Chromobox homolog 2 protein: A novel biomarker for predicting prognosis and Taxol sensitivity in patients with breast cancer

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Received March 5, 2015; Accepted November 10, 2016

DOI: 10.3892/ol.2016.5529

Abstract. Polycomb group (PcG) complexes modify histones to silence tumor suppressor genes, which exhibit an important function in tumorigenesis and progression. The chromobox (Cbx) protein family is a critical component of PcG-mediated repression. Cbx2, a member of the Cbx protein family, is hypothesized to exhibit a vital role in breast cancer. In the present study, immunohistochemical analysis using tissue microarrays was performed to determine the levels of Cbx2 protein expression in breast cancer. The association between Cbx2 expression and the clinical features and prognosis of 455 breast cancer patients was analyzed. In addition, the efficacy of Taxol was evaluated by comparing the survival of patients with high or low Cbx2 expression. The results revealed that Cbx2 expression was higher in cancer tissues compared with adjacent normal tissues. Furthermore, high Cbx2 expression was significantly associated with large tumor size, lymph node metastasis, high TNM stage and positive human epidermal growth factor receptor-2 (HER-2) status. Patients with high Cbx2 expression also exhibited a shorter mean overall survival (OS) time (74.37 months) compared with patients with low Cbx2 expression (77.37 months). Univariate analysis indicated that high Cbx2 expression increased the risk of mortality by 1.826-fold compared with low Cbx2 expression [hazard ratio (HR), 1.826; 95% confidence interval (CI), 1.069-3.116; P=0.027]. Among patients with high Cbx2 expression, the mean OS time of individuals treated with Taxol (71.01 months) was lower compared with patients that had not received Taxol treatment (78.43 months; log-rank test statistic, 13.03; P<0.001). However, no significant difference in OS time was identified in the low expression group. The results of the current study revealed that Cbx2 may present a novel biomarker for predicting the prognosis of breast cancer patients. Cbx2 may also represent a potential target for treatment due to its important function in Taxol treatment responses.

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Key words: chromobox2, chromobox protein, breast cancer, Taxol, survival

Introduction

Breast cancer is the most common type of cancer among women worldwide, accounting for 29% of novel cancer cases in 2014 (1). A total of 39,620 breast cancer mortalities were reported among women in 2013 in the USA despite constant breast cancer incidence (2). Considering the heterogeneity of breast cancer, diverse terms have been used to explain the underlying biological and pathological characteristics, responses to therapy and clinical outcomes (3). The molecular mechanisms of carcinogenesis are complex due to aberrant protein expression, gene changes and miRNA deregulation. Therefore, numerous studies have focused on screening for novel diagnostic and prognostic biomarkers and therapeutic targets in breast cancer (4-6).

Polycomb group (PcG) complexes mediate the inherent stability of cells. These proteins regulate the expression of numerous genes that control the maintenance, differentiation and proliferation of adult stem cells and cancer cells (7). Biochemical characterization has categorized PcG complexes into two subtypes: Polycomb repressive complex (PRC) 1 and PRC2 (8-10). The chromobox (Cbx) family comprises five members (Cbx2, Cbx4, Cbx6, Cbx7 and Cbx8) in mammals (11), and it is a component of PRC1. Numerous studies have indicated that the Cbx family is associated with cancer. High Cbx7 expression has been found to associate with ovarian clear cell adenocarcinoma, lymphomagenesis and gastric cancer (12-14). Cbx4 exerts a critical function in tumor angiogenesis by controlling the hypoxia-inducible factor-1α protein (15). However, the association between Cbx2 expression and cancer remains unclear. Recent evidence has

confirmed that the overexpression of Cbx2 results in the differentiation and exhaustion of hematopoietic stem cells (16). Notably, a number of malignant tumors with normal gene copy numbers demonstrate recurrent Cbx2 overexpression (17).

In the present study, Cbx2 protein expression was analyzed by immunohistochemistry (IHC) using tissue microarrays (TMAs) in an independent cohort of patients with breast cancer. Furthermore, the association between Cbx2 expression and the clinicopathological features, survival and chemotherapy outcomes of breast cancer patients were analyzed. The aim of the study was to determine whether Cbx2 presents a potential prognostic marker and alternative therapeutic target in breast cancer.

