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Diverse expression patterns of mucin 2 in colorectal cancer indicates its mechanism related to the intestinal mucosal barrier

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Abstract

BACKGROUND
Abnormal expression patterns of mucin 2 (MUC2) have been reported in a variety of malignant tumors and precancerous lesions. Reduced MUC2 expression in the intestinal mucosa, caused by various pathogenic factors, is related to mechanical dysfunction of the intestinal mucosa barrier and increased intestinal mucosal permeability. However, the relationship between MUC2 and the intestinal mucosal barrier in patients with colorectal cancer (CRC) is not clear.

AIM
To explore the relationship between MUC2 and intestinal mucosal barrier by characterizing the multiple expression patterns of MUC2 in CRC.

METHODS
Immunohistochemical staining was performed on intestinal tissue specimens from 100 CRC patients, including both cancer tissues and adjacent normal tissues. Enzyme-linked immunosorbent assays were performed on preoperative sera from 66 CRC patients and 20 normal sera to detect the serum levels of MUC2, diamine oxide (DAO), and D-lactate (D-LAC). The relationship between MUC2 expression and clinical parameters was calculated by the χ^2 test or Fisher's exact test. Prognostic value of MUC2 was evaluated by Kaplan-Meier curve and log-rank

tests.

RESULTS

Immunohistochemical staining of 100 CRC tissues showed that the expression of MUC2 in cancer tissues was lower than that in normal tissues (54% *vs* 79%, $P < 0.05$), and it was correlated with tumor-node-metastasis (TNM) stage and lymph node metastasis in CRC patients ($P < 0.05$). However, the serum level of MUC2 in CRC patients was higher than that in normal controls, and was positively associated with serum levels of human DAO ($\chi^2 = 3.957$, $P < 0.05$) and D-LAC ($\chi^2 = 7.236$, $P < 0.05$), which are the biomarkers of the functional status of the intestinal mucosal barrier. And the serum level of MUC2 was correlated with TNM stage, tumor type, and distant metastasis in CRC patients ($P < 0.05$). Kaplan-Meier curves showed that decreased MUC2 expression in CRC tissues predicted a poor survival.

CONCLUSION

MUC2 in tissues may play a protective role by participating in the intestinal mucosal barrier and can be used as an indicator to evaluate the prognosis of CRC patients.

Key Words: Colorectal cancer; Mucin 2; Mucin; Expression; Intestinal mucosal barrier; Prognosis

Core Tip: This study found that mucin 2 (MUC2) in intestinal tissues may play a protective role on the intestine and can be used as one of the indicators to evaluate the prognosis of patients with colorectal cancer (CRC). When the intestinal mucosal barrier function of patients with CRC is impaired, the serum level of MUC2 can reflect the severity of the damage. Therefore, in CRC patients with impaired intestinal mucosal barrier function, the serum level of MUC2 could reflect the severity of the damage, providing a potential mechanism for the development of therapeutic strategies for CRC patients.

INTRODUCTION

The incidence of colorectal cancer (CRC) ranks third in the world among the common malignant tumors, and the mortality ranks second[1]. Due to large changes in lifestyle and dietary habits, the incidence and mortality of CRC will continue to rise. In China, there are 300000 new CRC cases each year, with an annual average increase of 4.2%[2]. There are obvious gender and regional differences in the incidence and mortality rate of CRC cases, with the overall distribution being more in males than in females, and more in urban areas than in rural areas[3]. Therefore, CRC is one of the major diseases that seriously threatens human life and health, and the situation is quite serious.

The etiological mechanism of CRC tumorigenesis and development is extremely complex, involving a variety of genetic and environmental factors[4]. Among them, the impairment of intestinal barrier structure and function leads to a series of pathophysiological changes in the intestinal mucosa, which eventually evolves into tumor malignancy. Mucins (MUCs) are the main components of the mucus layer, which provides the first-line defense against infection and participates in the process of intercellular adhesion, intercellular communication, and immune regulation[5].

Among 27 reported MUC proteins, MUC2 is a secretory mucin involved in the formation of mucus, and is mainly secreted by goblet cells[6]. Reduced MUC2 expression in the intestinal mucosa, caused by various pathogenic microorganisms and/or toxic substances, induces apoptosis of intestinal mucosal epithelial cells and destroys the mechanical barrier of the intestinal mucosa, ultimately leading to increased intestinal mucosal permeability[7]. Interestingly, an abnormal expression pattern of MUC2 has been reported in a variety of malignant tumors and precancerous lesions, suggesting an important role for MUC2 in the occurrence and development of CRC[8, 9]. However, the expression of MUC2 is tissue- and cell-specific[10]. Kasprzak *et al*[8] reported a distinct expression pattern of MUC2 in mucinous *vs* non-mucinous colorectal adenocarcinoma[8], suggesting that diverse and specific mechanisms are involved in different types of CRC.

To verify the diverse expression patterns of MUC2 in CRC patients, the current study enrolled CRC patients and investigated MUC2 levels in both malignant tissue and serum to provide an underlying mechanism of MUC2 related to intestinal barrier function, and lay a foundation for further revealing the molecular mechanism of MUC2 involvement in the malignant biological behavior of CRC.

MATERIALS AND METHODS

Ethics statement
This study was approved by the Ethics Committee of the First Affiliated Hospital of Shantou University Medical College. All patients or their guardians signed written informed consent before the study. All experiments were conducted following the guidelines of the Ethics Review Committee.

Patient information
Cancer tissues and adjacent normal intestinal mucosal tissues (> 5 cm away from the tumor) were collected from 100 CRC patients who underwent radical resection at the Department of Gastrointestinal Surgery of the First Affiliated Hospital of Shantou University Medical College from January 2015 to December 2016. The inclusion criteria included: (1) Age from 18 to 90; (2) No medical history of blood, cardiovascular, or immune disease, or inflammatory bowel disease; (3) Pathological diagnosis of CRC; (4) Tumor-node-metastasis (TNM) stages I to III; and (5) Patients who underwent radical surgery. The exclusion criteria included: (1) Preoperative history of neoadjuvant chemotherapy or radiotherapy; (2) Binary or multivariate cancer; (3) Preoperative complications, such as malignant intestinal obstruction, perforation, or bleeding; (4) No standardized chemotherapy after the radical surgery; and (5) Incomplete clinico-pathological data.

