RESEARCH COMMUNICATION

Matrix Metalloproteinase-9 as a Prognostic Factor in Gastric Cancer: A Meta-Analysis

Qiong-Wen Zhang[1&], Lei Liu[1&*], Ru Chen[2&], Yu-Quan Wei[1], Ping Li[1], Hua-Shan Shi[1], Yu-Wei Zhao[1]

Abstract

Background: Matrix metalloproteinase-9 (MMP-9) is associated with disruption of basement membranes of blood vessels and promotion of metastasis through the lymphatics. However, its prognostic value for survival in patients with gastric cancer remains controversial. Method: We therefore conducted a meta-analysis of the published literature in order to clarify the impact of MMP-9. Clinical studies were selected for further analysis if they provided an independent assessment of MMP-9 in gastric cancer and reported analysis of survival data according to MMP-9 expression. Results: A total of 11 studies, covering 1700 patients, were included for meta-analysis. A summary hazard ratio (HR) of all studies and sub-group hazard ratios were calculated. The combined HR suggested that a positive MMP-9 expression had an impact on overall survival: 1.25 (95% confidence interval 1.11-1.40) in all eligible studies; 1.13 (1.06-1.20) in 8 studies detecting MMP-9 by immunohistochemistry; 1.36 (1.12-1.65) in 7 studies from Asia. Only one study for DFS showed a significant impact on disease free survival (HR 1.73, 95%CI 1.27-2.34). Conclusions: Our findings suggested that MMP-9 protein expression might be a factor for a poor prognosis in patients with gastric cancer. However, the association was rather weak, so that more prospective studies should further explore the prognostic impact of MMP-9 mRNA and correlations between MMP-9 and clinicopathological characteristics.

Keywords: Matrix metalloproteinase-9 - gastric cancer - meta-analysis; prognosis - survival

Introduction

Despite the incidence and mortality rate of gastric cancer have fallen over past several decades, gastric cancer is still the fourth most common cancer and the second leading cause of cancer-related death in the world (Crew and Neugut, 2006; Brenner et al., 2009; Jemal et al., 2011). In the past decades, intensive efforts have been made to identify tools to improve prognostication of gastric cancer, however, in clinical practice, we mostly only rely on clinic-pathological features to predict patients' outcome and these prognostic factors do not fully predict individual clinical outcome especially on patients after curative resection and/or node-negative patients (Harrison and Fielding, 1995; Allgayer et al., 1997; Zhao et al., 2010; Kim et al., 2011; Seshadri, et al., 2011). Therefore, to guide clinical practice and explain variability of survival, more prognostic markers are expected to be identified.

Before metastasizing, tumor cells have to complete a multistep progression including tumor cell detachment, local invasion, motility, et al, in which causative molecules such as matrix degradation enzymes can be regarded as prognostic factors (Yasui et al., 2005). Matrix metalloproteinase (MMPs) are a family of enzymes that are found in extracellular milieu of various tissues which play important roles in degrading extracellular matrix (ECM) and angiogenesis in tumor invasion and metastasis. Overexpression of MMPs can promote tumor cell detachments and metastasis which have been associated with malignancy with poor clinical outcome (Johansson et al., 2000; McCawley and Matrision, 2000; Stetler-Stevenson, 2001; Rundhaug, 2005). There are currently 26 known MMPs, which share a number of common structural and functional similarities, and differ in their substrate specificity (Park et al., 2000). Previous studies have suggested that MMP-9, a member of MMPs family, can degrade type IV collagen which is a major constituent of basement membranes of blood vessels and promote lymph node metastasis especially in gastric cancer (Ueda et al., 1996; Nabeshima et al., 2002). However, the function of MMP-9 is controversial. It is widely accepted that MMP-9 is associated with lymph node metastasis, while there is no final conclusion on whether it is associated with invasion, distant metastasis and TNM stage (Tang et al., 2008; Zhao et al., 2009). Therefore, the prognostic value of MMP-9 in gastric cancer is still unknown.

In this study, we sought to conduct a meta-analysis to estimate the prognostic importance of MMP-9 level for

[1]Department of Medical Oncology, Cancer Center, State Key Laboratory of Biotherapy, West China Hospital, Sichuan University, Chengdu, [2]Department of Oncology, The People's Hospital of Xinjiang Uygur Autonomous Region, Urumqi, China &Equal contributors
For correspondence: liuleihx@gmail.com

over survival (OS) and disease-free survival (DFS) among patients with gastric cancer, aiming to gain insights into whether MMP-9 could provide useful guidance in the biological understanding and treatment of gastric cancer.

