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Down-regulation of Barx2 predicts poor survival in colorectal cancer

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ABSTRACT

Human BarH-like homeobox 2 (Barx2), a homeodomain factor of the Bar family, has an important role in controlling the expression of cell adhesion molecules and has been reported in an increasing array of tumor types except colorectal cancer (CRC). The purpose of the current study was to characterize the expression of Barx2 and assess the clinical significance of Barx2 in CRC. First, we analyzed the expression of Barx2 in two independent public datasets from Oncomine. Subsequently, we evaluated Barx2 mRNA and protein expression by quantitative real-time PCR and western blotting, respectively. It was determined that Barx2 expression was lower in tumor tissues than in adjacent non-tumorous colorectal tissues of CRC patients, consistent with results from the public datasets. Subsequently, a tissue microarray containing 196 CRC specimens was evaluated for Barx2 expression by immunohistochemical staining. It was found that low expression of Barx2 significantly correlated with TNM stage, AJCC stage, differentiation, and relapse in patients with CRC. Patients with lower levels of Barx2 expression showed reduced disease-free survival and overall survival. Furthermore, a trend toward shorter overall survival in the patient group with Barx2-negative tumors independent of advanced AJCC stage and poor differentiation was determined by Kaplan-Meier survival analysis. Based on univariate and multivariate analyses, Barx2 expression was an independent prognostic factor for determining CRC prognosis. Taken together, low Barx2 expression was associated with the progression of CRC and could serve as a potential independent prognostic biomarker for patients with CRC.

1. Introduction

Colorectal cancer (CRC) is the third most common cancer and the fourth leading cause of cancer mortality worldwide [1]. Despite various advances in its diagnosis and treatment over the past decades, the prognosis for CRC patients remains poor partially owing to its high recurrence rate [2,3]. Tumor metastasis is a significant factor in the management of colorectal cancer and is a major obstacle to successful treatment [4]. To date, no biomarkers have been found that efficiently predict the prognosis of CRC in the clinics. Hence, identification and use of novel biomarkers associated with CRC for early detection and to assess malignancy is essential to improve prognosis in CRC patients.

As a homeodomain factor of the Bar family, BarH-like homeobox 2 (Barx2) regulates factors that control the expression of cell adhesion molecules and influences cellular differentiation in various developmental contexts [5,6]. The gene encoding human Barx2 maps to chromosome 11q25 and has four exons, ranging from 85 to 1099 bp [7]. As a transcription factor controlling cell adhesion and cytoskeleton remodeling [8], downregulation of Barx2 is associated with a number of solid tumors and is correlated with poor prognosis [9,10]. However, little is known about Barx2 in

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the colon; it is expressed in muscle cells of the muscularis externa and shows a graded pattern of expression in intestinal enterocytes, decreasing in the crypt-to-villous direction [11]. The clinicopathological significance of Barx2 and its prognostic value in CRC remains unknown.

In this study, the expression of Barx2 in CRC was investigated at both the mRNA and protein levels. Moreover, we analyzed Barx2 protein expression using a tissue microarray (TMA) with data from 196 patients to examine the correlation between expression and clinicopathologic parameters of CRC. The present study tested the hypothesis that Barx2 acts as a tumor suppressor; data regarding Barx2 expression in these patients will provide information for its usefulness as a biomarker for diagnosing and assessing the prognosis of CRC.

2. Materials and methods

2.1. Patients and tissue specimens

All patient-derived specimens were collected, formalin-fixed, paraffin-embedded, and used under protocols approved by the Institutional Review Boards of Shanghai General Hospital. No patients had received radiotherapy, chemotherapy or other related anti-tumor therapies before the surgery. Diagnosis was confirmed by at least two pathologists and the histology and clinical stages were classified according to the guidelines of NCCN2010. The clinicopathologic characteristics of patients are summarized in Table 1. Overall survival (OS) and disease-free survival (DFS) rates were defined as the interval between initial surgery and clinically or radiologically determined recurrence/metastasis and death, respectively. All patients provided informed consent according to a protocol approved by the Institutional Review Board of Shanghai General Hospital, School of Medicine, Shanghai Jiaotong University.