Peripheral blood was collected before surgery from 66 CRC patients who were diagnosed with CRC at the Department of Gastrointestinal Surgery of the First Affiliated Hospital of Medical College of Shantou University from January 2018 to December 2019. The inclusion criteria were almost the same as above, except that patients at stage IV were also recruited. The exclusion criteria were the same. For comparison, 20 normal subjects in the same period were recruited and their sera were collected in the Health Management Center of the First Affiliated Hospital of Shantou University Medical College.

Histology and immunohistochemistry
Formalin-fixed and paraffin-embedded CRC tissues were cut into 4-μm sections and stained with hematoxylin and eosin. Histopathological differentiation was made by two pathologists based on the World Health Organization criteria. TNM pathological staging was determined according to the staging manual of the American Joint Committee on Cancer[11].

Immunohistochemistry (IHC) was conducted as described before[12]. CRC and adjacent normal tissues were dewaxed in xylene, hydrated in a series of graded alcohols, and placed in a citric acid buffer for epitope retrieval. After immersion in 3% H_2O_2 solution to block endogenous peroxidase, the slides were incubated with anti-MUC2 monoclonal antibody (ab118964, Abcam, United Kingdom) at 4 ℃ overnight. Negative controls were treated with PBS instead of primary antibody. Haematoxylin was used for counterstaining.

Sections were visualized under a bright-field microscope (CKX41, Olympus, Japan) and evaluated independently by two investigators with no prior knowledge of the patient information. For tissue expression of MUC2, staining intensity was recorded as
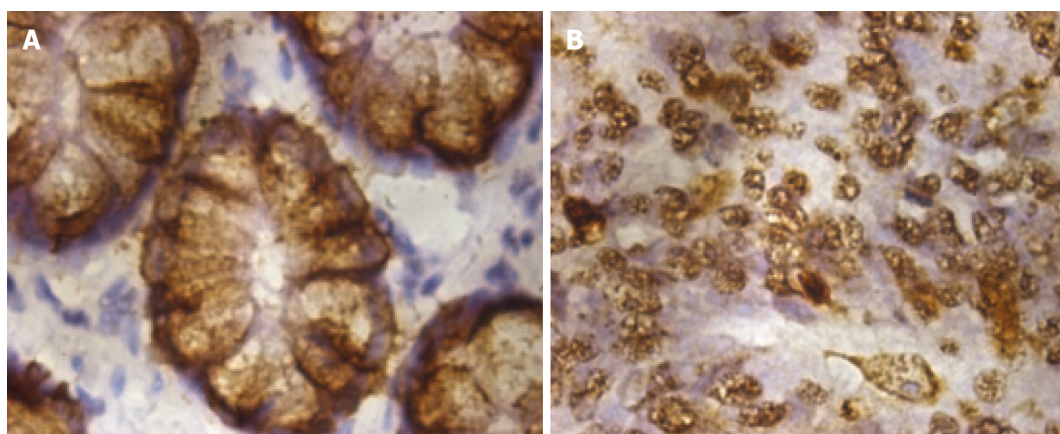
Figure 1 Mucin 2 is mainly located in the cytoplasm. A: Normal tissue; B: Colorectal cancer tissue. Magnification: × 1000.

0, 1, 2 and 3 for colorless, light yellow, brown yellow, and dark brown, respectively, and the percentage of positive cells < 5%, 6%-30%, 31%-60%, and 61%-100% was scored as 0, 1, 2, and 3 points, respectively. To evaluate the expression level of MUC2, the scores of staining intensity and the percentage of positive cells were added and defined as low expression (0-3) and high expression (4-6).

Enzyme-linked immunosorbent assay

The collected peripheral blood samples were centrifuged at 2000-3000 rpm for 15 min, and the supernatant serum was collected carefully and stored in -80 ℃. Human MUC2, human diamine oxide (DAO), and human D-lactate (D-LAC) enzyme-linked immunosorbent assay detection kits were purchased from Nanjing Senberga Biotechnology Co., Ltd., China. Experiments were carried out as previously described[13]. The standard curve was determined by the standard concentration and their corresponding absorbance (OD value). The actual concentrations of MUC2, DAO, and D-LAC were calculated based on the standard curves.

Follow-up and statistical analysis

Overall survival (OS) time was calculated in months from the date of diagnosis to the date of death of the patient or the last follow-up visit. Disease-free survival (DFS) time was determined by the date of relapse.

SPSS 21.0 statistical software was used to analyze the data. Enumerated data are expressed by the number of cases (N), and the measurement data that conformed to a normal distribution are expressed as the mean ± SD. The relationship between MUC2 and the patient's clinicopathological data was tested by χ^2 and Fisher's exact probability tests. The relationship between serum MUC2, DAO, and D-LAC was analyzed by the χ^2 test. The Kaplan-Meier survival curve and log-rank test were used to evaluate the association of MUC2 expression with prognosis. The difference between two groups was statistically significant at $P < 0.05$.

RESULTS

Expression of MUC2 is decreased in cancer tissues compared with normal tissues in CRC patients

To detect the location of MUC2 in intestinal tissues, IHC was performed and revealed that MUC2-positive staining was mainly located in the cytoplasm, both in normal tissues and CRC tissues (Figure 1). In normal tissues, the cytoplasm was diffusely and homogeneously positive, and the nucleus was not stained, while in cancer tissues, cells lost their normal morphology, adenoid structures were destroyed or had even disappeared, and heterotypic cancer cells could be detected.

