Medicine®

SYSTEMATIC REVIEW AND META-ANALYSIS

OPEN

Reduced NM23 Protein Level Correlates With Worse Clinicopathologic Features in Colorectal Cancers

A Meta-Analysis of Pooled Data

Tian Yang, MB, Bo-Zan Chen, PhD, Dan-Feng Li, MB, Huai-Ming Wang, MB, Xiao-Sheng Lin, MB, Hong-Fa Wei, MB, and Yong-Ming Zeng, BA

Abstract: The clinical value of a prominent metastasis suppressor, nonmetastatic protein 23 (NM23), remains controversial. In this study, we examined the correlation between NM23 protein levels and the clinicopathologic features of colorectal cancers (CRC), and assessed the overall prognostic value of NM23 for CRC.

Embase, PubMed, Web of Science, and other scientific literature databases were exhaustively searched to identify relevant studies published prior to June 31, 2015. The methodological qualities of selected studies were scored based on the critical appraisal skills program (CASP) criteria, as independently assessed by 2 reviewers. NM23 protein levels in tumor tissues of CRC patients were examined in relation to Dukes stage, differentiation grade, T-stage, lymph node metastasis status, and overall survival (OS). STATA software version 12.0 (Stata Corp, College Station, TX) was used for statistical analysis of data pooled from selected studies.

Nineteen cohort studies met the inclusion criteria for present study and contained a combined total of 2148 study subjects. Pooled odd ratios (ORs) for NM23 expression revealed that reduced NM23 protein levels in CRC tumor tissues correlated with Dukes stage C and D (OR = 1.89, 95% CI: 1.06–3.39, $P = 0.032$), poor differentiation grades (OR = 1.41, 95% CI: 1.03–1.94, $P = 0.032$), and positive lymph node metastasis status (OR = 3.21, 95% CI: 1.95–5.29, $P < 0.001$). On the other hand, no such correlations were evident with T-stage T3-4 (OR = 1.56, 95% CI: 0.60–4.06, $P = 0.367$) or OS (OR = 0.79, 95% CI: 0.58–1.08, $P = 0.138$).

Our analysis of pooled data found that NM23 expression is reduced in CRC tissues and low NM23 levels tightly correlate with higher Dukes stages, poorer differentiation grade, and positive lymph node metastases. However, NM23 levels did not influence the OS in CRC patients.

(*Medicine* 95(4):e2589)

Abbreviations: CASP = critical appraisal skills program, CBM = China BioMedicine, CI = confidence interval, CNKI = China National Knowledge Infrastructure, CRC = colorectal cancers, IHC = immunohistochemistry, MAPK = mitogen-activated protein kinase, NDPK = nucleoside diphosphate kinase, NM23 = nonmetastatic protein 23, ORs = odd ratios, OS = overall survival, SP = streptavidin-peroxidase.

Editor: Giuseppe Di Lorenzo.
Received: June 2, 2015; revised: November 4, 2015; accepted: December 29, 2015.
From the Department of Gastrointestinal Surgery, The First Affiliated Hospital, Shantou University Medical College, Shantou, China.
Correspondence: Yong-Ming Zeng, Department of Gastrointestinal Surgery, The First Affiliated Hospital, Shantou University Medical College, No. 57, Changping Road, Shantou 515041, China (e-mail: zeng290171@sina.com).
The authors have no conflicts of interest to disclose.

ISSN: 0025-7974
DOI: 10.1097/MD.0000000000002589

INTRODUCTION

Colorectal cancer (CRC) is a malignancy that originates from colon or the rectum as a result of abnormal proliferation of cells, which progresses to tumor invasion and metastasis. CRC is the third leading cause of cancer-related mortality in both males and females.[1,2] Approximately, 950,000 new CRC cases are diagnosed annually, accounting for 10% of the world-wide cancer incidence.[3,4] CRC causes close to 500,000 deaths each year and remains a major threat to human health in both developing and developed countries.[5,6] In China, the past few decades have witnessed a rapid growth in the disease incidence and mortalities associated with CRC.[6,7] The overall survival rate in CRC patients is currently around 50% and the poor prognosis of CRC is mainly attributed to the relapse and metastasis.[8] Risk of disease recurrence in CRC patients is currently largely predicted based on the extent of primary tumor spread, which is measured by Dukes stages as follows: Dukes Stage A (local infiltration), Dukes stage B (localized disease), Dukes stage C (lymph-node positive), and Dukes stage D (distant metastasis, extensive infiltration, or lymph-node positive). Dukes staging is a major guiding factor in tailoring treatment approaches and for long-term clinical management of CRC patients.[9] The prognosis of CRC is further aided by screening for a variety of biomarkers such as PCNA, AgNORs, Bcl-2 protein, Bax protein, and ras p21 protein.[10,11] Several previous studies showed that inactivating mutations or reduced expression of nonmetastatic protein 23 (NM23) correlate with tumor pathology and CRC disease prognosis.[11–13]

NM23 is a nucleoside diphosphate kinase (NDPK) that displays the activity of removing a terminal phosphate from nucleoside triphosphates and adds it to NDPs through a high-energy phosphohistidine intermediate.[13,14] This enzymatic activity of NM23 was initially thought to be important for maintenance of intracellular nucleotide homeostasis as a housekeeping function. Discoveries in multiple developmental model systems revealed NM23's participation in wide number cellular processes including proliferation, differentiation, embryonic development, gene regulation, apoptosis, and metastasis.[15,16] Important cellular functions of NM23 include its role in DNA binding, DNA cleavage, and epithelial cell integrity.[16,17] The path breaking discovery in the 1990s that NM23 is a tumor metastasis suppressor has significant implications to the design of cancer treatments. Altered expression of NM23 is observed in various tumors such as liver, ovarian, colon, breast, prostate,

and pancreatic carcinomas, suggesting an influential role for NM23 in tumor progression and clinical outcomes.[12,15,18] For example, low NM23 expression was associated with increased tumor aggressiveness and poor overall survival in CRC patients, indicating that reduced NM23 expression promotes tumor invasion and metastasis, leading to poor prognosis.[12,19] Although the exact mechanisms of metastasis suppression by NM23 remain a mystery, NM23 is known to inhibit the activities of important signaling pathways implicated in tumor invasion such as Ras/mitogen-activated protein kinase (MAPK) and transforming growth factor-β.[20,21] Similarly, NM23 inhibits the expression of matrix metalloproteinase MMP-2, which plays a crucial role in tumor invasion.[22] Furthermore, NM23 is capable of dominantly interfering with tumor invasion and migration via suppressing the cloning ability of carcinoma cells.[23] Thus, several authors have hypothesized that elevated expression of NM23 inhibits tumor cell invasion and metastasis, and thereby is associated with favorable disease prognosis.[12,18] Evidence supporting this hypothesis indeed exists and shows that reduced NM23 expression level is linked with low T-stage, aggressive metastatic behavior, and poor prognosis of CRC.[24,25] In direct contrast, a few studies found high NM23 expression in extremely aggressive tumors or found no significant correlation between NM23 expression and the clinical features of CRC.[13,26] To systematically examine the prognostic value of NM23 in CRC, we obtained a correlation between NM23 protein levels and the pathological characteristics of the tumors using pooled data from selected high-quality cohort studies.

