REG4 expression was a potential marker for carcinogenesis, aggressiveness and prognosis of gastric cancer: a meta and bioinformatics analysis

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ABSTRACT

REG4 is a potent activator of EGFR/Akt/AP-1 signaling pathway, and a potential marker for mucin-producing carcinoma and neuroendocrine tumors. Here, we performed a systematic meta and bioinformatics analysis through multiple online databases up to March 14, 2017. We found up-regulated REG4 expression in gastric cancer, compared with normal mucosa ($p < 0.05$). REG4 expression was positively with depth of invasion, lymph node metastasis, TNM staging and dedifferentiation of gastric cancer ($p < 0.05$). According to bioinformatics databases, REG4 mRNA expression was higher in gastric cancer than normal tissues ($p < 0.05$), and negatively correlated with histological grading of gastric cancer ($p < 0.05$). According to Kaplan-Meier plotter, we found that a higher expression of REG4 mRNA was positively correlated with overall and progression-free survival rates of the cancer patients with diffuse-type and N1-3 cancers or undergoing other adjuvant ($p < 0.05$), but versa for the M1 cancers ($p < 0.05$). These findings indicated that REG4 expression might be employed as a potential marker to indicate gastric carcinogenesis and subsequent progression, even prognosis.

INTRODUCTION

Regenerating (*Reg*) gene family belongs to the calcium-depending lectin gene super family, and its encoding protein acts as acute phase reactants, lectins, anti-apoptotic or growth agents [1]. REG4 is a potent activator of EGFR/Akt/AP-1 signaling pathway, and a potential marker for mucin-producing carcinoma and neuroendocrine tumors [2–4]. SP1, GATA6 and CDX2 are proved to be transcription factors of *REG4* gene, which is essential for cellular proliferation and tumorigenesis [5–7]. Bishnupuri et al. [8] found that REG4-induced mitogenesis was activated by Akt-GSK3β-β-catenin-TCF-4 signaling in colorectal cancer. REG4 also induced the expression of MMP-7 and MMP-9, which contributed to invasion of pancreatic cancer cells [9]. The pancreatic cancer secreted REG4, which promoted macrophage polarization to M2 through EGFR/Akt/CREB pathway [10].

Nanakin *et al.* [11] found that strong *REG4* mRNA was strongly expressed in inflamed epithelium, dysplasia and cancerous lesions of ulcerative colitis tissues, and correlated with the mRNA expression of *bFGF* and *HGF* that subsequently stimulated REG4 expression in colon cancer cells [12]. *REG4* mRNA was overexpressed in colorectal, and pancreatic, hepatocellular and prostate cancers [13–17]. Recently, it has been reported that *REG4* overexpression induces the phosphorylation of the EGFR and inhibits 5-fluorouracil- induced apoptosis in gastric cancer cells [18]. Our previous work showed that REG4 expression should be considered as a good biomarker for gastric intestinal metaplasia and dysplasia, and was especially related to the histogenic pathway of signet ring cell carcinoma [19]. Here, a meta- and bioinformatics analysis was carried out to explore the clinicopathological and prognostic significances of REG4 expression in gastric cancer.

RESULTS

Selection and characterization of the studies

Fourteen articles were selected for your meta-analysis from PubMed, Web of Science, BIOSIS,

SciFinder and CNKI (Figure 1 and Table 1). Four articles contain the samples of normal gastric mucosa [19–22], 14 do the correlation between REG4 expression and clinicopathological parameters of gastric cancer [2, 18–30], and 7 do the prognostic data of REG4 expression [18, 19, 23–26, 30].

The clinicopathological and prognostic significances of REG4 protein expression in gastric cancer

We found that REG4 expression was higher in gastric cancer than normal mucosa according to 692 cancers and 401 controls of 4 studies (Figure 2A, $p = 0.0005$). No distinct REG4 expression was observed in between male and female cancer patients (Figure 2B, $p > 0.05$). REG4 expression was positively linked to depth of invasion (Figure 2C, $p = 0.003$), lymph node metastasis (Figure 2D, $p = 0.03$), TNM staging (Figure 2E, $p = 0.04$), and dedifferentiation (Figure 2F, $p = 0.01$) of gastric cancer. There was no association between REG4 expression and favorable overall survival in gastric cancer patients (Figure 2G, HR = 1.00, 95% CI: 0.84–1.20, $p > 0.05$).

