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Original Article

Prognostic and clinical significance of STAT3 and MMP9 in patients with gastric cancer: a meta-analysis of a Chinese cohort

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Abstract: As signal transducer and activator of transcription 3 (STAT3)-mediated signaling cascade directly contributes to tumor metastasis, numerous agents targeting STAT3 are in clinical development. However, reported data on the prognostic impact of STAT3 expression vary considerably. We aim to quantitatively summarize available evidences for evaluating the association between STAT3 and STAT3-regulated target gene, matrix metalloproteinase 9 (MMP9), and the prognosis of Chinese patients with gastric cancer. Searches were applied to PubMed and the Chinese National Knowledge Infrastructure database without any language restriction. A total of 5,757 patients were included in the final analyses. All results favored an association between high STAT3 expression and poor 5-year overall survival (risk ratio = 1.845, 95% confidence interval [CI] = 1.027-3.315). The reduced survival was heavily influenced by advanced tumor invasion (OR = 2.885, 95% CI = 2.034-4.094), lymph node metastasis (OR = 5.349, 95% CI = 3.807-7.516), distant metastasis (OR = 5.873, 95% CI = 2.641-13.062), dedifferentiation (OR = 2.516, 95% CI = 1.814-3.491), tumor size (OR = 1.918, 95% CI = 1.246-2.954), and higher TNM stage (OR = 4.171, 95% CI = 2.840-6.126). Similar results were observed in the meta-analyses of MMP9, with the magnitude of effect OR > 2. Our findings indicate that STAT3 and MMP9, as measured by IHC, are associated with worse survival and potentially mark invasion and metastasis in gastric cancer, especially in Chinese patients. More significantly, these two biomarkers may be converted from candidates to the routine clinical evaluation to help predict the outcome of gastric carcinoma patients.

Keywords: Gastric cancer, STAT3, MMP9, prognostic factor

Introduction

Despite a decline in gastric cancer incidence in many Western countries, a report published in 2005 revealed that the disease remains the most common cancer in Eastern Asia [1-4]. Most patients with advanced disease die from complications by metastases rather than the primary tumor. Therefore, identifying novel markers involved in the key steps of metastasis would promote early prediction of recurrence and survival in such patients.

Growth and metastasis are often linked to angiogenesis in various cancers, including gastric cancer. More than 90% of solid tumors depend on a functional vascular network for their supply of oxygen and nutrients. Increasing evidence has indicated that tumor metabolism may be regulated by various growth factors/receptors and oncogenes, including vascular endothelial growth factor and receptor (VEGF/VEGFR), epidermal growth factor and receptor (EGF/EGFR), Src, Ras, etc [5]. Constitutive and aberrant activation of these factors often transmits signals through signal transducer and activator of transcription 3 (STAT3). Upon activation, STAT3 undergoes phosphorylation, homodimerization, nuclear translocation, and DNA binding, which subsequently leads to the transcription of various target genes, including Survivin, VEGF, matrix metalloproteinases

Table 1. Main characteristics of the 74 studies included in the final meta-analysis