Materials and methods

Patients and clinical samples. A total of 455 patients primarily diagnosed with breast cancer, who underwent surgery at The Affiliated Tumor Hospital of Harbin Medical University (Harbin, China) between March and December in 2006, were consecutively recruited for the present study. None of the patients had received any treatment prior to surgery. All patients were pathologically diagnosed with invasive ductal cancer according to the World Health Organization classification of breast tumors (18) and the median age of patients was 49 years (range, 25-78 years). Patients with a previous history of tumors, including recurrent tumors, metastatic disease and bilateral tumors, and patients who had previously received neoadjuvant treatment were excluded. A total of 455 tumor tissue specimens and 216 corresponding adjacent normal tissues located 5 cm from the cancer margin were archived from the Department of Pathology at the Affiliated Tumor Hospital of Harbin Medical University. A total of 216 paired tumor and normal breast tissues and 239 unpaired tumor tissues were collected for further study. Patient information regarding tumor size, pathological grade, lymph node status and chemotherapy treatment were obtained from medical records. Among the 309 patients with complete records of adjuvant chemotherapy regimen, 7 accepted Taxol cis-platinum treatment, 13 accepted taxol fluorouracil treatment, 43 accepted Taxol epirubicin treatment, 9 accepted nedaplatin cis-platinum treatment, 10 accepted nedaplatin fluorouracil treatment, 22 accepted nedaplatin epirubicin treatment, 143 accepted fluorouracil epirubicin cyclophosphamide treatment, 28 accepted epirubicin Taxol treatment and 34 accepted more than two regimens. According to regimens including Taxol, patients were split into two groups. One group contained 122 patients who received chemotherapy, including Taxol, and another group contained 187 patients who received chemotherapy without Taxol. All patients provided written informed consent for the use of their clinical specimens for medical research. This study was approved by the research medical ethics committee of Harbin Medical University.

Histology, TMA and IHC. The tissues obtained from surgical removal were rapidly fixed in 10% neutral buffered formalin. Subsequent to dehydration, clearing, infiltration and paraffin-embedding, the prepared tissue blocks were cut into 4-mm sections for hematoxylin and eosin staining. Three cores (2 mm in diameter) were obtained from each breast cancer sample and inserted into the recipient TMA blocks. A total of 216 invasive ductal carcinomas and corresponding normal breast tissue samples were inserted into three TMA blocks, and 239 unpaired cancer tissues were fixed in two TMA blocks. All TMA blocks were cut with a microtome to 4-μm sections and affixed to a slide treated with 5% poly-lysine.

Estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor-2 (HER-2) and tumor protein 53 (p53) expression and Ki-67 were routinely assayed by IHC, as previously described (19). Briefly, IHC staining was performed for ER and PR using 4-μm paraffin sections cut from TMA blocks. ER, PR and HER-2 markers were immunostained in a single process using hematoxylin and eosin stains and the following primary monoclonal antibodies: Mouse anti-human ER (1:100; ZM-0104), mouse anti-human PR (1:150; ZM-0215), mouse anti-human HER2 (1:100; ZM-0065) (Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd., Beijing, China), mouse anti-human Ki-67 (1:100; IR62661; Dako, Glostrup, Denmark) and mouse anti-human p53 (1:400; sc-47698; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) antibodies. Samples were incubated with the primary antibodies overnight at 4˚C, followed by incubation with a biotin-labeled goat anti-mouse immunoglobulin (Ig) G secondary antibody (SP-9002; Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd.) at room temperature for 30 min. Fluorescence *in situ* hybridization (FISH) assays were performed to determine HER-2 status in tumors with 2+ immunoreactivity according to guidelines (20). Tumor cells were considered to exhibit positive ER and PR expression when >10% of the tumor cell nuclei were stained in the three cores. Tumor cells were considered to exhibit HER-2 protein overexpression when >10% of cells exhibited strong membrane staining (3+) or positive signals in the FISH tests (21). The Ki-67 score was defined as the percentage of positively stained cells, regardless of the intensity, among the total number of invasive cells in the scored area (22). Positive staining for Ki-67 was defined when >10% of stained cells exhibited positivity. For p53, positive staining of <10% of tumor cells was defined as negative tumor expression, whereas staining of ≥10% tumor cells indicated positive tumor expression (23,24).

