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# The prognostic value of decreased NDRG1 expression in patients with digestive system cancers

## A meta-analysis

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**Abstract**

**Background:** Digestive system cancers are recognized as associated with high morbidity and mortality. It is generally accepted that N-myc downstream-regulated gene 1 (NDRG1) is aberrantly overexpressed or downregulated in digestive system cancers, and its prognostic value remains controversial. Accordingly, we herein conducted a meta-analysis to explore whether NDRG1 expression is correlated with overall survival (OS) and clinicopathological characteristics of patients with digestive system cancers.

**Methods:** We systematically searched PubMed, EMBASE, and Web of Science for eligible studies up to June 6, 2017. In all, 19 publications with 21 studies, were included.

**Results:** The pooled results showed that low NDRG1 expression was significantly associated with worse OS in colorectal cancer (pooled HR = 1.67, 95% CI: 1.22–2.28, $P < .001$) and pancreatic cancer (pooled HR = 1.87, 95% CI: 1–3.5, $P < .0001$). Moreover, the relationships between low NDRG1 expression and higher OS ratio of patients with liver cancer (pooled HR = 0.44, 95% CI: 0.32–0.62, $P = .009$) and gallbladder cancer (pooled HR = 0.56, 95% CI: 0.23–1.38, $P = .01$) were observed. Nevertheless, no significant association was observed between low NDRG1 expression and OS in gastric cancer (pooled HR = 0.81, 95% CI: 0.45–1.43, $P = .46$) or esophageal cancer (pooled HR = 0.76, 95% CI: 0.26–2.24, $P = .62$).

**Conclusion:** The prognostic significance of NDRG1 expression varies according to cancer type in patients with DSCs. Considering that several limitations existed in this meta-analysis, more studies are required to further assess the prognostic value of NDRG1 expression in patients with DSCs and relevant mechanisms.

**Abbreviations:** CI = confidence interval, DFS = disease-free survival, DSCs = digestive system cancers, ESCC = esophageal squamous cell carcinoma, HCC = hepatocellular carcinoma, HR = hazard ratio, NDRG1 = N-myc downstream-regulated gene 1, OS = overall survival.

**Keywords:** digestive system cancers, meta-analysis, N-myc downstream-regulated gene 1 (NDRG1), prognostic

## 1. Introduction

Digestive system cancers (DSCs) are one of the most deadly threats to humans due to the high morbidity and mortality rates.[1] Despite the identification of many biomarkers related to DSCs, it still be difficult to predict the prognoses of patients with digestive system malignancies, which depend on distant metastasis, lymph node invasion, and local recurrence.[2] Moreover, patients may experience diverse clinical outcomes and even they have similar patterns of lymph node metastases, TNM stage, and tumor differentiation.[3] Therefore, it is imperative to identify new credible prognostic markers to predict patient prognosis and o devise better therapies for patients with DSCs.

It is common knowledge that the NDRG1 protein is mainly expressed in epithelial cells in humans,[4] while some specific tissues including muscle, connective tissue, blood vessels, and most of the nervous system show without expression of NDRG1.[5] Concerning its subcellular localization, NDRG1 can be found in the nucleus, plasma membrane and cytoplasm depending on the cell type.[5] NDRG1 protein was initially identified as a predominantly cytoplasmic protein,[6] it has been shown to be involved in various biological functions including cell growth, differentiation, embryogenesis, development, lipid biosynthesis, myelination, stress and immunity responses.[7] Meanwhile, NDRG1 was also primarily known as a suppressor of metastasis, and demonstrated to suppress angiogenesis, cell proliferation and invasion processes in multiple cancers, including prostate cancer, pancreatic cancer, and colorectal cancer.[7–9] Inversely, some studies have reported that NDRG1 was overexpressed in various cancers such as hepatocellular carcinoma, gastric cancer, cervical cancer, renal cancer and squamous cell carcinoma, which indicates its tumorigenic effects.[7,10,11] Moreover, elevated NDRG1 expression was reported to promote proliferation and invasion in vitro, as

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well as tumor growth and angiogenesis in vivo in liver cancer and gastric cancer.[12–14] In addition to the effect of NDRG1 in tumorigenesis, many researchers have also investigated that NDRG1 might serve as a prognostic marker in cancer patients.[15–20] Specifically, numerous studies of DSCs had the limitations concerning tumor type, disparities in tumor stage and experimental schemes, and the prognostic role of NDRG1 in DSCs was inconsistent.[13–22] For instance, some previous studies showed that decreased NDRG1 expression was associated with better overall survival,[9,21,23,24] while others indicated that increased level of NDRG1 expression was correlated with poorer overall survival.[10,14,25,26] Even though the molecular functions of NDRG1 and its potential as a molecular target for cancer therapy have already been reviewed comprehensively,[10] its precise prognostic role in patients with DSCs has not been assessed in a systematic review with a meta-analysis, and as a result, its role is still controversial.

Therefore, we conducted this meta-analysis and systematic review to assess the influence of decreased NDRG1 expression on overall and disease-free survival, as well as the association between decreased NDRG1 expression and clinicopathological factors of patients with DSCs.

## 2. Materials and methods

### 2.1. Ethics and dissemination

Ethical approval and informed consent are not required, as the study will be a literature review and will not involve direct contact with patients or alterations to patient care.

### 2.2. Literature search strategy and study selection

To identify all the studies that assessed the association between NDRG1 expression and survival outcome of patients with digestive system cancers, 2 reviewers performed a comprehensive literature search in the following databases: PubMed, EMBASE, and Web of Science. The last search was updated on June 6, 2017. The publication language was restricted to English. Key words used were ("NDRG1" or "N-myc downstream regulated gene 1"), ("cancer" or "tumor" or "malignancy" or "carcinoma"), and ("prognosis or prognostic").

All the studies were included if they met the following inclusion criteria: the studies investigated the association between NDRG1 and overall survival (OS) of patients with digestive system cancers; relevant clinicopathologic characteristics were presented; tumor tissues from patients with digestive system cancers were used for the determination of NDRG1 expression; patients were grouped into high and low expression arms according to the NDRG1 expression level; sufficient information and data were available to calculate hazard ratios (HRs) with 95% confidence intervals (CIs).

The exclusion criteria for this study were as follows: studies that were published as reviews, abstracts, case reports, letters, or comments as well as duplicate studies; studies in which human cell lines or animals were used; studies that failed to provide the HRs with 95% confidence intervals or K-M survival curves used to calculate overall survival and disease-free survival.

### 2.3. Data extraction and quality assessment

All the candidate publications was extracted from each selected study independently by 2 independent investigator. The third review investigator was responsible for reconciling disagreements when the results were controversial. The following information was extracted: the first author's name, publication year, country, tumor type, number of patients, tumor stage, clinical and pathological features, cut-off value, OS, and DFS. If the results of both the univariate and multivariate analyses were provided in the studies, only the latter one was extracted due to its higher accuracy since multivariate analyses account for confounding factors. Three aspects including the selection of participants, comparability, and ascertainment of the outcome were assessed. A study with a score ≥6 was considered as high-quality study after the selected publication were evaluated with the Newcastle-Ottawa Scale (NOS) ranging from 0 (minimum) to 9 (maximum).

