SYSTEMATIC REVIEW

Prognostic value of matrix metalloproteinase-2 protein and matrix metalloproteinase-9 protein in colorectal cancer: a meta-analysis

Yusha Wang[1,2†], Yuhao Wei[1†], Jing Huang[3†], Xinke Li[4], Diqing You[5], Li Wang[2*] and Xuelei Ma[1*]

Abstract

Introduction Matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) are critical components of the extracellular matrix (ECM) in colorectal cancer (CRC). We aimed to evaluate the prognostic value of MMP-2 and MMP-9 in patients with CRC.

Methods We performed a meta-analysis of cohort studies with available data on the effect of MMP-2 and MMP-9 expression on both disease-free survival (DFS) and overall survival (OS) by the risk ratios (RRs) with their 95% confidence intervals (CIs). Studies were subgrouped based on the different tissue types, including cancer tissue and normal tissue, and the subgroup effect of MMP expression in different tissues was analyzed through meta-regression. To ensure the quality and reduce the risk of bias, the Newcastle–Ottawa Scale (NOS) was used to assess the included studies. A sensitivity analysis was randomly performed to assess the potential impact of each study on our results.

Results Eighteen trials were selected (Table 1) and included a total of 3944 patients. According to our primary meta-analysis, the expression of MMP-2 was significantly associated with a decrease in OS (RR = 1.75, 95% CI = 1.34 to 2.29, $P < 0.001$) and DFS (RR = 2.62, 95% CI = 1.25 to 5.49, $P < 0.001$), and the expression of MMP-9 was not significantly associated with a decrease in OS (RR = 1.48, 95% CI = 0.97 to 2.24, $P = 0.069$) or DFS (RR = 1.60, 95% CI = 0.87 to 2.94, $P = 0.133$). According to the subgroup analysis of MMPs in different tissues, high MMP-2 expression in cancer tissue (RR = 1.90, 95% CI = 1.29 to 2.79) and normal tissue (RR = 1.59, 95% CI = 1.17 to 2.17) were significant indicators of poor OS. High MMP-2 expression in cancer tissue was significant indicator of poor DFS (RR = 2.12, 95% CI = 1.09 to 4.11). MMP-9 expression was also associated with poor OS (RR = 1.40, 95% CI = 0.85 to 2.29), but the difference in OS between the high and low expression groups was not statistically significant.

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Conclusions High MMP-2 expression, especially in cancer tissue, is significantly associated with both poor DFS and poor OS in patients with CRC. High MMP-9 expression tended to indicate a poor prognosis of CRC but the correlation was not significant.

Keywords Matrix metalloproteinase-2 (MMP-2), Matrix metalloproteinase-9 (MMP-9), Colorectal cancer (CRC), Prognosis, Meta-analysis

Background

Colorectal cancer (CRC), recognized as the second most common cause of cancer mortality worldwide, has become a major global health burden [1]. Although only approximately 20% of patients with CRC are initially diagnosed with metastatic colorectal cancer (mCRC), up to 50% of patients with localized disease will eventually develop metastases [2]. Known for its high morbidity, mortality and distinctive evolution mechanism, mCRC has a poor clinical outcome, with a median overall survival (OS) of only 25–30 months after systemic therapy [3]. Treatment regimens include local resection, downstaging preoperative systemic therapy, extensive surgery, palliative chemotherapy, targeted therapy, and immunotherapy [4]. However, one of the significant challenges in CRC treatment is multidrug resistance (MDR), which refers to the ability of cancer cells to become resistant to a wide range of chemotherapies, making treatment increasingly difficult and often leading to treatment failure [5]. Hence, unraveling the molecular mechanism of colorectal cancer, identifying novel tumor targets, and developing personalized treatments are crucial research goals.

In recent years, the role of the extracellular matrix (ECM) as a possible predictive and prognostic marker in multiple types of malignant tumors, including colorectal cancer, has been explored [6]. A variety of molecules in the ECM, such as collagen, fibronectin, and matrix metalloproteinases (MMPs), have been shown to contribute to the cleavage of protein fibers and tissue remodeling. Among these, MMPs, which are the most important family of proteases, play an essential role in cleaving different components during the reconstruction of the ECM [7]. Furthermore, MMPs also regulate many biological functions by controlling the activity of growth factors, chemokines, and cell receptors [8]. In colorectal cancer, some studies have shown that MMPs are crucial for tumor invasion and metastasis, suggesting their potential role as diagnostic markers [9].

The analysis of MMP-2 and MMP-9 protein expression and its prognostic value in colorectal cancer could therefore be of particular value in the treatment setting. Several studies have revealed that the increased expression of MMP-2 and MMP-9 is closely related to the course of colorectal cancer, suggesting an association between MMP-2 and MMP-9 and poor prognosis in colorectal cancer patients [10, 11]. However, some investigators have reported that the MMP-9 gene inhibits β-catenin activity by stimulating Notch activation to lead to p21WAF1/CIP1 activation, thereby exerting a protective effect on CRC [12, 13]. Therefore, we performed a meta-analysis of available data to confirm these associations and address the heterogeneity of different reports on the prognostic role of MMP-2 and MMP-9 expression in CRC.

Methods

Research question

This meta-analysis of cohort trials was performed according to the MOOSE (Preferred Reporting Items for Systematic reviews and Meta-analyses of Observational Studies) recommendations. Ethical approval was not necessary since this study was not a human or animal experiment.

Literature search

We searched PubMed, Embase, and the Cochrane Library (all up to Jan 6, 2024) for published articles related to the expression of MMP-2 and MMP-9 in patients with CRC without any language restriction. The search strategy was designed by integrating the different expression levels of MMP-2 and MMP-9, which are diverse terms of CRC, and the required prognostic indicators. The detailed keywords used in the retrieval are listed in Supplementary Table 1.

Eligibility criteria

The inclusion criteria were as follows: (1) original articles on the association between the expression of MMP-2 and/or MMP-9 and the prognosis of CRC and (2) articles whose full text was available. The main exclusion criterion was the absence of hazard ratios (HRs) and/or risk ratios (RRs) of positive/high vs. negative/low expression for DFS, progression-free survival (PFS) or OS. In addition, reviews, meta-analyses, case reports, conference abstracts, and correspondence letters were excluded. When more than 1 article referring to the same trial was found, the most up-to-date and complete report was selected.