Cbx2 protein expression was evaluated using immunostained TMA slides from each core. IHC was conducted as follows: Briefly, antigen retrieval was performed using 10 mM sodium citrate (pH 6.0) and sections were washed with Tris-buffered saline. To block endogenous peroxidase activity, the sections were treated with 3% H_2O_2 for 10 min. Non-specific binding was blocked by incubation with 1% low lethal serum (Boster Inc., Wuhan, China) in PBS. Next, the slides were incubated with anti-CBX2 polyclonal antibodies (1:300; PA5-309961; Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 4˚C overnight. The slides were then incubated with goat anti-rabbit IgG secondary antibody (SP-9001; Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd.) at room temperature for 30 min. After washing with PBS three times, each section was treated with 300-500 ml diaminobenzidine working solution at room temperature for 5-10 min for visualization and then washed with distilled water.

IHC evaluation of Cbx2 protein expression. Immunostaining was evaluated by two breast pathologists from the The

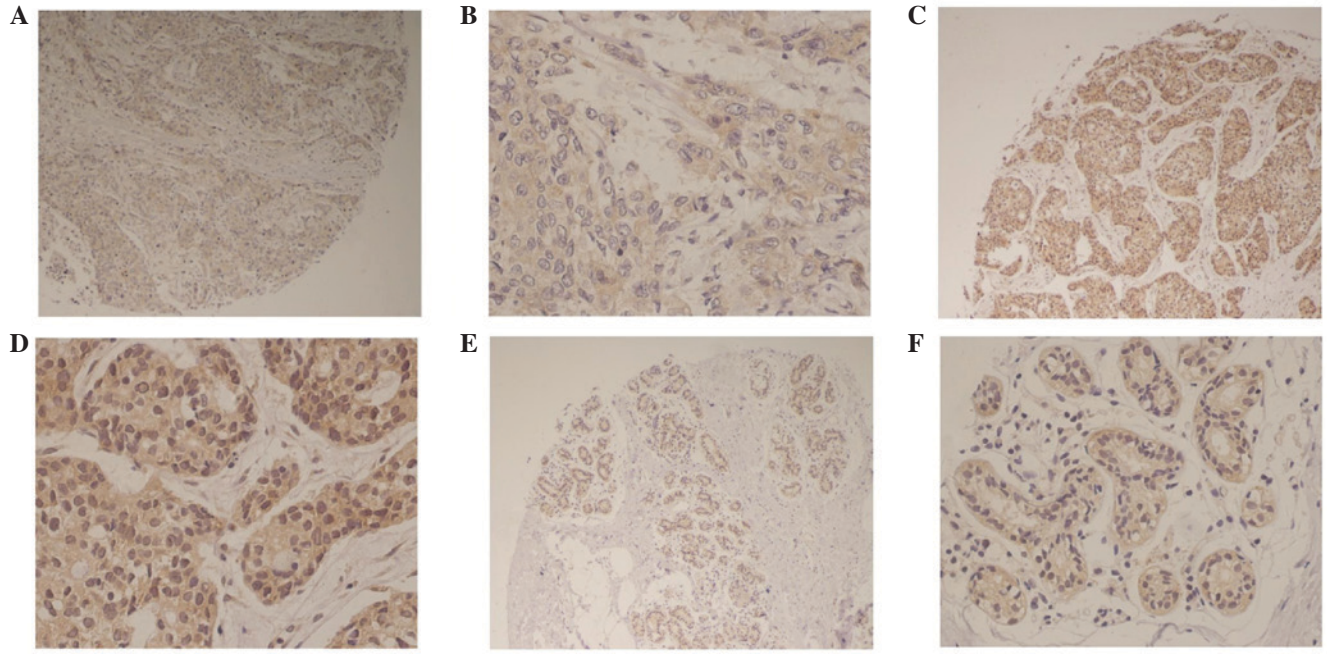

Figure 1. Immunohistochemical staining of Cbx2 in the normal breast and breast cancer tissues. (A) Low cytoplasmic Cbx2 expression in normal breast tissues (magnification, x100). (B) Low cytoplasmic Cbx2 expression in normal breast tissues (magnification, x400). (C) Low cytoplasmic Cbx2 expression in the specimens (magnification, x100). (D) Low cytoplasmic Cbx2 expression in the specimens (magnification, x400). (E) High cytoplasmic Cbx2 expression in cancer tissues (magnification, x100). (F) High cytoplasmic Cbx2 expression in cancer tissues (magnification, x400). Cbx2, chromobox2.

Affiliated Tumor Hospital of Harbin Medical University, who were blinded to the patient clinical outcomes. Scoring was performed using the semi-quantitative score method (25) to calculate the product of the percentage and intensity of positively stained tumor cells within the invasive tissue component. In the cytoplasm, staining intensity was graded as follows: 0, no staining; 1, weak staining (light yellow); 2, moderate staining (yellow brown); and 3, strong staining (brown). The percentage (0-100%) of staining was scored as follows: 0, no positive tumor cells; 1, <25% positive tumor cells; 2, 25-50% positive tumor cells; 3, 51-75% positive tumor cells; and 5, >75% positive tumor cells (25,26). The immunoreactive score (IRS) ranged between 0 and 12. An IRS score (IRS = staining percentage x staining intensity) of <6 was classified as low expression, whereas a score of >6 indicated high expression.

