

## Twist2 is a valuable prognostic biomarker for colorectal cancer

Hao Yu, Guang-Zhi Jin, Kai Liu, Hui Dong, Hua Yu, Ji-Cheng Duan, Zhe Li, Wei Dong, Wen-Ming Cong, Jia-He Yang

Hao Yu, Kai Liu, Ji-Cheng Duan, Zhe Li, Jia-He Yang, Department of Laparoscopy, Eastern Hepatobiliary Surgery Hospital, the Second Military Medical University, Shanghai 200438, China

Guang-Zhi Jin, Hui Dong, Hua Yu, Wei Dong, Wen-Ming Cong, Department of Pathology, Eastern Hepatobiliary Surgery Hospital, the Second Military Medical University, Shanghai 200438, China

**Author contributions:** Yu H, Jin GZ and Liu K contributed equally to this work: they performed the major experiments, analyzed the data and wrote the manuscript; Dong H and Cong WM evaluated the immunohistochemical staining; Yu H, Duan JC, Li Z and Dong W acquired the data; Cong WM and Yang JH designed the study together and critically reviewed the manuscript. **Supported by** National Natural Science Foundation of China, grant, No. 81201937 and 81070359

**Correspondence to:** Jia-He Yang, MD, PhD, Department of Laparoscopy, Eastern Hepatobiliary Surgery Hospital, the Second Military Medical University, No. 225, Changhai Road, Shanghai 200438, China. [ehbhjhyang@163.com](mailto:ehbhjhyang@163.com)

Telephone: +86-21-81875262 Fax: +86-21-81875262

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### Abstract

**AIM:** To investigate the significance of Twist2 for colorectal cancer (CRC).

**METHODS:** In this study, 93 CRC patients were included who received curative surgery in Eastern Hepatobiliary Surgery Hospital from January 1999 to December 2010. Records of patients' clinicopathological characteristics and follow up data were reviewed. Formalin-fixed, paraffin-embedded tissue blocks were used to observe the protein expression of Twist2 and E-cadherin by immunohistochemistry. Two independent pathologists who were blinded to the clinical information performed semi-quantitative scoring of immunostaining. A total score of 3-6 (sum of extent + intensity) was considered as Twist2-positive expression. The expression of E-cadherin

was divided into two levels (preserved and reduced). An exploratory statistical analysis was conducted to determine the association between Twist2 expression and clinicopathological parameters, as well as E-cadherin expression. Furthermore, the variables associated with prognosis were analyzed by Cox's proportional hazards model. Kaplan-Meier analysis was used to plot survival curves according to different expression levels of Twist2.

**RESULTS:** Twist2-positive expression was observed in 66 (71.0%) samples and mainly located in the cytoplasm. Forty-three (46.2%) samples showed reduced expression of E-cadherin. There were no significant correlations between Twist2 expression and any of the clinicopathological parameters. However, Twist2-positive expression was significantly associated with reduced expression of E-cadherin ( $P = 0.040$ ). Multivariate analysis revealed that bad M-stage [hazard ratio (HR) = 7.694, 95%CI: 2.927-20.224,  $P < 0.001$ ] and Twist2-positive (HR = 5.744, 95%CI: 1.347-24.298,  $P = 0.018$ ) were the independent risk factors for poor overall survival (OS), while Twist2-positive (HR = 3.264, 95%CI: 1.455-7.375,  $P = 0.004$ ), bad N-stage (HR = 2.149, 95%CI: 1.226-3.767,  $P = 0.008$ ) and bad M-stage (HR = 10.907, 95%CI: 4.937-24.096,  $P < 0.001$ ) were independently associated with poor disease-free survival (DFS). Survival curves showed a definite trend for Twist2-negative patients to have longer OS and DFS than Twist2-positive patients, not only overall, but also for patients in different stages, especially for DFS of patients in stage III ( $P = 0.033$ ) and IV ( $P = 0.026$ ).

**CONCLUSION:** Our data suggests, for the first time, that Twist2 is a valuable prognostic biomarker for CRC, particularly for patients in stage III and IV.

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**Key words:** Colorectal cancer; Prognostic biomarker; Twist2; Epithelial-mesenchymal transition; Immunohistochemistry

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## INTRODUCTION

Colorectal cancer (CRC) is one of the most common malignant tumors and continues to be one of the leading causes of cancer-related death worldwide<sup>[1]</sup>. Traditionally, the prognosis of patients with CRC is mainly evaluated by the tumor (T) node (N) metastasis (M) stage<sup>[2]</sup>. However, patients in the same stage frequently had different outcomes, despite similar postoperative treatments. There must be some unknown mechanisms affecting patients' outcome beyond the clinical stage. Although many efforts have been made to find biomarkers to predict CRC, truly effective clinical biomarkers are rare. Therefore, new and more effective biomarkers are still needed.

