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ORIGINAL ARTICLE

Retrospective Study

Correlation between the expressions of metastasis-associated factor-1 in colon cancer and vacuolar ATP synthase

Miao He, Zuo-Feng Cao, Li Huang, Wen-Juan Zhong, Xue-Ming Xu, Xiao-Li Zeng, Jing Wang

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Abstract

BACKGROUND

Clinical prognosis often worsens due to high recurrence rates following radical surgery for colon cancer. The examination of high-risk recurrence factors post-surgery provides critical insights for disease evaluation and treatment planning.

AIM

To explore the relationship between metastasis-associated factor-1 in colon cancer (MACC1) and vacuolar ATP synthase (V-ATPase) expression in colon cancer tissues, and recurrence rate in patients undergoing radical colon cancer surgery.

METHODS

We selected 104 patients treated with radical colon cancer surgery at our hospital from January 2018 to June 2021. Immunohistochemical staining was utilized to assess the expression levels of MACC1 and V-ATPase in these patients.

RESULTS

The rates of MACC1 and V-ATPase positivity were 64.42% and 67.31%, respectively, in colon cancer tissues, which were significantly higher than in paracancerous tissues ($P < 0.05$). Among patients with TNM stage III, medium to low differentiation, and lymph node metastasis, the positive rates of MACC1 and V-ATPase were significantly elevated in comparison to patients with TNM stage I-II, high differentiation, and no lymph node metastasis ($P < 0.05$). The rate of MACC1 positivity was 76.67% in patients with tumor diameters > 5 cm, notably higher than in patients with tumor diameters ≤ 5 cm ($P < 0.05$). We observed a positive correlation between MACC1 and V-ATPase expression ($r_s = 0.797$, $P < 0.05$). The

positive rates of MACC1 and V-ATPase were significantly higher in patients with recurrence compared to those without ($P < 0.05$). Logistic regression analysis revealed TNM stage, lymph node metastasis, MACC1 expression, and V-ATPase expression as risk factors for postoperative colon cancer recurrence (OR = 6.322, 3.435, 2.683, and 2.421; $P < 0.05$).

CONCLUSION

The upregulated expression of MACC1 and V-ATPase in colon cancer patients appears to correlate with clinicopathological features and post-radical surgery recurrence.

Key Words: Metastasis-associated factor-1 in colon cancer; Vacuolar ATP synthase; Colon cancer; Radical surgery; Recurrence

Core Tip: The abnormal expression of colon cancer metastasis-related factor-1 and vacuolar ATP synthase in colon cancer tissues is related to the clinicopathological characteristics of patients, and is related to the recurrence of colon cancer after radical resection.

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INTRODUCTION

Clinical prognosis often worsens due to high recurrence rates following radical surgery for colon cancer[1,2]. The examination of high-risk recurrence factors post-surgery provides critical insights for disease evaluation and treatment planning[3]. Metastasis-associated factor-1 in colon cancer (MACC1), a metastasis regulation-related factor, fosters epithelial cell invasiveness by increasing stromal infiltration depth, potentially causing metastasis and malignant tumor recurrence[4]. Similarly, the enzyme-binding ATP protease regulator, vacuolar ATP synthase (V-ATPase), regulates ATPase. This action enhances ATPase's binding activity to the tumor cell membrane, thereby optimizing tumor cell energy metabolism and exacerbating abnormal proliferation and division[5]. This study, involved 104 colon cancer patients treated at our hospital, and aimed to comprehensively explore risk factors for recurrence following radical colon cancer surgery. This study focused on the expression of MACC1 and V-ATPase and analyzed the relationship between their expression and the recurrence rate of colon cancer.

MATERIALS AND METHODS

General information

A total of 104 patients with colon cancer treated in our hospital from January 2015 to February 2017 were selected, including 56 males and 48 females. Their age ranged from 40 to 71 years old, with a median age of 54.50 years old.

The inclusion criteria were as follows: (1) Patients confirmed to have colon cancer through pathology from tissue samples; (2) Patients who underwent radical colon cancer surgery at our hospital; and (3) Patients who completed clinical follow-up treatment

The exclusion criteria included: (1) Patients receiving preoperative antitumor treatments such as radiotherapy; (2) Patients with other malignant tumors; and (3) Patients with other severe conditions such as autoimmune and metabolic diseases.

The staging of colon cancer refers to the standards in the TNM staging system for colorectal cancer (7th edition) by the American Joint Committee on Cancer/Union for International Cancer Control. Stage I is T1-2N0M0, Stage II is T3-4bN0M0, Stage III is T1-4bN1-2bM0, and Stage IV is T(any)N(any)M1a-1b[6,7].