Heterogeneity analysis

To evaluate the heterogeneity, we removed a study to observe the alteration in the pooled results of remaining studies (Figure 3). For instance, the pooled OR about relationship between REG4 expression and TNM staging was remarkably decreased if Wang's study was excluded (data not shown).

The clinicopathological and prognostic significances of REG4 mRNA expression in gastric cancers

Cho's, Chen's, Cui's and DEricco's datasets were used for bioinformatics analysis. It was found that REG4 mRNA expression was lower in gastric normal tissues than cancer, even intestinal-, diffuse- and mixed-type carcinomas (Figure 4A, $p < 0.05$). TCGA data showed that REG4 expression was negatively correlated with the histological grading of gastric cancers (Figure 4B, $p < 0.05$). As indicated in Figure 5, Kaplan-Meier plotter showed that a higher REG4 mRNA expression was positively related to both overall survival rate and

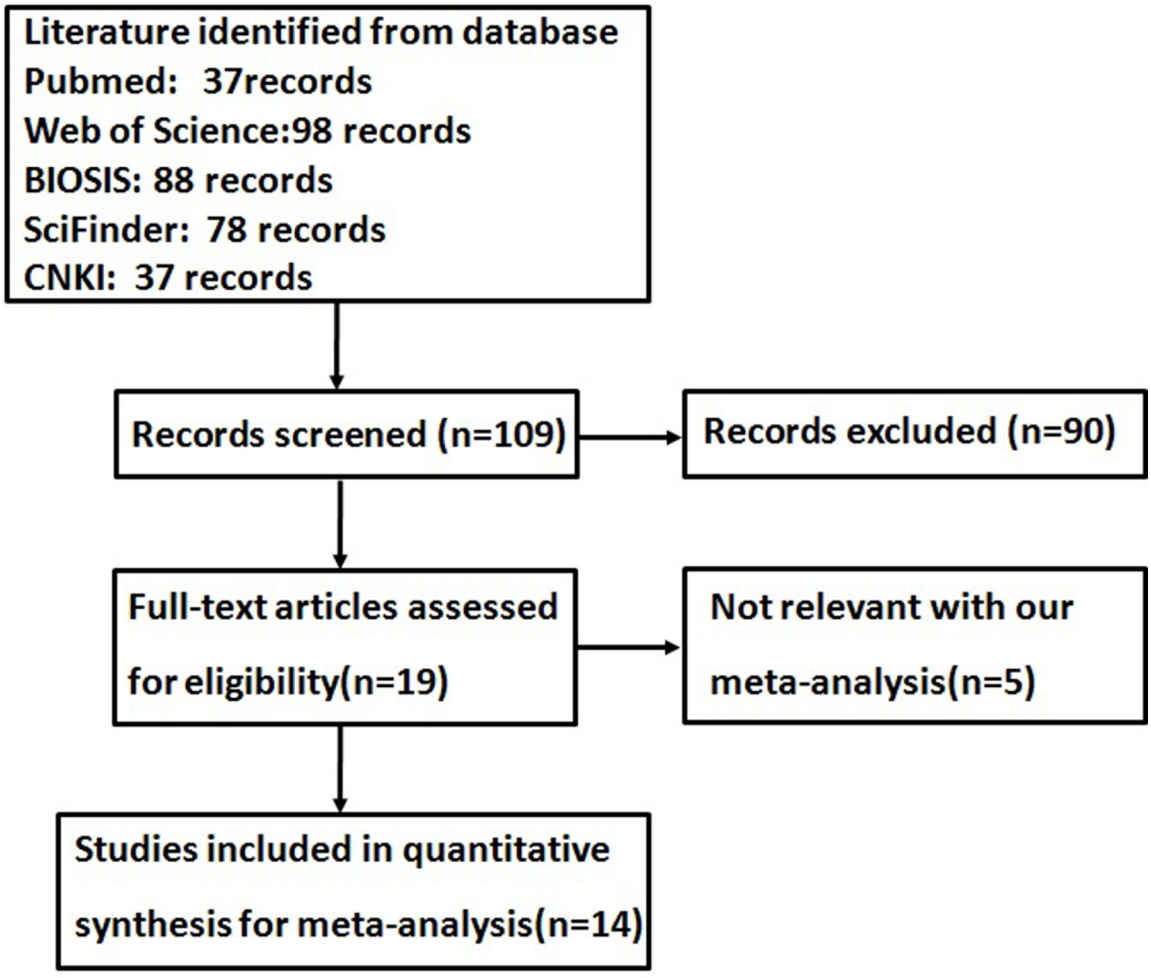

Figure 1: Flow diagram of the selection process in this meta-analysis.