Recently, Twist2 (Dermo1), a highly homologous protein of Twist1<sup>[3,4]</sup>, has attracted our attention. Koh *et al.*<sup>[5]</sup> reported that Twist2 could increase resistance to galectin-1-mediated-apoptosis, which facilitated the progression of some T-cells into tumors. Gasparotto *et al.*<sup>[6]</sup> found over-expression of Twist2 correlated with the poor prognosis of head and neck squamous cell carcinomas. Zhou *et al.*<sup>[7]</sup> suggested that Twist2 is associated with the invasion and metastasis of salivary adenoid cystic carcinoma. Li *et al.*<sup>[8]</sup> found that Twist2 is involved in the cervical malignant conversion and tumor metastasis. Twist2 is also considered an inducer of epithelial-mesenchymal transition (EMT)<sup>[9-11]</sup>, a well-known progression involved in embryogenesis<sup>[12,13]</sup>, tumor invasion and metastasis<sup>[14-18]</sup>, and drug resistance<sup>[19]</sup>. Evidently Twist2 is a significant biomarker for human tumors. However, until now, the relationship between Twist2 and CRC has remained unknown.

Therefore, we undertook the present investigation to determine the significance of Twist2 for CRC and to verify its function as an EMT inducer.

## MATERIALS AND METHODS

### Patients and tumor samples

Ninety-three CRC patients were included who underwent curative surgery in Eastern Hepatobiliary Surgery Hospital, the Second Military Medical University of China, from January 1999 to December 2010. The patients met the following criteria: no anti-cancer treatments were given before surgery; all the visible tumor nodules were resected (including the distant metastatic nodules); patients who died during surgery or from serious surgical complications were excluded; the resected nodules were identified as primary CRC or metastasis of CRC and the surgical margin was free of tumor cells by pathological examination; patients with lymphatic metastasis or/and

distant metastasis had received postoperative adjuvant chemotherapy, patients who died from non-CRC diseases or accidents were excluded; and the clinicopathological and follow-up data were available. All the formalin-fixed and paraffin-embedded primary CRC samples were obtained from the Department of Pathology of Eastern Hepatobiliary Surgery Hospital. All patients in this study gave written informed consent.

### Follow-up and postoperative treatment

Patients were followed up until death or until June 15, 2011. All patients were monitored by physical examination, routine blood tests [including serum carcinoembryonic antigen (CEA) concentration], chest X-ray and abdominal ultrasonography every 2 mo in the first year after surgery, and every 3-6 mo thereafter. A computed tomography scan (CT) or magnetic resonance imaging was performed every 6 mo or immediately when a recurrence/metastasis was suspected. If needed, a whole-body fludeoxyglucose positron emission tomography/CT was performed. The follow-up data were recorded during the postoperative examination in our hospital, while patients who were examined in another hospital were followed up by telephone or letter. Recurrence was determined by at least two imaging examination results. Once recurrent tumors were confirmed, further treatment was implemented, such as a second surgery and palliative chemotherapy. Disease-free survival (DFS) was defined as the period from the tumor resection until the tumor recurrence or the last observation. The overall survival (OS) was the interval between the surgery and death or the last follow-up examination.

### Immunohistochemistry

Immunohistochemistry was carried out as described previously<sup>[20]</sup>. Representative 4- $\mu$ m serial sections were prepared from 10% formalin-fixed, paraffin-embedded tissue blocks. To increase the immunoreactivity, microwave antigen retrieval was performed in citrate buffer (pH 6.0) for 5 min, then cooled the sections at room temperature for at least 30 min. Subsequently 3% hydrogen peroxide was used for 10 min to block endogenous peroxidase activity. After nonspecific binding sites were blocked for 30 min with goat serum, a monoclonal antibody against Twist2 (1:300, H00117581-M01, Abnova) and polyclonal antibodies against E-cadherin (1:100, BS1098, Bioworld Technology) were used to incubate the sections in a humid chamber at 4 °C overnight. Next, an EnVision Detection kit (GK500705, Gene Tech, China) was used to visualize tissue antigens. Sections were counterstained with hematoxylin for 5 min. Negative control sections were incubated with phosphate buffered solution instead of the primary antibody.

