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Original Article

Prognostic significance of matrix metalloproteinase 7 immunohistochemical expression in colorectal cancer: a meta-analysis

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Abstract: Matrix metalloproteinase 7 (MMP-7) was speculated to have a key role in the development and progression of human cancer. Considerable studies investigated the relationship between its expression and survival in colorectal cancer (CRC), but inconsistent results were obtained. The clinical significance of MMP-7 overexpression in CRC remains controversial. Therefore, in this article, we conducted a meta-analysis to analyze the prognostic value of MMP-7 in CRC. We searched studies in PubMed, Medline, and Web of Science databases until August 2014 to find relevant studies. A total of six high-quality studies met the inclusion criteria and 1631 patients were included in our study. Combined hazard ratios (HRs) suggested that MMP-7 overexpression had an unfavorable impact on overall survival (HR = 1.83, 95% CI: 1.24-2.71). Subgroup and sensitivity analyses further validated the role of MMP-7 as a predictor for prognosis. In conclusion, MMP-7 overexpression detected by immunohistochemistry indicated worse prognosis in CRC and may help to guide clinical therapy.

Keywords: Matrix metalloproteinase 7, prognosis, colorectal cancer, meta-analysis

Introduction

Colorectal cancer (CRC) is the third most frequent cancer and one of the leading causes of cancer deaths [1]. Although most of the stages I, II, and III CRCs are curable by surgical excision or combined with adjuvant chemotherapy, stage IV tumors are usually incurable [2]. Further understanding the biological mechanisms of the progression of this carcinoma and developing effective measures to intervene in this process are highly significant. Much attention has been recently focused on the molecular-based prognostic markers, which are complementary to the data obtained by pathological diagnosis and can be used to guide clinical therapy [3, 4]. Matrix metalloproteinases (MMPs) belong to a family of zinc-dependent endopeptidases. More than 25 human MMP members share common functional domains [5, 6]. Given that these MMPs are responsible for the breakdown of a vast number of protein targets, they are proposed to have a role in tissue degradation and remodeling in both normal and pathological processes [7]. MMPs directly regulate tumor cell invasiveness and metastasis [5]. This causal relationship between MMP overexpression and tumor progression has been generally validated by more recent studies [8]. Matrix metalloproteinase 7 (MMP-7, also known as matrilysin), an important MMP in carcinogenesis, is unregulated in colorectal neoplasms, especially in the invasive fronts [9]. Previous studies found that MMP-7 is expressed in 90% of colonic adenocarcinomas [10] and is one of the few MMPs that are secreted by tumor cells [11]. Various studies have been conducted to evaluate the associations between MMP-7 overexpression and CRC prognosis, but the results remain inconclusive. For example, Fang et al. reported that high MMP-7 expression is associated with worse CRC prognosis in Asian patients [12], whereas several other studies demonstrated that MMP-7 fails to act as a prog-

Table 1. Scale for quality assessment

Criteria	Score
Representativeness of cases	
Consecutive/random recruitment from case population	1
No method of selection stated	0
Ascertainment of colorectal cancer	
Histological confirmation at the department of pathology	2
Medical record	1
Not described	0
Sample size	
≥ 200	1
< 200	0
Immunohistochemistry examination	
Negative or positive control	1
Diagnosed by two experienced pathologists	1
Diagnosed under "blinded" condition	1
Follow-up of patients	
Describe follow-up time	1
Describe how many patients were lost to follow up or not available for statistical analysis	1
Total	10

nostic factor, because its expression is insignificantly associated with survival [13-15]. Therefore, an updated and systematic meta-analysis based on large samples is urgently needed to evaluate the associations between MMP-7 and CRC prognosis.

Materials and methods

Identification and eligibility of relevant studies

We searched studies in PubMed, Medline, and Web of Science databases until August 2014 using three search themes, which were combined using the Boolean operator "and." The first theme was ("colorectal neoplasms" OR "colorectal cancer" OR "colon cancer" OR "rectal cancer"); the second was ("MMP7" OR "MMP-7" OR "matrix metalloproteinase 7" OR "matrilysin"); and the third was ("prognosis"). The reviews were also artificially inspected to find related research. Using this search strategy, 50, 36, and 119 articles (a total of 205) were retrieved from PubMed, Medline, and Web of Science databases, respectively. After reading the abstracts and full texts, we selected six articles [12-24]. The inclusion criteria in this meta-analysis study were as follows: (1) the outcomes investigated in the literature were CRC; (2) immunohistochemistry (IHC) method was used to evaluate the relationships between MMP-7 expression and CRC prognosis; (3) sample size was more than 100 patients; (4) English language publications; and (5) adequate data were provided to measure hazard ratios (HRs) and their 95% confidence intervals (CIs).