2.2. Tissue microarray (TMA) construction

A total of 196 cases, including primary CRC tumors paired with normal mucosa, were retrieved from the archives of the Department of Pathology, Shanghai General Hospital, and were used to construct the TMA. Tissue morphology was validated by hematoxylin and eosin (H&E) staining and cores (2.0 mm diameter) were punched from the paraffin blocks. To ensure similar reaction conditions, paired cores punched from the same patient were spotted next to each other as previously reported [12]. All specimens were examined by two pathologists to prevent bias.

2.3. Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated from fresh CRC tissues and the adjacent normal mucosa using TRIzol reagent (TaKaRa, Japan) according to the manufacturer's instructions. Specimens were collected from the Department of General Surgery, Shanghai General Hospital during 2015. The RevertAid First Strand cDNA Synthesis Kit (Fermentas, USA) was used to reverse transcribe 2 μg of RNA according to the manufacturer's recommendations. qRT-PCR assays were performed using 4 μl of cDNA (1:10 dilution) and SYBR green (TaKaRa) in a total volume of 20 μl, using the ABI 7900 Real-time PCR System (ABI, USA). Primers sequences used for Barx2 detection were as follows: forward 5′- ATG ATC GAC GAG ATC CTC TC-3′ and reverse 5′- GCT TAA TGG TGG GGG TTC CG-3′. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control, and primer sequences were as follows: forward 5′- GGG AAG GTG AAG GTC GGA GT-3′ and reverse 5′- GGG GTC ATT GAT GGC AAC A-3′. Each PCR product was run in triplicate, and the relative Barx2 mRNA level was calculated by the $2^{-\Delta\Delta Ct}$ method.

Table 1
Clinicopathologic characteristics of 196 colorectal cancer patients.

Characteristics	N (%)
Age (yr)	
<65	78 (39.8)
>=65	118 (60.2)
Gender	
Male	85 (43.4)
Female	111 (56.6)
Tumor location	
Right	82 (41.8)
Transverse	19 (9.7)
Left	17 (8.7)
Sigmoid	78 (39.8)
T classification	
T 1	6 (3.1)
T 2	23 (11.7)
T 3	73 (37.2)
T 4	94 (48.0)
N classification	
N 0	105 (53.6)
N 1	57 (29.1)
N 2	34 (17.3)
M classification	
M 0	174 (88.8)
M 1	22 (11.2)
AJCC stage	
I	23 (11.7)
II	79 (40.3)
III	77 (39.3)
IV	17 (8.7)
Vascular invasion	
No	182 (92.9)
Yes	14 (7.1)
Differentiation	
Well	97 (49.5)
Moderate	69 (35.2)
Poor	30 (15.3)
Relapse	
No	116 (59.2)
Yes	73 (37.2)

AJCC American Joint Committee on Cancer.

2.4. Western blot (WB) analysis

Protein from tissue was extracted using RIPA lysis buffer with the inhibitor phenylmethanesulfonyl fluoride (Beyotime Biotechnology, Jiangsu, China) and protein concentration was measured using the BCA protein assay kit (Beyotime Biotechnology) according to the manufacturer's instructions. Equal amounts of protein (30 μg) were separated on 10% SDS-PAGE gels and transferred onto PVDF membranes (Millipore, Billeria, MA). After blocking with 5% shim milk in TBST buffer for 1.5 h at 27 °C, membranes were incubated overnight at 4 °C with an anti-Barx2 primary antibody (1:200 dilution; Santa Cruz, CA, USA). An anti-GAPDH antibody (1:1000 dilution; Santa Cruz, CA, USA) was used as the loading control. After incubation with a secondary antibody for 2 h at room temperature, proteins were detected using ECL regent (Millipore, Billeria, MA).

2.5. Immunohistochemical (IHC) staining

Slides were first baked at 60 °C for 2 h, and then slides were dewaxed in xylene, and rehydrated in graded series of ethanol followed by heat-induced antigen retrieval in 0.01 M sodium citrate buffer (PH 6.0) for 4 min. After blocking endogenous peroxidase with 3% H_2O_2, the TMA sections were then incubated with the primary antibody against Barx2 (1:100 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4 °C overnight. Following re-

warming for 45 min at 27 °C, sections were incubated with a HRP-conjugated secondary detection antibodies (Dako Cytomation, Glostrup, Denmark) at 27 °C for 30 min. Finally, the sections, were rinsed in PBS, incubated with DAB, counterstained with Mayer's hematoxylin, dehydrated, and mounted. Two investigators, blinded to patient prognosis, evaluated the staining and the sum of the staining intensity. The staining extent scores were used as the final staining score [13]. In the event of a discrepancy in scoring, the slides were simultaneously reexamined by two pathologists using a multi-head microscope until consensus was achieved.