METHODS

Data Sources and Keywords

All analyses were based on previous published studies; thus no ethical approval and patient consent are required. Computerized databases [Embase; China BioMedicine (CBM); China National Knowledge Infrastructure (CNKI); PubMed; and Web of Science]; updated in June 31; 2015 were searched exhaustively for cohort studies reporting the correlation between NM23 protein expression and the clinicopathologic features of CRC. The studies were retrieved by utilizing selected common keywords (''NM23 Nucleoside Diphosphate Kinases'' OR ''Nucleoside Diphosphate Kinase B'' OR ''NM23-H2 Nucleoside Diphosphate Kinase'' OR ''NM23 H2 Nucleoside Diphosphate Kinase'' OR ''Nucleoside Diphosphate Kinase C'' OR ''Nucleoside Diphosphate Kinase 3'' OR ''Nucleoside Diphosphate Kinase A'' OR ''NDP Kinase A'' OR ''Non-Metastatic Cells 1'' OR ''Non Metastatic Cells 1'' OR ''Granzyme A-activated DNase'' OR ''Granzyme A activated DNase'' OR ''Nm23-H1 Nucleoside Diphosphate Kinase'' OR ''Nm23 H1 Nucleoside Diphosphate Kinase'' OR ''Nm23 H1'' OR ''Nm23 H2'' OR ''NME1'') and (''Colorectal Neoplasms'' OR ''Rectal Neoplasms'' OR ''Colorectal Tumor'' OR ''Colorectal Carcinoma'' OR ''Colorectal Cancer'' OR ''large intestine cancer'' OR ''large intestine cancer'' OR ''large bowel carcinoma'' OR ''large intestine carcinoma'' OR ''large intestinal cancer'' OR ''large intestinal carcinoma'' OR ''large bowel cancer'' OR ''large colon cancer'' OR ''intestinal cancer'' OR ''intestinal carcinoma'' OR ''intestinal neoplasms'' OR ''intestine cancer'' OR ''intestine carcinoma'' OR ''intestine neoplasms'' OR ''bowel carcinoma'' OR ''bowel cancer'' OR ''bowel neoplasms'' OR ''Colonic Neoplasms'' OR ''Colonic Tumor'' OR ''Colonic Carcinoma'' OR ''Colonic Cancer'' OR ''Colon Neoplasms'' OR ''Colon Tumor'' OR ''Colon Carcinoma'' OR ''Colon Cancer'' OR ''Rectal Tumor'' OR ''Rectal Carcinoma'' OR ''Rectal Cancer'' OR ''Rectum Neoplasms'' OR ''Rectum Tumor'' OR ''Rectum Carcinoma'' OR ''Rectum Cancer''). The publication language was either Chinese or English. Bibliographies of related papers were further manually searched for additional relevant papers.

Inclusion and Exclusion Criteria

Published studies enrolled in our present meta-analysis fulfilled the following inclusion criteria: all patients must have CRC diagnosis confirmed by pathology;[27] studies must be human cohort studies; NM23 expression must be performed in CRC tissues with complete clinical and pathological data available; complete data must be available on the expression level of NM23 protein, sample number, Dukes stage, differentiation grade, T-stage, lymph node metastasis, and overall survival (OS) of CRC patients; quantification of NM23 expression level must include appropriate experimental standards for semiquantitative scoring by immunohistochemistry (IHC); the minimum number of samples must be at least 45, and published studies must have the full text available. Exclusion criteria were: nonhuman studies; letters, editorials, abstracts, reviews, case reports, expert opinions, and meta-analyses; insufficient data.

Data Collection

Two investigators performed the literature screening (TY and B-ZC) and data were extracted from selected studies. Relevant data extracted included surname of first author, publication date, country and ethnicity, sample size, sex and age of subjects, source of controls, and detection method for NM23 protein expression. Furthermore, the associations between NM23 expression and 5 different prognostic factors were examined. Previous studies reported these prognostic factors as reliable predictors of disease progression: Dukes stage, differentiation grade, T-stage, lymph node metastasis status, and OS. In case of any discrepancy in study selection or data extraction, the reviewers arrived at a consensus by consulting with a third investigator.

Quality Assessment

Quality assessment of selected studies was performed independently by 2 authors (TY and B-ZC). Any disagreement regarding the type and quality of the study was resolved by discussion. Checklists from the critical appraisal skills program (CASP) (http://www.phru.nhs.uk/pages/phd/resources.htm) were used to assess and assign a quality score to each study.

Statistical Analysis

Summary odd ratios (ORs) with 95% confidence interval (CI) were used to compare the NM23 expression levels of different models associated with the evaluation of Z test.[28] The ORs for NM23 protein expression were aggregated independently by 2 investigators (TY and B-ZC) utilizing STATA software, version 12.0 (Stata Corp, College Station, TX). A bilateral test was conducted with a P value of <0.05 considered statistically significant. Between groups comparison of ORs used the forest plots. Heterogeneity among the pooled studies was evaluated by Cochran Q-statistic and I^2 test.[29,30] Random-effects model was used when significant heterogeneity existed among studies ($P < 0.05$ or $I^2 > 50\%$); otherwise, a fixed-effects model was employed. Subgroup meta-analysis by ethnicity was conducted to explore potential effect modification. Sensitivity analysis was performed by sequential omission of each single study to evaluate whether removal of a single study altered the

overall study outcome. Funnel plot was performed to assess publication bias and symmetry of the funnel plot was further assessed by Egger linear regression test.[31]

RESULTS

Literature Screening and Study Selection

Figure 1 presents the study selection process. Computer-based database searches and complementary manual search retrieved a total of 742 relevant articles. Of these, 342 articles were retained after removing duplicates (n = 3) and studies unrelated to the research topic (n = 397). Additionally, 320 studies were excluded because they either did not include a homogenous population (n = 84), or did not report relevant outcomes (n = 198), or were letters, comments, and correspondences (n = 36), or consisted of a very small sample size (n = 2). In the next screening step, 19 out of 22 studies were identified as relevant to this meta-analysis, after 3 articles were eliminated for not supplying sufficient data. Full text was available for the 19 cohort studies that met the study selection criteria and these studies contained the required information on NM23 protein expression levels in correlation with Dukes stage, differentiation grade, T-stage, lymph node metastasis, or OS in CRC. All 19 studies were published between 1995 and 2015.[12,13,19,24–26,32–44] Baseline characteristics of the selected studies and their methodological qualities are shown in Table 1 and Figure 2, respectively. From the 19 studies, 12 studies were performed in Asian population and 7 studies were done in Caucasians, containing a combined total of 2148 subjects. NM23 protein levels in CRC patients with different Dukes stages, differentiation grades, T-stages, lymph node metastasis status, or OS were measured semiquantitatively in all the studies using streptavidin-peroxidase (SP) method.

