Expression of biogenesis of ribosomes BRX1 is associated with malignant progression and prognosis in colorectal cancer

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Background: Upregulated ribosome synthesis is associated with an increased risk of cancer onset. However, the role of biogenesis of ribosomes BRX1 (*BRIX1*), which is involved in the synthesis of ribosomal 60S subunits, in the progression and prognosis of colorectal cancer (CRC) is not clear.

Methods: Sixty CRC patients requiring surgical treatment were enrolled in the present prospective study. Patient characteristics were collected at admission. The CRC tissue samples and adjacent normal tissue samples from patients were collected for further quantitative reverse transcription-polymerase chain reaction and Western blot. All enrolled patients were followed up for 12 months, and overall patient survival during follow-up was recorded.

Results: The level of *BRIX1* mRNA in CRC tissues was higher compared with that in adjacent normal tissues (1.0 ± 0.5 *vs.* 5.5 ± 1.7, P<0.01). Similarly, the level of the BRIX1 protein in CRC tissues was significantly higher than that in adjacent normal tissues (1.0 ± 0.6 *vs.* 6.4 ± 2.1, P<0.01). On further analysis, we found that the levels of *BRIX1* mRNA and protein were positively correlated with tumor stage. Patients at stages III–IV had a higher expression of *BRIX1* mRNA and protein than at stages I–II. The Kaplan-Meier survival curve indicated that patients with higher level of the BRIX1 protein had a lower overall survival rate. The Cox proportional hazards model was used to identify tumor stage III or IV, poor differentiation, and elevated expression of the BRIX1 protein as risk factors for the overall survival of CRC patients.

Conclusions: *BRIX1* expression is positively correlated with CRC tumor stage; it also acts as a risk factor for the overall survival of CRC patients.

Keywords: *BRIX1* expression is positively correlated with CRC tumor stage; it also acts as a risk factor for the overall survival of CRC patients.

Introduction

Colorectal cancer (CRC) is one of the most common cancers in China. Both the incidence and mortality of CRC ranked fifth in 2015, with 276,000 new cases and 191,000 deaths, respectively (1). Surgical resection,

chemotherapy, and molecular targeted therapy are the major treatment options for CRC. However, 30–40% of patients who receive systematic treatment will relapse (2). The 5-year survival rate of CRC in China is around 55%, and the 5-year survival rate of patients with stage IV CRC is <10% (3). It is important that the therapeutic regime

is further improved to achieve a higher survival rate in patients.

Despite the rapid development of molecular targeted drugs in recent years, only several drugs, such as bevacizumab, cetuximab, and panitumumab, are currently recognized as molecular targeted drugs for CRC (4,5). According to the 2019 National Comprehensive Cancer Network guidelines for CRC, these three molecular targeted drugs are used as supplements for general chemotherapy programs and are only recommended for use in patients with the *KRAS* wild type (6). It has been suggested that these molecular targeted drugs cannot be dependently used for the treatment of CRC patients. The main limitation of current molecular targeted drugs is that they are targeted at only one or one kind of protein, whereas the expression of the specific protein in cancer cells is different due to their different progression and development. There are two solutions to this problem; one is to increase the type of drug targets, and the other is to find new targets to improve the inhibitory effects of tumors through interactions between different targets (7). The former has high risks due to the side-effects of drugs. The latter is the currently preferred option, because it can inhibit the growth of tumors more safely and effectively.

The ribosome synthesis superfamily plays an important role in the process of ribosome synthesis and rRNA binding, which can affect cell proliferation and differentiation (8). Growing evidence indicates that upregulated ribosome biogenesis is associated with an increased risk of cancer onset (9). The biogenesis of ribosomes BRX1 (*BRIX1*) gene, which is involved in the synthesis of ribosomal 60S subunits, is located in cytoband 5p13.2 and is expressed at highest in pulmonary alveoli (10). It has been reported that its expression is related to smoking habits and myocardial damage due to smoking and PM2.5 exposure (11,12). Studies on *BRIX1* in cancer are rare. Jung *et al.* reported the significantly increased expression of *BRIX1* in a variety of tumors (13). However, the relationship between the expression of *BRIX1* and the progression and prognosis of CRC is still unclear. In the present prospective study, we measured the expression of *BRIX1* in patients with CRC to determine the role of *BRIX1* in the progression of CRC and provide preliminary evidence for the development of novel molecular targeted drugs for CRC.

We present the following article in accordance with the MDAR reporting checklist (available at http://dx. doi. org/10. 21037/tcr-20-2564).