2.6. Statistical analysis

Data analysis was performed using the SPSS 22.0 statistical software package (SPSS, Chicago, IL, USA). Barx2 mRNA expression in fresh CRC tissues and normal colon mucosa was analyzed by a student's t-test. The χ^2 test or Fisher's exact test was appropriately used to determine the association between Barx2 expression and clinicopathological variables of CRC. Survival curves were plotted using the Kaplan-Meier method with the log-rank test employed to compare differences. The hazard ratio (HR), with a 95% confidence interval, in Cox proportional hazard regressions were applied to estimate the hazard risk of the individual factors DFS and OS. For all tests, a P-value < 0.05 was considered statistically significant.

3. Results

3.1. Decreased expression of Barx2 in CRC tissues

First, we analyzed the expression of Barx2 in two independent publicly available datasets from Oncomine (https://www.oncomine.org/resource/main.html) [14,15], and found that the mRNA expression of Barx2 in CRC tissues was markedly lower than that in matched normal mucosa (Fig. 1a–b). Subsequently, by qRT-PCR, the expression level of Barx2 was analyzed in 40 randomly selected paired specimens from Shanghai General Hospital, and showed significant down-regulation in CRC compared to that in paired non-tumor tissues (Fig. 1c), consistent with aforementioned observations. We then further analyzed the impact of aberrant expression of Barx2 at the RNA level on CRC patient survival using the public database from TCGA. However, no significant association was found between the expression of Barx2 (at the RNA level) and OS, regardless of classification (Fig. S1), suggesting that Barx2 expression might be associated with CRC tumorigenesis at the protein level rather than at the RNA level. WB analysis further confirmed that Barx2 was down-regulated in CRC tissues compared to that in the corresponding normal mucosa (Fig. 1d).

3.2. Correlation between Barx2 expression and clinicopathological characteristics of CRC

IHC analysis was conducted to investigate the correlation between Barx2 protein levels and clinical factors of CRC using a TMA that contained 196 cases of primary CRC tissues paired with normal mucosa. Barx2 was primarily localized in the nucleus of CRC cells; representative IHC staining of Barx2 in CRC tissue and the normal mucosa are summarized in Fig. 2. The rates of strong positive, weak positive, and negative staining in normal colorectal mucosa were 114/196 (58.2%), 66/196 (33.7%), and 16/196 (8.2%), respectively (Table 2). In contrast, low expression of Barx2 was observed in the

