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Matrix metalloproteinase-9 expression is a marker of aggressive esophageal carcinoma: a meta-analysis

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Summary. *Background:* Matrix metalloproteinase-9 (MMP-9) is the proteolytic enzyme which degrades type IV collagen in basement membrane causing tumor invasion and metastasis. The purpose of the study is to evaluate whether MMP-9 expression is associated with the aggressiveness of esophageal carcinoma. *Method:* A search was performed on PubMed, EMBASE, Google scholar and the Cochrane database until March 2014. A meta-analysis was conducted to investigate the association between MMP-9 expression and the clinicopathological characteristics of esophageal cancer. *Result:* The final analysis included 673 esophageal cancer patients from 9 studies. MMP-9 expression was determined by the immunochemistry on biopsy or surgical specimens. The pooled data demonstrated that positive MMP-9 expression was significantly associated with poorer tumor differentiation (OR = 1.71, 95% CI: 1.13, 2.58; p = 0.01), lymph node metastasis (OR = 1.80, 95% CI: 1.20, 2.71; p = 0.005) and vascular invasion (OR = 2.55, 95% CI: 1.54, 4.21; p = 0.003). However, there were no significant associations between MMP-9 expression and advanced tumor staging, depth of tumor invasion and lymphatic invasion. *Conclusion:* The results of the meta-analysis strongly indicate that MMP-9 expression could function as a valuable marker for predicting the aggressiveness of esophageal cancer.

Key words: matrix metalloproteinase-9, esophageal cancer, biomarker, meta-analysis

«L'espressione della matrice di metalloproteinasi-9 è un marcatore del carcinoma aggressivo esofageo: una meta-analisi»

Riassunto. *Background*: La matrice di metalloproteinasi-9 (MMP-9) è un enzima proteolitico che degrada il collagene di tipo IV nella membrana basale causando la dispersione del tumore e le metastasi. Lo scopo di questo studio è quello di valutare se l'espressione di MMP-9 è associata all'aggressività del carcinoma esofageo. *Metodi:* E' stata svolta una ricerca su PubMed, EMBASE, Google Scholar e sul database Cochrane fino a marzo 2014. E' stata condotta una meta-analisi al fine di indagare l'associazione tra l'espressione di MMP-9 e le caratteristiche clinico-patologiche del cancro esofageo. *Risultati*: L'analisi finale comprendeva 673 pazienti con cancro all'esofago provenienti da 9 studi. L'espressione di MMP-9 è stata determinata sulla base di analisi di immunoistochimica (IHC) su biopsie o campioni chirurgici. I dati comparati hanno dimostrato che l'espressione positiva di MMP-9 era significativamente associata alla scarsa differenziazione del tumore (OR = 1.71, 95% CI: 1.13, 2.58; p = 0.01), alle metastasi ai linfonodi (OR = 1.80, 95% CI: 1.20, 2.71; p = 0.005) e all'invasione vascolare (OR = 2.55, 95% CI: 1.54, 4.21; p = 0.003). Tuttavia, non si è riscontrata una associazione significativa tra l'espressione MMP-9 e la stadiazione avanzata del tumore, l'invasione più in profondità del tumore e l'invasione linfatica. *Conclusioni*: I risultati della meta-analisi indicano fortemente che l'espressione di MMP-9 potrebbe funzionare come valido marcatore per predire l'aggressività del cancro esofageo.

Parole chiave: espressione della matrice di metalloproteinasi-9, cancro esofageo, bio-marcatore, meta-analisi

Introduction

Esophageal carcinoma, including squamous cell carcinoma and adenocarcinoma is an aggressive malignancy with a poor prognosis. The main risk factors for squamous cell carcinoma include smoking, alcohol consumption, caustic injury of the esophagus, and frequent consumption of hot beverages. In addition, gastroesophageal reflux disease, obesity, and the decreasing prevalence of Helicobacter pylori infection may contribute to the development of adenocarcinoma of the esophagus. The majority of patients with esophageal carcinoma die within 1 year of diagnosis, and only 8-20% of patients are alive at 5 years (1-3). Most esophageal cancer patients present with advanced disease and more than 30% of patients have metastatic disease at the time of presentation (4-5).

