Int J Clin Exp Med 2017;10(5):7678-7687
www.ijcem.com /ISSN:1940-5901/IJCEM0048112

Original Article

Bbc3 is negatively correlated with epithelial-mesenchymal transition and is a prognostic biomarker for patients with rectal cancer

Hang Yuan*, Shiliang Tu*, Bo'an Zheng, Quanjin Dong

*Department of Coloproctologic Surgery, Zhejiang Provincial People's Hospital, Hangzhou, China. *Equal contributors.*

Received September 1, 2016; Accepted March 19, 2017; Epub May 15, 2017; Published May 30, 2017

Abstract: The role of Bbc3 in rectal cancer (RC) and correlation with Epithelial Mesenchymal Transition (EMT) remains unclear. The expression of Bbc3 in 30 fresh frozen RC and matched normal tissues were evaluated by using quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) and western blotting. Bbc3 expression was showed significantly higher in RC tissues than in corresponding normal tissues (P<0.001). Furthermore, IHC staining of Bbc3 and EMT markers (E-cadherin and N-cadherin) were performed using 140 formalin-fixed paraffin-embedded (FFPE) RC samples. The statistical analysis based on IHC evaluation demonstrated that Bbc3 aberrant expression was significantly associated with differentiation (*P*=0.028), lymph node metastasis (*P*=0.003) and TNM stage (*P*<0.001). Patients with low expression of Bbc3 have poor overall survival (OS) and disease-free survival (DFS), compared to those with high expression of Bbc3. Multivariate analysis indicated Bbc3 expression could predict the outcome of RC patients independently. Interestingly, we found Bbc3 expression was positively correlated with E-cadherin, but negatively correlated with N-cadherin expression, which was supported by analyzing TCGA data. In conclusion, our results suggested that Bbc3 is a potential prognostic biomarker for RC and its function may be negatively correlated with EMT transition.

Keywords: Rectal cancer, Bbc3, EMT, prognosis

Introduction

Colorectal cancer (CRC) remains one of the most common cancers worldwide [1]. In China, the incidence of CRC has been increasing in recent years [2]. The primary treatment for rectal cancer (RC) is resection of the primary tumor. Following that, patients are frequently administered adjuvant chemotherapy to eliminate cancer cells [3]. Although advanced methods of diagnosis, treatment, and prevention have been employed over the last few decades, the overall survival rate of patients with RC has not improved significantly [4-7]. The overall five-year survival is 50%-60% in European countries [8]. The Tumor Node Metastasis (TNM) staging system is widely used to predict prognosis, which is determined by the tumor invasion, lymph node metastasis and distal metastasis. However, stage-independent variability in patient outcome creates a need for novel prognostic markers to improve the prognostic evaluation and clinical management of patients with RC.

Evasion of apoptosis is a hallmark of human cancers that leads to cancer development, progression, and treatment resistance [9]. BH3-only proteins (Bbc3/PUMA, BIM, Bad, Bid, and Noxa) that are sensors of cellular apoptosis and can engage and inactivate pro-survival Bcl-2 family members, by inserting BH3 domain into a groove on the pro-survival proteins to negate their cytoprotective function [10, 11]. This interaction induces the cell to apoptosis by permeabilizing the outer mitochondrial membrane to enable cytochrome C release, which promotes caspase activation [12]. Studies indicate that BH3-only proteins bind promiscuously or selectively to pro-survival Bcl-2 proteins [12-14]. Bbc3 (p53 up-regulated modulator of apoptosis) has been shown to target all pro-survival

Table 1. Correlation of clinicopathological features of RC patients and Bbc3 expression

Clinicopathological features		Cases	Bbc3 expression			χ^2, *P*-value
			Negative	Moderately positive	Strongly positive	
Age (years)	≤60	65	14	28	23	χ^2=2.164, *P*=0.339
	>60	75	10	40	25	
Gender	Male	76	13	33	30	χ^2=2.213, *P*=0.331
	Female	64	11	35	18	
Tumor size (cm^2)	<3×3	23	5	13	5	χ^2=3.306, *P*=0.508
	3×5	97	14	47	36	
	>5×3	20	5	8	7	
Differentiation grade	High	37	6	16	15	χ^2=10.850 *P*=0.028
	Intermediate	84	10	45	29	
	Low	19	8	7	4	
Depth of invasion	Myometrial	29	4	13	12	χ^2=2.457, *P*=0.652
	Serosal	62	10	34	18	
	Outside of serosa	49	10	21	18	
Lymph node metastasis	No	84	7	45	32	χ^2=11.477, *P*=0.003
	Yes	56	17	23	16	
Histological type of adenocarcinoma	Papillary	25	3	11	11	χ^2=6.497, *P*=0.165
	Tubular	87	12	45	30	
	Mucinous	28	9	12	7	
TNM classification	I	21	4	6	11	χ^2=29.554, *P*<0.001
	II	63	3	39	21	
	III	47	11	20	16	
	IV	9	6	3	0	

protein and be potent inducers of apoptosis in vitro [13].

