Pathol. Oncol. Res. (2018) 24:45–57
DOI 10.1007/s12253-017-0195-5

ORIGINAL ARTICLE

Correlations of IGF-1R and COX-2 Expressions with *Ras* and *BRAF* Genetic Mutations, Clinicopathological Features and Prognosis of Colorectal Cancer Patients

Mei Jin[1] **· Zi-Wen Long**[2,3] **· Jing Yang**[1] **· Xiang Lin**[1]

Received: 23 February 2016 / Accepted: 9 January 2017 / Published online: 10 February 2017

Abstract This case-control study aims to investigate the correlations of insulin-like growth factor receptor 1 (IGF-1R) and cyclooxygenase 2 (COX-2) expressions with *Ras* and *BRAF* genetic mutations, clinicopathological features and prognosis of colorectal cancer (CRC) patients. A total of 213 CRC patients (case group) and 200 healthy individuals (control group) were selected from our hospital. *Ras (K-Ras/N-Ras)* and *BRAF* genetic mutations were detected by direct sequencing. The positive expression rates of IGF-IR and COX-2 in CRC and normal tissues were detected using immunohistochemistry. RT-qPCR and Western blotting were applied to detect the mRNA and protein expressions of IGF-IR and COX-2 in CRC tissues and normal tissues. Total mutation rate of *N-Ras*, *BRAF* and *K-Ras* in case group were 5.2%, 12.2% and 47.4%, respectively. The expressions of IGF-IR and COX-2 were higher in CRC tissues with *Ras* and *BRAF* mutations than in those without. CRC tissues with *Ras* mutation showed higher COX-2 expression than those with *BRAF* mutation. IGF-IR and COX-2 expressions were correlated to infiltration degree, lymphatic metastasis (in CRC tissues with and without *Ras* and *BRAF* mutations), and Dukes stages (only in CRC tissues with *Ras* and *BRAF* mutations). CRC patients with negative expressions of IGF-IR and COX-2 had significantly higher accumulative survival rate and longer mean survival duration than those with positive expressions of IGF-IR and COX-2. These findings indicate that IGF-1R and COX-2 expressions may be associated with *Ras* and *BRAF* genetic mutations, clinicopathological feature and prognosis of CRC patients.

Keywords Insulin-like growth factor receptor 1 · Cyclooxygenase 2 · Ras mutation · BRAF mutation · Clinicopathological feature · Prognosis

✉ Zi-Wen Long
rudolfstar@163.com

1 Department of Surgery, Shigatse People's Hospital, Shigatse, Tibet 85700, People's Republic of China

2 Department of Gastric Cancer Surgery, Fudan University Shanghai Cancer Center, Shanghai 200032, People's Republic of China

3 Department of Oncology, Shanghai Medical College, Fudan University, Shanghai 200032, People's Republic of China

Introduction

Colorectal cancer (CRC), a malignant disease of the alimentary canal, poses a major threat to human health [1]. In the United States, CRC is the third commonest malignancy and the second leading cause of cancer-associated death [2]. In Europe, an estimated 250,000 CRC patients are diagnosed annually, which accounts for almost 9% of all cancers diagnosed [3]. Ulcerative colitis is known to be a significant risk factor for CRC and up to 30% of patients with 40-year ulcerative colitis will develop as CRC [4]. Besides, tobacco smoking, lack of physical activity, red meat intake, low fruit and vegetable consumption also have some moderate associations with increased risk of CRC [5]. Currently, clinical surgery, radio- and chemo-therapy alone or in conjunction are the mainstays of treatment for CRC [6]. However, relapse and metastasis of the cancer following the conventional treatment frequently lead to poor survival [7]. Therefore, it is imperative to further study the pathogenesis of CRC and find out more effective therapies.

As a kind of transmembrane tetrameric protein encrypted by the proto-oncogene*IGF-1R,* insulin-like growth factor receptor-1 (IGF-1R) plays a critical role in development, proliferation, invasion and survival of cancer cells [8, 9]. Recently, strong association between dysregulated IGF signaling and many human cancers, such as colorectal, gastric and pancreatic cancer has aroused wide discussions [10, 11]. And the blocking of IGF bioactivity which may prevent cancer transformation has been recognized [12]. Cyclooxygenase2 (COX-2) is a key enzyme encoded by the *COX-2* gene situated on chromosome $1q^{25.2–25.3}$. It catalyzes the synthesis of prostaglandins and is inducible in reaction to some stimulating substances such as cytokines and growth factors [13]. COX-2 is implicated in many biologic processes such as inflammation and tumorigenesis [14]. Abnormal expression of COX-2 has been discovered to be correlated to a variety of premalignant and malignant epithelia, especially in gastrointestinal tract [15]. A previous study demonstrated that inhibition of COX-2 expression in gastric cancer may suppress and even reverse carcinogenesis [16]. Another study also found that, by knocking down COX-2 expression, the risk of colorectal cancer may be reduced [17]. *K-Ras* mutation is a predictive biomarker of resistance of anti-epidermal growth factor receptor (EGFR) therapy in patients with CRC [18–20] and activation of *Ras* (*K-Ras* or *N-Ras*) mutations in addition to *K-Ras* mutations in exon 2 have been regarded as negative predictive biomarkers for anti-EGFR therapy [21]. *BRAF*, whose gene located on chromosome 7, is the most potent activator of the MAK kinase pathway [22]. A study reported that *BRAF* mutation was correlated to poor prognosis of patients with advanced CRC [23]. However, there were few studies concerning expressions of IGF-1R and COX-2 in colorectal cancer patients with *Ras* and *BRAF* genetic mutations. On bases of previous studies, this study is designed to investigate the correlations ofIGF-1R and COX-2 expressions with *Ras* and *BRAF* genetic mutations, clinicopathological features and prognosis of CRC patients.

Materials and Methods

Ethnical Statement

This study was approved by the Ethical Committee of Fudan University Shanghai Cancer Center. All participants have provided written informed consents. This study was in strict accordance with the guidelines and principles of the Declaration of Helsinki [24].

Subjects

From March 2008 to March 2010, a total of 213 CRC patients (110 males and 103 females) who underwent surgery at the Department of Surgery of Fudan University Shanghai Cancer Center were included in this study as a case group. All CRC patients were confirmed by pathological examinations. *Ras* (*K-Ras*/*N-Ras*) and *BRAF* genetic mutation of all CRC patients were testified, according to which the patients were assigned into *Ras* genetic mutation, *BRAF* genetic mutation and wild type (no *Ras* or *BRAF* genetic mutation observed). Meanwhile, 200 healthy individuals were included as a control group. All of them were regarded as normal after full colonoscopy, without tumors of other body parts as well as diseases of digestive tract. Normal colonic mucosa confirmed by pathology was obtained from each of them after their agreements. In the case group, all patients (110 males and 103 females) aged 26 ~ 84 years with a mean age of 58.95 ± 9.21 years, and they have not received any anti-tumor treatment before hospitalization. In these 213 cases, there were 86 cases of ulcerative, 64 of protruded and 63 of infiltrative types; 78 of poor differentiation, 135 of moderate/well differentiation; 110 cases with lymphatic metastasis and 103 without. According to the Dukes staging of Colorectal Cancer in 1984 [25], there were 108 cases in stage A and B, and 105 cases in stage C and D. In the control group, there were 102 males and 95 females, aging from 25 to 83 with the mean age of 57.36 ± 9.17 years. All the patients had complete clinicopathologic data, and their pathological results of paraffin samples were reconfirmed by pathologists, with no one having received radiotherapy.

Detection of *Ras* and *BRAF* Genetic Mutations

K-Ras genetic mutation was mainly localized at codon family No. 12 and 13 in exon-2, while *N-Ras* genetic mutation was at codon family No. 12 and 13 in exon-2 and codon No. 61 in exon-3. As for *BRAF* genetic mutation, it was mainly at codon No. 600 in exon-15. The primer sequences needed was shown in Table 1. DNA FFPE Tissue Kit (Qiagen, Cat No.56404) was used to extract DNA of gene from tumor tissues, which was then amplified by polymerasechain reaction (PCR). *K-Ras*, *N-Ras* and *BRAF* genetic mutations were testified using direct sequencing, and the results was read by Chromes and DNAMAN software and compared using the NCBI Genome Databases. PCR reaction system: 2 μL10 × PCR buffer, 2 μL dNTP (2.5 m mol/L), 0.5uL primer upstream and 0.5uL downstream primer, 0.1 μL LA Taqenzyme, 2 μL DNA template, 13 μL sterile water, with the total reaction system being 20 μL. PCR reaction condition: denaturation for 5 min at 95 °C, annealing for 45 s at 56 °C, for 20s at 72 °C, 45 cycles, and extension for 5 min at 72 °C.