Study selection

Records retrieved via the search were imported in tabular format in a CSV file and screened for potential inclusion by 2 investigators independently in parallel. The full

texts of the articles deemed candidates for the meta-analysis were obtained and independently assessed for final inclusion by the 2 investigators in parallel. Discrepancies were resolved by discussion or by a third supervisor investigator.

Data extraction

Hazard ratio or risk ratio and 95% confidence interval (CI) data of MMP-2 and/or MMP-9-positive/high vs. negative/low-expression subgroups for DFS, PFS or OS for each study were independently extracted by 2 investigators. Specifically, DFS was defined as the time from treatment allocation to cancer relapse, death or the last follow-up. PFS was defined as the time from randomization or initiation of treatment to the occurrence of disease progression or death. OS was defined as the time from treatment allocation to death due to any cause or to the last follow-up. Due to the limited number of studies included in our meta-analysis, we decided to combine PFS and DFS to enhance the statistical power and obtain more reliable results. Considering the possible challenge in pooling results among different studies, we decided to use RRs throughout and convert HRs to RRs without exaggerating these results.

The following data were also collected: date of publication, first author, publishing journal, country or area, study design, detection methods, patient characteristics (sample source, number of patients, and sex ratio), median time of follow-up, number of positive/high and negative/low cases, type of Cox regression model for hazard ratio estimation (univariate vs. multivariate), and the aforementioned endpoints. All data were extracted from the eligible studies using a standard data-extraction form and then transferred into an Excel spreadsheet.

Quality assessment

To ensure that all the studies included in our meta-analysis were of high quality, we used a set of predefined criteria based on the Newcastle−Ottawa Scale (NOS) criteria to evaluate the studies; these evaluations were performed independently by 2 investigators in parallel. The criteria of the NOS include three aspects: (1) selection, 0–4; (2) comparability, 0–2; and (3) clinical outcome, 0–3. Total NOS scores range from 0 (lowest) to 9 (highest). According to the NOS, the included studies were classified into two levels: low-quality research with a score of 0–5 and high-quality research with a score of 6–9. A score of 6 or more was considered the inclusion criterion according to a discussion among all the investigators.

Statistical analysis

To evaluate the associations between the expression levels of MMPs and the survival outcomes of patients with CRC, pooled HRs with 95% CIs were evaluated for OS, PFS and DFS. To determine the associations between tumor tissue and normal tissue MMP-2 and MMP-9 expression and survival outcomes, reflecting their prognostic value in CRC, we conducted subgroup analyses. The heterogeneity and publication bias among the different studies were estimated by the I-squared test and visualized in a funnel plot. $I^2 > 50\%$ and $p < 0.05$ were considered indicators of obvious heterogeneity among the studies. For heterogeneity analysis, the random-effects model was utilized if $I^2 > 50\%$ or $p < 0.05$; otherwise, the fixed-effects model was used. Publication bias was visualized in a funnel plot and analyzed via Begg's test [14]. In our study, P values less than 0.05 were considered to indicate statistical significance. Analysis of the effect size of all the data were performed with RevMan 5.0 software, while heterogeneity was analyzed with Stata 12.0 software.

Results

Study selection

The initial search yielded 1610 articles, which were thoroughly reviewed for entry criteria (Fig. 1). Eighteen cohort studies were ultimately identified that met the inclusion criteria [10, 11, 15–30] (Table 1). Overall, a total of 3944 patients were analyzed.

Effect of MMP-2 expression on DFS and OS

Thirteen studies evaluated whether the expression of MMP-2 influences survival outcomes of patients with CRC. Nine studies were included for the OS analysis based on MMP-2 expression. Five studies were included for DFS analysis based on MMP-2 expression. Among them, we found that more than one cohort was analyzed in several studies, which were then analyzed separately. High MMP-2 expression was associated with a statistically significant decrease in OS in CRC patients (RR=1.75, 95% CI=1.34 to 2.29, *P*<0.001; Fig. 2A). Correspondingly, high MMP-2 expression was associated with significantly poorer DFS in CRC patients (RR=2.62, 95% CI=1.25 to 5.49, *P*<0.001; Fig. 2B). However, substantial heterogeneity was detected in the pooled HRs for both OS (I^2=60.4%, *P*=0.007) and DFS (I^2=71.6%, *P*=0.004).

Effect of MMP-9 expression on DFS and OS

We further evaluated the correlation between the expression of MMP-9 and the prognosis of CRC since its molecular structure and function are similar to those of MMP-2. Data for a total of 11 studies on cohorts meeting the criteria were extracted; 7 studies reported OS and 5 studies reported DFS. However, our analysis revealed no statistically significant associations between MMP-9 and OS (RR=1.48, 95% CI=0.97 to 2.24, *P*=0.069; Fig. 3A) or DFS (RR=1.60, 95% CI=0.87 to 2.94, *P*=0.133; Fig. 3B).
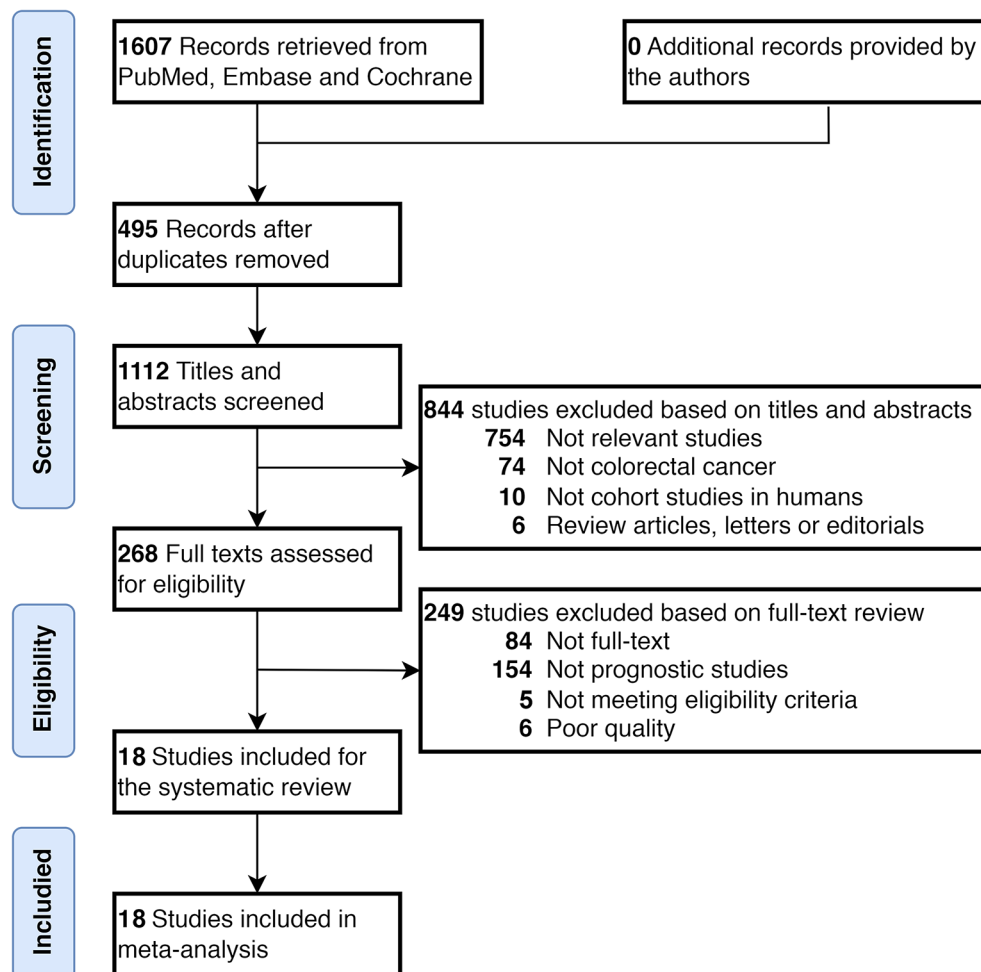
Fig. 1 Study selection flow diagram