The processes involved in tumor invasion and metastasis include tumor cell invasion to lymphatic and blood vessels, extravasations from the lymph and blood vessels, proliferation and induction of angiogenesis (6-8).

Matrix metalloproteinases (MMPs) are a large family of calcium-dependent-zinc containing endopeptidases, which are responsible for tissue remodeling and degradation of the extracellular matrix. Currently, 21 family members of MMPs have been identified in humans by cloning and sequencing (9, 10).

MMP-9 is also known as gelatinase B, 92kDa gelatinase or 92kDa type IV collagenase, which is produced by tumor cells (11, 12). MMP-9 has long been recognized as a key enzyme for proteolysis degradation of type IV collagen, which is the main component of basement membrane. Moreover, the expression of MMP-9 is involved in tumor angiogenesis by increasing the release of vascular endothelial growth factor, which is known to be a potent inducer of angiogenesis. These processes play a crucial role in malignant tumor growth, tumor invasion, and metastasis (13, 14).

Although several studies have attempted to assess the role of MMP-9 in the clinicopathological characteristics of esophageal cancer, there has been a significant disparity between the findings of these studies. In an attempt to clarify the relationship between MMP-9 expression and the clinicopathological characteristics of the patients, we conducted a meta-analysis based on the published literature to evaluate whether MMP-9 expression may be a biomarker for aggressive forms of esophageal cancer.

Methods

Data sources and search strategies

An electronic literature search was performed on PubMed, EMBASE, Google scholar and the Cochrane database. The search terms "Matrix metalloproteinase", "Matrix metalloproteinases-9 expression", and "esophageal cancer" were used as keywords to identify all studies published through March 2014 evaluating the correlation between MMP-9 expression and clinicopathological characteristics of esophageal cancer patients. The relative articles and references to selected article were also searched for additional studies for potential inclusion. This meta-analysis was performed according to the guidelines for preferred reporting items for systematic reviews and meta-analyses (PRISMA) 2009 (15).

Data extraction

The two reviewers independently extracted the following information from the studies selected: author's name, country of origin, year of publication, study design, number of patients, patient characteristics (age, sex), source of tissue for MMP-9 analysis, methods for MMP-9 assessment, antibodies and criteria for positive MMP-9 expression, number of positive MMP-9 expressions, clinicopathological characteristics and tumor staging. Extracted data were cross-checked to reach consensus and were entered in a computerized spreadsheet for analysis.

Study selection and eligibility criteria

Abstracts of the articles identified by searching were carefully reviewed. Studies were included if they had a prospective or retrospective cohort design. The inclusion criteria were as follows: (1) study published in English, (2) esophageal carcinoma proven by pathological diagnosis, (3) method described for measuring

MMP-9 expression and clear statement of the criteria for positive MMP-9 expression, (4) the association reported between MMP-9 expression and clinicopathological characteristics of patients. Studies were excluded if: (1) non-English articles, (2) review articles, (3) in-vitro studies, (4) insufficient in clinicopathological data for analysis.

The quality of the studies that were included in the meta-analysis was further evaluated using the Newcastle-Ottawa Scale. The maximum score possible was 9 points, which represents the highest methodological quality (16).

Statistical analysis

The meta-analysis was performed using Review Manager Software (RevMan version 5.2.6) provided by the Cochrane Collaboration (Nordic Cochrane center, Cochrane Collaboration; Copenhagen, Denmark). Cochran's chi-square-based Q-statistic test was applied to assess between-study heterogeneity. I^2 was used to test for heterogeneity between the studies included.

