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Research Article

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High expression of FABP4 in colorectal cancer and its clinical significance

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Abstract: Objective: To investigate the relationship between the fatty acid-binding protein 4 (FABP4) and colorectal cancer (CRC). Methods: Using an enzyme-linked immunosorbent assay (ELISA), we measured the expression of FABP4 in plasma of 50 patients who underwent surgery for CRC from October 2017 to May 2018 and 50 healthy controls. The content of the visceral fat area (VFA) as seen with abdominal computed tomography (CT) scanning was measured by ImageJ software. The expression levels of FABP4, E-cadherin, and Snail proteins in CRC and adjacent tissues were determined by immunohistochemistry. Results: The mean concentration of plasma FABP4 of CRC patients was higher than that of the control group (22.46 vs. 9.82 ng/mL; $P<0.05$). The concentration of plasma FABP4 was related to the tumor, node, metastatis (TNM) stage and lymph node metastasis and was independent of age, body mass index (BMI), tumor size and location, and the degree of differentiation of CRC. The concentration of plasma FABP4 was positively correlated with high VFA and lipoprotein-a (LPA) ($P<0.05$); but it was not correlated with total cholesterol (TG), total triglyceride (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), or apolipoprotein AI (Apo-AI). The expression of FABP4 protein in CRC tissues was positively correlated with the degree of CRC differentiation, tumor stage, and lymph node metastasis. The level of FABP4 protein was negatively correlated with E-cadherin protein ($r=-0.3292$, $P=0.0196$) and positively correlated with Snail protein ($r=0.5856$, $P<0.0001$). Conclusions: High LPA and VFA were risk factors for increased plasma FABP4 in CRC patients. FABP4 protein was highly expressed in CRC tissues and associated with TNM stage, differentiation, and lymph node metastasis of CRC. The level of FABP4 in CRC tissue was correlated with E-cadherin and Snail expression, suggesting that FABP4 may promote CRC progression related to epithelial-mesenchymal transition (EMT).

Key words: Fatty acid-binding protein 4 (FABP4); Colorectal cancer (CRC); Epithelial-mesenchymal transition

1 Introduction

Colorectal cancer (CRC) is the second most common cancer in women and the third most common cancer in men. Every year, there are 746 000 new cases of CRC in males and 614 000 in females worldwide (Furuhashi et al., 2014), representing a serious health threat. Due to its concealed onset and unclear pathogenesis, in the early stage of CRC, there are often no obvious symptoms. Most symptoms occur in the middle and late stages, and thus the prognosis is poor. At present, serum carcinoembryonic antigen (CEA) is used for the screening and follow-up of CRC in clinic, but its sensitivity and specificity are low. Therefore, using CEA to diagnose CRC does not produce satisfactory outcomes (Lee et al., 2012).

Fatty acid-binding protein 4 (FABP4), a carrier protein for fatty acids, is widely expressed in adipocytes, macrophages, dendritic cells, and microvascular endothelial cells. It participates in lipid transport, metabolism, and intracellular signal transduction. It is believed that FABP4 levels may be an effective circulating biomarker for some metabolic diseases such as obesity, metabolic syndrome, and type 2 diabetes. Recently, more and more studies have shifted the focus to the connection between FABP4 and tumors. A study on

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non-small-cell lung cancer found that the expression of FABP4 messenger RNA (mRNA) and protein in lung cancer tissues was significantly increased, and FABP4 was also associated with tumor stage and 5-year survival rate (Tang et al., 2016). However, FABP4 protein is lowly expressed in liver cancer and negatively correlated with tumor stage (Zhong et al., 2018). Recently, some researchers have studied FABP4 in CRC. Wang (2017) found different plasma FABP4 levels between a colorectal adenoma group and normal control group, but no significant difference between a colorectal carcinoma group and normal control group. Nieman et al. (2011) found that the overexpression of FABP4 in CRC tissues could promote tumor growth through activating the fatty acid oxidation pathway.

Epithelial-mesenchymal transition (EMT) is an important mechanism for initiating the invasion and metastasis of malignant tumors (Wan et al., 2020). Expressed in epithelial cells, E-cadherin can form tight junction barriers between cells and maintain epithelial cell polarity, stability, and integrity. When normal epithelial mucosal cells are damaged, the expression of E-cadherin decreases or even disappears. Snail protein is the earliest and most important transcription inhibitor found in the EMT pathway. Snail can down-regulate E-cadherin transcriptional activity, inhibit its expression, and promote distant metastasis of tumors by binding with E-box elements in the E-cadherin promoter region (Zheng et al., 2015). It can also weaken the tight junction of epithelial cells, making them lose polarity, enhancing their mobility, and then encouraging tumor cells to penetrate the mucosal barrier and to acquire distant invasive ability and metastasis. Jin et al. (2018) found that FABP4 could induce EMT through the protein kinase B (AKT)/glycogen synthase kinase-3β (GSK-3β)/Snail signaling pathway, and then promote metastasis. In this study, we investigated the expression of FABP4 in CRC patients and explored the possible effect of FABP4 on the occurrence of EMT in CRC.