### 2.4. Statistical analysis

The meta-analysis was performed using Stata SE12.0 (StataCorp, College Station, TX). HRs and 95% confidence intervals (95% CIs) were used to assess the prognostic value of NDRG1, and ORs (odds ratios) with 95% CIs were used to evaluate the association between NDRG1 expression and clinicopathological features of digestive system cancers. The sensitivity analysis was performed to assess the validity and reliability of the pooled overall survival in patients with a specific type of DSC. Chi-square-based $Q$ tests and $I^2$ statistics were applied to evaluate study heterogeneity, with $I^2 > 50\%$ and $P < .05$ indicating statistical heterogeneity. If no severe statistical heterogeneity was detected, a fixed-effects model was used to assess the pooled HRs; otherwise, a random-effects model was used.

## 3. Results

### 3.1. Study selection and study characteristics

A total of 173 articles were identified in PubMed, EMBASE, and Web of Science. Twenty duplicated articles were excluded. The remaining abstracts and full-texts of the references were meticulously reviewed, 19 publications, which included 21 studies, were finally determined to be eligible for the present pooled analysis of the prognostic value of NDRG1 in digestive system cancers (DSCs).[8–10,13–16,19–30] The inclusion of all publications was based on the selection criteria mentioned above and the detailed selection process is shown in Fig. 1.

The basic characteristics of the included studies are summarized in Table 1. Twenty-one studies with 2280 patients were totally included in the current meta-analysis, the sample size of which ranged from 47 to 240. Specifically, Kawahara et al and Koshiji et al conducted 2 studies of each, therefore, we marked them as Kawahara 1 and Kawahara 2[14]; Koshiji 1 and Koshiji 2.[22] All the included studies were published in English and the recruitment time of patients ranged from 1993 to 2010. There are 5 studies focused on gastric cancer,[13,14,16,23] 8 studies focused on colorectal cancer,[8,9,22,24,28–30] 3 studies focused on hepatocellular carcinoma (HCC),[10,25,26] 3 studies involved esophageal squamous cell carcinoma (ESCC),[15,19,21] 1 study involved pancreatic cancer[27] and 1 study involved gallbladder cancer,[20] among the 21 studies. Ten studies were performed in China, 7 studies were performed in Japan, and 4 studies were performed in the USA and Poland, regarding the population of the cases. The majority of studies on NDRG1 expression used IHC to detect NDRG1 protein, while 3 studies used qRT-PCR (Table 1). In addition, 18 studies reported cut-off values of NDRG1 expression, however, they were not consistent (Table 1). The sources of antibody included Sigma Aldrich, Santa Cruz, Abcam

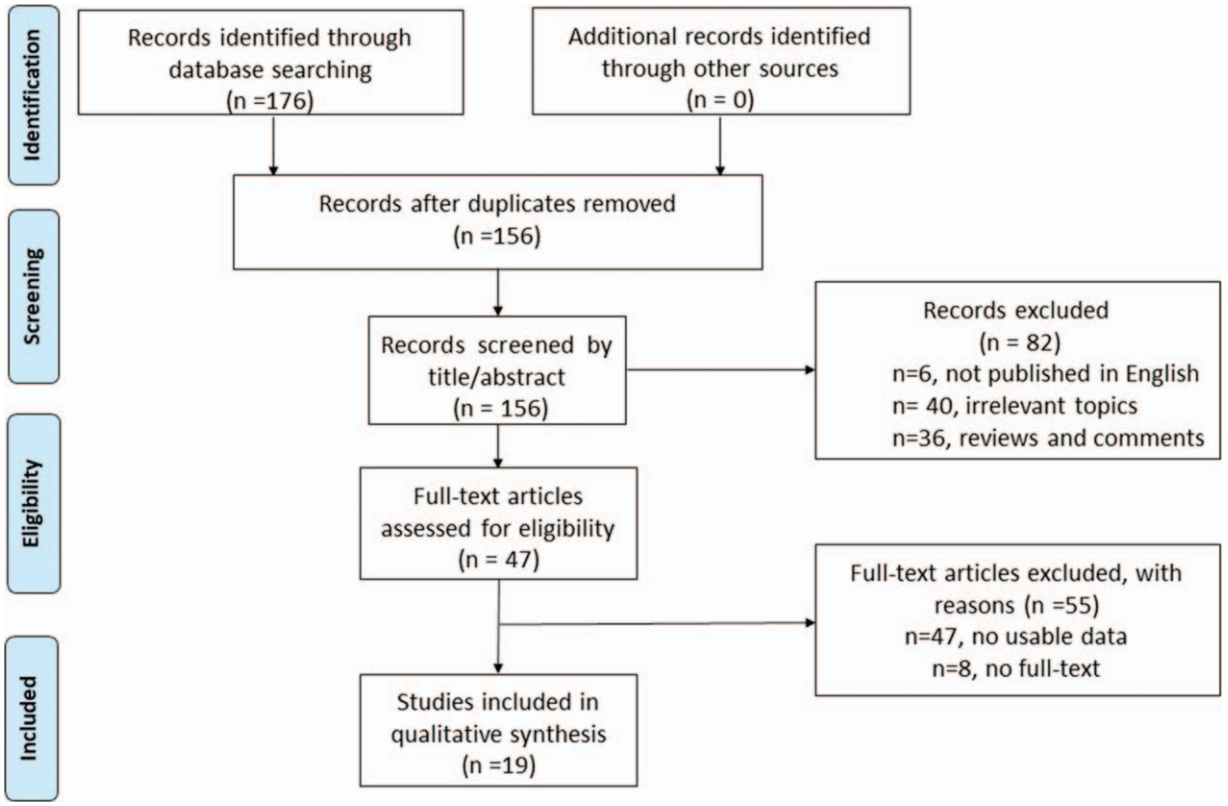


**Figure 1.** Flow diagram of study selection process.

(UK), Abnova (China), Cell Signaling Technology and Zhongshan Goldenbridge Biotechnology (China) (Table 1).

The study quality score was assessed using the modification of the Newcastle-Ottawa scale, in which the scores ranged from 5 to 7, which indicated that the quality of the included studies was moderate to high (Table 2).

### 3.2. Low NDRG1 expression and OS in digestive system cancers

The pooled result revealed that low NDRG1 expression was significantly associated with worse overall survival (OS) of patients with colorectal cancer (pooled HR = 1.67, 95% CI: 1.22–2.28, $P < .001$) (Fig. 2 and Table 3) and those with pancreatic cancer (pooled HR = 1.87, 95% CI: 1–3.5, $P < .0001$) (Table 3). Furthermore, the associations between low NDRG1 expression and better OS of patients with liver cancer (pooled HR = 0.36, 95% CI: 0.16–0.78, $P = .01$) (Fig. 3 and Table 3) or gallbladder cancer (pooled HR = 0.56, 95% CI: 0.23–1.38, $P = .01$) were observed (Table 3). However, no significant association was found between low NDRG1 expression and OS in gastric cancer (pooled HR = 0.81, 95% CI: 0.45–1.43, $P = .46$) (Fig. 4 and Table 3) and esophageal cancer (pooled HR = 0.76, 95% CI: 0.26–2.24, $P = .62$) (Fig. 5 and Table 3).