Data extraction and assessment of study quality

Two investigators (Yeting Hu and Weibo Xiang) independently extracted data, and disagreements were resolved through consensus. Data derived from the articles include the following: first author, year of publication, recruitment time, country of origin, gender of patients, cut-off value for high or low MMP-7 expression, antibody source, tumor characteristics, and survival data. If the aforementioned information was not reported in the study, items were treated as "NA (not available)." Study quality was assessed independently by two authors (Haiyan Chen and Yeting Hu) according to our modified criteria (**Table 1**). These criteria were developed to assess the quality of included studies, based on the representativeness of cases, ascertainment of CRC, IHC examination, and follow-up of patients [25-27]. The total scores ranged from 0 to 10, with higher scores indicating better quality.

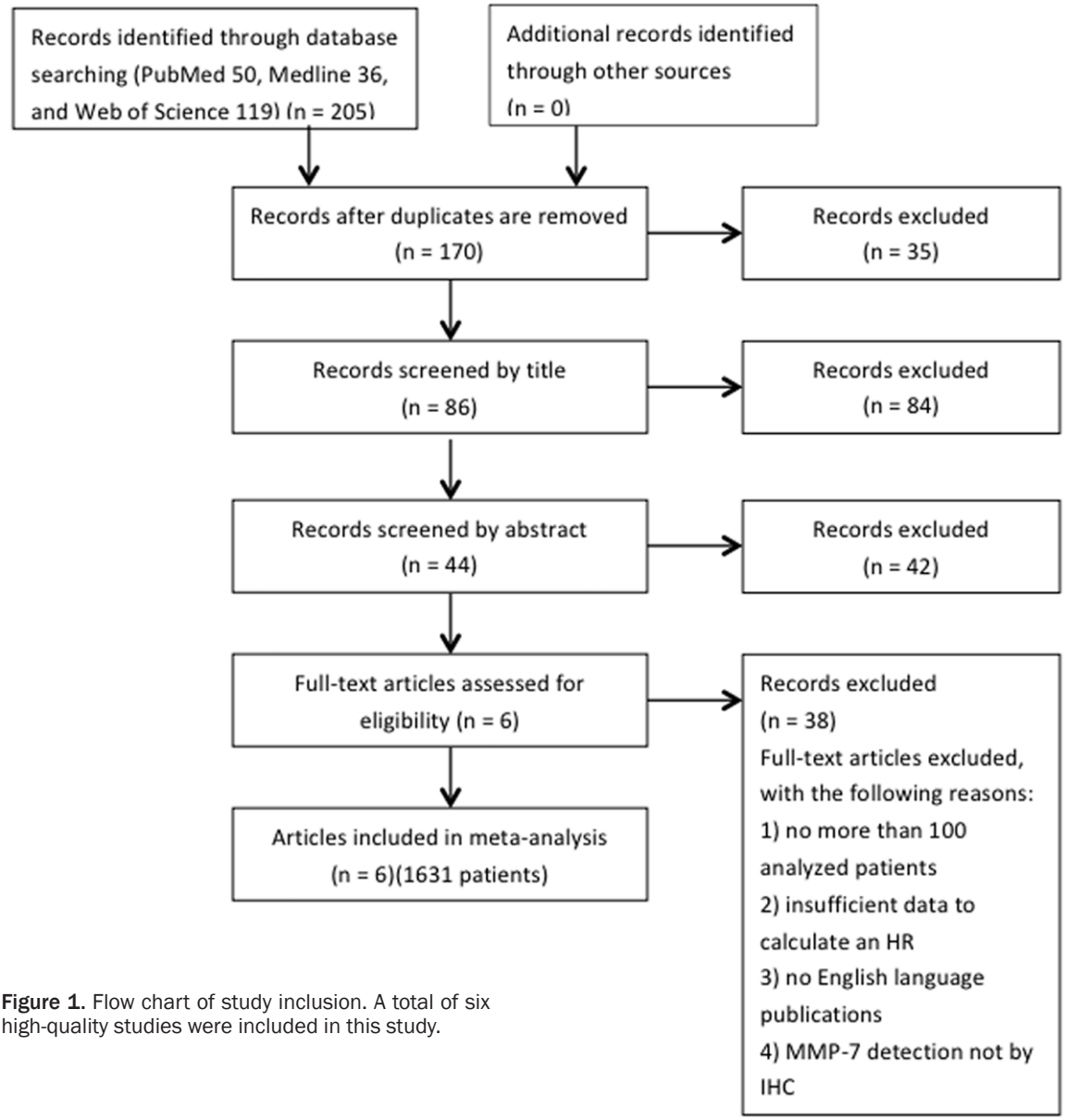

Figure 1. Flow chart of study inclusion. A total of six high-quality studies were included in this study.

Statistical methods

HRs, 95% CIs, and *P* values (Z test) were combined to quantitatively evaluate the impact of MMP-7 expression on survival outcomes. If HRs and their 95% CIs were given explicitly in articles, then we used them directly. Otherwise, they were calculated according to the methods described by Parmar and Tierney [28, 29]. Specifically, Kaplan-Meier curves were read by the digitizing software tool (Engauge Digitizer