The results also showed a high degree of study heterogeneity for OS (I^2=78.9%, *P*<0.001) and DFS (I^2=81.3%, *P*<0.001).

Effect of MMP-2 expression in different tissues on DFS and OS

To further evaluate the association between MMP-2 expression in different tissues and the clinical outcomes of CRC patients, we performed subgroup analyses of patients stratified according to MMP-2 expression in cancer tissue and normal tissue. The analysis revealed that MMP-2 expression in both cancer tissue (RR=1.90, 95% CI=1.29 to 2.79; Fig. 4A) and normal tissue (RR=1.59, 95% CI=1.17 to 2.17; Fig. 4A) significantly predicts poor OS in CRC patients. Moreover, we also independently evaluated the association between MMP-2 expression in cancer tissue and DFS (RR=2.12, 95% CI=1.09 to 4.11; Fig. 4B). The test for heterogeneity revealed positive associations of tissue MMP-2 expression with OS and DFS except for the normal tissue MMP-2 expression and OS (I^2=0.0%, *P*=0.825). However, there were not enough studies to analyze the effect of MMP-2 expression in normal tissue on DFS.

Effect of MMP-9 expression in cancer tissue on OS

According to the subgroup analysis, the expression of MMP-9 in cancer tissue was also associated with inferior OS, but this difference was not statistically significant (RR=1.40, 95% CI=0.85 to 2.29; Fig. 4C). A high degree of study heterogeneity was observed for this analysis (I^2=82%, *P*=0.000). However, few studies have analyzed the effect of MMP-9 expression in normal tissue on OS, and no study has reported the effect of MMP-9 expression in normal tissue on DFS.

Publication bias analysis and study quality assessment

All included studies were assessed for bias risk according to the NOS criteria, and all studies received an independent assessment and discussion score of 6 by 2 investigators, which further confirmed that the results were stable. Furthermore, a sensitivity analysis was randomly performed to assess the potential impact of each study on

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Table 1 Characteristics of the included studies

Author/year	Country	Study type	Total (Male: Female: unknown)	Follow-up (months)	NOS Score	Sample source	Cut-off value	Index	Number (high: low)	Outcome	Analysis type
Araújo, R. F., Jr, 2015	Brazil	Retrospective	108:72	60.0	8	Cancer Tissue	IHC staining > 25% cells	MMP-9	150:30	OS	Multi-variate
Araújo, R. F., Jr, 2015	Brazil	Retrospective	108:72	60.0	8	Cancer Tissue	IHC staining > 25% cells	MMP-2	153:27	OS	Multi-variate
A. M. Langers, 2008	Netherlands	Retrospective	124:91	>=120.0	8	Cancer Tissue	Protein level > 18.5 ng per mg protein	MMP-2	56:158	OS	Multi-variate
A. M. Langers, 2008	Netherlands	Retrospective	124:91	>=120.0	8	Cancer Tissue	Protein level > 125 ng per mg protein	MMP-9	14:166	OS	Multi-variate
A. M. Langers, 2012	Netherlands	Retrospective	113:85	>60.0	8	Normal Tissue	Protein level > 8.7 ng per mg protein	MMP-2	44:154	OS	Multi-variate
A. M. Langers, 2012	Netherlands	Retrospective	113:85	>60.0	8	Normal Tissue	Protein level > 1.6 ng per mg protein	MMP-9	150:48	OS	Multi-variate
Buhmeida, A, 2009	Finland	Retrospective	91:111	103.3 (median)	7	Cancer Tissue	IHC staining > 0	MMP-9	97:105	DFS	Multi-variate
Cho, Y. B, 2007	Korea	Retrospective	56:33	82.0 (median)	7	Cancer Tissue	IHC staining >=10% cells	MMP-9	33:56	DFS	Multi-variate
Deng, J, 2017	China	Retrospective	277:186	60.0 (median)	7	Cancer Tissue	IHC score >=4[a]	MMP-2	171:292	OS	Multi-variate
Dong, W, 2011	China	Retrospective	116:56	60.0	7	Cancer Tissue	IHC score >=6[b]	MMP-2	116:52	OS	Uni-variate
Hilska, M, 2007	Finland	Retrospective	162:189	NA	7	Normal Tissue	IHC score >=0.65[c]	MMP-2 Epithelial	44:307	OS	Multi-variate
Hilska, M, 2007	Finland	Retrospective	162:189	NA	7	Normal Tissue	IHC score >=0.65	MMP-2 Stromal	45:306	OS	Multi-variate
Inafuku, Y, 2009	Japan	Retrospective	49:60	NA	8	Cancer Tissue	IHC staining >=10% cells	MMP-2	32:77	DFS	Multi-variate
Inafuku, Y, 2009	Japan	Retrospective	49:60	NA	8	Normal Tissue	IHC staining >=10% cells	MMP-2	54:55	DFS	Multi-variate
M. Li, 2007	China	Retrospective	61:66	NA	6	Cancer Tissue	IHC score >=3[d]	MMP-2	21:106	DFS	Multi-variate
N. Salem, 2016	Saudi Arabia	Retrospective	63:55:9	NA	6	Cancer Tissue	IHC staining > 0	MMP-2	34:93	DFS	Multi-variate
N. Salem, 2016	Saudi Arabia	Retrospective	63:55:9	NA	6	Cancer Tissue	IHC staining > 0	MMP-9	46:81	DFS	Multi-variate
R. Peltonen, 2021	Finland	Retrospective	64:47	>=138.0	7	Cancer Tissue	IHC score >=2[e]	MMP-2	71:10	OS/DFS	Uni-variate
R. Peltonen, 2021	Finland	Retrospective	64:47	>=138.0	7	Cancer Tissue	IHC score >=2[f]	MMP-9	30:51	OS/DFS	Uni-variate
W. Wang, 2019	China	Retrospective	281:189	>=60.0	6	Cancer Tissue	IHC score >=6[g]	MMP-9	226:238	OS	Multi-variate