Expression Level of NM23 Protein in Pathological Features of CRC

Pooled ORs for NM23 protein expression, illustrated in Figure 3, revealed that low NM23 protein levels correlated with higher Dukes stage C and D (OR = 1.89, 95% CI: 1.06–3.39, $P = 0.032$), poor differentiation grade (OR = 1.41, 95% CI: 1.03–1.94, $P = 0.032$), and positive lymph node metastasis status (OR = 3.21, 95% CI: 1.95–5.29, $P < 0.001$) in CRC

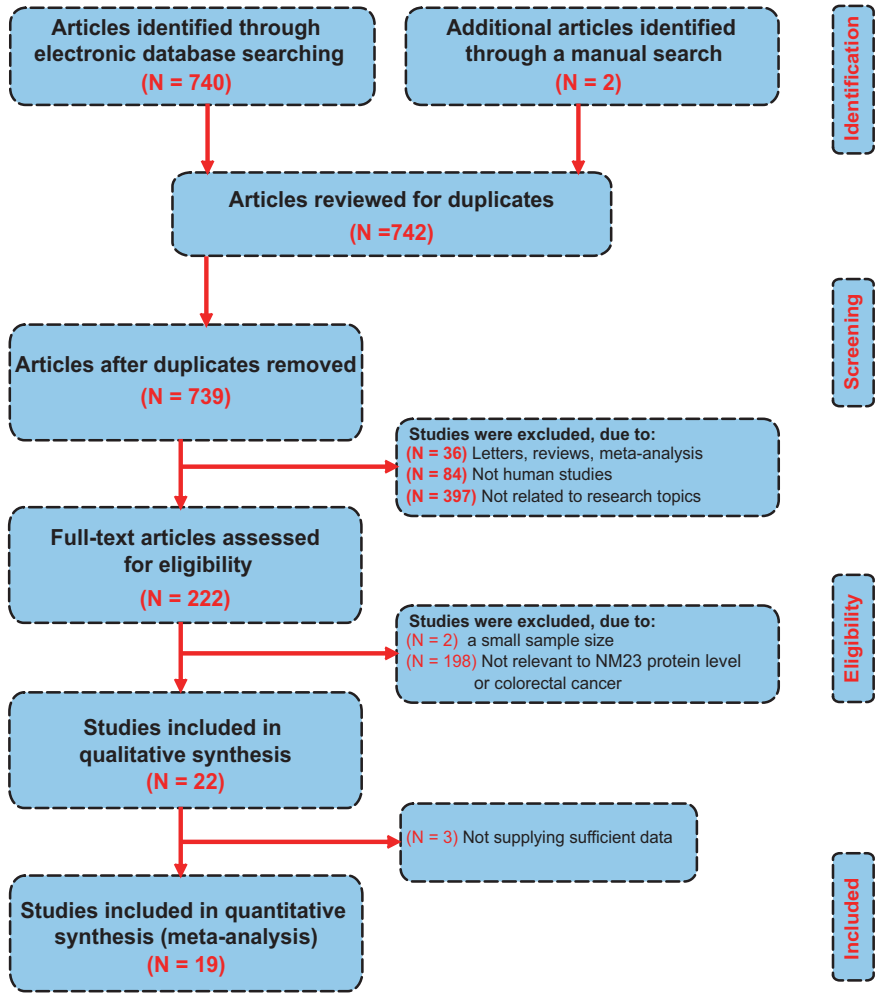

FIGURE 1. Flowchart illustrating the study search strategy and study selection. Nineteen cohort studies were eventually incorporated in this meta-analysis.

TABLE 1. The Baseline Characteristics of 19 Eligible Studies in Present Meta-Analysis

First Author	Year	Ethnicity	Number		Sample	Types of Adjustments	Included period (year)	Types of study designs	Length of follow-up for survival (months)
			Positive	Negative					
Zhang JH[37]	2015	Asians	63		Tissue	None of the patients underwent preoperative radiotherapy or chemotherapy	2010–2012	Retrospective	NR
Cui JJ[32]	2014	Asians	196		Tissue	None of the patients underwent preoperative radiotherapy or chemotherapy	2007–2014	Retrospective	Complete follow-up data
Jiao YH[33]	2014	Asians	120		Tissue	NR	2009–2013	Retrospective	Complete follow-up data
Li X[34]	2014	Asians	202		Tissue	None of the patients underwent preoperative radiotherapy or chemotherapy	2011–2013	Retrospective	NR
Peng T[35]	2014	Asians	88		Tissue	None of the patients underwent preoperative radiotherapy or chemotherapy	2008–2011	Retrospective	NR
Si R[36]	2014	Asians	243		Tissue	None of the patients underwent preoperative radiotherapy or chemotherapy	2006–2009	Retrospective	Complete follow-up data
Oliveira LA[12]	2010	Caucasians	82	11	Tissue	Patients have undergone radical surgery, and none of the patients underwent preoperative radiotherapy or chemotherapy	2001–2005	Retrospective	25.4 (1–47)
Soliani P[13]	2004	Caucasians	57	55	Tissue	Patients have undergone radical surgery, and none of the patients underwent preoperative radiotherapy or chemotherapy	1989–1992	Retrospective	60
Kapitanovic S[19]	2004	Caucasians	60	42	Tissue	Patients have undergone radical surgery, and none of the patients underwent preoperative radiotherapy or chemotherapy	NR	Retrospective	NR
Zhang JB[38]	2003	Asians	39	53	Tissue	Patients have undergone radical surgery, and none of the patients underwent preoperative radiotherapy or chemotherapy	1991–2002	Retrospective	NR
Sarris M[44]	2001	Caucasians	24	12	Tissue	NR	NR	Retrospective	NR
Lee JC[25]	2001	Asians	116	30	Tissue	Patients have undergone radical surgery, and none of the patients underwent preoperative radiotherapy or chemotherapy	1990–1994	Retrospective	54 (3–91)
Tabuchi Y[39]	1999	Asians	23	29	Tissue	Patients have undergone radical surgery, and none of the patients underwent preoperative radiotherapy or chemotherapy	1984–1988	Retrospective	NR
Gao Y[26]	2009	Asians	45		Tissue	Patients have undergone radical surgery, and none of the patients underwent preoperative radiotherapy or chemotherapy	2007–2010	Retrospective	NR

First Author	Year	Ethnicity	Number		Sample	Types of Adjustments	Included period (year)	Types of study designs	Length of follow-up for survival (months)
			Positive	Negative					
Dusonchet L[24]	2003	Caucasians	160		Tissue	Patients have undergone radical surgery, and none of the patients underwent preoperative radiotherapy or chemotherapy	1988–1992	Retrospective	71 (34–115)
Heys SD[40]	1998	Caucasians	81		Tissue	NR	1970–1990	Retrospective	NR
Cheah PY[41]	1998	Asians	141		Tissue	Patients have undergone radical surgery, and none of the patients underwent preoperative radiotherapy or chemotherapy	1991–1992	Retrospective	NR
Wang C[42]	1995	Asians	76		Tissue	NR	1982–1989	Retrospective	NR
Tannapfel A[43]	1995	Caucasians	100		Tissue	Patients have undergone radical surgery, and none of the patients underwent preoperative radiotherapy or chemotherapy	1991–1993	Retrospective	NR

NR = not reported.