Materials and Methods

Identification and Eligibility of Relevant Studies. A computer- aided literature search of Pubmed (MEDLINE) 1950-present and EMBASE was conducted. The search strategy was based on a combination of Medical Subject Headings (MeSH) and text words relating to "matrix metalloproteinase", "gastric cancer", "gastric carcinoma", "stomach cancer", "stomach carcinoma" and "gastric adenocarcinoma." Reference lists from identified primary studies and review articles were then searched to identify additional eligible studies missed by electronic search strategies.

Two independent reviewers (QWZ and LL) read titles and abstracts of all candidate articles. Articles that could not be categorized based on title and abstract alone were retrieved for full-text review. Articles were independently read and checked for inclusion criteria of articles in this study. Any disagreement in quality assessment and data collection was discussed and solved together.

Study inclusion/ exclusion criteria. Inclusion criteria for this study were as follows: (1) proven diagnosis of gastric cancer, (2) MMP-9 evaluation using ELISA or immunohistochemical method, (3) association of MMP-9 with overall survival (OS), and/or disease free survival (DFS). Reviews, letters to the editors, and articles published in a book or in languages other than English were excluded. We avoided duplication of data by examining the names of all authors and medical centers involved for each article. Authors that published multiple reports on the same sample were included once. We did not weight each study by a quality sore because no such sore had received general agreement for meta-analysis of observational studies (Altman, 2001).

Data extraction.Two authors (QWZ and LL) used a standard data collection form and carefully extracted information from each included studies. The following data were collected: (1) article data including publication date, first author's name and country; (2) demographic data regarding inclusion criteria, age, sex, number of patients and percentage of MMP-9 positive; (3) tumor data including staging and distant metastasis; (4) survival data including OS, DFS and follow-up period; (5) method of MMP-9 measurement, cut-off used for assessing MMP-9 positivity. Any differences in the data extraction were resolved together by two authors.

Statistical analysis.Hazard ratios (HRs) and its 95% confidence intervals (CIs) were used to estimate the association between MMP-9 and patient's prognosis. For those HRs that were not given directly in the published articles, the published data including the number of patients at risk in each groups, the total number of events and figures from original articles were used to estimate the HR according to the methods described by Parmar et al (Parmar et al., 1998). If the only exploitable survival data were in the form of figures, we read Kaplan-Meier curves

by Engauge Digitizer version 4.1 (free software downloaded from http://sourceforge.net) and extracted survival rate from them to reconstruct the HR and its standard error (SE). All the data analyses were performed with Stata version 11.0 (Stata Corporation, College Station, TX, USA) and we use Q-tests and P-values to estimate the heterogeneity. If P-value was greater than 0.05 which indicated a lack of heterogeneity among studies, a fixed-effects model was used to calculate the HR and its 95%CI according to the method of Mantel and Haenszel (Mantel and Haenszel, 1959). Otherwise, a random-effects model (the DerSimonian-Laird method) was used.By convention, an observed HR>1 implied a worse prognosis in the MMP-9 positive group. The impact of MMP-9 on survival was considered to be statistically significant if the 95%CI for the HR did not overlap 1.

Results

Study selection and characteristics. The results of the search strategy for studies were summarized in Figure 1. The abstracts and titles of primary 824 studies were identified for initial review using search strategies described below. 121 studies reported the survival data of MMPs other than MMP-9. 57 candidate studies were evaluated by full-text review. Of the candidate studies, 41 studies were excluded because of no survival data: 30 studies only discussed the relationship between MMP-9 and patients' clinical stages, 9 studies gave the conclusions of the studies without giving survival data, 2 studies only have the values of recurrent rate. 4 studies were excluded because identical cohorts of patients were used in other selected studies (Li et al., 2002; Zhao et al., 2005; Zhong et al., 2005; Zhong et al., 2009). One study was excluded because only patients with gastric stromal tumor were included in the study (Miao et al., 2007).