Methods

Patients and specimens

Patients who received surgical treatment for CRC between January 2018 and December 2018 at the Affiliated Nanjing Jiangbei Hospital of Nantong University were enrolled. In the present study, CRC was defined as tumors located between the ileocecal valve and dentate line. The following were excluded: (I) patients <18 years of age; (II) patients who refused to sign an informed consent form; (III) patients who were diagnosed with metastatic CRC; (IV) patients who had severe organ dysfunction; (V) patients who had life-threatening postoperative complications; and (VI) patients who had previously received surgical treatment for CRC. CRC tissue samples and adjacent normal tissue samples from patients were collected. Collected tissues were used for further quantitative reverse transcription–polymerase chain reaction (RT-PCR) and Western blot. All enrolled patients were followed up for 12 months. The outcome in the present study was overall survival of enrolled patients. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the ethics committee of the Affiliated Nanjing Jiangbei Hospital of Nantong University (No. J2017034), and informed consent was taken from all the patients.

Data collection

The following patient characteristics at admission were collected: age, sex, anatomical location, tumor stage, differentiation stage, and the level of carcinoembryonic antigen (CEA) and carbohydrate antigen 199 (CA199). Anatomical location was defined as right-sided colon, left-sided colon, and rectum. Tumor stage was defined as I, II, III, or IV stage, according to TNM stage. Differentiation stage was defined as well, moderate, or poor. The CEA level was defined as elevated (>5 ng/L) or normal (≤5 ng/L), and the CA199 level was defined as elevated (>37.00 μ/mL) or normal (≤37.00 μ/mL).

RNA isolation

The total RNA of collected tissues was extracted using a total RNA extraction kit (Solarbio). The total RNA was quantified using the qubit flex fluorometer (ThermoFisher Scientific) at 260 nm. The total RNA was then reverse-transcribed using the PrimeScript RT reagent kit (Takara Bio), according to the manufacturer's instructions.

Quantitative RT-PCR

The mRNA levels of *BRIX1* genes were quantified by the ABI PRISM 7500 sequence detection system (Applied Biosystems). The primer pair used in the present study was as follows: forward: CCCGTCTTCCAGTCTCCTAA; reverse: TGTCCAGGGTTTCCTTCG. All assays in the present study were performed in three duplicated repeats. The data were automatically collected and analyzed by SDS version 1.3.1 (Applied Biosystems).

Protein extraction

The protein in the tissue sample was extracted using a total protein extraction kit (Solarbio). Briefly, 100 mg tissue was washed with phosphate buffer saline and minced. The minced tissue was then immersed in lysis buffer for 2–4 hours and centrifuged at 12,000 ×g for 30 minutes at 4 ℃. The supernatant was collected and the protein was quantified using a bicinchoninic acid protein assay kit (ThermoFisher Scientific).

Western blot

In order to perform the Western blot assay, 20 mg protein was separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels and then transferred to polyvinylidene difluoride membranes. The membranes were blocked with 5% bovine serum albumin and incubated overnight with primary antibodies against BRIX1 (Sino Biological). The membranes were then incubated with secondary antibodies for 1 hour at room temperature. Glyceraldehyde-3-phosphate dehydrogenase was used as the reference. The blot was quantified using Image J software version 1.51 (National Institutes of Health).

Statistical analysis

Continuous variables in the present study were reported as mean ± standard deviation, and other categorical variables were reported as numbers with percentages. The differences of continuous variables and categorical variables between the two groups were analyzed using Mann-Whitney U-test and χ^2 test, respectively. GraphPad Prism 7 software (GraphPad Software) was used for the Kaplan-Meier survival curve, and the differences between the two groups were analyzed using log-rank test. Moreover, the Cox proportional hazards model was used to analyze the risk factors of overall survival in CRC patients. Differences were considered to be significant if P<0.05. The statistical analysis was performed using Stata16.0 (StataCorp LLC).

Results

Patient characteristics

Sixty CRC patients were enrolled in the present study. Their characteristics are shown in *Table 1*. There were 38 male patients and 22 female patients in the study. The mean age of the enrolled patients was 60.4±9.6 years. The tumors in 23, 15, and 22 patients were located at the right-sided colon, left-sided colon, and rectum, respectively. CRC patients at all stages were enrolled in the present study; 9 patients were stage I, 24 were stage II, 16 were stage III, and 11 were stage IV. Differentiation stage data were collected according to histopathological examinations. As a result, 16 tumors were found to be well differentiated, 31 were moderately differentiated, and 13 were poorly differentiated. The CEA and CA199 levels were also recorded. As seen in *Table 1*, the CEA level was elevated in 41 patients, and the CA199 level was elevated in 29 patients.