Epithelial-to-Mesenchymal Transition (EMT) is a process through which epithelial phenotype transit to mesenchymal phenotype, so the normal cells lose the cell polarity and connectivity with basement membrane and facilitate to resist apoptosis, invasion and migration. EMT is characterized by a cadherin switch which including low expression of E-cadherin and high expression of N-cadherin [14]. E-cadherin, a component of the epithelial adherens junction, has been widely known as a suppressor of motility and invasiveness of neoplastic epithelial cells in various types of tumors [15]. However, N-cadherin, a mesenchymal marker oppositely has been proved to be associated with invasion and metastasis [16]. Given the role of Bbc3 in apoptosis, Bbc3 may be associated with apoptosis regulated by EMT, however, few researches showed the internal relationship.

This study aimed to investigate the prognostic impact of Bbc3 in a Chinese cohort of RC patients and explore the association between Bbc3 and EMT specific markers.

Materials and methods

Patient

In this study, qRT-PCR and western blot were performed in 30 pairs of fresh frozen RC samples and paired normal mucosa. IHC experiments were further more employed to 140 FFPE RC tissues. All samples were obtained from patients who were diagnosed of RC clinicopathologically and underwent surgery treatment in the Department of Coloproctologic Surgery in Zhejiang Provincial People's Hospital (Hangzhou, China) between 2000 and 2010. Written informed consents were obtained from all the patients and this study was approved by the ethics committee of Zhejiang Provincial People's Hospital. Preoperative chemotherapy or radiotherapy was never applied in these patients. The clinicopathological characteristics of all the patients including age, gender, histological type, histological differentiation, and tumor size were summarized in **Table 1**. The median follow-up time of the patients was 57.06 months (range from 12 to 120 months). Cancer histological type and differentiation

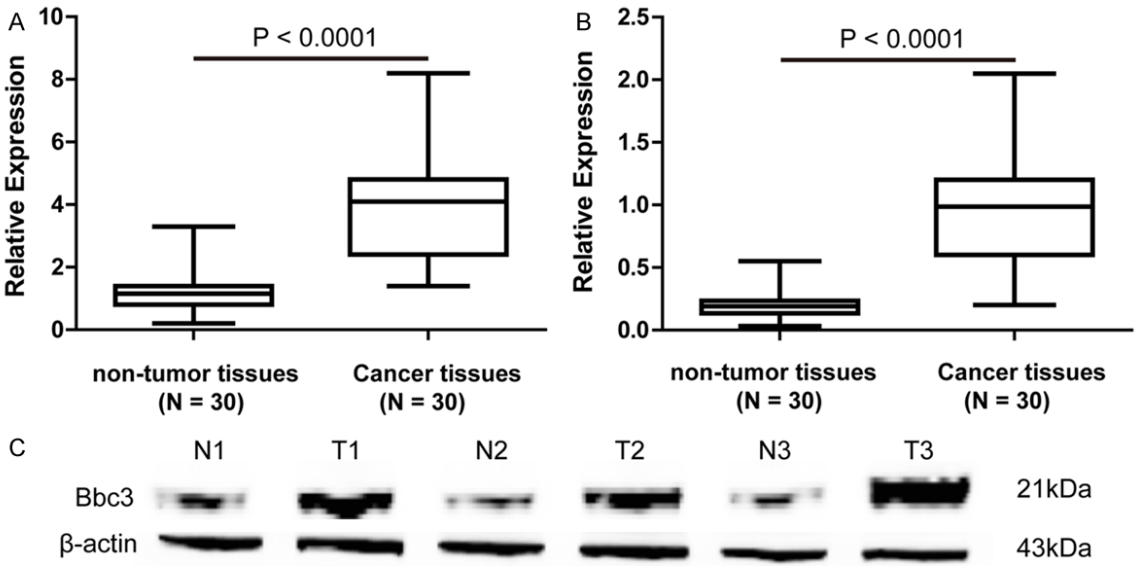

Figure 1. Expression of Bbc3 in RC tissues and non-tumor tissues. qRT-PCR was performed to evaluate the Bbc3 mRNA expression in RC tissues and adjacent normal mucosal tissues. A. The expression of Bbc3 was apparently higher in RC tissues than that in paired non-tumor tissues (P<0.0001, N=30). B and C. The result was confirmed by Western blot (P<0.0001, N=30).

were defined according to the recommendations of the World Health Organization. All the samples were fixed in 10% formalin and embedded in paraffin for further analysis. Tumor histologic grade was determined as defined by the American Joint Committee on Cancer Prognostic Factors Consensus Conference.