Immunohistochemistry

Fixed in formalin and embedded with paraffin, all specimens were cut into sections (each for 3 μm). Through deparaffin using xylene and dehydration using graded ethanol, the sections were put into citrate at pH 7.2 ~ 7.4 for antigen repairing. After the addition of dilute (1: 500)IGF-1R (Cell Signaling Technologies, Beverly, MA) and COX-2 (Santa Cruz Biotechnology, CA, USA) antibody and reaction at 4 °C

Table 1 Primer design amplified by polymerase chain reaction (PCR)

Genes	Primer sequence	Fragment (bp)
K-Ras		
exon 2	5′-TACTGGTGGAGTATTTGATAG-3′	248
	5′-TGGTCCTGCACCAGTAATATG-3′	
N-Ras		
exon 2	5′-GAACCAAATGGAAGGTCACACT-3′	243
	5′-CCTCACCTCTATGGTGGGATC-3′	
exon 3	5′-TAGCATTGCATTCCCTGTGGTT-3’	258
	5′-CCTGTAGAGGTTAATATCCGCAA-3’	
BRAF		
exon 15	5′-CATAATGCTTGCTCTGATAGGA-3’	211
	5′-CAATTTCTTACCATCCACAAAATG-3’	

BRAF, v-raf murine sarcoma viral oncogenehomolog B1

overnight, the sections were incubated at 37 °C for 1 h, and then washed using 0.01 mol/L phosphate-buffered saline (PBS). Subsequently, added with biotinylated second antibody (Boster Biotech Co. Ltd., Wuhan, China), the sections were incubated at 37 °C for 30 min. Then diaminobenzidine (DAB) was added for 10-min coloration. Hematoxylin was used for counterstaining and alcohol gradient for dehydration. Finally, the sections were sealed with neutral gum and observed under light microscope. The Image-Pro Plus software (Media Cybernetics, Inc., Bethesda, MD, USA) was employed to calculate the average light density values.

Staining results: IGF-1R-positive was mainly located in cell membrane and cytoplasm presenting yellow or dark brown-yellow. COX-2 mainly expressed in cell cytoplasm or membrane presenting brown-yellow or sepia. At high magnification, five horizons of each section were randomly selected. Using the couple score and semi quantitative method, the staining intensity (no staining is 0, slight staining 1 point, moderate staining 2 points, deep staining 3 points) and ratio of positive cells (0 ~ 5% refer to 0, 6 ~ 25% 1 point, 26 ~ 50% 2 points, 51 ~ 75% 3 points, over 75% 4 points) were determined. The final results were multiplied two scores mentioned above: 0 ~ 1 point, negative (−); 2 ~ 4 points, weak positive (+); 5 ~ 8 points, positive (++); >9 points, strong positive (+++).

Western Blotting

Protein was respectively extracted from the tissue samples and the protein concentration was measured according to the BCA kit (Boster Biotech Co. Ltd., Wuhan, China) instructions. Added with loading buffer (30 μg for each well) and boiled at 95 °C for 10 min, the protein was separated by polyacrylamide gel (10%) (Boster Biotech Co. Ltd., Wuhan, China) electropheresis with tube voltage of 80 V to 120 V. Wet transfer was run on using polyvinylidene fluoride (PVDF) membrane under 100 mV for 45 ~ 70 min. Then the reaction was blocked with 5% bovine serum albumin (BSA) at room temperature for 1 h. Following the addition of two primary antibodies IGF-1R (1:1000) (Cell Signaling Technologies, Beverly, MA) and COX-2 (1:1000) (Santa Cruz, CA), the membranes were incubated overnight at 4 °C. After being washed with Tris-buffered saline plus Tween (TBST) buffer for 3 times (5 min each time), the membranes were incubated with corresponding secondary antibodies at room temperature for 1 h and rinsed for 3 times again. With β-actin as an internal reference, chemiluminescence reagent and GelDocEZ imager (Bio-rad Laboratories, California, USA) were used for development. ImageJ software (National Institutes of Health, Bethesda, MD, USA) was employed for gray value analysis of target bands.

Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR)

Primer 5.0 software was employed to design primers (Table 2) according to gene sequence issued by Genbank. Total RNA were respectively extracted from the CRC tissues and normal tissues and reversely transcribed into cDNA following the kit instruction (Qiagen, Valencia, CA, USA). Ultraviolet spectrophotometer was used to calculate optical density (OD) value at wavelength of 260/280 for RNA concentration. The total

Table 2 The primers designed for real-time quantitative polymerase chain reaction (RT-qPCR)

Genes	Forward primer	Reverse primer
IGF-IR	5′-ACAGAGAACCCCAAGACTGAG-3’	5′-TGATGTTGTAGGTGTCTGCGG-3’
COX-2	5′-TTACAATGCTGACTATGGCTAC-3’	5′-CTGATGCGTGAAGTGCTG-3’
β-actin	5′-GTCCACCTTCCAGCAGATGTG-3’	5′-GCATTTGCGGTGGACGAT-3’

IGF-IR insulin-like growth factor-I receptor, *COX-2* cyclooxygenase-2 volume of PCR reaction was 20 μL: 10 μL 2 × SuperReal10μL, 0.6 μL upstream primers and 0.6 μL downstream primers, 4 μL reverse transcription product, 2 μL 50 × ROX, 2.8 μL RNase-free water. The PCR amplification was performed under initial denaturation at 95 °C for 15 min, then 40 cycles of denaturation at 95 °C for 10 s, annealing for 30 s and extension for 30 s. The β-actin was also used as an internal reference. The reliability of PCR results was evaluated by the dissolution curve with CT value (elbow values in amplification curve) obtained, and relative gene expression was determined through $2^{-\Delta\Delta}$Ct.

Follow-Up

With discharge time as initial period, the follow-up, ended in March, 2015, was conducted through the outpatient department, telephone or case reviewing. Month was the timing unit. Patients that were still alive at the end of follow-up were treated as censored data, and patients lost to follow-up were processed in accordance with the last statistical time. The death of patients was endpoint observation index, and Kaplan-Meier method was applied to calculate the mean survival time and cumulative survival rate. Time from CRC resection to death due to any cause was considered as overall survival (OS); time from surgery resection to recurrence or metastasis was considered as cumulative recurrence.

Statistical Analysis

Data analyses were conducted using SPSS 21.0 software (SPSS Inc., Chicago, IL, USA). Continuous data were displayed as mean ± standard deviation and testified by t test. Categorical data were expressed as percentage and rate, with chi-square test for comparison. Correlation analysis was tested by Pearson's correlation analysis. Kaplan-Meier method was used for survival analysis, which was tested by Log-rank. Prognostic factors were analyzed by the Cox regression model. $P < 0.05$ indicated significant difference.