BRIX1 level was significantly higher in CRC tissues

The *BRIX1* mRNA level in CRC tissues and adjacent normal tissues was measured using quantitative RT-PCR. As seen in *Figure 1A*, the *BRIX1* mRNA level in CRC tissues was higher than that in adjacent normal tissues (1.0±0.5 *vs.* 5.5±1.7, P<0.01). We then measured the BRIX1 protein level in both tissues. A representative diagram of the western blot is shown in *Figure 1B*. The BRIX1 protein level in CRC tissues was significantly higher than that in adjacent normal tissues (1.0±0.6 *vs.* 6.4±2.1, P<0.01) (*Figure 1C*). Furthermore, we divided all enrolled patients into two groups: stages I–II and stages III–IV. Stage III–IV patients were found to have a dramatically higher level of *BRIX1* mRNA and protein compared with stage I–II patients (*Figure 2A,B,C*; P<0.05).

BRIX1 level was related to the prognosis of CRC patients

The enrolled patients were divided into two groups based on the relative level of the BRIX1 protein in CRC tissues (>6 or ≤6). The Kaplan-Meier survival curve was used to compare the difference in the overall survival rate between

Table 1 Patient characteristics

Characteristics	Total
N	60
Sex	
Male	38
Female	22
Age (years)	60.4±9.6
Anatomical location	
Right-sided colon	23
Left-sided colon	15
Rectum	22
Tumor stage	
I	9
II	24
III	16
IV	11
Differentiation stage	
Well-differentiated	16
Moderately differentiated	31
Poorly differentiated	13
CEA	
Normal	19
Elevated	41
CA199	
Normal	31
Elevated	29

CA199, carbohydrate antigen 199; CEA, carcinoembryonic antigen.
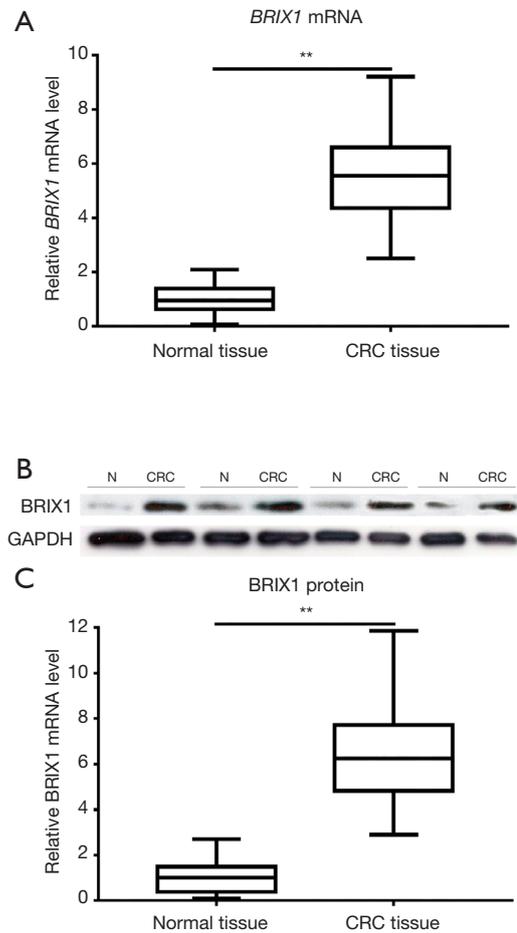
Figure 1 Biogenesis of ribosomes BRX1 (*BRIX1*) gene expression in colorectal cancer (CRC) tissues and normal tissues. (A) Relative mRNA level of *BRIX1* gene measured using quantitative reverse transcription-polymerase chain reaction; (B) representative figure of Western blot; (C) relative level of BRIX1 protein measured using Western blot. **, P<0.01. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

the two groups. As seen in *Figure 3*, the overall survival rate of patients with a high level of the BRIX1 protein was much lower than that of patients with a low level of the BRIX1 protein (P=0.022). The Cox proportional hazards model was further used to analyze the risk factors of overall survival in CRC patients. As shown in *Table 2*, three variables acted as risk factors of overall survival in CRC patients according to the multivariate analysis: tumor stage III or IV [P=0.044, hazards ratio (HR) =6.711], poor differentiation (P=0.022, HR =5.423), and elevated expression of the BRIX1 protein (P=0.048, HR =4.988).