Table 1: Main characteristics of eligible studies

First author	Year	Country	Ethnicity	AS	Cases	Control	Risk to cancer	Outcome	Quality
Oue N	2005	Japan	Asian	Self-making	143				8
Mitani Y	2007	Japan	Asian	R & D	161			No	8
Zheng HC	2009	Japan	Asian	R & D	372	93	Up	No	9
Yamagishi H	2009	Japan	Asian	R & D	63			No	8
Tao HQ	2011	China	Asian	R & D	192			Neg	8
Moon JH	2012	Korea	Asian	R & D	162			No	8
Suh YS	2012	Korea	Asian	R & D	450			Neg	8
Wang HX	2016	China	Asian	R & D	102			Neg	8
Ma H	2006	China	Asian	R & D	26				8
Wu WQ	2009	China	Asian	R & D	96				7
Dong YL	2012	China	Asian	R & D	147	147	Up		8
Liu ZH	2013	China	Asian	R & D	48				7
Dong YL	2013	China	Asian	R & D	101	89	Up		8
Yan W	2015	China	Asian	R & D	72	72	Up		8

AS, antibody source; Up, up-regulation; Neg, negative correlation.

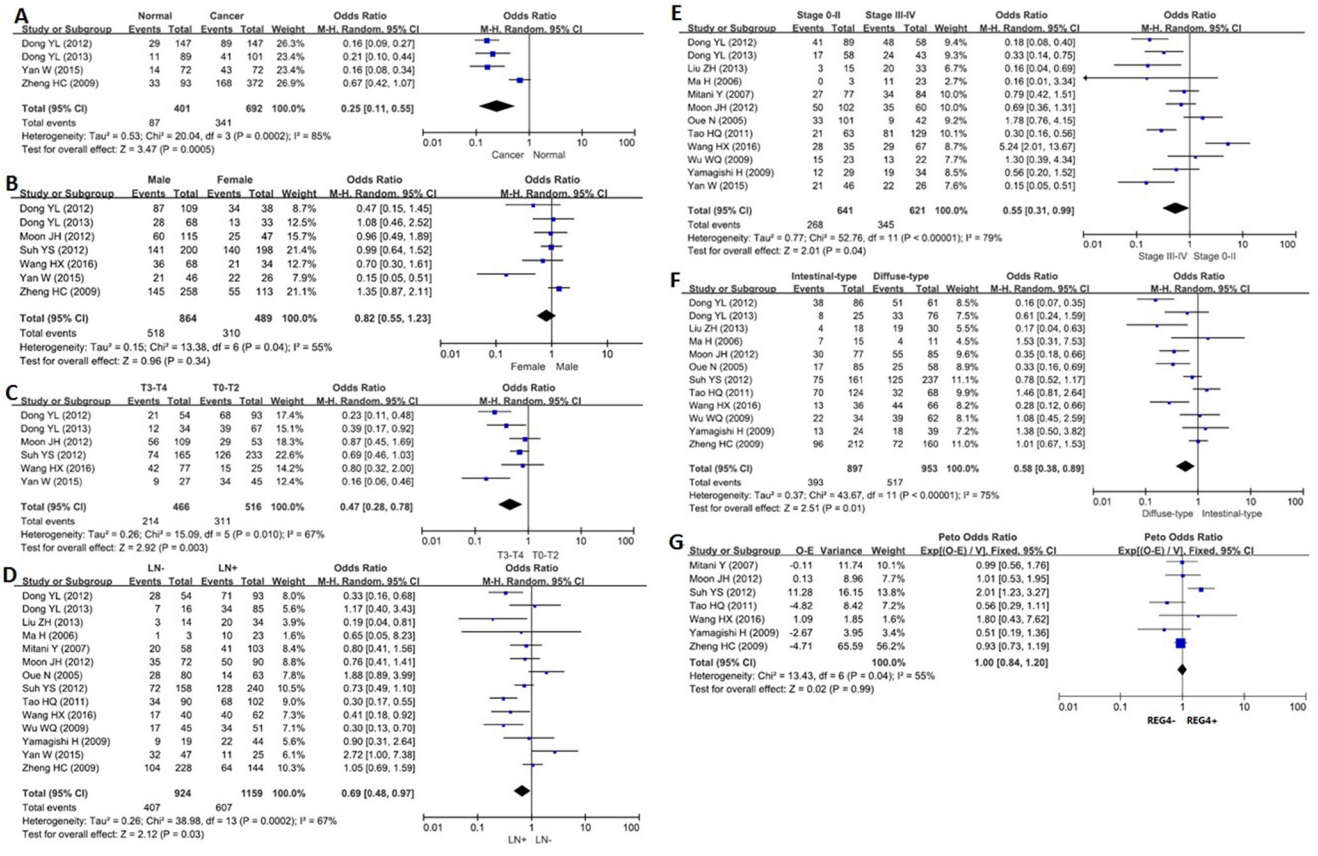

Figure 2: Forest plot for the relationship between REG4 expression and clinicopathological parameters of gastric cancer. (**A**) gastric carcinogenesis (cancer *vs* normal mucosa); (**B**) correlation between sex and REG4 expression (male *vs* female); (**C**) correlation between depth of invasion and REG4 expression (T0-2 *vs* T3–4); (**D**) correlation between lymph node metastasis (LN) and REG4 expression (LN- *vs* LN+); (**E**) correlation between TNM staging and REG4 expression (stage 0-II *vs* III-IV); (**F**) correlation between differentiation and REG4 (intestinal-type *vs* diffuse-type). (**G**) correlation between the prognosis and REG4 expression (REG4- *vs* REG4+).

progression-free survival rate of the patients with diffuse-type and N1-3 cancers or undergoing other adjuvant ($p < 0.05$), but versa for the M1 cancer patients ($p < 0.05$). Stage III, Her2-positive, and N2 cancer patients with REG4 mRNA hyperexpression had a high progression-free survival rate than those with its hypoexpression ($p < 0.05$). The similarity was seen for overall survival rate in Her2-positive cancer patients ($p < 0.05$).