The clinicopathological characteristics and the long-term survival rate outcomes of patients were analyzed using the Manzel-Haenszel method to generate a pooled odds ratio (OR) that represented the association of these outcomes with the expression of MMP-9. Dichotomous variables were analyzed by assessing the OR of an expression of MMP-9 in each clinicopathological parameter, along with 95% confidence intervals (CI). An OR > 1 suggested that MMP-9 expression was associated with the clinicopathological parameters and the point estimate of the OR was considered statistically significant at the $P < 0.05$ level if 95% CI did not include the value 1.

We adopted the random-effect model, which is a more conservative way of calculating OR assuming a high level of variation between studies and uses a weighted average of the effects reported in different studies to calculate levels of association. Publication bias was assessed by visual examination of a funnel plot; asymmetry was formally assessed using both Egger's linear regression test and the rank correlation test (Begg's test).

Results

The initial search identified a total of 258 potential articles. After screening, nine articles matched our inclusion criteria and were deemed suitable for this meta-analysis. The PRISMA diagram of the search process is shown in Figure 1. Reviews of data extraction showed 100% agreement between two reviewers. The pooled studies included 673 esophageal cancer patients (643 squamous cell carcinoma, 24 adenocarcinoma, and 6 undifferentiated carcinoma). None of the patients underwent pre-operative neo-adjuvant chemotherapy or radiation therapy.

All of these nine studies used the immunohistochemistry (IHC) method for measurement of MMP-9 expression. Tissue biopsies and surgical specimens were used as sources for analysis of MMP-9 expression. There was a difference in the criteria for the cutoff level to determine the positive MMP-9 expression in each study included, which varied from 10-30% staining to diffuse staining of IHC in tumor cells. The percentages of positive cases for MMP-9 expression detected by IHC were 55.27% (372 out of 673 patients). The Newcastle-Ottawa Scale confirmed the moderate to good quality of the studies included, their scores varying from six to eight points. The characteristics of the 9 studies included are shown in Table 1.

MMP-9 expression and tumor staging

Six studies (17-22) including 409 patients reported the association between MMP-9 expression and staging of esophageal cancer as outcomes. The purpose of this meta-analysis was to compare the expression of MMP-9 in early stages (stage I-II) with advanced stage cancer disease (stage III-IV). The percentage of MMP-9 expression in patients with stage I-II and stage III-IV groups was 53.1% (112/211) and 64.1% (127/198) respectively. There was moderate heterogeneity between the studies ($P = 0.05$, $I^2 = 56\%$). Forest plots illustrating the association between MMP-9 expression and tumor staging are given in figure 2.

The pooled analysis of the OR from all 409 patients indicated that MMP-9 expression was not significantly associated with advanced stage esophageal cancer (OR = 1.29, 95% CI = 0.57–2.91, P = 0.54).