IHC detection for Bbc3, E-cadherin and N-cadherin

IHC was performed to detect Bbc3, E-cadherin and N-cadherin. The degree of immunostaining was reviewed and scored independently by two pathologists who were blind to the clinicopathological parameters of patients. The percentage of positive cells was scored 0, 1, 2, or 3 for <25%, 25-50%, 50%-75% and >75%, respectively. Staining intensity were scored as 0, 1, 2, or 3 representing negative, weakly positive, mo-derately positive, and strongly positive, respectively. The final immunoreactivity score (IS) was calculated by multiplying the staining intensity and percentage of positive cells. The receiver operating characteristic (ROC) curve analysis was employed to define the cut-off value of Bbc3, E-cadherin and N-cadherin expression. IS of 0-1, 2-3, and 4-6 were rated as negative, moderately positive and strongly positive, respectively.

RNA extraction and qRT-PCR

Total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA) and reverse transcribed to complementary DNA (cDNA) using the ImProm-IITM Reverse Transcription System (Promega). Quantitative PCR was carried out with the ABI StepOne Real-Time PCR system (Applied Biosystems) using SYBR Green Realtime PCR Master Mix (Toyobo) according to the manufacturer's instructions. Relative gene expression were calculated using the $2^{-\Delta Ct}$ method. The housekeeping gene GAPDH was used as an internal control. The following primers were used: Bbc3: F: 5'-TCT CGG TGC TCC TTC ACT CTG-3' and R: 5'-CGT TTG GCT CAT TTG CTC TT-3'; GAPDH: F: 5'-TGA AGG TCG GAG TCA ACG G-3' and R: 5'-CTG GAA GAT GGT GAT GGG ATT-3'.

Western-blot

Western-blot was performed to measure Bbc3 expression in 30 pairs of fresh frozen RC tissue samples and paired normal controls. BCA protein assay (Pierce) was used to estimate protein concentrations. Equal amounts of cell lysates were resolved by electrophoresis using 12% gel and electrotransferred to nitrocellulose membrane. Then, the membrane was incu-

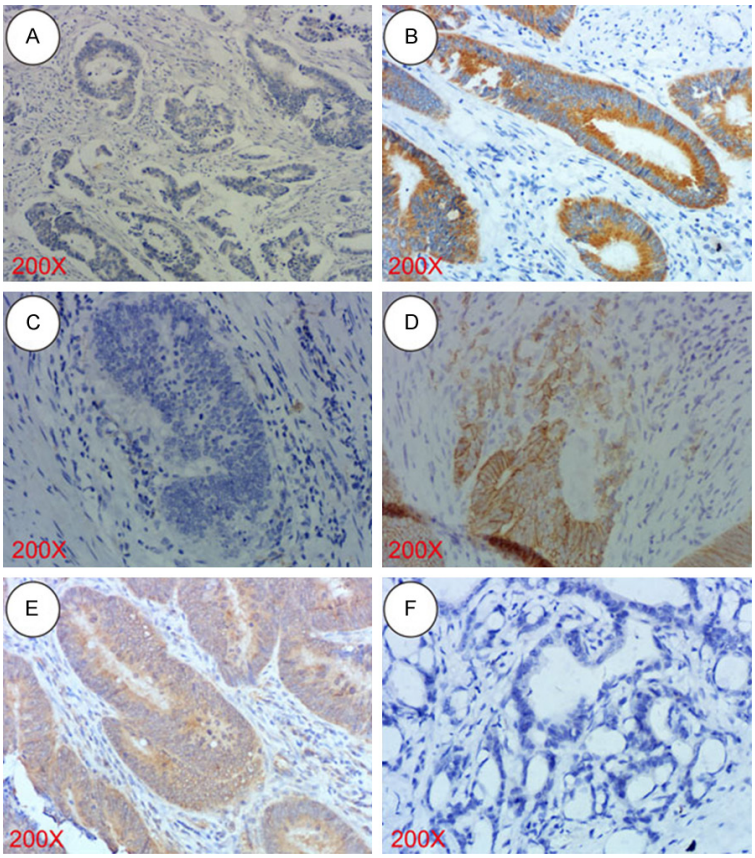

Figure 2. Representative IHC staining for Bbc3, E-cadherin and N-cadherin in RC tissues (×200). A. Bbc3 negative staining in RC tissues (×200). B. Bbc3 positive staining in RC tissues (×200). C. E-cadherin negative staining in RC tissues (×200). D. E-cadherin positive staining in RC tissues (×200). E. N-cadherin positive expression in RC tissues. F. N-cadherin negative expression in RC tissues (×200).

bated with anti-Bbc3 antibody (1:1000, Abcam) at 4°C overnight. β-actin antibody (1:2000, Abcam) was used as internal control. After washing for three times, the membrane was incubated with HRP-labeled Goat Anti-Rabbit IgG (1:2000) at room temperature for 2 hours. Chemiluminescence reagent (Abcam) was used to evaluate protein levels according to the manufacturer's instructions. Image J 1.43 software was used for quantitative analysis of Western-blot results.