2 Materials and methods

2.1 Collection and storage of plasma and tissue samples

The plasma and tissue specimens of 50 CRC patients who underwent surgery and 50 healthy volunteers were collected from Wuxi People's Hospital affiliated to Nanjing Medical University (Wuxi, China) between October 2017 and May 2018.

All blood samples were collected in ethylenediaminetetraacetic acid (EDTA) anticoagulation vacuum blood collection tubes (Improve Medical, Guangzhou, CHA) and stored at 4 ℃. All samples were centrifuged within 2 h after collection (4 ℃, 1000*g*, 15 min). Then the supernatant was placed in a dry Eppendorf (EP) tube and stored at −80 ℃. All specimens were fresh CRC and paracancerous tissues (normal mucosal tissues with a margin of more than 5 cm from the edge of the tumor). The specimens were placed in the prepared EP tubes treated with 0.1% diethyl pyrocarbonate (DEPC) water and stored in a liquid nitrogen tank in a low temperature refrigerator at −80 ℃.

All patients' surgical specimens were diagnosed by pathologists and no radiotherapy or chemotherapy had been conducted prior to the time of sampling. Exclusion criteria were: (1) inflammatory bowel disease or familial colon adenoma; (2) connective tissue diseases; (3) other malignant tumors; (4) acute phase of inflammatory diseases, especially sepsis.

2.2 Enzyme-linked immunosorbent assay (ELISA)

All reagents, working standards, and samples were placed at room temperature for at least 30 min. First, 100.0 μL of assay diluent was added to each well; then 50.0 μL of standard was added per standard well; next 2.5 μL of sample and 47.5 μL of calibrator diluent were added per sample well. The wells were covered with adhesive strips and incubated for 2 h at room temperature. Each well was filled with washing water buffer for washing, and the liquid was completely removed at each step. After the last wash, any remaining wash buffer was removed by aspirating or decanting. Human FABP4 (200 μL) was conjugated to each well and the wells were then covered with a new adhesive strip for 2 h at room temperature. Then, they were washed as before. Substrate solution (200 μL) was added to each well and the samples were incubated for 30 min at room temperature and kept from light. Stop solution was then added to each well. The optical density of each well was determined within 30 min using a microplate reader set to 450 nm.

2.3 Visceral fat measurement

ImageJ software (ImageJ64, SAAinc. lnk) was used to measure the subcutaneous fat area (SFA) and

visceral fat area (VFA) in umbilical computed tomography (CT) images of patients.

2.4 Immunofluorescence staining

The staining step was performed according to the streptavidin perosidase (SP) kit instructions (CW2035, CoWin Biosciences (CWBIO)). The primary antibody was FABP4 (1:100), in addition to E-cadherin (1:40 000) and Snail (1:1000) antibodies (Cell Signaling Technology, USA). The slides were deparaffinized and rehydrated using routine methods, and then boiled in citrate buffer (pH=6.0) to retrieve antigenicity. The endogenous peroxidase blocking solution was incubated at room temperature for 10 min and then washed. After incubation with goat serum for 1 h at room temperature, the slides were incubated with primary antibody FGF9 (1:100) overnight at 4 °C and then with the secondary antibody at room temperature for 1 h. Then horseradish peroxidase (HRP)-labeled streptavidin was added and the slides were incubated for 10 min at room temperature. Diaminobenzidine (DAB) chromogenic reagent was used for staining. Two senior pathologists calculated the expression of FABP4, E-cadherin, and Snail using the proportion score and intensity score. The proportion score of positive cells (*B*) was set as follows: 0, <5% positive cells; 1, 5%–<25% positive cells; 2, 25%–<50% positive cells; 3, 50%–<75% positive cells; and 4, ≥75% positive cells. The staining intensity score (*A*) was set as follows: 0, negative; 1, weak expression; 2, moderate expression; and 3, strong expression. The total score was calculated as $A \times B$. A total score of 1 was regarded as no expression. A total score ranging between 1 and 4 was regarded as a low level of expression; a total score ranging between 5 and 8 was classified as a medium level of expression; a total score ranging between 9 and 12 was classified as a high level of expression.