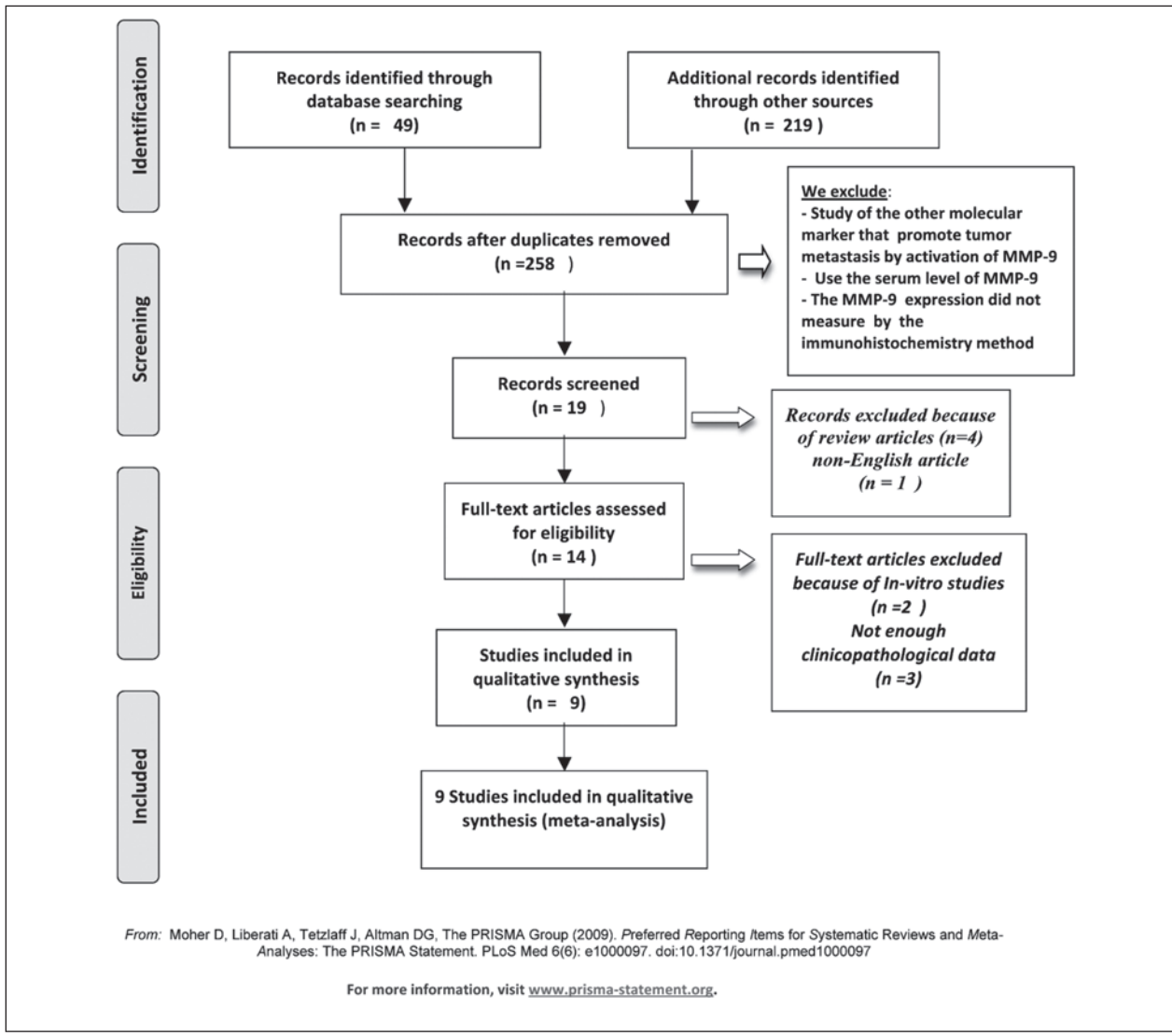

Figure 1. Selection process for inclusion of studies in the meta-analysis.

No evidence of publication bias was observed in either Egger's test (P=0.858) or the rank correlation test (P=0.624). The funnel plot of the association between MMP-9 expression and tumor staging shows a symmetrical distribution as illustrated in figure 3.

MMP-9 expression and clinicopathological parameters

The secondary aim of our study was to investigate the association between MMP-9 expressions in tumor tissue as detected by the IHC method, and the poor clinicopatholocial characteristics of esophageal cancer. The pooled data from all the studies included were carefully evaluated. We extracted the data which reported the association between MMP-9 expression and depth of tumor invasion, tumor differentiation, lymph node metastasis and lymphatic-vascular invasion with a view to analyzing the data.

Three studies (18, 22, 25) including 283 patients were conducted to access the association between the MMP-9 expression and the depth of tumor invasion (T1-2 versus T3-4). The pooled odds ratio indicated that MMP-9 expression was not significantly associated with the depth of tumor invasion (T3-4) (OR =

Table 1. Characteristics of 9 studies included in the meta-analysis.