Table 1 (continued)

Author/year	Country	Study type	Total (Male: Female: unknown)	Follow-up (months)	NOS Score	Sample source	Cut-off value	Index	Number (high: low)	Outcome	Analysis type
X. Z. Yang, 2017	China	Retrospective	95:84	54.2 (mean)	6	Cancer Tissue	IHC score >=156[h]	MMP-9	91:88	OS	Multi-variate
Y. Ogata, 2006	Japan	Retrospective	254:188	64/87 (median)[i]	8	Cancer Tissue	IHC staining >=10% cells	MMP-9	135:207	DFS	Multi-variate
Y. Zhang, 2012	China	Retrospective	136:80	>=36.0	7	Cancer Tissue	IHC staining >=30% cells	MMP-9	69:147	OS	Multi-variate
Z. Sundov, 2008	Croatia	Retrospective	95:57	60.5 (median)	6	Cancer Tissue	IHC staining >=10% cells	MMP-2	84:68	OS	Multi-variate
Z. G. Zhou, 2011	China	Retrospective	81:60	59.0 (median)	6	Cancer Tissue	IHC staining >0	MMP-2	99:42	OS/DFS	Multi-variate

NOS:Newcastle–Ottawa scale, NA: Not available, IHC:Immunohistochemistry

[a] Staining intensity was scored as 0 (none), 1 (weak), 2 (moderate), or 3 (strong). The staining percentage was scored as 0 (none), 1 (<10%), 2 (10–50%), 3 (51–80%), or 4 (>80%). Intensity × percentage=staining score (0–12)

[b] Staining intensity was scored as 0 (none), 1 (weak), or 2 (strong). The staining percentage was scored as 0 (10%), 1 (10–25%), 2 (26–50%), 3 (51–75%), or 4 (>75%). Intensity + percentage=staining score (0–7)

[c] Staining intensity was scored as 0 (<5% of the cytoplasm staining), 1 (1+: 5–15% moderate or strong or 15–100% weak), 2 (2+: 15–50% or 15–100% moderate), or 3 (3+: 0–100% strong). The weighted staining score was determined by adding the fraction of cells stained at an intensity of 1 + to the fraction of cells stained at an intensity of 2 + multiplied by 2. The fraction of cells stained at an intensity of 3 + multiplied by 3 was added

[d] Staining intensity was scored as 0 (-), 1 (+), 2 (++), or 3 (+++). The staining percentage was scored as 0 (none), 1 (1–5%), 2 (6–25%), 3 (26–50%), 4 (51–75%), or 5 (>75%). Intensity + percentage=staining score (0–8)

[e] Staining intensity was scored as 0 (none), 1 (mild), 2 (moderate), or 3 (strong)

[f] Staining intensity was scored as 0 (none), 1 (scattered granules in some cancer cells), 2 (granules in all or some cancer cells), or 3 (several granules in all cancer cells)

[g] Staining intensity was scored as 0 (none), 1 (mild), 2 (moderate), or 3 (strong). The staining percentage was ranked as 1 (0–25%), 2 (26–50%), 3 (51–75%) or 4 (76–100%). Intensity × percentage = staining score (0–12)

[h] Staining intensity was scored as 0 (none), 1 (mild), 2 (moderate), or 3 (strong). The percentage of stained cells was scored as 0–100. intensity × percentage=staining score (0-300)

A. Expression of MMP-2 on overall survival (OS) of CRC patients

Study (year)		Risk ratio (95% CI)	% Weight
Araújo, R. F., Jr, 2015		1.36 (1.00, 1.87)	14.51
Deng, J, 2017		1.58 (1.05, 2.39)	12.69
Hilska, M, 2007 (1)		1.34 (0.67, 2.65)	8.27
Hilska, M, 2007 (2)		1.55 (0.83, 2.91)	9.08
Dong, W, 2017		6.69 (2.88, 15.53)	6.48
A. M. Langers, 2008		1.42 (0.98, 2.06)	13.42
A. M. Langers, 2012		1.72 (1.14, 2.59)	12.69
R. Peltonen, 2021		0.79 (0.37, 1.69)	7.31
Z. G. Zhou, 2011		2.51 (1.19, 5.28)	7.54
Z. Sundov, 2008		3.63 (1.79, 7.36)	8.02
Overall (I-squared = 60.4%, p = 0.007) **Random-effect analysis**		1.75 (1.34, 2.29)	100.00
NOTE: Weights are from random effects analysis			