Study results. Finally, 11 studies (Sier et al., 1996; Zhang et al., 2003; Wang et al., 2005; Mrena et al., 2006; Zheng et al., 2006; De Mingo et al., 2007; Czyzewska et al., 2008; Chu et al., 2010; Peng et al., 2010; Ren et al., 2010; Yang et al., 2010) were eligible for meta-analysis. The main features of the eligible studies for MMP-9 were summarized in Table 1. The total number of patients included for meta-analysis was 1700, ranging from 37 to 330 per study. In total, 11 studies had data on OS,
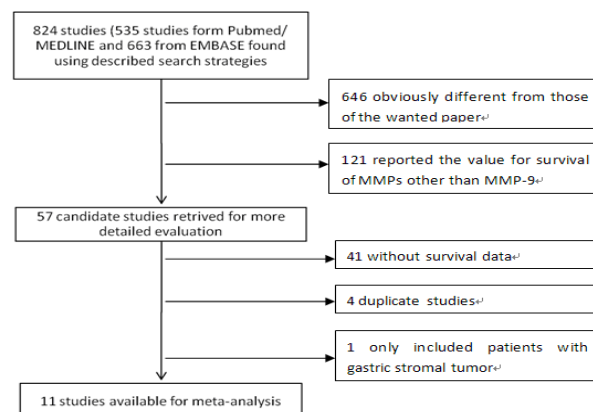

Figure 1. Methodological Flow Chart of the Systematic Review

Table 1. Main Characteristics of the Studies Relating MMP-9 to Patients' Prognosis

First author (years)	Country	N (Male %)	Age (years)	MMP-9 + (%)	Diameter >5cm (%)	Tumor grade III/IV (%)	Distant metastasis (%)	Technology	Antibody	Cut-off for MMP-9 +	Survival analysis	HR
Peng et a l(2010)	China	37 (NR)	NR	81.08	NR	72.97	21.62	IHC	Monoclonal antibody	>75%	OS	Survival curve
Chu et al (2010)	China	286 -81.12	NR	67.83	NR	56.29	9.79	IHC	Anti-rabbit polyclonal antibody	>5%	OS, DFS	Survival curve
Ren et al(2010)	China	189 -76.72	55	78.31	62.96	61.38	NR	IHC	Anti-mouse monoclonal antibody	>50%	OS	Estimated
Yang et al (2010)	China	118 -66.95	57.8	60.17	NR	71.19	46.61	IHC	Nucleotide probe	≥10%	OS	Estimated
Czyzewska et al (2008)	Poland	91 -68.13	62	41.76	NR	29.67	NR	IHC	Monoclonal antibody	>30%	OS	Survival curve
Mingo et al (2007)	Spain	44 -65.91	68	77.27	65.91	25	NR	ELISA	NR	42ng/mg	OS	Estimated
Zheng et al (2006)	Japan	234 -72.64	66.8	71.49	52.99	44.44	11.97	IHC	Anti-mouse antibody	>5%	OS	Reported in text
Mrena et al (2006)	Finland	330 -51.82	66	59.57	44.44	59	27.88	IHC	Anti-rabbit polyclonal antibody	>0%	OS	Estimated
Wang et al (2005)	China	65 -76.92	60.08	50.77	41.54	56.36	NR	IHC	Monoclonal	>25%	OS	Survival curve antibody
Zhang et al (2003)	China	256 -72.66	60	65.23	51.56	42.58	NR	IHC	Anti-mouse monoclonal antibody	≥5%	OS	Estimated
Sier et al (1996)	Netherland	50 -76	NR	80	44	32	8	ELISA	NR	7.25 arbitary units	OS	Estimated

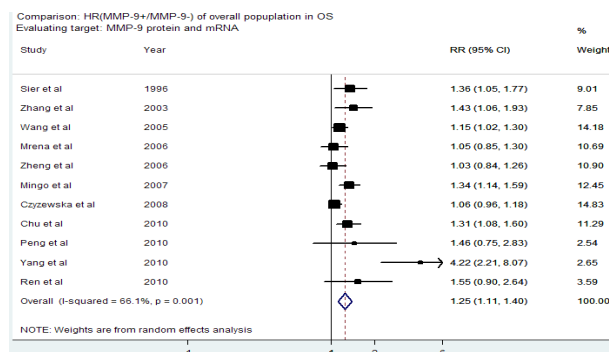Abbreviations: NR, not reported; IHC, immunohistochemistry

Figure 2. Results of Meta-analysis with All Evaluable Studies for OS. A HR>1 implies a worse OS for the group with increased MMP-9. The squared size is proportional to the number of patients included in each study. The centre of the lozenge gives the combined HR for the meta-analysis and its extremities the 95%CI