### 3.3. Low NDRG1 expression and clinicopathological factors in gastric cancer

Four studies reported the relationship between low NDRG1 expression and clinicopathological factors in gastric cancer,[9,19,20,25] including 3 studies that investigated tumor invasion depth, lymphatic invasion, TNM stage, age, and gender, while 2 studies that involved tumor differentiation and grade (Table 4). Except for gender ($I^2 = 0$, $P = .68$), significant heterogeneity was observed in age ($I^2 = 67$, $P = .05$), tumor invasion depth ($I^2 = 86$, $P = .0006$), lymphatic invasion ($I^2 = 88$, $P = .0002$), differentiation grade ($I^2 = 76$, $P = .004$), and tumor stage ($I^2 = 94$, $P < .0001$). Therefore, the random-effects model was employed for variables with the exception of gender, while the fixed-effects model was applied for gender (Table 4). Nevertheless, the pooled analysis showed no significance in the association between low NDRG1 expression and age (OR = 0.90, 95% CI: 0.35–2.29, $P = .82$), gender (OR = 0.59, 95% CI: 0.30–1.15, $P = .12$), invasion depth (OR = 1.59, 95% CI: 0.30–8.37, $P = .59$), lymphatic invasion (OR = 1.87, 95% CI: 0.37–9.5, $P = .45$), or tumor differentiation grade (OR = 3.34, 95% CI: 0.75–14.9, $P = .11$) (Table 4).

### 3.4. Low NDRG1 expression and clinicopathological factors in colorectal cancer

A total of 6 studies described the association between NDRG1 expression and clinicopathological factors in colorectal cancer,[9,10,16,22,24,28,30] including age, gender, tumor differentiation grade, and lymphatic invasion (Table 4). No significant heterogeneity was found between low NDRG1 expression and age ($I^2 = 32$, $P = .22$), gender ($I^2 = 49$, $P = .12$), or tumor differentiation grade ($I^2 = 43$, $P = .15$); therefore, a fixed-effects model was applied for analysis. Even so, significant heterogeneity

**Table 1**

**Baseline characteristics of studies included in the meta-analysis.**

| Cancer types | Author | Region | Time of recruitment | No. of patients | Test method | Definition of low expression | Rate of low NDRG1 expression | HR (95% CI) for OS | Antibody |
|---|---|---|---|---|---|---|---|---|---|
| Gastric cancer | Inagaki | Japan | 1994–1999 | 74 | IHC | The percentage of cancer cells with stained nuclei: ≤10% | 60.8% | 0.86 (0.68–0.92), UA | Santa Cruz |
| Gastric cancer | Jiang | China | 2001–2003 | 110 | IHC | Staining index (values 0–12) ≤4* | 73.6% | 1.72 (1.03–3.82), UA | Abnova |
| Gastric cancer | Kawahara1 | Japan | 2001–2004 | 65 | IHC | The percentage of cancer cells with strongly stained nuclei: ≤4% | 69.2% | 0.24 (0.11–0.51), MA | NR |
| Gastric cancer | Kawahara2 | Japan | 2001–2004 | 64 | IHC | The percentage of cancer cells with strongly stained nuclei: ≤4% | 92.2% | 0.48 (0.19–1.25), MA | NR |
| Gastric cancer | Chang | China | 2009–2010 | 112 | IHC | Staining index (values 0–12) ≤4* | 34.8% | 1.64 (0.78–3.45), UA | Cell Signaling Technology |
| Colorectal cancer | Koshiji1 | Japan | 1995–2003 | 80 | IHC | IHC score (values 0–3) = 0‡ | 45.5% | 1.01 (0.46–1.83), UA | Santa Cruz |
| Colorectal cancer | Koshiji2 | USA | 1995–2003 | 77 | IHC | IHC score (values 0–3) = 0‡ | 49.5% | 0.97 (0.57–1.69), UA | Santa Cruz |
| Colorectal cancer | Strzelczyk | Poland | 1996–2004 | 108 | qRT-PCR | ≤ Median expression levels | 49.0% | 2.01 (1.01–3.26), MA | Santa Cruz |
| Colorectal cancer | Mao | China | 2006–2007 | 240 | IHC | Staining index (values 0–12) ≤4* | 43.3% | 3.89 (2.05–7.37), UA | Sigma-Aldrich |
| Colorectal cancer | Zhi | China | 2006–2008 | 116 | IHC | Staining index (values 0–12) ≤1* | 66.4% | 1.95 (1.05–3.65), UA | NR |
| Colorectal cancer | Ma | China | 2008–2010 | 164 | IHC | Staining index (values 0–12) ≤4* | 65.9% | 1.80 (1.20–2.70), MA | Zhongshan Goldenbridge Biotechnology |
| Colorectal cancer | Shah | USA | 1991–1995 | 131 | IHC | The percentage of cancer cells with stained cytoplasmic and cell membrane: ≤30% | 42.7% | 1.27 (0.75–2.13), UA | NR |
| Colorectal cancer | Yang | China | NR | 97 | IHC | NR | 41.2% | 2.30 (0.57–9.24), UA | Sigma Aldrich |
| Hepatocellular carcinoma | Chua | USA | NR | 59 | qRT-PCR | ≤ The median tumor/normal ratio | 81.4% | 0.42 (0.18–0.91), MA | Applied Genomics Inc. |
| Hepatocellular carcinoma | Cheng | China | NR | 143 | IHC | Weak to moderate intensity and less than 50% of tumor cells staining | 48.3% | 0.16 (0.07–0.34), UA | Santa Cruz |
| Hepatocellular carcinoma | Xu | China | NR | 180 | IHC | Staining index (values 0–12) ≤4* | 50% | 0.59 (0.39–0.89), MA | NR |
| Pancreatic cancer | Maruyama | Japan | 1991–1998 | 65 | IHC | IHC score (values 0–3) = 0, 1§ | 58.5% | 1.87 (1.03–3.61), UA | NR |
| Gallbladder carcinoma | Zhang | China | 1998–2009 | 138 | IHC | Staining index (values 0–12) ≤1* | 36.3% | 0.56 (0.21–1.27), MA | Abcam |
| Esophageal squamous cell carcinoma | Ai | China | 2006–2008 | 86 | IHC | NR | 62.7% | 0.32 (0.18–0.59), UA | Abcam |
| Esophageal squamous cell carcinoma | Ando | Japan | 1996–2001 | 47 | qRT-PCR | NR | 42.6% | 2.65 (1.14–8.07), UA | NR |
| Esophageal squamous cell carcinoma | Sohda | Japan | 1983–2002 | 124 | IHC | Staining intensity of cytoplasm in tumor cells ≤ in normal epithelium | 44.4% | 0.63 (0.26–0.98), UA | NR |

CI = confidence interval, HR = hazard ratio, MA = univariate analysis, NDRG1 = N-myc downstream-regulated gene 1, NR = not reported, OS = overall survival, UA = multivariate analysis.

* Staining index (values 0–12) = staining intensity (0–3) × proportion of immune-positive cells (0 = 5%, 1 = 6–25%, 2 = 26–50%, 3 = 51–75%, and 4 = > 75%).

† No staining, 0; weak staining, 1+; moderate staining, 2+; and strong staining, 3+ in >10% of cancer cells.