.0644 — 1 — 15.5

B. Expression of MMP-2 on disease free survival (DFS) of CRC patients

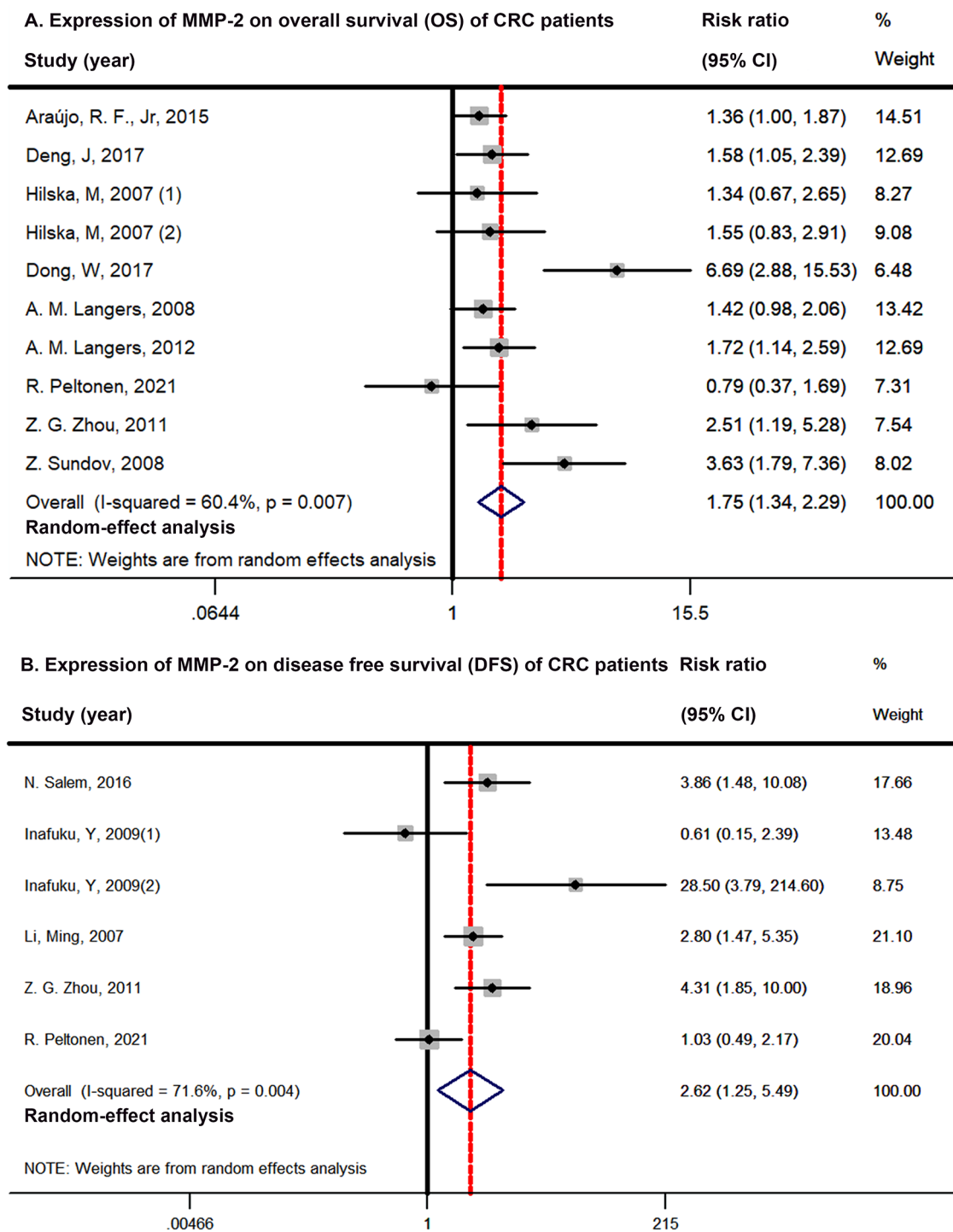| Study (year)		Risk ratio (95% CI)	% Weight
N. Salem, 2016		3.86 (1.48, 10.08)	17.66
Inafuku, Y, 2009(1)		0.61 (0.15, 2.39)	13.48
Inafuku, Y, 2009(2)		28.50 (3.79, 214.60)	8.75
Li, Ming, 2007		2.80 (1.47, 5.35)	21.10
Z. G. Zhou, 2011		4.31 (1.85, 10.00)	18.96
R. Peltonen, 2021		1.03 (0.49, 2.17)	20.04
Overall (I-squared = 71.6%, p = 0.004) **Random-effect analysis**		2.62 (1.25, 5.49)	100.00
NOTE: Weights are from random effects analysis			

.00466 — 1 — 215

Fig. 2 Effect of MMP-2 expression on the DFS and OS of CRC patients

our results. The results indicated that some studies significantly influenced the observed association between the expression of MMP-2 and MMP-9 and poor survival outcomes (Fig. 5). Notably, one study had opposite results compared to all other included studies, which led to a negative change in overall statistical significance for the MMP-9-related analysis. According to this article, high