### Evaluation of immunohistochemistry

Two independent pathologists (Dong H and Cong WM), who were blinded to clinical information, assessed the expression of Twist2 and E-cadherin semiquantitatively.

**Table 1 Relationship between Twist2 expression and clinicopathological characteristics *n* (%)**

Characteristics	Total	Twist2 expression		<i>P</i> value
		Negative	Positive	
Gender				
Female	52 (55.9)	18 (66.7)	34 (51.5)	0.182 <sup>1</sup>
Male	41 (44.1)	9 (33.3)	32 (48.5)	
Age				
< 59	41 (44.1)	13 (48.1)	28 (42.4)	0.614 <sup>1</sup>
≥ 59	52 (55.9)	14 (51.9)	38 (57.6)	
T-stage				
T1-2	17 (18.3)	5 (18.5)	12 (18.2)	1.000 <sup>2</sup>
T3-4	76 (81.7)	22 (81.5)	54 (81.8)	
N-stage				
N0	52 (55.9)	17 (63.0)	35 (53.0)	0.381 <sup>1</sup>
N1-2	41 (44.1)	10 (37.0)	31 (47.0)	
M-stage				
M0	52 (55.9)	17 (63.0)	35 (53.0)	0.381 <sup>1</sup>
M1	41 (44.1)	10 (37.0)	31 (47.0)	
Tumor differentiation				
Moderate/good	83 (89.2)	26 (96.3)	57 (86.4)	0.271 <sup>2</sup>
Poor	10 (10.8)	1 (3.7)	9 (13.6)	
Vascular invasion				
No	56 (60.2)	17 (63.0)	39 (59.1)	0.729 <sup>1</sup>
Yes	37 (39.8)	10 (37.0)	27 (40.9)	
Tumor location				
Rectum	19 (20.4)	4 (14.8)	15 (22.7)	0.390 <sup>1</sup>
Colon	74 (79.6)	23 (85.2)	51 (77.3)	
CEA level (ng/mL)				
≤ 5	48 (51.6)	12 (4.4)	36 (54.5)	0.376 <sup>1</sup>
> 5	45 (48.4)	15 (55.6)	30 (45.5)	

<sup>1</sup>Pearson's  $\chi^2$  test; <sup>2</sup>Fisher's exact test. T-, N-, M-stage are tumor, node, and metastasis stage (6<sup>th</sup> edition), performed according to the American Joint Committee on Cancer; CEA: Serum carcinoembryonic antigen.

Twist2 staining was observed only in the cytoplasm of CRC tumor cells (described in the results); therefore, the nucleolus staining was not evaluated. Cytoplasmic staining of Twist2 was scored according to its extent and intensity (extent + intensity), similar to the methods described previously<sup>[21-24]</sup>. The extent of staining was graded as follows: 0 for < 15% positive cells, 1 for 15%-30%, 2 for 30%-60% and 3 for more than 60% positive cells. The intensity of staining was scored on the following scale: 0, no staining; 1, weak staining; 2, moderate staining; 3, strong staining. The total score was 0 to 6 when summed (extent + intensity) together. Subsequently, a total score of 0-2 was considered to be a negative/low expression, while a score of 3-6 was considered as positive/high expression. For E-cadherin, the scoring was determined as previous studies<sup>[25,26]</sup>. Preserved expression of E-cadherin was defined where tumor cells were stained as strongly and homogeneously as normal epithelial cells. Heterogeneous staining, weaker staining or completely negative staining of E-cadherin was considered as reduced expression.

### Statistical analysis

Pearson's  $\chi^2$  test and Fisher's exact test (wherever was applicable) were performed to determine the relationship between Twist2 expression and clinicopathological parameters and E-cadherin expression. The prognostic

factors for OS and DFS were examined by both univariate and multivariate analyses (Cox's proportional hazards model). Survival curves were plotted by Kaplan-Meier analysis and by a log rank test. A *P* value < 0.05 (two-sided) was considered statistically significant. All statistical analysis were performed using SPSS version 19 (SPSS Inc., Chicago, IL, United States).

## RESULTS

Patients' clinicopathological characteristics are shown in Table 1. The mean age was 58.9 years, ranging from 16 to 81. Forty-one patients had distant metastasis (M1); all the metastatic nodes were in the liver. The median follow-up period was 32 mo (range 6-144 mo). At the last follow-up, 55 patients had tumor recurrence, including one in rectal anastomotic, two with pelvic metastasis, three in the lung, one in both the liver and the lung and the other 48 only in the liver. Thirty patients had died. The OS and DFS rates were 82.2% and 61.0% at 1 year, 71.3% and 42.4% at 3 years, and 66.2% and 30.3% at 5 years, respectively.