version 4.1), which converted graphs into data. We ran this process three times and took the mean for further analysis. If *P* was less than 0.05, then the study was considered to be statistically significant. Heterogeneity across studies was evaluated using a Chi-square-based Q statistical test [30]. I^2 was calculated as $\frac{Q - df}{Q} \times 100\%$, where *df* was degrees of freedom (number of studies minus 1) and ranged from 0% to 100%. High I^2 values indicated a high degree of heterogeneity. If I^2 was less than 50%, then the study was considered to be homogeneous, and fixed-effects model (FEM) was used to calculate HRs. Otherwise, we chose the random-effects model (REM) for heterogeneous samples ($I^2 \geq 50\%$) [30, 31]. We

Table 2. Characteristics of studies included in the meta-analysis

First author	Recruit time	Ethnicity	Sample size	Histopathology cancer type	Definition of MMP7 high expression	MMP7 high expression (%)	HR estimation	Quality score
Fang	1995-2003	Asian	509	Mixed	> 10% or moderate/strong staining	88.9%	HR for OS	8
Koskensalo	1989-1998	European	545	Adenocarcinoma	> 50% of stained cells	19.3%	HR for OS	8
Ougolkov	1991-1993	Asian	202	Adenocarcinoma	> 10% of stained cells	19.0%	HR for OS	8
Yang	2004-2007	Asian	118	Adenocarcinoma	Any reddish brown staining	61.0%	Survival curve for OS	8
Adachi	1988-1993	Asian	113	Adenocarcinoma	> 30% of stained cells	41.6%	Survival curve for OS	7
Yeh	1995-2005	Asian	144	Adenocarcinoma	Any reddish brown staining	32.6%	Survival curve for OS	6