MMP-9 expression in primary tumors indicates a better prognosis in patients with CRC, especially after resection of colorectal liver metastases. Through comparison, we found that the patients enrolled had both primary colorectal tumors and liver metastases, which implies that liver metastasis and surgery may change the expression of MMP-9 in the extracellular matrix. Similarly, the
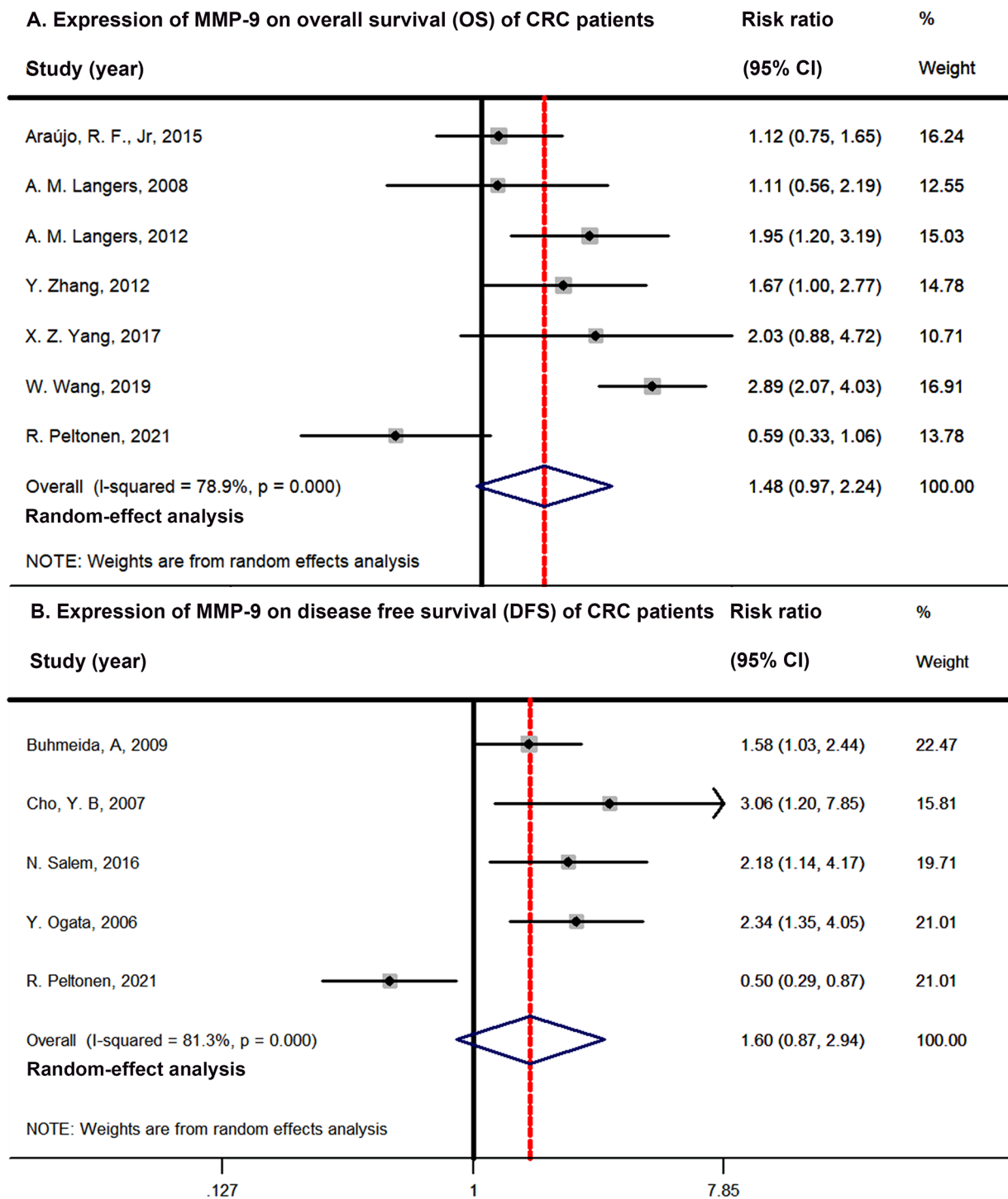
A. Expression of MMP-9 on overall survival (OS) of CRC patients

Study (year)	Risk ratio (95% CI)	% Weight
Araújo, R. F., Jr, 2015	1.12 (0.75, 1.65)	16.24
A. M. Langers, 2008	1.11 (0.56, 2.19)	12.55
A. M. Langers, 2012	1.95 (1.20, 3.19)	15.03
Y. Zhang, 2012	1.67 (1.00, 2.77)	14.78
X. Z. Yang, 2017	2.03 (0.88, 4.72)	10.71
W. Wang, 2019	2.89 (2.07, 4.03)	16.91
R. Peltonen, 2021	0.59 (0.33, 1.06)	13.78
Overall (I-squared = 78.9%, p = 0.000) Random-effect analysis	1.48 (0.97, 2.24)	100.00

NOTE: Weights are from random effects analysis

B. Expression of MMP-9 on disease free survival (DFS) of CRC patients

Study (year)	Risk ratio (95% CI)	% Weight
Buhmeida, A, 2009	1.58 (1.03, 2.44)	22.47
Cho, Y. B, 2007	3.06 (1.20, 7.85)	15.81
N. Salem, 2016	2.18 (1.14, 4.17)	19.71
Y. Ogata, 2006	2.34 (1.35, 4.05)	21.01
R. Peltonen, 2021	0.50 (0.29, 0.87)	21.01
Overall (I-squared = 81.3%, p = 0.000) Random-effect analysis	1.60 (0.87, 2.94)	100.00

NOTE: Weights are from random effects analysis

.127   1   7.85

Fig. 3 Effect of MMP-9 expression on the DFS and OS of CRC patients

subgroup sensitivity analysis showed that the pooled RR for MMP-9 was influenced the most by this study. When this study was excluded from the analysis, MMP-9 was a significant biomarker for poor outcomes in CRC patients (Supplementary Fig. 1).