Follow-up. All patients were advised to attend follow-up examinations every 4-6 months for the first 5 years following surgery, and every 12 months thereafter. All patients were regularly followed up until mortality or the study end date (30 December, 2012). Prognosis was recorded by the Center of Medical Records at The Affiliated Tumor Hospital of Harbin Medical University. Overall survival (OS) time was assessed for prognostic analysis.

Statistical and survival analyses. All statistical analyses were performed using SPSS 13.0 statistical software (SPSS, Inc., Chicago, IL, USA). The difference in Cbx2 expression between breast cancer tissues and normal tissues was assessed using the Mann-Whitney U test. The association between Cbx2 and patient clinicopathological features was analyzed using the χ^2 test. OS time was determined as the time from surgery to the date of mortality or last follow-up. Kaplan-Meier analysis was used to estimate OS time. Univariate analysis was performed using the log-rank test. Univariate and multivariate Cox proportional hazard models were used to assess clinicopathological prognostic factors affecting OS. P<0.05 was considered to indicate a statistically significant difference.

Results

Cbx2 protein expression in cancer and normal breast tissues. Cbx2 expression was identified in the cytoplasm of breast cancer tissues (Fig. 1). Cbx2 expression was also identified in the cytoplasm of matched adjacent normal tissues (Fig. 1A and B). Representative immunohistochemical images demonstrate low and high Cbx2 expression (Fig. 1C-F).

A total of 216 paired cancer tissues and matched adjacent normal tissues were obtained for analysis. Cbx2 protein expression was identified in 199/216 tumor tissues and 196/216 adjacent tissues. Notably, in 15 (6.94%) paired cancer and adjacent normal tissues, Cbx2 expression was higher in normal tissues compared with cancer tissues. However, in 175 (81.01%) normal tissues, Cbx2 expression was lower compared with in cancer tissues. A total of 26 (12.04%) paired tissues exhibited equal Cbx2 expression. The median and mean IRS of 455 tumor tissues were 6.00 and 6.21, respectively, whereas the median and mean IRS of the 216 matched normal adjacent tissues were 3.00 and 3.48, respectively. The protein expression of Cbx2 was significantly higher in tumor tissues compared with adjacent normal tissues (P<0.001). Using an IRS of 6 as the cut-off value for high Cxb2 expression, 53.89% (245/416) tumor tissues and 11.11% (24/216) adjacent normal tissues exhibited high Cbx2 expression (P<0.001).