First author	Year of publication	language	Study from PubMed	Number of patients (M/F)	Median age (years)	Antibody used for the evaluation	Cutoff for MMP9 positivity (%)	Blinded reading	Reader (s) (n)	Survival analysis	Results
Liang, et al	2000	Chinese	NO	25/11	60.5	FIK, Japan, 1:100	-	-	-	-	-
Li, et al	2000	Chinese	YES	-	-	Santa Cruz, USA, 1:80	-	-	-	-	-
Zhang, et al	2000	Chinese	YES	82/26	54.8	Maixin Fuzhou, China, 1:150	> 0	-	-	OS	negative
Wang, et al	2001	Chinese	NO	28/12	58.6	Dako, Denmark + I18	-	-	-	-	-
Zhou, et al	2001	Chinese	NO	28/19	54.6	Maixin, Fuzhou, China	> 10%	-	-	-	-
Li, et al	2002	Chinese	YES	186/70	-	Maxim, USA	> 25%	-	-	OS	negative
Xue, et al	2002	Chinese	NO	28/19	54.6	Maixin Fuzhou, China	> 10%	-	-	-	-
Guan, et al	2002	Chinese	YES	64/36	58	Maixin Fuzhou, China	-	-	-	OS	negative
Wang, et al	2003	Chinese	NO	48/26	-	NeoMarkers, USA, 1:1	> 10%	-	-	-	-
Jiang, et al	2003	Chinese	NO	62/25	55.6	Zhongshan, Beijing, China	> 10%	-	-	-	-
Shen, et al	2003	Chinese	NO	30/10	57.5	Maixin Fuzhou, China	> 0	YES	2	-	-
Zuo, et al	2003	Chinese	NO	39/28	56.5	Maxim, USA	> 25%	-	-	-	-
Sun, et al	2003	Chinese	NO	36/24	42	Zhongshan, Beijing, China, 1:300	-	-	-	-	-
Gao, et al	2004	Chinese	NO	32/9	53	Maixin Fuzhou, China	> 5%	-	-	-	-
Chen, et al	2004	Chinese	NO	59/21	60	Maixin Fuzhou, China	> 10%	-	-	-	-
Li, et al	2004	Chinese	NO	-	-	Maixin Fuzhou, China	> 10%	-	-	-	-
Wang, et al	2004	Chinese	NO	43/20	55.6	Maixin Fuzhou, China	> 30%	-	-	-	-
Lu, et al	2005	Chinese	NO	140/120	53	Maixin Fuzhou, China	> 25%	YES	2	-	-
Xie, et al	2005	Chinese	NO	47/23	48.6	Zhongshan, Beijing, China	> 5%	-	-	-	-
Wu, et al	2005	Chinese	NO	67/38	53	Changdao, Shanghai, China	> 0	-	-	OS	negative
Gao, et al	2005	Chinese	NO	56/31	56.5	Maixin Fuzhou, China	> 25%	-	-	-	-
Zhang, et al	2005	Chinese	YES	45/39	52.6	RIBOBIO, Wuhan, China	> 5%	-	-	-	-
Chen, et al	2006	Chinese	NO	-	-	-	> 0	-	-	-	-
Zhu, et al	2006	Chinese	NO	61/19	56	RIBOBIO, Wuhan, China, 1:200	> 25%	-	-	OS	negative
Gao, et al	2006	Chinese	NO	48/22	51	Maixin Fuzhou, China	> 5%	-	-	-	-
Tang, et al	2006	Chinese	NO	91/37	58	Maxim, USA	> 25%	YES	2	-	-
Wu, et al	2006	Chinese	NO	44/16	59.6	Maixin Fuzhou, China	> 10%	-	-	OS	negative
Ye, et al	2006	Chinese	NO	54/26	47.6	Maixin Fuzhou, China	> 10%	-	-	-	-
Feng, et al	2006	Chinese	NO	-	55	Maixin Fuzhou, China	> 10%	-	-	-	-
Yan, et al	2006	Chinese	NO	44/11	56.4	Santa Cruz, USA	> 0	-	-	-	-
Lv, et al	2006	Chinese	NO	49/34	55	Zymed, USA	> 5%	-	-	-	-
Yu, et al	2006	Chinese	NO	32/20	48.6	Maixin Fuzhou, China	> 0	YES	2	-	-
Sun, et al	2006	Chinese	NO	67/29	62	NeoMarkers, USA, 1:100	> 4%	-	-	-	-
Gao, et al	2006	Chinese	NO	26/14	55.2	Changdao, Shanghai, China	> 5%	-	-	-	-
Liu, et al	2006	Chinese	NO	55/19	60.9	ZSGB-BIO, Beijing, China	-	-	-	-	-
Hu, et al	2006	Chinese	YES	50/26	48. 3	Maixin Fuzhou, China	> 5%	-	-	-	-
Wang, et al	2007	Chinese	NO	96/24	59.14	Santa Cruz, USA, 1:1	> 0	-	-	-	-