Results

Ras (*K-Ras*/*N-Ras*) and *BRAF* Genetic Mutations in CRC Patients

Among these 213 CRC patients, the total mutation rate of *K-Ras* gene was 47.4% (101/213), with 76 mutation cases localized at codon No.12 in exon-2 (35.7%) and 25 cases at codon

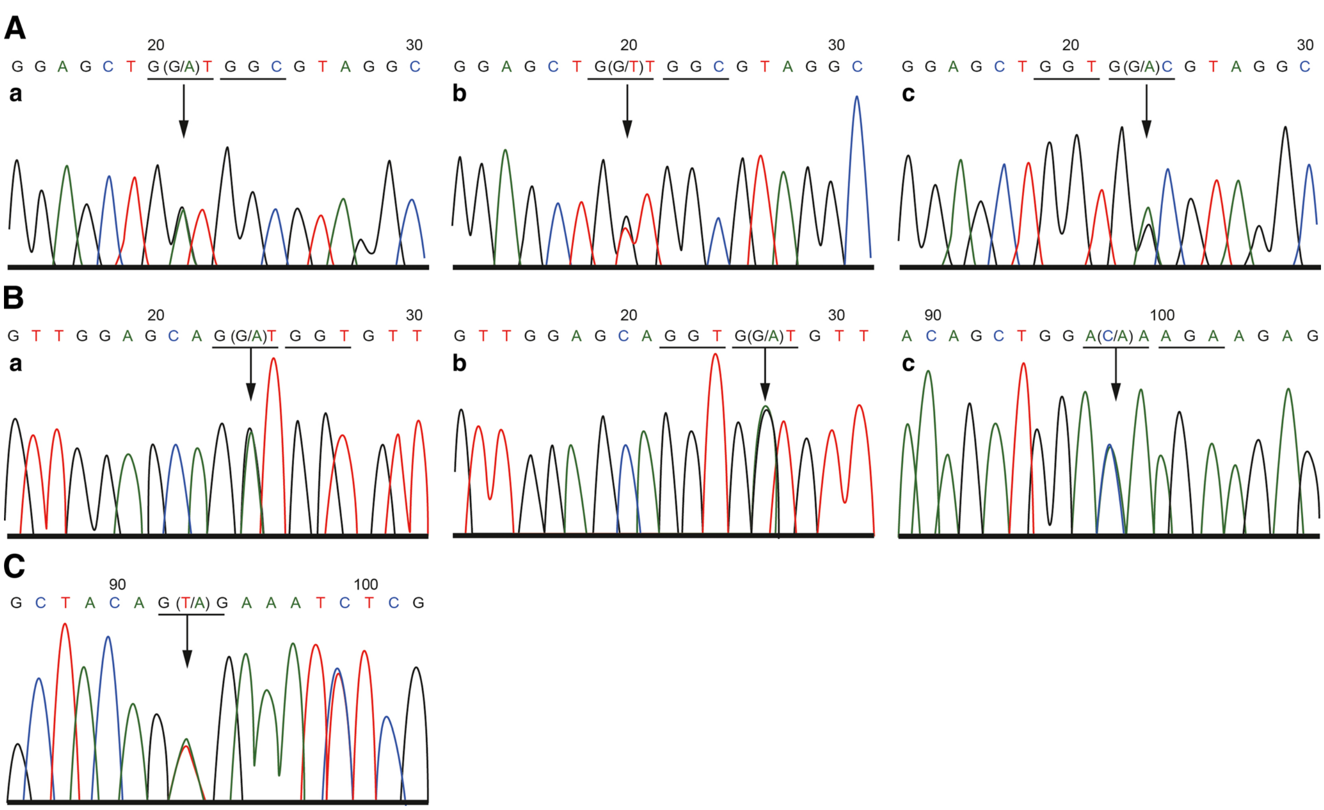

Fig. 1 Sequence of *Ras* (*K-Ras*/*N-Ras*) and *BRAF* genetic mutations. Note: (**a-a**)codon No.12 in exon-2 of *K-Ras* gene GGT > GAT; (**a-b**) codon No.12 in exon-2 of *K-Ras* gene GGT > GTT; (**a-c**): codon No.13 in exon-2 of *K-Ras* gene GGC > GAC; (**b-a**) codon No.12 in exon-2 of *N-Ras* geneGGT > GAT; (**b-b**) codon No.13 in exon-2 of *N-Ras* gene GGT > GAT; (**b-c**): codon No.61 in exon-3 of *N-Ras* gene ACA > AAA; (**c**) codon No.51 in exon-15 of *BRAF* gene GTG > GAG; base changes are indicated by the arrows

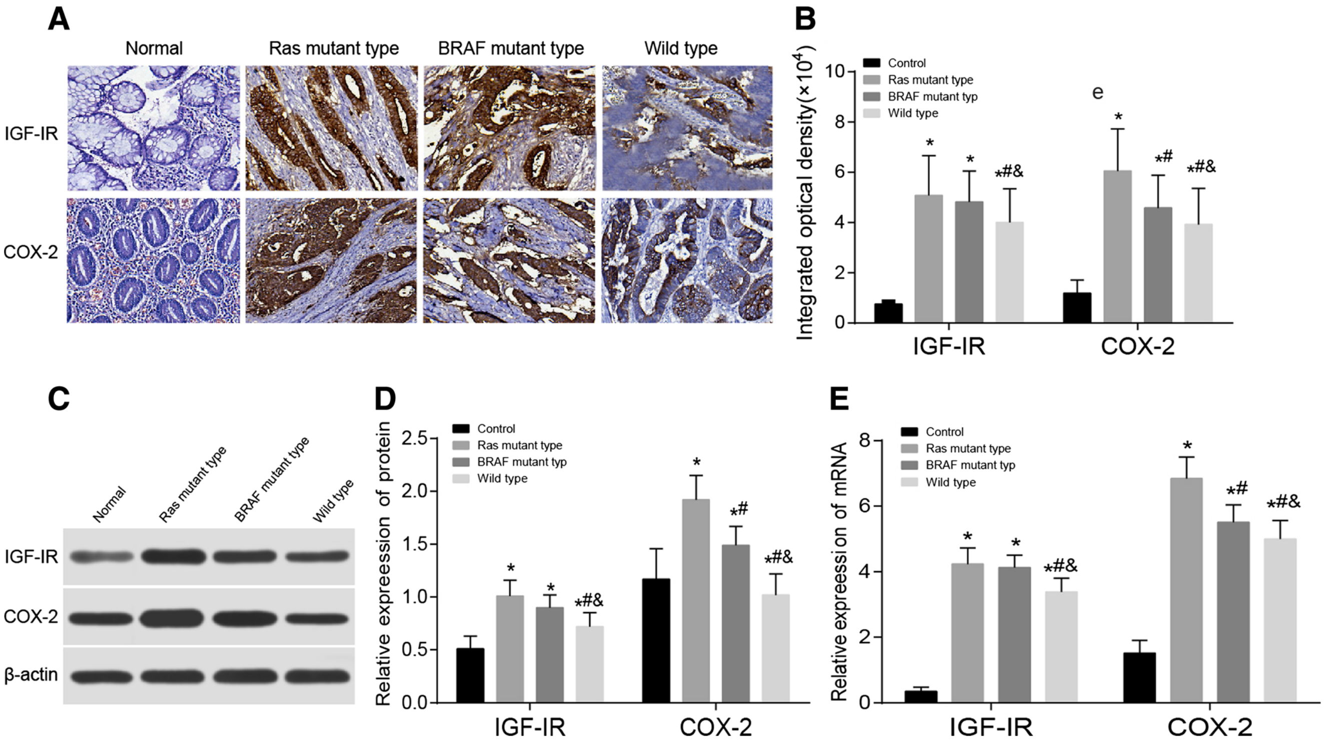

Fig. 2 IGF-IR and COX-2 expressions in normal tissues and colorectal cancer tissues. Note: (**a**) Immunohistochemistry of IGF-IR and COX-2 expressions in normal tissues and colorectal cancer tissues; (**b**) Comparisons of IGF-IR and COX-2 expressions in normal tissues and colorectal cancer tissues with different genetic mutations; (**c**) IGF-IR and COX-2 expressions in normal tissues and colorectal cancer tissues detected by Western blotting; (**d**) Comparisons of the protein expressions of IGF-IR and COX-2 in normal tissues and colorectal cancer tissues with different genetic mutations; (**e**) Comparisons of the mRNA expressions of IGF-IR and COX-2 in normal tissues and colorectal cancer tissues with different genetic mutations; *, $P < 0.05$ compared with the control group; #, $P < 0.05$ compared with patients with *Ras* mutation; &, $P < 0.05$ compared with patients with *BRAF* mutation

No.13 at exon-2 (11.7%) (Fig. 1a); the total mutation rate of *N-Ras* gene was 5.2% (11/213), with 3 mutation cases localized at codon No. 12 in exon-2 (1.4%), 2 cases at codon No.13 in exon-2 (0.9%) and 6 cases at codon No. 61 in exon-3 (2.8%), among which there were 2 cases exhibiting both *K-Ras* and *N-Ras* genetic mutation (Fig. 1b). The total mutation rate of *BRAF* gene was 12.2% (26/213) (Fig. 1c). No patient showed both *BRAF* and *K-Ras*/*N-Ras* genetic mutation in the present study. In total, there were 112 cases showing *Ras* genetic mutation, 26 showing *BRAF* genetic mutation and 75, wild type (no mutation found in the two genes mentioned above).