FIGURE 2. Risk of publication bias summary: review authors' judgements about each risk of bias item for each included study.

patients. By contrast, no significant correlation was detected between NM23 protein levels and T-stage T3-4 (OR = 1.56, 95% CI: 0.60–4.06, $P = 0.367$) or OS (OR = 0.79, 95% CI: 0.58–1.08, $P = 0.138$). Subgroup analysis based on ethnicity revealed that in Asian CRC patients, NM23 protein levels in Dukes stages A and B were markedly higher than in patients with Dukes stages C and D (OR = 3.38, 95% CI: 1.07–10.67, $P = 0.038$) (Figure 4). Consistent with this, in Caucasian population, reduced NM23 protein expression was observed in low-differentiation grade CRC compared with high-differentiation grade CRC (OR = 1.71, 95% CI: 1.06–2.76, $P = 0.027$). A statistically significant correlation between NM23 protein levels and tumor differentiation stage was observed in Asian population and Caucasian population (all $P < 0.05$). Interestingly, subgroup analysis based on ethnicity also revealed that markedly reduced expression level of NM23 protein correlated with positive lymph node metastasis and poor OS in CRC patients in Asian population (all $P < 0.05$), but a similar relationship was not detected in Caucasians (all $P > 0.05$).

Sensitivity Analysis and Publication Bias

The significance of the pooled OR was not affected by omitting any single study, which highlighted the lack of publication bias and supports the credibility of the results

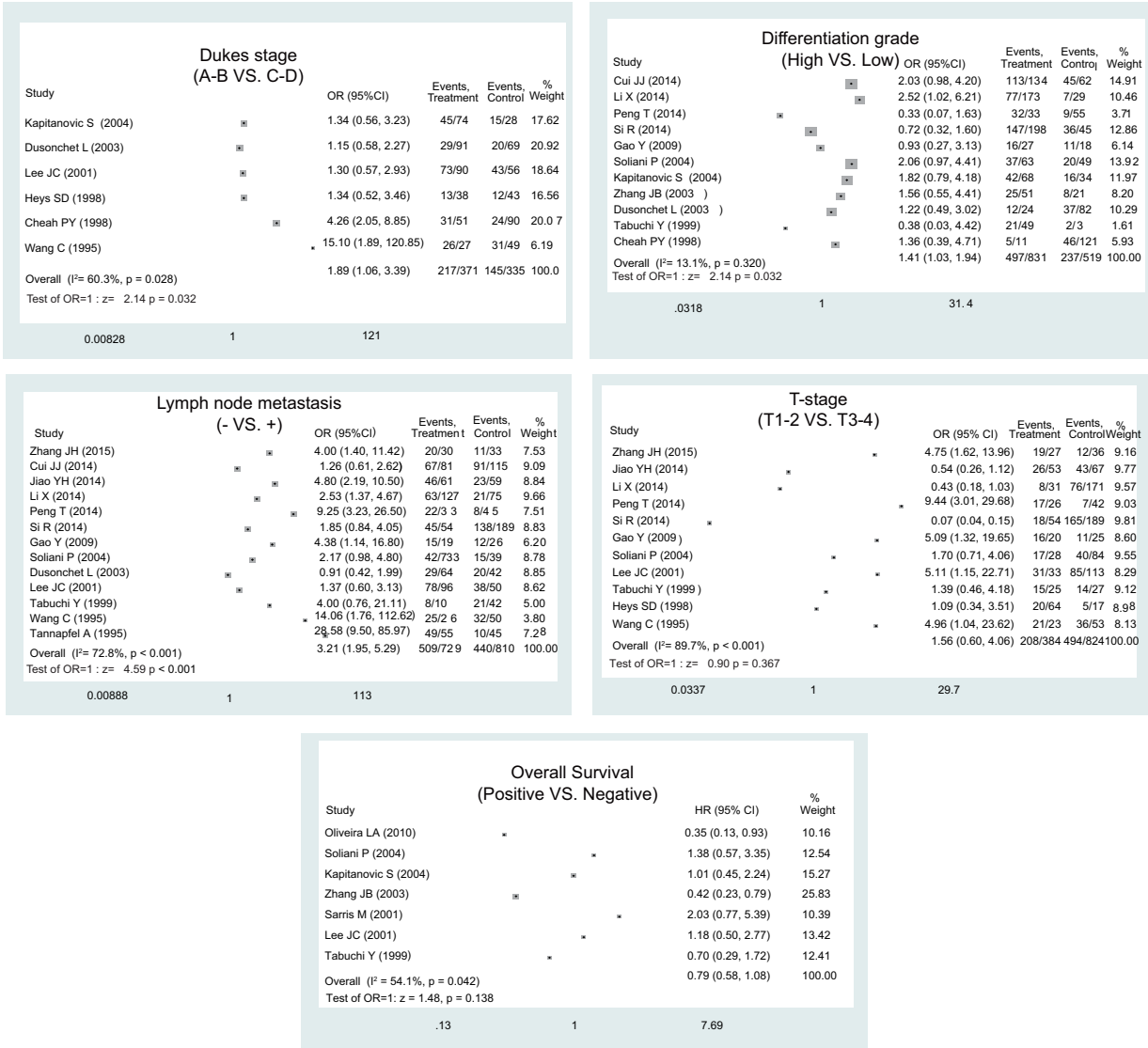

FIGURE 3. Forest plots based on odd ratios with 95% confidence interval of individual studies and pooled data detailing the association of the NM23 expression with the clinicopathological features and prognosis of colorectal cancer patients in overall analysis.

(Figure 5). The graphical output of the funnel plots of 19 selected studies showed symmetry and Egger test suggested no publication bias (all $P > 0.05$) (Figure 6).

