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Serum Levels of TRIM72 Are Lower among Patients with Colon Cancer: Identification of a Potential Diagnostic Marker

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Colon cancer is one of the most common malignancies causing the majority of cancer-related deaths worldwide. The tripartite motif family protein 72 (TRIM72), also known as mitsugumin 53, acts as an E3 ubiquitin ligase. TRIM72 is involved in insulin resistance and metabolic syndrome, which are risk factors of colon cancer. However, the correlation between TRIM72 and colon cancer remains unknown. In the present study, we explored the expression profile of TRIM72 in colon cancer tissues and the diagnostic value of serum TRIM72 in colon cancer. The receiver operating characteristic (ROC) curves were applied for evaluating the diagnostic value of serum TRIM72. We thus found that immunoreactive TRIM72 levels were significantly lower in colon cancer tissues than those in normal colon tissues. Moreover, serum TRIM72 levels were significantly lower in colon cancer patients than those in healthy volunteers. Importantly, the lower serum TRIM72 levels were associated with advanced clinical stage, lymph node, and distant metastases in colon cancer patients. The ROC curve analysis showed that serum TRIM72 has a superior diagnostic value (the area under the curve (AUC) = 0.829) than the traditional tumor biomarkers, carcinoembryonic antigen (CEA) (AUC = 0.707) and carbohydrate antigen 19-9 (CA199) (AUC = 0.750), and the combination of TRIM72 with CEA and CA199 showed the best diagnostic value for colon cancer (AUC = 0.928). In conclusion, serum TRIM72 may be a potential biomarker for the diagnosis and the prognosis of colon cancer.

Keywords: biomarker; colon cancer; diagnosis; receiver operating characteristic; tripartite motif family protein 72
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Introduction

Colon cancer is one of the major malignancies among humans, accounting for 10% of all new cancer cases worldwide (Torre et al. 2015). In the past 2 decades, the prevalence of colon cancer has increased at an alarming rate; it is now the 5th most common cancer and the 6th deadliest cancer in China (Chen et al. 2016a). Most cases of colon cancer have a better prognosis if they are diagnosed at an early stage, and the overall 5-year survival rate can reach 80-90% (Wang et al. 2015). However, there are still no effective diagnostic methods for colon cancer because a flexible colonoscopy is not available in routine physical examinations, and the faecal occult blood test has a low specificity for colon cancer. Serological tumor biomarkers, carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA199), are useful for detecting colon cancer, but they have a low specificity or sensibility (Zhao et al. 2015; Zhong et al. 2015). Thus, there is an urgent need to develop valuable diagnostic biomarkers for the rapid detection of colon cancer.

The tripartite motif family protein 72 (TRIM72), also known as mitsugumin 53 (MG53), is one of the tripartite motif (TRIM) family members. TRIM72 consists of 477 amino acid residues with the typical tripartite motif, including a RING finger, a zinc binding moiety (B-box), and a coiled-coil structure (RBCC) at its N-terminus, as well as a SPRY domain at its C-terminus (Ozato et al. 2008; Park et al. 2009). Originally, TRIM72 was viewed as an essential component of the cell membrane repair machinery (Cai et al. 2009), and was involved in cardiac ischemic preconditioning and post-conditioning by activating the PI3K-AKT

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and ERK signaling pathways (Ham and Mahoney 2013). Because the RING finger domain is a characteristic signature of the E3 ubiquitin ligases, which mediates protein ubiquitination, recent studies have suggested that TRIM72 could act as an E3 ligase to target the insulin receptor (IR) and insulin receptor substrate-1 (IRS-1) and promote their ubiquitin-dependent degradation, leading to insulin resistance and metabolic syndrome (Song et al. 2013; Yi et al. 2013). It is widely accepted that the occurrence of colon cancer is closely correlated with type 2 diabetes mellitus (T2DM), metabolic syndrome, and insulin resistance (Zaafar et al. 2014; Shah and Patel 2016; de Kort et al. 2017). Additionally, recent research has shown that E3 ligase-mediated ubiquitylation plays a vital role in the development of colon cancer (Liu et al. 2017). These findings prompted us to hypothesize that TRIM72 may be associated with the progression of colon cancer.

In this study, we aimed to investigate the expression patterns and diagnostic value of TRIM72 in colon cancer patients. We found that both TRIM72 expression levels in cancer tissues and in the serum of colon cancer patients were significantly lower compared with the normal controls. More important, the lower serum TRIM72 levels were associated with a more advanced clinical stage, lymph node and distant metastases in colon cancer patients. Moreover, serum TRIM72 was more effective in diagnosis of colon cancer than traditional tumor biomarkers CEA and CA199. These results collectively suggested that serum TRIM72 may be a new biomarker for the diagnosis of colon cancer.