IGF-IR and COX-2 Expressions in CRC and Normal Tissues

According to immunohistochemistry, IGF-1R-positivereactant presented yellow or dark brown-yellow in cell membrane and cytoplasm after being stained; while COX-2-positivereactant presented brown-yellow or sepia in cell cytoplasm. There were significantly higher IGF-IR and COX-2-positive rates in CRC tissues, than in normal tissues (all $P < 0.05$) (Fig. 2a-b). In CRC tissues with different genetic mutations, it could be observed that IGF-IR and COX-2-positive rates were remarkably higher in CRC tissues with *Ras* and *BRAF* genetic mutations than those with wild-type *Ras* and *BRAF*, with higher COX-2 expression in *Ras* genetic mutation than in *BRAF* genetic mutation (all $P < 0.05$), however, there was no significant difference in IGF-IR-positive expression rate between *Ras* and *BRAF* genetic mutations ($P > 0.05$) (Fig. 2a-b). According to Western blotting, higher protein expressions of IGF-IR and COX-2 were found in CRC tissues than in normal tissues (all $P < 0.05$). In terms of CRC tissues of different types, there were higher protein expressions of IGF-IR and COX-2 in CRC tissues of *Ras* and *BRAF* genetic mutation than in those with wild type, with higher expression of COX-2 in CRC tissues with *Ras* genetic mutation than in *BRAF* gentic mutation (all $P < 0.05$), however, no significant difference was found in IGF-IR expression between *Ras* and *BRAF* genetic mutations ($P > 0.05$) (Fig. 2c-d). According to RT-qPCR, mRNA expressions of IGF-IR and COX-2 were much higher in CRC tissues than in normal tissues (all $P < 0.05$); besides, mRNA expression of IGF-IR and COX-2 were higher in CRC tissues with *Ras* and *BRAF* genetic mutations than in those with wild type, with higher much expression of COX-2 in *Ras* genetic mutation than in *BRAF* genetic mutation (all $P < 0.05$), however, IGF-IR

Table 3 Associations between expressions of IGF-IR and COX-2 proteins and clinicopathologic features of CC patients with *Ras* gene mutation

Clinicopathologic features	Number	IGF-IR expression		χ2	*P*	COX-2 expression		χ2	*P*
		Positive	Negative			Positive	Negative		
Gender									
Male	58	45	13	0.492	0.807	46	12	0.084	0.959
Female	54	39	15			44	10		
Age									
≥ 60	65	51	14	0.99	0.32	54	11	0.726	0.696
< 60	47	33	14			36	11		
Tumor location									
Left colon	49	39	10	0.98	0.322	39	10	0.032	0.984
Right colon	63	45	18			51	12		
Tumor size									
≥ 5	54	43	11	1.192	0.275	41	13	1.297	0.523
< 5	58	41	17			49	9		
Infiltration degree									
Inside serosa	47	29	18	7.638	0.006	32	15	7.727	0.021
Outside serosa	65	55	10			58	7		
Differentiation degree									
Poor differentiation	43	35	8	1.523	0.217	38	5	2.841	0.242
Moderate/high differentiation	68	49	20			52	17		
Dukes stages									
A + B	56	36	20	6.857	0.009	37	19	14.48	0.001
C + D	56	48	8			53	3		
Lymphatic metastasis									
Yes	54	47	7	8.058	0.005	49	5	7.123	0.028
No	58	37	21			41	17		
Type									
Ulcerative	47	32	15	2.119	0.347	24	23	0.762	0.683
Protruded	38	30	8			23	15		
Infiltrative	27	22	5			15	12		

IGF-IR insulin-like growth factor-I receptor, *COX-2* cyclooxygenase

mRNA expression remained statistically same between *Ras* and *BRAF* genetic mutations ($P > 0.05$) (Fig. 2e)

Correlations of IGF-IR and COX-2 Expressions with Clinicopathological Features of CRC Patients

For CRC patients with *Ras* or *BRAF* genetic mutation, the protein expressions of IGF-IR and COX-2 were not correlated with age, gender, tumor location, tumor size, differentiation degree and pathologic gross types (all $P > 0.05$), however, they were correlated to Dukes stages, infiltration degree and lymphatic metastasis (all $P < 0.05$). For patients with wild-type *Ras* and *BRAF* gene, protein expressions of IGF-IR and COX-2 were not correlated with age, gender, tumor location, tumor size, differentiation degree, Dukes stages and pathologic gross types (all $P > 0.05$), hut they were associated with infiltration degree and lymphatic metastasis (all $P < 0.05$) (Tables 3, 4 and 5).

After Pearson correlation analysis, it was found that there was positive relation between IGF-IR and COX-2 in CRC patients with *Ras* and *BRAF* genetic mutations ($r = 0.272$, $P < 0.05$; $r = 0.329$, $P < 0.05$) and those with wild-type *Ras* and *BRAF* ($r = 0.160$, $P < 0.05$) (Fig. 3) ($P < 0.05$). Furthermore, chi-square test for 122 cases of positive co-expression of IGF-1R and COX-2 and 23 cases of negative co-expression showed that co-expression of IGF-1R and COX-2 was associated with infiltration degree, lymphatic metastasis and Dukes stages of CRC patients with *Ras* and *BRAF* genetic mutations (all $P < 0.05$), however, it was only correlated to infiltration degree and lymphatic metastasis when it came to CRC patients with wild-type *Ras* and *BRAF* gene (all $P < 0.05$) (Tables 6, 7 and 8).

Correlations of IGF-IR and COX-2 Expressions with the Prognosis of CRC Patients

During the follow-up of five-year survival, twenty-one patients were lost to the follow-up, among which there were 10 cases with *Ras* genetic mutation, 3 cases with *BRAF* genetic mutation and 8 with wild type. The last statistical time was considered as the survival of lost patients. Five-year survival rates of patients with negative co-expression of IGF-IR and COX-2, IGF-IR-positive expression, COX-2-positive expression and positive co-expression of IGF-IR and COX-2 were respectively 70.6%, 57.2%, 54.1% and 33.6% with the mean survival time being respectively 52 months, 48 months, 46 months and 41 months. The cumulative survival rate and mean survival duration of CRC patients with negative co-expression of IGF-1R and COX-2 were significantly higher than those with COX-2, IGF-IR-positive expression, COX-2-

Table 4 Associations between expressions of IGF-IR and COX-2 proteins and clinicopathologic features of CC patients with *BRAF* gene mutation

Clinicopathologic features	Number	IGF-IR expression		χ2	P	COX-2 expression		χ2	P
		Positive	Negative			Positive	Negative		
Gender									
Male	12	10	2	1.192	0.551	10	2	2.081	0.353
Female	14	9	5			8	6		
Age									
≥ 60	14	10	4	0.465	0.495	9	5	0.348	0.84
< 60	12	9	3			9	3		
Tumor location									
Left colon	15	11	4	0.078	0.78	8	7	4.026	0.122
Right colon	11	8	3			10	1		
Tumor size									
≥ 5	9	6	3	0.288	0.592	6	3	0.042	0.979
< 5	17	13	4			12	5		
Infiltration degree									
Inside serosa	10	5	5	4.398	0.036	4	6	6.518	0.011
Outside serosa	16	14	2			14	2		
Differentiation degree									
Poor differentiation	14	11	3	0.465	0.495	11	3	1.242	0.537
Moderate/high differentiation	12	8	4			7	5		
Dukes stages									
A + B	10	4	6	9.036	0.003	4	6	6.518	0.011
C + D	16	15	1			14	2		
Lymphatic metastasis									
Yes	16	14	2	4.398	0.036	15	1	11.74	0.001
No	9	5	5			3	7		
Type									
Ulcerative	10	8	2	0.502	0.778	8	2	5.447	0.066
Protruded	4	3	1			3	1		
Infiltrative	12	8	4			4	8		

IGF-IR insulin-like growth factor-I receptor, *COX-2* cyclooxygenase

positive expression and positive co-expression of IGF-IR and COX-2 ($P < 0.05$) (Fig. 4a).