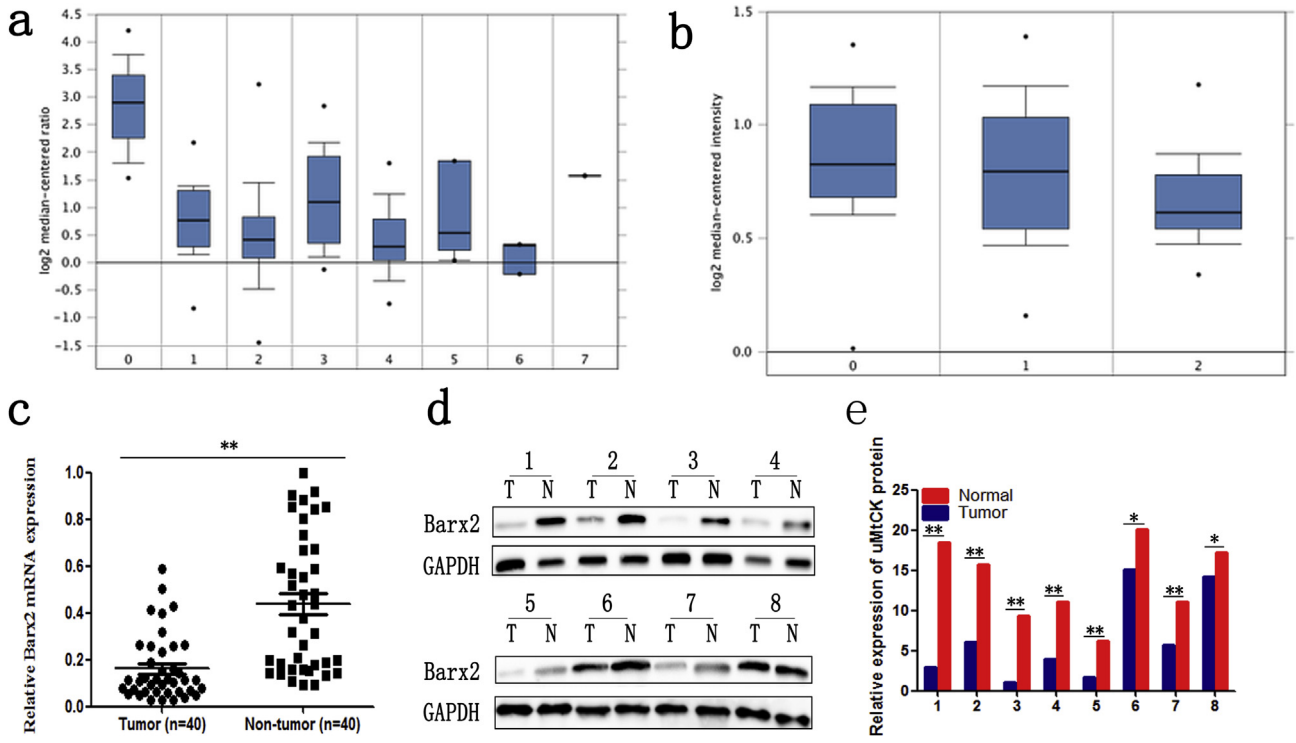

Fig. 1. Expression of Barx2 in CRC tissues and paired normal mucosa. a. Barx2 expression at the RNA level in TCGA colorectal samples grouped by no value (0), cecum adenocarcinoma (1), colon adenocarcinoma (2), colon mucinous adenocarcinoma (3), rectal adenocarcinoma (4), rectal mucinous adenocarcinoma (5), rectosigmoid adenocarcinoma (6), and rectosigmoid mucinous adenocarcinoma (7). **b**. Barx2 expression at the RNA level in the Skrzypczak Colorectal dataset grouped by no value (0), colorectal carcinoma (1) and colorectal adenocarcinoma (2). **c**. Quantitative real-time PCR detection of relative expression of Barx2 in 40 human CRC tissues and paired non-tumor mucosa. **d**. Western blot analysis of Barx2 protein expression in eight representative paired CRC tissues, with GAPDH used as the loading control. **e**. Quantification of western blot analysis of Barx2 protein levels, normalized to levels of GAPDH; $^{*}P < 0.05$, $^{**}P < 0.01$, compared to 3T.

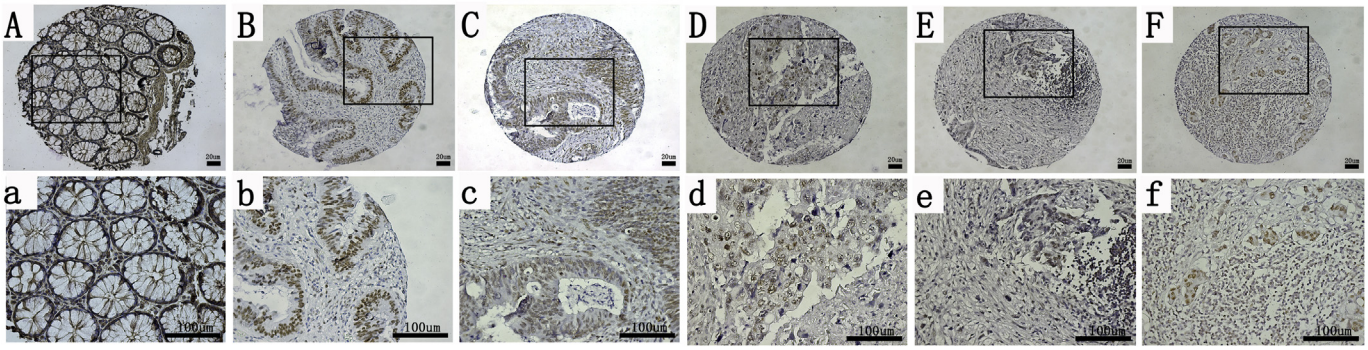

Fig. 2. Representative immunohistochemistry staining of Barx2 in CRC tissues and non-tumor colorectal mucosa. A-a. Strong Barx2 staining in non-tumor colorectal mucosa; **B-b**. Moderate Barx2 staining in well-differentiated CRC **C-c**. Weak Barx2 staining in moderately differentiated CRC; **D-d**. Negative Barx2 staining in poorly differentiated CRC; **E-e**: Negative Barx2 staining in metastatic lymph node; **F-f**. Elevated Barx2 staining in lymph node without metastasis. **A**–**F**. Original magnification, ×50; **a**–**f**. original magnification, ×200.