Representative images of MUC2 staining in adjacent and cancer tissues are shown in Figure 2. Interestingly, the percentage of tissues with high MUC2 expression was significantly decreased from 79% in adjacent normal tissues to 54% in CRC tissues (Figure 3). The difference in MUC2 expression between normal and cancer tissues was statistically significant ($P < 0.01$; Table 1)

Table 1 Comparison of mucin 2 expression in normal and cancer tissues of patients with colorectal cancer (cases)

Group	MUC2 expression		χ^2	*P* value
	Low	High		
Normal tissues	21	79	14.028	< 0.01[1]
Cancer tissues	46	54		

[1]Indicates that the difference was statistically significant, which confirmed that the expression difference of mucin 2 (MUC2) in normal tissues and cancer tissues was statistically significant, and the high expression rate of MUC2 in cancer tissues was lower than that in normal tissues. MUC2: Mucin 2.
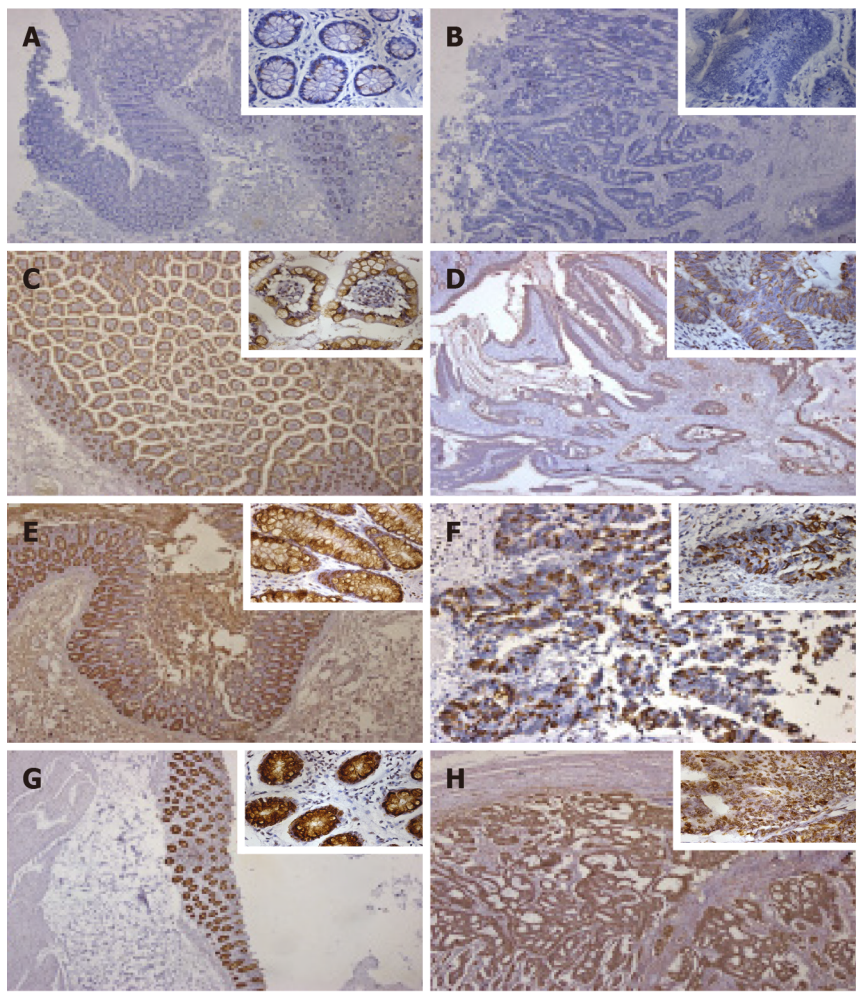
Figure 2 Representative pictures of immunohistochemical staining for mucin 2 in normal and cancer tissues of patients with colorectal cancer. A and B: Negative expression; C and D: Weak expression; E and F: Moderate expression; G and H: Strong expression. A, C, E, and G: Normal tissue; B, D, F, and H: Cancer tissue. Magnification: × 100 and × 400 (located in the upper right corner of each image).

Tissue MUC2 level is negatively associated with TNM stage and lymph node status in patients with CRC

Clinicopathological analysis showed that tissue MUC2 expression (low *vs* high) was not significantly associated with the age at diagnosis, gender, tumor location, tumor size, depth of invasion, degree of differentiation, or tumor type (Table 2). In comparison with tumors at stage III, tumors at stages I and II showed significantly more tissue MUC2 expression (χ^2 = 13.963, *P* < 0.05). Importantly, low expression of tissue MUC2 was more frequently observed in patients with lymph node metastasis (χ^2 = 12.538, *P* < 0.05) (Table 2).

Table 2 Relationship between expression of mucin 2 and clinicopathological parameters in patients with colorectal cancer, *n* (%)

Clinicopathological parameter	*n* (%)	MUC2 expression		χ^2	*P* value
		Low, *n* (%)	High, *n* (%)		
Age, yr					
≤ 60	48	22 (45.8)	26 (54.2)	0.001	0.974
> 60	52	24 (46.2)	28 (53.8)		
Gender					
Male	52	23 (44.2)	29 (55.8)	0.137	0.712
Female	48	23 (47.9)	25 (52.1)		
Tumor location					
Rectum and anus	48	21 (43.8)	27 (56.3)	0.188	0.664
Colon	52	25 (48.1)	27 (51.9)		
TNM stage					
I-II	57	17 (29.8)	40 (70.2)	13.963	< 0.01
III	43	29 (67.4)	14 (32.6)		
Maximum tumor diameter					
< 5 cm	49	23 (46.9)	26 (53.1)	0.034	0.854
≥ 5 cm	51	23 (45.1)	28 (54.9)		
Depth of invasion					
Non-immersed serosa	22	8 (36.4)	14 (63.6)	1.054	0.304
Immersed serosa	78	38 (48.7)	40 (51.3)		
Degree of differentiation					
High-moderate	93	42 (45.2)	51 (54.8)	F[1]	0.700
Low	7	4 (57.1)	3 (42.9)		
Tumor type					
Mucinous adenocarcinoma	13	5 (38.5)	8 (61.5)	0.342	0.559
Non-mucinous adenocarcinoma	87	41 (47.1)	46 (52.9)		
Lymph node metastasis					
No	56	17 (30.4)	39 (69.6)	12.538	< 0.01
Yes	44	29 (65.9)	15 (34.1)		

[1]Fisher exact test was used when the expected frequency was less than 1. Due to the small number of patients with stage I, in order to reduce bias, we combined the stage I with stage II patients for analysis. Similarly, there was only one patient with highly differentiated colorectal cancer, so the patient was combined with patients with moderately differentiated disease. It can be seen from the above table that the expression of mucin 2 is correlated with tumor-node-metastasis stage and lymph node metastasis in colorectal cancer patients. MUC2: Mucin 2; TNM: Tumor-node-metastasis.