Materials and Methods

Patients and specimens

Colon cancer tissues and matched normal tissues were obtained from 60 patients diagnosed with colon cancer at Nanfang Hospital, Southern Medical University in Guangzhou, China. All of the samples were previously processed following routine formalin fixation and paraffin embedding protocols and then stored at –20°C before use. To measure serum TRIM72 levels in colon cancer patients and healthy controls, we obtained 60 samples of serum from the colon cancer patients and 40 samples of serum from healthy volunteers at Nanfang Hospital, from August 2016 to December 2016. All of the colon cancer patients were diagnosed by histological examination, and all of the healthy volunteers were recruited without any health problems during health checkups at Nanfang Hospital. All of the serum samples were stored at –80°C before further analysis. The relevant clinical data of all of the patients are available in Table 1. Ethics approval was granted by the Ethics Committee of Nanfang Hospital in Guangzhou, China, and all of the methods used were in accordance with the approved guidelines. Written informed consent was required from all patients and healthy volunteers to enroll in the study.

Immunohistochemistry

We examined expression levels of TRIM72 in the colon tissue through immunohistochemical staining as described previously (Chen et al. 2016b). Briefly, paraffin-embedded 5-μm sections from the colon tissue was deparaffinized in xylene and rehydrated through an alcohol gradient. Then the slides were immersed in distilled water containing 3% hydrogen peroxide for 10 minutes to quench endogenous peroxidase activity. Antigen retrieval was performed by microwave treatment in a 0.01 M citrate buffer (pH = 6.0) for 30 minutes. The sections were blocked using a 5% bovine serum albumin for 40 minutes to prevent unspecific reactions. Subsequently, the sections were incubated with a primary antibody against TRIM72 (1:100 dilution; Abcam, Cambridge, USA) at 4°C overnight, followed by a secondary antibody (1:500 dilution) at room temperature for 1 hour. Finally, the sections were stained using a DAB substrate kit (Boster, China) and counter-stained by hematoxylin.

Five pictures of each section were randomly captured. The expression of TRIM72 in each section was assessed under a light microscope (Nikon, Japan), using 400 × magnification, by two experienced pathologists who were blinded to the other pathologist's assessment. The intensity was assessed using a modified quickscore method on a scale of 0 (no staining), 1 (weak staining, light yellow), 2 (moderate staining, yellowish brown), and 3 (strong staining, brown). The extent of the TRIM72 staining was defined as the percentage of positive TRIM72 immunostained cells among the total stromal cells and was scored on a scale of 0 (0-1%), 1 (1-10%), 2 (10-50%), and 3 (50-100%) according to the previous study (Henry et al. 2007). An overall protein expression score (overall score range 0-9) was calculated by multiplying the intensity with the positivity scores. The average score of the 5 random pictures evaluated by the 2 pathologists was taken as the final staining score.

Measurement of serum TRIM72, CEA, and CA199 levels

Serum samples were prepared by centrifugation according to standard protocols and aliquoted and stored at –80°C until assayed. The levels of TRIM72 were quantitatively determined using commercial enzyme-linked immunosorbent assays (ELISA) kits (Xinle, Shanghai, China) according to the manufacturer's instructions. The detection range for TRIM72 is 0 pg/mL to 1,600 pg/mL. Serum levels of cancer markers CEA and CA199 were measured using electrochemiluminescence immunoassay (ECLIA) kits (Siemens, Berlin, Germany) on a Roche Cobas e601 fully automatic electrochemistry luminescence immunity analyzer (Roche, Basel, Switzerland). Each test included a standard control (coefficient of variation, CV < 5%).

Statistical analysis

All of the statistical analysis was performed using the SPSS 20.0 software (SPSS Inc., Chicago, IL, USA) and GraphPad software 6.0 (GraphPad Software, Inc., La Jolla, CA, USA). Data were expressed as the mean ± standard deviation (SD). The comparisons between the two groups were analyzed by a student's t-test. To analyze the relationship between the serum TRIM72 levels and the clinicopathological characteristics, a chi-square test was employed for categorical variables except where a small sample size (< 5) required the use of Fisher's exact test. Receiver operating characteristic (ROC) curves were used to evaluate the diagnostic value of serum TRIM72 for colon cancer and determine the cutoff values. Additionally, $P < 0.05$ was considered statistically significant.

Results

Expression of TRIM72 in colon cancer tissues

Alterations in gene copy number and mRNA expres-

Table 1. Clinicopathologic characteristcs of the colon cancer patients and their correlation with serum TRIM72 expression.

