Int J Clin Exp Med 2019;12(6):7717-7724
www.ijcem.com /ISSN:1940-5901/IJCEM0091700

Original Article

Tissue inhibitor matrix metalloproteinase 1 polymorphisms associated with colorectal cancer prognosis in a Han Chinese cohort

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Received January 23, 2019; Accepted March 11, 2019; Epub June 15, 2019; Published June 30, 2019

Abstract: Purpose: Tissue inhibitor of metalloproteinase-1 (TIMP-1) is an inhibitor of matrix metalloproteinases, known to play a crucial role in tumorigenesis. It has significant predictive value for patients with colorectal cancer (CRC). However, its precise clinical applicability remains unknown. The current study investigated association between TIMP-1 gene polymorphisms and survival in CRC patients. Method: This study enrolled 118 endometrial cancer patients that underwent surgery for CRC between January 2011 and July 2015. Patients were genotyped for TIMP-1 polymorphism (rs4898, 372 T/C). In addition, TIMP-1 levels were assessed using enzyme-linked immunosorbent assay kits. Results: Higher plasma TIMP-1 levels were observed in patients with TIMP-1 rs4898 T allele, compared to patients with non-T alleles (P<0.001). There was significant association with vascular invasion (P=0.002), lymph node metastasis (P=0.038), distant metastasis (P=0.049), TNM stage (P=0.003), Dukes' stage (P=0.045), and recurrence (P=0.005). Kaplan-Meier analysis demonstrated that TIMP-1 rs4898 T allele was associated with lower cumulative overall survival (OS) and recurrence-free survival (RFS) (P<0.001, P<0.001, respectively). Cox proportional hazards modeling showed the TIMP-1 rs4898 T allele to be an independent prognostic predictor for OS (HR=2.607, 95% CI=1.158-5.870, P=0.021) and RFS (HR=2.667, 95% CI=1.194-5.959, P=0.017) in CRC patients. Conclusion: TIMP-1 rs4898 polymorphisms are associated with unfavorable clinicopathological factors, indicating potential clinical predictive value for CRC patient prognosis.

Keywords: Tissue inhibitor matrix metalloproteinase 1, polymorphism, prognosis, colorectal cancer, biomarker

Introduction

Colorectal cancer (CRC) is the third most common cancer, with high mortality rates, especially for patients in developing countries. A total of 1.4 million individuals are diagnosed with CRC every year, with almost 693,900 patients dying from the disease [1]. As with other cancers, the probability of survival strongly depends on CRC staging at the time of diagnosis. Early detection significantly improves prognosis and reduces CRC-related mortality [2]. Several studies have sought to develop effective diagnostic tests for early detection, searching for ways to mitigate cancer progression [3-5]. Colorectal carcinogenesis has been associated with many genetic alterations and aberrant protein expression [6, 7]. However, genetic mechanisms underlying CRC have not been fully elucidated. Hence, identification of genetic markers is critical in designing optimal prognostic tools for long-term survival in CRC patients.

Tissue inhibitor matrix metalloproteinase-1 (TIMP-1) is a 28-kDa glycoprotein, belonging to the tissue inhibitor of metalloproteinase (MMP) family [8]. TIMP-1 is upregulated by epidermal growth factor via ERK and MAPK pathways. It plays an important role in progression and metastasis of CRC and may induce cyclin D1 expression, stimulating cancer cell proliferation in an MMP-independent manner [5, 9, 10]. Several studies have demonstrated that the cell proliferative effects of TIMP-1 depend on its ability to inhibit MMPs [11, 12]. TIMP-1 can exert anti-apoptotic effects by binding to the CD63/integrinβ1 complex or by phosphorylating AKT and BAD [13-15]. Not surprisingly, ab-

normally elevated plasma TIMP-1 levels are observed in patients with CRC, indicating considerable diagnostic and prognostic value for patients with CRC [16, 17]. However, due to difficulties in defining an ideal cut-off value for plasma TIMP-1 levels, there has been a lack of consensus regarding its clinical applicability. Thus, it is necessary to find a more objective and quantifiable standard, assisting clinicians that adopt TIMP-1 as a diagnostic or prognostic marker. Interestingly, a functional single-nucleotide polymorphism (SNP) (rs4898, 372 T/C) has been reported to enhance expression of TIMP-1 and increase the risk of developing certain diseases [18]. TIMP-1 overexpression can lead to a significant increase in gene expression related to proliferation, apoptosis, and signal transduction [19, 20]. However, association between rs4898 and CRC patient survival has not been investigated.