Online datasets and statistical analyses

Bbc3, E-cadherin and N-cadherin mRNA expression data of CRC (level3) were obtained from The Cancer Genome Atlas (TCGA) for association studies. All the data were analyzed using IBM SPSS 21.0 software. Normally distributed continuous variables were described as the mean ± SD. Means were compared by either a paired sample t-test or one-way anova. Chi-squared (χ^2) test or Fisher's exact test was used where appropriate. Kaplan-Meier method was employed for univariate survival data analysis. Cox's proportional hazard regression model was used for the multivariate survival analyses. The correlation between Bbc3 and E-cadherin/N-cadherin expression was measured using Spearman correlation coefficient. *P*-value <0.05 was considered as statistically significant.

Results

RC patients had higher level of Bbc3

First, mRNA expression levels of Bbc3 in RC tissues and matched normal mucosa were quantified by qRT-PCR. Results showed that Bbc3 was significantly higher in RC patients than in matched normal tissues (P<0.0001) (**Figure 1A**). Moreover, this result was further confirmed by Western blot (P<0.0001) (**Figure 1B** and **1C**).

Bbc3 expression was negatively with prognosis-related clinical parameters

To investigate the association between Bbc3 expression and clinicopathological features, IHC staining of Bbc3 were performed using 140 FFPE RC tissues. Representative IHC staining for Bbc3 in RC tissues (×200) were shown in **Figure 2A** and **2B**. Statistical analysis showed that the expression of Bbc3 protein significantly correlated with grade of differentiation (P=0.028), lymph node metastasis (P=0.003) and TNM classification (P<0.001), but not with patients' age, gender, tumor size, histological type and depth of invasion.

Bbc3 was associated with favorable prognosis of rectal cancer

RC patients with high expression of Bbc3 had a 5-year survival rate of 77.3%, compared with

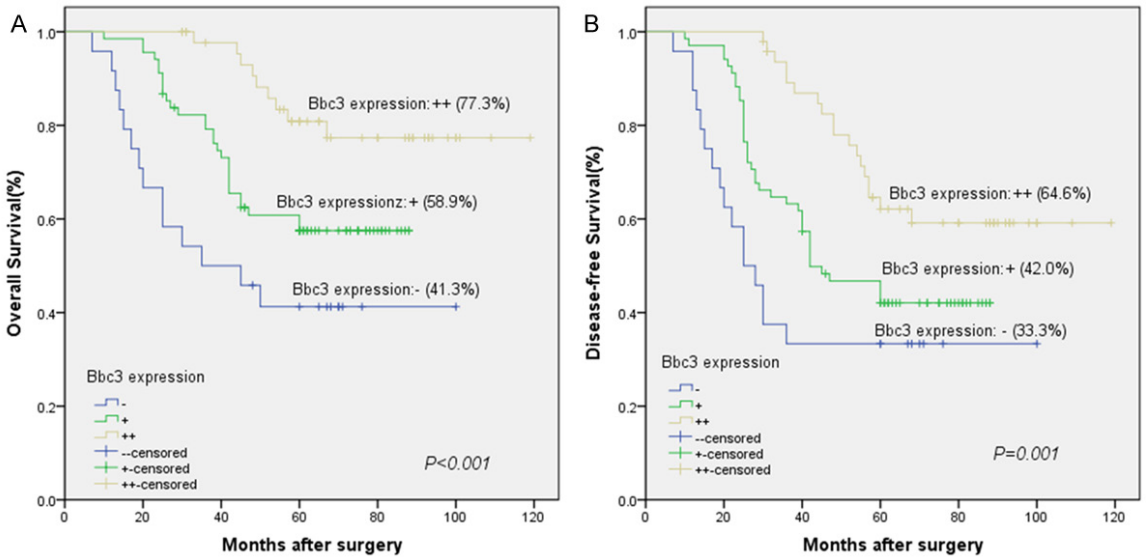

Figure 3. The Kaplan-Meier graph for 140 RC patients stratified according to the immunoreactivity score. A. The overall survival rate for RC patients with high expression of Bbc3 was higher than those with low expression (P<0.001). B. The disease-free survival rate for RC patients with high expression of Bbc3 was higher than those with low expression (P<0.001).

