E. Medical experimentation: international rules and practice. Law Rev 1989; 19: 1-10.

26) Cheung SY, Boey YJ, Koh VC, Thike AA, Lim JC, Iqbal J, Tan PH. Role of epithelial-mesenchymal transition markers in triple-negativebreast cancer. Breast Cancer Res Treat 2015; 152: 489-98

27) Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, Dowsett M, Fitzgibbons PL, Hanna WM, Langer A, McShane LM, Paik S, Pegram MD, Perez EA, Press MF, Rhodes A, Sturgeon C, Taube SE, Tubbs R, Vance GH, van de Vijver M, Wheeler TM, Hayes DF; American Society of Clinical Oncology/College of American Pathologists. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. Arch Pathol Lab Med 2007; 131: 18-43.

28) Karantza V. Keratins in health and cancer: more than mere epithelial cell markers. Oncogene 2011; 2: 127-138.

29) Ma L, Wen ZS, Liu Z, Hu Z, Ma J, Chen XQ, Liu YQ, Pu JX, Xiao WL, Sun HD, Zhou GB. Over expression and small molecule-triggered down regulation of CIP2A in lung cancer. PLoS One 2011; 6: e20159.

30) Yu HC, Chen HJ, Chang YL, Liu CY, Shiau CW, Cheng AL, Chen KF. Inhibition of CIP2A determines erlotinib-induced apoptosis in hepatocellular carcinoma. Biochem Pharmacol 2013; 85: 356-366.