DISCUSSION

We conducted a systematic review to obtain a relationship between NM23 protein level and CRC clinicopathologic features, with the aim of assessing the value of NM23 as a prognostic indicator. Results from this study strongly suggest that NM23 expression is markedly reduced in CRC tumor tissue and low NM23 levels correlated with higher Dukes stages, poor tumor differentiation grade, and positive lymph node metastasis, implying that NM23 might be used as a critical biomarker to characterize tumor aggressiveness and for CRC prognosis. Although altered serum levels of a prominent NM23 family member, NM23-H1, were observed in several hematological malignancies and in neuroblastoma, which correlated with treatment outcomes, the clinical relevance of NM23 in CRC tumor tissues is not fully understood. *NM23* is the first-discovered metastasis suppressor gene, which does not influence primary tumor growth but is a powerful inhibitor of metastatic spread of tumors. Overexpression of NM23 confers antimetastatic effects in CRC model in a dominant fashion and reduced expression or loss of NM23 in CRC tissues or cell lines is strongly associated with highly invasive activities and higher Dukes stages.[45] Several theories exist on how NM23 expression is reduced or lost in various tumors. In CRC, microsatellite instability is caused by loss of DNA mismatch repair and occurs in 15% of all colorectal cancers. Sequence variants of mismatch repair protein (hMSH2) are directly related to CRC pathogenesis in familial CRC patients. Interestingly, NM23 expression is influenced by hMSH2 variants through genomic instability and DNA

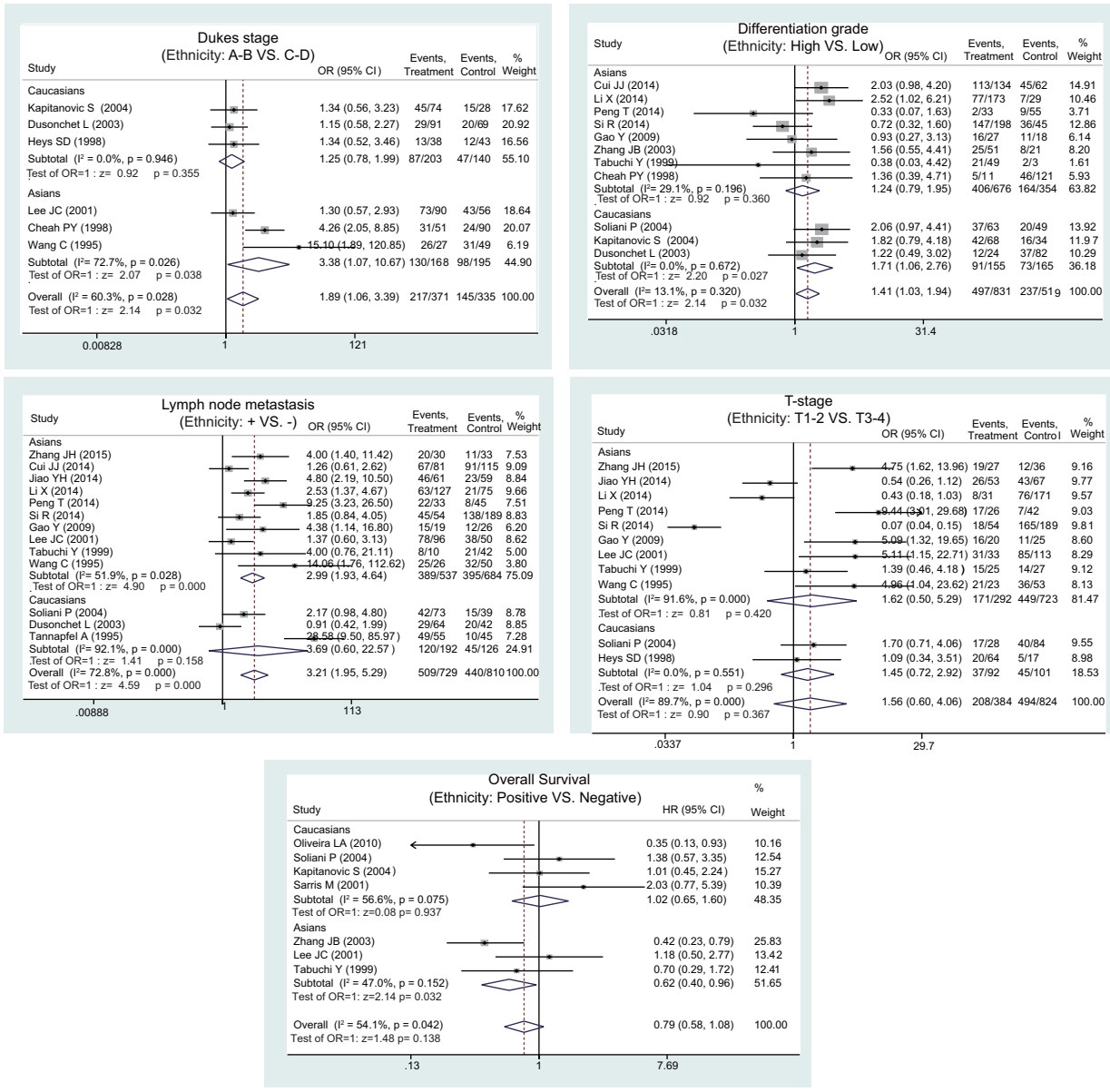

FIGURE 4. Ethnic-stratified subgroup analysis investigating the effect of the NM23 expression on colorectal cancer.

replication errors, leading to reduced expression or loss of NM23 protein, promoting tumor metastasis.[46] It was well established that favorable prognosis in CRC depends on depth of tumor infiltration, lymph node involvement, and the extent of metastatic spread. NM23 expression is inhibited in lymph node positive CRC tumors, suggesting that NM23 protein level serves as a prognostic factor in predicting the disease course and the overall survival in CRC patients.[11] We propose that low NM23 expression results in poor prognostic outcome in CRC due to the disruption of normal functioning of biological pathways regulated by NM23, such as the pathways controlling cell adhesion, epithelial integrity, and cell invasion.[18] Our meta-analysis results are consistent with our hypothesis and confirm that NM23 protein expression level has a remarkable value in predicting CRC progression since its expression levels are tightly correlated with critical tumor parameters such as Dukes stage, differentiation grade, and lymph node metastasis. In agreement with our results, Nobili et al[47] noted that high NM23 expression level accounted for disease-free survival in CRC patients and was an important prognostic indicator, along with other biomarkers. By contrast, multiple previous studies reported that NM23 expression in CRC has no correlation with tumor progression and patient clinical outcomes. For example, using immunohistochemistry to measure NM23 protein levels, Dusonchet et al[24] showed that the survival curve in patients with NM23-positive CRC tumors was not statistically different from NM23-negative patients, challenging the relevance of NM23 expression to tumor progression and clinical outcomes. In view of such contradicting data in the literature, our present meta-analysis was designed to evaluate the clinical