Figure 3. Results of the Meta-analysis with Studies Evaluating MMP-9 Protein for OS. A HR>1 implies a worse OS for the group with increased MMP-9. The squared size is proportional to the number of patients included in each study. The centre of the lozenge gives the combined HR for the meta-analysis and its extremities the 95%CI

and only onestudy (Chu et al., 2010) (n=323) on PFS. 6 reports originated from China, one from Japan, and 4 from Europe. The reported median age of patients ranged from 55 to 68 years across the eligible studies. Men accounted for 69.87% of the enrolled patients across the 10 studies with gender information. The positive rate of MMP-9 ranged from 50.77% to 81.08% in the included 11 studies. The percentage of tumors with diameters more than 5cm was reported in 6 studies (Sier et al., 1996; Zhang et al., 2003; Wang et al., 2005; Mrena et al., 2006; Zheng et al., 2006; Ren et al., 2010) ranging from 41.54% to 62.96%. Percentage of patients with stage III and IV was reported in all studies (from 29.67% to 72.97%) and distant metastasis in 7 studies (Sier et al., 1996; Mrena et al., 2006; Zheng et al., 2006; De Mingo et al., 2007; Chu et al., 2010; Peng et al., 2010; Yang et al., 2010) (from 8% to 46.61%). Immunohistochemistry was the mostly common technique used to detect MMP-9 expression with formalin-fixed, paraffin-embedded tissue specimens, in two trails (Sier C et al., 1996; De Mingo et al., 2007), authors used ELISA to assess MMP-9. Different antibodies were used to detect MMP-9 expression with immunohistochemistry: three (Zhang et al., 2003; De Mingo et al., 2007; Ren et
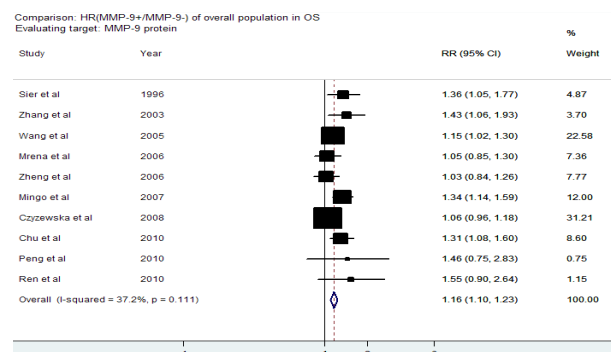
al., 2010) used an anti-mouse antibody and two (Mrena et al., 2006; Chu et al., 2010) used an anti-rabbit antibody, five (Zhang et al., 2003; Wang et al., 2005; Czyzewska et al., 2008; Peng et al., 2010; Ren et al., 2010) used a monoclonal antibody and two (Mrena et al., 2006; Chu et al., 2010) used an polyclonal antibody. In one trial (Yang et al., 2010), authors evaluated positive expression of MMP-9 mRNA with MMP-9 (MK 1540) nucleotide probe (Boster Biological Technology Limited Company, Wuhan, China) to analyze its prognostic value. The cut-off value for definition of MMP-9 positive ranged from more than 0% to 75%. Of all eligible studies for OS analysis, HR values were estimated by the survival data provided in 5 studies (Sier et al., 1996; Zhang et al., 2003; Mrena et al., 2006; De Ming et al., 2007; Yang et al., 2010) and by survival curve in 5 studies (Wang et al., 2005; Czyzewska et al., 2008; Chu et al., 2010; Peng et al., 2010; Ren et al., 2010), however, only one study (Zheng et al., 2006) provided the final adjusted RR.

Meta-analysis. The main meta-analyses result (overall population and OS) is shown in Figure 2. For the overall population, worse OS (HR 1.25, 95%CI 1.11-1.40) was observed among patients considered MMP-9 positive.
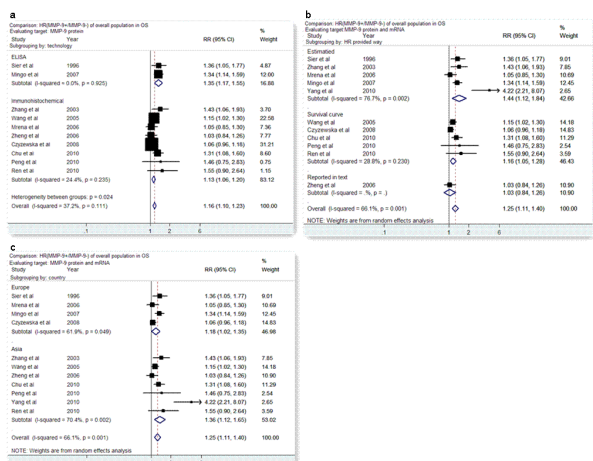
Figure 4. Results of the Meta-analysis with Studies Evaluating MMP-9 for OS in Subgroups. a, Sub-grouped by technology (ELISA/immunohistochemical). b, Sub-grouped by HR provided way (Estimated/survival curve/reported in text).c, Sub-grouped by country (Asia/Europe). A HR>1 implies a worse OS for the group with increased MMP-9. The squared size is proportional to the number of patients included in each study. The centre of the lozenge gives the combined HR for the meta-analysis and its extremities the 95%CI
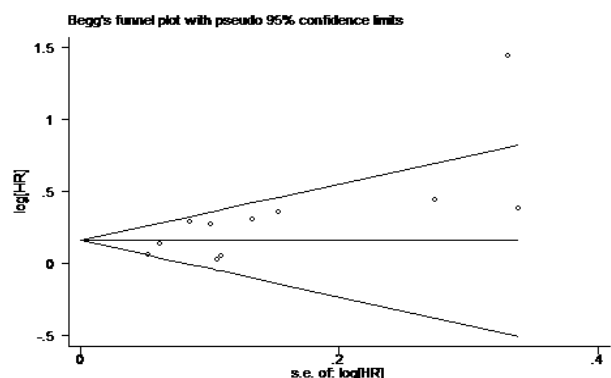