### Twist2 and E-cadherin expression in CRC

Although some previous investigations found Twist2 was expressed in both the cytoplasm and the nucleus in several tumors<sup>[7,8,11,27]</sup>, we found Twist2 was mainly expressed in the cytoplasm in CRC, not in the nucleus (Figure 1). A similar expression pattern of Twist2 was found in hepatocellular carcinoma (HCC)<sup>[28]</sup>. By semiquantitative analysis, 66 (71.0%) of the 93 primary CRC tissue samples were positive for Twist2 expression, while the other 27 (39.0%) were negative. Twist2 expression was generally low in normal colon mucosa compared with the cancer tissues. For E-cadherin, as described previously<sup>[29,30]</sup>, normal epithelial cells were strongly and homogeneously stained in the membrane, while tumor cells were stained mainly in the membrane and occasionally in the cytoplasm (Figure 2). E-cadherin was considered as reduced in 43 (46.2%) patients. The other 50 (53.8%) patients were preserved.

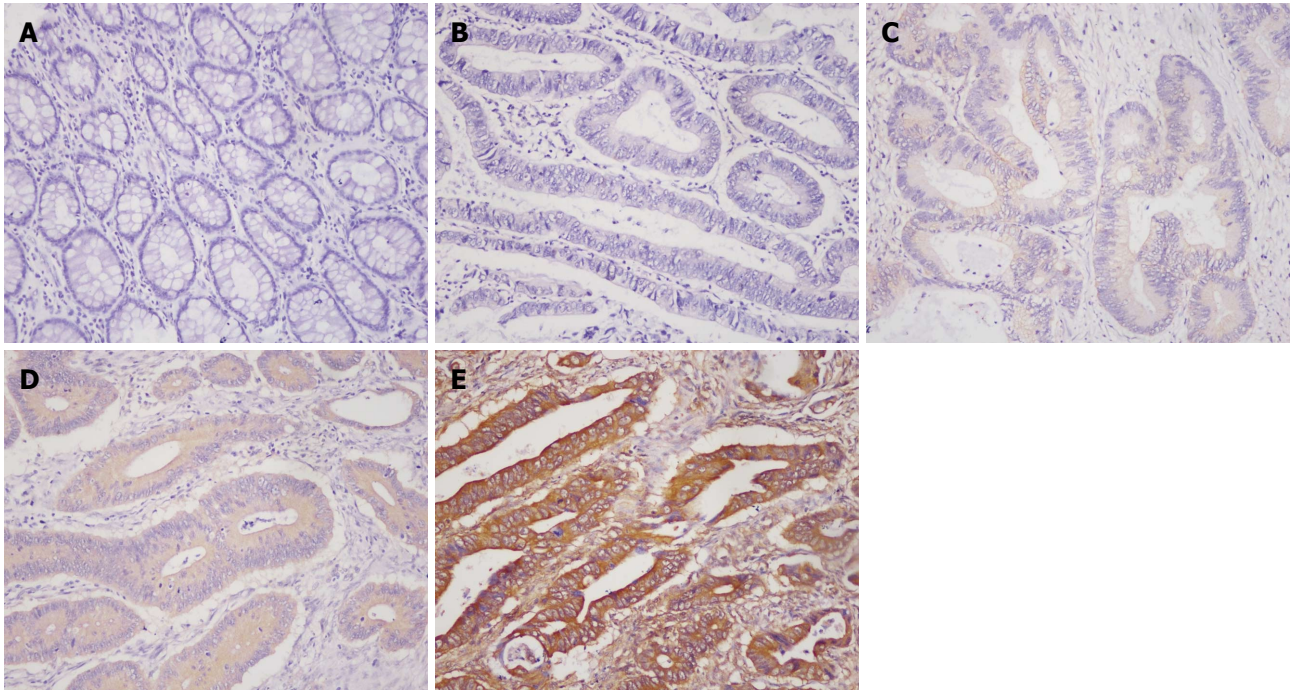
### Relationship between Twist2 expression and clinicopathological parameters and E-cadherin expression

As shown in Table 1, we did not find that Twist2 expression correlated with any of the clinicopathological parameters (gender, age, T-stage, N-stage, M-stage, tumor differentiation, vascular invasion, Tumor location and serum CEA level, all *P* > 0.05). When the relationship between Twist2 and E-cadherin expression was analyzed, we found a significant correlation: Twist2-positive patients showed a higher percentage of reduced E-cadherin than Twist2-negative ones (53.0% *vs* 29.6%, *P* = 0.040, Table 2).