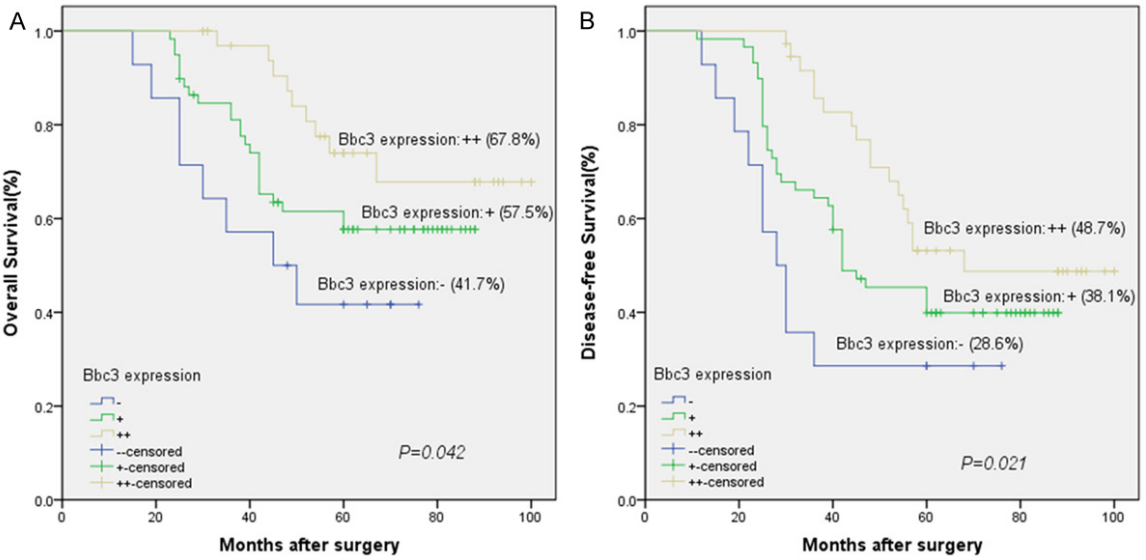

Figure 4. Kaplan-Meier graph for the overall survival (A) and disease-free survival (B) of 110 intermediate RC patients with low and high Bbc3 expression (P=0.042 and 0.021 respectively).

58.9%, 41.3% for RC patients with low or negative expression of Bbc3. The survival rates were significantly different among the three groups and strong expression of Bbc3 was associated with a better survival rate (*P*<0.001). The association between Bbc3 expression and clinical outcome (OS) in RC patients was shown in **Figure 3A**. Similar result was observed in the disease-free survival analysis (*P*<0.001) (**Figure 3B**). Furthermore, patients in the intermediate stages (II and III stage) were analyzed specially to detect the value of Bbc3 level in predicting the prognosis of these patients. The results demonstrated that patients in stage II and III with high expression of Bbc3 had a better overall and disease-free survival rate than those with low expression (*P*=0.042 and 0.021, respectively) (**Figure 4A** and **4B**).

Table 2. Correlation of Bbc3 expression and EMT markers in RC tissues

EMT markers	Bbc 3			r	*P*-value
	Negative	Moderately positive	Strongly positive		
E-cadherin					
Negative	16	21	8	0.350	*P*<0.001
Low expression	4	25	14		
High expression	4	22	26		
N-cadherin					
Negative	3	15	24	-0.402	*P*<0.001
Low expression	9	33	21		
High expression	12	20	3		

Cox proportional hazards regression models were applied to determine independent prognostic factors. As shown in **Table 3**, univariate analysis indicated that overall survival of RC patients was significantly associated with lymph node metastasis (*P*<0.01), Bbc3 expression (*P*<0.01), depth of invasion (*P*<0.01), TNM classification (*P*<0.01) and histological type (*P*=0.047). In multivariate analysis, we found that lymph node invasion (*P*=0.003), TNM classification (*P*<0.001) and Bbc3 expression level (*P*=0.033) were independent predictive factors in RC patients. Univariate analysis also performed Univariate analysis for DFS was also performed. The results showed DFS was significantly associated with depth of invasion, histological type, lymph node metastasis, TNM classification, and Bbc3 expression (*P*<0.001, *P*=0.035, *P*<0.001, *P*<0.001 and *P*<0.001, respectively). While in multivariate analysis, the association with Bbc3 expression (*P*=0.006), lymph node metastasis (*P*=0.001), TNM classification (*P*<0.001) were significant.

Bbc3 expression was positively associated with E-cadherin, but negatively associated with N-cadherin in RC tissues

IHC was performed to examine the expression of two well-known EMT markers (E-cadherin and N-cadherin). Representative IHC staining for E-cadherin and N-cadherin in RC tissues (×200) were shown in **Figure 2C**, **2D** for E-cadherin and **Figure 2E**, **2F** for N-cadherin, respectively. As we can see from **Table 2**, in 48 patients with high expression of Bbc3, low expression of E-cadherin was only found in 8 patients, compared with high expression of N-cadherin in 3 patients. Nonparametric Spearman rank correlation coefficient analysis indicated that Bbc3 expression was positively associated with E-cadherin (r=0.350, P<0.001) but negatively associated with N-cadherin (r=-0.402, P<0.001). These results were further validated by analyzing gene expression data of CRC obtained from TCGA (**Figure 5**, for association with E-cadherin and N-cadherin, Spearman r=0.14 and 0.12, with P=0.008 and 0.02, respectively).

Discussion

Clinical and pathologic tumor staging insufficiently addresses tumor heterogeneity and, therefore, cannot account for interpatient variability in clinical outcome. Prognostic markers are needed to stratify RC patients to direct treatment and prevention. Abundant evidences support the role of apoptotic regulatory proteins in tumor progression and metastasis [17]. Previous studies indicated that Bbc3 was important mediator of apoptosis induced by a number of chemotherapeutics and radiation [18-21]. A recent study suggested Bbc3 can provide prognostic information for stage II and III colon cancer patients receiving 5-fluorouracil-based adjuvant chemotherapy [22]. But its role in Chinese RC patients remains inconclusive. In this study, we found the expression of Bbc3 was significantly up-regulated in RC tissues in comparison to that in normal mucosa, suggesting its repression may be an important step during RC tumorigenesis [23].