Figure 5. Funnel Graph for the Assessment of Potential Publication Bias in Studies of MMP-9 Expression in Patients with Gastric Cancer. The funnel graph plots logHR against the standard error of the logHR. The result of the Egger's test for publication bias show no significant (P=0.604)

The result of the test for heterogeneity was significant (p=0.001), so it was possible to go further in categorizing the trials. The first subgroup analysis was performed according to evaluating targets. One study for MMP-9 mRNA was excluded and 10 studies for MMP-9 protein were assessable (Figure 3). Result was significantly in favor of MMP-9 positivity with an HR of 1.16 (95%CI 1.10-1.23). To analyze different methods on evaluating MMP-9, Fig 4a showed an HR of 1.35 (95%CI 1.17-1.55) in ELISA and an HR of 1.13 (95%CI 1.06-1.20) in immunohistochemistry. It is necessary to analyze the value of MMP-9 sub-grouped by HR provided way. In Fig 4b, the provided way of reporting in text showed an non statistically significant HR of 1.03 (95%CI 0.84-1.26), however, the estimated and survival curve ways showed significant HRs (HR 1.44, 95%CI 1.12-1.84 and HR 1.16, 95%CI 1.05-1.28). At last, subgroup analysis was performed according to countries. In Asian countries, with 7 out of 11 studies evaluable, showed a significant HR of 1.36 (95%CI 1.12-1.65), and European countries showed

an HR of 1.18 (95%CI 1.02-1.65).

Publication bias statistics by using the methods of Egger et al. (1977) and Begg and Mazumdar (1994) were as follows: bias coefficient -0.04 [-0.22, 0.14] (p=0.604), which suggests an absence of publication bias in all studies (Figure 5).

Discussion

This meta-analysis showed that MMP-9 overexpression is associated with worse overall survival in patients with gastric cancer. However, our conclusion should be discussed from several aspects. The overall link between MMP-9 and survival, although stastistically significant was rather weak, with a global HR of 1.25. The similar conclusion was showed on the meta-analysis including selectively the studies evaluating MMP-9 protein with a HR of 1.16. The only one study which evaluated MMP-9 mRNA reported a HR of 4.22. In general, we consider HR > 2 strongly predictive (Ferlay et al., 2008). These results indicated that MMP-9 mRNA more predictive than protein for OS. As the incidence and mortality rate of gastric cancer are high in Eastern Asian countries especially China and Japan (Yang, 2006; Seo et al., 2007; Vidal et al., 2008), we performed analysis in subgroup of different continents. The MMP-9 is a poor prognostic factor for survival in gastric cancer patients not only in Eastern Asian (China and Japan) but also Europe (Poland, Netherland, Finland and Spain) and strengthens of the link between MMP-9 and OS were so close in different areas (HR 1.16, HR 1.36).

In the 11 eligible studies, only one of these studies (Chu et al., 2010) had evaluated the correlation between MMP-9 and PFS, and this study used immunohistochemistry method to measure the fresh gastric cancer specimens from 286 patients. The result of this study showed that there was a significant correlation between the level of MMP-9 and PFS (HR 1.73, 95%CI 1.27-2.34). However, for OS, the link between MMP-9 and OS was weaker (HR 1.31, 95%CI 1.08-1.60), which indicated that MMP-9 appeared more predictive for DFS than OS. The 2 studies (Seo et al., 2007; Vidal et al., 2008) which only statistical data on recurrent rate could be obtained showed that high level of MMP-9 might predict a high risk for tumor recurrence (HR 1.14, 95CI 0.86-1.15; HR 1.59, 95%CI 1.12-2.82). As recurrent rate and PFS have different value on predicting patients' prognosis, more prospective studies are needed to determine the prognostic utility of MMP-9 in PFS.