Through the heterogeneity test, we chose random effect models if $I^2 > 50\%$. The publication bias in our study was assessed by Begg's test and funnel plots, all of which were generated with Stata software [31]. Funnel plots were used to assess publication bias for the main outcome, which was the effect of MMP-2 expression on DFS and OS, and the secondary outcome, which was the effect of MMP-9 expression on DFS and OS in our meta-analysis (Supplementary Fig. 2). The same was true for the
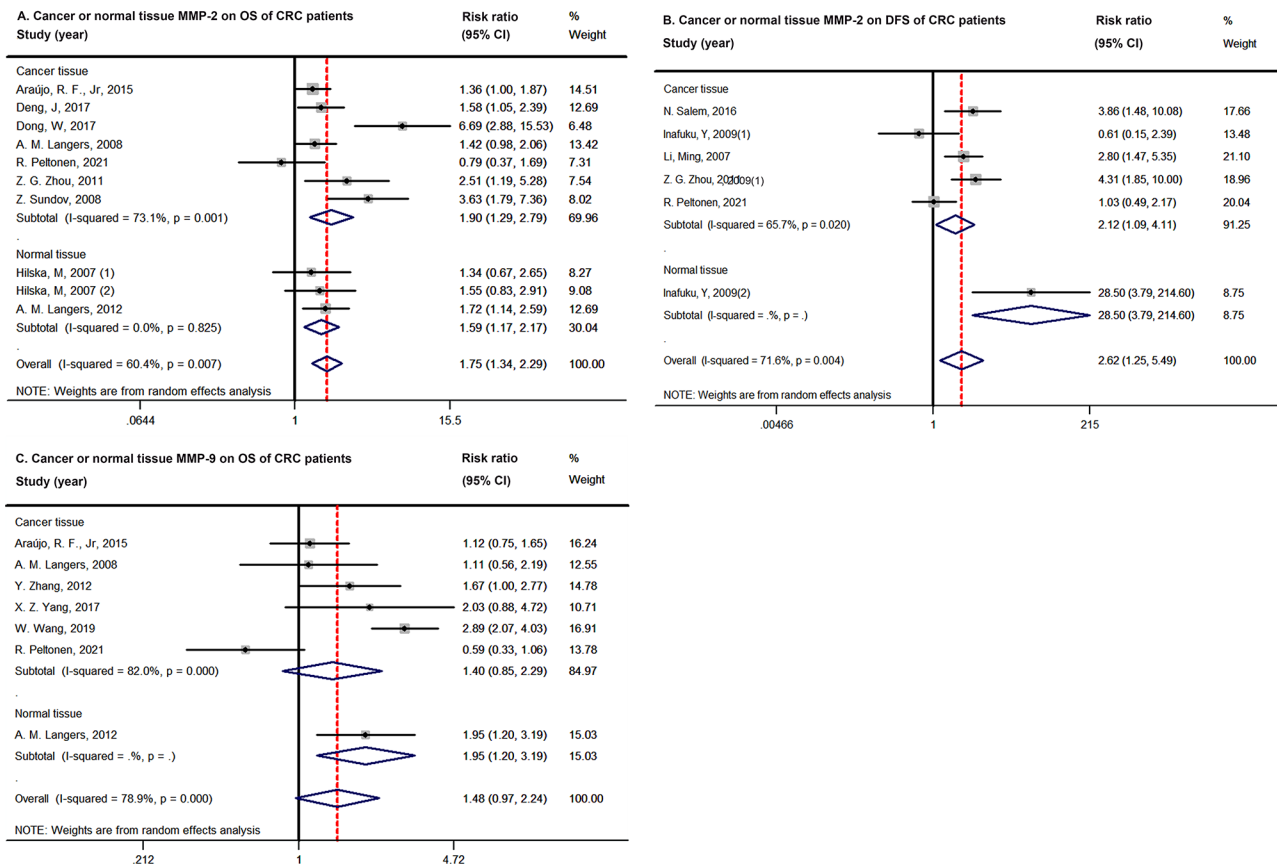
Fig. 4 Effect of MMP-2 and MMP-9 expression in different tissues on the DFS and OS of CRC patients

subgroup analysis, which involved assessment of the correlation of MMP-2 and MMP-9 expression in different tissues and the clinical outcomes of patients with CRC (Supplementary Fig. 3). The relatively significant asymmetry for both MMP-2 and MMP-9 was shown by Begg's test, with an overall p value greater than 0.151. Regarding subgroup analysis, Begg's test also indicated that no publication bias existed in the pooled analysis.

Discussion

In 2020, there were an estimated 1,148,515 new colorectal cancer (including anal cancer) diagnoses and 576,858 related deaths worldwide, making it the second most common cause of death among malignancies [1]. Unfortunately, disease recurrence and metastases after curative surgery with or without adjuvant chemotherapy contribute to many of these mortalities. Biomarkers that can predict local recurrence or distant metastasis should be identified, and drug targets should be explored to guide more personalized treatment. In addition, understanding the roles of these molecules in processes such as tumor growth, local invasion, and distant metastasis may help to elucidate the pathogenesis of CRC. MMP-2 and MMP-9, which are known as gelatinases, can digest type IV collagen and gelatin in the ECM. Previous studies have shown a positive correlation between the expression of MMP-2 and MMP-9 and tumor invasion and metastasis in CRC. To increase the likelihood of high-quality assessments of MMP-2 and MMP-9 in CRC, we searched for cohort studies with survival data and found 18 studies in which the effects of the expression of these MMPs on DFS, PFS, and/or OS were investigated.

MMP-2 plays a significant role in angiogenesis and affects cell adhesion by enhancing tumor invasion and distant metastasis through the degradation of collagen type IV in the ECM [17]. High MMP-2 expression is related to high-grade tumor stage, lymph node metastasis, and poor survival in CRC patients [19, 32, 33]. Similarly, the detrimental effect of MMP-2 expression on DFS and OS was evident in our study. Increased MMP-2 expression in both cancer and normal tissues was observed to be associated with worse outcomes CRC patients, highlighting its role as a potential prognostic biomarker for CRC. However, the relationship between MMP-2 expression and DFS in normal tissues was not analyzed because of the lack of studies. Overall, according to the RR, MMP-2 in cancer tissue may be a better index for predicting survival in CRC patients.
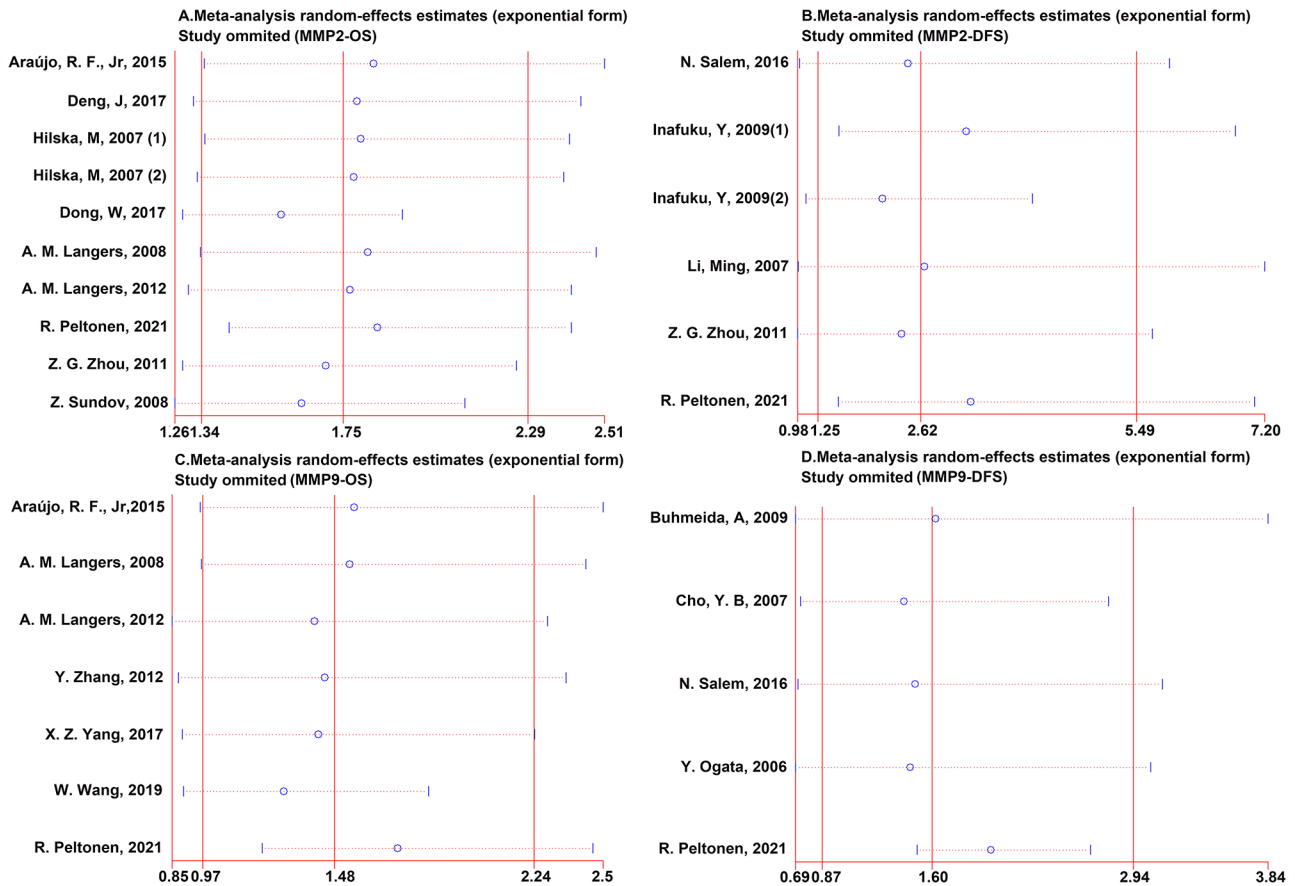
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Fig. 5 Sensitivity plots of studies included in the meta-analysis