Discussion

The *BRIX1* gene has been known to be involved in the synthesis of ribosomal 60S subunits. However, its function in the progression of cancer, especially CRC, has not been investigated. The aim of the present study was to determine the relationship between the expression of *BRIX1* and the progression and prognosis of CRC patients. The results indicated that CRC tissues expressed more *BRIX1* mRNA and protein than normal tissues. Moreover, the expressions of *BRIX1* mRNA and protein were much higher in CRC tissues from patients with stages III–IV CRC than stages I–
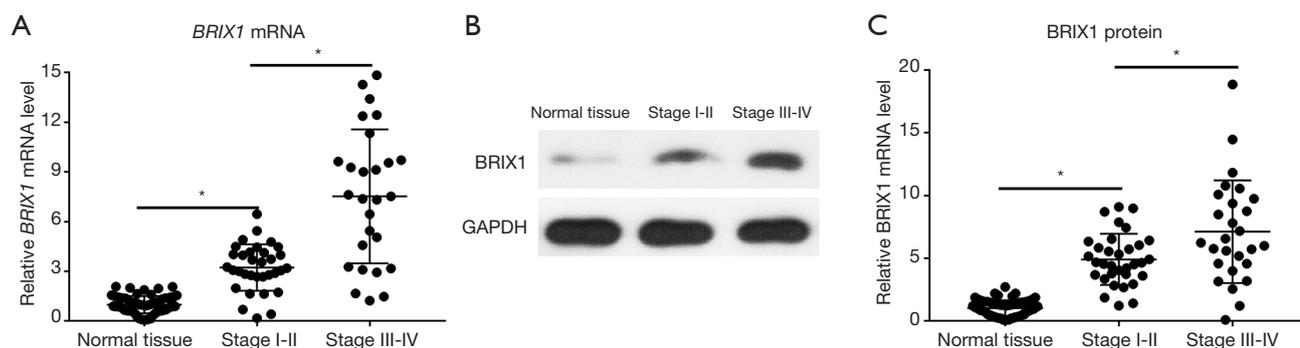
Figure 2 Association of expression of biogenesis of ribosomes BRX1 (*BRIX1*) gene with different stages of colorectal cancer. (A) Relative mRNA level of *BRIX1* gene measured using quantitative reverse transcription-polymerase chain reaction; (B) representative figure of Western blot; (C) relative level of the BRIX1 protein was measured using Western blot. *, P<0.05.
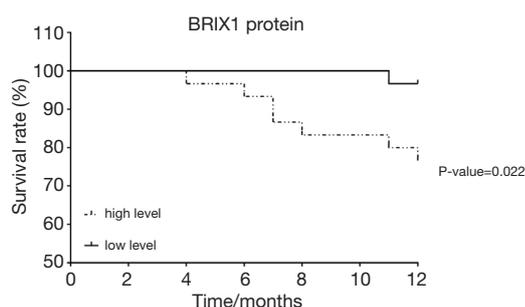

Figure 3 Kaplan-Meier survival was used to compare overall survival in groups with high and low levels of the BRIX1 protein according to the log-rank test.

II CRC. An elevated level of the BRIX1 protein also acted as a risk factor of mortality in CRC patients. In the present study, we preliminarily discussed the role of *BRIX1* in CRC and laid a foundation for future research of new therapeutic targets for CRC.

As an energy-intensive process in normal cells, protein synthesis is strictly regulated (14). Ribosome is essentially a supramolecular complex of RNA and protein, thus ribosome synthesis is also a part of protein synthesis (15). Four steps are needed for protein synthesis––initiation, elongation, termination, and ribosome recycling—and the step of initiation is the key step for protein synthesis. *BRIX1* is involved in the synthesis of ribosomal 60S subunits, which is required at the last stage of initiation to assemble an elongation-competent 80S ribosome (16). To some degree, *BRIX1* plays an important role as a key regulatory factor in protein and ribosome synthesis. However, ribosome synthesis is usually abnormal in cancer cells.