Study (first author)	Country	Year of publication	Number of patient	Age (years)	Male	Histology	Source of MMP-9 analysis	Method for MMP-9 analysis	Antibody used for MMP-9 analysis	Criteria for MMP-9 expression positive	MMP -9 Expression positive (%)	Staging	Matching	Newcastle Ottawa Quality score
El-shahat M	Egypt	2004	38	34-67	26	ESCC 24 EADC 8 UDEC 6	Tissue from biopsy specimen	Immunohisto chemistry	Anti MMP-9 [MMP-9(92Kd Collegenase IV)Ab-9],Neo marker lab.,USA	Diffuse staining in cytoplasm of carcinoma cells	71.0%	I - IV	a,b,c,d,e,f	6
Gu ZD	China	2005	208	38-78 (mean 60)	162	ESCC	Tissue from surgical specimen	Immunohisto chemistry	Mouse antihuman MMP-9 antibody (15W2,1:20), Novocastra lab.	Staining > 10% of carcinoma cells	49.0%	I - III	a,b,c,d,e,f,g,i	7
Tanioka Y	Japan	2003	55	44-85 (mean 65.2)	41	ESCC 54 EADC 1	Tissue from surgical specimen	Immunohisto chemistry	Antihuman MMP-9,First fine Chemical,Japan	Staining > 10% of carcinoma cells	47.3%	T1a - T2 N0 - N1	a,b,c,e,f,g,h,i	8
Ohashi K	Japan	2000	148	NA	NA	ESCC	Tissue from surgical specimen	Immunohisto chemistry	Anti MMP-9 (56-2A4,1:20) ,Fuji chemical industries,Japan	Staining > 50% of invading nest area	52.7	T1a - T2 N0 - N1	a,b,c,e,f,g,h,i	7
Li Y	China	2009	58	60 (mean)	36	ESCC	Tissue from surgical specimen	Immunohisto chemistry	Mouse antihuman MMP-9 antibody, Zhongshan Bio Co.,China	Staining > 10% of carcinoma cells	60.3%	I - III	a,b,c,d,e,f,g	7
Samantaray S	India	2004	58	NA	40	ESCC	44 Tissue from surgical specimen 14 Tissue from biopsy specimen	Immunohisto chemistry	Mouse anti MMP-9 monoclonal antibody (1:100),Oncogene Research,Mass.,USA	Staining in tumor cell cytoplasm and /or stromal fibroblasts	55.0%	T1 - T4 N0 - N1	a,b,c,d,e,f,g	6
Murray G	U.K.	1998	46	34-70	29	ESCC	Tissue from surgical specimen	Immunohisto chemistry	Anti MMP-9 monoclonal antibody (2C3,950W, 1:100),British Biotech plc.,U.K.	Diffuse staining in cytoplasm of carcinoma cells	70.0%	IIa - IV	a,b,c,d,e	6
Koyama H	Japan	2000	30	59-76	25	ESCC	Tissue from surgical specimen	Immunohisto chemistry	Anti MMP-9 monoclonal antibody (56-2A4),Nichirei Co.,Japan	Staining > 30% of carcinoma cells	60.0%	I - IV	a,b,c,d,e,g,h,i	6
Mroczko B	Poland	2008	32	49-77	28	ESCC 17 EADC 15	Tissue from surgical specimen	Immunohisto chemistry	Mouse antihuman MMP-9 antibody (15W2,1:40), Novocastra lab.	Semi-quantitative scale : 0 = no reaction, 1= weak reaction ,2 = moderate reaction, 3 = intense reaction	81.0%	I - IV	a,b,c,d,e,g	7

Abbreviation: ESCC = Esophageal squamous cell carcinoma, EADC = Esophageal adenocarcinoma, UDEC = Undifferentiated esophageal carcinoma, NA = not available, a = age, b = sex, c = histological type, d = TNM staging, e = depth of tumor invasion, f = tumor differentiation, g = lymph node metastasis, h = lymphatic invasion, i = vascular invasion