For patients with *Ras* mutation, five-year survival rates of those showing negative expressions of IGF-IR and COX-2, positive expression of IGF-IR, positive expression of COX-2 and positive expressions of IGF-IR and COX-2 were 81.8%, 52.9%, 36.4% and 38.4% respectively; and their mean survival durations were respectively 55 months, 45 months, 46 months and 42 months. It suggested that patients with negative expressions of IGF-IR and COX-2 had higher cumulative survival rate and mean survival duration than those with positive expression of IGF-IR, positive expression of COX-2 and positive expressions of IGF-IR and COX-2 ($P < 0.05$) (Fig. 4b)

For patients with *BRAF* mutation, five-year survival rates of those showing negative expressions of IGF-IR and COX-2, positive expression of IGF-IR, positive expression of COX-2 and positive expressions of IGF-IR and COX-2 were 50.0%, 66.7%, 75.0% and 26.7% respectively; and their mean survival durations were respectively 50 months, 48 months, 50 months and 41 months. Patients with negative expressions of IGF-IR and COX-2 had remarkably higher cumulative survival rate and mean survival duration than those with positive expressions of IGF-IR and COX-2 ($P < 0.05$), however they didn't significantly differ from those with positive expression of IGF-IR and those with positive expression of COX-2 in terms of cumulative survival rate and mean survival duration (Fig. 4c).

For patients with wild-type *BRAF* and *Ras,* five-year survival rates of those showing negative expressions of IGF-IR and COX-2, positive expression of IGF-IR, positive expression of COX-2 and positive expressions of IGF-IR and COX-2 were 75.0%, 61.1%, 73.3% and 50.0% respectively, and their mean survival rates durations were respectively 54 months, 51 months, 54 months and 50 months. Patients with negative expressions of IGF-IR and COX-2 had remarkably higher cumulative survival rate and mean survival duration than those with positive expressions of IGF-IR and COX-2 ($P < 0.05$), however they showed no significantly difference in cumulative survival rate and mean survival duration from those with positive expression of IGF-IR and those with positive expression of COX-2 (Fig. 4d).

Multiple Regression Analysis of Overall Survival Duration and Recurrence of Patients with CRC

Mutation type, infiltration degree, lymphatic metastasis and Dukes stages, IGF-IR-positive expression, COX-2-positive expression and positive co-expression of IGF-IR and COX-2 were concluded into COX regression model of survival

Table 5 Associations between expressions of IGF-IR and COX-2 proteins and clinicopathologic features of CC patients with wild-type *Ras* and *BRAF* gene

Clinicopathologic features	Number	IGF-IR expression		χ2	*P*	COX-2 expression		χ2	*P*
		Positive	Negative			Positive	Negative		
Gender									
Male	37	24	13	0.007	0.997	25	10	0.136	0.935
Female	38	25	13			27	13		
Age									
≥ 60	38	21	17	3.449	0.063	25	13	0.455	0.797
< 60	37	28	9			27	10		
Tumor location									
Left colon	34	20	14	1.164	0.281	22	12	0.626	0.731
Right colon	41	29	12			30	11		
Tumor size									
≥ 5	30	18	12	0.628	0.428	21	9	0.01	0.995
< 5	45	31	14			31	14		
Infiltration degree									
Inside serosa	30	15	15	5.19	0.023	15	15	8.793	0.012
Outside serosa	45	34	11			37	8		
Differentiation degree									
Poor differentiation	33	25	8	2.827	0.093	26	7	2.447	0.29
Moderate/high differentiation	42	24	18			26	16		
Dukes stages									
A + B	37	21	16	2.372	0.124	25	12	0.107	0.948
C + D	38	28	10			27	11		
Lymphatic metastasis									
Yes	42	33	9	7.386	0.007	35	7	8.799	0.012
No	33	16	17			17	16		
Type									
Ulcerative	29	19	10	2.706	0.258	9	20	1.979	0.372
Protruded	22	17	5			9	13		
Infiltrative	24	13	11			12	12		

IGF-IR insulin-like growth factor-I receptor, *COX-2* cyclooxygenase

duration and CRC recurrence, and the results showed that mutation type, infiltration degree, Dukes stages, IGF-IR-positive expression, COX-2-positive expression and positive co-expression of IGF-IR and COX-2were the risk factors of CRC (all $P < 0.05$) (Table 9). And the accumulative recrudescent time was associated with mutation types, lymphatic metastasis, IGF-IR or COX-2-positive expression and co-expression of IGF-IR and COX-2 (all $P < 0.05$) (Table 10).

Discussion

Currently, the incidence of CRC worldwide remains an upward trend, but the molecular pathogenesis of this disease is not clear. This study was designed to investigate the expressions of IGF-1R and COX-2 in CRC patients with *Ras* and *BRAF* genetic mutations and further to lay a foundation for the treatment of CRC toward molecular target.

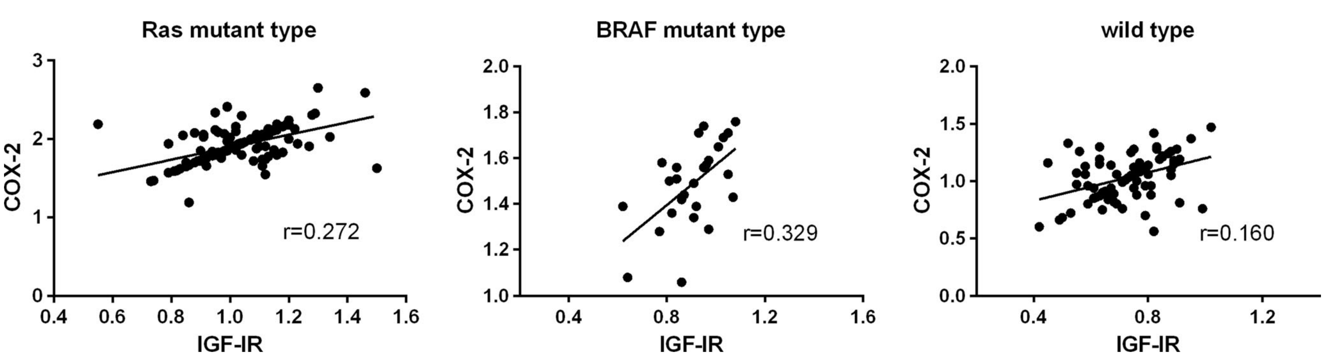

Fig. 3 Pearson's correlation analysis of the protein and mRNA expressions of IGF-IR and COX-2 with colorectal cancer patients with different genetic mutations. Note: r, correlation coefficients; $r > 0$ stands for positive correlation; $r < 0$ stands for negative correlation

Table 6 Association of positive co-expression of IGF-IR and COX-2 with infiltration degree, lymphatic metastasis and Dukes stages of colorectal cancer patients with *Ras* gene mutation

	Positive co-expression of IGF-IR and COX-2		χ2	*P*
	Positive (number)	Negative (number)		
Infiltration degree				
Inside serosa	24	10		
Outside serosa	49	1	13.36	<0.001
Dukes stage				
A + B	26	9		
C + D	47	2	8.396	0.004
Lymphatic metastasis				
Yes	44	2		
No	29	9	6.837	0.009

IGF-IR insulin-like growth factor-I receptor, *COX-2* cyclooxygenase

IGF-1R and COX-2 expressions in the CRC tissues were found significantly higher than those in the normal tissue. Shen et al. announced that IGF-1R was often up-regulated in colorectal cancer [9]. Elzagheid et al. also found high expression of COX-2 in colorectal cancer, and its higher levels was correlated to Dukes staging and lymphatic metastasis [26], which is in consistency with results of our study. Besides, it was found in the study that patients with *Ras and BRAF* genetic mutations showed higher mRNA and protein expressions of IGF-IR and COX-2 than those with wild-type. Activation IGF-1R signaling contributes to survival, proliferation, metastasis, angiogenesis and resistance to anti-cancer therapies in a number of human malignancies, with CRC included [27] Co-expression of the EGFR and IGF-IR is common in CRC patients [28], from which a positive correlation between them could be known. Activating mutations in *Ras* in exon 2 have been indicated as negative biomarkers for predicting anti-EGFR therapy [21]. Hence it can be concluded that *Ras* mutation promoted EGFR, which was positively correlated with IGF-IR. *BRAF* mutation is a crucial step in malignant transformation within the methylated pathway to CRC [29]. *BRAF*, activating mutation, could result in activation of the MAPK pathway, and MAPK activation is one of the resistance to IGF-IR tyrosine kinase inhibitors [27], which indicated that *BRAF* mutation activated MARP, further promoting IGF-IR. Due to the fact that the IGF-IR may activate specific pathways that were previously shown to lead to COX-2 induction in CRC [30], *Ras* and *BRAF* mutation enhanced IGF-IR, thus promoting expression of COX-2.