‡ 0 = negative staining, +1 = weak staining or moderate to intense staining in the peripheral region of <10% of the cancer nests; +2 = moderate staining in most of the cancer cells or intense staining in the peripheral regions in 10–40% of the cancer nests; +3 = intense staining in almost all the cancer cells.

§ 0 = no positive cells, 1 = <30% positive cancer cells, 2 = 30–80% positive cancer cells, and 3 = >80% positive cancer cells.

‖ Score 0 = no staining at all, 1 = nuclear expression in less than 10% of the cancer cells, 2 = nuclear expression in more than 0% of the cancer cells.

¶ 0 = no staining, 1 = dotted pattern staining, 2 = weak or moderate circumferential staining in >10% of the tumor cells, 3 = strong circumferential staining in >10% of the tumor cells.

# The numeric values in quantitative analysis were obtained using the image capture system, Automated Cellular Imaging System.

was observed in studies that reported lymphatic invasion ($I^2 = 80$, $P = .007$); therefore, a random-effects model was applied (Table 4). The pooled analysis revealed no significant relationship between decreased NDRG1 expression and age (OR = 1.02, 95% CI: 0.73–1.42, $P = .92$), gender (OR = 1.02, 95% CI: 0.75–1.39, $P = .88$), or lymphatic invasion (OR = 1.29, 95% CI: 0.47–3.55, $P = .62$), while low NDRG1 expression was obviously related to poor tumor differentiation grade (OR = 2.16, 95% CI: 1.35–3.46, $P = .001$) (Table 4).

### 3.5. NDRG1 expression and clinicopathological factors in hepatocellular carcinoma

Three studies reported the relationship of NDRG1 expression and clinicopathological factors in hepatocellular carcinoma.[10,14,26] Age, gender, venous invasion, and tumor stage were all reported in 2 studies, while tumor differentiation grade was described in 3 studies (Table 4).

No significant heterogeneity was detected in the studies regarding venous invasion ($I^2 = 39$, $P = .20$), differentiation grade ($I^2 = 19$, $P = .29$), or tumor stage ($I^2 = 17$, $P = .27$); therefore, a fixed-effects model was applied. Nevertheless, significant heterogeneity was detected in studies that involved age ($I^2 = 57$, $P = .13$) and gender ($I^2 = 83$, $P = .01$); therefore, a random-effects model was used. The pooled results showed that low NDRG1 expression was significantly correlated with venous invasion (OR = 0.24, 95% CI: 0.12–0.49, $P < .0001$), tumor differentiation grade (OR = 0.43, 95% CI: 0.27–0.69, $P = .0005$), and tumor stage (OR = 0.45, 95% CI: 0.29–0.70, $P < .0004$). However, no significant relationship was found between low NDRG1 expression and age (OR = 0.86, 95% CI: 0.42–1.75, $P = .13$) or gender (OR = 0.49, 95% CI: 0.06–4.12, $P = .51$).

**Table 2**

**The Newcastle-Ottawa Scale (NOS) quality assessment of the enrolled studies.**

| | Selection | | | | Comparability | Outcome | | | |
|---|---|---|---|---|---|---|---|---|---|
| Study ID | Representativeness of the exposed cohort | Selection of the nonexposed cohort | Ascertainment of exposure | Demonstration that outcome of interest was not present at start of study | Comparability of cohorts on the basis of the design or analysis (study adjusts for age,* sex*) | Assessment of outcome | Was follow-up long enough for outcomes to occur | Adequacy of follow up of cohorts | Total |
| Inagaki 2009 | — | — | — | * | * | * | * | * | 5 |
| Jiang 2010 | — | — | — | * | ** | * | * | * | 6 |
| Kawahara 2011 | — | — | — | * | * | * | * | * | 5 |
| Chang 2014 | — | — | — | * | ** | * | * | * | 6 |
| Koshiji 2007 | — | — | — | * | * | * | * | * | 5 |
| Strzelczyk 2009 | — | — | * | * | ** | * | * | * | 7 |
| Mao 2013 | — | — | * | * | ** | * | * | * | 7 |
| Zhi 2016 | — | — | — | * | * | * | * | * | 5 |
| Ma 2016 | * | — | * | * | ** | * | * | * | 8 |
| Shah 2005 | — | — | * | * | ** | * | * | * | 7 |
| Yang 2017 | — | — | — | * | ** | * | * | * | 6 |
| Chua 2007 | — | — | * | * | ** | * | * | * | 7 |
| Cheng 2011 | — | — | — | * | ** | * | * | * | 6 |
| Xu 2016 | * | — | * | * | ** | * | * | * | 8 |
| Maruyama 2006 | — | — | — | * | * | * | * | * | 5 |
| Zhang 2011 | * | — | * | * | ** | * | * | * | 8 |
| Ai 2016 | — | — | — | * | ** | * | * | * | 6 |
| Ando 2006 | — | — | — | * | * | * | * | * | 5 |
| Sohda 2009 | — | — | — | * | ** | * | * | * | 6 |

*Means that the included study is given a score for a corresponding item of quality assessment.
**Means that the included study is given two scores for a corresponding item of quality assessment.

### 3.6. Sensitivity analysis

The sensitivity analyses were performed to evaluate the stability of the pooled results for OS in gastric cancer (Fig. 6A), colorectal cancer (Fig. 6B), ESCC (Fig. 6C), and HCC (Fig. 6D). The results of the sensitivity analyses showed that the pooled HRs for OS did not change substantially, which indicates that the conclusions from our meta-analysis were relatively reliable.