DISCUSSION

Bishnupuri et al. [4, 31] found that REG4 was a potent activator of the EGFR/Akt/AP-1 signaling pathway, and protected colon cancer cells against apoptotic induction or normal intestinal crypt cells from irradiation-induced apoptosis via up-regulating the expression of Bcl-2, Bcl-xl and survivin. Chen et al. [32] found that REG4 overexpression and the recombinant protein inhibited cell apoptosis, and enhanced G_2/S progression, proliferation, migration and invasion with Wnt5a, p70s6k, survivin and VEGF hyperexpression and Bax hypoexpresion. Additionally, REG4 promoted migration and invasion of colon cancer cells in both autocrine and paracrine manners, which was blocked by anti-REG4 antibody [33]. Takehara et al. [34] found that REG4 knockdown caused a decrease in cell viability, and recombinant REG4 enhanced growth of pancreatic cancer cells in a dose-dependent manner. In prostate cancer cells, REG4 downregulation was found to induce p21 expression, which negatively regulated Cyclin D1 and blocked the G_1/S transition [35]. Therefore, we performed a meta- and bioinformatics analysis to clarify the clinicopathological and prognostic significances of REG4 expression in gastric cancer.

Serum REG4 level was significantly higher in esophageal, gastric and pancreatic cancer patients than that in controls [36–38]. Consistent with the data about ovarian cancer, colon cancer, glioma, pancreatic cancer, gallbladder cancer, and prostate cancer [32, 38–42], we found that REG4 expression was higher in gastric cancer than normal mucosa, and positively correlated with depth of invasion, lymph node metastasis, TNM staging and differentiation of gastric cancer, suggesting that REG4 overerexpression promoted gastric carcinogenesis and subsequent progression. The higher REG4 expression

