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*Original Article*

# Retrospective study of the roles of NDRG1 expression in gastric cancer: a meta- and bioinformatics analysis

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**Abstract:** NDRG1 (N-myc downstream-regulated gene 1) is the first member discovered in the NDRG family. It is believed to function as an inhibitor gene of tumors. This study performed a detailed and retrospective document retrieval through PubMed, Web of Science, and CNKI updated through July 10, 2017. It was found that levels of NDRG1 expression were decreased in gastric cancer, compared to normal mucosa ($p<0.05$). Expression of NDRG1 was negatively correlated with depth of invasion, lymph node metastasis, distant metastasis, TNM staging, and de-differentiation of gastric cancer ($p<0.05$). A positive association between NDRG1 expression and favorable overall survival was detected in patients with gastric cancer ($p<0.0001$). Bioinformatics analysis demonstrated that *NDRG1* mRNA expression was higher in gastric cancer than in homologous normal tissues, evenly stratified into different type of intestinal-, diffuse-, mixed-, tubular-, and mucinous-type carcinomas ($p<0.05$). Moreover, less invasion and lighter TNM staging had hyperexpression of *NDRG1* mRNA ($p<0.05$). According to KM plotter database, expression of *NDRG1* was significantly correlated with overall survival or progression-free survival rates of patients with gastric cancer, depending on the subgrouping ($p<0.05$). These consequences indicate that NDRG1 expression might be employed as a potential biomarker to indicate gastric carcinogenesis and subsequent progression, even prognosis.

**Keywords:** NDRG1, gastric cancer, biomarker, meta-analysis, bioinformatics analysis

## Introduction

As the inaugural member of the NDRGs family, NDRG1, also termed Cap43, was firstly identified as a predominantly-expressed protein in various organisms and encoded by N-myc down-stream regulated gene 1 (NDRG1) [1, 2]. It has been considered a type of inhibitor gene of tumors, whose putative function is suppression of tumor metastasis. The protein encoded by this gene is a cytoplasmic protein involved in stress response, hormone response, cell growth, and differentiation. NDRG1 is located on the long arm of human chromosome 8, spanning 60,085 bp. It includes 16 exons and 15 introns and encodes a 2997-bp transcript [3, 4]. Increasing evidence has demonstrated that NDRG1 has an anti-oncogenic impact in a large array of malignancies because it can inhibit angiogenesis by stopping migration, proliferation, invasion, and cell cycle, while enhancing apoptotic sensitivity of cancer cells [5-11]. The protein is necessary for p53-mediated caspase activation and apoptosis. Mutations of this gene are a cause of Charcot-Marie-Tooth disease type 4D. Expression of this gene may be a prognostic indicator for several types of cancer. NDRG1 inhibits "stemness" of colorectal cancer, via downregulation of nuclear β-catenin and CD44, and inhibits TGF-β-induced EMT, via its inhibitory effects on EMT transcription factors, such as snails and slugs [12, 13]. Dixon et al. [14] found that NDRG1 might suppress metastasis of prostate cancer cells by targeting Dp44mT in PI3K/AKT/ERK signaling pathways. Sharleen et al. [15] found that NDRG1 inhibited ErbB signaling by suppressing formation of epidermal growth factor receptor (EGFR)/human epidermal growth factor receptor 2 (HER2) and HER2/HER3 heterodimers and by downregulating EGFR expression. These findings indicate that loss of downregulation of NDRG1 expression may be employed as a potential marker to indicate invasion and metastasis of carcinoma.

Since its discovery in 2000, a total of 391 articles concerning NDRG1 have been published in the PubMed database up through July 10th,

2017. According to documents, NDRG1 expression was downregulated in breast cancer [6], prostate cancer [16], pancreatic cancer [17], esophageal cancer [18] and colorectal cancer [19], but upregulated in hepatoma [20], lung cancer [21], kidney cancer [22], bladder cancer [23], and cervical cancer [24]. NDRG1 downregulation has been positively associated with aggressive behavior and poor prognosis of hepatocellular carcinoma [25]. Therefore, this was a retrospective study by means of meta- and bioinformatics analysis to illuminate clinicopathological and prognostic significances of expression of NDRG1 in gastric cancer.

## Materials and methods

### *Identification of eligible studies and data extraction*

Articles were searched in PubMed, Web of Science, and CNKI up through May 10, 2017. The following search terms were used: NDRG1 OR AND (gastric OR stomach) AND (cancer OR carcinoma OR adenocarcinoma). This search was performed without language restriction or publication years. Inclusion criteria for studies were as follows: (1) Gastric cancer patients; (2) Expression of NDRG1 by immunohistochemical staining; and (3) Papers comparing expression of NDRG1 with pathobiological behaviors or prognosis of gastric cancer by immunohistochemistry. Exclusion criteria for studies included: (1) Abstracts, case reports, reviews, and meetings; (2) Redundant publications; and (3) Western blot, qRT-PCR, cDNA microarray, or transcriptomic sequencing for NDRG1 expression.

### *Data extraction*

Information from all eligible publications was extracted by two reviewers (Xiao XJ and Zheng HC), independently. The following information was included in each study: name of first author, publication year, country, antibody information, numbers of cases and controls, expression alteration, and follow-up outcomes. For survival analysis, Engauge Digitizer software was used to extract data from Kaplan-Meier curves. Hazard ratios and their corresponding 95% confidence intervals were calculated accurately. Any disagreement was eliminated through discussion until the two reviewers reached an agreement.

### *Quality score assessment*

Two independent reviewers (Xiao XJ and Zheng HC) assessed the quality of selected studies according to Newcastle Ottawa Scale (NOS) (http://www.ohri.ca/programs/clinical_epidemiology/oxford.htm). The method consisted of sample selection, comparability, and ascertainment of outcomes.