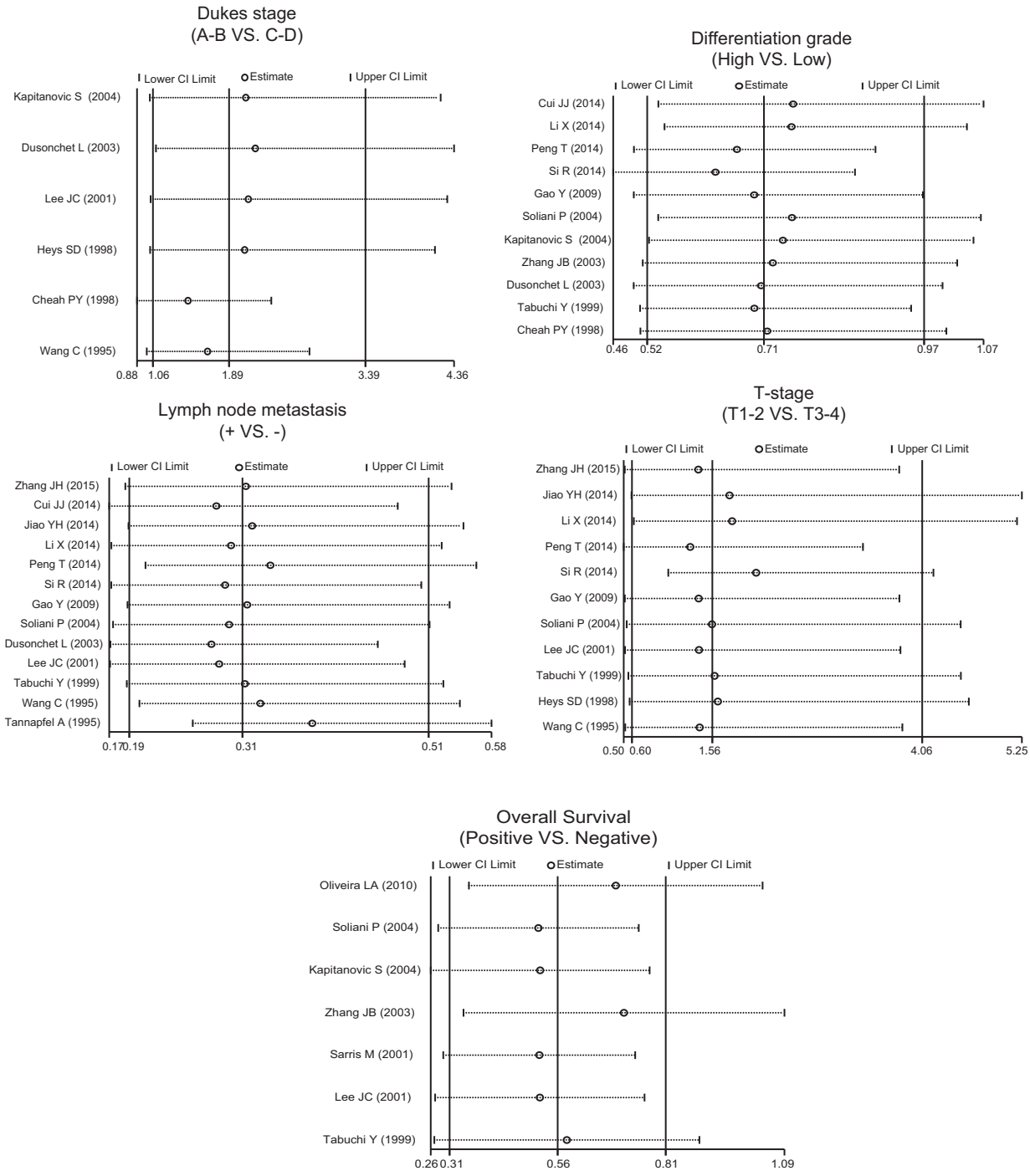

FIGURE 5. Sensitivity analysis to investigate the association of NM23 expression and colorectal cancer clinicopathological features and prognosis.

significance of NM23 protein level in CRC tumors. Our data indeed suggest that NM23 levels influence the CRC disease course.

To address other influencing factors, such as ethnicity, that may affect our results on the relationship between NM23 expression level and CRC pathological features, we performed subgroup analysis. In Asians, NM23 expression levels showed negative correlation with Dukes stages, with low NM23 levels being tightly associated with higher Dukes stages. On the other hand, in Caucasians, low NM23 levels correlated with poor differentiation grades, but such relationship was not observed in Asian population. Interestingly, although low NM23 expression levels correlated with positive lymph node metastasis and poor OS in Asian population, a statistically significant relationship