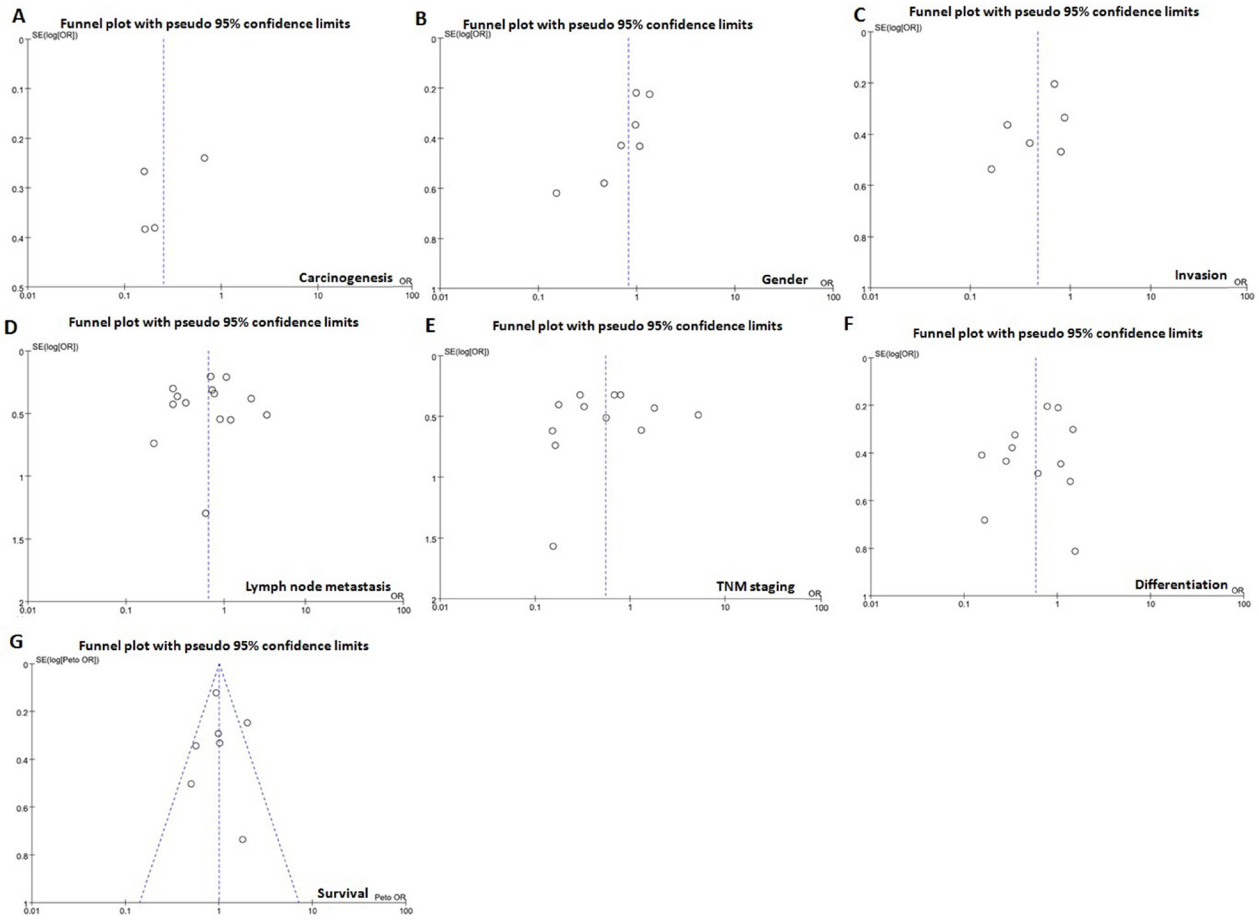

Figure 3: Funnel plot for publication bias test between REG4 expression and gastric carcinogenesis or progression. The bias was analyzed about risk degrees of REG4 expression in gastric mucosa (**A**) for gastric carcinogenesis. Additionally, it was tested between REG4 expression and clinicopathological features of gastric cancer, including age (**B**), depth of invasion (**C**), lymph node metastasis (**D**), TNM staging (**E**), and differentiation (**F**) and prognosis (**G**).

in diffuse- than intestinal-type carcinoma might be due to REG4 overexpression in mucinous and signet ring cell carcinoma [19]. In agreement with our finding, Lehtinen et al. [43] identified REG4 as a potential biomarker with specificity for the mucinous ovarian cancer subtype, and Chen et al. [32] found that higher *REG4* mRNA expression was observed in mucinous than serous carcinomas. All 14 studies have used anti-REG4 antibody from R&D System Corp and ensured the consistence. The inconsistence might be due to immunohistochemical approaches, criteria for positive staining, statistical analysis, and subjects and samples (e.g. tissue microarray of small size).