Pan, et al	2007	Chinese	NO	54/33	51.6	ZSGB-BIO, Beijing, China	> 0	-	-	-	-
Hu, et al	2007	Chinese	NO	44/16	57	Maixin Fuzhou, China	> 40%	-	-	-	-
Wang, et al	2007	Chinese	NO	36/18	58	Maixin Fuzhou, China	> 10%	-	-	-	-
Song, et al	2007	Chinese	NO	37/17	60.4	1:100	> 0	-	-	-	-
Zhang, et al	2007	Chinese	NO	87/12	58.2	Santa Cruz, USA, 1:60	> 0	-	-	-	-
Yuan, et al	2007	Chinese	NO	43/17	60.0	ZSGB-BIO, Beijing, China	> 10%	-	-	-	-
Zhou, et al	2008	Chinese	NO	48/19	56.5	Maixin Fuzhou, China	> 30%	-	-	-	-
Guo, et al	2008	Chinese	NO	30/15	60.4	Maixin Fuzhou, China	> 10%	-	-	-	-
Zhang, et al	2008	Chinese	NO	78/42	61	Maixin Fuzhou, China	> 10%	-	-	-	-
Chen, et al	2008	Chinese	NO	48/12	58.6	ZSGB-BIO, Beijing, China	> 5%	-	-	-	-
Ni, et al	2008	Chinese	NO	34/20	60.4	Zhongshan, Beijing, China	0	-	-	-	-
Zhang, et al	2008	Chinese	NO	32/18	54	-	> 10%	-	-	-	-
Li, et al	2008	Chinese	NO	32/13	56.3	NeoMarkers, USA	> 5%	-	-	-	-
Zhen, et al	2008	Chinese	NO	42/18	57	-	> 25%	-	-	-	-
Li-Yu Lee, et al	2008	English	YES	52/36	-	Lab Vision Corporation, Fremont, CA, 1:50	> 10%	-	-	-	-
Chen, et al	2009	Chinese	NO	34/20	60.4	-	0	-	-	-	-
Zhu, et al	2009	Chinese	NO	68/36	49.7	Maixin, Fuzhou, China	0	-	-	-	-
Zhao, et al	2009	Chinese	YES	-	-	Santa Cruz, USA	-	-	-	-	-
Peng, et al	2010	English	YES	-	-	Santa Cruz, USA, 1:300	-	-	-	OS and DFS	negative
Chu, et al	2011	English	YES	232/54	-	Abcan, HK, 1:200	> 5%	-	-	OS	negative
Yang, et al	2011	English	YES	37/17	-	Maixin Fuzhou, China, 1:200	-	-	-	DFS	negative
Zheng, et al	2007	Chinese	NO	51/39	56	Maixin Fuzhou, China	> 5%	-	-	-	-
Han, et al	2007	English	YES	-	-	Santa Cruz, USA, 1:100	> 0%	YES	2	-	-
Deng, et al	2008	Chinese	NO	37/23	44.7	Santa Cruz, USA, 1:300	> 0%	YES	2	-	-
Hu, et al	2008	Chinese	NO	32/8	63.5	RIBOBIO, Wuhan, China, 1:100	> 10%	YES	2	-	-
Song, et al	2008	Chinese	NO	120/30	62	ZSGB-BIO, Beijing, China	> 10%	-	-	-	-
Sun, et al	2008	Chinese	NO	-	-	1:50	-	-	-	-	-
Li, et al	2009	Chinese	NO	41/18	62.8	Cell signaling, USA, 1:100	> 0%	YES	2	-	-
Zhang, et al	2009	Chinese	NO	56/35	60	CST, USA	> 5%	-	-	-	-
Cai, et al	2010	Chinese	NO	30/26	57.5	RIBOBIO, Wuhan, China	> 10%	YES	2	-	-
Shang, et al	2010	Chinese	NO	23/17	60	Maixin Fuzhou, China, 1:100	> 5%	-	-	-	-
Deng, et al	2010	English	YES	37/16	55	Santa Cruz, USA, 1:100	-	YES	2	OS	negative
Deng, et al	2012	Chinese	NO	42/38	47	Santa Cruz, USA, 1:300	> 40%	-	-	-	-
Yan, et al	2012	Chinese	NO	35/20	51	Bioss, Beijing, China, 1:100	> 5%	-	-	-	-
Xiong, et al	2012	English	YES	176/86	-	Dako, Denmark, 1:20	> 15%	YES	2	OS	negative
Du, et al	2013	Chinese	NO	46/14	55.6	1:100	> 10%	-	-	-	-
Jia, et al	2013	English	YES	34/14	-	1:20	-	-	-	OS	negative

OS, overall survival; Positive, inverse relationship between specific protein expression and survival; Negative, no relationship. 'Reader' are readers of the histologic slides, 'blinded reading' means that readers of the slides without knowledge of the clinical outcome, and '-' corresponds to missing data.

Table 2. Meta-analysis of STAT3 and MMP9 expressions on gastric cancer

Stratification of gastric cancer	MMP9							STAT3						
	Number of studies	Total patients	Model	OR (95% CI)	*P*-value	I^2 for heterogeneity	*P*-value for bias	Number of studies	Total patients	Model	OR (95% CI)	*P*-value	I^2 for heterogeneity	*P*-value for bias
Gastric cancer -normal gastric mucosa	33	4367	Random	14.713 (9.623-22.496)	0.000	74.10%	0.014	13	1256	Fixed	13.535 (10.087-18.162)	0.000	29.50%	0.039
5-year survival	8	862	Random	1.515 (1.236-1.856)	0.000	60.10%	0.102	3	363	Random	1.845 (1.027-3.315)	0.04	71.10%	0.35
The depth of invasion	40	3252	Fixed	3.731 (3.148-4.424)	0.000	34.10%	0.000	11	725	Fixed	2.885 (2.034-4.094)	0.000	38.20%	0.011
Lymph node status	51	3957	Fixed	3.818 (3.285-4.436)	0.000	28.00%	0.057	12	1187	Fixed	5.349 (3.807-7.516)	0.000	47.50%	0.128
Distant metastasis	16	1322	Fixed	3.180 (2.236-4.524)	0.000	0.00%	0.437	4	333	Fixed	5.873 (2.641-13.062)	0.000	30.80%	0.325
TNM stage	28	2534	Fixed	3.733 (3.086-4.514)	0.000	49.60%	0.041	10	832	Fixed	4.171 (2.840-6.126)	0.000	44.40%	0.075
Age	10	943	Fixed	1.106 (0.837-1.461)	0.479	40.20%	0.710	9	815	Fixed	1.048 (0.743-1.479)	0.789	0.00%	0.567
Sex	22	1920	Fixed	1.130 (0.911-1.402)	0.266	0.00%	0.456	12	991	Fixed	1.344 (0.971-1.860)	0.074	0.00%	0.181
Size	14	1085	Fixed	1.493 (1.154-1.931)	0.002	14.80%	0.689	6	611	Fixed	1.918 (1.246-2.954)	0.003	0.00%	0.056
Histological differentiation	44	3485	Random	1.451 (1.124-1.872)	0.004	56.10%	0.000	13	1027	Fixed	2.516 (1.814-3.491)	0.000	47.80%	0.041

OR, odd ratio; RR, risk ratio; CI, confidence interval.

