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Original Article

Correlation of HER2 and FOXM1 in human colorectal carcinoma and its clinical significance

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Abstract: Background: The human epidermal growth factor receptor 2 (HER2) and transcription factor forkhead box protein M1 (FOXM1) are proto-oncogenes and have expressed in various kinds of human solid malignancies. However, the clinical significance of HER2 and FOXM1 in colorectal cancer (CRC) remains controversial, and there is no related report of the connection between HER2 and FOXM1 in colorectal cancer. This study aims to investigate the clinical values based on the connection of HER2 with FOXM1 in colorectal cancer. Material/Methods: We retrospectively investigated HER2 and FOXM1 expressions of 130 paraffin embedded CRC and their adjacent paraneoplastic tissues through immunohistochemical assay. Western blot and RT-PCR assays were applied to measure the relative expressions of HER2 and FOXM1 in mRNA and the protein levels of 30 fresh CRC and the adjacent paraneoplastic tissues. Results: HER2 and FOXM1 expressions were significantly higher in colorectal cancer than those in paraneoplastic tissues ($P<0.001$). Similarly, the relative expression levels of HER2 and FOXM1 in mRNA and protein were also significantly higher in CRC tissues than those in paraneoplastic tissues ($P<0.05$). The HER2 expression was closely correlated with tumor size, degree of differentiation, presence of vascular invasion, lymph node metastasis, distant metastases, advanced TNM stage, and prognosis ($P<0.05$). The FOXM1 expression was related to tumor invasion, vascular invasion, lymph node metastasis, distant metastases, TNM stage, and prognosis ($P<0.05$). Besides, FOXM1 was an independent prognostic factor in CRC. There was a significant correlation between FOXM1 and HER2 expressions in colorectal cancer ($r=0.335$; $P<0.01$). Conclusions: The overexpressions of HER2 and FOXM1 protein in colorectal cancer correlate with their clinicopathological characteristics and prognosis. HER2 and FOXM1 are important diagnostic markers for colorectal cancer. Furthermore, FOXM1 may be a potential target for therapy especially in HER2-targeted therapy-resistant cancers.

Keywords: Colorectal cancer, HER2, FOXM1, clinical significance, targeted therapy

Introduction

Colorectal cancer (CRC) is a common malignant tumor in the digestive system, the third most common cancer and the fourth leading cause of cancer-associated mortality worldwide [1]. The morbidity and mortality of colorectal cancer in China both rank the fifth, and have seriously threatened people's health [2]. Presently, the treatment of colorectal cancer is still mainly relying on the surgery supplemented by chemotherapy, but the therapeutic effects are far from satisfaction, especially for the patients with distant metastasis [3]. The biological behavior of colorectal cancer is complex and easily subject to recurrence, metastasis and resistance to chemotherapeutic drugs [4, 5]. Therefore, understanding the mechanism of carcinogenesis and development of colorectal cancer, and identifying specific markers for targeted therapy are great significances for patients' treatment in addition to the surgery and adjuvant chemotherapy.

HER2, a 185KD transmembrane tyrosine kinase receptor mapped onto chromosome 17p21 and a member of the epidermal growth factor receptor (EGFR) family [6], plays an important role in cell growth, differentiation and apoptosis. It contains three structural domains: a transmembrane domain, an N-terminal extracellular domain, and an intracellular tyrosine kinase domain. HER2 is the only receptor with no direct binding ligand, but it can form het-

Table 1. Relationship between HER2, FOXM1 and clinic pathological factors in 130 CRC

Varieties	n	HER2				FOXM1			
		Positive	Negative	χ^2	P	Positive	Negative	χ^2	P
Age (years)									
≤60	67	14	53	1.471	0.225	43	24	0.470	0.493
>60	63	19	44			44	19		
Gender									
Male	82	24	58	1.768	0.184	57	25	0.673	0.412
Female	48	9	39			30	18		
Tumor site									
Colon	68	18	50	0.089	0.766	50	18	2.811	0.094
Rectum	62	15	47			37	25		
Tumor Size (cm)									
<5	73	13	60	5.046	0.025	44	29	3.325	0.068
≥5	57	20	37			43	14		
CEA (ug/l)									
<5	88	19	69	2.070	0.150	58	30	0.127	0.722
≥5	42	14	28			29	13		
CA19-9 (U/mL)									
<40	111	26	85	1.542	0.214	71	40	3.004	0.083
≥40	19	7	12			16	3		
Vascular invasion									
Negative	98	12	86	36.290	<0.001	60	38	5.841	0.016
Positive	32	21	11			27	5		
Degree of differentiation									
Well/moderate	96	20	76	4.014	0.045	62	34	0.908	0.341
Poor	34	13	21			25	9		
Depth of tumor invasion									
T1-2	25	5	20	0.474	0.491	9	16	13.371	<0.001
T3-4	105	28	77			78	27		
Lymph node metastasis									
N0	75	13	62	8.207	0.017	39	36	17.845	<0.001
N1	38	16	22			33	5		
N2	17	4	13			15	2		
Distant metastases									
M0	116	22	94	23.433	<0.001	74	42	4.767	0.029
M1	14	11	3			13	1		
TNM staging									
I	19	2	17	27.953	<0.001	5	14	25.572	<0.001
II	49	6	43			29	20		
III	48	14	34			40	8		
IV	14	11	3			13	1		

erodimer with other human epidermal growth factor receptor (HER) members. HER2 that involved heterodimerization is the most potent signal transduction pathway among all dimmers formed by the HER family. Additionally, the over-expression of HER2 has been found in breast cancer, gastric cancer, esophageal cancer and other malignant tumors [7-9].