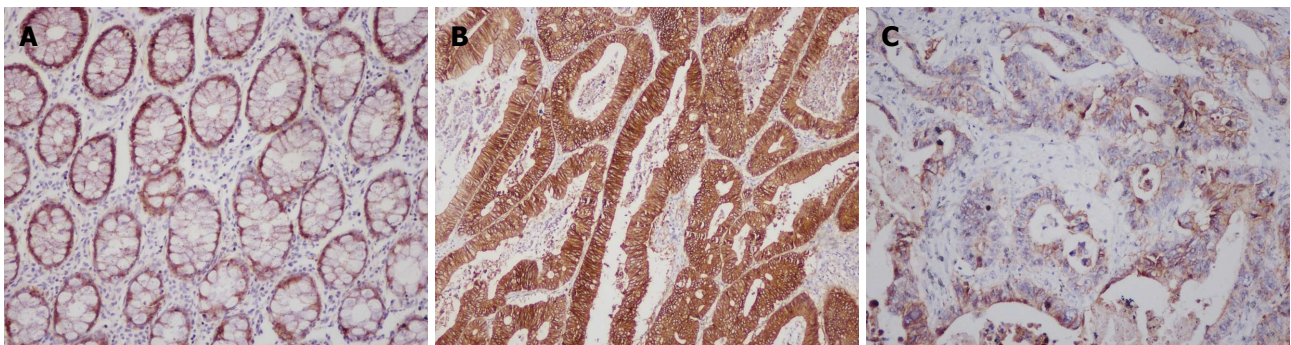
### Prognostic analysis

For OS on univariate analysis, bad N-stage (lymph node metastasis), bad M-stage (distant metastasis), vascular invasion, serum CEA level (> 5 ng/mL) and Twist2-positive





**Figure 1** Immunohistochemical images of Twist2. A: Negative staining in the normal mucosa; B: Negative; C: Weak; D: Moderate; E: Strong cytoplasmic staining in colorectal cancer (200× magnification).



**Figure 2** Immunohistochemical images of E-cadherin. A: Strong and homogeneous staining in normal mucosa; B: Preserved expression in colorectal cancer (CRC); C: Reduced expression in CRC (200× magnification).

Twist2 expression	E-cadherin expression		P value
	Preserved (n = 50)	Reduced (n = 43)	
Positive (n = 66)	31 (47.0)	35 (53.0)	0.040
Negative (n = 27)	19 (70.4)	8 (29.6)	

were significantly associated with poor survival (all  $P < 0.05$ , Table 3). When adjusted by multivariate analysis by Cox's proportional hazard model, bad M-stage [hazard ratio (HR) = 7.694, 95%CI: 2.927-20.224,  $P < 0.001$ ] and Twist2-positive (HR = 5.744, 95%CI: 1.347-24.298,  $P = 0.018$ ) were considered to independent risk factors for poor OS.

We also analyzed the risk factors for DFS (Table 4). The result of univariate analysis was similar to OS: bad N-stage, bad M-stage, vascular invasion, serum CEA

level ( $> 5$  ng/mL) and Twist2-positive were risk factors for poor DFS (all  $P < 0.05$ ). After adjustment, multivariate analysis revealed bad N-stage (HR = 2.149, 95%CI: 1.226-3.767,  $P = 0.008$ ), bad M-stage (HR = 10.907, 95%CI: 4.937-24.096,  $P < 0.001$ ) and Twist2-positive (HR = 3.264, 95%CI: 1.455-7.375,  $P = 0.004$ ) were independent risk factors for poor DFS, while vascular invasion and serum CEA level were not.

Survival curves plotted according to different expression levels of Twist2 are shown in Figure 3. Significantly, Twist2-negative patients had a higher 5-year OS (86.2% *vs* 59.6%,  $P = 0.015$ , Figure 3A) and 5-year DFS (55.4% *vs* 24.8%,  $P = 0.012$ , Figure 3B) than the Twist2-positive patients. Interestingly, further analysis of the value of Twist2 for CRC patients in different stages showed that for patients in stage I - II ( $n = 34$ ), there were no differences in OS or DFS (both  $P > 0.05$ , Figure 3C and D). For patients in stage III ( $n = 18$ ) and IV ( $n = 41$ ), Kaplan-