Deregulated ribosome biogenesis was first found in CRC in the 1980s; genes encoding ribosome-related proteins were overexpressed in CRC cell lines and human tissues (17,18). Since then, many studies have pointed out the abnormal expression of several genes encoding ribosome-related proteins in CRC (19,20). The upregulated expression of ribosome-related proteins promotes the development, migration, and proliferation of CRC cells (20). In their study, Wang *et al.* reported that the knockdown of RPS24, which is responsible for the synthesis of a ribosome-related protein, inhibits the migration and proliferation of CRC cell *in vitro* (21). The increase of ribosome-related proteins can further induce increased ribosome synthesis. They found that, similar to our study, the expression of *BRIX1* increased in CRC, which meant an upregulation of ribosome synthesis. According to the results from the Cox proportional hazards regression analysis, the upregulation of ribosome synthesis accelerated the development of CRC and increased patient mortality during follow-up.

Some commonly used chemotherapeutics for CRC, such as 5-fluorouracil (5-FU) and oxaliplatin, have been reported to interfere with not only DNA synthesis but also ribosome synthesis (22,23). 5-FU is able to combine with all kinds of RNA to alter the metabolism of RNA and reduce the biogenesis of ribosome (22). Oxaliplatin is also able to capture the components related to ribosome synthesis in CRC cells (23). The interference of these drugs on ribosome synthesis may also play a role in the treatment of CRC. However, this warrants further investigation.

As previously reported, several gene mutations in CRC may contribute to the upregulation of ribosome synthesis. The most common mutation related to ribosome

Table 2 Univariate and multivariate Cox proportional hazards regression analysis of risk factors of survival in colorectal cancer patients

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	P value	HR	95% CI	P value
Elevated BRIX1	8.231	1.034–58.763	0.041	4.988	1.018–21.119	0.048
Age (years)	1.029	0.968–1.094	0.357	–	–	–
Sex	0.598	0.150–2.393	0.468	–	–	–
Rectal cancer	1.195	0.531–2.691	0.667	–	–	–
Tumor stage III or IV	9.517	1.170–77.402	0.035	6.711	1.097–36.184	0.044
Poor differentiation	7.138	1.700–29.977	0.007	5.423	1.272–23.124	0.022
Elevated CEA	0.766	0.183–3.206	0.715	–	–	–
Elevated CA199	0.615	0.147–2.575	0.506	–	–	–

BRIX1, biogenesis of ribosomes BRX1; CA199, carbohydrate antigen 199; CEA, carcinoembryonic antigen; CI, confidence interval; HR, hazard ratio.

synthesis in CRC is the *MYC* mutation. There are three kinds of RNA polymerases involved in ribosome synthesis through the transcription of rRNA. The *MYC* mutation was found to induce the transcriptions of all three RNA polymerases and further stimulate rRNA synthesis (24,25). Another common mutation in CRC is found in *KRAS*. The expression of *KRAS* leads to the activation of the mitogen-activated protein kinase signaling pathway, and further participates in the regulation of ribosome synthesis. Moreover, *KRAS* mutation was found to increase the expression of ribosome-related proteins and the translation of mRNA (26). Drugs have been developed to target these two mutations. CX-3543 and CX-5461, two specific RNA polymerase I inhibitors, have proved to be effective in some hematologic and solid malignancies in I/II clinical trials (9). They can also inhibit the proliferation of CRC, which has defects in DNA damage repair (27). *KRAS* mutation was once considered untreatable with drugs. Fortunately, Canon *et al.* developed the first *KRAS* inhibitor, AMG 510, in 2019 (28). It was reported to cure mice with isogenic *KRAS* tumors, and demonstrated anti-tumor activity in the first dosing cohort in clinical trials (28). However, the effects of AMG 510 on ribosome synthesis are unclear. According to the results of the present study, *BRIX1* may be a novel target for the treatment of CRC.

The present study had some limitations. First, we used a relatively small sample size, so further analysis of a deeper relationship between *BRIX1* and CRC would be difficult. Second, immunohistochemistry was not performed in the present study. Immunohistochemistry may be beneficial to determine the location of the BRIX1 protein aggregation in cancer cells and to improve our knowledge of the mechanism of how BRIX1 affects the progression and development of CRC. Third, some parameters of surgical treatment may also affect the prognosis of patients, and this will need to be improved in future research.

Conclusions

The results of the present study indicate that BRIX1 expression was elevated in CRC tissues than in adjacent normal tissues. The elevated expression of *BRIX1* was also positively correlated with CRC tumor stage and contributed to a lower overall survival rate for CRC patients.

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Footnote

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org/10. 21037/tcr−20−2564). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the ethics committee of the Affiliated Nanjing Jiangbei Hospital of Nantong University (No. J2017034) and informed consent was taken from all the patients.

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