FOXM1, an oncogenic transcription factor of the Forkhead family, is widely expressed in embryonic tissues. The encoding gene consist-

ing of 10 exons and located in chromosome 12p13-3 has an evolutionarily conserved DNA-binding domain with 110 amino acid long [10], this domain is called winged-helix or forkhead domain. There are three different splicing isoforms, namely FOXM1A, FOXM1B and FOXM1C. Among them, FOXM1B and FOXM1C are highly expressed in cancer tissues, and play critical roles in transcriptional activation and carcinogenesis. However they are much less expressed in normal tissues. On the contrary, FOXM1A is much less expressed in cancer tissues, and plays a transcriptionally inactive effect. Therefore, the preferential expressions of FOXM1B and FOXM1C are important for carcinogenesis. Furthermore, the functions of FOXM1 affect the cell cycle by activating the expression of downstream molecules, for example vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs). FOXM1 also plays a critical role in cell cycle progression by regulating the transition from G1 to S phase and from G2 to M phase. It is one of the most frequently overexpressed genes in human malignant tumors, causing carcinogenesis by accelerating the process of cell cycle [11].

HER2 and FOXM1 have many similar biological characteristics. Recent studies have suggested that the expression of FOXM1 is regulated by HER2 in the breast cancer and gastric cancer [12, 13]. However, the relationship between HER2 and FOXM1 in colorectal cancer has not been reported. In this study, we intend to investigate the expression levels and clinical significances of HER2 and FOXM1 in colorectal cancer, and further analyze their correlation.

Materials and methods

Patients and tissue samples

130 CRC tissues and the corresponding paraneoplastic tissues (within 5 cm from the tumor edge), paraffin-embedded, selected from the First Affiliated Hospital of Anhui Medical University from January 2012 to December 2013, underwent surgical resection (**Table 1**). Besides, 30 tumor tissues and the matched paraneoplastic tissues, collected from the First Affiliated Hospital of Anhui Medical University between October 2016 to December 2016, underwent surgical resection and were immediately frozen in liquid nitrogen and stored at -80°C refrigerator. This study was approved by the ethics committee of the First Affiliated Hospital of Anhui Medical University. The inclusion criteria were: 1) all patients were informed and their written consents were obtained prior to surgery, 2) all patients had complete clinic-pathological information; 3) tumors were confirmed to be colorectal adenocarcinoma pathologically. None of these patients had received preoperative chemotherapy or radiotherapy. The criteria for histological classification accorded with the 8th American Joint Committee on Cancer (AJCC). Detailed clinicopathological characteristics of the patients are summarized in **Table 1**. The postoperative follow-up was carried after surgery until December 2016, and the median follow-up period is 32.91 months (ranging from 5 to 36 months). For patients who remained alive, the survival data were recorded as 36 months.

Immunohistochemistry

The expressions of HER2 and FOXM1 were detected through analyses with 4 μm-thick sections of formalin-fixed and paraffin-embedded tissues. The tissue sections were deparaffinized in dimethylbenzene and rehydrated with graded ethanol. The antigen retrieval was carried out by citrate buffer solution (pH 6.0) in microwave oven for 15 min. Then the 0.3% hydrogen peroxide was used to block the activity of endogenous peroxidase for 10 min. After that, tissue sections were incubated with rabbit monoclonal antibody against human HER2 (dilution 1:250, Abcam, US) and FOXM1 (dilution 1:150, Abcam, US) at 4°C overnight. In the next day, they were incubated by a secondary goat anti-rabbit antibody (PV6000, ZSGB-BIO Biotechnology, China) for 30 min. Finally, sections were incubated in 3, 30-diaminobenzidine tetrahydrochloride and counterstained with 0.1% hematoxylin. In each case, the positive controls were set with invasive breast carcinomas tissues replacing the CRC tissues. For negative controls, the antibodies were substituted by PBS. The IHC scores were independently evaluated by two experienced pathologists, who were blinded to patients' clinic-pathological features.

The HER2 expressions were scored on four groups according to Hofmann criterion [14]. A) negative (0): no membranous reactivity or staining in <10% of tumour cells; B) weakly positive