2.5 Statistical analysis

All results were represented as the median± interquartile range for non-normally distributed data using SPSS software (IBM SPSS statistics, Version 21.0). The Mann-Whitney *U* test was used to determine the statistical significance of continuous data. Chi-square test, a contingency table, or the Fisher exact test was used to determine the statistical significance of categorical data. Multiple stepwise regression analysis was used to test the association between FABP4 and the other factors. Correlations were detected using the Spearman's test. A *P*-value of <0.05 was considered to be statistically significant in all analyses.

3 Results

3.1 Concentration of plasma FABP4 in CRC

We collected blood samples from 50 CRC patients and 50 healthy volunteers for the plasma FABP4 test. Plasma FABP4 concentration in the CRC group was significantly higher than that in the normal control group ((20.19±15.87) ng/mL vs. (8.69±5.26) ng/mL, $P<0.0001$; Fig. 1).

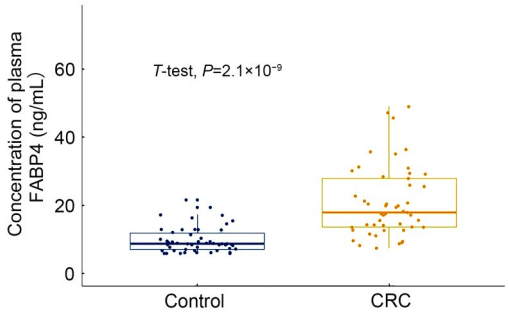

Fig. 1 FABP4 plasma concentration in colorectal cancer (CRC) patients and normal control. Scatter plot describes the mean and standard error of the mean (*n*=50). A *P*-value of <0.05 is considered to be statistically significant.

3.2 Correlation of plasma FABP4 levels and CRC

A total of 33 males and 17 females with CRC were recruited in our study for testing. The plasma FABP4 concentration in female CRC patients was higher than that in male CRC patients ((25.92±12.44) ng/mL vs. (14.11±21.34) ng/mL, $P<0.05$). The plasma FABP4 concentration in the non-lymph node metastasis group was higher than that in the lymph node metastasis group ((25.53±16.27) ng/mL vs. (15.62±8.33) ng/mL, $P<0.05$). The plasma FABP4 concentration in the tumor, node, metastatis (TNM) stage I+II group was higher than that in the III+IV group ((25.53±16.27) ng/mL vs. (15.62±8.33) ng/mL, $P<0.05$). There was no significant correlation between plasma FABP4 concentration and age, tumor location or size, or CRC differentiation ($P>0.05$; Table 1).

Table 1 Correlations between plasma FABP4 level and characteristics of patients with colorectal cancer (CRC)

Variable	*n*	FABP4 (ng/mL)#	*P* value
Gender			
Male	33	14.11±21.34	0.0007
Female	17	25.92±12.44	
Age			
⩽60 years	11	14.56±16.53	0.4697
>60 years	39	20.36±14.89	
Differentiation			
Low	15	17.52±12.85	0.5629
Medium	28	30.93±20.58	
High	7	17.89±15.26	
Lymphatic metastasis			
Yes	25	15.62±8.33	0.0045
No	25	25.53±16.27	
TNM stage			
I+II	25	25.53±16.27	0.0045
III+IV	25	15.62±8.33	

P values for survival were determined with the Mann-Whitney *U* test. # Data were expressed as median±interquartile range.

3.3 Correllation of FABP4 plasma concentration with VFA and lipid metabolism in CRC patients

The concentration of plasma FABP4 in VFA of >100 cm^2 was higher than that in VFA of ⩽100 cm^2 ((26.71±15.91) ng/mL vs. (15.62±9.36) ng/mL, $P<0.05$). The concentration of plasma FABP4 in lipoprotein-a (LPA) of >300 mg/L was higher than that in LPA of ⩽300 mg/L ((25.92±26.79) ng/mL vs. (20.39±15.50) ng/mL, $P<0.05$). There was no significant difference in plasma FABP4 among different groups based on body mass index (BMI), total cholesterol (TG), total triglyceride (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), or apolipoprotein AI (Apo-AI). However, monofactor analysis found that plasma FABP4 was associated with high LPA and high VFA (Table 2; Fig. 2). We established a multivariate linear regression using FABP4 as a dependent variable, and TC, LPA, BMI, and VFA as independent variables. Stepwise analysis showed that the regression equation was y=0.138VFA+0.045LPA−7.297, suggesting that VFA and LPA were independent correlative factors of serum FABP4 (Table 3).