In our study, the expressions of IGF-1R and COX-2 were found to have close associations with infiltration degree, Dukes stage and lymphatic metastasis for patients with *Ras and BRAF* genetic mutations. Yamamoto et al. have discovered that over-expression of IGF-1R was associated with differentiation degree, disease stage, lymphatic metastasis, overall survival and recurrence in patients with non-small cell lung cancer [8], which can further strengthen the reliability of our results. Therefore, it can be concluded that IGF-1R and COX-2 have synergistic influence in the CRC occurrence, development and migration. Interestingly, we found that IGF-1R and

Table 7 Association of positive co-expression of IGF-IR and COX-2 with infiltration degree, lymphatic metastasis and Dukes stages of colorectal cancer patients with *BRAF* gene mutation

	Positive co-expression of IGF-IR and COX-2		χ2	*P*
	Positive (number)	Negative (number)		
Infiltration degree				
Inside serosa	2	3		
Outside serosa	13	1	6.193	0.013
Dukes stage				
A + B	1	3		
C + D	14	1	8.872	0.003
Lymphatic metastasis				
Yes	14	1		
No	1	3	8.872	0.003

IGF-IR insulin-like growth factor-I receptor, *COX-2* cyclooxygenase

Table 8 Association of positive co-expression of IGF-IR and COX-2 with infiltration degree, lymphatic metastasis and Dukes stages of colorectal cancer patients with wild-type *Ras* and *BRAF*

	Positive co-expression of IGF-IR and COX-2		χ2	*P*
	Positive (number)	Negative (number)		
Infiltration degree				
Inside serosa	6	6		
Outside serosa	28	2	10.44	0.001
Dukes stage				
A + B	14	5		
C + D	20	3	1.189	0.276
Lymphatic metastasis				
Yes	28	2		
No	6	6	10.44	0.001

IGF-IR insulin-like growth factor-I receptor, *COX-2* cyclooxygenase

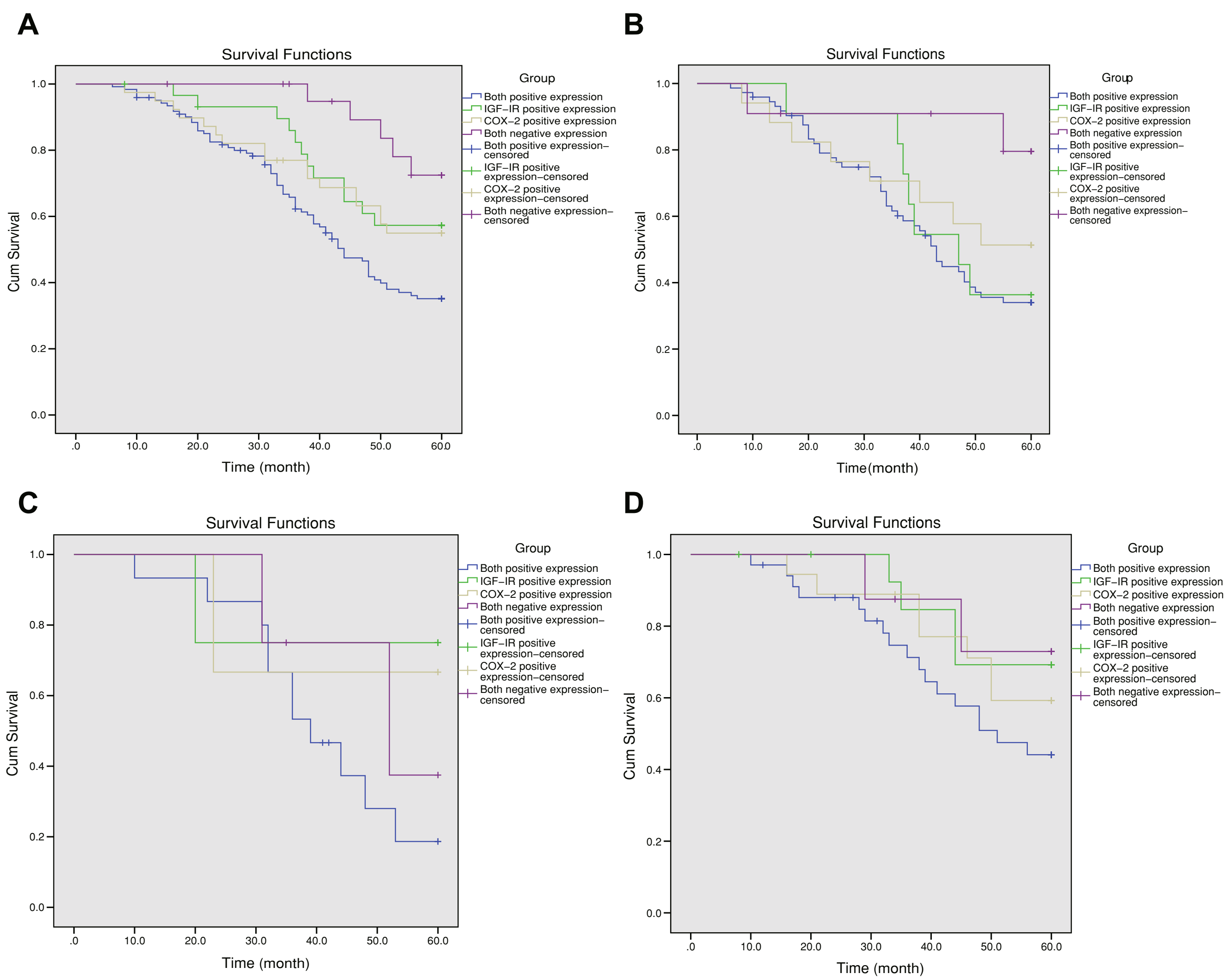

Fig. 4 Kaplan-Meier survival curves for overall survival of colorectal cancer patients with positive or negative expressions of IGF-IR or COX. Note: (**a**) Comparison of overall survival duration among patients with different expressions of IGF-IR and COX-2; (**b**) Comparison of survival duration among patients with *Ras* mutation who shows different expressions of IGF-IR and COX-2; (**c**) Comparison of survival duration among patients with *BRAF* mutation who shows different expressions of IGF-IR and COX-2; (**d**) Comparison of survival duration among patients with wild-type *Ras* and *BRAF* who shows different expressions of IGF-IR and COX-2

Table 9 Cox regression analysis of colorectal cancer patients' total survival time

	B	SE	Wald	Sig.	Exp(B)	95.0% CI for Exp(B)	
						Lower	Upper
Mutation type	0.48	0.216	4.951	0.026	1.616	1.059	2.466
Dukes stages	0.428	0.199	4.625	0.032	1.533	1.039	2.264
Lymphatic metastasis	-0.313	0.2	2.437	0.119	0.731	0.494	1.083
Infiltration degree	0.462	0.21	4.853	0.028	1.586	1.052	2.392
IGFI-positive expression	0.556	0.241	5.311	0.021	1.743	1.087	2.796
COX2-positive expression	0.668	0.266	6.294	0.012	1.951	1.157	3.289
Positive co-expression of IGFI and COX-2	0.709	0.208	11.598	0.001	2.033	1.351	3.058

B regression coefficient, *SE* standard error, *Wald* variance, *Sig* significance, *Exp(B)* constant, *CI* confidence interval, *CI* confidence interval, *IGF-IR* insulin-like growth factor-I receptor, *COX-2* cyclooxygenase