Variables	n (%)	TRIM72 (pg/mL)		P value
		≥ 47.32 (n = 30)	< 47.32 (n = 30)	
Sex				0.390[b]
Male	43	23 (38.3%)	20 (33.3%)	
Female	17	7 (11.7%)	10 (16.7%)	
Age, y				0.417[b]
≥ 50	39	18 (30.0%)	21 (35.0%)	
< 50	21	12 (20.0%)	9 (15.0%)	
T Stage (tumor size and invasiveness)				0.095[a]
T1 + T2 + T3	11	8 (13.3%)	3 (5.0%)	
T4	49	22 (36.7%)	27 (45.0%)	
N Stage (lymph node metastasis)				0.002[b,**]
N0	23	17 (28.3%)	6 (10.0%)	
N1	14	8 (13.3%)	6 (10.0%)	
N2 + N3	23	5 (8.3%)	18 (30.0%)	
M Stage (distant metastasis)				0.003[b,**]
M0	23	17 (28.3%)	6 (10.0%)	
M1	37	13 (21.7%)	24 (40.0%)	
Tumor Stage				0.001[a,***]
I + II	16	14 (23.3%)	2 (3.3%)	
III + IV	44	16 (26.7%)	28 (46.7%)	
Dukes' Stage				0.006[a,**]
A + B	20	15 (25.0%)	5 (8.3%)	
C + D	40	15 (25.0%)	25 (41.7%)	
Degree of differentiation				0.687[a]
Poor	6	2 (3.3%)	4 (6.7%)	
Moderate	48	25 (41.7%)	23 (38.3%)	
High	6	3 (5.0%)	3 (5.0%)	
CEA (μg/L)				0.002[b,**]
≥ 5.0	34	11 (18.3%)	23 (38.3%)	
< 5.0	26	19 (31.7%)	7 (11.7%)	
CA199 (U/mL)				0.436[b]
≥ 37.0	27	12 (20.0%)	15 (25.0%)	
< 37.0	33	18 (30.0%)	15 (25.0%)	

[a]P values were determined using Fisher's exact test for categorical variables.
[b]P values were determined using Chi-square test for categorical variables.
$P < 0.05$ was considered statistically significant.
**$P < 0.01$, ***$P \leq 0.001$.

sion play an important role in the tumorigenesis of various cancers. Thus, we analyzed alterations in the TRIM72 gene using the Cancer Genome Atlas (TCGA) gene alteration database, available from cBioPortal. We found that 6% of the 195 colon cancer patients had an amplified gene copy number or up-regulated mRNA of TRIM72 (Fig. 1A). To further explore TRIM72 expression in colon cancer, we examined 60 pairs of human colon cancer tissues and matched normal tissues by immunohistochemical staining. As shown in Fig. 1B, C, the stain intensity of TRIM72 was greater in the extracellular matrix and the stromal cells of normal colon tissues, while was significantly lower in those of colon cancer samples. Moreover, statistical analysis demonstrated that the average score of TRIM72 expression value in human colon cancer tissues (0.71 ± 0.25) was significantly lower than that in normal colon tissues (2.05 ± 0.36) (Fig. 1D).

Serum levels of TRIM72 among colon cancer patients

Given the above results and that TRIM72 can be detected in human serum (Lemckert et al. 2016), we measured the serum expression of TRIM72 in colon cancer