### *Bioinformatics analysis*

Oncomine database (www.oncomine.org), a cancer microarray database and web-based data mining platform for new discoveries in genome-wide expression analyses, was used to analyze individual gene expression level of *NDRG1*. Differences in *NDRG1* mRNA levels between gastric tumor tissues and corresponding normal tissues were compared, elaborately, and all data were log-transformed. Median centered per array and standard deviations were normalized to one per array. Expression (RNA-seqV2) and clinicopathological information of 411 gastric cancer patients were gathered from the Cancer Genome Atlas (TCGA) database by TCGA-assembler in R software. This study integrated the original data, analyzed expression of *NDRG1* in gastric cancer, and compared it with clinicopathological and prognostic information of patients with gastric cancer. In addition, the prognostic significance of *NDRG1* mRNA was analyzed using Kaplan-Meier plotter (http://kmplot.com).

### *Statistics analysis*

Statistical analysis was carried out, as described previously [26]. Briefly, Chi-squared test was used to evaluate HWE in control groups of each study. Odds ratios with 95% confidence intervals were applied to assess strength of association between NDRG1 expression and cancer risk. Z test was applied to determine the statistical significance of pooled OR. $I^2$ test was applied to quantify heterogeneity, which was subdivided into three components related to low, moderate, and high degrees of heterogeneity, respectively equal to cut-off values of 25%, 50%, and 75%. If the heterogeneity had no differences, a fixed effects model (Mantel-Haenszel method) was applied. Moreover, a random effects model (DerSimonian and Laird method) was employed excluding prognostic analysis. This meta-analysis was performed

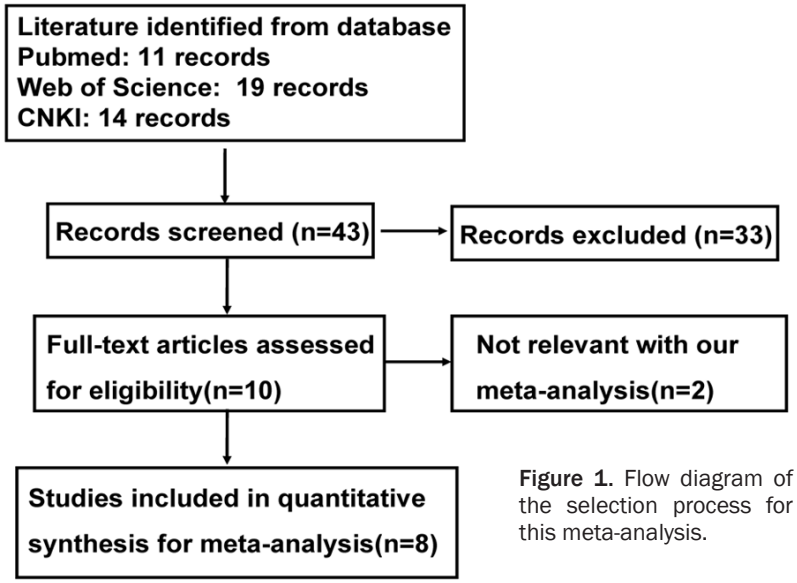



**Figure 1.** Flow diagram of the selection process for this meta-analysis.

with Review Manager 5.2 and data from TCGA database was managed with SPSS 17.0. In addition, two-sided $p < 0.05$ is considered statistically significant.

## Results

*Characteristics of eligible studies*

As shown in **Figure 1** and **Table 1**, a total of 8 articles about the relationship between NDRG1 protein expression and cancer risk, including clinicopathological and prognostic parameters of gastric cancer, were selected for this study from PubMed, Web of Science, and CNKI (Chinese). Only 8 articles contained samples of normal gastric mucosa [27-34]. These contained the information of comparison between NDRG1 expression and clinicopathological characteristics of gastric cancer, including sex, age, depth of invasion, lymph node metastasis, distant metastasis, TNM staging, and differentiation. Additionally, the prognostic significance of NDRG1 expression was explored in 3 articles [28, 29, 33].

*Association between NDRG1 expression and cancer susceptibility of gastric mucosa or dysplasia*

This study compared differences of NDRG1 expression between normal gastric mucosa and cancer in 5 studies, with 403 cancers and 400 controls. Results revealed that NDRG1 expression was downregulated in gastric cancer, compared to normal mucosa (**Figure 2A**, p=0.001).

*Correlation of NDRG1 expression with clinicopathological parameters of gastric cancer*

As shown in **Figure 2B** and **2C**, there was no association between expression of NDRG1 and sex or age of patients with gastric cancer (p>0.05). Higher NDRG1 expression was also found in $T_{is-2}$ than $T_{3-4}$ gastric cancer (**Figure 2D**, p=0.05). Cancer patients with lymph node metastasis and distant metastasis showed lower NDRG1 expression than those without lymph node and distant metastasis (**Figure 2E**, **2F**, p<0.01). NDRG1 expression was negatively associated with TNM staging (**Figure 2G**, p<0.05). Well-differentiated gastric carcinomas had a higher expression of NDRG1 than poorly-differentiated ones (**Figure 2H**, p<0.05).

*Association between NDRG1 expression and survival rates of gastric cancer*

As demonstrated in **Figure 2I**, pooled results from 3 datasets indicated a significantly positive association between NDRG1 expression and favorable overall survival in patients with gastric cancer (HR = 0.45, 95% CI: 0.30-0.65, p<0.05).

*Publication bias*

Heterogeneity was tested as shown in **Figure 3**. Sensitivity analysis was performed to evaluate an individual study's influence on pooled results by deleting one single study each time from pooled analysis. As a result, lymph node metastasis and depth of invasion results of NDRG1 expression in Inagaki's study had great significance on *p*-values and pooled OR. When this study was excluded, the *p*-value had no statistical significance and heterogeneity testing showed a significant decrease (data not shown).