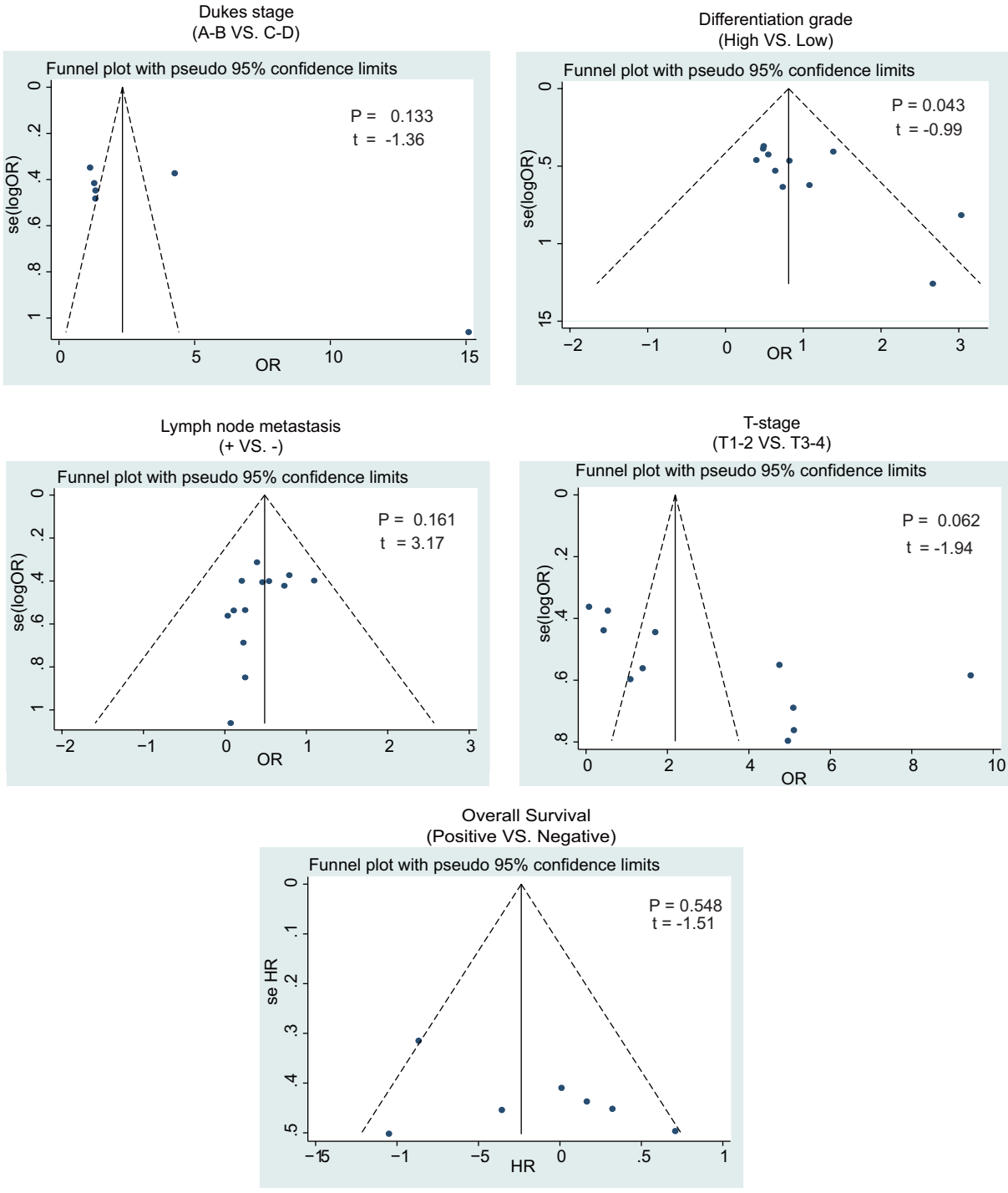

FIGURE 6. Publication biases detection to examine the relationship between NM23 expression and colorectal cancer, which highlighted the lack of publication bias and supports the credibility of the results.

was not seen in Caucasian population, implying that differences due to ethnicity should be considered while choosing specific biomarkers and ethnic differences could potentially have a strong influence on the clinical outcomes in CRC. However, larger sample size studies are warranted to further explore this issue. Nevertheless, the prognostic value of NM23 was not affected by subgroup analysis, since the overall clinical relevance of NM23 did not change substantially in both Asians and Caucasians. Sensitivity analysis did not draw diverse conclusions from pooled estimates, indicating that results we obtained were relatively stable. Finally, our study supports the clinical applications of NM23 as a biomarker and provides

strong evidence to show that NM23 expression can serve as an independent and critical indicator to assess the clinical and pathologic characteristics of CRC, which is important in guiding prognosis and improving the clinical outcomes of CRC.

We support NM23 as a critical biomarker for predicting rapid tumor progression and metastasis in CRC patients, which is a major finding of this study. However, this study has limitations that should be taken into consideration when interpreting our data. First, although we did not detect asymmetry in funnel plots and found no evidence of publication bias in Egger test, publication bias may be inevitable since studies without statistically significant outcomes remain unpublished. Thus, the pooled results may be an overestimate. Second, in this meta-analysis, we searched limited databases restricted to Chinese and English publications, which reduces the statistical power of the pooled estimate. Third, this meta-analysis mainly focused on the relationship between NM23 protein expression and clinicopathological features and prognosis of CRC based on semiquantitative approaches which describe ''increase'' and ''decrease'' or ''positive'' and ''negative'' as measured factors. Unfortunately, in such approaches a cut-off value of NM23 cannot be established and the results are influenced by individual variations in the interpretation of different observers, which is a major drawback and limits immediate clinical applications. Fourth, majority of patients from the nineteen included studies received radical surgery without chemo-radiotherapy. Therefore, subgroup analysis based on treatment regimen was not performed. The largely uniform treatment approach excludes potential differences due to treatment effects on the correlation between altered NM23 expression and OS in CRC patients, which may become apparent in a more diverse patient group. Finally, we could not ascertain a relationship between NM23 expression and T-stage of CRC patients, a result that is different from data published by several previous studies in the field. However, sensitivity and publication bias analyses suggest our results are credible and reliable. In this respect, other factors might play a role in influencing CRC prognosis, such as Dukes stage, differentiation grade, and lymph node metastasis.

In conclusion, the present meta-analysis revealed that NM23 expression is markedly reduced in CRC tissues and reduced expression or loss of NM23 tightly correlated with higher Dukes stage, poor differentiation grade, and positive lymph node metastasis in CRC patients, suggesting that NM23 is a highly valuable biomarker in CRC diagnosis and prognosis. Nonetheless, further studies are needed to confirm our findings in a larger patient population and conduct focused studies toward the clinical applications of NM23 for diagnosis and prognosis in CRC patients.

ACKNOWLEDGMENT

We would like to acknowledge the helpful comments on this paper received from our reviewers.

REFERENCES

1. Siegel R, Desantis C, Jemal A. Colorectal cancer statistics, 2014. *CA Cancer J Clin.* 2014;64:104–117.
2. Lam CS, Cheung AH, Wong SK, et al. Prognostic significance of CD26 in patients with colorectal cancer. *PLoS One.* 2014;9:e98582.
3. Theodoratou E, Montazeri Z, Hawken S, et al. Systematic meta-analyses and field synopsis of genetic association studies in colorectal cancer. *J Natl Cancer Inst.* 2012;104:1433–1457.
4. Jemal A, Siegel R, Xu J, et al. Cancer statistics, 2010. *CA Cancer J Clin.* 2010;60:277–300.
5. Zhao J, Li W, Zhu D, et al. Association of single nucleotide polymorphisms in MTHFR and ABCG2 with the different efficacy of first-line chemotherapy in metastatic colorectal cancer. *Med Oncol.* 2014;31:802.
6. Sun K, Deng HJ, Lei ST, et al. miRNA-338-3p suppresses cell growth of human colorectal carcinoma by targeting smoothened. *World J Gastroenterol.* 2013;19:2197–2207.
7. Sun K, Wang W, Zeng JJ, et al. MicroRNA-221 inhibits CDKN1C/p57 expression in human colorectal carcinoma. *Acta Pharmacol Sin.* 2011;32:375–384.
8. Takii Y, Shimada Y, Moriya Y, et al. A randomized controlled trial of the conventional technique versus the no-touch isolation technique for primary tumor resection in patients with colorectal cancer: Japan Clinical Oncology Group Study JCOG1006. *Jpn J Clin Oncol.* 2014;44:97–100.
9. Jorissen RN, Gibbs P, Christie M, et al. Metastasis-associated gene expression changes predict poor outcomes in patients with Dukes Stage B and C colorectal cancer. *Clin Cancer Res.* 2009;15:7642–7651.
10. Yantiss RK, Goodarzi M, Zhou XK, et al. Clinical, pathologic, and molecular features of early-onset colorectal carcinoma. *Am J Surg Pathol.* 2009;33:572–582.
11. Elagoz S, Egilmez R, Koyuncu A, et al. The intratumoral microvessel density and expression of bFGF and nm23-H1 in colorectal cancer. *Pathol Oncol Res.* 2006;12:21–27.
12. Oliveira LA, Artigiani-Neto R, Waisberg DR, et al. NM23 protein expression in colorectal carcinoma using TMA (tissue microarray): association with metastases and survival. *Arq Gastroenterol.* 2010;47:361–367.
13. Soliani P, Ziegler S, Romani A, et al. Prognostic significance of nm23 gene product expression in patients with colorectal carcinoma treated with radical intent. *Oncol Rep.* 2004;11:1193–1200.
14. Lee MJ, Xu DY, Li H, et al. Pro-oncogenic potential of NM23-H2 in hepatocellular carcinoma. *Exp Mol Med.* 2012;44:214–224.
15. Markowska J, Bar J, Madry R, et al. The expression of BRCA1, P53, KAI1, and Nm23 in ovaries of BRCA1 mutation carriers after prophylactic adnexectomy. *Arch Gynecol Obstet.* 2013;288:839–844.
16. Boissan M, Dabernat S, Peuchant E, et al. The mammalian Nm23/NDPK family: from metastasis control to cilia movement. *Mol Cell Biochem.* 2009;329:51–62.
17. Amendola R, Martinez R, Negroni A, et al. DR-nm23 expression affects neuroblastoma cell differentiation, integrin expression, and adhesion characteristics. *Med Pediatr Oncol.* 2001;36:93–96.
18. Boissan M, De Wever O, Lizarraga F, et al. Implication of metastasis suppressor NM23-H1 in maintaining adherens junctions and limiting the invasive potential of human cancer cells. *Cancer Res.* 2010;70:7710–7722.
19. Kapitanovic S, Cacev T, Berkovic M, et al. nm23-H1 expression and loss of heterozygosity in colon adenocarcinoma. *J Clin Pathol.* 2004;57:1312–1318.
20. Seong HA, Jung H, Ha H. NM23-H1 tumor suppressor physically interacts with serine-threonine kinase receptor-associated protein, a transforming growth factor-beta (TGF-beta) receptor-interacting protein, and negatively regulates TGF-beta signaling. *J Biol Chem.* 2007;282:12075–12096.
21. Hartsough MT, Morrison DK, Salerno M, et al. Nm23-H1 metastasis suppressor phosphorylation of kinase suppressor of Ras via a histidine protein kinase pathway. *J Biol Chem.* 2002;277:32389–32399.