Table 2 Correlations between FABP4 plasma concentration and lipid metabolism, VFA, and BMI in CRC patients from mono-factor analysis

Variable	*r*	*P* value
TG	0.157	0.277
TC	0.231	0.106
LDL	0.150	0.297
HDL	0.122	0.397
LPA	0.465	0.001
Apo-AI	0.130	0.368
BMI	0.270	0.058
VFA	0.544	<0.001

P values for survival were determined with the Spearman's test. Apo-AI: apolipoprotein AI; BMI: body mass index; CRC: colorectal cancer; HDL: high-density lipoprotein; LDL: low-density lipoprotein; LPA: lipoprotein-a; TG: triglyceride; TC: total cholesterol; VFA: visceral fat area.

Table 3 Correlations between FABP4 plasma concentration and lipid metabolism, VFA, and BMI in CRC patients from multivariate linear regression

Parameter	*B*	95% CI	*t*	Sig
Constant	−7.297	(−16.929, 2.334)	−1.524	0.134
LPA	0.045	(0.024, 0.066)	4.369	<0.001
VFA	0.138	(0.084, 0.192)	5.161	<0.001

BMI: body mass index; CI: confidence interval; CRC: colorectal cancer; LPA: lipoprotein-a; VFA: visceral fat area; Sig: signficance.

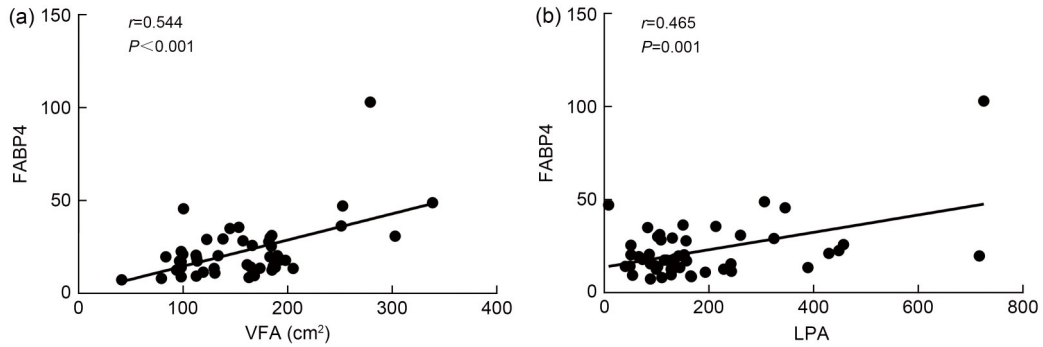

Fig. 2 Positive corrections between plasma FABP4 and VFA (a) and between plasma FABP4 and LPA (b). LPA: lipoprotein-a; VFA: visceral fat area.

3.4 Positive correlation of FABP4 protein expression and CRC

FABP4 protein was mainly expressed in the cytoplasm and nucleus of CRC epithelial cells (Fig. 3). The expression rate of FABP4 protein was 82% (41/50) in CRC tissues and 18% (9/50) in normal adjacent tissues (Table 4). The expression of FABP4 protein in CRC was significantly higher than that in normal adjacent tissues (χ^2=40.960, P<0.001).

The positive expression of FABP4 protein was higher in low-differentiated tissues than in high-differentiated tissues (χ^2=6.730, P=0.035). The positive expression of FABP4 protein in TNM stage III+IV was higher than that in stage I+II in CRC (χ^2=4.878, P=0.027). We also found that FABP4 expression with lymph node metastasis was higher than that without lymph node metastasis of CRC (χ^2=4.878, P=0.027). Thus, the expression of FABP4 protein was correlated with tumor differentiation, lymph node metastasis, and TNM stage, but not correlated with age, gender, tumor location, or tumor size (P=0.644, 0.263, 0.591, and 0.287, respectively; Table 5).

3.5 Expression of E-cadherin and Snail protein in CRC

E-cadherin protein was mainly expressed on the cell membrane (Fig. 4). As shown in Table 4, the expression rate in the adjacent normal tissues was 96% (48/50), significantly higher than the rate in CRC tissues (42%, 21/50; χ^2=34.081, P<0.001). Snail protein was mainly expressed in the nucleus of cancer tissues (Fig. 5). As shown in Table 4, the expression rate of

Table 4 Expression of FABP4, E-cadherin, and Snail proteins

Variable	n	FABP4		E-cadherin		Snail	
		−	+	−	+	−	+
CRC	50	9	41	29	21	10	40
Normal	50	41	9	2	48	34	16
χ^2		40.960		34.081		23.377	
P value		<0.001		<0.001		<0.001	

CRC: colorectal cancer.