*Clinicopathological significance of NDRG1 mRNA expression in gastric cancer*

Deng's, Chen's, and TCGA's datasets were used to perform bioinformatics analysis. It was dem-

**Table 1.** Main characteristics of eligible studies

| First author | Year | Country | Ethnicity | Antibody source | Cases | Control | Risk to cancer | Out come | Quality |
|---|---|---|---|---|---|---|---|---|---|
| Akihiko K | 2011 | Japan | Asian | Benchmark XT | 129 | | Down | Pos | 8 |
| Chang XJ | 2014 | China | Asian | CST | 112 | 112 | Down | Pos | 8 |
| Chang XJ | 2015 | China | Asian | CST | 101 | 101 | Down | | 8 |
| Jiang KW | 2010 | China | Asian | Abnova | 110 | 110 | Down | Pos | 8 |
| Jiao ZG | 2009 | China | Asian | Santa | 54 | 67 | | | 9 |
| Chang XJ | 2013 | China | Asian | CST | 20 | 20 | | | 9 |
| Inagaki Y | 2009 | Japan | Asian | Santa | 96 | | | | 9 |

Note: down, downregulated; Pos, positive correlation; Pos, positive correlation.

onstrated that *NDRG1* mRNA expression was higher in gastric cancer tissues than normal tissues, even stratified into different types of intestinal-, diffuse-, mixed-, tubular- and mucinous-type carcinomas (**Figures 4A-5J**, $p<0.05$). TCGA's and Chen's data showed higher NDRG1 expression in intestinal-type than diffuse-type adenocarcinoma (**Figure 4K**, **4L**, $p<0.05$). According to TCGA data, NDRG1 expression was negatively correlated with depth of invasion and TNM staging of gastric cancer (**Figure 4M** and **4N**, $p<0.05$).

*Prognostic significance of NDRG1 mRNA expression in gastric cancer*

According to the KM plotter, high *NDRG1* mRNA expression showed a negative correlation with overall and progression-free survival rates of stage I cancer patients (**Figure 5A**, **5B**, $p<0.05$), while it was the same for overall survival in patients with poorly-differentiated adenocarcinoma (**Figure 5C**, $p<0.05$). Overall survival rates of patients with $T_3$, $T_4$, $N_2$ or $N_3$ cancer had a positive correlation with *NDRG1* mRNA expression (**Figure 5D-G**, $p<0.05$). Positive association between *NDRG1* mRNA expression and progression-free prognosis was also observed in stage II cancer patients (**Figure 5H**, $p<0.05$).

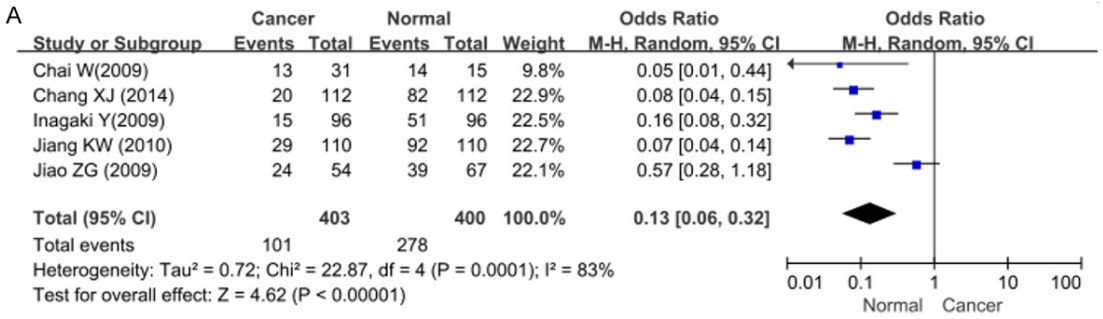
## Discussion

Gastric cancer is one of the most common human cancers. Despite significant improvement made in surveillance and treatment of gastric cancer, it remains a devastating disease with poor prognosis. Metastasis is still the most destructive impediment for the long-term survival of cancer patients [35]. Many studies have revealed that level of NDRG1 expression is inversely correlated with tumor metastasis, suggesting that NDRG1 may act as a metastasis suppressor [13-15, 36, 37]. Guan et al. [38] found that NDRG1 suppressed metastasis by downregulating MMPs, which degraded extracellular matrix and adhesion molecules, such as β-catenin and E-cadherin. Chen et al. [39] found that NDRG1 inhibited TGF-β-induced epithelial-mesenchymal transition and restored β-catenin and E-cadherin levels. To investigate association between clinicopathological and prognostic significance and NDRG1 expression, this study performed a retrospective analysis of 8 studies, meeting specific inclusion criteria and with moderate to high quality NOS scores.

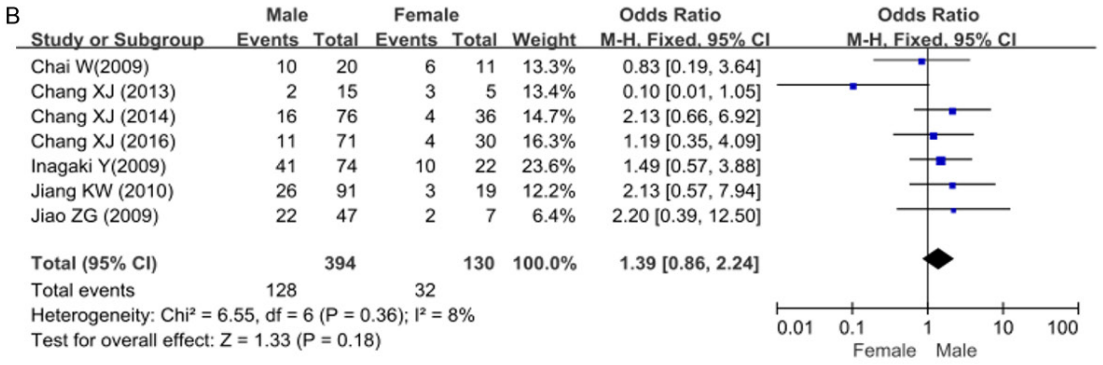
Consistent with systematic data about hepatoma, lung, kidney, bladder carcinoma, and uterine cervix [20-24], it was found that expression of NDRG1 was downregulated in gastric cancer, compared to gastric mucosa in a recent study, suggesting that NDRG1 hypoexpression might contribute to gastric carcinogenesis. Wang et al. [40] demonstrated that mRNA levels of *NDRG1* were significantly upregulated in cancer samples compared with normal colorectal samples. This is in line with the present bioinformatics findings. This result is not surprising since mRNA levels do not usually predict corresponding protein levels. There is a long distance from mRNA to functional protein by translation and so many factors can regulate post-translational modification.