Table 3. Ongoing studies evaluating anti-STAT3 and anti–MMP9 therapeutic strategies

	Study	sponsor	Phase/setting	Experimental arm (s)
MMP9	NCT00783523	University of California, San Francisco	Arteriovenous Malformations; Cavernous Angiomas; Brain Aneurysms; complete	Doxycycline or Placebo
	NCT00695851	Ambrilia Biopharma, Inc	Phase 1; Prostate Cancer; complete	PCK3145
	NCT00538967	Leiden University Medical Center	Aortic Aneurysm, Abdominal; Phase 2	Doxycycline
	NCT00126204	Barnes-Jewish Hospital	Aortic Aneurysm; completed	Doxycycline
	NCT00001683	National Cancer Institute (NCI)	Lymphoma Melanoma Neoplasm Metastasis Renal Cell Carcinoma: Phase 1	COL-3
STAT3	NCT01563302	Isis Pharmaceuticals	Advanced Cancers, DLBCL and Lymphoma; Phase 1/2	ISIS-STAT3Rx
	NCT01663571	New York University School of Medicine	Cutaneous T Cell Lymphoma	-
	NCT01839604	AstraZeneca	Advanced Adult Hepatocellular, Carcinoma Hepatocellular Carcinoma Metastatic; Phase 1	AZD9150
	NCT01066663	Dana-Farber Cancer Institute	Chronic Lymphocytic Leukemia Small Lymphocytic Leukemia; Phase 1/2	Pyrimethamine
	NCT01009437	Masonic Cancer Center, University of Minnesota	Breast Cancer; Phase 1/2	Ritonavir + therapeutic conventional surgery
	NCT01445405	National Cancer Institute (NCI)	Carcinoma, Squamous Head and Neck Cancer Oral Cancer Laryngeal Cancer Pharyngeal Cancer; Phase 1	Bortezomib (Velcade, PS-341), Cetuximab and Cisplatin; Procedure: Radiation Therapy
	NCT00735930	National Cancer Institute (NCI)	relapsed or refractory B-cell chronic lymphocytic leukemia or small lymphocytic lymphoma; Phase 1	Alvocidib + lenalidomide
	NCT00955812	M.D. Anderson Cancer Center	Advanced Cancer Solid Tumor; phase 1	OPB-31121
	NCT00105950	GlaxoSmithKline	Neoplasms, Breast; phase 2	Lapatinib
	NCT00655499	Groupe Cooperateur Multidisciplinaire en Oncologie (GERCOR)	Colorectal Cancer	-
	NCT00113217	M.D. Anderson Cancer Center	Renal Cell Carcinoma Kidney Cancer; phase 2	Bevacizumab

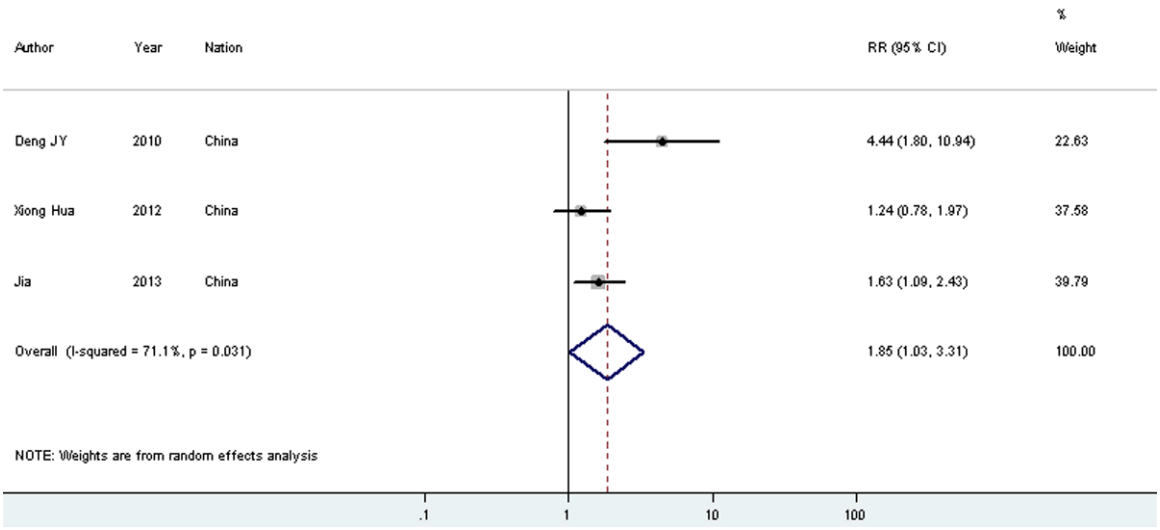

Figure 1. Meta-analysis on the relation between STAT3 expression and 5-year overall survival (OS).