**Table 3** Univariate and multivariate analysis of the prognostic factors for overall survival

Prognostic factors	Univariate analysis			Multivariate analysis		
	HR	95%CI	P value	HR	95%CI	P value
Gender						
Female	1					
Male	1.011	0.490-2.085	0.977	NA	NA	NA
Age						
< 59	1					
≥ 59	1.579	0.750-3.326	0.229	NA	NA	NA
T-stage						
T1-2	1					
T3-4	1.118	0.493-2.534	0.789	NA	NA	NA
N-stage						
N0	1					
N1-2	2.172	1.056-4.468	0.035	NS	NS	NS
M-stage						
M0	1			1		
M1	6.324	2.659-15.041	< 0.001	7.694	2.927-20.224	< 0.001
Tumor differentiation						
Moderate/good	1					
Poor	1.290	0.491-3.384	0.229	NA	NA	NA
Vascular invasion						
No	1					
Yes	3.398	1.601-7.211	0.001	NS	NS	NS
Tumor location						
Rectum	1					
Colon	1.188	0.517-2.729	0.685	NA	NA	NA
CEA level (ng/mL)						
≤ 5	1					
> 5	3.173	1.475-6.827	0.003	NS	NS	NS
Twist2 expression						
Negative	1			1		
Positive	4.964	1.181-20.863	0.029	5.744	1.347-24.298	0.018

Multivariate analysis included adjustment for N-stage, M-stage, vascular invasion, serum carcinoembryonic antigen level and Twist2 expression. T: Tumor; N: Node; M: Metastasis; HR: Hazard ratio; NA: Not available; NS: Not significant.

Meier curves showed a clear trend that Twist2-negative patients had a more favorable outcome. Although the differences in OS were not statistically significant (both  $P > 0.05$ , Figure 3E and G), we found significant differences in DFS for both stage III and IV ( $P = 0.033$  and  $P = 0.026$  respectively, Figure 3F and H).

## DISCUSSION

This study, which investigated the significance of Twist2 protein expression in CRC, identified some variables that affected the patients' prognosis. Bad N-stage, bad M-stage (liver metastasis in our study), vascular invasion, serum CEA level ( $> 5$  ng/mL) and Twist2-positive were valuable predictors for both OS and DFS by univariate analysis. After adjustment by multivariate analysis, bad M-stage and Twist2-positive remained as independent risk factors for poor OS, while bad N-stage, bad M-stage and Twist2-positive were the independent risk factors for poor DFS. Twist2-positive was identified to be an independent risk factor for both poor OS and DFS. Kaplan-Meier analysis showed that patients with Twist2-negative expression had significantly longer OS and DFS than the positive pa-