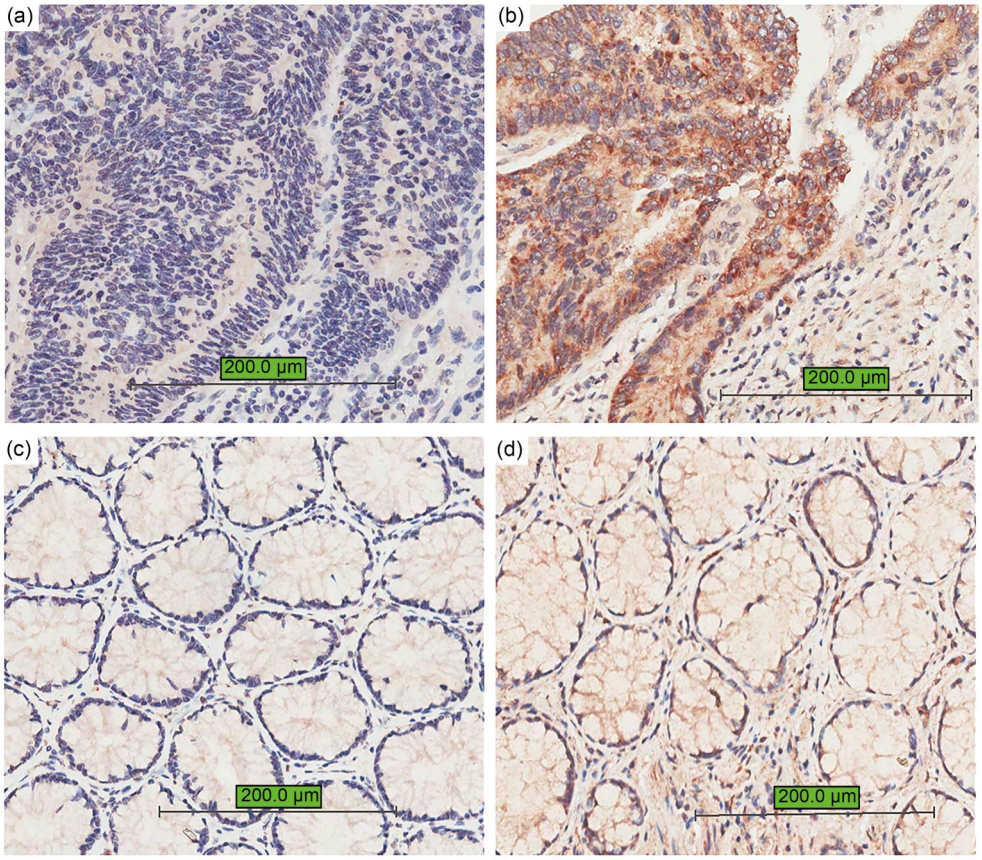

Fig. 3 Immunohistochemical analyses of FABP4 expression. (a) Negative expression in colorectal cancer (CRC); (b) Positive expression in CRC; (c) Negative expression in normal tissues; (d) Positive expression in normal tissues.

Table 5 Relationship between FABP4 protein and pathology of CRC

Variable	*n*	FABP4					χ^2	*P* value
		−	+	++	+++	Positive rate (%)		
Total	50	9	21	15	5	82.0		
Age								
⩽60 years	11	3	2	4	2	72.7	0.214	0.644
>60 years	39	6	19	11	3	84.6		
Gender								
Male	33	4	15	10	4	65.9	1.252	0.263
Female	17	5	6	5	1	70.6		
Location								
Colon	29	4	12	9	4	86.2	0.288	0.591
Rectum	21	5	9	6	1	76.2		
Size								
>3 cm	8	3	2	2	1	62.5	1.133	0.287
⩽3 cm	42	6	19	13	4	85.7		
Differentiation								
Low	15	2	1	8	4	86.7	6.730	0.035
Medium	28	3	18	6	1	89.3		
High	7	4	2	1	0	42.9		
Lymph node metastasis								
Yes	25	1	11	11	2	96.0	4.878	0.027
No	25	8	10	4	3	68.0		
TNM stage								
I+II	25	8	10	4	3	68.0	4.878	0.027
III+IV	25	1	11	11	2	96.0		

CRC: colorectal cancer; TNM: tumor, node, metastatis.