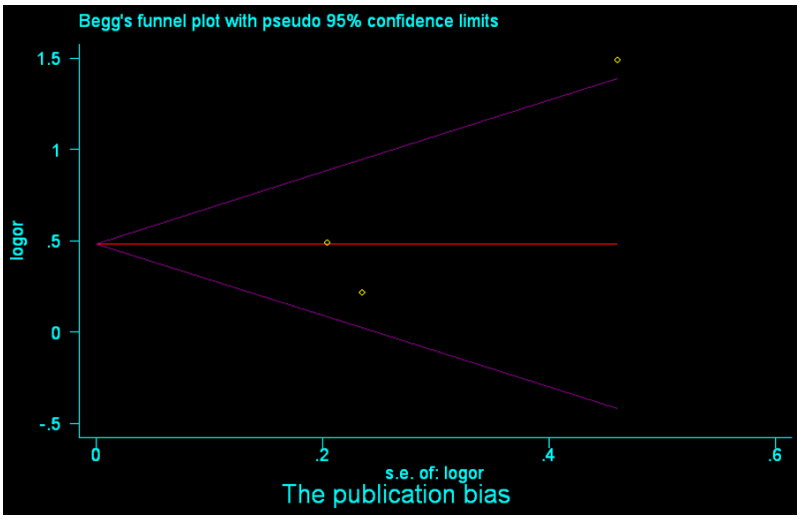

Figure 2. Begg's funnel plot analysis of STAT3 to detect publication bias for overall survival (OS).

(MMPs), E-cadherin, etc. to regulate cell proliferation, survival, angiogenesis, metastasis, immune evasion, inflammation, and drug resistance in a tumor microenvironment [6-8]. Among these, MMP9, one of the most important members of MMP, is well known to degrade the extracellular matrix (ECM) and basement membrane (BM), thus promoting disease progression in various cancers through increased migration, invasion, metastasis, and angiogenesis [9]. High levels of MMP9 have been shown to strongly correlate with tumor aggressiveness and poor prognosis in various human cancers [10]. Currently, routine phase 1 and phase 2 clinical trials that target STAT3 function or expression have been completed, including trials of ISIS-STAT3Rx for the treatment of advanced cancers expressing STAT3 and the effects of OPB-31121 on solid tumor (Clinical Trials: NCT01563302, NCT00955-812; http://clinicaltrials.gov/). In addition, positive results from clinical practice have further reinforced the interest of drug development targeting STAT3-mediated signaling pathway.

However, despite the clinical development of anti-STAT3 therapies, whether STAT3 overexpression has any prognostic and clinical values remains controversial. Deng *et al.* reported that STAT3 overexpression was associated with lymph node metastasis in gastric cancer [11]. However, Xiong *et al.* found that increased levels of STAT3 did not relate to differentiation and tumor-node-metastasis [12]. It is unclear whether the conflicting results from these investigations are due to their limited sample size or genuine heterogeneity. Almost two-thirds of gastric cancer cases are estimated to occur in Asia, especially in China, where the date from the 7th Chinese