Table 2
Expression of Barx2 in normal colon mucosa and primary cancerous tissues.

Tissue sample	N	Expression of Barx2			*P* value
		Negative (n, %)	Weak positive (n, %)	Strong positive (n, %)	
Normal mucosa	196	16 (8.2)	66 (33.7)	114 (58.2)	<0.001[a]
Tumor tissue	196	93 (47.4)	73 (37.2)	30 (15.3)	

[a] Significant difference in the expression of Barx2 between normal colon mucosa and cancerous tissues.

Table 3
Association between Barx2 expression and clinicopathological characteristics in colorectal cancer (n = 196).

Variable	N	Barx2 expression			*P* value
		Negative (93)	Weak positive (73)	Strong positive (30)	
Age (yr)					
<65	78	40 (51.3%)	25 (32.1%)	13 (16.7%)	0.473
>=65	118	53 (44.9%)	48 (40.7%)	17 (14.4%)	
Gender					
Male	85	38 (44.7%)	34 (40.0%)	13 (15.3%)	0.762
Female	111	55 (49.5%)	39 (35.1%)	17 (15.3%)	
Tumor location					
Right	82	41 (50.0%)	29 (35.4%)	12 (14.6%)	0.513
Transverse	19	10 (52.6%)	6 (31.6%)	3 (15.8%)	
Left	17	8 (47.1%)	9 (52.9%)	0 (0.0%)	
Sigmoid	78	34 (43.6%)	29 (37.2%)	15 (19.2%)	
T classification					
T 1	6	0 (0.0%)	3 (50.0%)	3 (50.0%)	<0.001[a]
T 2	23	7 (30.4%)	9 (39.1%)	7 (30.4%)	
T 3	73	20 (27.4%)	35 (47.9%)	18 (24.7%)	
T 4	94	66 (70.2%)	26 (27.7%)	2 (2.1%)	
N classification					
N 0	105	39 (37.1%)	43 (41.0%)	23 (21.9%)	0.001[a]
N 1	57	28 (49.1%)	23 (40.4%)	6 (10.5%)	
N 2	34	26 (76.5%)	7 (20.6%)	1 (2.9%)	
M classification					
M 0	174	77 (44.3%)	68 (39.1%)	29 (16.7%)	0.037[a]
M 1	22	16 (72.7%)	5 (22.7%)	1 (4.5%)	
AJCC stage					
I	23	5 (21.7%)	11 (47.8%)	7 (30.4%)	0.006[a]
II	79	31 (39.2%)	32 (40.5%)	16 (20.3%)	
III	77	46 (59.7%)	25 (32.5%)	6 (7.8%)	
IV	17	11 (64.7%)	5 (29.4%)	1 (5.9%)	
Vascular invasion					
No	182	83 (45.6%)	69 (37.9%)	30 (16.5%)	0.109
Yes	14	10 (71.4%)	4 (28.6%)	0 (0.0%)	
Differentiation					
Well	97	32 (33.0%)	42 (43.3%)	23 (23.7%)	<0.001[a]
Moderate	69	39 (56.5%)	23 (33.3%)	7 (10.1%)	
Poor	30	22 (73.3%)	8 (26.7%)	0 (0.0%)	
Relapse					
No	116	47 (40.5%)	44 (37.9%)	25 (21.6%)	0.018[a]
Yes	73	40 (54.8%)	28 (38.4%)	5 (6.8%)	

[a] Significant difference.