Table I. Association between Cbx2 expression and patient clinicopathological features.

Parameter	n	Cbx2 expression		P-value
		Low, n (%)	High, n (%)	
Age, years				0.441
<50	249	119 (47.8)	130 (52.2)	
≥50	206	91 (44.2)	115 (55.8)	
Tumor size, cm				<0.001
<2	101	65 (64.4)	36 (35.6)	
≥2	353	144 (40.8)	209 (59.2)	
Pathological stage				0.286
I	40	23 (57.5)	17 (42.5)	
II	121	56 (46.3)	65 (53.7)	
III	285	126 (44.2)	159 (55.8)	
LNM				0.008
Negative	210	111 (52.9)	99 (47.1)	
Positive	245	99 (40.4)	146 (59.6)	
TNM stage				<0.001
I	91	75 (82.4)	16 (17.6)	
II	225	131 (58.2)	94 (41.8)	
III	139	4 (2.9)	135 (97.1)	
Ki-67, %				0.080
<10	147	77 (52.4)	70 (47.6)	
≥10	305	133 (43.6)	172 (56.4)	
HER-2 status				0.048
Negative	363	176 (48.5)	187 (51.5)	
Positive	92	34 (37.0)	58 (63.0)	
ER status				0.162
Negative	242	104 (43.0)	138 (57.0)	
Positive	212	105 (49.5)	107 (50.5)	
PR status				0.174
Negative	180	76 (42.2)	104 (57.8)	
Positive	275	134 (48.7)	141 (51.3)	
p53 status				0.717
Negative	79	35 (44.3)	44 (55.7)	
Positive	376	175 (46.5)	201 (53.5)	
Subtype				0.388
HER-2	92	37(40.2)	55 (59.8)	
Luminal A	85	44 (51.8)	41 (48.2)	
Luminal B	234	111 (47.4)	123 (52.6)	
Triple negative	44	18 (40.9)	26 (59.1)	

Cbx2, chromobox2; LNM, lymph node metastasis; TNM, tumor node metastasis; HER-2, human epidermal growth factor-2; ER, estrogen receptor; PR, progesterone receptor; p53, tumor protein 53.

Association between Cbx2 protein expression and patient clinicopathological features. A total of 455 tumor tissues were included in the present study. The median IRS of Cbx2 expression was 6, which was used as a cut-off for high expression. Based on this cut-off value, 46.15% (210/455) and 53.85% (245/455) tumor tissues exhibited low and high Cbx2 cytoplasmic expression, respectively. The expression of cytoplasmic Cbx2 was found to significantly associate with tumor size (P<0.001), lymph node metastasis (P=0.008), tumor node metastasis (TNM) classification of malignant tumors (18) stage (P<0.001) and positive HER-2 status (P=0.048) (Table I).

Association between Cbx2 expression and prognosis of patients with breast cancer. A total of 403 patients were

Table II. Univariate and multivariate cox regression analyses of overall survival time in breast cancer patients.

	Univariate analysis			Multivariate analysis		
Parameter	HR	95% CI	P-value	HR	95% CI	P-value
Tumor size, cm (≥2/<2)	2.361	1.074-5.189	0.033	1.231	0.542-2.795	0.619
Pathological stage (I/II/III)	1.778	1.095-2.888	0.020	1.399	0.838-2.336	0.199
TNM stage (positive/negative)	3.671	1.989-6.777	<0.001	3.427	1.363-8.614	0.009
LNM (positive/negative)	3.069	2.006-4.696	<0.001	1.025	0.389-2.699	0.960
Ki-67 status (positive/negative)	1.920	1.040-3.545	0.037	1.673	0.866-3.233	0.126
PR status (positive/negative)	0.579	0.350-0.956	0.033	0.878	0.333-2.314	0.792
Subtype						
HER-2 status (positive/negative)	Reference			Reference		
Luminal A	0.878	0.433-1.781	0.718	1.264	0.409-3.909	0.684
Luminal B	0.488	0.259-0.919	0.026	0.698	0.250-1.948	0.493
Triple negative	1.108	0.478-2.569	0.810	1.608	0.677-3.823	0.282
Cbx2 expression (high/low)	1.826	1.069-3.116	0.027	1.790	1.048-3.056	0.236

HR, hazard ratio; CI, confidence interval; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor-2; p53, tumor protein 53; Cbx2, chromobox2.