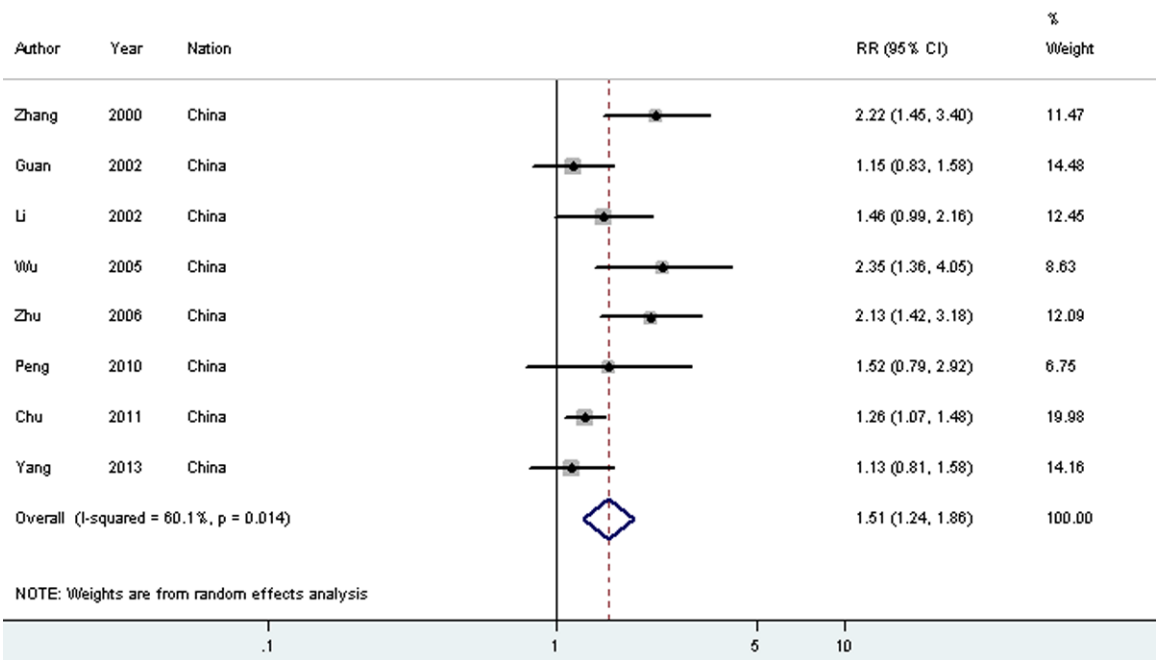

Figure 3. Meta-analysis on the relation between MMP9 expression and 5-year overall survival (OS).

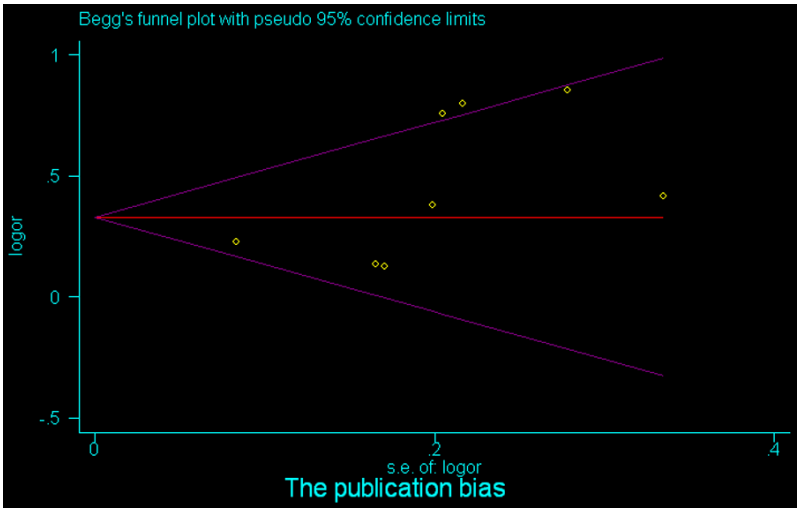

Figure 4. Begg's funnel plot analysis of MMP9 to detect publication bias for overall survival (OS).

Symposium on Medical Oncology and Chinese Cancer Registry Annual Report predicted a 1.6% annual rate of gastric cancer incidence in 2015 [13]. Therefore, we herein presented a meta-analysis on the prognostic impact of STAT3 and STAT3-regulated MMP9 abnormal expression in Chinese patients with gastric cancer. We believe that understanding the relationship between gene expression profiles and prognosis may allow more rational development of therapeutic strategies against these two markers in clinical practice.

Materials and methods

Study identification and selection

The present meta-analysis was conducted according to the statement on preferred reporting items for systematic reviews and meta-analyses [12, 14, 15]. PubMed and the Chinese National Knowledge Infrastructure (CNKI) databases were searched for studies evaluating the expression of STAT3 and MMP9 in gastric cancer from 1995 to 2013. Subject heading terms such as STAT3, prognosis, and gastric cancer or all other synonyms for gastric cancer were used to screen for potentially relative studies. Similar searching process was performed for MMP9.

Inclusion criteria were as follows: (1) study selection was based on the association between STAT3 or MMP9 and prognosis in humans; (2) protein expression was evaluated

via immunohistochemical (IHC) methods; (3) data were collected from Chinese study cohorts; and (4) data were available for the number of cases and controls, patients' age, sex, tumor size, venous invasion, lymph node status, distant metastasis, TNM stage, histo-differentiation, and 5-year overall survival (OS). Citation lists of the retrieved articles were manually reviewed to ensure sensitivity of the search strategy.

Data collection and compilation

Two authors (Chen J and Liu XX) independently extracted information from search results using predefined forms. Information collected included an article's first author name, year of publication, nation, language, the cut-off values for determining STAT3 and MMP9 positivity, blinded reading, the numbers of controls and cases, association data between STAT3/MMP9 expression and 5-year OS, and the number of events in each category of STAT3/MMP9 expression on different clinicopathological factors as described above. In most cases, survival data were extracted from Kaplan-Meier curves.

Owing to the applicable clinical characteristics, each examined parameters were divided into two groups: well and moderate differentiation vs. poor and undifferentiation, T1 and T2 vs. T3 and T4, stage I and II vs. stage III and IV, tumors larger than 5 cm in size vs. those of less than 5 cm, and above vs. below 60 years of age. Disagreement was resolved by consensus in all items.