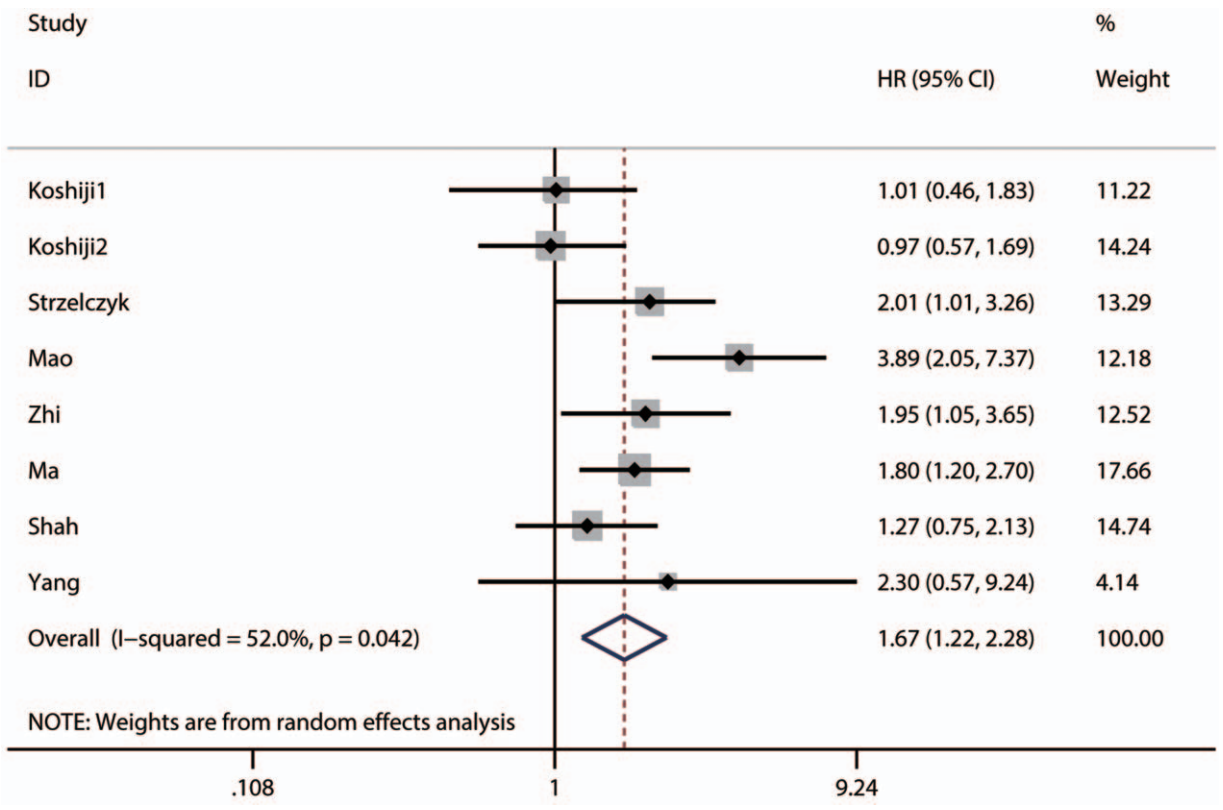


**Figure 2.** Forest plot of pooled HR for the association between low NDRG1 expression and OS of patients with colorectal cancer.

**Table 3**

**Results of pooled hazard ratios of overall survival of patients with low NDRG11 expression level.**

| Cancer type | No. of studies | No. of patients | Pooled HR (95% CI) | *P* | Heterogeneity | | |
|---|---|---|---|---|---|---|---|
| | | | | | $I^2$ (%) | *P* | Model |
| Colorectal cancer | 8 | 1013 | 1.67 (1.22—2.28) | .001 | 52 | .042 | Random effects |
| Pancreatic cancer | 1 | 65 | 1.87 (1.00–3.50) | .05 | — | — | — |
| Hepatocellular carcinoma | 3 | 382 | 0.44 (0.32–0.62) | .009 | 75.8 | .016 | Random effects |
| Gallbladder carcinoma | 1 | 138 | 0.56 (0.23–1.38) | .207 | — | — | — |
| Gastric cancer | 5 | 425 | 0.81 (0.45–1.43) | .46 | 79.3 | .001 | Random effects |
| Esophageal cancer | 3 | 257 | 0.76 (0.26–2.24) | .62 | 84.8 | .001 | Random effects |

CI = confidence interval, HR = hazard ratio.

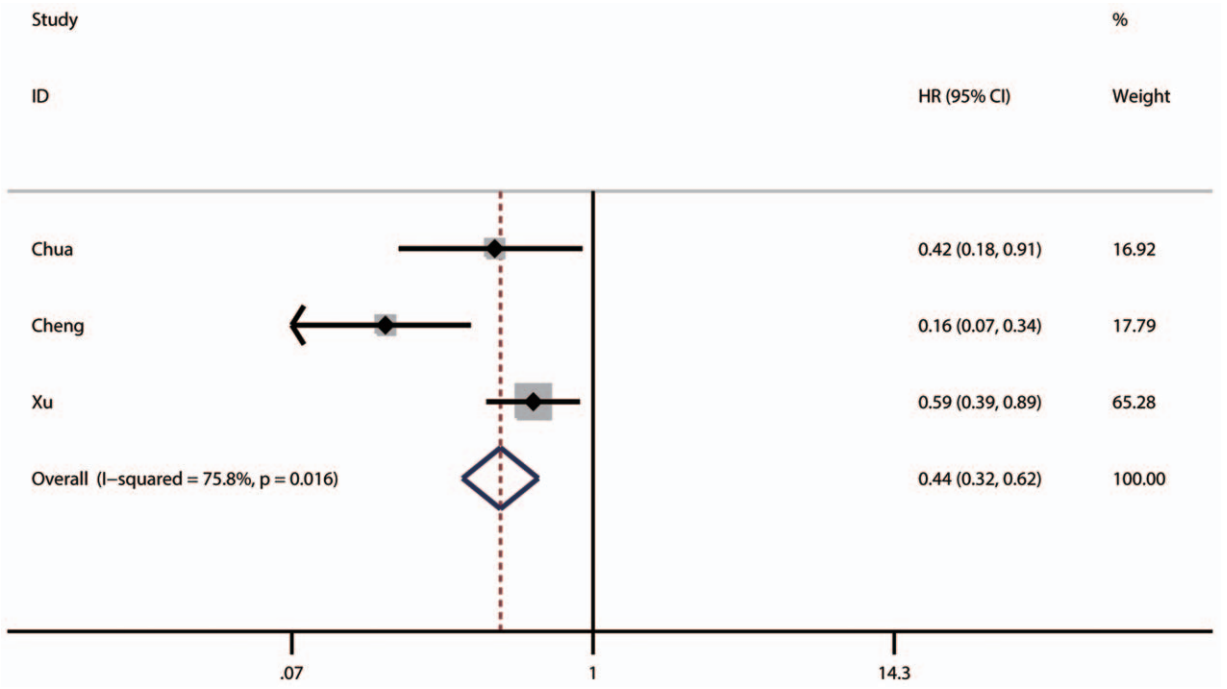


**Figure 3.** Forest plot of pooled HR for the association between low NDRG1 expression and OS of patients with liver cancer.

## 4. Discussion

It has been reported that the effect of NDRG1 in the carcinogenesis of digestive system cancers (DSCs) is conflicting[7,10]; therefore, its prognostic value in patients with DSCs is also inconsistent and remains unknown.[8–10,13,14,16,19–27,29] Despite that a previous study comprehensively reviewed the molecular functions of NDRG1 and its potential as a molecular target for cancer therapy, the impact of low NDRG1 expression on the prognosis of patients with DSCs has not been fully explored. Hence, we herein combined 19 publications that included 21 studies and 2280 patients to perform the first meta-analysis that has evaluated the association of NDRG1 with overall survival (OS) of patients with DSCs.[8–10,13–16,19–30] The purpose was to provide a comprehensive and relatively reliable conclusion. In addition, we also explored the relationship between low NDRG1 expression and clinicopathological features of DSCs.

The pooled results of the meta-analysis revealed that low NDRG1 expression was significantly associated with worse overall survival (OS) in colorectal cancer (pooled HR = 1.67, 95% CI: 1.22–2.28, $P < .001$) and pancreatic cancer (pooled HR = 1.87, 95% CI: 1–3.5, $P < .0001$). However, associations between better OS and low NDRG1 expression in liver cancer (pooled HR = 0.36, 95% CI: 0.17–0.78, $P = .01$) and gallbladder cancer (pooled HR = 0.56, 95% CI: 0.23–1.38, $P = .01$) were also observed. However, the results showed that low NDRG1 expression was not related to OS in gastric cancer (pooled HR = 0.81, 95% CI: 0.45–1.43, $P = .46$) and esophageal cancer (pooled HR = 0.76, 95% CI: 0.26–2.24, $P = .62$). Moreover, sensitivity analyses demonstrated that the pooled HRs evaluating the prognostic value of decreased NDRG1 expression in gastric cancer, colorectal cancer, esophageal cancer, and liver cancer were not significantly altered, which indicates that the pooled results were robust. Considering the above findings, we hypothesized that the prognostic value of decreased NDRG1 expression varies according to cancer type in patients with DSCs.