COX-2 expressions showed a significant positive correlation to each other in the CRC cells. Wu et al. demonstrated that IGF-1 increased the activity of $\alpha 5\beta 1$ integrin (a key molecular for chondrosarcoma cell migration) via the IGF-1R, IKK a/b, NF-kB-dependent and PI3K/Akt pathways [31]. Liu et al. observed that the over-expression of COX-2 promoted the mRNA expression of $\alpha 2\beta 1$ integrin, which has been revealed to play a critical role in metastasis of chondrosarcoma cells [32]. The two studies mentioned above can explain that the expressions of IGF-1R and COX-2 in CRC cells were in positive correlation to each

The overall survival of patients suggested that the infiltration degree, Dukes stage, lymphatic metastasis, IGF-1R and COX-2 expression, and positive co-expression of the two were risk factors influencing the prognosis of CRC patients. The cumulative recurrence time of patients demonstrated the association within filtration degree and positive co-expression of IGF-1R and COX-2. IGF-1/IGF-1R can promote proliferation and invasiveness and suppress apoptosis of cancer cells, including CRC cells, through activation of the PI3K/AKT signaling [10]. Jairamet et al. also reported that IGF-1R mediated down-stream proliferating and survival pathways in normal and malignant cells such as the PI3K/Akt and Ras signaling leading to enhanced human CRC cell proliferation, thus inhibiting IGF-1R may attenuate cell proliferation [6]. In many cancers such as colorectal, breast cancers and gastric, high expression of IGF-1R has been reported to show resistance to therapy, exhibiting poor clinical outcome [11]. COX-2 was recognized to stimulate growth, enhance angiogenesis and metastasis in breast tumor cells [33], it also has been associated with poor survival and identified as an independent factor for bad prognosis of gastric cancer [34].

In Lee's study, tumor invasion is associated with poor prognosis in human CRC [35]. In Zare et al.'s study, lymph node ratio of cancer metastasis is closely correlated to the prognosis of CRC patients [36]. The both previous studies confirm the result of the study. It was also found that compared with those with positive expression of IGF-1R or COX-2 alone, or positive co-expression of the two, patients with negative co-expression of IGF-1R and COX-2 showed the highest cumulative and mean survival. Therefore, expressions of IGF-1R and COX-2 can be potential marker for the treatment and prognosis of CRC.

In summary, our findings provided evidence that IGF-1R and COX-2 expressions may be associated with *Ras* and *BRAF* genetic mutations, clinicopathological feature and prognosis of CRC patients. Thus, IGF-1R and COX-2 proteins are likely to be molecular markers for the clinical treatment and prognosis of CRC patients with *Ras* and *BRAF* genetic mutations. However, in the study, the case number of CRC patients with wild-type *Ras* and *BRAF* is limited, which should be further explored to confirm the association of wild-type *Ras* and *BRAF* with expressions of IGF-1R and COX-2. And future researches focusing on genetic transcriptions of IGF-1R and COX-2 and catalysis of active proteins may be done to explore the effect of IGF-1R and COX-2 on CRC.

Table 10 Cox regression analysis of colorectal cancer patients' reoccurrence

	B	SE	Wald	Sig.	Exp(B)	95.0% CI for Exp(B)	
						Lower	Upper
Mutation type	0.462	0.227	4.144	0.042	1.587	1.017	2.477
Dukes stages	0.389	0.211	3.387	0.066	1.476	0.975	2.233
Lymphatic metastasis	0.453	0.212	4.575	0.032	1.573	1.039	2.383
Infiltration degree	0.416	0.22	3.568	0.059	1.515	0.984	2.332
IGFI-positive expression	0.631	0.263	5.734	0.017	1.879	1.121	3.148
COX2-positive expression	0.758	0.291	6.784	0.009	2.135	1.206	3.778
Positive co-expression of IGFI and COX-2	0.794	0.224	12.501	<0.001	2.212	1.424	3.434

B regression coefficient, *SE* standard error, *Wald* variance, *Sig* significance, *Exp(B)* constant, *CI* confidence interval, *CI* confidence interval, *IGF-IR* insulin-like growth factor-I receptor, *COX-2* cyclooxygenase

Acknowledgements We would like to acknowledge the helpful comments on this paper received from our reviewers.

Author Contributions Mei Jin, Zi-Wen Long, Jing Yang and Xiang Lin designed the study, collated the data, designed and developedthe database, carried out data analyses and produced the initial draft of the manuscript. Mei Jin andZi-Wen Long contributed to revise the manuscript. All authors have read and approved the final submitted manuscript.

Compliance with Ethical Standards

Conflict of Interest None.