Eguchi et al. [44] found that the patients with a higher REG4 level showed an unfavorable response to chemotherapy and radiotherapy. Reportedly, REG4 expression was positively linked to the worse prognosis of the patients with colon cancer and adenoid cystic carcinoma [39, 45]. REG4 expression might be considered as an independent factor of the worse prognosis of ovarian cancer, rectal cancer, glioma, and advanced gallbladder carcinoma as [32, 40, 41, 46]. Our meta-analysis showed that REG4 expression was not linked to the prognosis of the patients with gastric cancer. Our bioinformatics analysis indicated that REG4 mRNA expression was positively associated with overall and progression-free survival rates of the patient with diffuse-type and N1-3 cancers or undergoing other adjuvant, while versa for M1 cancer. The paradoxical findings should be explained by distinct methodologies (immunohistochemistry and transcriptomic sequencing), tissue specificity and different subgroupings of gastric cancers.

In summary, there existed up-regulated REG4 expression during gastric carcinogenesis at both mRNA and protein levels. REG4 protein expression was positively correlated with aggressive behaviors of gastric cancer. *REG4* mRNA was considered as a good prognostic marker of the patients with diffuse-type, N1-3 and M1 cancers or undergoing other adjuvant. The following disadvantages are mentioned in our study, including publication bias from published results being predominantly positive, subjects bias from limited kinds of the patient populations, and performance bias from data extraction of survival data using software.