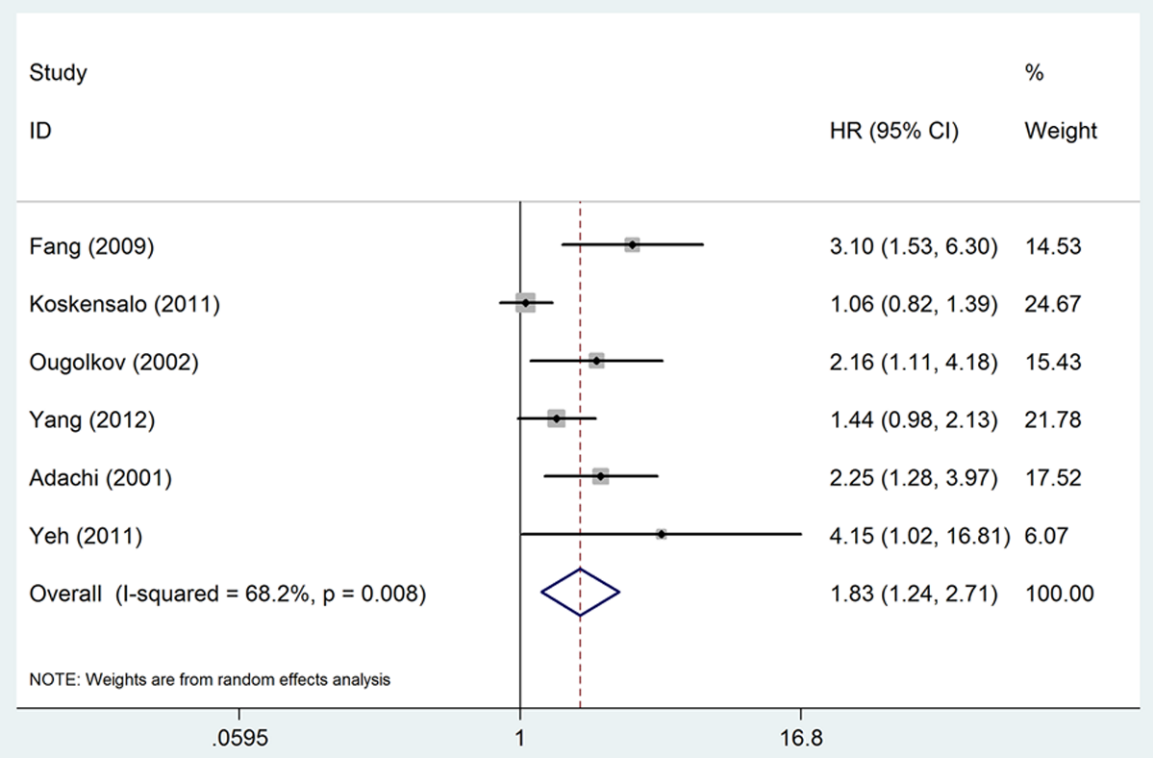

Figure 2. Forrest plot of HRs for the association of MMP-7 overexpression with OS. HR = 1.83 (95% CI: 1.24-2.71) (Z test) indicates that high MMP-7 expression is significantly associated with worse prognosis.

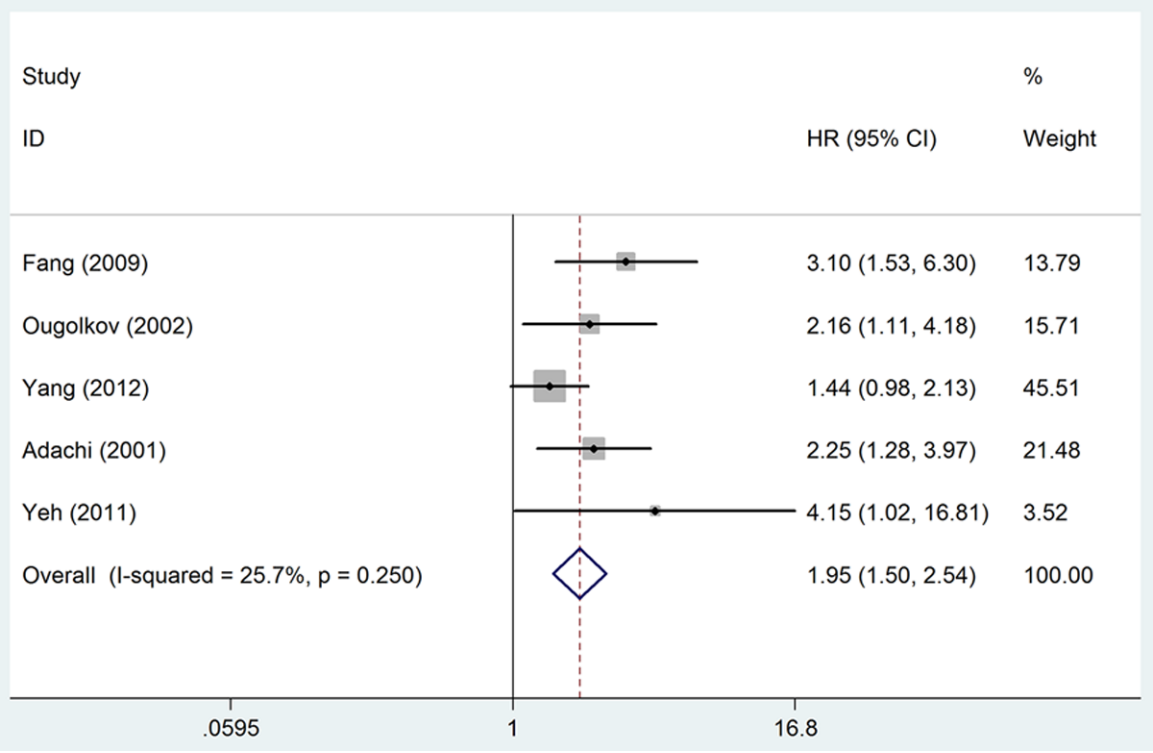

Figure 3. Forrest plot of HRs for the relation of MMP-7 expression with OS in Asian countries.