Statistical analysis

Three categories of stratified models were analyzed. The first stratified multivariate model was performed to confirm whether STAT3/MMP9 highly expressed in gastric cancer patients compared to normal gastric mucosa. The second outcome meta-analysis aimed to measure the impact of STAT3/MMP9 expression on survival by estimating the risk ratio (RR) between the positive and negative groups. The third analysis was to examine the prognostic value of STAT3/MMP9 expression in various clinical factors, such as age, sex, tumor size, location and histo-differentiation, depth of invasion, vascular invasion, lymph node status, distant metastasis, and TNM stage.

Statistical Analysis System Software (STATA SE 9.0) was used to combine collected data for meta-analyses. All studies were assessed by RR or odds ratio (OR) using different models as previously described [16]. The Egger's linear regression test and Begg's test were performed to examine publication bias. All statistical analyses were two-sided, and a *P*-value of less than 0.05 was considered significant.

Results

Study description

We identified 74 studies [12, 17-89] that employed IHC assay for assessing the association between STAT3/MMP9 expression and prognosis in Chinese patients with gastric cancer. A total of 5,757 patients were included in those studies. Thirteen out of 16 studies compared the expression of STAT3 between gastric cancer and normal gastric mucosa, whereas 3 out of 16 studies evaluated the impact of STAT3 expression on OS. For all patients, measurements were obtained from the primary tumor, and all specimens were collected before chemotherapy or radiotherapy. The main features of eligible studies included in our meta-analyses and their results are summarized in **Tables 1**, **2**.

Correlation between STAT3 expression and prognostic and clinical values

The combined results showed that STAT3 expression in Asian patients with gastric cancer was significantly higher in 13 studies (717 patients and 539 controls, OR = 13.535, 95% CI = 10.087-18.162, $P < 0.001$ (**Table 2**, Supplementary Table 1). High levels of STAT3 correlated with poor OS in 3 studies (363 patients) (RR = 1.845, 95% CI = 1.027-3.315, $P = 0.04$ (**Table 2**, **Figures 1** and **2**). Subgroup analysis revealed that increased STAT3 expression was associated with invasion depth (11 studies, 725 patients, OR = 2.885, 95% CI = 2.034-4.094, $P < 0.001$), lymph node metastasis (12 studies, 1,187 patients, OR = 5.349, 95% CI = 3.807-7.516, $P < 0.001$), distant metastasis (4 studies, 333 patients, OR = 5.873, 95% CI = 2.641-13.062, $P < 0.001$), TNM stage (10 studies, 832 patients, OR = 4.171, 95% CI = 2.840-6.126, $P < 0.001$), tumor size (6 studies, 611 patients, OR = 1.918, 95% CI = 1.246-2.954, $P = 0.003$), and histological differentiation (13 studies, 1,027

patients, OR = 2.516, 95% CI = 1.814-3.491, *P* < 0.001) (**Table 2**, Supplementary Table 1).

Correlation between MMP9 expression and prognostic and clinical values

When compared to normal controls, MMP9 overexpression was associated with worse outcomes for gastric cancer patients in 33 studies (2,652 patients and 1,715 controls, OR = 14.713, 95% CI = 9.623-22.496, *P* < 0.001 (**Table 2**, Supplementary Table 1). Such results from the pooled analysis were statistically significant for the detrimental 5-year OS in 8 studies (862 patients) (RR = 1.515, 95% CI = 1.236-1.856, *P* < 0.001 (**Table 2**, **Figure 3** and **4**). In addition, the reduced survival was heavily influenced by the depth of invasion (40 studies, 3,252 patients, OR = 3.731, 95% CI = 3.148-4.424, P < 0.001), lymph node metastasis (51 studies, 3,957 patients, OR = 3.818, 95% CI = 3.285-4.436, *P* < 0.001), distant metastasis (16 studies, 1,322 patients, OR = 3.180, 95% CI = 2.236-4.524, *P* < 0.001), TNM stage (28 studies, 2,534 patients, OR = 3.733, 95% CI = 3.086-4.514, *P* < 0.001), histological differentiation (44 studies, 3,485 patients, OR = 1.451, 95% CI = 1.124-1.872, *P* = 0.004), and tumor size (14 studies, 1,085 patients, OR = 1.493, 95% CI = 1.154-1.931, *P* = 0.002) (**Table 2**, Supplementary Table 1).

Assessment of publication bias

Our results indicated no evidence of publication bias for most subgroup analyses (**Table 2**). The potential bias for case-control study (P_{bias} = 0.039), invasion depth (P_{bias} = 0.011), and histo-differentiation (P_{bias} = 0.041) of the STAT3 analyses could be ruled out by the Begg's and Egger's tests (**Table 2**). Similar results were observed in the MMP9 analyses regarding case-control study (P_{bias} = 0.014), invasion depth (P_{bias} = 0.000), histo-differentiation (P_{bias} = 0.000), and TNM stage (P_{bias} = 0.041) (**Table 2**).