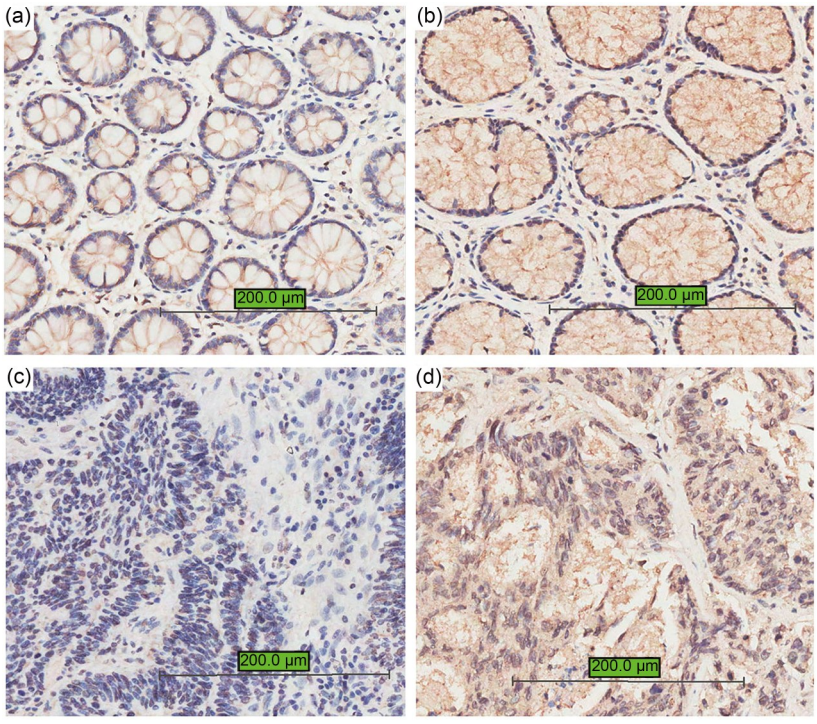

Fig. 4 Immunohistochemical analyses of E-cadherin expression. (a) Negative expression in normal tissues; (b) Positive expression in normal tissues; (c) Negative expression in colorectal cancer (CRC); (d) Positive expression in CRC.

Snail in CRC was 80% (40/50), also significantly higher than that in the adjacent normal tissues (32%, 16/50; χ^2=23.377, P<0.001).

3.6 Correlation of FABP4 with Snail and E-cadherin proteins in CRC

In the E-cadherin-positive staining group, seven samples showed negativity for FABP4. Two samples had both FABP4 and E-cadherin-negative staining. In the Snail-negative group, positivity for FABP4 was observed in four samples, and negativity for FABP4 was seen in six samples. Further Spearman's correlation analysis revealed that FABP4 had a positive correlation with Snail and a negative correlation with E-cadherin, with correlation coefficient (r) values of 0.5856 and −0.3292, respectively (Figs. 6a and 6b). E-cadherin had a negative correlation with Snail (r=−0.3371, P=0.0167; Fig. 6c).

4 Discussion

FABP4 is a lipid chaperone protein that is mainly expressed in adipocytes, macrophages, dendritic cells, and microvascular endothelial cells. It can specifically bind to free fatty acids and transport fatty acids, inhibit fat decomposition, and thereby affect lipid transport, cholesterol balance, and TC formation. Our study