The primary aim of the present study was to determine the existence of association between TIMP-1 rs4898 variations, plasma TIMP-1 levels, and CRC patient survival in a Han Chinese cohort after surgery.

Materials and methods

Patient characteristics and clinical samples

This retrospective and observational study was performed in Zhejiang Hospital, including a total of 189 patients undergoing CRC surgery between January 2011 and July 2015. All CRC patients were Han Chinese and diagnosed, independently, by at least two pathologists, based on the American Joint Committee on Cancer. Patients that received pre-surgical chemotherapy or radiotherapy were excluded from this study, resulting in a total of 152 CRC patients qualifying for study inclusion. Blood samples were collected immediately before surgery. They were stored at 80°C for future DNA and protein extraction. In addition, patient age, gender, tumor location, vascular invasion, lymph node metastasis, distant metastasis, TNM stage, Dukes' stage, and histologic grades were recorded.

SNP detection and plasma TIMP-1 levels

Genomic DNA extraction was performed using the AllPrep DNA/RNA Mini kit (Qiagen, Venlos, The Netherlands), based on manufacturer instructions. SNP genotyping was performed using the Sequenom Mass ARRAY single-nucleotide polymorphism genotyping platform (Sequenom, San Diego, CA, USA) [21]. Quality control was performed, excluding individual SNPs or samples with genotype efficiency call rates <95% and SNP assays with poor-quality spectrum/cluster plots. Plasma TIMP-1 levels were measured using enzyme-linked immunosorbent assay kits (Abcam, Cambridge, MA, USA), using plasma samples prepared according to manufacturer instructions. Plasma TIMP-1 levels were expressed as ng/mL. All tests were performed in a blinded manner by two experienced technicians.

Ethics approval

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Research Ethics Committee of Zhejiang Hospital. Written informed consent was obtained from all patients and family members. The Research Ethics Board considered this study a minimal risk experiment.

Statistical analysis

Data analyses were performed using SPSS v20.0 (SPSS, USA), with two-sided tests used for all analyses. Descriptive variables are expressed as medians (range) and categorical variables are expressed as numbers (percentage). Comparisons of TIMP-1 levels between the groups were performed using the Wilcoxon-Mann-Whitney test. Categorical variables were assessed using Chi-squared or nonparametric tests, as appropriate. Association of each variable with recurrence-free survival (RFS) and overall survival (OS) was assessed using Kaplan-Meier survival estimates and log-rank tests, along with extraction of Kaplan-Meier survival curves. Subsequent multivariate Cox proportional hazards regression analyses were performed using variables derived from univariate analysis. Hazard ratios (HRs) and corresponding 95% confidence intervals (CIs) were calculated as measures for the prognostic value of selected variables. $P<0.05$ indicates statistical significance.

Results

TIMP-1 SNP determination and association with TIMP-1 plasma levels

A total of 152 patients that underwent CRC surgery were evaluated in this study. The pa-

Table 1. TIMP-1 gene determination and association with plasma levels of TIMP-1

TIMP-1 rs4898	Gender (n)		TIMP-1 (ng/ml)				
	Male	Female	Mean	Median	Range	Z-value	*P*-value
Without T allele	49 (C allele)	10 (CC)	225.3	236.4	75.1-318.5	-3.828	<0.001
With T allele	72 (T allele)	9 (CT)+12 (TT)	311.8	267.2	100.9-708.9		

TIMP-1: Tissue inhibitors of metalloproteinases-1; *P*-value is based on the Wilcoxon-Mann-Whitney test.

Table 2. Correlation between TIMP-1 rs4898 genetic polymorphisms and clinicopathological characteristics