Chen et al. [41] found that NDRG1 expression was positively correlated with large tumor size, portal vein invasion, TNM staging, AFP level of ≥400 U/l, intrahepatic metastasis, recurrence, and poor patient survival in hepatocellular cancer patients. However, present findings demonstrated that NDRG1 expression was inversely linked to deep invasion, TNM staging, lymph node metastasis, distant metastasis, and de-differentiation of gastric cancer at either *mRNA* or protein levels, in agreement with reports on breast cancer, prostate, pancreas, esophagus,

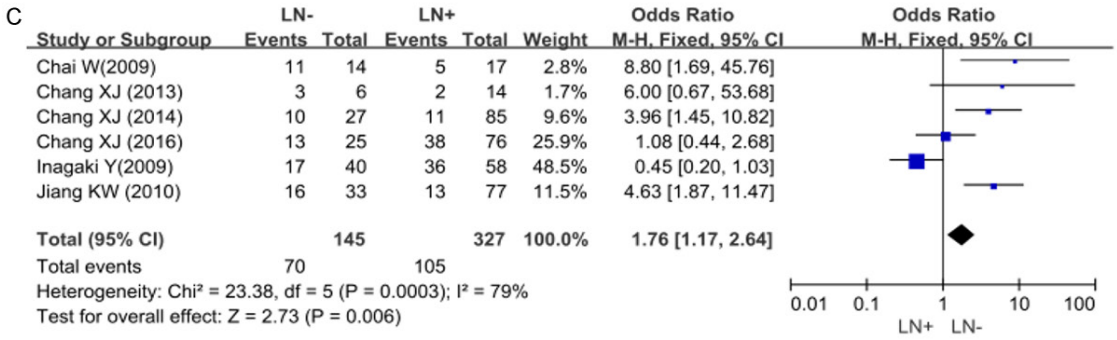
A


Cancer
Normal
Odds Ratio
Odds Ratio
Study or Subgroup Events Total Events Total Weight M-H, Random, 95% CI
M-H, Random, 95% CI
Chai W(2009) 13 31 14 15 9.8% 0.05 [0.01, 0.44]
Chang XJ (2014) 20 112 82 112 22.9% 0.08 [0.04, 0.15]
Inagaki Y(2009) 15 96 51 96 22.5% 0.16 [0.08, 0.32]
Jiang KW (2010) 29 110 92 110 22.7% 0.07 [0.04, 0.14]
Jiao ZG (2009) 24 54 39 67 22.1% 0.57 [0.28, 1.18]
Total (95% CI) 403 400 100.0% 0.13 [0.06, 0.32]
Total events 101 278
Heterogeneity: Tau² = 0.72; Chi² = 22.87, df = 4 (P = 0.0001); I² = 83%
Test for overall effect: Z = 4.62 (P < 0.00001)
0.01 0.1 1 10 100
Normal Cancer

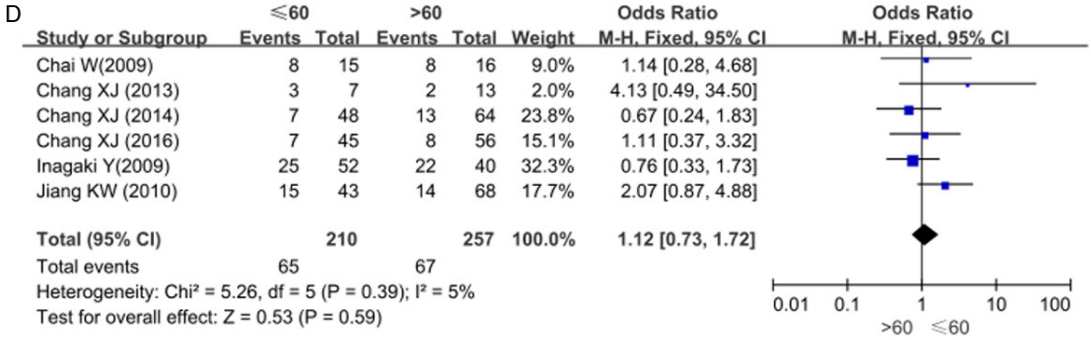
B


Male
Female
Odds Ratio
Odds Ratio
Study or Subgroup Events Total Events Total Weight M-H, Fixed, 95% CI
M-H, Fixed, 95% CI
Chai W(2009) 10 20 6 11 13.3% 0.83 [0.19, 3.64]
Chang XJ (2013) 2 15 3 5 13.4% 0.10 [0.01, 1.05]
Chang XJ (2014) 16 76 4 36 14.7% 2.13 [0.66, 6.92]
Chang XJ (2016) 11 71 4 30 16.3% 1.19 [0.35, 4.09]
Inagaki Y(2009) 41 74 10 22 23.6% 1.49 [0.57, 3.88]
Jiang KW (2010) 26 91 3 19 12.2% 2.13 [0.57, 7.94]
Jiao ZG (2009) 22 47 2 7 6.4% 2.20 [0.39, 12.50]
Total (95% CI) 394 130 100.0% 1.39 [0.86, 2.24]
Total events 128 32
Heterogeneity: Chi² = 6.55, df = 6 (P = 0.36); I² = 8%
Test for overall effect: Z = 1.33 (P = 0.18)
0.01 0.1 1 10 100
Female Male