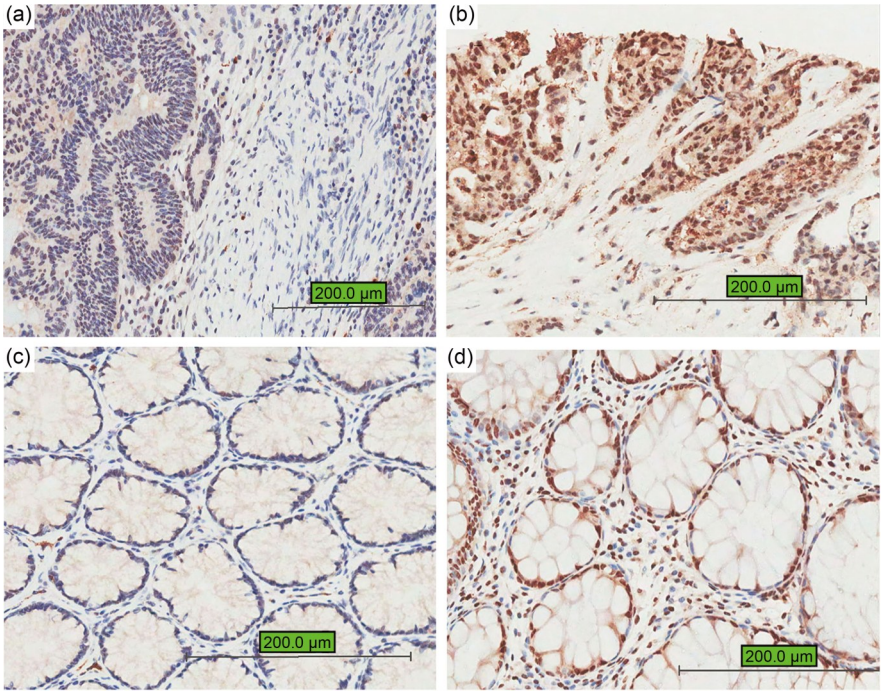

Fig. 5 Immunohistochemical analyses of Snail expression. (a) Negative expression in colorectal cancer (CRC); (b) Positive expression in CRC; (c) Negative expression in normal tissues; (d) Positive expression in normal tissues.

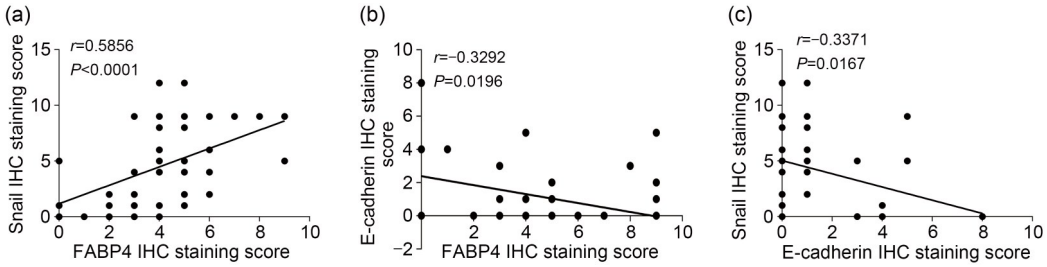

Fig. 6 Graphical representation of the positive correction of FABP4 with Snail (a), negative correction of FABP4 with E-cadherin (b), and negative correction of E-cadherin with Snail (c) in CRC. CRC: colorectal cancer; IHC: immunohistochemistry.

showed that the concentration of plasma FABP4 in CRC patients was significantly higher than that in healthy controls (P<0.05). This is consistent with the results of Zhang et al. (2019), who found that serum FABP4 concentrations in CRC patients were higher than those of the control group and decreased significantly two weeks after surgery. In our study, the expression of plasma FABP4 in TNM stage I+II was higher than that in stage III+IV; the concentration of plasma FABP4 in the non-lymph node metastasis group was higher than that in the lymph node metastasis group. We speculate that these results could be attributed to more participation of plasma FABP4 in lipid transport and metabolism in CRC stage III+IV, and more FABP4 being consumed and utilized by tumor tissues, resulting in low plasma FABP4 concentration in circulating blood.

FABP4 is widely expressed throughout the body, but mainly in adipocytes (Furuhashi et al., 2014). Studies have confirmed that FABP4 positively regulates pre-adipocyte differentiation and stimulates its maturation (Gan et al., 2015), which in turn affects lipid transport and the cholesterol level. Our study showed different plasma FABP4 concentrations between VFA and LPA groups. Spearman's correlation analysis revealed that plasma FABP4 in CRC patients was positively correlated with LPA and VFA (P=0.001 and P<0.001), but not correlated with TG, TC, LDL, HDL, or Apo-AI (P>0.05). A multivariate linear stepwise regression model showed that VFA and LPA are both significant influencing factors of plasma FABP4. The relationships of plasma FABP4 concentration with VFA and LPA in CRC are rarely reported and the mechanism remains unclear. The possible explanation could be as follows: since visceral fat mainly distributes in mesentery and omentum, it has more active metabolism. It secretes various proteins such as adipokines (adiponectin, endotrophin, leptin, and fatty acid-binding protein), inflammatory cytokines (interleukin-6 (IL-6), IL-8, tumor necrosis factor (TNF)), angiogenic factors, and extracellular matrix components, which promote the proliferation and spread of tumor cells through vascularization mediated by vascular endothelial growth factor (Liesenfeld et al., 2015). A previous study showed that the accumulation of visceral fat is a strong determinant of insulin resistance and hyperinsulinemia (Nam et al., 2010), which induces hyperglycemia, increases insulin-like growth factors, stimulates the proliferation of intestinal epithelial cells, and then promotes the occurrence and development of CRC. LPA is a special lipoprotein composed of a hydrophobic core rich in sterol lipids, TG, phospholipids, and cholesterol. LPA has been confirmed as an independent risk factor for coronary heart disease and proven to be associated with cardiovascular disease (Kooijman et al., 2010). However, few studies have reported the association between LPA and CRC. LPA could reverse the pro-apoptotic behavior of 5-fluorouracil-induced apoptosis in colon cancer cell line HT29. This anti-apoptotic effect is mainly achieved by affecting the related proteins in the mitochondrial apoptosis pathway, inhibiting the expression of proapoptotic protein Bax and up-regulating the anti-apoptotic protein Bcl-2 (Gao, 2013).