References

1. Ahmed FE, Ahmed NC, Vos PW, Bonnerup C, Atkins JN, Casey M, Nuovo GJ, Naziri W, Wiley JE, Mota H, Allison RR (2013) Diagnostic microRNA markers to screen for sporadic human colon cancer in stool: I. Proof of principle. Cancer Genomics Proteomics 10(3):93–113
2. Hari DM, Leung AM, Lee JH, Sim MS, Vuong B, Chiu CG, Bilchik AJ (2013) AJCC cancer staging manual 7th edition criteria for colon cancer: do the complex modifications improve prognostic assessment? J Am Coll Surg 217(2):181–190. doi:10.1016/j.jamcollsurg.2013.04.018
3. Labianca R, Nordlinger B, Beretta GD, Brouquet A, Cervantes A, Group EGW (2010) Primary colon cancer: ESMO clinical practice guidelines for diagnosis, adjuvant treatment and follow-up. Ann Oncol 21(Suppl 5):v70–v77. doi:10.1093/annonc/mdq168
4. Jiang R, Wang H, Deng L, Hou J, Shi R, Yao M, Gao Y, Yao A, Wang X, Yu L, Sun B (2013) IL-22 is related to development of human colon cancer by activation of STAT3. BMC Cancer 13:59. doi:10.1186/1471-2407-13-59
5. Johnson CM, Wei C, Ensor JE, Smolenski DJ, Amos CI, Levin B, Berry DA (2013) Meta-analyses of colorectal cancer risk factors. Cancer Causes Control 24(6):1207–1222. doi:10.1007/s10552-013-0201-5
6. Vanamala J, Reddivari L, Radhakrishnan S, Tarver C (2010) Resveratrol suppresses IGF-1 induced human colon cancer cell proliferation and elevates apoptosis via suppression of IGF-1R/Wnt and activation of p 53 signaling pathways. BMC Cancer 10:238. doi:10.1186/1471-2407-10-238
7. Ding J, Zhang ZM, Xia Y, Liao GQ, Pan Y, Liu S, Zhang Y, Yan ZS (2013) LSD1-mediated epigenetic modification contributes to proliferation and metastasis of colon cancer. Br J Cancer 109(4):994–1003. doi:10.1038/bjc.2013.364
8. Yamamoto T, Oshima T, Yoshihara K, Nishi T, Arai H, Inui K, Kaneko T, Nozawa A, Adachi H, Rino Y, Masuda M, Imada T (2012) Clinical significance of immunohistochemical expression of insulin-like growth factor-1 receptor and matrix metalloproteinase-7 in resected non-small cell lung cancer. Exp Ther Med 3(5):797–802. doi:10.3892/etm.2012.493
9. Shen K, Liang Q, Xu K, Cui D, Jiang L, Yin P, Lu Y, Li Q, Liu J (2012) MiR-139 inhibits invasion and metastasis of colorectal cancer by targeting the type I insulin-like growth factor receptor. Biochem Pharmacol 84(3):320–330. doi:10.1016/j.bcp.2012.04.017
10. Ma J, Sawai H, Matsuo Y, Ochi N, Yasuda A, Takahashi H, Wakasugi T, Funahashi H, Sato M, Takeyama H (2010) IGF-1 mediates PTEN suppression and enhances cell invasion and proliferation via activation of the IGF-1/PI3K/Akt signaling pathway in pancreatic cancer cells. J Surg Res 160(1):90–101. doi:10.1016/j.jss.2008.08.016
11. Wu J, Yu E (2014) Insulin-like growth factor receptor-1 (IGF-IR) as a target for prostate cancer therapy. Cancer Metastasis Rev 33(2–3):607–617. doi:10.1007/s10555-013-9482-0
12. King H, Aleksic T, Haluska P, Macaulay VM (2014) Can we unlock the potential of IGF-1R inhibition in cancer therapy? Cancer Treat Rev 40(9):1096–1105. doi:10.1016/j.ctrv.2014.07.004
13. Cheng J, Fan XM (2013) Role of cyclooxygenase-2 in gastric cancer development and progression. World J Gastroenterol 19(42):7361–7368. doi:10.3748/wjg.v19.i42.7361
14. Akutsu Y, Hanari N, Yusup G, Komatsu-Akimoto A, Ikeda N, Mori M, Yoneyama Y, Endo S, Miyazawa Y, Matsubara H (2011) COX2 expression predicts resistance to chemoradiotherapy in esophageal squamous cell carcinoma. Ann Surg Oncol 18(10):2946–2951. doi:10.1245/s10434-011-1645-z
15. Roelofs HM, Te Morsche RH, van Heumen BW, Nagengast FM, Peters WH (2014) Over-expression of COX-2 mRNA in colorectal cancer. BMC Gastroenterol 14:1. doi:10.1186/1471-230X-14-1
16. Lehingue Y, Urtizberea JA (1986) Tuberculosis control in Malawi. Time distribution of cessation of treatment and proposals for reorientation of the program. Bull Soc Pathol Exot Filiales 79(2):259–265
17. Rahman M, Selvarajan K, Hasan MR, Chan AP, Jin C, Kim J, Chan SK, Le ND, Kim YB, Tai IT (2012) Inhibition of COX-2 in colon cancer modulates tumor growth and MDR-1 expression to enhance tumor regression in therapy-refractory cancers in vivo. Neoplasia 14(7):624–633
18. Amado RG, Wolf M, Peeters M, Van Cutsem E, Siena S, Freeman DJ, Juan T, Sikorski R, Suggs S, Radinsky R, Patterson SD, Chang DD (2008) Wild-type kras is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol 26(10):1626–1634. doi:10.1200/JCO.2007.14.7116
19. Douillard JY, Siena S, Cassidy J, Tabernero J, Burkes R, Barugel M, Humblet Y, Bodoky G, Cunningham D, Jassem J, Rivera F, Kocakova I, Ruff P, Blasinska-Morawiec M, Smakal M, Canon JL, Rother M, Oliner KS, Wolf M, Gansert J (2010) Randomized, phase iii trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (folfox4) versus folfox4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the prime study. J Clin Oncol 28(31):4697–4705. doi:10.1200/JCO.2009.27.4860
20. Peeters M, Price TJ, Cervantes A, Sobrero AF, Ducreux M, Hotko Y, Andre T, Chan E, Lordick F, Punt CJ, Strickland AH, Wilson G, Ciuleanu TE, Roman L, Van Cutsem E, Tzekova V, Collins S, Oliner KS, Rong A, Gansert J (2010) Randomized phase iii study of panitumumab with fluorouracil, leucovorin, and irinotecan (folfiri) compared with folfiri alone as second-line treatment in patients with metastatic colorectal cancer. J Clin Oncol 28(31):4706–4713. doi:10.1200/JCO.2009.27.6055
21. Douillard JY, Oliner KS, Siena S, Tabernero J, Burkes R, Barugel M, Humblet Y, Bodoky G, Cunningham D, Jassem J, Rivera F, Kocakova I, Ruff P, Blasinska-Morawiec M, Smakal M, Canon JL, Rother M, Williams R, Rong A, Wiezorek J, Sidhu R, Patterson SD (2013) Panitumumab-folfox4 treatment and ras mutations in colorectal cancer. N Engl J Med 369(11):1023–1034. doi:10.1056/NEJMoa1305275
22. Xing M (2015) Braf mutation and thyroid cancer recurrence. J Clin Oncol 33(22):2482–2483. doi:10.1200/JCO.2015.61.4016
23. Richman SD, Seymour MT, Chambers P, Elliott F, Daly CL, Meade AM, Taylor G, Barrett JH, Quirke P (2009) Kras and braf mutations in advanced colorectal cancer are associated with poor prognosis but do not preclude benefit from oxaliplatin or irinotecan: results from the mrc focus trial. J Clin Oncol 27(35):5931–5937. doi:10.1200/JCO.2009.22.4295
24. Mastroleo I (2015) Post-trial obligations in the declaration of Helsinki 2013: classification, reconstruction and interpretation. Dev World Bioeth. doi:10.1111/dewb.12099

25. Sarma DP (1988) Dukes' classification of rectal cancer. South Med J 81(3):407–408
26. Elzagheid A, Emaetig F, Alkikhia L, Buhmeida A, Syrjanen K, El-Faitori O, Latto M, Collan Y, Pyrhonen S (2013) High cyclooxygenase-2 expression is associated with advanced stages in colorectal cancer. Anticancer Res 33(8):3137–3143
27. Huang F, Chang H, Greer A, Hillerman S, Reeves KA, Hurlburt W, Cogswell J, Patel D, Qi Z, Fairchild C, Ryseck RP, Wong TW, Finckenstein FG, Jackson J, Carboni JM (2015) Irs2 copy number gain, kras and braf mutation status as predictive biomarkers for response to the igf-1r/ir inhibitor bms-754807 in colorectal cancer cell lines. Mol Cancer Ther 14(2):620–630. doi:10.1158/1535-7163.MCT-14-0794-T
28. Cunningham MP, Essapen S, Thomas H, Green M, Lovell DP, Topham C, Marks C, Modjtahedi H (2006) Coexpression of the igf-ir, egfr and her-2 is common in colorectal cancer patients. Int J Oncol 28(2):329–335
29. Kalady MF, Dejulius KL, Sanchez JA, Jarrar A, Liu X, Manilich E, Skacel M, Church JM (2012) Braf mutations in colorectal cancer are associated with distinct clinical characteristics and worse prognosis. Dis *Colon rectum* 55(2):128–133. doi:10.1097/DCR.0b013e31823c08b3
30. Stoeltzing O, Liu W, Fan F, Wagner C, Stengel K, Somcio RJ, Reinmuth N, Parikh AA, Hicklin DJ, Ellis LM (2007) Regulation of cyclooxygenase-2 (cox-2) expression in human pancreatic carcinoma cells by the insulin-like growth factor-i receptor (igf-ir) system. Cancer Lett 258(2):291–300. doi:10.1016/j.canlet.2007.09.009
31. Wu CM, Li TM, Hsu SF, Su YC, Kao ST, Fong YC, Tang CH (2011) IGF-I enhances alpha5beta1 integrin expression and cell motility in human chondrosarcoma cells. J Cell Physiol 226(12):3270–3277. doi:10.1002/jcp.22688
32. Liu JF, Fong YC, Chang CS, Huang CY, Chen HT, Yang WH, Hsu CJ, Jeng LB, Chen CY, Tang CH (2010) Cyclooxygenase-2 enhances alpha2beta1 integrin expression and cell migration via EP1 dependent signaling pathway in human chondrosarcoma cells. Mol Cancer 9:43. doi:10.1186/1476-4598-9-43
33. Kim HS, Moon HG, Han W, Yom CK, Kim WH, Kim JH, Noh DY (2012) COX2 overexpression is a prognostic marker for stage III breast cancer. Breast Cancer Res Treat 132(1):51–59. doi:10.1007/s10549-011-1521-3
34. Thiel A, Mrena J, Ristimaki A (2011) Cyclooxygenase-2 and gastric cancer. Cancer Metastasis Rev 30(3–4):387–395. doi:10.1007/s10555-011-9312-1
35. Lee Y C YCN and, Hsu T (2013) Abstract 1146: Stk4 downregulation promotes tumor invasion/migration and is associated with poor prognosis in human colon cancer. Cancer Res 73 (8 Supplement): 1146–1146
36. Zare Mirzaei A, Abdorrazaghi F, Lotfi M, Kazemi Nejad Band Shayanfar N (2015) Prognostic value of lymph node ratio in comparison to lymph node metastases in stage iii colon cancer. Iran J Pathol 10(2):127–135