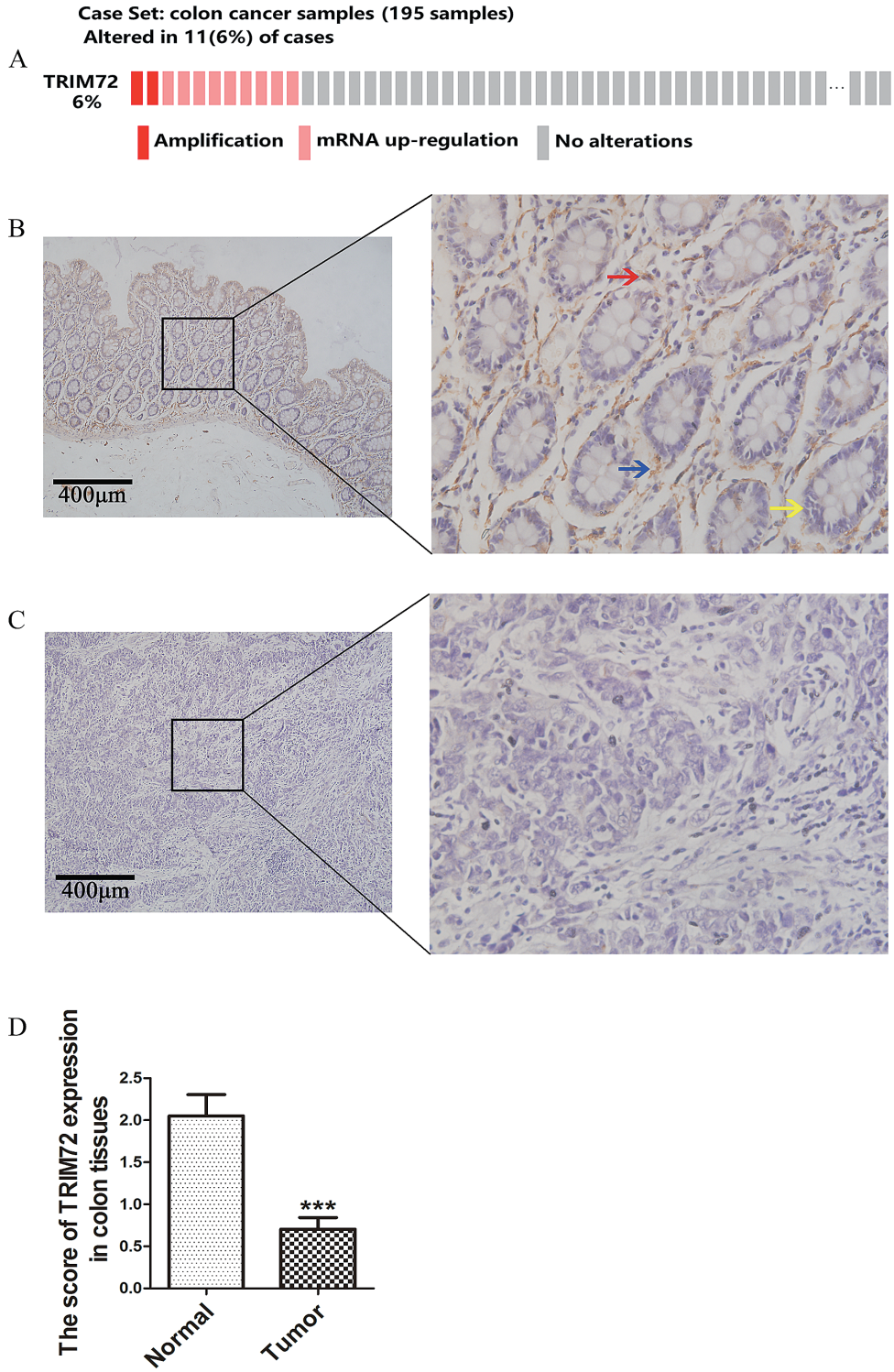

Fig. 1. TRIM72 expression in the colon cancer and colon normal tissues.
(A) TRIM72 gene alteration was analyzed using the Cancer Genome Atlas (TCGA) gene alteration database. Each column represents an individual patient, and different colors distinguish the types of alteration in the TRIM72 gene (red: gene amplification; pink: mRNA up-regulation; grey: no alteration). (B) Representative images of the TRIM72 immunohistochemical staining for normal colon tissues. The red arrow indicates the TRIM72-positive stromal cell; the blue arrow indicates the TRIM72-positive extracellular matrix; and the yellow arrow indicates the TRIM72-negative normal colon cell. The staining intensity score is 2 and the positive cell percentage score is 2; thus, the final score is 4 (calculated by multiplying the staining intensity score by the positive cell percentage score). (C) Representative images of the TRIM72 immunohistochemical staining for colon cancer tissues. The staining intensity score is 0 and the positive cell percentage score is 0, so the final score is 0. (D) Comparison of the immunohistochemical scores of TRIM72 expression in colon cancer tissues (n = 60) and normal tissues (n = 60).
***P < 0.001 compared with the control group.

patients and healthy controls using a commercially available ELISA kit. We found that serum levels of TRIM72 were significantly lower among colon cancer patients (60.57 ± 53.8 pg/mL) compared with healthy controls (143.80 ± 78.1 pg/mL) ($P < 0.001$), which was in accordance with the aforementioned immunohistochemical results (Fig. 2). In contrast, there was no significant difference in serum TRIM72 levels ($P = 0.960$) between gastric cancer and healthy controls (data not shown), which suggests that serum TRIM72 might have a specific diagnostic value for colon cancer.