The results of Meta-analysis are considered as gold standards by authors worldwide (Stewart and Parmar, 1993; Hayes et al., 2001; Tong et al., 2011). However, some kind of potential bias still exists between studies and cannot be completely eliminated. First of all, we included only literatures published in English, leading to other languages, such as Japanese, Spanish, inaccessible for data aggregation. A phenomenon called the "file-drawer problem" revealed that positive studies are apt to be published in English, while negative studies are often published in native languages or even not submitted by the journal (Earleywine, 1993; Egger et al., 1997), which could overestimation of the prognostic significance of

MMP-9 in gastric cancer in our meta-analysis. Second, variability in definitions, outcomes, measurements, and experimental procedures may contribute to heterogeneity between studies (Simon and Altman, 2001; Kyzas et al., 2005). For example, technology to evaluate MMP-9 is a potential source of bias. We therefore dichotomized the meta-analysis into two subgroups: ELISA and immunohistochemistry, and the analysis reveals similar features in different subgroups (Figure 4a). Furthermore, different antibodies were used for immunodetection of MMP-9 across the studies: monoclonal antibody in 5 studies (Zhang et al., 2003; Wang et al., 2005; Czyzewska et al., 2008; Peng et al., 2010; Ren et al., 2010), polyclonal antibody in 2 studies (Chu et al., 2010; Yang et al., 2010), anti-rabbit antibody in 2 studies (Mrena et al, 2006; Chu et al., 2010), anti-mouse antibody in 3 studies (Zhang et al., 2003; Zheng et al., 2006; Ren et al., 2010). Some studies even did not clarify the antibody used in detail. There was also potential bias from the antibody concentration, because the intensity of the antibody is correlated to its concentration which could influence the positive rate of MMP-9. However, antibody concentrations were reported only in 6 studies (Zhang et al., 2003; Mrena et al., 2006; Zheng et al., 2006; Czyzewska et al., 2008; Peng et al., 2010; Chu et al., 2010), and it differed from 1:10 to 1:5000. The follow-up duration and end-point of the study are also potential bias between different studies. However, we did not define the time end points such as the 1-year survival rate and 5-year survival rate, because the designs of the follow-up duration and end-point in the included 11 studies were quite different. Third, another potential source of bias is related to the method for extrapolating HR. Three methods were defined in our study, and subgroup analysis showed that MMP-9 was a significant prognostic factor in estimated and survival curve groups. On the other hand, the only HR reported in the text showed MMP-9 no significant value for OS (Figure 4b).

Considerable attention should be paid to other prognostic factors other than MMP-9. MMP-9 might be a potential prognostic marker in gastric cancer, but the correlation between MMP-9 expression and traditional prognostic factors such as clinical stage or differentiation is still needed to study. The prognostic value of other biomarkers should be further examined such as angioenin, interleukin 10 and 8, platelet-derived endothelial growth factor, fibroblast growth factor, angiopoetins, thrombospodin (Reinmuth et al., 2003), andother MMPs which play their complex role in angiogenesis and invasion together (Johansson et al., 2000; Jackson, 2002). Some of the studies included in the meta-analysis had already addressed a significant association of OS with other key biomarkers, such as type IVcollegen, microvascular density (MVD) (Peng et al., 2010), c-Jun (Ren et al., 2010), vascular endothelial growth factor (VEGF) (Yang et al., 2010), tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) (De Mingo et al., 2007) and MMP-2 (Wang et al., 2005).

Acknowledgements

References

Allgayer H, Heiss MM, Schildberg FW (1997). Prognostic factors in gastric cancer. *Br J Surg*, **84**, 1651-64.

Altman DG (2001). Systematic reviews of evaluations of prognostic variables. *BMJ*, **323**, 224-8.

Begg C, Mazumdar M (1994). Operating characteristics of a rank correlation test for publication bias. *Biometrics*, **50**, 1088-101.

Brenner H, Rothenbacher D, Arndt V (2009). *Epidemiology of stomach cancer.Cancer Epidemiology*, **472**, 467-77.

Chu D, Zhang Z, Li Y, et al (2010). Matrix metalloproteinase-9 is associated with disease-free survival and overall survival in patients with gastric cancer. *Int J Cancer*, **129**, 887-95.

Crew KD, Neugut AI (2006). Epidemiology of gastric cancer. *World J Gastroenterol*, **12**, 354-62.