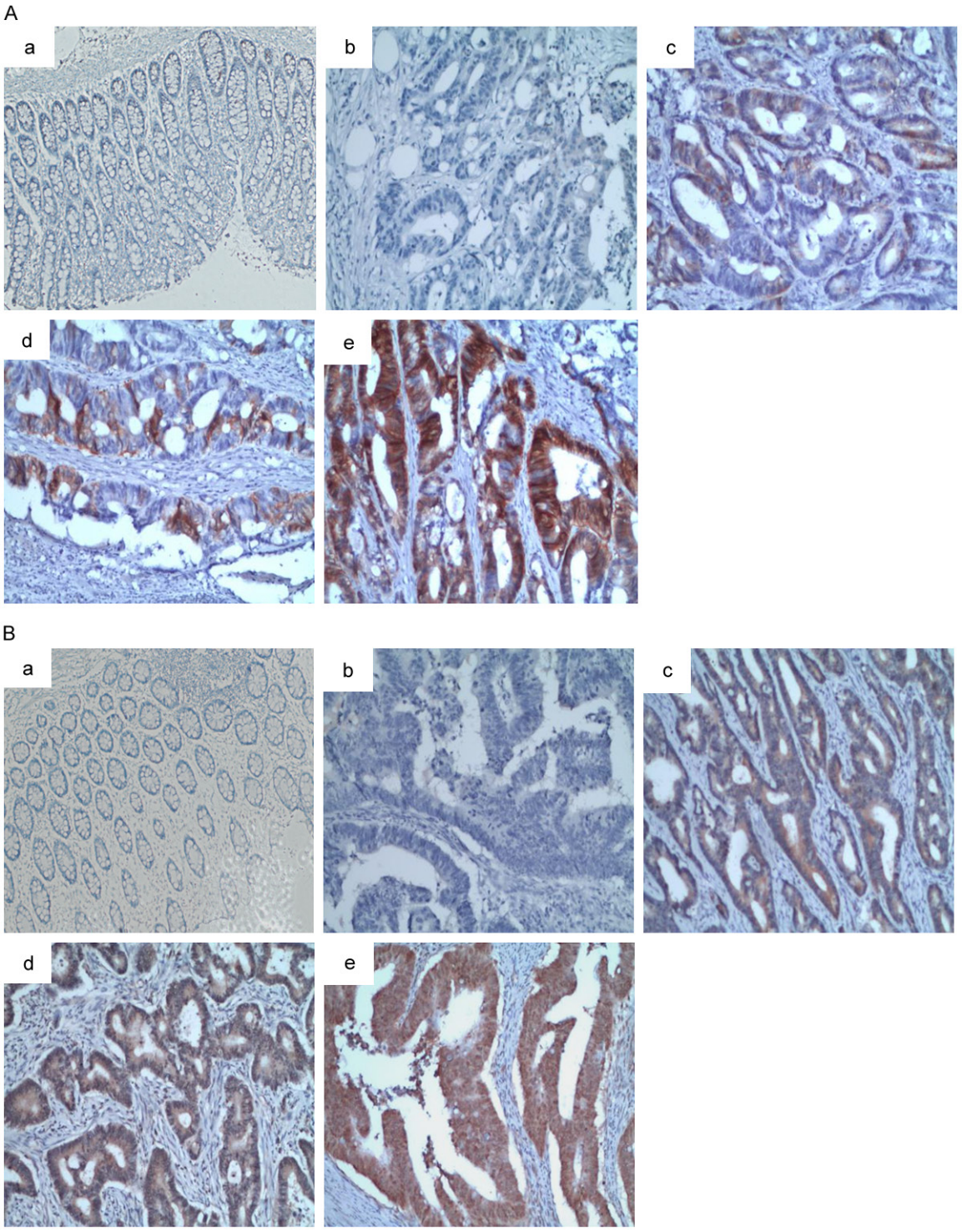

Figure 1. (A) Immunohistochemical analysis of HER2 protein expressions in human colorectal cancer tissues (b-e) and adjacent paraneoplastic tissues (a). The staining of HER2 protein was mainly located in the membrane and lightly in the cytoplasm of tumor cells. (b) negative staining (0); (c) weak staining (1+); (d) moderate staining (2+); (e) strong staining (3+). (B) Immunohistochemical analysis of FOXM1 protein expressions in human colorectal cancer tissues (b-e) and adjacent paraneoplastic tissues (a). The staining of FOXM1 protein was mainly located in the nucleus and cytoplasm of tumor cells. (b) negative staining (0); (c) weak staining (1+); (d) moderate staining (2+); (e) strong staining (3+).

Table 2. Different expression of HER2 and FOXM1 in CRC cancer tissues and the corresponding paraneoplastic tissues

Varieties	HER2		χ^2	P	FOXM1		χ^2	P
	Positive	Negative			Positive	Negative		
Cancer tissues	33	97	21.991	<0.001	87	43	68.991	<0.001
Paraneoplastic tissues	6	124			21	109		

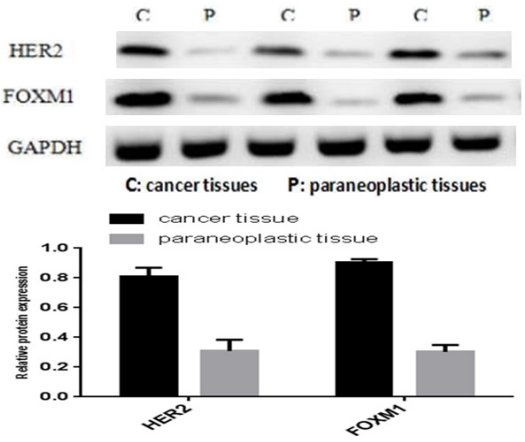

Figure 2. Analysis of HER2 and FOXM1 protein expressions in 30 cases of CRC tissues and the adjacent paraneoplastic tissues. The average relative expressions of HER2 in CRC tissues and the adjacent paraneoplastic tissues were 0.809±0.060 and 0.309±0.075 (t=6.033, P=0.026); and the average relative expression of FOXM1 in CRC tissues and the adjacent paraneoplastic tissues were 0.903±0.024 and 0.303±0.046 (t=9.092, P=0.012).

(1+): barely perceptible partial membranous reactivity ≥10% of tumor cells; C) moderate positive (2+): weak to moderate complete membranous staining in ≥10% of tumor cells; and D) strongly positive (3+): strong complete membranous staining in ≥10% of tumor cells. The expressions of FOXM1 were classified into four groups, based on the staining intensity and number of positive cells [15]. The staining intensities were scored as follows: 0=no staining; 1=light staining; 2=moderate staining; and 3=dark staining. The proportions of positive cells were scored as follows: 0=no staining; 1=0%-25%; 2=25%-50%; 3=50%-75%; and 4=75%-100%. Then we multiplied them to obtain overall scores. The overall scores of ≤3, 4-5, 6-7 and >8 were respectively termed as negative (0), weakly positive (1+), moderate positive (2+) and strongly positive (3+). For the HER2 and FOXM1, IHC 0 and 1+ were considered low expression while IHC 2+ and 3+ were considered overexpression.

Western blot analysis

30 paired colorectal cancer tumor tissues and matched paraneoplastic tissues were first lysed with moderate phenylmethanesulfonyl fluorid and Lysate, and then centrifuged supernatant repeatedly to extract protein. Amounts of total protein extracts were determined via bicinchoninic acid (BCA) assays. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and transferred to a polyvinylidene fluoride membranes. Next, membranes were blocked in 5% dried skimmed milk buffer for 60 min. After that, rabbit monoclonal antibodies were used: anti-HER2 (dilution 1:1000; Abcam, US), Anti-FOXM1 (dilution 1:500; Abcam, US), and anti-GAPDH (dilution 1:3000; CWBIO Biotechnology, China) at 4°C overnight. The next day, the membranes were incubated with a horseradish peroxidase conjugated secondary antibody (ZSGB-BIO Biotechnology, China) for 60 min. The results were detected by an enhanced chemiluminescence detection system (GE healthcare, UK). To ensure experimental accuracy, all reactions were performed in triplicate. The gray ratios of HER2/GAPDH and FOXM1/GAPDH were used to reflect their relative expression levels. The results were expressed by mean ± SD.