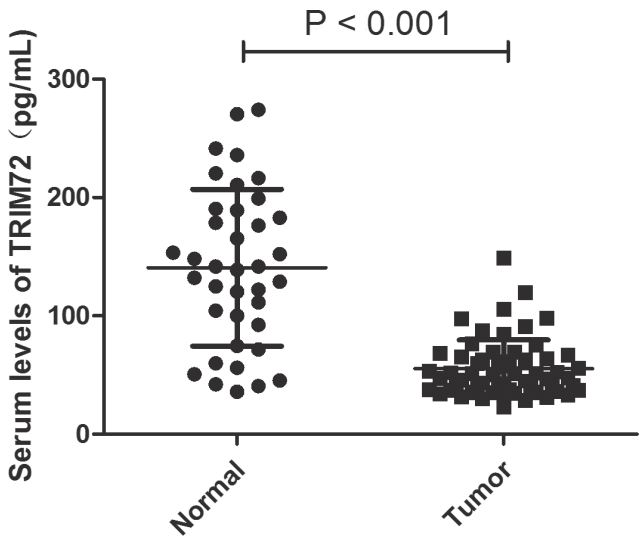

Fig. 2. Serum levels of TRIM72 in colon cancer patients.
Serum TRIM72 was measured by enzyme-linked immunosorbent assay (ELISA). The statistical differences of serum TRIM72 between colon cancer patients (n = 60) and healthy controls (n = 40) were analyzed by the student's t-test. Each symbol indicates the individual serum TRIM72 levels, and the horizontal lines indicate the mean values ± SD.
$P < 0.001$ compared with the control group.

Association between serum TRIM72 and clinicopathological characteristics

To determine whether the serum levels of TRIM72 are correlated with colon cancer progression, we analyzed the relationship between TRIM72 levels and the clinicopathological characteristics of colon cancer patients. All of the samples were classified into 2 groups with lower or higher serum levels of TRIM72 according to the median serum level of TRIM72 (47.32 pg/mL). Statistical analyses revealed that lower serum levels of TRIM72 were associated with tumor lymph node metastasis ($P = 0.002$), distant metastasis ($P = 0.003$), advanced tumor clinical stage ($P = 0.001$), advanced Dukes' stage ($P = 0.006$), and higher serum CEA levels ($P = 0.002$) (Table 1). However, no significant correlations were observed between serum TRIM72 levels and other clinicopathological features including sex, age, tumor size, degree of differentiation, and serum CA199 levels.

Diagnostic value of serum TRIM72 in colon cancer

Next, we explored whether serum TRIM72 has the diagnostic value for colon cancer by using the ROC curve analysis. It was noteworthy that the area under the curve (AUC) of TRIM72 was 0.829 (95% CI: 0.745-0.912), which was significantly higher than that of CEA (AUC = 0.707) and CA199 (AUC = 0.750). Besides, TRIM72 showed a high sensitivity (81.7%) for colon cancer with a positive predictive value (PPV) of 85.5% based on the optimal cutoff value (90.024 pg/mL), according to the coordinates of the ROC curve analysis (Fig. 3A, Table 2). Furthermore, the ROC curve analyses for combined biomarkers were compared. We found that the combined AUC of CEA and CA199 was 0.808, which was even lower than the AUC of TRIM72 alone (AUC = 0.829) (Tables 2 and 3).

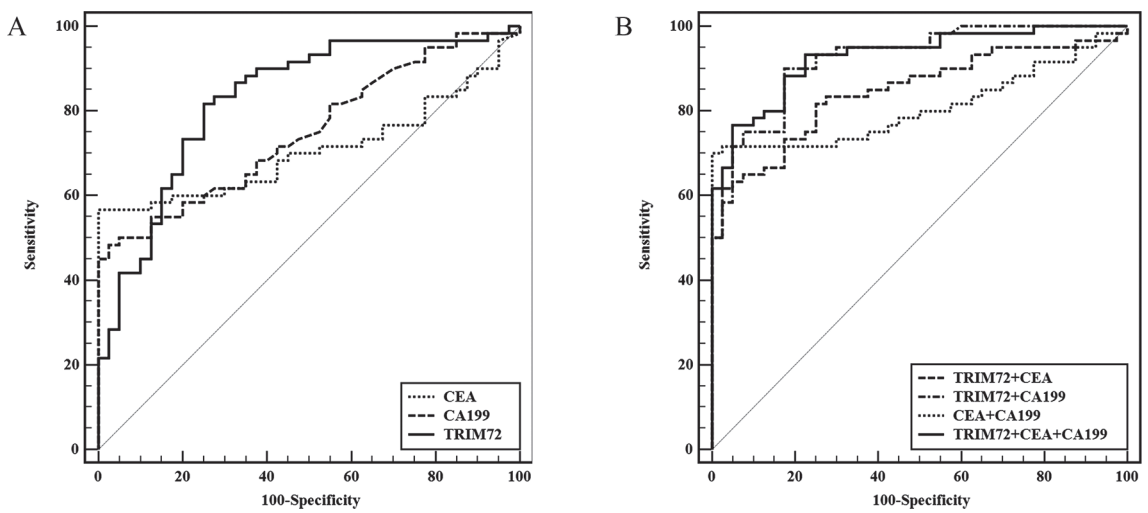

Fig. 3. The ROC curve analyses of TRIM72, CEA, and CA199.
(A) ROC curve analyses of serum TRIM72, CEA, or CA199. (B) ROC curve analyses for the combination of TRIM72, CEA, and CA199.