**Table 4** Univariate and multivariate analysis of the prognostic factors for disease-free survival

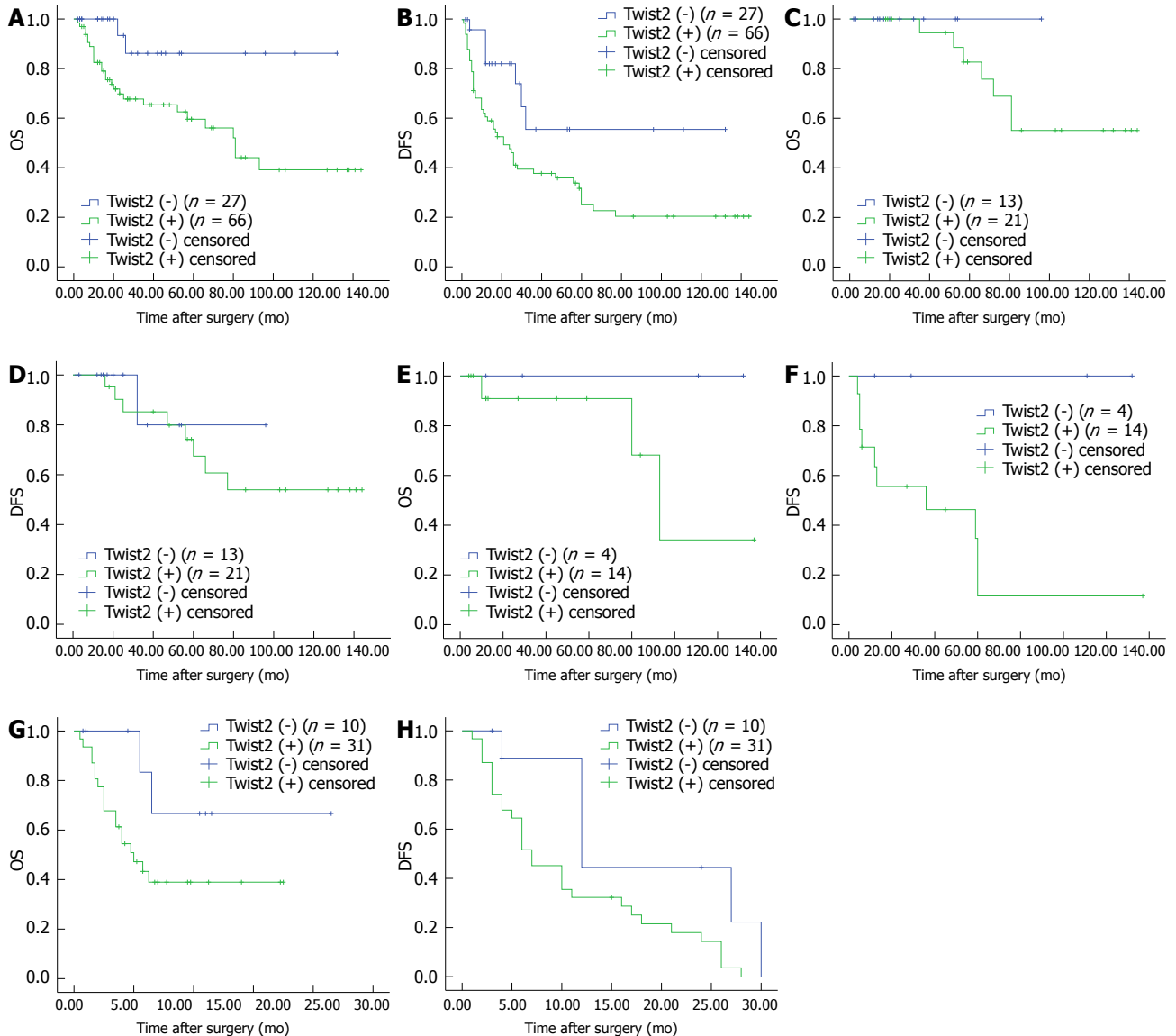
Prognostic factors	Univariate analysis			Multivariate analysis		
	HR	95%CI	P value	HR	95%CI	P value
Gender						
Female	1					
Male	1.001	0.587-1.706	0.998	NA	NA	NA
Age						
< 59	1					
≥ 59	1.060	0.621-1.809	0.831	NA	NA	NA
T-stage						
T1-2	1					
T3-4	1.258	0.833-1.899	0.276	NA	NA	NA
N-stage						
N0	1			1		
N1-2	2.511	1.468-4.295	0.001	2.149	1.226-3.767	0.008
M-stage						
M0	1			1		
M1	11.737	5.442-25.313	< 0.001	10.907	4.937-24.096	< 0.001
Tumor differentiation						
Moderate/good	1					
Poor	1.140	0.537-2.418	0.733	NA	NA	NA
Vascular invasion						
No	1					
Yes	3.246	1.874-5.625	< 0.001	NS	NS	NS
Tumor location						
Rectum	1					
Colon	1.557	0.810-2.992	0.184	NA	NA	NA
CEA level (ng/mL)						
≤ 5	1					
> 5	2.958	1.692-5.172	< 0.001	NS	NS	NS
Twist2 expression						
Negative	1			1		
Positive	2.632	1.184-5.809	0.017	3.264	1.455-7.375	0.004

Multivariate analysis included adjustment for N-stage, M-stage, vascular invasion, serum carcinoembryonic antigen level and Twist2 expression. T: Tumor; N: Node; M: Metastasis; HR: Hazard ratio; NA: Not available; NS: Not significant.

tients. When considering the prognostic value of Twist2 for CRC patients in different stages, we observed a trend for Twist2-negative patients to have a more favorable prognosis compared with Twist2-positive patients, especially for the patients in stage III and IV. Although the  $P$  values for OS in stage III and IV didn't reach significance, the  $P$  values for DFS in stage III and IV were statistically significant.

To the best of our knowledge, our study is the first report on the prognostic value of Twist2, based on the protein level, for human CRC. Currently, there is a lack of clinical biomarkers for effectively and routinely predicting CRC, especially for the patients in stage IV. Therefore, the findings of this study are very useful, as we found that Twist2 could be an effective biomarker for predicting the prognosis of CRC, even for patients in stage IV. Furthermore, the expression of Twist2 protein is easily detected by immunohistochemistry. For these reasons, Twist2 is potentially an extremely useful clinical biomarker for predicting the prognosis of CRC patients.