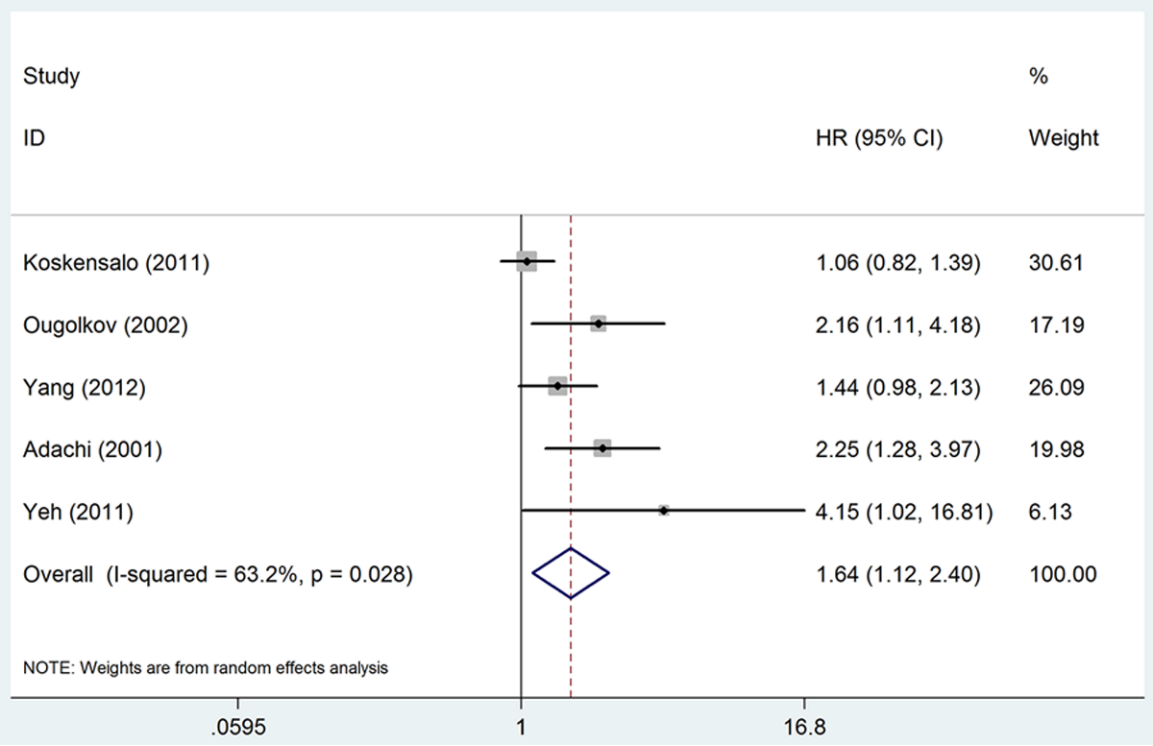

Figure 4. Forrest plot of HRs for the relation of MMP-7 expression with OS in adenocarcinoma patients.

also conducted sensitivity analysis to ensure the feasibility of our results, in which we moved one study at a time and calculated the remaining HRs. All analyses were performed using STATA version 12.0 (Stata Corporation, College Station, Texas, USA). Egger's [32] and Begg's [33] tests were used to calculate the potential risks of publication bias. In addition, *t*-test suggested by Egger was used to indicate the intercept significance of the publication bias ($P > 0.05$ was considered as no publication bias).