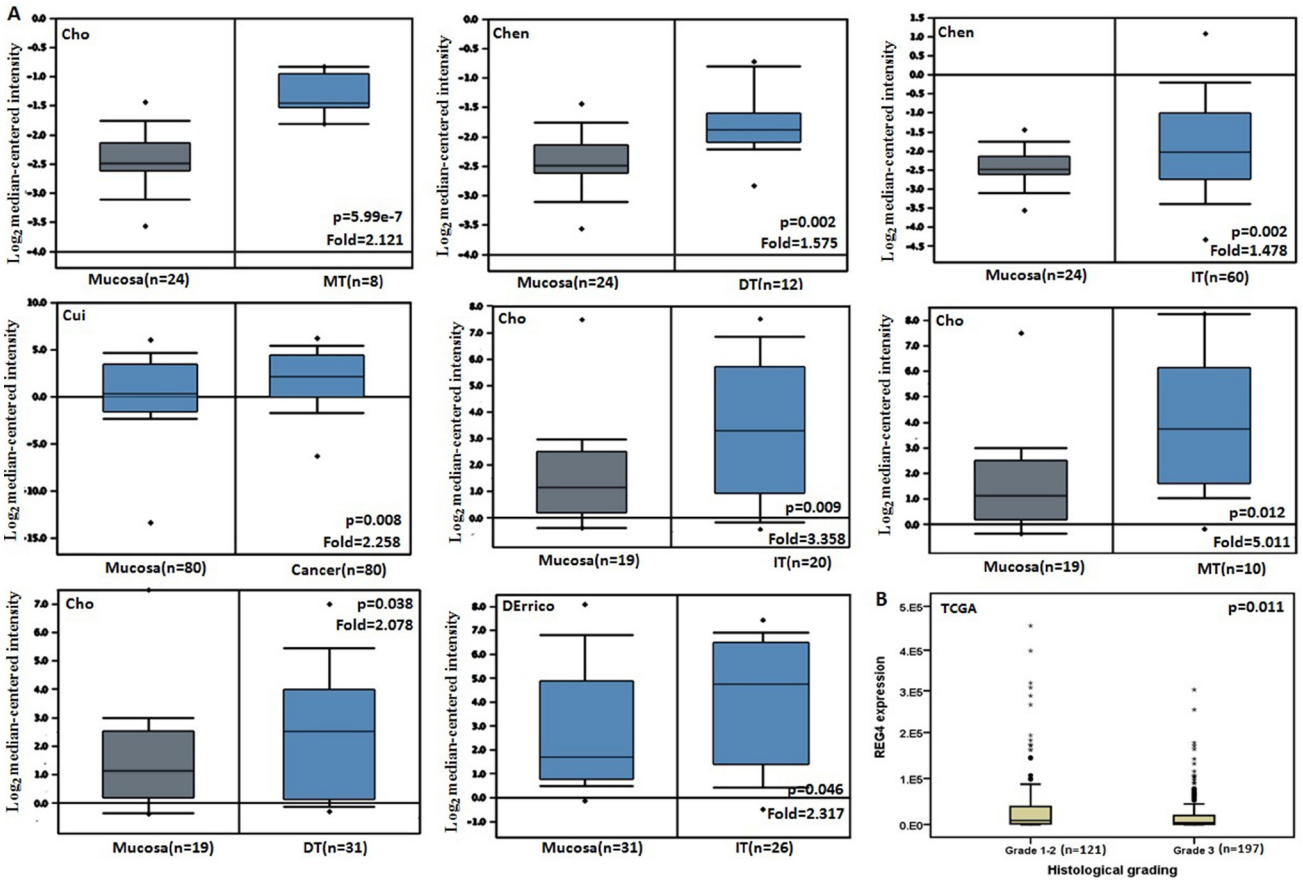

Figure 4: *REG4* mRNA expression in gastric cancer and its clinicopathological significance. Cui's DErrico's, Chen's and Cho's datasets were employed for Bioinformatics analysis to analyze *REG4* mRNA expression during gastric carcinogenesis. A higher *REG4* mRNA expression was detectable in gastric cancer than that in normal gastric mucosa, even stratified into intestinal-(IT), diffuse-(DT), and mixed-type (MT) carcinomas by Lauren's classification (**A**) ($p < 0.05$). TCGA database showed that *REG4* mRNA was more expressed in G1–2 than G3 cancers (**B**) ($p < 0.05$).

MATERIALS AND METHODS

Identification of eligible studies and data extraction

We performed a publication search using PubMed, Web of Science, BIOSIS, SciFinder and CNKI updated on March 14, 2017. The following search terms were used: (REG4 OR REG IV) AND (gastric OR stomach) AND (cancer OR carcinoma OR adenocarcinoma). Searching was done without restriction on language or publication years. Inclusion criteria for studies: (1) articles to observe the alteration in REG4 expression in gastric cancer by immunohistochemistry; (2) papers to compare REG4 expression with pathobiological behaviors and prognosis of gastric cancer by immunohistochemistry. Exclusion criteria included: (1) abstract, comment, review and meeting; (2) duplication of the previous publications; (3) Western blot, RT-PCR, cDNA microarray, or transcriptomic sequencing for REG4 expression; (4) lack of sufficient information.

Data extraction

Based on the inclusion criteria, two reviewers (HC Zheng and S Zhao) independently extracted information from all eligible publications. The following information was included in each study: name of first author, year of publication, country, ethnicity, antibody source, numbers of cases and controls, expression alteration, and follow-up outcome. Regarding survival analysis, we used Engauge Digitizer software to extract data from Kaplan-Meier curves and calculated the Hazard ratios (HR) and their corresponding 95% confidence intervals (CI). Any disagreement was resolved through discussion until the two reviewers reached a consensus.

Quality score assessment

Two reviewers (HC Zheng and S Zhao) independently assessed the quality of the included studies according to Newcastle Ottawa Scale (NOS) (http://www.ohri.ca/programs /clinical_ epidemiology/oxford.htm). The scale consists of three components related to sample selection, comparability and ascertainment of outcome.

Bioinformatics analysis

The individual gene expression level of REG4 was analyzed using Oncomine (www. oncomine.org), a cancer microarray database and web-based data mining platform for a new discovery from genome-wide expression analyses. We compared the differences in REG4 mRNA