RNA extraction and RT-PCR

A semi-quantitative RT-PCR approach was used to assess mRNA levels of HER2 and FOXM1 in this study. Total RNA was extracted from 30 paired colorectal cancer tumor tissues and the matched paraneoplastic tissues with TRIzol reagent (Invitrogen, Carlsbad, CA, US) according to the manufacturer's instructions. The reverse transcription was performed via a RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, San Jose, CA, US) according to the manufacturer's instructions. The following primer sequences were used: HER2 sense, 5'-CTGTTTGCCGTGCCACCCTGAGT-3' and anti-

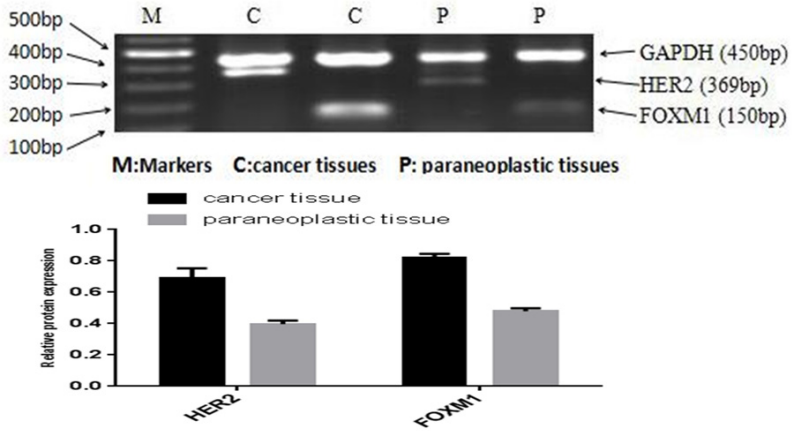

Figure 3. Analysis of HER2 and FOXM1 mRNA expressions in 30 cases of CRC tissues and the adjacent paraneoplastic tissues. The average relative expressions of HER2 in CRC tissues and the adjacent paraneoplastic tissues were 0.690±0.063 and 0.396±0.023 (t=29.821, P<0.001); and the average relative expressions of FOXM1 in CRC tissues and the adjacent paraneoplastic tissues were 0.819±0.026 and 0.479±0.019 (t=13.886, P=0.005).

Table 3. Correlation between HER2 and FOXM1 expressions

FOXM1	HER2		p	R
	Positive	Negative		
Positive	31	56	<0.01	0.335
Negative	2	41		

sense, 5'-CTTCTGCTGCCGTCGCTTGATGAG-3'; FOXM1 sense, 5'-GGAGGAAATGCCACACTTAGCG-3' and antisense, 5'-TAGGACTTCTTGGGTCTTGGGGTG-3'; GAPDH sense, 5'-ACCACAGTCCATGCCATCAC-3' and antisense, 5'-TCCACCACCCTGTTGCTG-3'. PCR products were separated via electrophoresis on 2.0% agarose. Images were captured via the Gel-Doc image analysis system (Bio-Rad). To ensure experimental accuracy, all reactions were performed in triplicate. The gray ratios of HER2/GAPDH and FOXM1/GAPDH were used to reflect their relative expression levels. The results were expressed by mean ± SD.

Statistical analysis

All statistical analyses were carried out via the software package SPSS 16.0 (Chicago, IL, US). The results of IHC of HER2 and FOXM1 expressions and their relationships with clinic-pathological parameters were analyzed via chi-square statistical or Fisher's exact test. The spearman correlation test was used to assess the correlation between HER2 and FOXM1. The student's t test was used to test the differences between the subgroups after RT-PCR and Western blot. Survival curves were plotted via the Kaplan-Meier method, and compared by the log-rank test. Univariate and multivariate survival analyses were conducted via the Cox proportional hazards regression model. A value of $P<0.05$ was considered as statistical significance for all tests.

Results

Overexpressions of HER2 and FOXM1 in colorectal cancer tissues

HER2 was positively stained in the membrane and cytoplasm, while FOXM1 was mainly expressed in the nucleus and cytoplasm of colorectal tissues (**Figure 1A**, **1B**). The results showed that the positive rate of HER2 was 25.38% (33/130) in cancer tissues of CRC and 4.62% (6/130) in the adjacent paraneoplastic tissues. The difference of HER2 expressions between the two groups was statistically significant (χ^2=21.991, P<0.001, **Table 2**). Besides, higher HER2 expressions were found to be closely correlated with larger tumor sizes, poorer degrees of differentiation, presence of vascular invasions, lymph node metastases, distant metastases and advanced TNM stages (P<0.05, **Table 1**). However, there was no correlation between the HER2 expression and the age, gender, tumor site, CEA, CA19-9 or depth of tumor invasion (P>0.05, **Table 1**). Furthermore, FOXM1 overexpressions were observed in 87 cases (66.92%) among the 130 colorectal cancer tissues; however, much lower expressions of FOXM1 were observed in paraneoplastic tissues (16.15%, 21/130). The FOXM1 expressions in CRC cancer tissues were significantly higher than those in the corresponding paraneoplastic tissues (χ^2=68.991, P<0.001, **Table 1**). The FOXM1 expressions correlated significantly with deeper tumor invasions, presence of vascular invasions, lymph node metastases, distant metastases and advanced TNM stages (P<0.05, **Table 1**). The FOXM1 expression was not found to be associated with age,