Results

Description of studies

Based on the title, 86 eligible articles met the inclusion criteria (**Figure 1**). We scrutinized the abstracts and full-texts and selected six studies for this meta-analysis. The characteristics of these studies are summarized in **Table 2**. A total of 1631 CRC patients were enrolled and exhibited relationships between MMP-7 expression and prognostic outcomes of interests. The sample sizes of the included studies ranged from 113 to 545 patients, and the studies were conducted in China, Japan, and Finland. IHC was used to detect MMP-7 expression in all of the publications. In addition, the qualities of these six studies were assessed according to the scale for quality assessment (**Table 1**). These studies were consecutively or randomly selected from case population. Moreover, they clearly mentioned that the diagnosis of CRC was confirmed by histology. Considering that studies with small sample size may have a high risk of false-positive association, we only included studies with more than 100 patients. A total of 83.3% (5/6) of studies described the IHC control, such as examination with negative or positive control and diagnosis by two experienced pathologists or under "blinded" condition. Moreover, half of the studies described the follow-up of patients. In summary, all the included studies obtained scores of 6 or more (**Table 2**), implying that they were all of high quality.

Impact of MMP-7 expression on prognosis of CRC

Among the six studies on overall survival (OS), a large heterogeneity ($I^2 = 68.2\%$) was observed. Thus, the REM was used to pool the results. The combined HR estimate for OS was 1.83

Tabel 3. HRs (95% CI) of sensitivity analysis for MMP-7 overexpression on OS

Study omitted	Estimated HR (95% CI)	*P* value	Heterogeneity		
			I^2 (%)	*P* value	Model used
Fang	1.64 (1.12-2.40)	0.011	63.20%	0.028	REM
Koskensalo	1.95 (1.50-2.54)	0.000	25.70%	0.250	FEM
Ougolkov	1.80 (1.16-2.79)	0.009	71.70%	0.007	REM
Yang	2.50 (1.20-3.50)	0.009	74.50%	0.003	REM
Adachi	1.76 (1.14-2.73)	0.011	69.30%	0.011	REM
Yeh	1.73 (1.17-2.56)	0.006	70.30%	0.009	REM

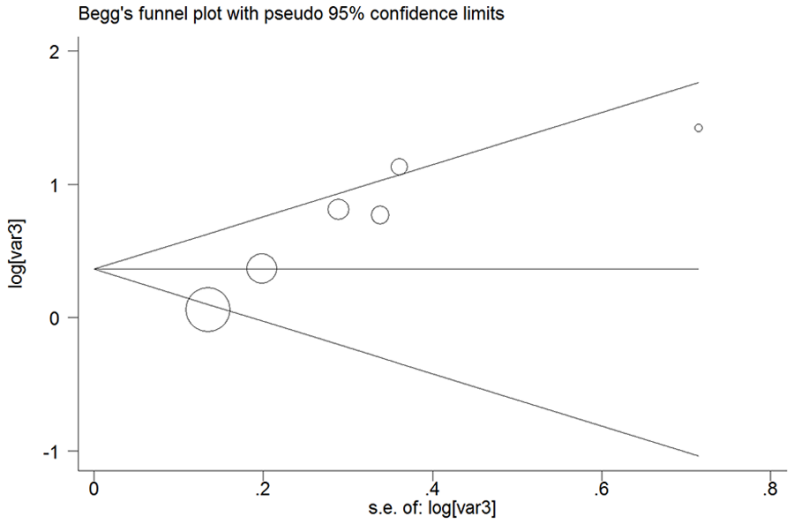

Figure 5. Begg's funnel plot for MMP-7 overexpression and OS. (Begg's test).

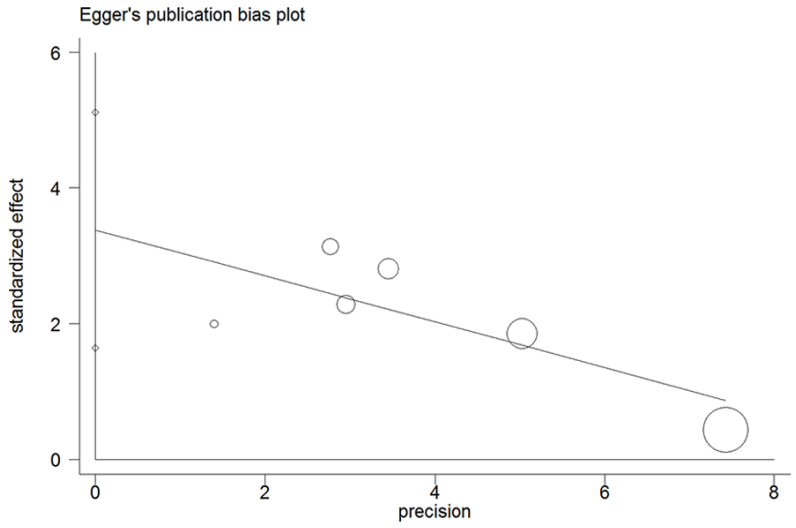

Figure 6. Egger's regression asymmetry plot for MMP-7 overexpression and OS. (Egger's test).