Our previous study showed Bbc3 expression was related to the prognosis of CRC patients [24]. But recently, rectal cancer (RC) and colon cancer (CC) were thought as two different diseases, having different biological behaviors and different prognosis. To determine its clinical significance in RC patients, IHC was performed and the results showed that Bbc3 expression was negatively with prognosis-related clinical parameters, including differentiation grade, lymph node metastasis and TNM stages (all *P*<0.001). Kaplan-Meier analyses and Cox proportional hazard model analyses were performed to investigate whether Bbc3 could be

Table 3. Univariate analysis and multivariate analysis for factors influencing the overall survival of RC patients

Variables	Univariate analysis				Multivariate analysis			
	β value	HR	95% CI	*P* value	β value	HR	95% CI	*P* value
Age (≤60 vs >60)	-0.191	0.826	0.474-1.439	0.500			NR	
Gender	-0.114	0.893	0.512-1.555	0.688			NR	
Tumor size (<3×3 vs 3×5 vs >5×3)	0.266	1.305	0.782-2.176	0.308			NR	
Differentiation grade (high vs intermediate vs low)	-0.228	0.796	0.505-1.256	0.327			NR	
Depth of invasion (myometrial vs serosal vs outside of serosa)	0.692	1.997	1.320-3.022	0.001	0.366	1.441	0.917-2.266	0.113
Lymph node metastasis	1.975	7.208	3.724-13.952	0.000	-2.778	0.062	0.010-0.380	0.003
Histological type (papillary vs tubular vs mucinous)	0.325	1.384	1.005-1.908	0.047	0.243	1.275	0.894-1.820	0.180
TNM classification (I vs II vs III vs IV)	2.195	8.981	4.884-16.514	0.000	4.133	62.340	12.554-309.562	0.000
Bbc3 expression (negative vs low expression vs high expression)	-0.779	0.459	0.307-0.686	0.000	-0.450	0.638	0.422-0.965	0.033

CI: confidence interval. NR, variable was not included in the resultant model; HR, hazard ratio. *Significant difference (P<0.05).

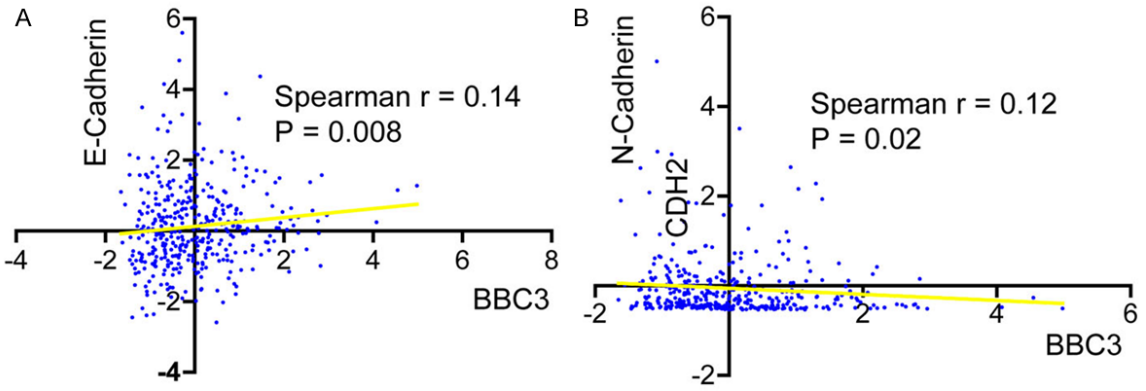

Figure 5. Bbc3 expression is positively correlated with E-cadherin, while negatively correlated with N-cadherin. A. Bbc3 is positively correlated with E-cadherin (Spearman r=0.14, P=0.008). B. Bbc3 is negatively correlated with N-cadherin (Spearman r=0.12, P=0.02). mRNA expression data are obtained from the Cancer Genome Atlas (TCGA, N=382).

an independent prognostic marker for RC patients. The result showed that overall survival rate was significantly higher in patients with high levels of Bbc3 than those with low expression of Bbc3, and Bbc3 could be an important independent prognostic factor for RC patients. Our findings is in consistence with a tumor suppressor role of Bbc3 [25]. A potential mechanism by which absent or reduced expression of Bbc3 can negatively affect prognosis is suggested by the observation that defects in apoptosis enable tumor cells to survive and grow intravascularly [17]. TNM classification has been reported not to be suitable to predict outcome in patients with intermediate levels of disease, as same pathologic stage suffer different clinical outcomes [26]. So, patients with stage II and III regarding as intermediate levels were applied to evaluate the effect of Bbc3 overexpression on the prognosis. Our data showed low expression of Bbc3 achieved a poorer prognosis, even in the same pathologic stage. Therefore, Bbc3 biomarker may be a useful tool for patients to make clinical decision with respect to administration of adjuvant therapy or follow-up.