Experimental methods

Paraffin sections were prepared, dehydrated, and subsequently incubated with 3% H_2O_2 for 20 min at room temperature. The goat serum was washed with phosphate buffer for 3 times, 5 min each time, and the goat serum diluted with phosphate buffer was blocked for 5 min. After pouring off the serum without washing, 5 mL of primary antibody (sourced from Thermo Fisher China, concentration:1:1000) was added. The mixture was incubated at 37 ℃ for 2 h or refrigerated at 4 ℃ for overnight incubation. It was then washed three times with phosphate buffer, each wash lasting 5 min. Next, 3 mL of biotin fluorescence-labeled secondary antibody (sourced from Thermo Fisher China, concentration:1:2000) was added, followed by a 20-30 min incubation at 37 ℃. After three 5-min phosphate buffer washes,

Table 1 Comparison of metastasis-associated factor-1 and vacuolar ATP synthase expression in colon cancer and paracancerous tissues

Group	Cases	MACC1 positive expression (%)	V-ATPase positive expression (%)
Colon cancer	104	67 (64.42)	70 (67.31)
Paracancerous tissue	104	20 (19.23)	8 (7.69)
χ^2		43.647	78.851
P value		0.000	0.000

MACC1: Metastasis-associated factor-1; V-ATPase: Vacuolar ATP synthase.

Streptavidin/HRP horseradish-labeled streptavidin was added. After incubation at 37 ℃ for another 20-30 min and three more phosphate buffer washes of 5 min each, the enhanced HRP-DAB substrate chromogenic kit (PA110) was used for development. This was followed by rinsing with tap water, restraining, and sealing[8].

Statistical processing

This study adopted SPSS 22.0 software to conduct statistical analysis, used χ^2 test to compare the counting data, and applied logistic regression analysis to implement multivariate analysis. Inspection level a = 0.05.

RESULTS

Comparison of MACC1 and V-ATPase expression in colon cancer and paracancerous tissues

The positive expression rates of MACC1 and V-ATPase in colon cancer tissues were significantly higher than those in paracancerous tissues ($P < 0.05$). See Table 1 for more details.

The Relationship between the expression of MACC1, V-ATPase and clinicopathological features of colon cancer

The positive expression rates of MACC1 and V-ATPase in patients with TNM stage III, medium and low differentiation, and lymph node metastasis were significantly higher than those in patients with stage I-II, high differentiation and no lymph node metastasis ($P < 0.05$); MACC1 positive expression rates of patients with tumor diameter > 5 cm were significantly higher than those of patients with tumor diameter ≤ 5 cm ($P < 0.05$). See Table 2 for more details.

Correlation analysis

The expressions of MACC1 and V-ATPase in colon cancer tissues were positively correlated ($r_s = 0.797$, $P < 0.05$). See Table 3 for more details.

Comparison of the expressions of MACC1 and V-ATPase in colon cancer tissues between patients with postoperative recurrence and patients without postoperative recurrence

As of September 2019, a total of 72 patients had recurrence, and 32 patients had no recurrence; the positive expression rates of MACC1 and V-ATPase in colon cancer tissues of patients with recurrence were significantly higher than those of patients without recurrence ($P < 0.05$), as shown in Table 4.

Multivariate analysis

The study used clinicopathological features of the patients and the expressions of MACC1 and V-ATPase as independent variables, and used the recurrence as the dependent variable for Logistic regression analysis. The analysis results showed that TNM staging, lymph node metastasis, MACC1 expression and V-ATPase expression were risk factors for postoperative recurrence (OR = 6.322, 3.435, 2.683 and 2.421, $P < 0.05$). See Table 5 for more details.

DISCUSSION

The recurrence of colon cancer post-radical surgery is intricately linked to factors such as the excised tumor lesion's completeness, the biological activity of tumor cells, and the self-proliferation traits of residual tumor cells[9,10]. For patients with poorly differentiated tumor cells or in advanced clinical stages, the risk of recurrence may progressively rise post-surgery, correspondingly increasing the mortality rate[11,12]. Currently, reliable indicators to assess the risk of post-surgical recurrence in colon cancer are scarce. While postoperative clinicopathological staging or immunohistochemical indicators can offer some degree of predictability, their reliability remains insufficient. Imaging techniques can aid in predicting recurrence; however, most patients are usually in the intermediate to advanced disease stages when recurrence is clinically diagnosed, limiting the assessment's early recurrence value[13,14].