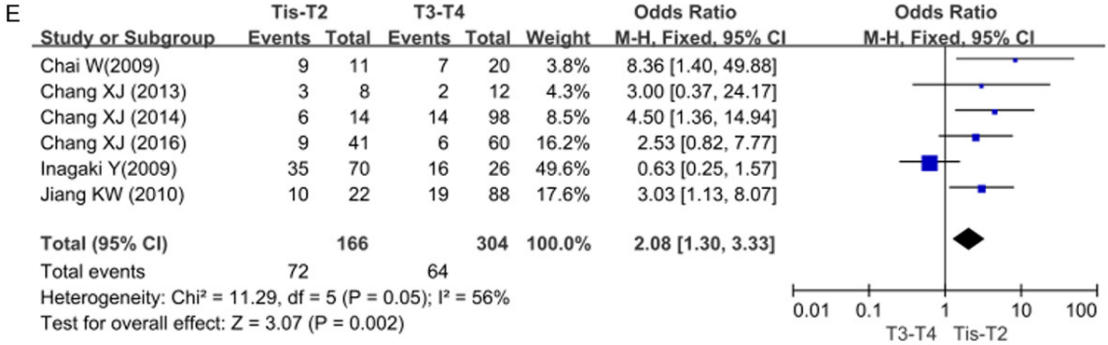
C


LN-
LN+
Odds Ratio
Odds Ratio
Study or Subgroup Events Total Events Total Weight M-H, Fixed, 95% CI
M-H, Fixed, 95% CI
Chai W(2009) 11 14 5 17 2.8% 8.80 [1.69, 45.76]
Chang XJ (2013) 3 6 2 14 1.7% 6.00 [0.67, 53.68]
Chang XJ (2014) 10 27 11 85 9.6% 3.96 [1.45, 10.82]
Chang XJ (2016) 13 25 38 76 25.9% 1.08 [0.44, 2.68]
Inagaki Y(2009) 17 40 36 58 48.5% 0.45 [0.20, 1.03]
Jiang KW (2010) 16 33 13 77 11.5% 4.63 [1.87, 11.47]
Total (95% CI) 145 327 100.0% 1.76 [1.17, 2.64]
Total events 70 105
Heterogeneity: Chi² = 23.38, df = 5 (P = 0.0003); I² = 79%
Test for overall effect: Z = 2.73 (P = 0.006)
0.01 0.1 1 10 100
LN+ LN-

D


≤60
>60
Odds Ratio
Odds Ratio
Study or Subgroup Events Total Events Total Weight M-H, Fixed, 95% CI
M-H, Fixed, 95% CI
Chai W(2009) 8 15 8 16 9.0% 1.14 [0.28, 4.68]
Chang XJ (2013) 3 7 2 13 2.0% 4.13 [0.49, 34.50]
Chang XJ (2014) 7 48 13 64 23.8% 0.67 [0.24, 1.83]
Chang XJ (2016) 7 45 8 56 15.1% 1.11 [0.37, 3.32]
Inagaki Y(2009) 25 52 22 40 32.3% 0.76 [0.33, 1.73]
Jiang KW (2010) 15 43 14 68 17.7% 2.07 [0.87, 4.88]
Total (95% CI) 210 257 100.0% 1.12 [0.73, 1.72]
Total events 65 67
Heterogeneity: Chi² = 5.26, df = 5 (P = 0.39); I² = 5%
Test for overall effect: Z = 0.53 (P = 0.59)
0.01 0.1 1 10 100
>60 ≤60

E


Tis-T2
T3-T4
Odds Ratio
Odds Ratio
Study or Subgroup Events Total Events Total Weight M-H, Fixed, 95% CI
M-H, Fixed, 95% CI
Chai W(2009) 9 11 7 20 3.8% 8.36 [1.40, 49.88]
Chang XJ (2013) 3 8 2 12 4.3% 3.00 [0.37, 24.17]
Chang XJ (2014) 6 14 14 98 8.5% 4.50 [1.36, 14.94]
Chang XJ (2016) 9 41 6 60 16.2% 2.53 [0.82, 7.77]
Inagaki Y(2009) 35 70 16 26 49.6% 0.63 [0.25, 1.57]
Jiang KW (2010) 10 22 19 88 17.6% 3.03 [1.13, 8.07]
Total (95% CI) 166 304 100.0% 2.08 [1.30, 3.33]
Total events 72 64
Heterogeneity: Chi² = 11.29, df = 5 (P = 0.05); I² = 56%
Test for overall effect: Z = 3.07 (P = 0.002)
0.01 0.1 1 10 100
T3-T4 Tis-T2