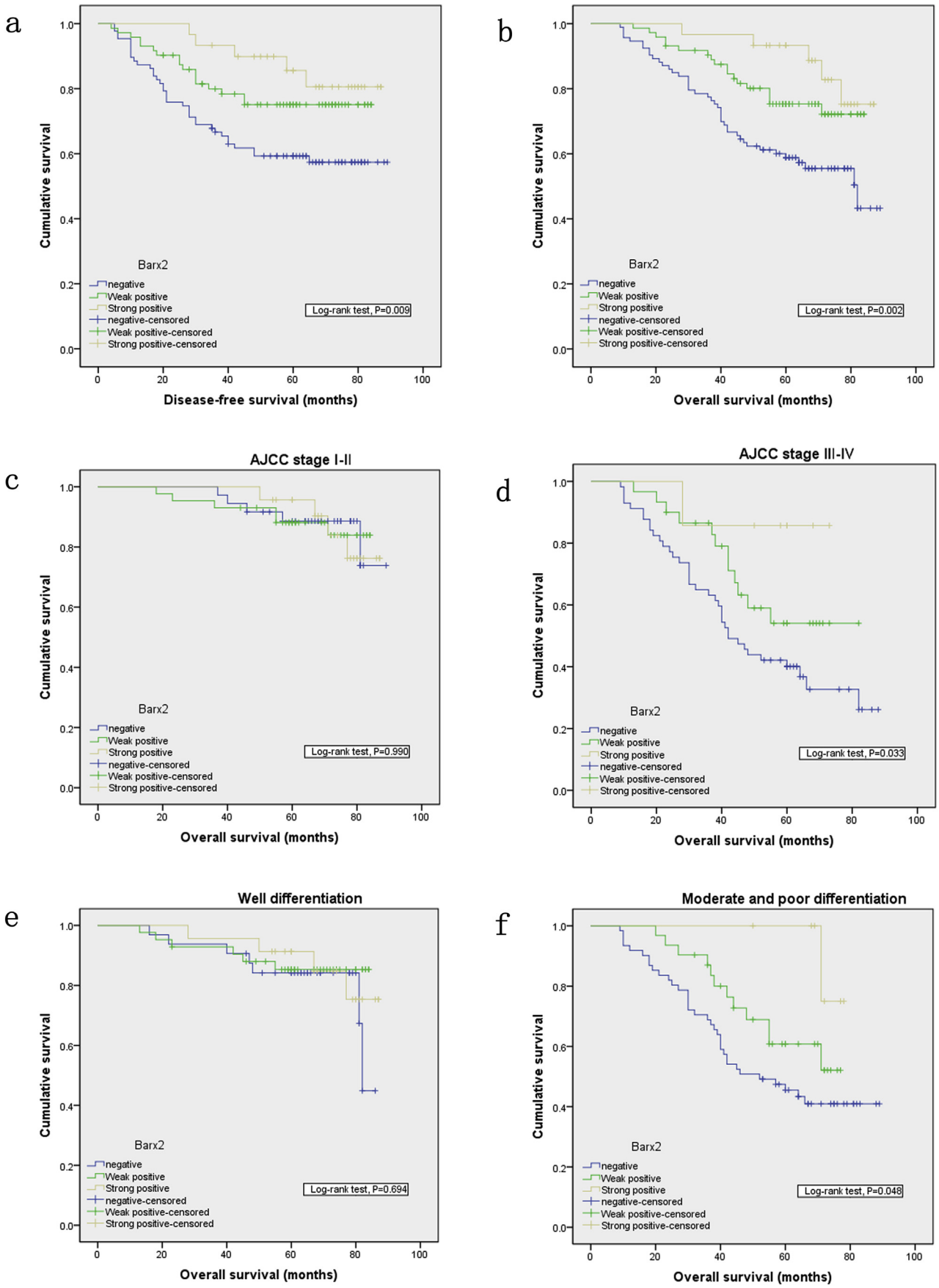

Fig. 3. The prognostic significance of Barx2 in CRC patients assessed by Kaplan-Meier analysis. DFS (**a**) and OS (**b**) were significantly poorer in patients with Barx2-negative expression than in those with Barx2-positive expression. Comparisons of OS between patients with Barx2-negative expression and those with Barx2-positive expression in an early AJCC stage (I-II) cohort and in an advanced AJCC stage (III-IV) cohort (**c-d**) and in patients with well-differentiated tumor and in those with moderate and poor differentiation (**e-f**). *P*-values were calculated by the log-rank test and $P < 0.05$ denoted significance.

Table 4
Univariate and multivariate analysis of overall survival in colorectal cancer.