Table 2 The relationship between the expression of metastasis-associated factor-1, vacuolar ATP synthase and clinicopathological features of colon cancer

Clinicopathologcal features	Cases	MACC1 positive expression (%)	χ^2	*P* value	V-ATPase positive expression (%)	χ^2	*P* value
Age (years)							
≤ 55 years old	54	33 (61.11)	0.538	0.463	35 (64.81)	0.317	0.573
> 55 years old	50	34 (68.00)			35 (70.00)		
Gender							
Male	56	35 (62.50)	0.196	0.658	40 (71.43)	0.936	0.333
Female	48	32 (66.67)			30 (62.50)		
Tumor site							
Left colon	50	32 (64.00)	0.008	0.931	36 (72.00)	0.964	0.326
Right colon	54	35 (64.81)			34 (62.96)		
TNM staging							
Phase I-II	65	34 (52.31)	11.101	0.001	35 (53.85)	14.275	0.000
Phase III	39	33 (84.62)			35 (89.74)		
Degree of differentiation							
High differentiation	31	12 (38.71)	12.74	0.000	11 (35.48)	20.327	0.000
Medium and low differentiation	73	55 (75.34)			59 (80.82)		
Lymph node metastasis							
Yes	49	40 (81.63)	11.973	0.001	42 (85.71)	14.266	0.000
No	55	27 (49.09)			28 (50.91)		
Vascular infiltration							
Yes	41	24 (58.54)	1.023	0.312	26 (63.41)	0.466	0.496
No	63	43 (68.25)			44 (69.84)		
Nervous system infiltration							
Yes	32	22 (68.75)	0.378	0.539	21 (65.63)	0.059	0.807
No	72	45 (62.50)			49 (68.06)		
Tumor diameter							
> 5 cm	60	46 (76.67)	9.276	0.002	40 (66.67)	0.026	0.871
≤ 5 cm	44	21 (47.73)			30 (68.18)		

MACC1: Metastasis-associated factor-1; V-ATPase: Vacuolar ATP synthase.

Our study analyzed MACC1 and V-ATPase-two factors integral to tumor cell gene regulation and energy metabolism-providing a dependable recurrence risk prediction model for clinical use. We chose to examine MACC1 and V-ATPase expression due to their influence on the, regulation of colon cancer cell proliferation.

MACC1, a metastasis regulation-related factor, contains serine and sulfhydryl protein structures. These can impact the activity of tumor cell membrane-bound proteins *via* phosphorylation. MACC1's activation on G protein-coupled receptors in tumor cells can heighten the abnormal transcriptional activation of nuclear DNA in colon cancer cells. As an ATPase protein-binding factor, MACC1's; effect on adenosine triphosphate can boost ATP synthesis in tumor cells, the synthetic division of tumor cell spindles, and tumor cell proliferation[15]. Certain researchers have analyzed MACC1 expression in patients with colon cancer proposing that an elevated MACC1 positive expression rate may increase the risk of colon cancer[16,17]. On the other hand, studies on V-ATPase are sparse, with, most resorting to univariate analysis. To better understand these variables' relationships, we conducted a correlation study.

We discovered that the positive expression rates of MACC1 and V-ATPase proteins in colon cancer lesions significantly exceeded those in paracancerous tissues. This suggests that higher expression of these two proteins might impact the onset or progression of colon cancer. Such high expression is primarily driven by the activation of the transcriptional regulatory signaling pathway in colon cancer cells. This influences the synthesis rates of adenosine triphosphate and

Table 3 Correlation analysis

MACC1 expression	V-ATPase expression		r_s	*P* value
	Positive	Negative		
Positive	63	4	0.797	0.000
Negative	7	30		

MACC1: Metastasis-associated factor-1; V-ATPase: Vacuolar ATP synthase.

Table 4 Comparison of the expression of metastasis-associated factor-1 and vacuolar ATP synthase in colon cancer tissues between patients with postoperative recurrence and patients without postoperative recurrence

Group	Cases	MACC1 positive expression (%)	V-ATPase positive expression (%)
Recurrence	72	52 (72.22)	57 (79.17)
No recurrence	32	15 (46.88)	13 (40.63)

MACC1: Metastasis-associated factor-1; V-ATPase: Vacuolar ATP synthase.

Table 5 Results of logistic regression analysis

Factor	β	SE	Walds	*P* value	OR (95%CI)
TNM staging	1.844	0.411	20.130	0.000	6.322 (2.825-14.148)
Lymph node metastasis	1.234	0.315	15.346	0.000	3.435 (1.853-6.369)
MACC1 expression	0.987	0.264	13.977	0.000	2.683 (1.599-4.502)
V-ATPase expression	0.884	0.221	16.000	0.000	2.421 (1.570-3.733)

MACC1: Metastasis-associated factor-1; V-ATPase: Vacuolar ATP synthase.

guanosine triphosphate, enhances ATP supply, and ultimately impacts the tumor cells' metastasis and adhesion capabilities.