Table 2. Diagnostic values of serum TRIM72, CEA, or CA199.

	AUC	Cutoff Value	Sensibility (%)	Specificity (%)	PPV (%)	NPV (%)	95% CI
TRIM72	0.829	90.024 pg/mL	81.7	75.0	85.5	69.4	0.745-0.912
CEA	0.707	5.015 μg/L	56.7	100.0	100.0	56.2	0.605-0.810
CA199	0.750	32.466 U/mL	40.0	100.0	100.0	48.1	0.657-0.843

AUC, the area under the curve; PPV, positive predictive value; NPV, negative predictive value; 95% CI, 95% confidence interval.

Table 3. Diagnostic values of combined detection of serum TRIM72, CEA, and CA199.

	AUC	Sensitivity (%)	Specificity (%)	95% CI
CEA + CA199	0.808	71.7	82.5	0.717-0.880
TRIM72 + CA199	0.922	85.0	82.5	0.851-0.966
TRIM72 + CEA	0.846	73.3	82.5	0.760-0.911
TRIM72 + CEA + CA199	0.928	88.3	82.5	0.858-0.970

AUC, the area under the curve; 95% CI, 95% confidence interval.

Additionally, both the combined AUC of TRIM72 and CA199 (AUC = 0.922) or TRIM72 and CEA (AUC = 0.846) was significantly higher than the combined AUC of CEA and CA199 (AUC = 0.808). Moreover, the combination of TRIM72, CEA, and CA199 yielded the highest AUC value (AUC = 0.928) (Fig. 3B, Table 3). Together, the results demonstrate that serum TRIM72 might have potential value in the diagnosis of colon cancer.

Discussion

TRIM72 was previously identified as a cardiac and skeletal muscle-specific protein, which plays a key role in facilitating plasma membrane repair in cardiac and skeletal muscle cells. Multiple studies have focused on the biological characterization of TRIM72 and its function in the pathogenesis, as well as the underlying mechanisms in various diseases (Cai et al. 2009; Ham and Mahoney 2013; Song et al. 2013; Yi et al. 2013). However, little is known about the role of TRIM72 in cancer. In this study, for the first time, we determined the expression and diagnostic value of TRIM72 for colon cancer.

First, we examined the expression patterns of TRIM72 in colon cancer tissues and the serum of colon cancer patients. The immunohistochemical staining results showed that the expression levels of the immunoreactive TRIM72 were markedly lower in colon cancer tissues as compared with normal controls (Fig. 1B-D). Besides, serum TRIM72 levels in colon cancer patients were also significantly lower than in healthy controls (Fig. 2). Tumor suppressors, like Caveolin-1 and Slit2, are highly expressed in the normal stromal cells while gradually suppressed during tumor growth (Williams et al. 2003; Trimmer et al. 2011; Chang et al. 2012). Additionally, we demonstrated that serum TRIM72 levels were negatively associated with tumor stage and metastasis. Therefore, we speculate that TRIM72 might also function as a suppressor for tumor progression and the distant metastasis of colon cancer. The immunohistochemical results showed that TRIM72 was located in the extracellular matrix and stromal cells but not in the normal colon cells or colon cancer cells (Fig. 1B, C). Thus, we speculate that the TRIM72 protein in the extracellular matrix might be expressed and secreted by the stromal cells in normal colon tissue or colon cancer tissue. Many cell types, including cardiac and skeletal muscle cells and colon stromal cells, can produce TRIM72 (Weisleder et al. 2012; Liu et al. 2015); therefore, TRIM72 in serum might be derived from a different source of cells. Moreover, we identified that 6% of 195 colon cancer patients contained an amplified gene copy number or up-regulated mRNA of TRIM72, which suggests that the TRIM72 expression in colon cancer might be lower through protein-level regulation rather than through gene-level regulation. However, the exact involvement, origin, and regulation of TRIM72 in colon cancer patients are still not clear and require further study.

Biomarkers for colon cancer have been extensively explored over the past decades. CEA and CA199 are considered the most frequently used biomarkers for clinical colon cancer screenings, but have a low specificity or sensibility (Perkins et al. 2003; McKeown et al. 2014). Recently, TRIM72 was regarded as a biomarker for myocardial injury (Lemckert et al. 2016). Herein, we have demonstrated a significant difference in the serum levels of TRIM72 between colon cancer patients and healthy controls, and that