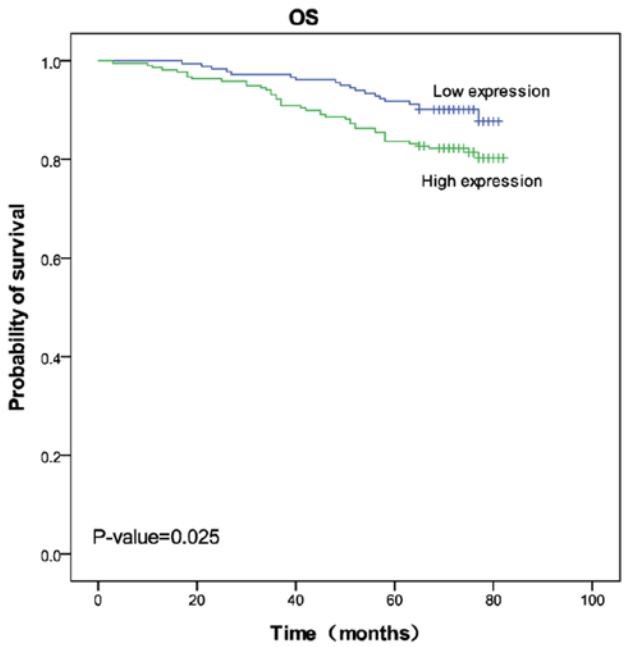

Figure 2. Kaplan-Meier survival analysis revealed high expression of chromobox2 indicates poor overall survival in patients with breast cancer. OS, overall survival.

followed up and 52 patients were lost to follow-up. During the follow-up period, 10.93% (20/183) and 18.64% (41/220) patients in the low and high Cbx2 expression groups succumbed to the disease, respectively. The mean survival times were 77.37 months (range, 75.66-79.07 months) and 74.29 months (range, 71.94-76.64 months) in the low and high Cbx2 expression groups, respectively. Kaplan-Meier 5-year survival curves were stratified for Cbx2 expression and the results revealed that high Cbx2 expression was associated with poor prognosis (log-rank test statistic, 5.032; P=0.025; Fig. 2).

Univariate and multivariate Cox regression analyses were performed to evaluate the association between Cbx2 expression and clinicopathological features on patient prognosis. Variables such as tumor size, pathological stage, lymph node metastasis, TNM stage, Ki-67 status, PR status and molecular subtype were included in multivariate analyses, which were associated with survival of patients with breast cancer as identified by the log-rank test. Univariate Cox analysis demonstrated significantly shorter OS time in patients with a large tumor size [(hazard ratio (HR), 2.361; 95% confidence interval (CI), 1.074-5.189; P=0.033], positive PR status (HR, 0.579; 95% CI, 0.350-0.956; P=0.033), positive Ki-67 status (HR, 1.920; 95% CI, 1.040-3.545; P=0.037) and high Cbx2 expression (HR, 1.826; 95% CI, 1.069-3.116; P=0.027) (Table II). The multivariate Cox proportional hazard model revealed that only high TNM stage (HR=3.427; 95% CI=1.363-8.614; P=0.009) was independently associated with poor survival (Table II).

Effect of Taxol in patients expressing Cbx2. A total of 309 patients underwent chemotherapy treatment. Among these patients, 122 received Taxol and 187 received an alternative chemotherapy regimen without Taxol (including nedaplatin *cis*-platinum, nedaplatin fluorouracil, nedaplatin epirubicin and fluorouracil epirubicin cyclophosphamide). Among patients with high Cbx2 expression, the mean OS time of patients receiving Taxol treatment (71.01 months) was significantly shorter compared with patients receiving treatment without Taxol (78.43 months) (P<0.001). However, in the low Cbx2 expression group, no significant difference in the mean OS time of patients was identified between those treated with Taxol (76.45 months) and those without Taxol treatment (78.41 months) (P=0.296). These results indicated that patients with high Cbx2 expression do not exhibit sensitivity to chemotherapy programs that include Taxol (Fig. 3).