The finding that decreased expression of NDRG1 differently associated with patients' OS in differently DSCs made sense to us due to several possible mechanisms. First, NDRG1 expression

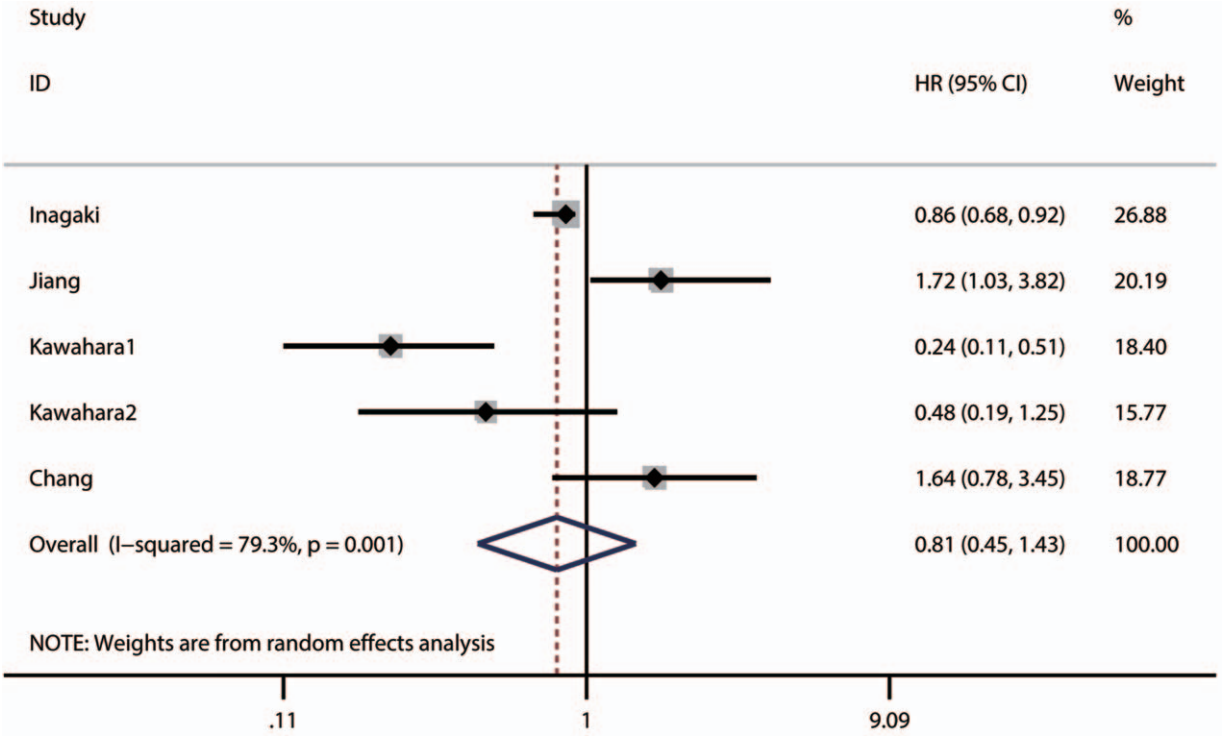


**Figure 4.** Forest plot of pooled HR for the association between low NDRG1 expression and OS of patients with gastric cancer.

levels throughout the digestive system are different. It is expressed in esophagus, gastric, small intestine, colon, and rectum but it has been reported no expression found in liver.[12,26] For those tissues with high expression of NDRG1, it may play important roles in keeping the cells in their normal status. In those tissues where NDRG1 is weakly expressed or no expression, changes of NDRG1 expression may make a relatively dismal effect on cells. Second, it has been reported that NDRG1 is involved in the regulation of various cellular functions,[31] thus it is a target protein and/or mediator protein for multiple signaling pathways.

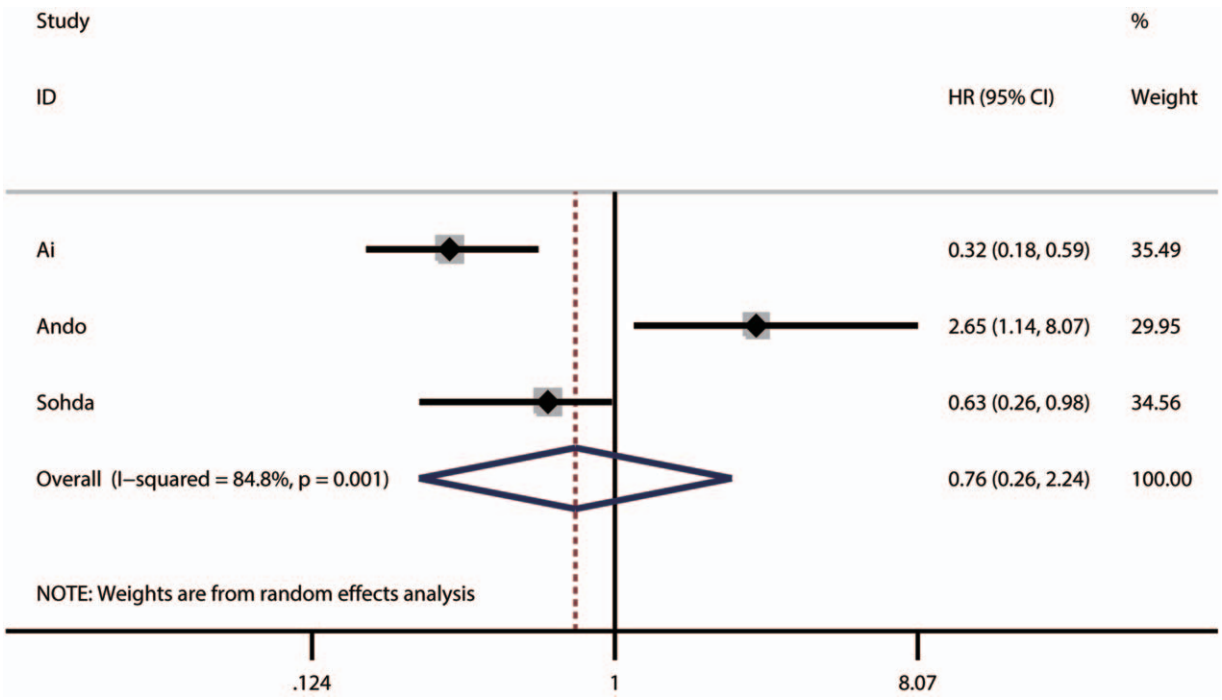


**Figure 5.** Forest plot of pooled HR for the association between low NDRG1 expression and OS of patients with gastric cancer.