lower serum TRIM72 levels are associated with the advanced tumor stage and the metastasis of colon cancer. Thus, we wonder whether serum TRIM72 could be a biomarker for the diagnosis of colon cancer. The ROC curve analysis demonstrated that the AUC of TRIM72 (AUC = 0.829) was significantly higher than the AUC of CEA (AUC = 0.707) or CA199 (AUC = 0.750). Additionally, the combined AUC of TRIM72, CEA, and CA199 was as high as 0.928, which far surpasses the combined AUC of CEA and CA199 (AUC = 0.808). These findings suggest that TRIM72 may be a new serum biomarker for the diagnosis of colon cancer. However, an expanded number of serum samples are needed to verify the above findings. The early diagnosis and treatment of colon cancer is of great value to improve the survival rates of patients; thus, we will focus on the early diagnostic value of serum TRIM72 in the future. Interestingly, we collected 27 serum samples from gastric cancer patients and further demonstrated that there was no significant difference in serum TRIM72 levels between gastric cancer patients and healthy controls (P = 0.960), which may suggest the specificity of serum TRIM72 in diagnosing colon cancer. However, the serum levels of TRIM72 in patients with other tumor types require further investigation.

In conclusion, we have provided the first evidence that serum TRIM72 levels are significantly lower among colon cancer patients and that serum TRIM72 may be a potential biomarker for diagnosing colon cancer. However, the roles and the regulatory mechanisms involved should be further elucidated in future studies.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

References

Cai, C., Masumiya, H., Weisleder, N., Matsuda, N., Nishi, M., Hwang, M., Ko, J.K., Lin, P., Thornton, A., Zhao, X., Pan, Z., Komazaki, S., Brotto, M., Takeshima, H. & Ma, J. (2009) MG53 nucleates assembly of cell membrane repair machinery. *Nat. Cell Biol*., **11**, 56-64.

Chang, P.H., Hwang-Verslues, W.W., Chang, Y.C., Chen, C.C., Hsiao, M., Jeng, Y.M.,Chang, K.J., Lee, E.Y., Shew, J.Y. & Lee, W.H. (2012) Activation of robo1 signaling of breast cancer cells by slit2 from stromal fibroblast restrains tumorigenesis via blocking pi3k/akt/*β*-catenin pathway. *Cancer Res.*, **72**, 4652-4661.

Chen, W., Zheng, R., Baade, P.D., Zhang, S., Zeng, H., Bray, F., Jemal, A., Yu, X.Q. & He, J. (2016a) Cancer statistics in China, 2015. *CA Cancer J. Clin*., **66**, 115-132.

Chen, Z., Gao, P. & Li, Z. (2016b) Expression of G protein-coupled receptor 56 is an unfavorable prognostic factor in osteosarcoma patients. *Tohoku J. Exp. Med.*, **239**, 203-211.

de Kort, S., Masclee, A.A.M., Sanduleanu, S., Weijenberg, M.P., van Herk-Sukel, M.P.P., Oldenhof, N.J.J., van den Bergh, J.P.W., Haak, H.R. & Janssen-Heijnen, M.L. (2017) Higher risk of colorectal cancer in patients with newly diagnosed diabetes mellitus before the age of colorectal cancer screening initiation. *Sci. Rep.*, **7**, 46527.

Ham, Y.M. & Mahoney, S.J. (2013) Compensation of the AKT signaling by ERK signaling in transgenic mice hearts overexpressing TRIM72. *Exp. Cell Res*., **319**, 1451-1462.

Henry, L.R., Lee, H.O., Lee, J.S., Kleinszanto, A., Watts, P., Ross, E.A., Chen, W.T. & Cheng, J.D. (2007) Clinical implications of fibroblast activation protein in patients with colon cancer. *Clin. Cancer Res.*, **13**, 1736-1741.

Lemckert, F.A., Bournazos, A., Eckert, D.M., Kenzler, M., Hawkes, J.M., Butler, T.L., Ceely, B., North, K.N., Winlaw, D.S., Egan, J.R. & Cooper, S.T. (2016) Lack of MG53 in human heart precludes utility as a biomarker of myocardial injury or endogenous cardioprotective factor. *Cardiovasc. Res*., **110**, 178-187.

Liu, J., Zhu, H., Zheng, Y., Xu, Z., Li, L., Tan, T., Park, K.H., Hou, J., Zhang, C., Li, D., Li, R., Liu, Z., Weisleder, N., Zhu, D., Lin, P. & Ma, J. (2015) Cardioprotection of recombinant human mg53 protein in a porcine model of ischemia and reperfusion injury. *J. Mol. Cell. Cardiol.*, **80**, 10-19.

Liu, L., Wong, C.C., Gong, B. & Yu, J. (2017) Functional significance and therapeutic implication of ring-type E3 ligases in colorectal cancer. *Oncogene*, **37**, 148-159.