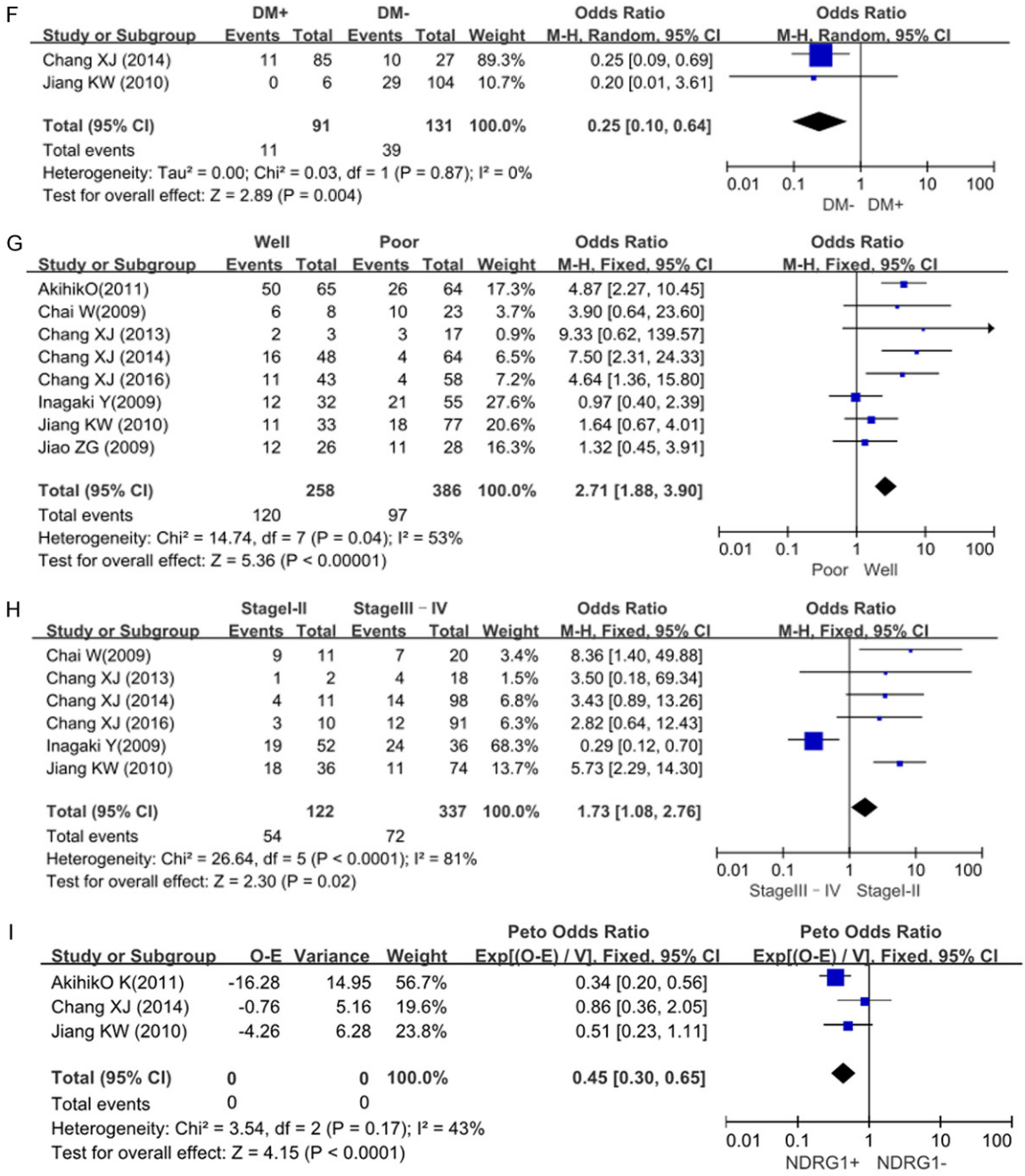



**Figure 2.** Forest plot for the relationship between NDRG1 expression and clincopathological parameters of gastric cancer. A. Gastric carcinogenesis (cancer *vs* normal mucosa); B. Correlation between sex and NDRG1 expression (male *vs* female); C. Correlation between age and NDRG1 expression (≤60 years *vs* >60 years); D. Correlation between depth of invasion and NDRG1 expression ($T_{is-2}$ *vs* $T_{3-4}$); E. Correlation between lymph node metastasis (LN) and NDRG1 expression (LN- *vs* LN+); F. Correlation between distant metastasis (DM) and NDRG1 expression (DM- *vs* DM+); G. Correlation between TNM staging and NDRG1 expression (0-II *vs* III-IV); H. Correlation between differentiation and NDRG1 expression (well-differentiated *vs* poorly-differentiated); I. Correlation between survival rate and NDRG1 expression.

and colorectal cancers [6, 16-19]. These findings indicate that NDRG1 hypoexpression is positively linked to invasion and progression of gastric cancer.

NDRG1 overexpression has been positively associated with better overall survival of patients with colorectal cancer [19]. Here, the present meta-analysis demonstrated that NDRG1