Czyzewska J, Guzinska-Ustymowicz K, Kemona A, et al (2008). The expression of matrix metalloproteinase 9 and cathepsin B in gastric carcinoma is associated with lymph node metastasis, but not with postoperative survival. *Folia Histochem Cytobiol*, **46**, 57-64.

De Mingo M, Moran A, Sanchez-Pernaute A, et al (2007). Expression of MMP-9 and TIMP-1 as prognostic markers in gastric carcinoma. *Hepatogastroenterology*, **54**, 315-9.

Earleywine M (1993). The file drawer problem in the meta analysis of the subjective responses to alcohol. *Am J Psychiatry*, **150**, 1435-6.

Egger M, Smith GD, Schneider M, et al (1977). Bias in meta-analysis detected by a simple, graphical test. *BMJ*, 629-34.

Egger M, Zellweger-Zahner T, Schneider M, et al (1997). Language bias in randomized controlled trials published in English and German. *Lancet*, **350**, 326-9.

Ferlay J, Parkin DM, Steliarova-Foucher E (2010). Estimates of cancer incidence and mortality in Europe in 2008. *Eur J Cancer*, **46**, 765-81.

Harrison JD, Fielding JW (1995). Prognostic factors for gastric cancer influencing clinical practice. *World J Surg*, **19**, 496-500.

Hayes DF, Isaacs C, Stearns V (2001). Prognostic factors in breast cancer: current and new predictors of metastasis. *J Mammary Gland BiolNeoplasia*, **6**, 375-92.

Jackson C (2002).Matrix metalloproteinases and angiogenesis. *Current Opinion in Nephrology and Hypertension*, **11**, 295-9.

Jemal A, Bray F, Melissa M, et al (2011). Global cancer statistics. *CA Cancer J Clin*, **61**, 69-90.

Johansson N, Ahonen M, Kahari VM (2000). Matrix metalloproteinases in tumor invasion. *CMLS Cell Mol Life Sci*, **57**, 5-15.

Kim BS, Cho SW, Min SK, et al (2011). Differences in prognostic factors between early and advanced gastric cancer. *Hepatogastroenterology*, **58**, 1032-40.

Kyzas PA, Cunha IW, Ioannidis JP (2005). Prognostic significance of vascular endothelial growth factor immunohistochemical expression in head and neck squamous cell carcinoma: a meta-analysis. *Clin Cancer Res*, **11**, 1434-40.

Li L, Zhang S, Lin H, et al (2002). Relationship of expression unbalance of matrix metalloproteinase and tissue inhibitor of metalloproteinase to invasiveness and metastasis in gastric carcinomas. *Ai Zheng*, **21**, 305-310.

Mantel N, Haenszel W (1959).Statistical aspects of the analysis of data from retrospective studies of disease. *J Natl Cancer Inst*, **22**, 719-48.

McCawley LJ, Matrisian LM (2000). Matrix metalloproteinases: multifunctional contributors to tumor progression. *Mol Med Today*, **6**, 149-56.

Miao R, Li L, Wang Y, et al (2007). Expression and clinical significance of matrix metalloproteinase in gastric stromal tumor. *Zhonghua Wai Ke Za Zhi*, **45**, 1196-8.

Mrena J, Wiksten J-P, Nordling S, et al (2006). MMP-2 but not MMP-9 associated with COX-2 and survival in gastric cancer. *J ClinPathol*, **59**, 618-23.

Nabeshima K, Inoue T, Shimao Y, et al (2002). Matrix metalloproteinases in tumor invasion: role for cell migration. *Pathol Int*, **52**, 255-64.

Park HI, Ni J, Gerkema FE, et al (2000). Identification and characterization of human endometase (matrix metalloproteinase-26) from endometrial tumor. *J Biol Chem*, **275**, 20540-4.

Parmar MK, Torri V, Stewart L (1998). Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. *Stat Med*, **17**, 2815-34.

Peng C, Liu X, Liu X, et al (2010). Co-evolution of cancer microenvironment reveals distinctive patterns of gastric cancer invasion: laboratory evidence and clinical significance. *J Transl Med*, **8**, 101.

Reinmuth N, Parikh AA, Ahmad SA, et al (2003). Biology of angiogenesis in tumors of the gastrointestinal tract. *Microsc Res Tech*, **60**, 199-207.

Ren T, Wu J, Zhou Y, et al (2010). c-Jun and MMP-9 protein expression and the biologic action in gastric carcinoma. *Chin J Clin Oncol*, **37**, 19-22.

Rundhaug JE (2005). Matrix metalloproteinases and angiogenesis. *J Cell Mol Med*, **9**, 267-85.