	Total (n=152)	TIMP-1 rs4898		χ^2-value	*P*-value
		CC (n=59)	CT/TT (n=93)		
Age					
<65	82	34 (57.6%)	48 (51.6%)	0.526	0.468
≥65	70	25 (42.4%)	45 (48.4%)		
Gender					
Female	31	10 (16.9%)	21 (22.6%)	0.705	0.401
Male	121	49 (83.1%)	72 (77.4%)		
Tumor location					
Right	44	20 (33.9%)	24 (25.8%)	3.787	0.052
Transverse	53	24 (40.7%)	29 (31.2%)		
Left+Others	55	15 (25.4%)	40 (43.0%)		
Vascular invasion					
Yes	22	2 (3.4%)	20 (21.5%)	9.570	0.002
No	130	57 (96.6%)	73 (78.5%)		
Lymph node metastasis					
Yes	31	7 (11.9%)	24 (25.8%)	4.322	0.038
No	121	52 (88.1%)	69 (74.2%)		
Distant metastasis					
Yes	24	5 (8.5%)	19 (20.4%)	3.881	0.049
No	128	54 (91.5%)	74 (79.6%)		
TNM stage					
I+II	72	37 (62.7%)	35 (37.6%)	9.106	0.003
III+IV	80	22 (37.3%)	58 (62.4%)		
Dukes' stage					
A+B	67	32 (54.2%)	35 (37.6%)	4.037	0.045
C	85	27 (45.8%)	58 (62.4%)		
Differentiation					
Well	54	23 (39.0%)	31 (33.3%)	1.427	0.232
Moderate	63	26 (44.1%)	37 (39.8%)		
Poor	35	10 (16.9%)	25 (26.9%)		
Recurrence					
Yes	30	5 (8.5%)	25 (26.9%)	7.721	0.005
No	122	54 (91.5%)	68 (73.1%)		

TIMP-1: Tissue inhibitors of metalloproteinases-1; TNM stage: Tumor-node-metastasis stage; *P*-value is based on the Chi-squared test and nonparametric test, as appropriate.

tient cohort consisted of 121 males (79.6%) and 31 females (20.4%), with a median age of 62.5 years (range: 25-89 years). Of these, 44 (28.9%) patients experienced CRC recurrence, while 30 (19.7%) died during the follow-up period. This study initially assessed TIMP-1 gene sequence and TIMP-1 plasma levels. Calculated frequencies were 42.6% and 57.4% for C and T alleles, respectively. TIMP-1 allele distribution was significantly associated with TIMP-1 expression levels. As shown in **Table 1**, the median TIMP-1 level for patients with the T allele was 267.2 ng/mL (ranging from 100.9 to 708.9 ng/mL). It was 236.4 ng/mL (ranging from 75.1 to 318.5 ng/mL) for patients without the T allele. This indicates that patients carrying the T allele had higher plasma TIMP-1 levels than patients without the T allele (P<0.001).

Association between TIMP-1 rs4898 polymorphisms and clinical features of colorectal cancer

Patients were categorized based on the presence or absence of the T allele [18]. Chi-squared and nonparametric tests were used to estimate the impact of rs4898 polymorphisms on clinical features. As shown in **Table 2**, the TIMP-1 rs4898 polymorphism was significantly asso-

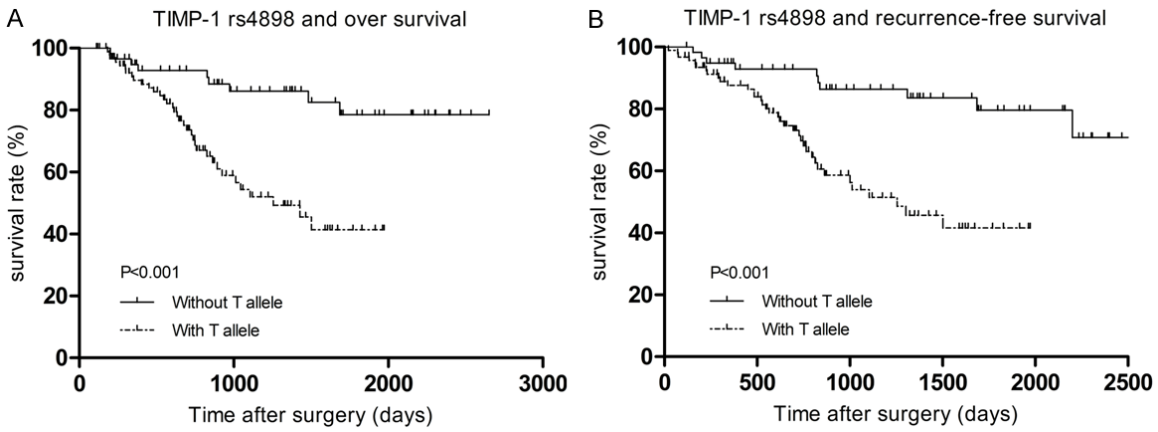

Figure 1. Kaplan-Meier survival estimates for (A) overall survival (OS) and (B) recurrence-free survival (RFS) in patients with the rs4898 polymorphism.