As a member of the MMP family with complex domains, MMP-9 is capable of degrading a variety of components in the ECM, such as decorin, fibrillin, and collagen [34]. The overexpression of MMP-9 has been found to be a biomarker predictive of poor prognosis in various malignancies, including colorectal cancer. In contrast, several studies have reported that MMP-9 can suppress β-catenin, reduce reactive oxygen species (ROS) levels, and decrease DNA damage, suggesting its protective role in CRC [12, 13, 35]. In our study, although there was a poor prognosis in MMP-9-positive CRC patients, the difference in prognosis between these patients and MMP-9-negative patients was not statistically significant. Specifically, we observed that one study had opposite results compared to all other included studies. Therefore, the negative results may be related to the findings of the outlier study or to the protective role of MMP-9, which has been previously reported. This indicates the need for more studies. Generally, MMP-9 had no statistically significant effect on the clinical outcomes of CRC patients, which may indicate that the expression of MMP-2 is a better prognostic biomarker than the expression of MMP-9.

As MMPs are widely expressed in the ECM, MMP-2 and MMP-9 may have different characteristics and functions according to their location and relative expression. In our subgroup analysis, we analyzed the effect of MMPs, including MMP-2 and MMP-9, in normal tissue and cancer tissue on the prognosis of CRC. Notably, cancer tissue was inferred to be a more appropriate sample source for the detection of MMPs since the expression levels were more closely related to prognosis. However, MMP-9 expression in tumor tissue was not significantly associated with poor OS in CRC patients. The effect of MMP-9 in normal tissue on the prognosis of CRC was not assessed due to insufficient data in the included studies. Based on these limited results, we can infer that the expression of MMP-2 in tumor tissues has prognostic value and that the prognostic value of MMP-9 expression in different tissues cannot be confirmed. Overall, our study has shown that high expression of MMP-2 and MMP-9 tend to be associated with poor prognosis in CRC patients. Exploring related inhibitors may be a promising treatment for CRC. Recent advances have highlighted the ability of natural small molecules to inhibit tumor growth through various mechanisms, including the modulation of signaling pathways and inhibition of MMPs [36]. For

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instance, berberine and apigenin derivatives inhibit the activity and expression of MMP-2 and MMP-9 and demonstrate significant promise in cancer treatment [37, 38]. Future research should focus on the application of MMPs inhibitors in CRC treatment.

Several limitations of the present meta-analysis should be discussed. Given the relatively moderate number of included studies, the conclusion that MMP-2 is a prognostic indicator of cancer should be recognized with caution. In addition, given its retrospective nature, the grade of evidence was inferior to that of randomized controlled trials. Furthermore, the reason for the high heterogeneity may include the following factors. First, patient characteristics, including age, sex, tumor grade, complications and treatment regimens, were not taken into consideration. Second, the detection methods used for IHC among different laboratories and the cutoff values used vary. Third, there are not enough studies to demonstrate the relationship between the expression of MMP-9 and its expression in different tissues and the prognosis of colorectal cancer patients. Similarly, there is a lack of studies exploring the effect of MMP-2 in normal tissue on prognosis. Moreover, not only do MMP-2 and MMP-9 have prognostic value in CRC, but other MMPs, such as matrix metalloprotein-1 (MMP-1), matrix metalloprotein-7 (MMP-7), matrix metalloprotein-8 (MMP-8) and matrix metalloprotein-13 (MMP-13), are also potential prognostic markers [39–42].

Conclusions

The present meta-analysis demonstrated a significant association between high MMP-2 expression and a poor prognosis in CRC patients, especially in cancer tissue. Although we expected to that MMP-9 would have the same predictive effect, we only observed a nonsignificant trend. Considering the paucity of studies included, larger samples and well-designed prospective confirmation studies of these findings are warranted.

Abbreviations
CRC	Colorectal cancer
CI	Confidence interval
DFS	Disease-free survival
ECM	Extracellular matrix
HR	Hazard ratio
IHC	Immunohistochemistry
mCRC	Metastatic colorectal cancer
MMPs	Matrix metalloproteins
MDR	Multidrug resistance
NOS	Newcastle–Ottawa Scale
NA	Not available
OS	Overall survival
PFS	Progression-free survival
ROS	Reactive oxygen species
RR	Risk ratio

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