	Overall survival			
	Univariate analysis		Multivariate analysis	
	HR (95%CI)	*P* value	HR (95%CI)	*P* value
Age (yr)	0.992 (0.974–1.011)	0.418		
Gender	1.453 (0.873–2.419)	0.150		
Tumor location	1.080 (0.903–1.292)	0.397		
T classification	2.696 (1.739–4.178)	0.001[a]	1.490 (0.917–2.421)	0.108
N classification	3.701 (2.679–5.112)	0.024[a]	2.340 (1.402–3.905)	0.001[a]
M classification	4.005 (2.563–5.176)	0.001[a]	2.886 (1.945–3.101)	<0.001[a]
UICC stage	5.069 (3.388–7.586)	0.004[a]	0.497 (0.257–0.963)	0.038[a]
Vascular invasion	4.274 (2.213–8.255)	0.084		
Differentiation	2.840 (2.049–3.937)	<0.001[a]	1.286 (0.842–1.964)	0.244
Relapse	3.663 (2.556–4.573)	<0.001[a]	2.004 (1.375–3.772)	<0.001[a]
Barx2	0.513 (0.348–0.758)	0.001[a]	0.920 (0.557–1.518)	0.006[a]

HR hazard ratio; CI confidence interval.
[a] P < 0.05 indicate that the 95% CI of HR was not including 1.

majority of CRC tissues, with strong staining in 30/196 (15.3%) specimens, weak staining in 73/196 (37.2%) specimens, and negative staining in 93/196 (47.4%) specimens (Table 2). Furthermore, in the available lymph nodes (LN) of CRC patients, significantly lower positive Barx2 staining was observed in metastatic LNs compared to those without metastasis (Fig. 2e–f).

The relationship between Barx2 expression and clinicopathologic features in CRC is summarized in Table 3. Downregulation of Barx2 in CRC was markedly correlated with T classification ($P < 0.001$), N classification ($P = 0.001$), M classification ($P = 0.037$), AJCC stage ($P = 0.006$), histological differentiation ($P < 0.001$), and tumor relapse ($P = 0.018$). However, no significant association was found between Barx2 expression and other clinical parameters, such as age, gender, tumor location, or vascular invasion, in the present study ($P > 0.05$ for all, Table 3). Taken together, these data indicate that downregulation of Barx2 is associated with an aggressive CRC phenotype.

3.3. Association between Barx2 expression and prognosis in CRC patients after curative surgery

To test the prognositic value of Barx2 for CRC, disease-free survival (DFS) and overall survival (OS) were assessed by Kaplan-Meier survival analysis and these parameters were compared using the log-rank test. As shown in Fig. 3a–b, patients with lower Barx2 expression had poorer DFS ($P = 0.009$) and OS ($P = 0.002$) than those with higher Barx2 expression. To further confirm the association between Barx2 expression and CRC metastases or local relapse, regardless of clinical stage, we performed subgroup analyses for OS according to AJCC stages and histological differentiation. Interestingly, a statistical difference was observed between positive and negative Barx2 staining groups for both low-stage (I-II; $P = 0.990$) and advanced-stage (III-IV; $P = 0.033$) CRC patients (Fig. 3c–d). In addition, in CRC patients with moderate and poor differentiation, lower Barx2 expression exhibited an obvious relationship with decreased OS ($P = 0.048$; Fig. 3f), whereas in patients with well-differentiated tumor, down-regulated Barx2 expression did not apparently affect OS ($P = 0.694$, Fig. 3e).

Subsequently, based on univariate Cox regression analyses, T classification, N classification, M classification, AJCC stage, differentiation, tumor relapse, and Barx2 expression were significantly associated with both OS and DFS (Tables 4 and 5). Furthermore, multivariate analysis, used to analyze parameters with significance based on univariate analyses, demonstrated that Barx2 expression remained an independent prognostic factor for increased disease recurrence and decreased survival in CRC (Tables 4 and 5).

4. Discussion

Homeobox genes have been reported to be essential for the correct positioning and differentiation of tissues and organs in diverse species [16,17]. All homeobox genes encode a 61-amino acid DNA binding structure called the homeodomain, that act as transcription factors to control the activity of other genes [18–20].

Table 5
Univariate and multivariate analysis of disease-free survival in colorectal cancer.