Serum levels of MUC2 are elevated in CRC patients, and positively associated with the levels of DAO and D-LAC in serum

Serum DAO and D-LAC are indicators of intestinal mucosal barrier permeability and integrity[14,15]. As expected, in patients with CRC, the serum levels of DAO and D-LAC were significantly increased compared with those in normal controls (Figure 4A and B), indicating impaired intestinal mucosal barrier function and increased intestinal permeability in CRC patients (Table 3). Interestingly, serum levels of MUC2 were also higher than those in normal controls (Figure 4C) and closely related to the serum levels of DAO and D-LAC (Table 4).

Table 3 Comparison of serum levels of diamine oxide, D-lactate, and mucin 2 between colorectal cancer patients and normal control (mean ± SD)

Group	*n* (%)	DAO (pg/mL)	D-LAC (μg/L)	MUC2 (ng/L)
Normal control	20	158.21 ± 15.98	973.69 ± 128.08	305.98 ± 31.50
CRC	66	185.40 ± 25.49	1216.93 ± 204.20	364.58 ± 48.30
P value		< 0.01	< 0.01	< 0.01

There were 66 cases of colorectal cancer (CRC) and 20 cases of normal controls. Serum levels of mucin 2, diamine oxide, D-lactate in CRC patients were higher than those in the normal control group. MUC2: Mucin 2; DAO: Diamine oxide; D-LAC: D-lactate; CRC: Colorectal cancer.

Table 4 Relationship between serum levels of mucin 2, diamine oxide, and D-lactate in patients with colorectal cancer (cases)

MUC2	DAO		D-LAC	
	Low	High	Low	High
Low	15	8	16	7
High	17	26	15	28
χ^2	3.957		7.236	
P value	0.047		0.007	

The measured mucin 2 (MUC2) level of P95 in the normal population was taken as the normal reference range, and those beyond P95 were regarded as increased, otherwise as decreased. Based on this, colorectal cancer patients were divided into groups with high and low levels of MUC2. Similarly, the number of cases in diamine oxide (DAO) and D-lactate (D-LAC) groups with high and low levels could be obtained. Serum MUC2 levels were positively correlated with DAO and D-LAC levels. Low: Low serum level; High: High serum level; MUC2: Mucin 2; DAO: Diamine oxide; D-LAC: D-lactate.
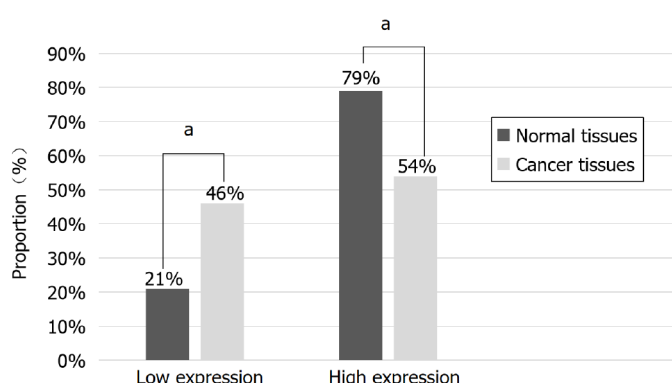
Figure 3 Histogram of mucin 2 expression in cancer and normal tissues of patients with colorectal cancer. According to the comprehensive score of staining intensity and positive cell percentage, 0-3 was classified as low expression and 4-6 as high expression. [a]$P < 0.05$.

Serum MUC2 is positively associated with TNM stage and distant metastasis in patients with CRC

As shown in Table 5, the higher the TNM stage in CRC patients, the higher the serum MUC2 level (*P* = 0.033). Importantly, the percentage of patients with high serum levels of MUC2 was dramatically increased in CRC patients with distant metastasis compared with those without (100.0% *vs* 59.6%, *P* = 0.022). The tumor type was also found to be related to the expression of serum MUC2 in CRC patients. CRC patients with mucinous adenocarcinoma had higher serum MUC2 levels than non-mucinous CRC patients (*P* < 0.01). However, serum MUC2 level was not associated with age at diagnosis, gender, tumor size, tumor location, depth of invasion, degree of differentiation, or lymph node metastasis (Table 5).

Low expression of MUC2 in cancer tissues predicts a poor survival in CRC patients

Kaplan-Meier curve analyses with log-rank test revealed that tissue MUC2 expression was significantly associated with DFS (*P* = 0.032) (Figure 5A) and OS (*P* = 0.037)

Table 5 Relationship between serum levels of mucin 2 and clinicopathological parameters in patients with colorectal cancer, *n* (%)

| Clinicopathological parameter | *n* (%) | Serum MUC2 expression | | χ^2 | *P* value |
		Low, *n* (%)	High, *n* (%)		
Age, yr					
≤ 60	30	11 (36.7)	19 (63.3)	0.080	0.777
> 60	36	12 (33.3)	24 (66.7)		
Gender					
Male	38	12 (31.6)	26 (68.4)	0.422	0.516
Female	28	11 (39.3)	17 (60.7)		
Tumor location					
Rectum and anus	24	10 (47.1)	14 (58.3)	0.772	0.380
Colon	42	13 (31.0)	29 (65.2)		
TNM stage					
I-II	36	16 (44.4)	20 (55.6)	6.687	0.033
III	21	7 (33.3)	14 (66.7)		
IV	9	0 (0.0)	9 (100.0)		
Maximum tumor diameter					
< 5 cm	33	12 (36.4)	21 (63.6)	0.067	0.796
≥ 5 cm	33	11 (33.3)	22 (66.7)		
Depth of invasion					
Non-immersed serosa	12	5 (41.7)	7 (58.3)	0.300	0.584
Immersed serosa	54	18 (33.3)	36 (66.7)		
Degree of differentiation					
High-moderate	60	21 (38.3)	39 (61.7)	F[1]	1.000
Low	6	2 (33.3)	4 (66.7)		
Tumor type					
Mucinous adenocarcinoma	14	8 (57.1)	6 (42.9)	21.241	< 0.01
Non-mucinous adenocarcinoma	52	15 (32.7)	37 (67.3)		
Lymph node metastasis					
No	36	16 (44.4)	20 (55.6)	3.212	0.073
Yes	30	7 (23.3)	23 (76.7)		
Distant metastasis					
No	57	23 (40.4)	34 (59.6)	F[1]	0.022
Yes	9	0 (0.0)	9 (100.0)		