Table 3. Influence of clinicopathological factors on recurrence-free survival (RFS) and overall survival (OS) in univariate Cox analysis

Parameter	OS		RFS	
	χ^2-value	*P*-value	χ^2-value	*P*-value
Age (<65/≥65)	0.101	0.751	0.184	0.668
Gender (Male/Female)	0.381	0.537	0.126	0.723
Tumor location (Right/Transverse/Left+Others)	0.404	0.817	0.616	0.735
Vascular invasion (Yes/No)	35.174	<0.001	39.410	<0.001
Lymph node metastasis (Yes/No)	20.538	<0.001	24.942	<0.001
Distant metastasis (Yes/No)	34.467	<0.001	37.873	<0.001
TNM stage (I+II/III+IV)	25.537	<0.001	25.708	<0.001
Dukes' stage (A+B/C)	2.327	0.127	2.120	0.145
Differentiation (Well/Moderate/Poor)	32.583	<0.001	32.505	<0.001
TIMP-1 rs4898 gene (without T/with T)	14.604	<0.001	15.457	<0.001

TNM stage: Tumor-node-metastasis stage; TIMP-1: Tissue inhibitors of metalloproteinases-1; *P*-value is based on the univariate Cox analysis.

ciated with vascular invasion (P=0.002), lymph node metastasis (P=0.038), distant metastasis (P=0.049), TNM stage (P=0.003), Dukes' stage (P=0.045), and recurrence (P=0.005), suggesting that the rs4898 T allele may be associated with unfavorable clinicopathological features in CRC patients (P>0.05). However, there were no significant differences between patients with the T allele and those without in terms of age, gender, tumor location, and differentiation (P>0.05).

Prognostic value for survival of TIMP-1 rs4898 polymorphisms

Kaplan-Meier survival analysis, along with log-rank testing, was used to assess the relationship between clinicopathological factors and patient prognosis. Notably, vascular invasion, lymph node metastasis, distant metastasis, advanced TNM stage, poor tumor differentiation, and TIMP-1 rs4898 T allele (**Figure 1A**, **1B**) were associated with lower cumulative OS and RFS (P<0.05, **Table 3**). All influencing parameters were then included for multivariate Cox proportional hazard regression analysis. In addition, age and gender were included in the analysis model for necessary adjustments. Results demonstrated that the TIMP-1 rs4898 T allele was associated with significantly reduced OS and RFS, identifying it as a significant independent predictor for patient prognosis (HR=2.607, 95% CI=1.158-5.870, P=0.021; HR=2.667, 95% CI=1.194-5.959, P=0.017, respectively). As expected, advanced TNM stage significantly affected the OS of CRC patients

Table 4. Influence of clinicopathological factors on overall survival (OS) and recurrence-free survival (RFS) in multivariate Cox regression analysis

	Hazard ratio (95% CI)	*P*-value
OS		
TIMP-1 rs4898 gene (1=without T, 2=with T)	2.607 (1.158-5.870)	0.021
TNM stage (1=I+II, 2=III+IV)	2.998 (1.006-8.936)	0.049
RFS		
TIMP-1 rs4898 gene (1=without T, 2=with T)	2.667 (1.194-5.959)	0.017
Distant metastasis (1=Yes, 2=No)	0.337 (0.123-0.926)	0.035

TIMP-1: Tissue inhibitors of metalloproteinases-1; TNM stage: Tumor-node-metastasis stage; *P*-value is based on multivariate Cox regression analysis.

following surgery (HR=2.998, 95% CI=1.006-8.936, P=0.049). Similar results were found for distant metastasis on RFS (HR=0.337, 95% CI=0.123-0.926, P=0.035) (**Table 4**).

Discussion

Growing evidence has demonstrated that proteins aberrantly regulated in tumors, including STYK1, DBC1, and TIMP-1, may be regarded as early diagnostic prognostic markers and therapeutic targets [5, 22, 23]. TIMP-1 is one these proteins that is highly upregulated in CRC. However, data regarding its clinic applicability for CRC patients has not been properly investigated. The current study was conducted to specifically investigate the roles of TIMP-1 genetic variations for robust CRC patient prognosis after surgery.