	Disease-free survival			
	Univariate analysis		Multivariate analysis	
	HR (95%CI)	*P* value	HR (95%CI)	*P* value
Age (yr)	0.978 (0.578–1.656)	0.935		
Gender	1.348 (0.793–2.291)	0.270		
Tumor location	1.142 (0.944–1.382)	0.171		
T classification	2.536 (1.616–3.979)	<0.001[a]	1.675 (0.991–2.831)	0.054
N classification	2.691 (2.637–5.187)	0.037[a]	2.439 (1.404–4.236)	0.002[a]
M classification	6.241 (4.347–8.602)	<0.001[a]	4.705 (3.692–5.512)	<0.001[a]
UICC stage	3.966 (2.604–6.040)	<0.008[a]	0.401 (0.188–0.851)	0.017[a]
Vascular invasion	4.645 (2.328–9.267)	0.079		
Differentiation	2.556 (1.815–3.599)	<0.001[a]	1.185 (0.805–1.742)	0.389
Relapse	4.345 (3.602–5.880)	<0.001[a]	2.162 (1.745–4.041)	<0.001[a]
Barx2	0.551 (0.369–0.823)	0.004[a]	0.914 (0.553–1.510)	0.025[a]

HR hazard ratio; CI confidence interval.
[a] P < 0.05 indicate that the 95% CI of HR was not including 1.

As a homeobox transcription factor [21], human Barx2 could regulate the expression of cell adhesion molecules (CAMs) including NCAM and cadherin 6 [5,9], suggesting that it is involved in cell processes such as cell aggregation, formation of intercellular contacts, and cell fusion. To date, human Barx2 has been described as a tumor suppressor gene, and downregulation of Barx2 expression was shown to correlate with invasiveness and poor clinical outcome in several solid tumors [9,10]. As reported, Barx2 is expressed throughout the gut and is located in epithelial cells of the proliferative and differentiative regions of the stomach, esophagus, and intestine [11]. However, the Barx2 expression pattern and its correlation with clinicopathological parameters and prognosis in CRC remain unknown. In the present study, we demonstrated that the expression of Barx2 was much lower in CRC tissues than in corresponding human normal mucosa, and that downregulation of Barx2 was a novel independent prognostic biomarker for CRC.

In our study, consistent with the results of two publicly available datasets from Oncomine, we confirmed that the expression of Barx2 was markedly downregulated in CRC tissues compared to that in adjacent normal colorectal mucosa at both the transcriptional and posttranscriptional levels. Furthermore, according to IHC analysis in the TMA, strong positive expression of Barx2 was more apparent in normal colorectal mucosa (58.2%, 114/196) compared to that in primary CRC specimens (15.3%, 30/196). In addition, Barx2 positive staining was markedly reduced in metastatic LNs compared to that in those without metastasis, suggesting that lower expression of Barx2 might be associated with CRC metastasis.

Previous studied of ovarian cancer and primary hepatocellular carcinoma showed that low expression of Barx2 was significantly correlated with metrics of tumor malignancy and poor prognosis [9,10]. Herein, we discovered a similar phenomenon in which low expression of Barx2 in CRC was strikingly associated with various clinicopathological characteristics, such as T classification, N classification, M classification, AJCC stage, histological differentiation, and tumor relapse. In addition, based on Kaplan-Meier survival analysis, we demonstrated that Barx2-negative tumors were associated with poorer DFS and OS, when compared to Barx2-positive tumors. Subsequently, both univariate and multivariate analysis verified that Barx2 expression was an independent prognostic marker for DFS and OS in CRC patients after curative surgery. Most importantly, for the first time, we showed that aberrant downregulation of Barx2 was markedly correlated with shorter OS in cased with advanced AJCC stage and poor tumor differentiation. These discoveries further confirmed that downregulation of Barx2 contributes to an aggressive CRC phenotype.

In summary, we demonstrated for the first time that aberrant downregulation of Barx2 is significantly correlated with an aggressive phenotype and poor prognosis in human CRC, suggesting that Barx2 might serve as a novel independent biomarker and potential therapeutic target for this disease. Barx2 was reported to be involved in Wnt Signaling and ras/raf signaling [22,23]; in this study, we did not extensively study the mechanism associated with downregulation of Barx2 in CRC; this requires further investigation.

Disclosure of interest

The authors confirm that there are no conflicts of interest regarding this article.

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Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2016.07.091

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2016.07.091

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