Reduced expression of E-cadherin, which is a hallmark of EMT<sup>[14]</sup> and plays a significant role in multi-stage carcinogenesis<sup>[31]</sup>, generally represents a common



**Figure 3** Kaplan-Meier analysis of overall survival and disease-free survival, according to the expression levels of Twist2. A, B: All patients (A, OS,  $P = 0.015$  and B, DFS,  $P = 0.012$ ); C, D: Patients in stage I - II (C, OS,  $P = 0.351$  and D, DFS,  $P = 0.652$ ); E, F: Patients in stage III (E, OS,  $P = 0.178$  and F, DFS,  $P = 0.033$ ); G, H: Patients in stage IV (G, OS,  $P = 0.101$  and H, DFS,  $P = 0.026$ ). OS: Overall survival; DFS: Disease-free survival.

feature of EMT inducers if these biomarkers were also upregulated<sup>[8]</sup>. In this study, Twist2-positive expression was significantly associated with reduced expression of E-cadherin, which supports the view that Twist2 is an EMT inducer in CRC. Unfortunately, we did not find a significant correlation between Twist2 expression and the adverse biological behaviors of CRC (bad T, N, M-stage, poor differentiation and vascular invasion). Thus, the mechanism remains unclear. However, other prognostic biomarkers share similar features with Twist2, such as vimentin<sup>[32]</sup>, a-smooth muscle actin<sup>[33]</sup> and S100A4<sup>[29]</sup> for CRC, and osteopontin for HCC<sup>[34]</sup>.

Considering the previous reports and the present study, several mechanisms probably contribute to the function of Twist2. Crucially, as an inducer of EMT, Twist2 can activate the EMT program, which is frequently involved in tumor progression and correlates with acquisi-

tion of therapeutic resistance<sup>[14-19]</sup>. In addition, hypoxia may participate in Twist2 function, as Zhou *et al*<sup>[7]</sup> found that positive expression of hypoxia-inducible factor-2 $\alpha$  was significantly associated with Twist2 overexpression in salivary adenoid cystic carcinoma. Furthermore, Twist2 also correlates with methylation<sup>[35,36]</sup> and cancer stem cell self-renewal<sup>[11]</sup>, as well as drug resistance<sup>[37]</sup>, which may explain the different outcomes of patients in the same stage. As EMT, cancer stem cells and drug resistance together comprise an axis of evil during tumor progression<sup>[19]</sup>, we speculate that Twist2 is a key component of this axis. In summary, the mechanism of Twist2's function in CRC is likely to be complex rather than simple.

In conclusion, the results of this study suggest that Twist2 is an independent prognostic factor for CRC. In particular, Twist2 exhibits a prognostic value for CRC in stage III and IV. Future studies with larger samples and



functional experiments are needed to confirm the function of Twist2 in CRC.

## COMMENTS

### Background

Colorectal cancer (CRC) is one of the most common malignant tumors and continues to be one of the most common causes of cancer death worldwide. It is important to identify biomarkers to predict patients' outcomes. Twist2 is a potential prognostic biomarker, but its value for CRC is unknown.

### Research frontiers

Twist2 is a regulatory factor of epithelial-mesenchymal transition, a well-known progression involved in embryogenesis, tumor invasion, metastatic dissemination and acquisition of therapeutic resistance. Hypoxia, methylation, cancer stem cell self-renewal and drug resistance correlate with Twist2 function. Therefore, Twist2 is a potential prognostic biomarker for tumors, and its prognostic value has also been identified for head and neck squamous cell carcinomas.

### Innovations and breakthroughs

This study revealed that Twist2 was overexpressed in CRC at the protein level. Twist2-positive expression correlated with the poor prognosis of CRC, particularly for patients in stage III and IV (tumor-node-metastasis stage).

### Applications

These results suggest that overexpression of Twist2 can probably serve as a prognostic factor for patients with CRC.

### Terminology

EMT is an important change in cell phenotype, which allows the escape of epithelial cells from the structural constraints imposed by tissue architecture, and was first recognized as a central process in early embryonic morphogenesis. Over recent decades, a series of studies have identified the involvement of EMT in solid tissue epithelial cancers' invasiveness and metastasis.

### Peer review

This study investigated Twist2 expression in 93 CRC patients and evaluated its value as a prognostic biomarker based on relapse and survival data of patients. The results indicate that Twist2 could be used as an effective prognostic biomarker for CRC. This paper is generally well designed and the result looks reliable.

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