**Table 4**

**Results of meta-analysis of high NDRG1 expression level and clinicopathological features in gastric cancer, colorectal cancer, and hepatocellular carcinoma.**

| Stratified analysis | No. of studies | No. of patients | Pooled OR (95% CI) | *P* | Heterogeneity | | |
|---|---|---|---|---|---|---|---|
| | | | | | $I^2$ (%) | *P* | Model |
| Gastric cancer | | | | | | | |
| Age (≥60 vs <60) | 3 | 296 | 0.90 (0.35–2.29) | .82 | 67 | .05 | Random effects |
| Gender (male vs female) | 3 | 296 | 0.59 (0.30–1.15) | .12 | 0 | .68 | Fixed effects |
| Invasion depth (T3 + T4 vs T1 + T2) | 3 | 296 | 1.59 (0.30–8.37) | .59 | 86 | .0006 | Random effects |
| Lymphatic invasion (yes vs no) | 3 | 295 | 1.87 (0.37–9.51) | .45 | 88 | .0002 | Random effects |
| Differentiation grade (poorly vs well and moderately) | 2 | 222 | 3.34 (0.75–14.9) | .11 | 76 | .04 | Random effects |
| Tumor stage (III + IV vs I + II) | 3 | 314 | 0.56 (0.05–6.37) | .64 | 94 | <.0001 | Random effects |
| Colorectal cancer | | | | | | | |
| Age (≥60 vs <60) | 5 | 658 | 1.02 (0.73–1.42) | .92 | 20 | .29 | Fixed effects |
| Gender (male vs female) | 6 | 789 | 1.02 (0.75–1.39) | .88 | 44 | .11 | Fixed effects |
| Lymphatic invasion (yes vs no) | 4 | 418 | 1.29 (0.47–3.55) | .62 | 80 | .002 | Random effects |
| Differentiation grade (poorly vs well and moderately) | 4 | 519 | 2.16 (1.35–3.46) | .001 | 43 | .15 | Fixed effects |
| Liver cancer | | | | | | | |
| Age (≥60 vs <60) | 2 | 323 | 0.86 (0.42–1.75) | .68 | 57 | .13 | Random effects |
| Gender (male vs female) | 2 | 331 | 0.49 (0.06–4.12) | .51 | 83 | .01 | Random effects |
| Venous invasion (yes vs no) | 2 | 201 | 0.24 (0.12–0.49) | <.0001 | 39 | .20 | Fixed effects |
| Differentiation grade (poorly vs well and moderately) | 3 | 381 | 0.43 (0.27–0.69) | .0005 | 19 | .29 | Fixed effects |
| Tumor stage (III + IV vs I + II) | 2 | 323 | 0.45 (0.29–0.70) | .0004 | 17 | .27 | Fixed effects |
| Esophageal cancer | | | | | | | |
| Gender (male vs female) | 2 | 210 | 0.977 (0.403–2.369) | .959 | 32.3 | .224 | Fixed effects |
| Invasion depth (T3 + T4 vs T1 + T2) | 2 | 210 | 0.154 (0.072–0.330) | <.0001 | 17.3 | .271 | Fixed effects |
| Lymphatic invasion (yes vs no) | 2 | 210 | 0.254 (0.124–0.519) | <.0001 | 0 | .92 | Fixed effects |
| Tumor stage (III + IV vs I + II) | 2 | 210 | 0.182 (0.011–3.147) | .241 | 87.5 | .005 | Random effects |

CI = confidence interval, ORs = odds ratios.

Those signaling pathways play different roles during carcinogenesis in different cancers. If the signaling pathway that NDRG1 regulates or involves is pivotal for the carcinogenesis, then it is very possible for NDGR1 to be associated with the OS for the patients and vice versa. More studies are needed to investigate the functions of NDRG1 in different cancers to fulfill the requirement of precisely explaining the prognostic value of NDRG1.

In addition, we also investigated the relationship of NDRG1 expression and clinicopathological characteristics to further validate the pooled results of the association between OS and NDRG1 expression. We assessed the associations between low NDRG1 expression and clinicopathological characteristics in gastric cancer, colorectal cancer, hepatocellular carcinoma (HCC), and esophageal squamous cell carcinoma (ESCC), considering that the biology, pathology, clinical courses, and treatments vary enormously among different types of DSCs. However, low expression of NDRG1 was not evaluated in pancreatic cancer and gallbladder carcinoma due to limited data on the clinicopathological features. The results showed no correlation between NDRG1 expression and the clinicopathological characteristics of gastric cancer, which is inconsistent with the pooled results for OS in gastric cancer. Without any doubt, this result should also be interpreted with caution. First, the prognostic value and association of NDRG1 with the clinicopathological features of gastric cancer might vary with the subcellular localization of NDRG1 expression and subtypes of gastric cancer. Nonetheless, only the study by Kawahara et al[14] specifically explored the impacts of 2 factors on the prognostic value of NDRG1 in gastric cancer. Inversely, the in vitro studies verified that NDRG1 overexpression inhibited cell proliferation and invasiveness and induced G1 cell cycle arrest and early apoptosis in gastric cancer. This suggests that NDRG1 may act as a tumor suppressor gene, and its expression upregulation can cause favorable prognosis.[16,23] Similarly, decreased NDRG1 expression in colorectal cancer was significantly associated with poor tumor differentiation grade, suggesting that NDRG1 may also act as a tumor suppressor gene. Moreover, it was demonstrated that *NDRG1* overexpression could inhibit the invasion, metastasis and epithelial–mesenchymal transition (EMT) of colorectal cancer via multiple pathways, including NF-κB and nuclear β-catenin signaling pathways.[8,9] Additionally, some studies found that N-myc downstream-regulated gene 1 could promote apoptosis in colorectal cancer cells by enhancing ubiquitination of Bcl-2[30] and upregulation of death receptor 4,[32] and meanwhile some recent researches have also verified that NDRG1 could play anti-tumor roles by inhibiting the ErbB signaling pathway through restraining the formations of epidermal growth factor receptor (EGFR)/human epidermal growth factor receptor 2 (HER2) and HER2/HER3 heterodimers, as well as promoting the degradation of EGFR.[33] Paradoxically, a recent study by Kim et al[34] demonstrated that downregulation of NDRG1 could cause the resensitization of radioresistant rectal cancer cells by causing more DNA double-strand breakages, indicating that NDRG1 act as a protumor factor in rectal cancer. In HCC, up to date nearly all relevant literatures suggested that NDRG1 acted as a tumorigenic element by promoting HCC cell migration, invasion, and growth.[25,35,36] Furthermore, several mechanisms have been investigated to explain the tumorigenic effects of NDRG1. For instance, it has been demonstrated that NDRG1 could trigger numerous