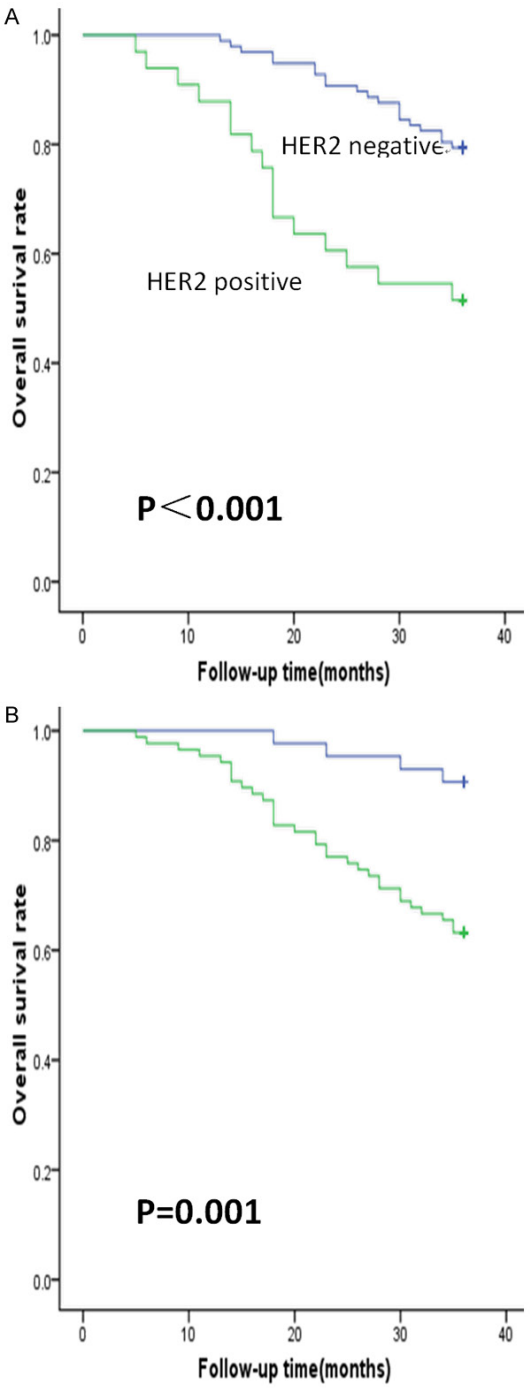

Figure 4. Kaplan-Meier survival analysis comparing HER2 (A) and FOXM1 (B) positivity with HER2 and FOXM1 negativity in colorectal cancer. The CRC patients with overexpression of HER2 and FOXM1 had lower 3-year survival rates. (51.5% vs. 79.4%, χ^2=13.277, P<0.001; 63.2% vs. 90.7%, χ^2=10.630, P=0.001, respectively).

gender, tumor site, size, CEA, CA19-9 or degree of differentiation (P>0.05, **Table 1**). The results suggested that HER2 and FOXM1 might play important roles in CRC progression. Moreover, we compared the levels of HER2 and FOXM1 expressions in 30 paired CRC tissues via RT-PCR and western blot analyses. The results showed that the relative expressions of HER2 and FOXM1 in tumor tissues were significantly higher than those in the adjacent paraneoplastic tissues in mRNA and protein level (P<0.05. **Figures 2**, **3**).

Correlation between HER2 and FOXM1 expressions in colorectal cancer

31 out of the 33 HER2 positive specimens were also positive for FOXM1 by immunohistochemistry. There was a significant correlation between the HER2 and FOXM1 expressions in colorectal cancer (r=0.335; P<0.01) (**Table 3**). Furthermore, we also analyzed HER2 and FOXM1 expressions in 30 paired CRC tissues by RT-PCR and Western blot, and found the HER2 overexpressions were associated with increased levels of FOXM1 expressions. Conversely, the tissues samples devoid of HER2 expressions also lacked FOXM1 expressions. Thus, there is a significant association between the HER2 and FOXM1 expressions at transcription and translation levels.

Survival analysis of colorectal cancer patients in 3 years after surgery

Patients with HER2 and FOXM1 positive expressed exhibited significantly lower 3-year survival rates compared with those with HER2 and FOXM1 negative expressed (χ^2=13.277, P<0.001, and χ^2=10.630, P=0.001, respectively, **Figure 4**). In univariate survival analyses, as expected, CEA, CA19-9, degrees of differentiation, vascular invasions, lymph node metastases, distant metastases and advanced TNM stages, FOXM1 expressions and HER2 expressions both affected the overall survivals (P< 0.05, **Table 4**). The multivariate analyses revealed that only CEA, distant metastases and FOXM1 expressions were verified to be independent prognostic factors while the HER2 overexpression was not independently associated with the overall survival (P=0.120, **Table 5**).

Discussion

The HER2 overexpression were observed in 33 of 130 (25.38%) CRC samples, significantly

Table 4. Results of univariate analyses of 130 CRC patients' survivals by Cox regression model

Varieties	n	95.0% CI	OR	P
Age (years)				
≤60/>60	67/63	0.810-3.049	1.571	0.181
Gender				
Male/Female	82/48	0.722-2.731	1.415	0.301
Tumor site				
Colon/Rectum	68/62	0.501-1.855	0.964	0.913
Tumor Size (cm)				
<5/≥5	73/57	0.643-2.379	1.236	0.525
CEA (ug/l)				
<5/≥5	88/42	2.446-9.577	4.840	<0.001
CA19-9 (U/mL)				
<40/≥40	111/19	2.005-8.084	4.026	<0.001
Vascular invasion				
Negative/Positive	98/32	1.114-4.258	2.178	0.023
Degree of differentiation				
Well and moderate/poor	96/34	1.065-4.070	2.082	0.032
Depth of tumor invasion				
T1-2/T3-4	25/105	0.787-6.296	2.226	0.131
Lymph node metastasis				
Negative/Positive	75/55	1.222-4.674	2.390	0.011
Distant metastases				
M0/M1	116/14	4.491-18.902	9.214	<0.001
TNM staging				
I-II/III-IV	68/62	1.622-6.983	3.365	0.001
HER2				
Negative/Positive	97/33	1.640-6.129	3.171	0.001
FOXM1				
Negative/Positive	43/87	1.679-13.439	4.750	0.003