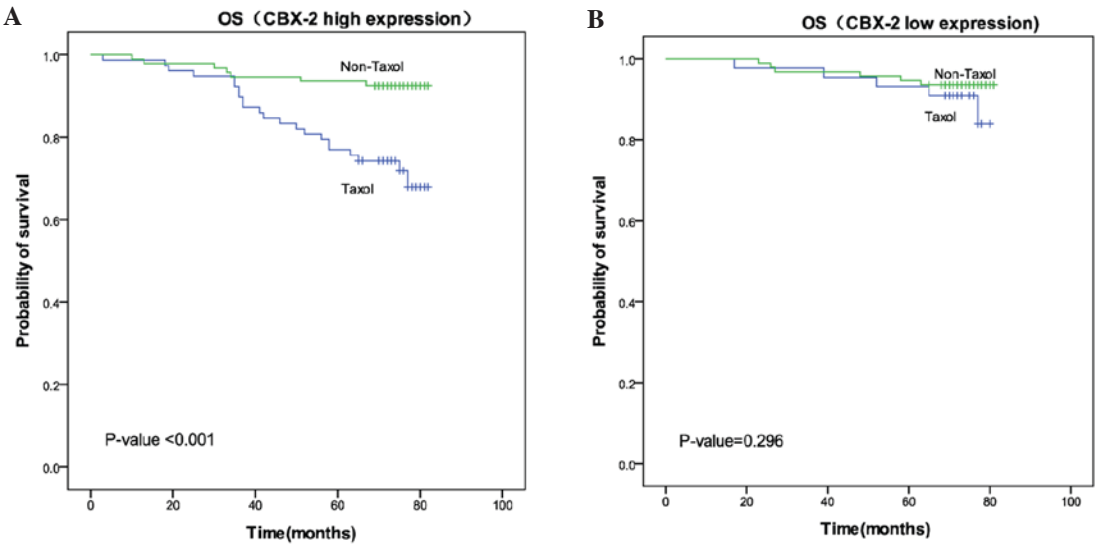

Figure 3. Kaplan-Meier survival analysis curves showing OS times of breast cancer patients in the (A) high and (B) low Cbx2 expression groups, treated with or without Taxol. OS, overall survival; Cbx2, chromobox2.

Discussion

The results of the present study demonstrated that breast cancer tissues exhibited higher levels of Cbx2 expression compared with normal tissues (53.85 vs. 11.11%). Aberrant expression of Cbx2 at the mRNA level has been observed in colon, breast, stomach and lung cancer (27). In the present study, Cbx2 protein expression was associated with certain clinicopathological features in breast cancer patients. High Cbx2 expression was found to associate with a large tumor size (P<0.001), and this finding was consistent with the results of a previous oral squamous cell carcinoma study (28). In addition, Cbx2 was significantly associated with lymph node metastasis (P=0.008) and a positive HER-2 status (P=0.048). A positive association was also identified between Cbx2 expression and TNM stage (P<0.001). At present, the function of Cbx2 in tumorigenesis remains unclear, however emerging evidence has indicated that the Cbx2 protein exhibits a critical role in cancer initiation and progression (29,30).

Cbx2 is a primary member of the Cbx protein family, and is a component of the PRC1 complex that regulates chromatin. PRC1 exhibits enzymatic activity to modify histones and repress the transcription of target genes (31,32). The ability of PRC1 to promote proliferation may be associated with PcG activity in cancer (33). The Cbx2 protein is a major component involved in the recruitment of PRC1 proteins to mitotic chromosomes (34). Cbx2 also directly regulates the expression of the cyclin-dependent kinase inhibitor, p21, and dominantly controls the expression of the INK4A/ARF locus, which is extremely important for human hematopoietic cell proliferation (35). The overexpression of Cbx2 results in the differentiation and exhaustion of hematopoietic stem cells (16). Previous studies regarding the mechanism of Cbx2 in regulating hematological stem cell differentiation have been conducted, however, few studies have investigated the function of Cbx2 in solid tumors (16,36). The present study provides a basis for future functional studies to identify molecular mechanisms by which Cbx2 may promote tumor initiation and progression.