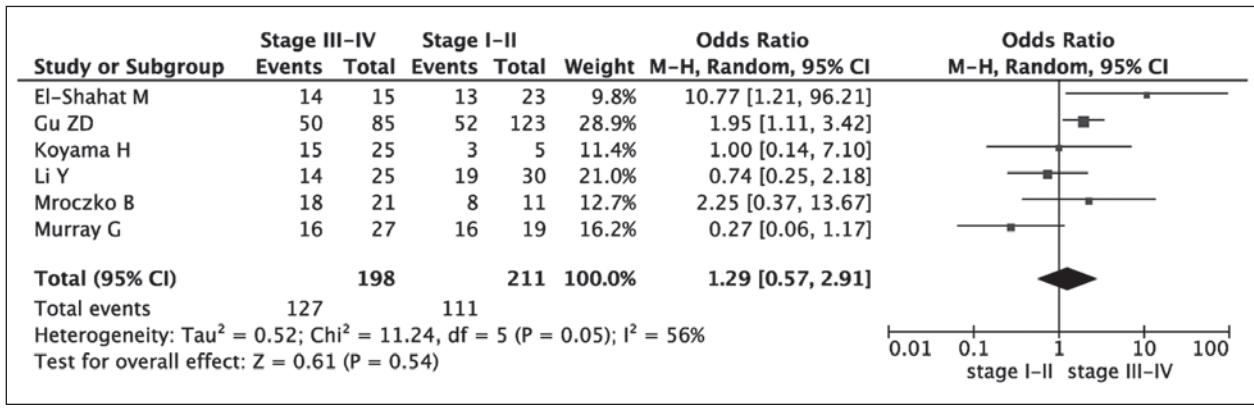

Figure 2. Forest plots of the association between MMP-9 expression and tumor staging.

0.06, 95% CI =-0.06–0.20, P = 0.35). There was no significant heterogeneity between the studies (P= 0.69, I^2 = 0%) and no evidence of any significant publication bias was observed on either Egger's test (P=0.493) or the rank correlation test (P=0.602).

A total of six studies (17-19) including 490 patients were enrolled to access the association between MMP-9 expression and tumor differentiation (poor tumor differentiation versus good/moderate tumor differentiation). The pooled odds ratio indicated that MMP-9 expression was significantly associated with poorer tumor differentiation (OR = 1.71, 95% CI =1.31–2.58, P = 0.01). There was no significant heterogeneity between the studies (P= 0.43, I^2 = 0%) and no evidence of a significant publication bias was observed on either Egger's test (P=0.836) or the rank correlation test (P=0.851).

A total of seven studies (18, 19, 21, 23-25) including 501 patients had been conducted to assess the association between MMP-9 expression and lymph node metastasis. The pooled odds ratio indicated that MMP-9 expression was significantly associated with lymph node metastasis (OR = 1.80, 95% CI =1.20–2.71, P = 0.005). There were no significant heterogeneity between the studies (P= 0.37, I^2 = 8%) and no evidence of any significant publication bias was observed on either Egger's test (P=0.663) or the rank correlation test (P=0.453).

Three studies (21, 24, 25) including 160 patients had been conducted to assess the association between