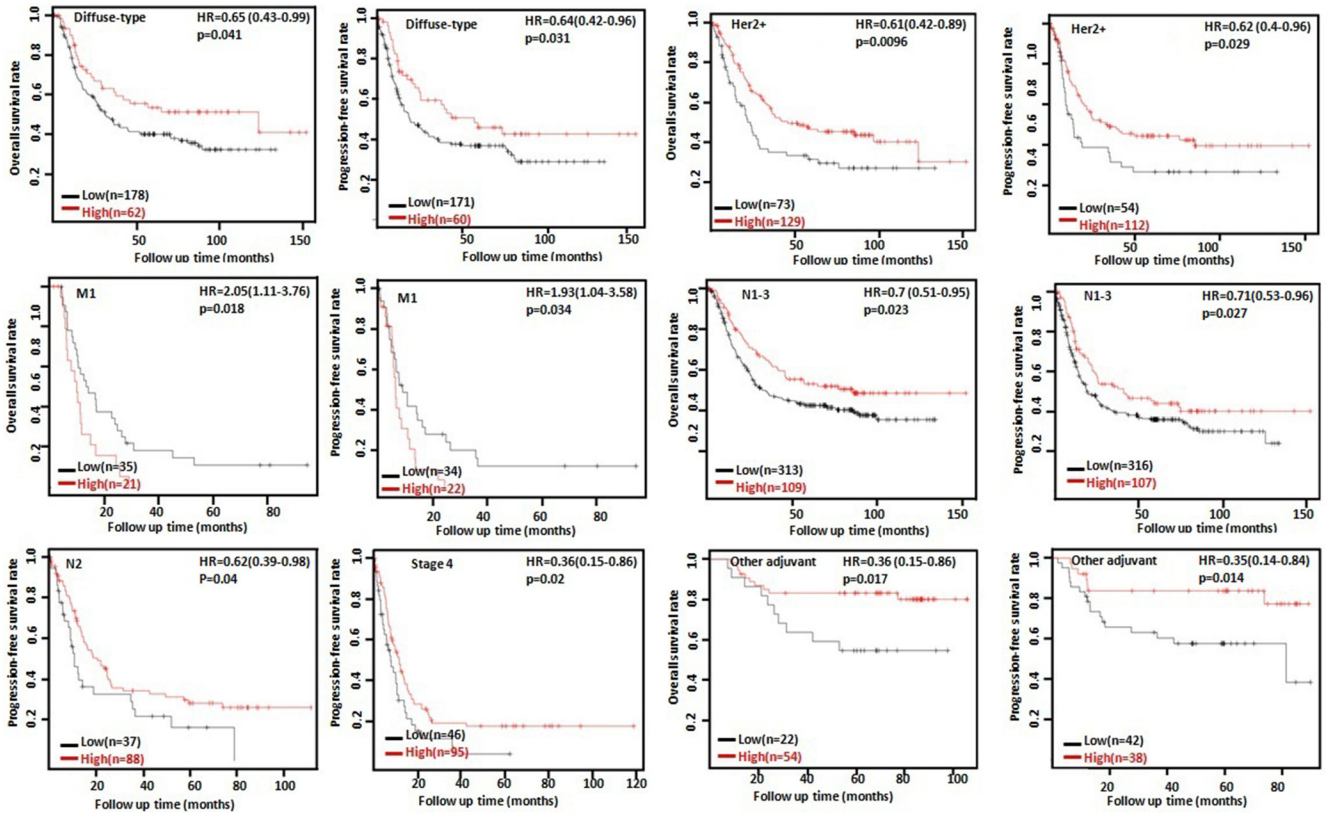

Figure 5: The prognostic significance of *REG4* mRNA in the patients with gastric cancer. According to the data from Kaplan-Meier plotter, *REG4* mRNA expression was positively related to either overall or progression-free survival rate of the patients with gastric cancer except M1 cancer. HR, hazard ratio.

level between gastric normal tissue and cancer. All data were log-transformed, median centered per array, and standard deviation normalized to one per array. The expression (RNA-seqV2) and clinicopathological data of 392 gastric cancer patients were downloaded from the Cancer Genome Atlas (TCGA) database by TCGA-assembler in R software. We integrated the raw data, analyzed REG4 expression in gastric cancer, and compared it with clinicopathological and prognostic data of the patients with gastric cancer. Additionally, the prognostic significance of REG4 mRNA was also analyzed using Kaplan- Meier plotter (http://kmplot.com).

Statistics analysis

HWE was evaluated using Chi-square test in control groups of each study. Strength of association between REG4 expression and cancer risk was assessed by odds ratios with 95% confidence intervals. Statistical significance of the pooled OR was determined by *Z* test. If there was no significant heterogeneity, the fixed effect model (Mantel-Haenszel method) would be employed. Otherwise, the random effect model (DerSimonian and Laird method) would be used excluding prognostic analysis. Heterogeneity effect was then quantified by I^2 test, which was subdivided into low, moderate and high degrees of heterogeneity according to the cut-off values of 25%, 50% and 75% respectively. Publication bias was evaluated by funnel plot and quantified by Begg's test and Egger's test to assess funnel plot asymmetry. Meta-analyses were performed with Revman software 5.3 and data from TCGA database was dealt with SPSS 10.0 software using student *t* test. Two-sided $p < 0.05$ was considered as statistically significant.

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CONFLICTS OF INTEREST

The authors have declared that no conflicts of interest exist.

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