(95% CI: 1.24-2.71, *P* = 0.002) (**Figure 2**). We also performed subgroup analysis to show the relationship between high MMP-7 expression and OS in Asian countries (HR = 1.95, 95% CI: 1.50-2.54, *P* = 0.000) and adenocarcinoma patients (HR = 1.64, 95% CI: 1.12-2.40, *P* = 0.011) (**Figures 3** and **4**). All these results suggested that MMP-7 overexpression was correlated with worse prognosis of CRC. In addition, to verify the above conclusion, we used a sensitivity analysis. The estimated HRs ranged from 1.64 (95% CI: 1.12-2.40) to 2.50 (95% CI: 1.20-3.50) (**Table 3**), confirming the role of MMP-7 as a predictor for prognosis.

Publication bias

Funnel plot was used to assess the publication bias. Begg's and Egger's funnel plot for the six studies did not reveal significant evidence of asymmetry (*P* = 0.133 and 0.081, respectively). The funnel plot suggested low risk of publication bias (**Figures 5** and **6**).

Discussion

MMPs are matrix-degrading enzymes that contribute to all stages of tumor progression. MMPs are involved in the cleavage of extracellular matrix components, such as fi-bronectin, collagen type IV, laminin, elastin, entactin, and

cartilage proteoglycan aggregates [6, 7, 34]. MMPs facilitate tumor cells to move freely through the stroma for metastasis to distant sites [34]. Therefore, MMPs may be bound at docking sites on cancer cells and perhaps arm the cancer cells with the proteases required for invasion [6]. Among these MMPs, MMP-7 is the only one expressed in tumor cells, but not in normal colonic epithelial cells [34]. Carole et al. [35] found that MMP-7 not only possesses an important role in late-stage tumor progression, but is also required for the formation of intestinal adenomas. All these observations indicate that MMP-7 expression may have effects on premalignant cells, which confirm the causal role of MMP-7 in CRC tumorigenesis. Furthermore, recent studies found that MMP-7 inhibitors reduce the number of intestinal polyps [11] and MMP-7 may be a validated anticancer drug target. Therefore, our meta-analysis examines the association between MMP-7 overexpression and the prognosis of CRC patients.

Our analysis included six high-quality studies, which comprise 1631 CRC patients. We found that high MMP-7 expression was significantly associated with increasing mortality risk. Specifically, HR was 1.83 (95% CI: 1.24-2.71, P = 0.002) for OS. Our study concurred with previous study that MMP-7 expression was associated with prognosis in CRC and patients with high MMP-7 expression had poor survival.

In this meta-analysis based on heterogeneity assessments, we used REM or FEM to deal with highly significant heterogeneity among the six studies. To further reduce heterogeneity, we only included research with methods of IHC. However, differences of antibody sources and dilutions, evaluation standards, and other inevitable factors were observed. Given the limited data, we did not perform stratified analysis according to the previously mentioned heterogeneous factors. Moreover, although extrapolating HRs from survival curves was repeated three times, they may still be less credible than direct analysis of variance. Thus, potential errors caused by humans or calculators may exist. The studies included in our meta-analysis were restricted only to articles published in English, which probably produced additional bias. Considering that limitations existed in this meta-analysis, adequately prospective studies with large sample size are required to further assess the precise effect of MMP-7 overexpression on CRC. However, our studies still provide valuable references for the research of MMP-7 and its relationships with prognosis and other clinical features.

In summary, this meta-analysis showed that high MMP-7 expression was correlated with worse CRC prognosis, and using MMP-7 as a molecular marker may become a routine part of patient management in CRC, which can help define therapeutic and follow-up protocols. However, larger and well-designed studies in various areas should be performed to further validate these results.

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Disclosure of conflict of interest

None.

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