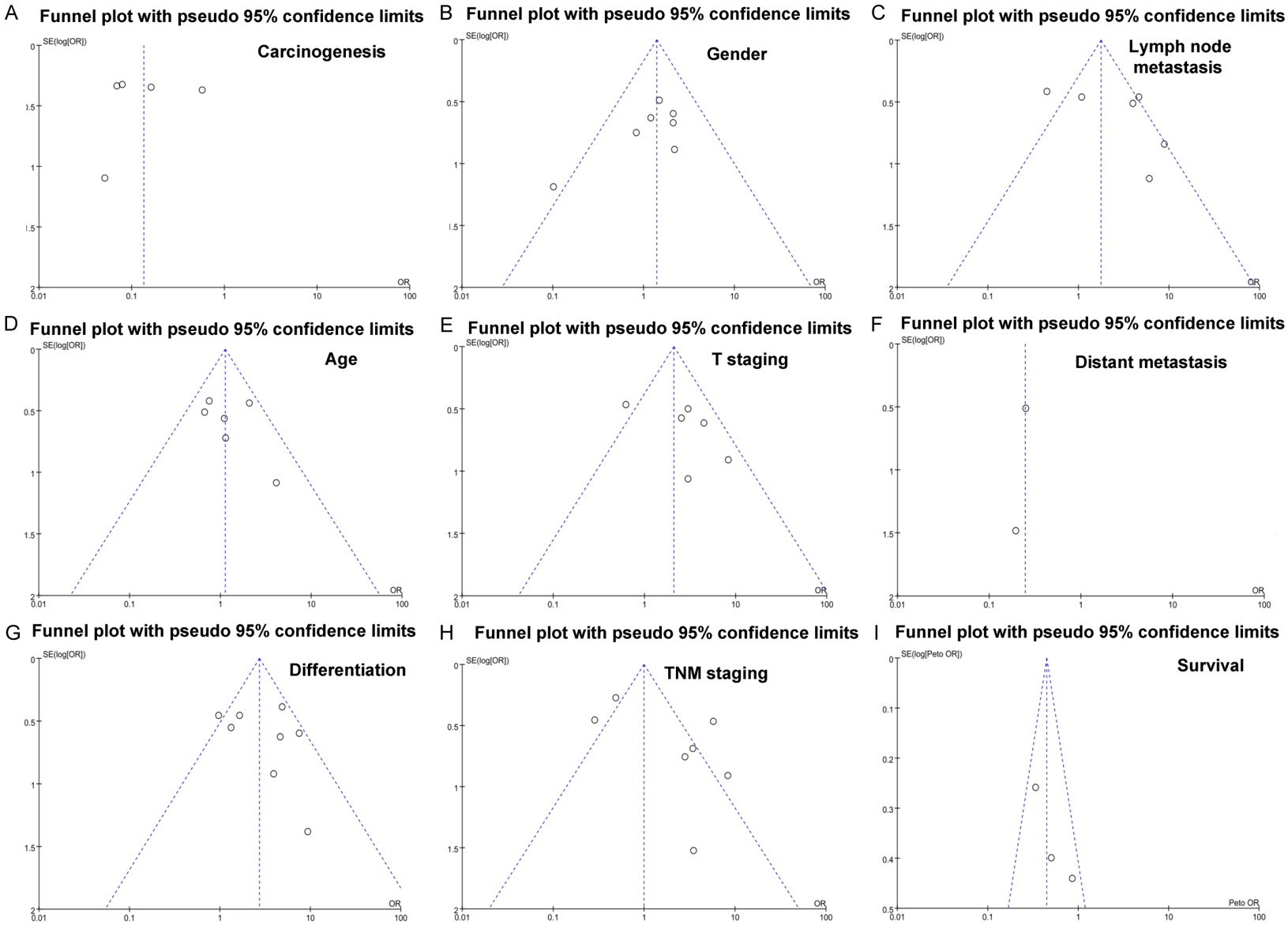



**Figure 3.** Funnel plot for publication bias test between NDRG1 expression and gastric carcinogenesis and subsequent progression. Bias was analyzed about risk degrees of NDRG1 expression in gastric mucosa (A) for gastric carcinogenesis. Additionally, it was tested between NDRG1 expression and clinicopathological features of gastric cancer, including sex (B), age (C), depth of invasion (D), lymph node metastasis (E), distant metastasis (F), TNM staging (G), differentiation (H) and prognosis (I).