Discussion

The recurrence and metastasis in gastric cancer remain a formidable obstacle for therapy and one of the main causes of high mortality. Prognostic factors such as clinicopathological features cannot fully predict individual clinical outcome, especially in patients receiving curative resection and/or with node negativity [90-92]. Therefore, identification of new prognostic markers may be useful in guiding surveillance and explaining survival variability for personalized therapy [93]. In the present report, we introduced two potential biomarkers, STAT3 and MMP9, and precisely estimated their prognostic and clinicopathological significances in Chinese patients with gastric cancer.

STAT3 and STAT3-regulated MMP9 overexpression has been implicated in the etiology of most solid tumors in many studies. They are thought to play key roles in the signaling of tumor proliferation, metastasis, and angiogenesis. Therapeutic agents targeting these factors are currently under development. In this study, we meta-analyzed published data on the expression of STAT3 and MMP9 between gastric cancer and normal gastric mucosa. We also investigated their association with survival and other clinical features in gastric cancer using information from studies. Only studies with IHC evaluation of STAT3 and MMP9 expression were selected to maintain the consistency in the evaluation process among different studies.

Our results demonstrated that STAT3 overexpression occurred at a median frequency of 54.1% in gastric cancer. Patients with high levels of STAT3 often experienced worse outcomes, with a meta-risk for OS (RR = 1.845). Subgroup analysis confirmed that the reduced survival was strikingly correlated with increased dedifferentiation, large tumor size, tumor invasion, lymph node spread, distant metastasis, and advanced TNM stage, which suggested an increased biological aggressiveness and a greater possibility of systemic diffusion. Tumor metastasis is a complex multi-step process, which may allow cancer cells to detach from their lattice to become migratory and invasive. STAT3, a latent self-signaling transcription factor, has been implicated to be the hallmark of tumor invasion and metastasis in a wide variety of human malignancies. Yadav *et al.* reported that interleukin-6 promoted head and neck tumor metastasis by inducing epithelial-mesenchymal transition via the activation of STAT3 signaling [94]. Additionally, we also confirmed that increased MMP9 expression by IHC studies was linked to poor 5-year OS in gastric cancer patients. The higher odds of death at 5 years was 1.515, with the magnitude of effect OR being > 2 for the main stratified meta-analyses of clinical factors. MMP9, one of the

STAT3-regulated responsive genes, not only contributes directly to epithelial-mesenchymal transition through ECM and BM degradation but also regulates tumor angiogenesis, which may offer a possible explanation for the observed strong statistical association of STAT3/MMP9 overexpression with advanced tumor invasion, lymph node spread, distant metastasis, and TNM stage. Our findings therefore suggested that these two markers might have potential prognostic and clinical values, and that they could be included in the routine clinical practice to predict the outcome of individual patient with gastric carcinoma.

Our analyses presented two critical findings. First, STAT3 and MMP9 overexpression was associated with worse outcomes, suggesting that each protein may be a potential therapeutic target. In fact, multiple studies evaluating anti-STAT3 and anti-MMP9 therapeutic strategies are ongoing (**Table 3**, http://www.clinicaltrials.gov). Second, STAT3/MMP9 expression was significantly different between gastric carcinomas and non-neoplastic mucosa, and such expression was associated with prognostic and clinical factors. Our findings emphasize the values in identifying surrogate markers. We also believe that STAT3 and MMP9 act synergistically in gastric tumor proliferation, metastasis, and angiogenesis. Detection of STAT3 and MMP9 in gastric cancer biopsies may be important to determine an optimal clinical treatment option and achieve a reasonable prognosis assessment.

In conclusion, the present investigation revealed that STAT3 and MMP9 overexpression was associated with a worse survival in gastric cancer patients and potentially indicated disease invasion and metastasis, especially in a Chinese population. Our results suggested that the development of targeting strategies against these proteins could be a reasonable therapeutic approach. Otherwise, these markers may be included in the routine clinical practice for a better prognostic prediction.

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Disclosure of conflict of interest

None.

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Supplementary Table 1. Main characteristics of protein expressions on prognostic factors

Stratification	Type	MMP9			STAT3		
		Number of studies	Total	Number	Number of studies	Total	Number
Case-Control	carcinomas	33	4367	2652	13	1256	717
	Non-neoplastic mucosa			1715			539
Overall 5-year survival	Mortal	8	862	548	3	551	119
	Survival			314			244
The depth of invasion	T3 + T4	40	3252	1887	11	725	446
	T1 + T2			1365			279
Lymph node status	Positive	51	3957	2363	12	1187	609
	Negative			1594			578
Distant metastasis	Positive	16	1322	273	4	333	66
	Negative			1049			267
TNM stage	III + IV	28	2534	1418	10	831	470
	I + II			1116			361
Histological differentiation	Poorly	44	3485	1834	13	426	519
	Well/moderate			1651			508
Size	≥ 5 cm	14	1085	566	6	611	327
	< 5 cm			519			284
Sex	Male	22	1920	1376	12	995	658
	Female			544			337
Age	> 60	10	943	457	9	817	489
	≤ 60			486			328