higher than those in paraneoplastic tissues. A close association was found between the HER2 expressions and the following factors: tumor sizes, degrees of differentiation, vascular invasions, lymph node metastases, distant metastases and TNM stages. In addition, the HER2 overexpression was found to be associated with poor survival, but it was not an independent prognostic factor. HER2 is a proto-oncogene, and plays an important role in cell growth, survival, and differentiation [6]. After dimerization, HER2 can signal through migtogen-activated protein kinase (MAPK) pathway and phosphatidylinositol 3-kinase (PI3K) pathways. Based on the previous studies, positive rates of HER2 overexpression in CRC were diverse, and the clinical significance and prognostic values remain controversial. For example, Heppner et al. [16] revealed that HER2 overexpression were observed in 1.6% of 1645 patients with CRC and significantly correlated with advanced TNM stages and lymph node metastases; while there was no significant connection between the HER2 expressions and the patients' overall survivals. Park et al. [17] reported HER2 overexpression in 47.4% of 137 patients and found the patients with HER2 overexpression showed high postoperative recurrence rates and poor 3-year and 5-year survival rates. The overexpression of HER2 was an independent prognostic factor by multivariate analysis. Moreover, Tu et al. [18] demonstrated HER2 overexpression in 11.6% of 878 patients with CRC using immunohistochemistry and stated that only the TNM stage connected with the HER2 protein levels. Based upon our study and these previous findings, the positive expression rates and the clinical significance of HER2 in colorectal cancer were controversial. It might be caused by the following reasons. 1) Some tissue may degraded. The resected specimens frozen in liquid nitrogen should be within 30 min. 2) The expressions of HER2 might be different not only between the right hemicolon and the left hemicolon, but also between the colon and the recta. 2) Most research samples were small and the statistical significance was not certain. Therefore, the expression of HER2 in colorectal cancer demands further large-scale sample research.

We also found that FOXM1 overexpressions closely correlated with deeper tumor invasions, vascular invasions, lymph node metastases, distant metastases and TNM stages. The FOXM1 expression was an independent prognostic factor in CRC. These results suggested that FOXM1 plays an important role in progression, invasion and metastasis of CRC. As a transcription factor. Its main function is to accelerate the process of tumor cell cycle [10, 19]. Through transcriptional regulation of multiple

Table 5. Results of multivariate analyses of 130 CRC patients' survivals by Cox regression model

Varieties	n	95.0% CI	OR	P
CEA (ug/l)				
<5/≥5	88/42	1.587-7.876	3.535	0.002
CA19-9 (U/mL)				
<40/≥40	111/19	0.584-3.600	1.450	0.423
Vascular invasion				
Negative/Positive	98/32	0.143-1.134	0.402	0.085
Degree of differentiation				
Well and moderate/poor	96/34	0.947-4.394	2.039	0.069
Lymph node metastasis				
Negative/Positive	75/55	0.330-5.662	1.366	0.667
Distant metastases				
M0/M1	116/14	1.159-12.317	3.778	0.027
TNM staging				
I-II/III-IV	68/62	0.250-7.125	1.336	0.735
HER2				
Negative/Positive	97/33	0.837-4.716	1.987	0.120
FOXM1				
Negative/Positive	43/87	1.173-10.737	3.548	0.025

genes, it can realize G1/S and G2/M conversion, promote the formation of tumor vessels by transcriptional activation of VEGF [20], and promote tumor invasion and metastasis by transcriptional activation of MMP2 and MMP9 [21]. FOXM1 is closely related to a variety of classical signaling pathways, like mitogen-activated protein kinases (MAPK) signaling pathway via activating Raf-MEK-MAPK cascade to enhance the activation effect on cyclingB1 promoter [22]. Besides, there is also an interaction between FOXM1 and PI3K/Akt signaling pathway [23].

FOXM1, as one of the most frequently expressed genes in human malignant tumors, is expressed in a variety of malignant tumors [24-26]. Similarly, FOXM1 has been found to promote the development of colorectal cancer by accelerating the cell cycle. Yoshida et al. [27], through mouse model experiment, found mice with FOXM1b gene were easier to suffer from colon cancer than the control mice, suggesting FOXM1 might play an important role in the pathogenesis of colon cancer. In addition, Li et al. [28] discovered FOXM1 overexpressed in colorectal cancer was related to the depth of invasion, regional lymph node metastasis and TNM stage, and was also closely associated with prognosis and metastasis of colorectal cancer patients. FOXM1-positive patients were more likely to suffer from vascular and neural invasion. Similarly, Uddin et al. [29] found that the expression of FOXM1 was closely related with Ki-67 and MMP-9, and the expressions of FOXM1 in cancer tissues were higher than those in adenoma tissues. To sum up, FOXM1 is indeed overexpressed in colorectal cancer, but the expression rate and the relationship with clinical parameters also demand larger sample data to verify.

It has been previously shown that a significant association existed between the HER2 and FOXM1 expressions in breast cancer and gastric cancer [12, 13]. In the present study, we detected the correlation of HER2 and FOXM1 in CRC tissues based on the mRNA and protein levels, and found a significant association between the HER2 and FOXM1 expressions ($P<0.05$). HER2 and FOXM1 have many similar biological characteristics. For example, both HER2 and FOXM1 are involved in the signal pathways of Ras-MAPK and PI3K-Akt, and they are also closely related to tumor angiogenesis, invasion and metastasis. Recently, some studies have showed FOXM1 was a key target of trastuzumab, lapatinib and gefitinib [30-32]. Carr et al. [30] revealed that FOXM1 overexpression conferred resistance to the HER2 monoclonal antibody Herceptin. Similarly, stable overexpression of FOXM1 in HER2-overexpressed cell lines effectively diminished trastuzumab sensitivity, increased colony formation, and inhibited trastuzumab-induced cytotoxicity. Treatment of HER2-positive breast cancer cells with thiostrepton, a selective inhibitor of FOXM1 mRNA, increased sensitivity to lapatinib [33]. These findings have confirmed that FOXM1 is a principal determining factor of anti-HER2 therapeutics sensitivity, and FOXM1 and HER2 might be closely correlated. It is consistent to our findings. The PI3K-Akt signaling pathway is frequently hyperactivated in tumorigenesis, the HER2 overexpression can activate the PI3K-Akt pathway by inhibiting the anti-oncogene PTEN, leading to FOXO3a inactivation [34]. Some researchers have confirmed