[1]Fisher exact test was used when the expected frequency was less than 1. Due to the small number of patients with stage I, in order to reduce bias, we combined the stage I with stage II patients for analysis. Similarly, there was only one patients with highly differentiated colorectal cancer, so this patient was combined with the patients with moderately differentiated disease. Serum levels of mucin 2 was correlated with tumor-node-metastasis stage, tumor type, and distant metastasis in colorectal cancer patients. MUC2: Mucin 2; TNM: Tumor-node-metastasis.

(Figure 5B) in all CRC patients. Decreased tissue MUC2 level predicted a poor prognosis of CRC patients. During the 5-year follow-up period, the recurrence was 40.0% in patients with low expression of MUC2 and 18.5% in patients with high expression of MUC2 (χ^2 = 5.485, *P* < 0.05) (Figure 5C).
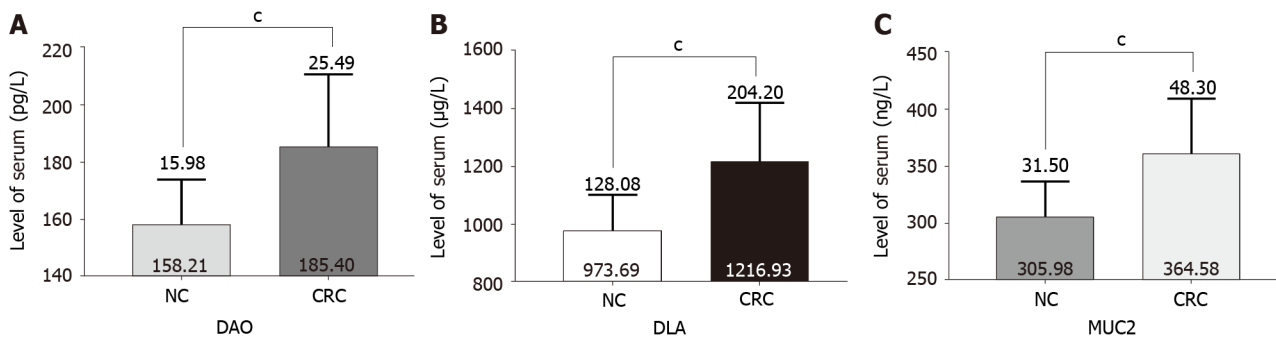
Figure 4 Comparison between the levels of serum diamine oxide, D-lactate, and mucin 2 in colorectal cancer patients and normal controls. A: The level of serum diamine oxidase in colorectal cancer (CRC) patients and normal controls (NC); B: The level of serum D-lactate in CRC patients and NC; C: The level of serum mucin 2 in CRC patients and NC. The numbers at the bottom of the bar chart represent the mean, and the numbers above the error line represent the standard deviation. [c]$P < 0.001$. CRC: Colorectal cancer; NC: Normal controls; DAO: Diamine oxidase; D-LAC: D-lactate; MUC2: Mucin 2.
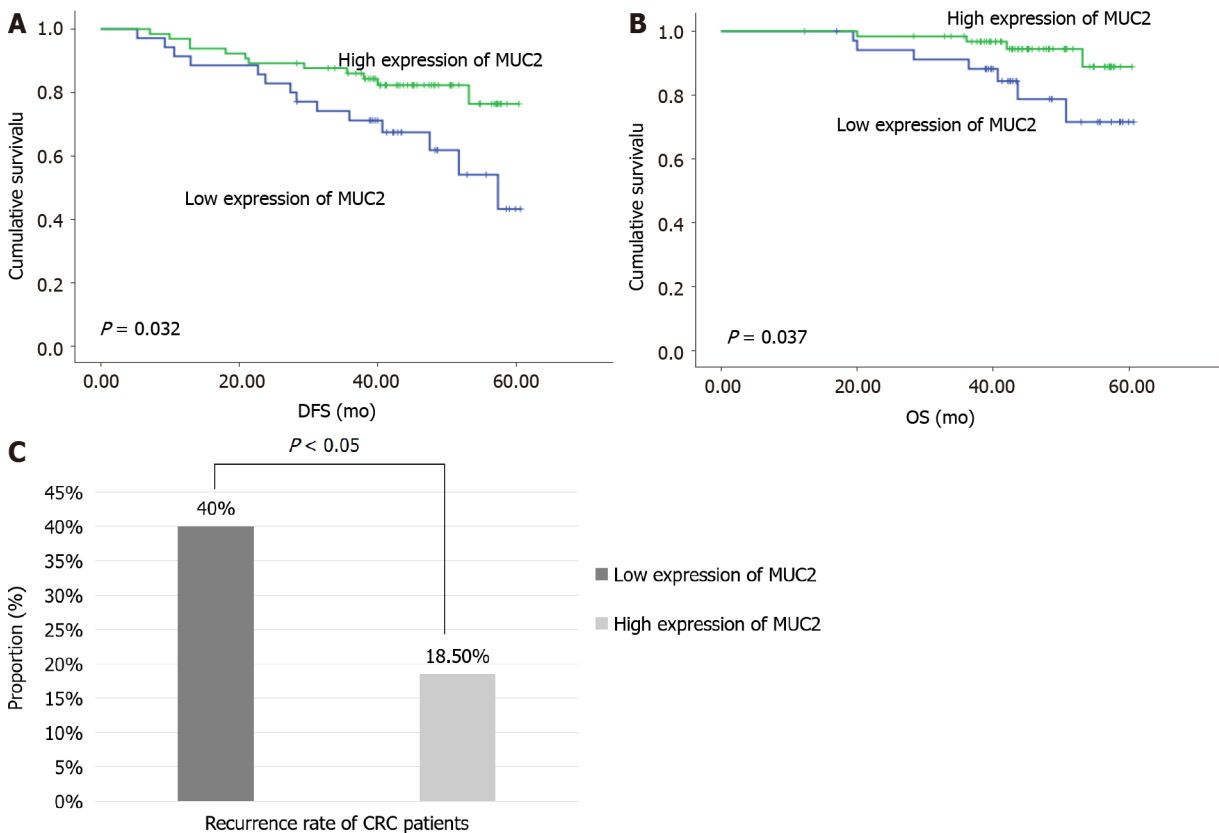
Figure 5 Prognostic value of tissue mucin 2 expression in colorectal cancer patients. A: Correlation between tissue mucin 2 (MUC2) expression and disease-free survival in colorectal cancer (CRC) patients; B: Correlation between tissue MUC2 expression and overall survival in CRC patients; C: Histogram of the recurrence rate of CRC patients with different tissue levels of MUC2 expression. DFS: Disease-free survival; OS: Overall survival. MUC2: Mucin 2; CRC: Colorectal cancer.