Seo YS, Park JJ, Kim JH, et al (2007). Usefulness of MMP-9/ TIMP-1 in predicting tumor recurrence in patients undergoing curative surgical resection for gastric carcinoma. *Dig Dis Sci*, **52**, 753-9.

Seshadri RA, Jayanand SB, Ranganathan R (2011). Prognostic factors in patients with node-negative gastric cancer: an Indian experience. *World J SurgOncol*, **9**, 48

Sier C, Kubben F, Ganesh S, et al (1996). Tissue levels of matrix metalloproteinases MMP-2 and MMP-9 are related to the overall survival of patients with gastric carcinoma. *Br J Cancer*, **74**, 413-7.

Simon R, Altman DG (2001). Statistical aspects of prognostic factor studies in oncology. *Br J Cancer*, **69**, 979-85.

Stetler-Stevenson WG (2001).The role of matrix metalloproteinases in tumor invasion, metastasis, and angiogenesis. *Cancer Metastasis: Biological and Clinical Aspects*, **10**, 383-92.

Stewart LA, Parmar MK (1993). Meta-analysis of the literature or of individual patient data: is there a difference? *Lancet*, **341**, 418-22.

Tang Y, Zhu J, Chen L, et al (2008). Associations of matrix metalloproteinase-9 protein polymorphisms with lymph node metastasis but not invasion of gastric cancer. *Clin Cancer Res*, **14**, 2870-7.

Tong J, Sun X, Cheng H, et al (2011). Expression of p16 in non-small cell lung cancer and its prognostic significance: a meta-analysis of published literatures. *Lung Cancer*, **74**, 155-63.

Ueda Y, Imai K, Tsuchiya H, et al (1996). Matrix metalloproteinase 9 (gelatinase B) is expressed in multinucleated giant cells of human giant cell tumor of bone and is associated with vascular invasion. *Am J Pathol*, **148**, 611-22.

Vidal O, Soriano-Izquierdo A, Pera M, et al (2008). Positive VEGF immunostaining independently predicts poor prognosis in curative resected gastric cancer patients: results of a study assessing a panel of angiogenic markers. *J GastrointestSurg*, **12**, 1005-14.

Wang J, Tian X, Wang S, et al (2005). Correlation between expression of matrix metalloproteinase-2, matrix metalloproteinase-9 and angiogenesis in gastric cancer. *Chin J Cancer Res*, **17**, 283-7.

Yang L. Incidence and mortality of gastric cancer in China (2006). *World J Gastroenterol*, **12**, 17-20.

Yang Q, Ye Z, Zhang J, et al (2010). Expression of matrix metalloproteinase-9 mRNA and vascular endothelial growth factor protein in gastric carcinoma and its relationship to its pathological features and prognosis. *Anatomical Record*, **293**, 2012-9.

Yasui W, Oue N, Aung PP, et al (2005). Molecular-pathological prognostic factors of gastric cancer: a review. *Gastric Cancer*, **8**, 86-94.

Zhang S, Li L, Lin J, et al (2003). Imbalance between expression of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in invasiveness and metastasis of human gastric carcinoma. *World J Gastroenterol*, **9**, 899-904.

Zhao D, Xu H, Li K, et al (2010). Prognostic factors for patients after curative resection for proximal gastric cancer. *J Huazhong Univ Sci Technolog Med Sci*, **30**, 530-5.

Zhao ZS, Wang YY, Ye ZY, et al (2009). Prognostic value of tumor-related molecular expression in gastric carcinoma. *Pathol Oncol Res*, **15**, 589-96.

Zhao Z, Zhou J, Yao G, et al (2005). Correlative studies on bFGF mRNA and MMP-9 mRNA expressions with microvascular density, progression, and prognosis of gastric carcinomas. *World J Gastroenterol*, **11**, 3227-33.

Zheng H, Takahashi H, Murai Y, et al (2006). Upregulated EMMPRIN/CD 147 might contribute to growth and angiogenesis of gastric carcinoma: a good marker for local invasion and prognosis. *Br J Cancer*, **95**, 1371-8.

Zhong S, Wang Y, Ye Z, et al (2009). Prognostic value of tumor-related molecular expression in gastric carcinoma. *Pathol Oncol Res*, **15**, 589-96.

Zhong S, Yao G, Ru G, et al (2005). Relationship between bFGF mRNA and MMP-9 mRNA expression in gastric carcinoma and their clinicopathological features as well as patients survival. *Zhong Hua Wai Ke Za Zhi*, **43**, 169-73.