there is an antagonism between FOXO3a and FOXM1 [30, 32, 35]. For example, McGovern et al. [32] found that gefitinib could repress the expression of FOXM1 by activating FOXO3a in gefitinib-sensitive breast carcinoma cell lines. Moreover, Myattet et al. [36] found that FOXO3a was also regulated by the mitogenactivated protein kinase (MAPK) and extracellular signal-related kinase (ERK) pathways. Therefore, FOXM1 might be a downstream target of the HER2-PI3K-Akt-FOXO3a and HER2-Raf-MAPK-FOXO3a signaling pathways.

In recent years, molecule-targeted therapies have played an important role in the treatment of patients with advanced colorectal cancer. For example, cetuximab (an epidermal growth factor receptor inhibitor) and bevacizumab (a vascular endothelial growth factor inhibitor) have been widely used in clinical treatment and achieved good results for patients suffering from colorectal cancer [37, 38]. Although HER2-targeted inhibitor trastuzumab has not been used in the clinical treatment of colorectal cancer, its widespread application in breast cancer and gastric cancer offers a basis for its use in advanced colorectal cancer patients who cannot undergo radical surgery. At present, some patients have appeared to have resistance to HER2 targeted drugs. Fortunately, based upon previous works and our findings of the many common effects of the FOXM1 and HER2, FOXM1 might be the downstream target of HER2 signaling. Inhibiting the expression of FOXM1 can increase the sensitivity of HER2-targeted drugs. Therefore, the inhibition of FOXM1 expression will provide a new strategy for the treatment of colorectal cancer, especially for the colorectal cancers with resistance to HER2-targeted therapy.

In conclusion, our study suggests that HER2 and FOXM1 are overexpressed in colorectal cancer, and may function as important diagnostic markers for colorectal cancer. Our results have revealed that there is a positive correlation between HER2 and FOXM1, and they may serve as useful molecular biomarkers and potential therapeutic targets for colorectal cancer.

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Disclosure of conflict of interest

None.

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References

[1] Yu J, Wu WK, Li X, He J, Li XX. Novel recurrently mutated genes and a prognostic mutation signature in colorectal cancer. Gut 2015; 64: 636-45.

[2] Chen W, Zheng R, Baade PD, Zhang S, Zeng H. Cancer statistics in China, 2015. CA Cancer J Clin 2016; 66: 115-32.

[3] Brenner H, Kloor M, Pox CP. Colorectal cancer. Lancet 2014; 383: 1490-502.

[4] Budinska E, Popovici V, Tejpar S, D'Ario G, Lapique N, Sikora KO. Gene expression patterns unveil a new level of molecular heterogeneity in colorectal cancer. J Pathol 2013; 231: 63-76.

[5] Muzny DM, Bainbridge MN, Chang K, Dinh HH, Drummond JA, Fowler G. Comprehensive molecular characterization of human colon and rectal cancer. Nature 2012; 487: 330-37.

[6] Tai W, Mahato R, Cheng K. The role of HER2 in cancer therapy and targeted drug delivery. J Control Release 2010; 146: 264-75.

[7] Solomon JP, Dell'Aquila M, Fadare O, Hasteh F. Her2/neu status determination in breast cancer: a single institutional experience using a dual-testing approach with immunohistochemistry and fluorescence in situ hybridization. Am J Clin Pathol 2017; 147: 432-37.

[8] Lei Y, Huang J, Zhao Q, Jiang N, Xu H, Wang Z, Li H, Zhang S, Sun Z. The clinicopathological parameters and prognostic significance of HER2 expression in gastric cancer patients: a meta-analysis of literature. World J Surg Oncol 2017; 15: 68.

[9] Janjigian YY, Braghiroli MI. Current progress in human epidermal growth factor receptor 2 targeted therapies in esophagogastric cancer. Surg Oncol Clin N Am 2017; 26: 313-24.

[10] Alvarezfernández M, Medema RH. Novel functions of FoxM1: from molecular mechanisms to cancer therapy. Front Oncol 2013; 3: 30.

[11] Barger CJ, Zhang W, Hillman J, Stablewski AB, Higgins MJ, Vanderhyden BC, Odunsi K, Karpf AR. Genetic determinants of FOXM1 overexpression in epithelial ovarian cancer and functional contribution to cell cycle progression. Oncotarget 2015; 6: 27613-27.

[12] Francis RE, Myatt SS, Krol J, Stablewski AB, Higgins MJ, Vanderhyden BC, Odunsi K, Karpf AR. FoxM1 is a downstream target and marker