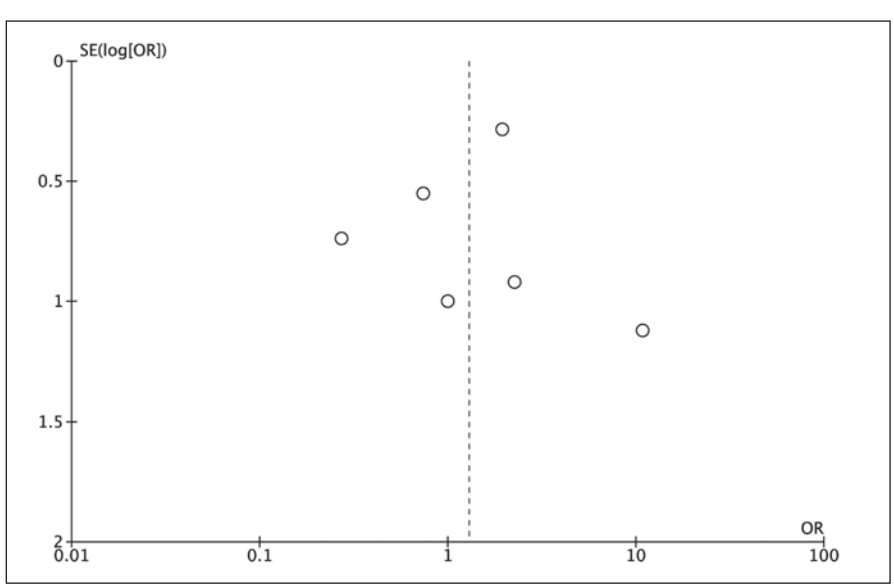

Figure 3. Funnel plot of the association between MMP-9 expression and tumor staging.

MMP-9 expression and lymphatic invasion. The pooled odds ratio indicated that MMP-9 expression was not significantly associated with lymphatic invasion (OR=2.90, 95% CI=0.66–12.68, P = 0.16) with the evidence of substantial heterogeneity between the studies (P=0.03, I^2=71). No evidence of any significant publication bias was observed on either Egger's test (P=0.461) or the rank correlation test (P=0.602).

A total of four studies (18, 21, 24, 25) including 368 patients were designed to assess the association between MMP-9 expression and vascular invasion. The pooled odds ratio indicated that MMP-9 expression was significantly associated with vascular invasion (OR = 2.55, 95% CI =1.54–4.21, P = 0.0003). There was no heterogeneity between the studies (P= 0.85, I^2 = 0%) and no evidence of a significant publication bias was observed on either Egger's test (P=0.793) or the rank correlation test (P=0.497). Table 2 summarizes the meta-analysis results on the association between MMP-9 expression and clinicopathological parameters.

Discussion

Esophageal cancer affects more than 450,000 people worldwide, and by nature has highly aggressive behavior. The prognosis in patients with esophageal carcinoma has improved significantly over the past few decades but remains poor with an overall 5-year survival of 37% in localized disease and 3% in advanced stage disease respectively. The proportion of patients with in situ and local cancer at the time of diagnosis remains lower than 30% (26, 27).

The results of previous studies concerning the clinic-pathological characteristics which were represented as predictors of unfavorable prognosis in esophageal cancer patients, including tumor size, poor tumor differentiation, depth of tumor invasion, lymph node metastasis, perineural invasion, lymphatic and vascular invasion (28-30). Lymph node metastasis is the most important prognostic factor in esophageal cancer. The overall 5-year survival rate after surgical resection is 70% -92% for patients without nodal involvement, but only 18-47% for patients with nodal metastasis (31).

MMP-9 is involved in the processes of tumor growth and metastasis by degradation of the main component of the extracellular matrix in basement membrane such as type IV collagen, laminin, entactin, proteoglycans, and glycosaminoglycan (32). Additionally, MMP-9 is involved in the induction of angiogenesis of tumor cells. These processes play a crucial role in tumor proliferation, tumor cell invasion and distant metastasis (33, 34).

The results of pooled data in this meta-analysis including 673-esophageal cancer patients from 9 studies demonstrated that the expression of MMP-9 was significantly associated with poorer tumor differentiation, lymph node metastasis and vascular invasion, which were an unfavorable clinicopathological characteristics in esophageal cancer patients. However, the expression of MMP-9 was not significantly associated with the depth of tumor invasion (T3-4), advanced tumor staging (stage III-IV) or lymphatic invasion. The evidence from our study supports the view that the expression of MMP-9 in tumor tissue could potentially be a valuable marker for the aggressiveness of esophageal cancer.