DISCUSSION

The protein encoded by the *MUC2* gene is the most abundant secreted mucin, covering the surface of the intestinal mucosa in the form of a gel and forming the skeleton of the mucus layer, protecting the intestine in many ways[16]. Recently, Javitt *et al*[17] presented an integrated structural analysis of the intestinal mucin MUC2, and revealed that the mucin assembly mechanism and its adaptation for hemostasis provide the foundation for rational manipulation of barrier function and coagulation[17]. On the other hand, CRC shows multiple complex pathologies based on the impaired structure and function of the intestinal mucosal barrier, and is associated with the disordered expression and dysfunction of mucins[18]. However, controversial findings of MUC2 function in the occurrence and development of CRC have required further invest-

igation to uncover the underlying mechanisms. We showed that MUC2 expression was decreased in carcinomas, compared with adjacent normal tissues, but CRC patients had higher serum levels of MUC2 compared with normal controls.

MUC2 plays an important role in maintaining the homeostasis of the intestinal environment and protecting susceptible bacteria from pathogenic microorganisms and/or toxic substances[19]. We detected decreased MUC2 in CRC tissue, compared to adjacent intestinal tissues, which may be related to the suppressed immune function in intestinal homeostasis. Importantly, decreased MUC2 expression was found to be associated with advanced CRC stage, suggesting a tumor-suppressive role in the development of CRC. In CRC patients with lymph node metastasis, the tissue MUC2 level was also lower than that in CRC patients without lymph node metastasis, further suggesting a potential protective role of MUC2 in lymph node metastasis in CRC patients. Although statistical significance was not found in the relationship between tissue MUC2 expression and tumor size, depth of invasion, or degree of differentiation, our results indicate that a more severe malignant biological behavior was more likely found in CRC tissues with low MUC2 expression, demonstrating a potential protective role of MUC2 in the development of CRC.

The expression of MUC2 in CRC tissues is reported to be related to the histopathological types of CRC. The expression of MUC2 in mucinous adenocarcinoma is increased, while that in non-mucinous adenocarcinoma is decreased[8,20]. In this study, patients with mucinous adenocarcinoma tended to have a higher proportion of patients with high expression of MUC2 than those with non-mucinous adenocarcinoma. However, the underlying roles of MUC2 in different types of CRC are still unclear and need further investigation.

Li *et al*[21] reviewed the prognostic and clinicopathological significance of MUCs in CRC, and demonstrated that upregulated MUC2 expression is associated with a better OS, while upregulated MUC1 expression is associated with a poor OS[21]. Elzagheid *et al*[22] reported that loss of MUC2 expression is associated with disease recurrence and tumor location. However, in multivariate survival analysis, MUC2 lost its power as an independent predictor of DFS and disease-specific survival[22]. To verify the prognostic value of MUC2 expression in CRC patients, we used Kaplan-Meier curve analyses and showed that low expression of tissue MUC2 was associated with a poor DFS and OS in CRC patients, which may be related to the high recurrence rate exhibited by CRC patients with low tissue MUC2 levels.

To investigate the potential function of secreted MUC2 in CRC patients, this study further analyzed the expression of serum MUC2 in CRC patients and normal controls. As serum DAO and D-LAC are biomarkers of the functional status of the intestinal mucosal barrier[23], we also monitored serum DAO and D-LAC levels. Interestingly, the serum level of MUC2 was positively related with serum DAO and D-LAC levels, which indicates dysfunction of intestinal mucosal barriers and increased intestinal permeability in CRC patients. The increased serum level of MUC2 may be associated with the progress of tumor infiltration.

The increased serum levels of MUC2 may be related to the fact that cancer cells gradually destroy the mucus layer, intestinal mucosal epithelial cells, and tight junctions, which result in the destruction of the MUC2 skeletal structure in the mucus layer, the apoptosis of intestinal mucosal epithelial cells, and abnormal expression and distribution of tight junction proteins. Decreased MUC2 in the intestinal mucosa and the damage of the intestinal barrier could result in invasion of various pathogenic microorganisms and toxic substances in the intestinal cavity to further aggravate the damage of the intestinal barrier and constitute a vicious circle, increasing intestinal mucosal permeability and promoting the translocation of MUC2 from epithelial cells to the blood.

CONCLUSION

This study found that MUC2 in intestinal tissues may play a protective role in the intestine and can be used as one of the indicators to evaluate the prognosis of patients with CRC. When the intestinal mucosal barrier function of patients with CRC is impaired, the serum level of MUC2 can reflect the severity of damage. Subsequent studies can further investigate the role of MUC2 in the malignant transformation of colorectal inflammatory diseases, cancer cell proliferation, invasion, metastasis, and the mechanism of resistance to chemotherapeutic drugs at the molecular level.