McKeown, E., Nelson, D.W., Johnson, E.K., Maykel, J.A., Stojadinovic, A., Nissan, A., Avital, I., Brucher, B.L. & Steele, S.R. (2014) Current approaches and challenges for monitoring treatment response in colon and rectal cancer. *J. Cancer*, **5**, 31-43.

Ozato, K., Shin, D.M., Chang, T.H. & Morse, H.C. (2008) TRIM family proteins and their emerging roles in innate immunity. *Nat. Rev. Immunol*., **8**, 849-860.

Park, E.Y., Kwon, O.B., Jeong, B.C., Yi, J.S., Lee, C.S., Ko, Y.G. & Song, H.K. (2009) Crystal structure of PRY-SPRY domain of human TRIM72. *Proteins*, **78**, 790-795.

Perkins, G.L., Slater, E.D., Sanders, G.K. & Prichard, J.G. (2003) Serum tumor markers. *Am. Fam. Physician*, **68**, 1075-1082.

Shah, N.R. & Patel, B.M. (2016) Secoisolariciresinol diglucoside rich extract of L. usitatissimum prevents diabetic colon cancer through inhibition of CDK4. *Biomed. Pharmacother*., **83**, 733-739.

Song, R., Peng, W., Zhang, Y., Lv, F., Wu, H.K., Guo, J., Cao, Y., Pi, Y., Zhang, X., Jin, L., Zhang, M., Jiang, P., Liu, F., Meng, S., Zhang, X., et al. (2013) Central role of E3 ubiquitin ligase MG53 in insulin resistance and metabolic disorders. *Nature*, **494**, 375-379.

Torre, L.A., Bray, F., Siegel, R.L., Ferlay, J., Lortet-Tieulent, J. & Jemal, A. (2015) Global cancer statistics, 2012. *CA Cancer J. Clin*., **65**, 87-108.

Trimmer, C., Sotgia, F., Whitaker-Menezes, D., Balliet, R.M., Eaton, G., Martinez-Outschoorn, U.E., Pavlides, S., Howell, A., Iozzo, R.V., Pestell, R.G., Scherer, P.E., Capozza, F. & Lisanti, M.P. (2011) Caveolin-1 and mitochondrial SOD2 (MnSOD) function as tumor suppressors in the stromal microenvironment: a new genetically tractable model for human cancer associated fibroblasts. *Cancer Bio. Ther.*, **11**, 383-394.

Wang, J., Du, Y., Liu, X., Cho, W.C. & Yang, Y. (2015) MicroRNAs as regulator of signaling networks in metastatic colon cancer. *Biomed Res. Int.*, **2015**, 823620.

Weisleder, N., Takizawa, N., Lin, P., Wang, X., Cao, C., Zhang, Y., Tan, T., Ferrante, C., Zhu, H., Chen, P.J., Yan, R., Sterling, M., Zhao, X., Hwang, M., Takeshima, M., et al. (2012) Recombinant MG53 protein modulates therapeutic cell membrane repair in treatment of muscular dystrophy. *Sci. Transl. Med.*, **4**, 139ra85.

Williams, T.M., Cheung, M.W., Park, D.S., Razani, B., Cohen, A.W., Muller, W.J., Di Vizio, D., Chopra, N.G., Pestell, R.G. & Lisanti, M.P. (2003) Loss of caveolin-1 gene expression accelerates the development of dysplastic mammary lesions in tumor-prone transgenic mice. *Mol. Biol. Cell*, **14**, 1027-1042.

Yi, J.S., Park, J.S., Ham, Y.M., Nguyen, N., Lee, N.R., Hong, J., Kim, B.W., Lee, H., Lee, C.S., Jeong, B.C., Song, H.K., Cho, H., Kim, Y.K., Lee, J.S., Park, K.S., et al. (2013) MG53-induced IRS-1 ubiquitination negatively regulates skeletal myogenesis and insulin signalling. *Nat. Commun.*, **4**, 2354.

Zaafar, D.K., Zaitone, S.A. & Moustafa, Y.M. (2014) Role of metformin in suppressing 1,2-dimethylhydrazine-induced colon cancer in diabetic and non-diabetic mice: effect on tumor angiogenesis and cell proliferation. *PloS One*, **9**, e100562.

Zhao, S., Mei, Y., Wang, Y., Zhu, J., Zheng, G. & Ma, R. (2015) Levels of CEA, CA153, CA199, CA724 and AFP in nipple discharge of breast cancer patients. *Int. J. Clin. Exp. Med*., **8**, 20837-20844.

Zhong, W., Yu, Z., Zhan, J., Yu, T., Lin, Y., Xia, Z.S., Yuan, Y.H. & Chen, Q.K. (2015) Association of serum levels of CEA, CA199, CA125, CYFRA21-1 and CA72-4 and disease characteristics in colorectal cancer. *Pathol. Oncol. Res*., **21**, 83-95.