According to the results of this meta-analysis, which demonstrated that MMP-9 expression is sig-

Table 2. Association between MMP-9 expression and clinicopathological parameters of esophageal cancer patients

Clinicopathological parameters	No. of studies	n	OR	95%CI	P Value	Heterogeneity		Eager's test	Rank-correlation test
						I2	P		
Depth of tumor invasion	3	283	0.06	-0.07-0.20	0.35	0%	0.69	0.493	0.602
Poorer tumor differentiation	6	490	1.17	1.31-2.58	0.01*	0%	0.43	0.836	0.851
Lymph node metastasis	7	501	1.80	1.20-2.71	0.005*	8%	0.37	0.663	0.453
Lymphatic invasion	3	160	2.90	0.66-12.68	0.16	71%	0.03	0.461	0.602
Vascular invasion	4	368	2.55	1.54-4.21	0.0003*	0%	0.85	0.793	0.497

OR = Odds ratio, 95%CI = 95% Confidential interval

* Statistical significant

nificantly associated with lymph node metastasis it is nevertheless not significantly associated with the depth of tumor invasion. This finding could be applied to clinical practice by using endoscopic ultrasonography (EUS), computed tomography (CT) and ^{18}F-Fluoro-2-deoxy- D-glucose positron emission tomography (FDG-PET) preoperatively in order to maximize the accuracy of lymph node metastasis evaluation. Particularly in cases where MMP-9 expression is detected in superficial cancers of the esophagus.

The limitations of this meta-analysis include 1) only articles published in English were included in the meta-analysis, which may lead to publication bias. However, the statistical evaluation revealed that there was no evidence of publication bias encountered in this meta-analysis. 2) our results were somewhat complicated by the fact that heterogeneity was identified in our meta-analysis of the association between MMP-9 expression and tumor staging and lymphatic invasion.

Given this, it seems likely that the heterogeneity that we did observe in the meta-analysis can be explained by using a different source of tissue specimens for MMP-9 analysis, different types of antibodies and differing criteria used for the cutoff level to determine the positive MMP-9 expression. However, to compensate for this effect, we adopted a random-effects model to calculate the OR, which affords a more conservative result in situations where significant heterogeneity occurs.

In addition, none of the studies included reported the long-term survival of esophageal cancer patients. Thus, the analysis of the association between MMP-9 expression and survival of the patients was impossible to evaluate in this study.

Furthermore, the expression of MMP-9 in cancer cells may have a potential role as a novel therapeutic target for the treatment of human cancers. There are several matrix metalloproteinase inhibitors (MMPIs) that have been evaluated by clinical trials in cancer therapy such as Batimastat and Marimastat, which are relatively nonspecific, inhibiting the activity of MMP-1, -2, -3, -7, and -9 (35, 36).

Bramhall SR *et al.* conducted a randomized, double blind control study to evaluate the therapeutic efficacy of Marimastat in patients with advanced gastric cancer. The results demonstrated a survival benefit in the Marimastat group (P=0.07, hazard ratio=1.23). The median overall survivals in the Marimastat and the control group were 5.2 months and 4.5 months with 2-year survival of 9% and 3% respectively. (37) This evidence suggests that the expression of MMP-9 is an essential therapeutic target in cancer treatment. However, the therapeutic efficacy of MMPIs on treatment of esophageal cancer should be evaluated in a randomized control study in the future.

Conclusion

The meta-analysis demonstrates a significant association between MMP-9 expression and poor clinicopathological parameters such as poor tumor differentiation, lymph node metastasis and vascular invasion. We found strong evidence that MMP-9 could function as a marker to predict the aggressiveness of esophageal cancer.

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