In patients with TNM stage III, medium and low differentiation, and lymph node metastasis, MACC1 and V-ATPase expression rates were significantly higher compared to patients with stage I-II, high differentiation, and no lymph node metastasis. This indicates that the expression of these two factors can markedly influence the prognosis of clinicopathological processes in patients with colon cancer. High expression of MACC1 primarily impacts clinical staging, lymph node metastasis, or tumor cell differentiation because it can affect the epithelial-mesenchymal transition process, intensify tumor cell infiltration and metastasis, and ultimately advance TNM staging. V-ATPase's influence on related pathological characteristics chiefly stems from its capacity to affect tumor cells' energy metabolism rate, leading to the compromised release of tumor cell differentiation and maturation-inducing factors, thereby promoting medium and low differentiation of tumor cells[18].

Further studies have also demonstrated that in colon cancer patients, the MACC1 expression level significantly rises with clinical staging progression. This increase is notably pronounced for patients in the advanced or terminal stages of colon cancer[19,20]. Our correlation analysis revealed a positive correlation between MACC1 and V-ATPase expression in colon cancer tissues, suggesting a collaborative role of MACC1 and V-ATPase in colon cancer progression. In patients who experienced recurrence, the positive expression rates of MACC1 and V-ATPase proteins markedly increased and surpassed those in non-recurrence patients. This statistically significant difference implies that high MACC1 and V-ATPase protein expression can influence colon cancer recurrence. However, the specific underlying mechanism remains unclear, but it could involve MACC1 and V-ATPase impacting the activity of residual tumor cells, leading to an enhanced self-proliferation capacity and ultimately promoting colon cancer recurrence. Risk factor analysis further identified TNM stage, lymph node metastasis, MACC1 expression, and V-ATPase expression as risk factors for postoperative recurrence, underscoring the influence of MACC1 and V-ATPase on colon cancer recurrence.

Colon cancer's development is governed by numerous cytokines. Clinical studies have largely focused on single-factor regulation, the functionality of which can be swayed by various environmental relationships. A multifactor correlation analysis could offer greater value for clinical diagnosis. In this study, we jointly examined MACC1 and V-ATPase's clinical value in this disease, using the patients' clinicopathological characteristics and MACC1 and V-ATPase expression as independent variables, and recurrence as the dependent variable for logistic regression analysis. The results suggested these two indicators might pose as risk factors for postoperative recurrence in colon cancer patients. High MACC1

expression could foster the metastasis of various tumor cells, although the specific mechanism of action remains unelucidated. MACC1 protein could not only augment tumor metastasis by regulating Met transcription but also modulate cell metastasis by activating the Akt/β-catenin signaling pathway or promoting the secretion of matrix metalloproteinases. Overexpression of V-ATPase in tumor cells plays a crucial role in maintaining the cytoplasm's alkaline environment, stimulating tumor cell growth, enhancing the extracellular acidic environment, promoting cell invasive growth and metastasis, and inducing the invasive phenotype of tumor cells. Thus, our study can serve as a reference for clinical prediction of the postoperative recurrence in colon cancer patients. However, there were some limitations of this study. The patients were selected from one single center, and the sample size was limited. The results of this study need to be confirmed by further studies.

CONCLUSION

In summary, the elevated expression of MACC1 and V-ATPase in colon cancer patients is associated with the clinicopathological features and post-radical surgery recurrence of colon cancer, and warrants further investigation.

ARTICLE HIGHLIGHTS

Research background

Clinical prognosis often worsens due to high recurrence rates following radical surgery for colon cancer. The examination of high-risk recurrence factors post-surgery provides critical insights for disease evaluation and treatment planning.

Research motivation

The factors influencing the recurrence of colon cancer after surgery remains unclear.

Research objectives

To explore the relationship between metastasis-associated factor-1 in colon cancer (MACC1) and vacuolar ATP synthase (V-ATPase) expression in colon cancer tissues, and recurrence rate in patients undergoing radical colon cancer surgery.

Research methods

We selected 104 patients treated with radical colon cancer surgery at our hospital from January 2018 to June 2021. Immunohistochemical staining was utilized to assess the expression levels of MACC1 and V-ATPase in these patients.

Research results

The positive rates of MACC1 and V-ATPase were significantly higher in patients with recurrence compared to those without. Logistic regression analysis revealed TNM stage, lymph node metastasis, MACC1 expression, and V-ATPase expression as risk factors for postoperative colon cancer recurrence.

Research conclusions

The upregulated expression of MACC1 and V-ATPase in colon cancer patients appears to correlate with clinicopathological features and post-radical surgery recurrence.

Research perspectives

This study can serve as a reference for clinical prediction of the postoperative recurrence in colon cancer patients.

FOOTNOTES

Author contributions: He M, Cao ZF, Huang L, Zhong WJ, and Wu XM designed the research study; Zeng XL, Wang J, He M and Cao ZF performed the research; Cao ZF, Huang L, Zhong WJ and Xu XM analyzed the data and wrote the manuscript; All authors have read and approve the final manuscript.

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