of HER2 overexpression in breast cancer. Int J Oncol 2009; 35: 57-68.
[13] Li X, Tang D, Yao Y, Qi W, Liang J. Clinical significance and positive correlation of FoxM1 and Her-2 expression in gastric cancer. Clin Exp Med 2014; 14: 447-55.
[14] Valtorta E, Martino C, Sartore-Bianchi A. Assessment of a HER2 scoring system for colorectal cancer: results from avalidation study. Mod Pathol 2015; 28: 1481-91.
[15] Chu XY, Zhu ZM, Chen LB, Wang JH, Su QS, Yang JR, Lin Y, Xue LJ, Liu XB, Mo XB. FOXM1 expression correlates with tumor invasion and a poor prognosis of colorectal cancer. Acta Histochem 2012; 114: 755-62.
[16] Heppner BI, Behrens HM, Balschun K, Haag J, Krüger S, Becker T, Röcken C. HER2/neu testing in primary colorectal carcinoma. Br J Cancer 2014; 111: 1977-84.
[17] Park DI, Kang MS, Oh SJ, Kim HJ, Cho YK. HER-2/neu overexpression is an independent prognostic factor in colorectal cancer. Int J Colorectal Dis 2007; 22: 491-7.
[18] Tu J, Yu Y, Liu W, Chen S. Significance of human epidermal growth factor receptor 2 expression in colorectal cancer. Exp Ther Med 2015; 9: 17-24.
[19] Halasi M, Gartel AL. FOX(M1) news--it is cancer. Mol Cancer Ther 2013; 12: 245-54.
[20] Kuda M, Kohashi K, Yamada Y, Maekawa A, Kinoshita Y, Nakatsura T, Iwamoto Y, Taguchi T, Oda Y. FOXM1 expression in rhabdomyosarcoma: a novel prognostic factor and therapeutic target. Tumour Biol 2016; 37: 5213-23.
[21] Wen N, Wang Y, Wen L, Zhao SH, Ai ZH, Wang Y, Wu B, Lu HX, Yang H, Liu WC, Li Y. Overexpression of FOXM1 predicts poor prognosis and promotes cancer cell proliferation, migration and invasion in epithelial ovarian cancer. J Transl Med 2014; 12: 134.
[22] Koo CY, Muir KW, Lam WF. FOXM1: from cancer initiation to progression and treatment. Biochim Biophys Acta 2012; 1819: 28-37.
[23] Chen Y, Wang BC, Xiao Y. PI3K: a potential therapeutic target for cancer. J Cell Physiol 2012; 227: 2818-21.
[24] Shang R, Pu M, Li Y, Wang D. FOXM1 regulates glycolysis in hepatocellular carcinoma by transactivating glucose transporter 1 expression. Oncol Rep 2017; 37: 2261-9.
[25] Takata A, Takiguchi S, Okada K, Takahashi T, Kurokawa Y, Yamasaki M, Miyata H, Nakajima K, Mori M, Doki Y. Clinicopathological and prognostic significance of FOXM1 expression in esophageal squamous cell carcinoma. Anticancer Res 2014; 34: 2427-32.
[26] Li X, Qiu W, Liu B, Yao R, Liu S, Yao Y, Liang J. Forkhead box transcription factor 1 expression in gastric cancer: FOXM1 is a poor prognostic factor and mediates resistance to docetaxel. J Transl Med 2013; 11: 204.
[27] Yoshida Y, Wang IC, Yoder HM, Davidson NO, Costa RH. The Forkhead box M1 transcription factor contributes to the development and growth of mouse colorectal cancer. Gastroenterology 2007; 132: 1420-31.
[28] Li D, Wei P, Peng Z, Huang C, Tang H, Jia Z, Cui J, Le X, Huang S, Xie K. The critical role of dysregulated FOXM1-PLAUR signaling in human colon cancer progression and metastasis. Clin Cancer Res 2013; 19: 62-72.
[29] Uddin S, Ahmed M, Hussain A, Abubaker J, Al-Sanea N, AbdulJabbar A, Ashari LH, Alhomoud S, Al-Dayel F, Jehan Z, Bavi P, Siraj AK, Al-Kuraya KS. Genome-wide expression analysis of Middle Eastern colorectal cancer reveals FOXM1 as a novel target for cancer therapy. Am J Pathol 2011; 178: 537-47.
[30] Carr JR, Park HJ, Wang Z, Kiefer MM, Raychaudhuri P. FoxM1 mediates resistance to herceptin and paclitaxel. Cancer Res 2010; 70: 5054-63.
[31] Gayle SS, Castellino RC, Buss MC, Nahta R. MEK inhibition increases lapatinib sensitivity via modulation of FOXM1. Curr Med Chem 2013; 20: 2486-99.
[32] Mcgovern UB, Francis RE, Peck B, Guest SK, Wang J, Myatt SS, Krol J, Kwok JM, Polychronis A, Coombes RC, Lam EW. Gefitinib (Iressa) represses FOXM1 expression via FOXO3a in breast cancer. Mol Cancer Ther 2009; 8: 582-91.
[33] Peake BF, Nahta R. Resistance to HER2-targeted therapies: a potential role for FOXM1. Breast Cancer Manag 2014; 3: 423-31.
[34] Gomes AR, Zhao F, Lam EW. Role and regulation of the forkhead transcription factors FOXO3a and FOXM1 in carcinogenesis and drug resistance. Chin J Cancer 2013; 32: 365-70.
[35] Karadedou CT, Gomes AR, Chen J, Petkovic M, Ho KK, Zwolinska AK, Feltes A. FOXO3a represses VEGF expression through FOXM1-dependent and -independent mechanisms in breast cancer. Oncogene 2012; 31: 1845-58.
[36] Myatt SS, Lam EW. The emerging roles of forkhead box (Fox) proteins in cancer. Nat Rev Cancer 2007; 7: 847-59.
[37] Snoeren N, Voest EE, Bergman AM, Dalesio O, Verheul HM, Tollenaar RA, van der Sijp JR, Schouten SB, Rinkes IH, van Hillegersberg R. A randomized two arm phase III study in patients post radical resection of liver metastases of colorectal cancer to investigate bevacizumab in combination with capecitabine plus oxaliplatin (CAPOX) vs CAPOX alone as adjuvant treatment. BMC Cancer 2010; 10: 545.
[38] Tang XM, Chen H, Liu Y, Huang BL, Zhang XQ, Yuan JM, He X. The cardiotoxicity of cetuximab as single therapy in Chinese chemotherapy-refractory metastatic colorectal cancer patients. Medicine (Baltimore) 2017; 96: e5946.