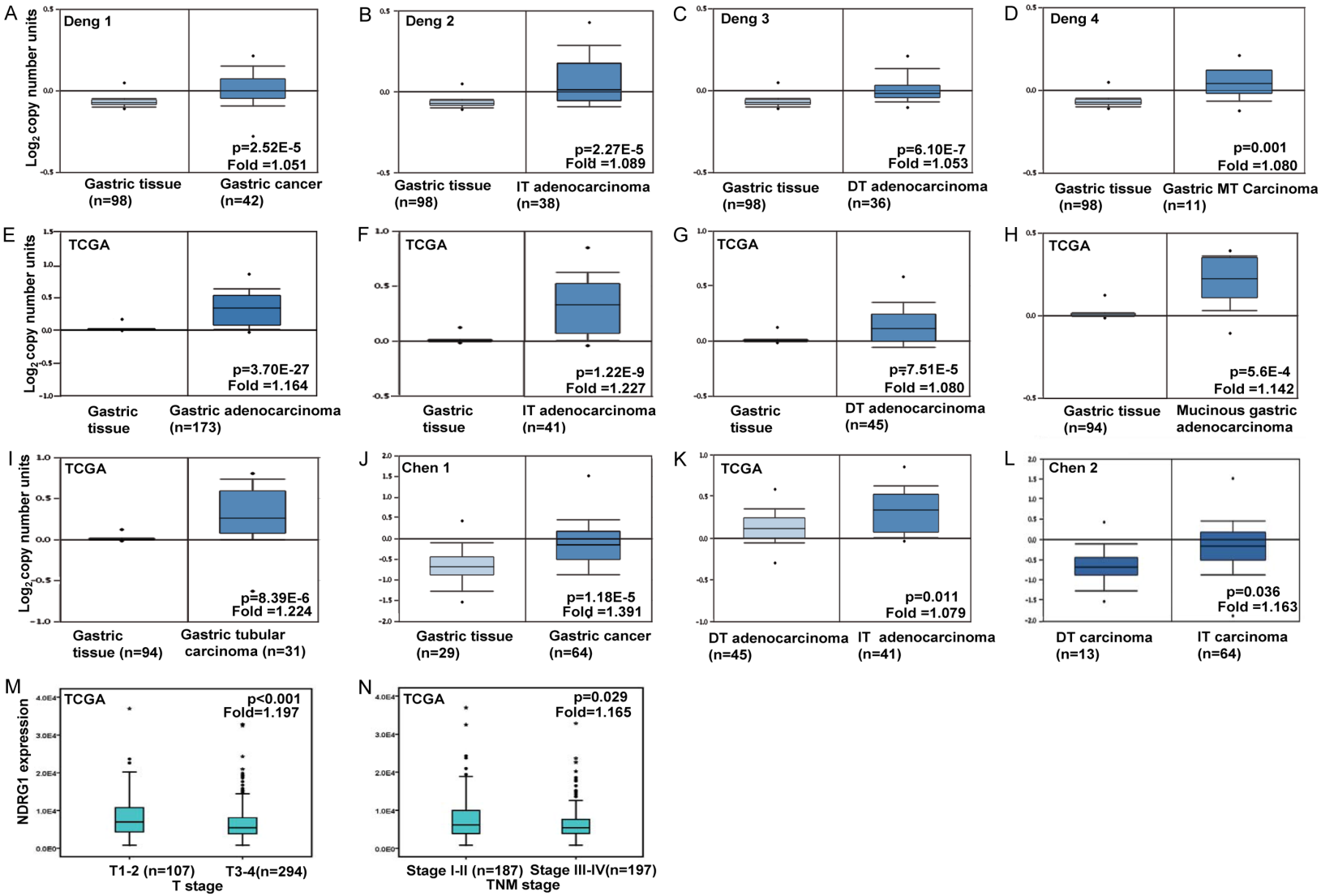



**Figure 4.** *NDRG1* mRNA expression in gastric carcinogenesis and subsequent progression. Deng's, Chen's, and TCGA datasets were employed for bioinformatics analysis to analyze *NDRG1* mRNA expression during gastric carcinogenesis. A higher NDRG1 expression was detectable in gastric cancer than that in normal gastric mucosa (A, E, J, $p<0.05$), even stratified into intestinal- (IT: B, F), diffuse- (DT: C, G), mixed-type (MT: D), mucinous carcinomas (H), and tubular carcinomas (I). TCGA and Chen's databases showed that *NDRG1* was more expressed in intestinal- than diffuse-type gastric adenocarcinoma (K and L, $p<0.05$). According to the TCGA database, *NDRG1* expression was negatively related to depth of invasion (M, $p<0.05$) and TNM staging (N, $p<0.05$) of gastric cancer.

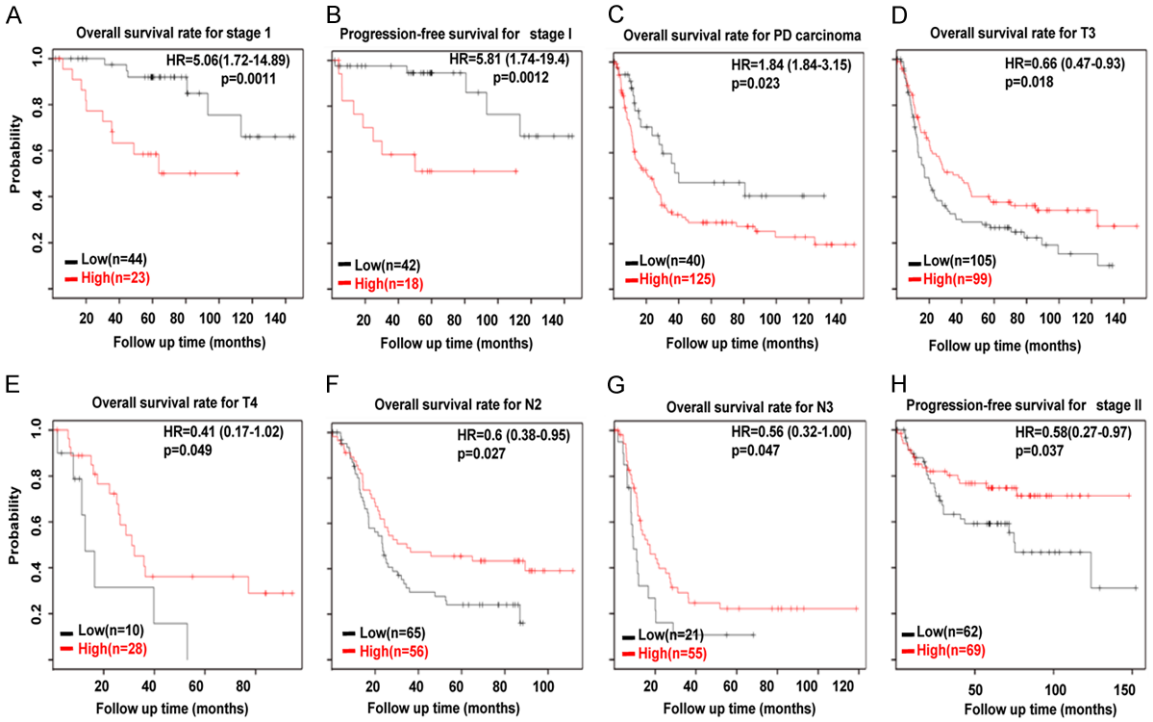



**Figure 5.** Prognostic significance of *NDRG1* mRNA expression in gastric cancer. KM plotter was employed to analyze correlation between *NDRG1* mRNA expression and overall or progression-free survival rates of patients with gastric cancer. PD: poorly-differentiated; HR: hazard ratio.

expression had a significantly positive association with favorable overall survival in patients with gastric cancer. This is consistent with findings confirming that NDRG1 expression might be considered a good potential biomarker for good prognosis of gastric cancer patients. Bioinformatics data confirmed that *NDRG1* mRNA expression was significantly associated with overall and progress-fee survival rates of patients with gastric cancer, in contrast to reports concerning colorectal cancer [19] and gastric cancer [28]. This inverse phenomenon might be due to the distinct sensitivity of different methodologies. Bioinformatics analysis depends on RNA sequencing, while the latter two experiments are mainly based on immunohistochemistry. Inagaki et al. [32] found that high expression of cytoplasmic NDRG1 was positively correlated with overall survival in patients of gastric cancer, while nuclear was negatively correlated with overall survival in patients of gastric cancer, suggesting that the distinct localization of NDRG1 expression in cancer cells might have different prognostic significance.

In conclusion, NDRG1 expression was downregulated in gastric carcinogenesis, but inversely for mRNA levels. It was negatively correlated with deep invasion, TNM staging, and dedifferentiation of gastric cancer at both mRNA and protein levels. NDRG1 expression might be employed as a good potential biomarker for good prognosis of gastric cancer patients. Several limitations should be pointed out concerning the present retrospective analysis. First, potential publication bias stems from published results being predominantly positive. Second, patient populations were limited because the patients came only from Asia. Third, only three articles contained survival data and all of them were extracted from survival curves, which may introduce subjective bias. Fourth, sample sizes were small. This may have limited the power to detect association in some articles.

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## Disclosure of conflict of interest

None.

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