22. Cheng S, Alfonso-Jaume MA, Mertens PR, et al. Tumour metastasis suppressor, nm23-beta, inhibits gelatinase A transcription by interference with transactivator Y-box protein-1 (YB-1). *Biochem J.* 2002;366:807–816.
23. Palmieri D, Horak CE, Lee JH, et al. Translational approaches using metastasis suppressor genes. *J Bioenerg Biomembr.* 2006;38:151–161.
24. Dusonchet L, Corsale S, Migliavacca M, et al. Nm23-H1 expression does not predict clinical survival in colorectal cancer patients. *Oncol Rep.* 2003;10:1257–1263.
25. Lee JC, Lin YJ, Chow NH, et al. Reappraisal of the role of NM23-H1 in colorectal cancers. *J Surg Oncol.* 2001;76:58–62.
26. Gao Y, Liu YQ, Cao WK, et al. Effects of allicin on invasion and metastasis of colon cancer LoVo cell line in vitro. *Nat Med J Chin.* 2009;89:1382–1386.
27. Garrett CR, Hassabo HM, Bhadkamkar NA, et al. Survival advantage observed with the use of metformin in patients with type II diabetes and colorectal cancer. *Br J Cancer.* 2012;106:1374–1378.
28. Chen H, Manning AK, Dupuis J. A method of moments estimator for random effect multivariate meta-analysis. *Biometrics.* 2012;68:1278–1284.
29. Jackson D, White IR, Riley RD. Quantifying the impact of between-study heterogeneity in multivariate meta-analyses. *Stat Med.* 2012;31:3805–3820.
30. Peters JL, Sutton AJ, Jones DR, et al. Comparison of two methods to detect publication bias in meta-analysis. *JAMA.* 2006;295:676–680.
31. Zintzaras E, Ioannidis JP. HEGESMA: genome search meta-analysis and heterogeneity testing. *Bioinformatics.* 2005;21:3672–3673.
32. Cui JJ, Lu QG, Wang AL. Expression and clinical significances of p53and nm23 in colorectal carcinoma. *J Colorectal Surg.* 2014;20:45–47.
33. Jiao YH, Wang XY, Liu YT. The expression and clinical significance of vascular endothelial growth factor-C and nm23-H1 in colorectal carcinoma. *Chin J Postgrad Med.* 2014;37:20–23.
34. Li X, Long HA, Xiao XL, et al. Expression and clinicopathological significance of D2-40 and nm23H1 in colorectal carcinoma. *J Luzhou Med College.* 2014;37:456–460.
35. Peng T. The expression of Her-2 and nm23H1 in colorectal cancer and its clinical significance. *Chin Community Doctors.* 2014;30:5–6+8.
36. Si R, Wu SH, He S, et al. Clinical significance of expression of p85a, nm23 and MMP-2 in primary colorectal cancer and lymph node metastasis. *World J Gastroenterol.* 2014;22:5623–5630.
37. Zhang JH, Zhang F, Zhang XL, et al. Expression and clinical significance of Cox-2, nm23 and MMP-3 in small cell carcinoma of rectum. *Hebei Med J.* 2015;37:497–500.
38. Zhang JB, Chen L, Han F, et al. Overexpression of c-erbB-2 and hypoexpression of nm23 relating to poor prognosis of colorectal carcinoma. *Chin J Path.* 2003;32:115–119.
39. Tabuchi Y, Nakamura T, Kuniyasu T, et al. Expression of nm23-H1 in colorectal cancer: no association with metastases, histological stage, or survival. *Surg Today.* 1999;29:116–120.
40. Heys SD, Langlois N, Smith IC, et al. NM23 gene product expression does not predict lymph node metastases or survival in young patients with colorectal cancer. *Oncol Rep.* 1998;5:735–739.
41. Cheah PY, Cao X, Eu KW, et al. NM23-H1 immunostaining is inversely associated with tumour staging but not overall survival or disease recurrence in colorectal carcinomas. *Br J Cancer.* 1998;77:1164–1168.
42. Wang C, Zhang X, Yang F. nm23 gene product/NDPK expression and its clinical significance in human colorectal carcinoma. *Chin J Path.* 1995;24:356–358.
43. Tannapfel A, Kockerling F, Katalinic A, et al. Expression of nm23-H1 predicts lymph node involvement in colorectal carcinoma. *Dis Colon Rectum.* 1995;38:651–654.
44. Sarris M, Lee CS. nm23 protein expression in colorectal carcinoma metastasis in regional lymph nodes and the liver. *Eur J Surg Oncol.* 2001;27:170–174.
45. Tripkovic A, Tripkovic I, Tomic S, et al. The role of nm23 gene in colorectal carcinogenesis. *Acta Clin Croat.* 2012;51:43–49.
46. Wu HW, Gao LD, Wei GH. hMSH2 and nm23 expression in sporadic colorectal cancer and its clinical significance. *Asian Pac J Cancer Prev.* 2013;14:1995–1998.
47. Nobili S, Napoli C, Landini I, et al. Identification of potential pharmacogenomic markers of clinical efficacy of 5-fluorouracil in colorectal cancer. *Int J Cancer.* 2011;128:1935–1945.