To the best of our knowledge, the data showed for the first time Bbc3 expression level positively correlated with E-cadherin expression but negatively correlated with N-cadherin expression, suggesting a potential involvement of Bbc3 in EMT phenotype. EMT has been widely accepted that promoting metastasis which is one of the most important factors influencing patient prognosis [27]. We detected the protein expression of EMT markers (E-cadherin and N-cadherin) in RC tissues by IHC and analyzed the association with Bbc3. Our data was in accordance with previous researches in other tumors. Researchers found Bbc3 might cooperate with p21 to prevent EMT in mammary epithelial cells [28]. A recent study suggested overexpression of Bbc3 activated by miR-22 inhibited EMT in renal cell carcinoma [29]. Many studies showed EMT could confer abilities of anti-apoptosis and chemo/radiotherapy resistance [18, 30]. Above all, it is reasonable that the loss of Bbc3 expression may be involved in progression of RC by promoting EMT. However, the specific regulatory role of Bbc3 in EMT need subsequent further assays in vitro and vivo.

In conclusion, our study demonstrates that low Bbc3 expression may be associated with the development of RC and have potential utility as prognostic biomarker for Chinese patients with RC. Furthermore, Bbc3 can also be an ideal prognostic biomarker for patients with intermediate levels of RC, may assist in the risk stratification of RC patients and may be useful in therapeutic decision making. In particular, the present study showed for the first time that Bbc3 may be associated with EMT phenotype in RC. Subsequent further studies based on cellular and animal model are warranted to investigate the molecular mechanism of Bbc3 in EMT.

Acknowledgements

This work is supported by Zhejiang Medicine and Health Science & Technology (No. 2009-

A019 and 2014KYA024). We thank Zhejiang Province Key Laboratory of gastrointestinal disease for assisting in the preparation of experiment.

Informed consent was obtained from all individual participants included in the study.

Disclosure of conflict of interest

None.

Address correspondence to: Quanjin Dong, Department of Coloproctologic Surgery, Zhejiang Provincial People's Hospital, Hangzhou, China. E-mail: 13858039619@163.com

References

[1] Siegel R, Desantis C and Jemal A. Colorectal cancer statistics, 2014. CA Cancer J Clin 2014; 64: 104-117.

[2] Meng WJ, Yan H, Zhou B, Zhang W, Kong XH, Wang R, Zhan L, Li Y, Zhou ZG and Sun XF. Correlation of SATB1 overexpression with the progression of human rectal cancer. Int J Colorectal Dis 2012; 27: 143-150.

[3] Chen LT and Whang-Peng J. Current status of clinical studies for colorectal cancer in Taiwan. Clin Colorectal Cancer 2004; 4: 196-203.

[4] Bohanes P, LaBonte MJ, Winder T and Lenz HJ. Predictive molecular classifiers in colorectal cancer. Semin Oncol 2011; 38: 576-587.

[5] Cummings LC and Cooper GS. Colorectal cancer screening: update for 2011. Semin Oncol 2011; 38: 483-489.

[6] Meyerhardt JA. Beyond standard adjuvant therapy for colon cancer: role of nonstandard interventions. Semin Oncol 2011; 38: 533-541.

[7] Thompson C, Leong S and Messersmith W. Promising targets and drugs in development for colorectal cancer. Semin Oncol 2011; 38: 588-597.

[8] Coleman MP, Gatta G, Verdecchia A, Esteve J, Sant M, Storm H, Allemani C, Ciccolallo L, Santaquilani M and Berrino F. EUROCARE-3 summary: cancer survival in europe at the end of the 20th century. Ann Oncol 2003; 14 Suppl 5: v128-149.

[9] Hanahan D and Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144: 646-674.

[10] Sattler M, Liang H, Nettesheim D, Meadows RP, Harlan JE, Eberstadt M, Yoon HS, Shuker SB, Chang BS, Minn AJ, Thompson CB and Fesik SW. Structure of Bcl-xL-Bak peptide complex: recognition between regulators of apoptosis. Science 1997; 275: 983-986.

[11] Liu X, Dai S, Zhu Y, Marrack P and Kappler JW. The structure of a Bcl-xL/Bim fragment complex: implications for bim function. Immunity 2003; 19: 341-352.

[12] Kuwana T, Bouchier-Hayes L, Chipuk JE, Bonzon C, Sullivan BA, Green DR and Newmeyer DD. BH3 domains of BH3-only proteins differentially regulate Bax-mediated mitochondrial membrane permeabilization both directly and indirectly. Mol Cell 2005; 17: 525-535.