ARTICLE HIGHLIGHTS

Research background

At present, several studies have reported abnormal expression patterns of mucin 2 (MUC2) in cancerous lesions, including colorectal cancer (CRC). However, as a member of the intestinal mucosal mechanical barrier, the relationship between MUC2 and the intestinal mucosal barrier in patients with CRC remains unclear. Revealing this association will help us more fully understand the role of MUC2 in CRC.

Research motivation

Although many studies have proved that intestinal mucosal barrier function is impaired and MUC2 expression is abnormal in patients with CRC, the direct relationship between MUC2 and intestinal mucosal barrier has rarely been studied. The main problem to be solved in this study is to clarify this relationship and lay a foundation for further studies on the molecular mechanism of MUC2 involvement in CRC.

Research objectives

This study aimed to explore abnormal expression patterns of MUC2 and the relationship between MUC2 and intestinal mucosal barrier by characterizing the multiple expression patterns of MUC2 in CRC. The findings will provide a basis for further study of the pathogenesis of MUC2 in the process of intestinal mucosal barrier damage in CRC.

Research methods

Immunohistochemical staining was performed on cancer tissue and normal tissue samples from 100 patients with CRC to evaluate the expression of MUC2 in two different tissues, and these patients were followed for 12-60 mo to understand the overall survival (OS) and disease-free survival (DFS). Preoperative serum levels of MUC2, diamine oxide (DAO), and D-lactate (D-LAC) in 66 patients with CRC were detected by enzyme-linked immunosorbent assay and compared with those in 20 normal controls, so as to evaluate the damage of intestinal mucosal barrier in patients with CRC. The statistical methods involved in this study include χ^2 test, Fisher's exact test, Kaplan-Meier curve, and log-rank tests.

Research results

Immunohistochemical staining results showed that the expression of MUC2 in cancer tissues was lower than that in normal tissues (54% *vs* 79%, $P < 0.05$), and the expression of MUC2 was correlated with tumor-node-metastasis (TNM) stage and lymph node metastasis in CRC patients ($P < 0.05$), but not significantly related to the patient's age, sex, tumor location, size, depth of invasion, or degree of differentiation. The serum levels of MUC2, DAO, and D-LAC in patients with CRC were higher than those in normal people ($P < 0.05$), and were positively associated with serum levels of human DAO ($\chi^2 = 3.957$, $P < 0.05$) and D-LAC ($\chi^2 = 7.236$, $P < 0.05$), which are the biomarkers of the functional status of the intestinal mucosal barriers. It was suggested that the intestinal mucosal barrier was damaged, and MUC2 can also be used as a new evaluation index. The serum levels of MUC2 were correlated with TNM stage, tumor type, and distant metastasis in CRC patients ($P < 0.05$). It seems to be a trend that patients with higher malignancy and later stage of tumors have higher serum MUC2 levels. Survival analysis showed that decreased expression of MUC2 in CRC tissues predicted a poor survival. The expression of MUC2 in tissues was significantly correlated with DFS ($P = 0.032$) and OS ($P = 0.037$). And the recurrence rate of patients with low expression of MUC2 was higher than that of patients with high expression of MUC2 (40% *vs* 18.5%, $\chi^2 = 5.485$, $P < 0.05$).

Research conclusions

MUC2 in the intestinal tissue may play a protective role on the intestine, which can be used as an indicator to evaluate the prognosis of CRC patients. Intestinal mucosal barrier function of CRC patients is impaired, and the serum MUC2 level can reflect the severity of the damage.

Research perspectives

Future researchers can further study the molecular mechanism of MUC2 in the process of intestinal mucosal barrier damage, which may reveal the pathological mechanism of CRC from a new perspective and provide a basis for the development of new targeted

therapy drugs. In addition, related research can also be carried out in inflammatory bowel disease.

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REFERENCES

1   **Schreuders EH**, Ruco A, Rabeneck L, Schoen RE, Sung JJ, Young GP, Kuipers EJ. Colorectal cancer screening: a global overview of existing programmes. *Gut* 2015; **64**: 1637-1649 [PMID: 26041752 DOI: 10.1136/gutjnl-2014-309086]

2   **Xing XL**, Yao ZY, Zhang T, Zhu N, Liu YW, Peng J. MicroRNA-Related Prognosis Biomarkers from High-Throughput Sequencing Data of Colorectal Cancer. *Biomed Res Int* 2020; **2020**: 7905380 [PMID: 32964043 DOI: 10.1155/2020/7905380]

3   **Zahnd WE**, Josey MJ, Schootman M, Eberth JM. Spatial accessibility to colonoscopy and its role in predicting late-stage colorectal cancer. *Health Serv Res* 2021; **56**: 73-83 [PMID: 32954527 DOI: 10.1111/1475-6773.13562]

4   **Gonzalez-Villarreal CA**, Quiroz-Reyes AG, Islas JF, Garza-Treviño EN. Colorectal Cancer Stem Cells in the Progression to Liver Metastasis. *Front Oncol* 2020; **10**: 1511 [PMID: 32974184 DOI: 10.3389/fonc.2020.01511]

5   **Loktionov A**, Soubieres A, Bandaletova T, Mathur J, Poullis A. Colorectal cancer detection by biomarker quantification in noninvasively collected colorectal mucus: preliminary comparison of 24 protein biomarkers. *Eur J Gastroenterol Hepatol* 2019; **31**: 1220-1227 [PMID: 31498281 DOI: 10.1097/MEG.0000000000001535]

6   **Pigny P**, Van Seuningen I, Desseyn JL, Nollet S, Porchet N, Laine A, Aubert JP. Identification of a 42-kDa nuclear factor (NF1-MUC5B) from HT-29 MTX cells that binds to the 3' region of human mucin gene MUC5B. *Biochem Biophys Res Commun* 1996; **220**: 186-191 [PMID: 8602841 DOI: 10.1006/bbrc.1996.0378]