TIMPs are endogenous tissue-specific inhibitors that bind and inhibit MMPs, maintaining homeostasis of extracellular matrix (ECM) components [24]. Recent studies have revealed that they can exert their biological activities in an MMP-independent manner [8, 10]. Imbalances between TIMPs and MMPs have been demonstrated to play a role in the development of G1 malignancies [25]. Moreover, several studies have demonstrated that high TIMP-1 plasma levels in CRC patients leads to poor prognosis. Dysregulated TIMP-1 expression may lead to carcinoma progression by inhibiting the matrix-degrading properties of endopeptidases or through FAK-PI3K/AKT and MAPK pathways [4, 5, 7, 17]. Additionally, several studies have come to a similar conclusion through genetic level analyses [26]. There is growing evidence that genetic alterations in TIMP-1 play a role in the pathogenesis and progression of certain diseases [27]. The gene encoding TIMP-1 is located at chromosome Xp11.23-11.4. TIMP-1 rs4898 polymorphisms account for the vast majority of known gene polymorphisms [28]. Despite previous efforts, the exact genetic mechanisms by which TIMP-1 rs4898 exerts its function are still unknown, especially concerning the relationship between alleles and associated diseases [26, 29]. However, there is general agreement that patients with the rs4898 T allele have higher TIMP-1 plasma levels and that gender differences for disease risk susceptibility may exist [18, 30]. This agrees with the results of the current study. Patients carrying the TIMP-1 rs4898 T allele had higher plasma TIMP-1 levels and lower cumulative OS and RFS. However, gender differences could not be found for this effect, suggesting that TIMP-1 rs4898 polymorphisms may be an applicable and independent prognostic factor for CRC.

SNPs are the most common form of inherited variation in humans, accounting for more than 90% of all known polymorphisms. Many functional polymorphisms have been demonstrated to be useful for disease diagnosis and treatment, indicating that SNPs can help identify individual differences in complex diseases and drug responses [31]. For example, microRNA-149 polymorphisms [32] and interleukin-17A polymorphisms [33] have been associated with CRC risk. In addition, a previous study demonstrated that TIMP-1 rs4898 polymorphisms were associated with TIMP-1 expression, which in-turn was associated with risk of certain diseases [18]. Given the importance of SNPs in predicting tumor occurrence and progression, it is reasonable to speculate that TIMP-1 rs4898 polymorphisms may be valuable for CRC patient prognosis. In the present study, the TIMP-1 rs4898 T allele was found to be associated with vascular invasion, lymph node metastasis, advanced TNM stage, and advanced Dukes' stage in CRC. This suggests that is a useful biomarker for prediction of clinicopathological outcomes in CRC patients.

Research into developing safe and effective systemic anti-cancer treatment strategies has been extensively explored. Metastasis and chemoresistance are major challenges for CRC anti-cancer treatments. Phenotypic variations in CRC patients have been associated with clinical response to post-surgical chemotherapy and OS [20]. There is evidence that functional polymorphisms in DNA repair and metabolism-related genes may be associated with chemoresistance in CRC patients after chemotherapy [34, 35]. Coincidentally, TIMP-1 gene deficiency has been found to increase tumor cell sensitivity to chemotherapy-induced apoptosis. Modulators of TIMP activity have been successfully used to decrease TIMP-1 levels to improve prognosis in certain disease models [36, 37]. Relating these findings to present results, it appears that TIMP-1 rs4898 polymorphisms, a natural regulator of TIMP-1 expression, may help establish effective treatment for favorable prognosis of patients with CRC.

The current study had several limitations, however. First, the sample size was relatively small and patients were of Han Chinese ethnicity only. Second, only one SNP in the TIMP-1 gene was analyzed. Despite its strong linkage disequilibrium with other TIMP-1 polymorphisms, several gene interaction effects may have been overlooked. Third, this study determined the relationship between TIMP-1 gene polymorphisms and TIMP-1 expression in patients with CRC. However, this study did not investigate the association between TIMP-1 gene polymorphisms and TIMP activity or investigate its behavior in patients with other diseases. Studies using larger patient cohorts with different types of colorectal diseases should be performed to validate present findings. In addition, more attention should be paid to understanding specific differences between TIMP-1 gene polymorphisms, determining their relationship with gene expression and protein activity.

In conclusion, this is the first study to suggest that TIMP-1 rs4898 polymorphisms may be able to predict CRC patient clinical prognosis. Patients with the TIMP-1 rs4898 T allele showed higher plasma TIMP-1 levels, unfavorable clinicopathological outcomes, and lower cumulative survival rates. These novel findings indicate that TIMP-1 rs4898 polymorphisms act as an independent prognostic factor for CRC patient survival and may be potential therapeutic targets.

Disclosure of conflict of interest

None.

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