[13] Chen L, Willis SN, Wei A, Smith BJ, Fletcher JI, Hinds MG, Colman PM, Day CL, Adams JM and Huang DC. Differential targeting of prosurvival Bcl-2 proteins by their BH3-only ligands allows complementary apoptotic function. Mol Cell 2005; 17: 393-403.

[14] Willis SN, Fletcher JI, Kaufmann T, van Delft MF, Chen L, Czabotar PE, Ierino H, Lee EF, Fairlie WD, Bouillet P, Strasser A, Kluck RM, Adams JM and Huang DC. Apoptosis initiated when BH3 ligands engage multiple Bcl-2 homologs, not Bax or Bak. Science 2007; 315: 856-859.

[15] Woynarowski JM, Faivre S, Herzig MC, Arnett B, Chapman WG, Trevino AV, Raymond E, Chaney SG, Vaisman A, Varchenko M and Juniewicz PE. Oxaliplatin-induced damage of cellular DNA. Mol Pharmacol 2000; 58: 920-927.

[16] Lowe SW and Lin AW. Apoptosis in cancer. Carcinogenesis 2000; 21: 485-495.

[17] Wong CW, Lee A, Shientag L, Yu J, Dong Y, Kao G, Al-Mehdi AB, Bernhard EJ and Muschel RJ. Apoptosis: an early event in metastatic inefficiency. Cancer Res 2001; 61: 333-338.

[18] Plotz M and Eberle J. BH3-only proteins: possible proapoptotic triggers for melanoma therapy. Exp Dermatol 2014; 23: 375-378.

[19] Sun C, Hobor S, Bertotti A, Zecchin D, Huang S, Galimi F, Cottino F, Prahallad A, Grernrum W, Tzani A, Schlicker A, Wessels LF, Smit EF, Thunnissen E, Halonen P, Lieftink C, Beijersbergen RL, Di Nicolantonio F, Bardelli A, Trusolino L and Bernards R. Intrinsic resistance to MEK inhibition in KRAS mutant lung and colon cancer through transcriptional induction of ERBB3. Cell Rep 2014; 7: 86-93.

[20] Drury LJ, Wendt MK and Dwinell MB. CXCL12 chemokine expression and secretion regulates colorectal carcinoma cell anoikis through Bim-mediated intrinsic apoptosis. PLoS One 2010; 5: e12895.

[21] Ni B, Ma Q, Li B, Zhao L, Liu Y, Zhu Y and Chen Q. Phenylarsine oxide induces apoptosis in Bax- and Bak-deficient cells through upregulation of Bim. Clin Cancer Res 2012; 18: 140-151.

[22] Sinicrope FA, Rego RL, Okumura K, Foster NR, O'Connell MJ, Sargent DJ and Windschitl HE. Prognostic impact of bim, puma, and noxa expression in human colon carcinomas. Clin Cancer Res 2008; 14: 5810-5818.

[23] Greenhough A, Wallam CA, Hicks DJ, Moorghen M, Williams AC and Paraskeva C. The pro-

apoptotic BH3-only protein Bim is downregulated in a subset of colorectal cancers and is repressed by antiapoptotic COX-2/PGE(2) signalling in colorectal adenoma cells. Oncogene 2010; 29: 3398-3410.

[24] Yuan H, Tu SL and He XJ. [Relationship between PUMA and BIM expression in colorectal cancer and tumor invasion, metastasis and prognosis]. Zhonghua Wai Ke Za Zhi 2013; 51: 547-551.

[25] Hata AN, Yeo A, Faber AC, Lifshits E, Chen Z, Cheng KA, Walton Z, Sarosiek KA, Letai A, Heist RS, Mino-Kenudson M, Wong KK and Engelman JA. Failure to induce apoptosis via BCL-2 family proteins underlies lack of efficacy of combined MEK and PI3K inhibitors for KRAS-mutant lung cancers. Cancer Res 2014; 74: 3146-3156.

[26] Schneider NI and Langner C. Prognostic stratification of colorectal cancer patients: current perspectives. Cancer Manag Res 2014; 6: 291-300.

[27] Lee SJ, Yang CS, Kim DD, Kang YN, Kwak SG, Park JB, Cho CH and Park KK. Microenvironmental interactions and expression of molecular markers associated with epithelial-to-mesenchymal transition in colorectal carcinoma. Int J Clin Exp Pathol 2015; 8: 14270-14282.

[28] Zhang Y, Yan W, Jung YS and Chen X. PUMA cooperates with p21 to regulate mammary epithelial morphogenesis and Epithelial-To-Mesenchymal transition. PLoS One 2013; 8: e66464.

[29] Zhang S, Zhang D, Yi C, Wang Y, Wang H and Wang J. MicroRNA-22 functions as a tumor suppressor by targeting SIRT1 in renal cell carcinoma. Oncol Rep 2016; 35: 559-567.

[30] Reed JC. Proapoptotic multidomain Bcl-2/Bax-family proteins: mechanisms, physiological roles, and therapeutic opportunities. Cell Death Differ 2006; 13: 1378-1386.