The current study indicated that Cbx2 expression associates with the prognosis of breast cancer patients. At the final follow-up, the mortality rates in the high and low Cbx2 expression groups were 18.64 and 10.93%, respectively. The mean survival time in the high Cbx2 expression group (74.29 months) was significantly shorter than that in the low expression group (77.37 months). High Cbx2 expression was also significantly associated with poor OS (HR, 1.826; 95% CI, 1.069-3.116; P=0.027). Considering the poor prognosis of patients with high Cbx2 expression, we hypothesize that Cbx2 may present an important predictor of breast cancer prognosis. By integrating multiple platforms on several biological levels, Clermont *et al* (27) demonstrated that an increased Cbx2 copy number associates with increased Cbx2 expression, which is significantly associated with poor OS. Due to the heterogeneity observed in the progression and outcome of breast cancer, the identification of more predictive biomarkers is required to guide clinical treatment.

Taxol is a microtubule-stabilizing drug approved by the Food and Drug Administration for the treatment of breast cancer (37). A previous study revealed that breast cancer patients that received nab-paclitaxel neoadjuvant chemotherapy exhibited a pathological complete response rate of 48.1% (38). Despite the high response rate, certain patients exhibit low or no response to Taxol. Thus, additional effective biomarkers are required to identify which patients would benefit from Taxol therapy. In the current study, the OS time of patients treated with Taxol was significantly lower than that of patients who did not receive Taxol treatment in the high Cbx2 expression group. However, no significant difference in OS time was identified between patients treated with or without Taxol in the low Cbx2 expression group. The association between Cbx2 expression and survival of breast cancer patients treated with fluorouracil was also analyzed in the present study. No significant difference was identified between the OS time of patients with and without fluorouracil

treatments in the high or low Cbx2 expression groups (data not provided). Thus, Cbx2 may present a specific biomarker for Taxol resistance in breast cancer patients. Numerous studies have been conducted to identify marker resistance or sensitivity to Taxol. These studies identified various candidates, including adenosine triposphate-binding cassette subfamily C member10, microRNA and solute carrier genes (39-41). However, these studies did not identify a validated biomarker to predict which patients would benefit from Taxol therapy. Cbx2 may present a promising indicator for predicting the use of Taxol in breast cancer chemotherapy. However, further clinical studies are required to verify these results.

The present study demonstrated the association between Cbx2 expression and breast cancer. However, certain points require further study. Firstly, dynamic localization of the nuclear-cytoplasmic and/or sub-nuclear distribution of members of the Cbx family occurs during the maternal-to-embryonic transition (42). The present study revealed that Cbx2 was predominantly expressed in the cytoplasm. Nuclear proteins under normal conditions are frequently overexpressed in the cytoplasm in various human cancers, including hepatocellular carcinoma, melanoma, papillary thyroid carcinoma and ductal breast carcinoma (43,44). Secondly, tumorigenesis is a dynamic evolutionary process that promotes genetic heterogeneity and produces a complex combination of random and nonrandom aberrations. The current study investigated Cbx2 expression at the protein level and thus, results must be confirmed by integrating multiple platforms on several biological levels (DNA-RNA-protein). Thirdly, all samples in the current study were recruited from a single hospital. Due to the heterogeneity of breast cancer, future studies which include individuals of different ethnicities in the patient cohort are required to validate the results of the present study. Furthermore, studies which aim to elucidate the molecular mechanisms underlying Cbx2 protein expression and its function in tumorigenesis are required.

In conclusion, in the present study Cbx2 protein expression levels were inversely associated with prognosis in breast cancer patients. Cbx2 expression was associated with clinical features, including positive lymph node metastasis status, large tumor size and positive HER-2 status. Therefore, Cbx2 may present a novel biomarker for the selection of an appropriate chemotherapy regimen for breast cancer patients.

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