7   **Liu Y**, Yu X, Zhao J, Zhang H, Zhai Q, Chen W. The role of MUC2 mucin in intestinal homeostasis and the impact of dietary components on MUC2 expression. *Int J Biol Macromol* 2020; **164**: 884-891 [PMID: 32707285 DOI: 10.1016/j.ijbiomac.2020.07.191]

8   **Kasprzak A**, Siodła E, Andrzejewska M, Szmeja J, Seraszek-Jaros A, Cofta S, Szaflarski W. Differential expression of mucin 1 and mucin 2 in colorectal cancer. *World J Gastroenterol* 2018; **24**: 4164-4177 [PMID: 30271081 DOI: 10.3748/wjg.v24.i36.4164]

9   **Luo C**, Cen S, Ding G, Wu W. Mucinous colorectal adenocarcinoma: clinical pathology and treatment options. *Cancer Commun (Lond)* 2019; **39**: 13 [PMID: 30922401 DOI: 10.1186/s40880-019-0361-0]

10   **Pyo JS**, Ko YS, Kang G, Kim DH, Kim WH, Lee BL, Sohn JH. Bile acid induces MUC2 expression and inhibits tumor invasion in gastric carcinomas. *J Cancer Res Clin Oncol* 2015; **141**: 1181-1188 [PMID: 25475007 DOI: 10.1007/s00432-014-1890-1]

11   **Hou Y**, Hou L, Liang Y, Zhang Q, Hong X, Wang Y, Huang X, Zhong T, Pang W, Xu C, Zhu L, Li L, Fang J, Meng X. The p53-inducible CLDN7 regulates colorectal tumorigenesis and has prognostic significance. *Neoplasia* 2020; **22**: 590-603 [PMID: 32992138 DOI: 10.1016/j.neo.2020.09.001]

12   **Liu J**, Wei XL, Huang WH, Chen CF, Bai JW, Zhang GJ. Cytoplasmic Skp2 expression is associated with p-Akt1 and predicts poor prognosis in human breast carcinomas. *PLoS One* 2012; **7**: e52675 [PMID: 23300741 DOI: 10.1371/journal.pone.0052675]

13   **Liu C**, Zha Z, Zhou C, Chen Y, Xia W, Wang YN, Lee HH, Yin Y, Yan M, Chang CW, Chan LC, Qiu Y, Li H, Li CW, Hsu JM, Hsu JL, Wang SC, Ren N, Hung MC. Ribonuclease 7-driven activation of ROS1 is a potential therapeutic target in hepatocellular carcinoma. *J Hepatol* 2021; **74**: 907-918 [PMID: 33031845 DOI: 10.1016/j.jhep.2020.09.030]

14   **Wang H**, He C, Liu Y, Zhao H, Long L, Gai X. Soluble dietary fiber protects intestinal mucosal barrier by improving intestinal flora in a murine model of sepsis. *Biomed Pharmacother* 2020; **129**: 110343 [PMID: 32593968 DOI: 10.1016/j.biopha.2020.110343]

15   **Honzawa Y**, Nakase H, Matsuura M, Chiba T. Clinical significance of serum diamine oxidase activity in inflammatory bowel disease: Importance of evaluation of small intestinal permeability. *Inflamm Bowel Dis* 2011; **17**: E23-E25 [PMID: 21225906 DOI: 10.1002/ibd.21588]

16   **Cobo ER**, Kissoon-Singh V, Moreau F, Holani R, Chadee K. MUC2 Mucin and Butyrate Contribute to the Synthesis of the Antimicrobial Peptide Cathelicidin in Response to Entamoeba histolytica- and Dextran Sodium Sulfate-Induced Colitis. *Infect Immun* 2017; **85** [PMID: 28069814 DOI: 10.1128/IAI.00905-16]

17   **Javitt G**, Khmelnitsky L, Albert L, Bigman LS, Elad N, Morgenstern D, Ilani T, Levy Y, Diskin R,

Fass D. Assembly Mechanism of Mucin and von Willebrand Factor Polymers. *Cell* 2020; **183**: 717-729.e16 [PMID: 33031746 DOI: 10.1016/j.cell.2020.09.021]

18   **Gan GL**, Liu J, Chen WJ, Ye QQ, Xu Y, Wu HT, Li W. The Diverse Roles of the Mucin Gene Cluster Located on Chromosome 11p15.5 in Colorectal Cancer. *Front Cell Dev Biol* 2020; **8**: 514 [PMID: 32695780 DOI: 10.3389/fcell.2020.00514]

19   **Cobo ER**, Kissoon-Singh V, Moreau F, Chadee K. Colonic MUC2 mucin regulates the expression and antimicrobial activity of β-defensin 2. *Mucosal Immunol* 2015; **8**: 1360-1372 [PMID: 25921338 DOI: 10.1038/mi.2015.27]

20   **Shen P**, Yang S, Sun H, Li G, Wu B, Ji F, Sun T, Zhou D. SCF/c-KIT Signaling Increased Mucin2 Production by Maintaining Atoh1 Expression in Mucinous Colorectal Adenocarcinoma. *Int J Mol Sci* 2018; **19** [PMID: 29786668 DOI: 10.3390/ijms19051541]

21   **Li C**, Zuo D, Liu T, Yin L, Li C, Wang L. Prognostic and Clinicopathological Significance of MUC Family Members in Colorectal Cancer: A Systematic Review and Meta-Analysis. *Gastroenterol Res Pract* 2019; **2019**: 2391670 [PMID: 31933627 DOI: 10.1155/2019/2391670]

22   **Elzagheid A**, Emaetig F, Buhmeida A, Laato M, El-Faitori O, Syrjänen K, Collan Y, Pyrhönen S. Loss of MUC2 expression predicts disease recurrence and poor outcome in colorectal carcinoma. *Tumour Biol* 2013; **34**: 621-628 [PMID: 23179399 DOI: 10.1007/s13277-012-0588-8]

23   **Guo YY**, Liu ML, He XD, Jiang CQ, Liu RL. Functional changes of intestinal mucosal barrier in surgically critical patients. *World J Emerg Med* 2010; **1**: 205-208 [PMID: 25214969]