It has been reported that FABP4 protein is highly expressed in cholangiocarcinoma (Nie et al., 2017), breast cancer (Guaita-Esteruelas et al., 2017), and ovarian cancer (Nieman et al., 2011), but lowly expressed in hepatocellular carcinoma (Zhong et al., 2018). In our study, the expression of FABP4 protein in CRC tissues was significantly higher than that in adjacent normal tissues. FABP4 protein overexpression in CRC tissues was significantly correlated with lymph node metastasis, tumor differentiation, and TNM stage (P<0.05), suggesting that FABP4 might promote CRC infiltration and metastasis. There were no significant differences in FABP4 protein expression based on participants' age or gender, or on the size and location of tumors. However, Zhang et al. (2019) found that tumors with high or low FABP4 expression have no significant correlation with distant organ and lymph node metastasis or TNM classification. FABP4 is a potential therapeutic target for metastatic prostate cancer (Uehara et al., 2014) and bladder cancer (Mathis et al., 2018). Tang et al. (2016) reported that high FABP3 or FABP4 protein expression as well as high concurrent FABP3 and FABP4 protein expression in non-small-cell lung cancer was all significantly associated with higher TMN stage.

The five-year prognostic survival rate of early CRC is close to 90% (Miller et al., 2019), while the five-year survival rate of late CRC is less than 10% (Moriarity et al., 2016). Nearly 90% of CRC patients died of metastasis (Li et al., 2017). EMT is considered

to be an important mechanism for initiating invasion and metastasis of malignant tumors (Wan et al., 2020). Our study confirmed that the positive expression of E-cadherin protein in normal tissues was higher than that in CRC tissues. The positive expression of Snail protein in CRC tissues was higher than that in normal tissues adjacent to cancer. Our Spearman's correlation analysis showed that the expression of FABP4 protein was negatively correlated with E-cadherin protein (r=−0.3292, P=0.0196) and positively correlated with Snail protein in CRC (r=0.5856, P<0.0001). E-cadherin protein was also negatively correlated with Snail protein level (r=−0.3371, P=0.0167). The results suggest that FABP4 promotes malignant characteristics and metastasis of CRC highly likely to be related to EMT. Jin et al. (2018) found that the level of FABP4 protein was significantly increased in cervical carcinoma tissues and that FABP4 could activate Snail protein expression through the AKT/GSK-3β pathway in human cervical cancer cells.

In summary, plasma FABP4 concentrations were higher in CRC patients than in the control group and positively correlated with VFA and lipid LPA. The expression of FABP4 protein in CRC tissues was increased and correlated with tumor differentiation, lymphatic metastasis, and TNM stage. FABP4 protein was positively correlated with Snail protein and negatively correlated with E-cadherin protein. We hypothesize that FABP4 may be involved in tumor invasion and metastasis of CRC, and at least in part, related to the EMT pathway. In the future, we will continue to expand the clinical sample size to verify our clinical results and the potential mechanism. We believe that the pathway between FABP4 and CRC should be studied more deeply.

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Author contributions

Min XIA, Yan ZHANG, and Wenjia ZHANG conceived and designed the experiments. Yan ZHANG wrote and edited the manuscript. Yan ZHANG, Zhujun XIE, Wenying TIAN, and Tianyue ZHU performed the experiments. Yan ZHANG, Wenjia ZHANG, and Zhujun XIE analyzed the data. Min XIA, Fangmei AN, and Qiang ZHAN revised the manuscript. All authors have read and approved the fnal manuscript and, therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Yan ZHANG, Wenjia ZHANG, Min XIA, Zhujun XIE, Fangmei AN, Qiang ZHAN, Wenying TIAN, and Tianyue ZHU declare that they have no conflict of interest.

The present study was approved by the Ethics Committee of Wuxi People's Hospital Affiliated to Nanjing Medical University (Wuxi, China) and with the Helsinki Declaration of 1975, as revised in 2008 (5). All the patients and volunteers provided written informed consent for participation in this study. Additional informed consent was obtained from all patients for which identifying information is included in this article.

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