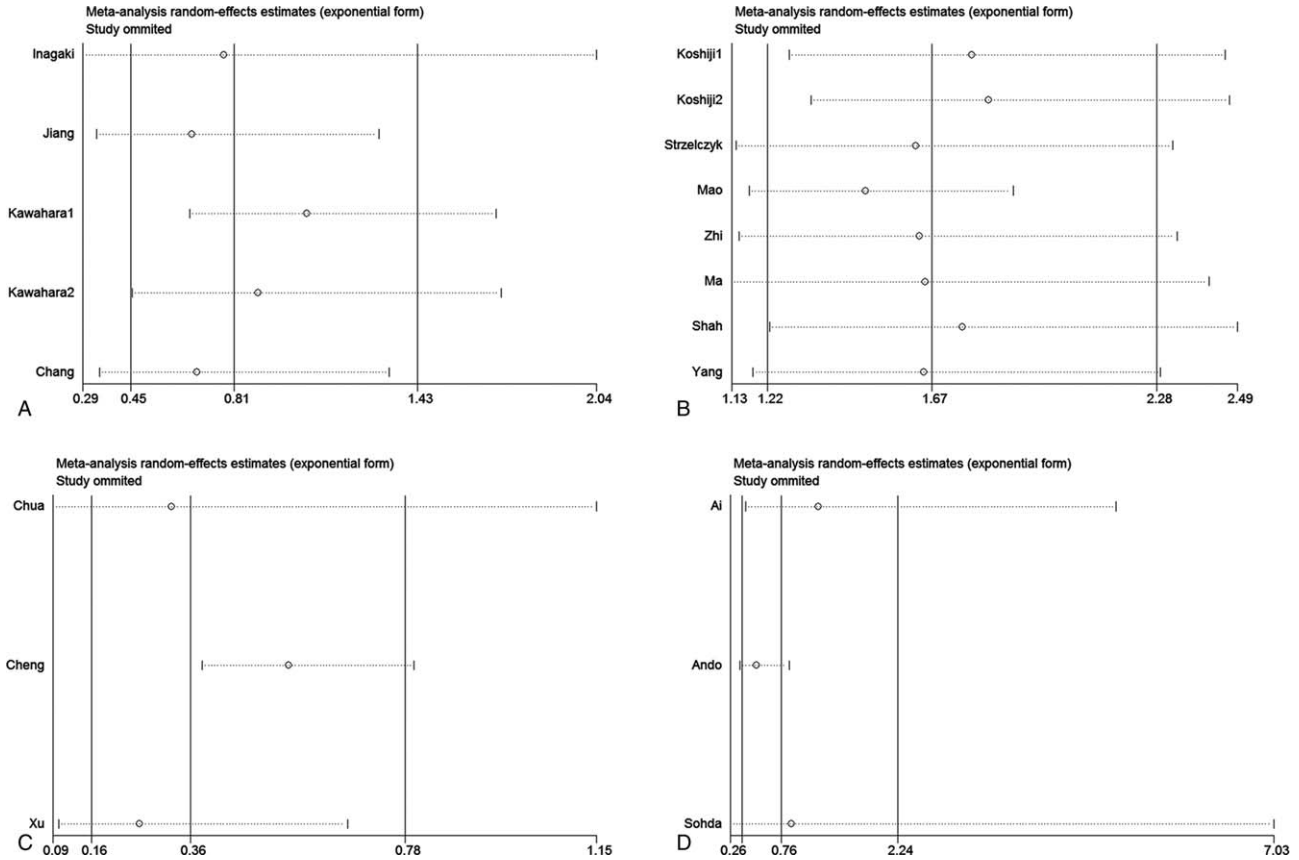


**Figure 6.** The sensitivity analyses for OS in gastric cancer (A), colorectal cancer (B), ESCC (C) and liver cancer (D).

oncogenic signaling pathways in cancer cells, including the AKT, EGF, ErbB, Wnt/β-catenin, MAPK, and Jak-STAT pathways.[25,37–39] A most recent study by Sevinsky et al[40] reported that high NDRG1 expression was associated with worse prognosis in breast cancer patients, and they demonstrated that NDRG1 promoted breast cancer aggressiveness by regulating the fate of lipids in cells. In addition, some evidence hold on that NDRG1 plays critical role in activating the stress-induced, prosurvival autophagic pathway in cancer cells.[41,42] More interestingly, recently Luo et al[43] demonstrated that NDRG1 can promote HCC progression by regulating tumor microenvironment. They found that forkhead box Q1 (FOXQ1)/NDRG1 axis in HCC cells could activate pSTAT6/C-C motif chemokine ligand 26 (CCL26) signaling, thus recruiting hepatic stellate cells (HSCs), the main cellular source of cancer associated fibroblast, which is a well-known microenvironment contributor for HCC progression.[43] Overall, the biological functions of NDRG1 in tumors may vary according to tumor type, which is consistent with the results of our meta-analysis. In future, more studies are required to uncover the concrete mechanisms for the anti- or protumor effects of NDRG1 in different tumors, so as to develop NDRG1 as a therapeutic target.

We aware that our study may have several significant limitations. First, it may have introduced publication bias since that only English publications were considered in this meta-analysis. Second, no pooling analysis could be used to synthetically assess the prognostic value of NDRG1 expression in these 2 cancer type due to that only a single study on gallbladder carcinoma and pancreatic cancer was identified through thorough literature search. Additionally, although the current meta-analysis focused on comprehensively assessing the prognostic value of NDRG1 expression in digestive system tumors, no eligible studies referred to biliary tumor. Therefore, more studies are warranted to further explore the prognostic value of NDRG1 expression in biliary tumor, gallbladder carcinoma, and pancreatic cancer. Third, there are many differences in tumor biology between right and left colon cancers. Moreover, it has been considered that the primary colorectal tumor location (right and left) is closely associated with response to chemotherapy and long-term prognosis in patients, especially for metastatic colorectal cancer.[44] Therefore, it will be more reasonable to analyze the prognostic value of NDRG1 in left and right colorectal cancer, respectively. However, all the included studies about colorectal cancer did not analyze the prognostic value of NDRG1 in left and right colorectal cancer, respectively, thus we cannot obtain relevant data to conduct the pooled analysis in this regard. Fourth, the definitions of overexpression of NDRG1 were inconsistent throughout various studies, which probably may partly account for the heterogeneity among the included studies. Fifth, despite that HRs with 95% CIs in most of the included studies were produced by a multivariate analysis, variables added into the Cox proportional hazard models varied from study to study. It may be one of sources of heterogeneity in this meta-analysis as well. Sixth, NDRG1 staining was heterogeneous and it can be detected in nuclei, cytoplasm and cell membrane. However, the majority of the included studies did not assess the prognostic value of NDRG1 expressed in nuclear, cytoplasm or membrane, respectively. This

may also introduce bias into our meta-analysis in a degree, thus weakening the reliability of the pooled analysis. Hence, future studies are required to evaluate the prognostic value of nuclear, cytoplasmic, and membranous NDRG1 expression, respectively. Last but not least, only few eligible studies provided available data for synthetically assessing the associations of low NDRG1 expression with the clinicopathological parameters, which may reduce the reliability of those pooled results due to the limitation of small sample size.

In conclusion, the prognostic significance of NDRG1 expression varies according to cancer type in patients with DSCs. Low NDRG1 expression was significantly associated with worse OS in colorectal cancer and pancreatic cancer, while it was closely related to better OS in liver cancer and gallbladder cancer. However, in gastric cancer and esophageal cancer, no associations of NDRG1 expression with prognosis were found. Considering that several limitations existed, more studies are required to further assess the prognostic value of NDRG1 expression in patients with DSCs and relevant mechanism.

## Author contributions

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**Methodology:** Hai-Peng Liu, Ze-Ping Huang.
**Software:** Ze-Ping Huang.
**Supervision:** Fu-Rong Wang, Xiao Chen.
**Writing – original draft:** Kang Chen.
**Writing – review & editing:** Xiao-hong Liu, Fu-Rong Wang, Xiao Chen.

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