J Huazhong Univ Sci Technol［Med Sci］
DOI 10.1007/s11596-017-1686-z
37(1):1-10,2017

The Role of NM23 in Patients with Colorectal Cancer: A Systematic Review and Meta-Analysis

Wei HAN (韩 玮)[1†], Jun MA (马 俊)[2†], Fang CAO (曹 方)[1], Cong ZHANG (张 聪)[2], Rong ZHU (朱 荣)[1], Yong-wei HU (胡永伟)[1], Min-bin CHEN (陈敏斌)[3], Hou-zhong DING (丁厚中)[1#]
[1]*Department of General Surgery, Kunshan First People's Hospital Affiliated to Jiangsu University, Kunshan 215300, China*
[2]*Department of Urinary Surgery, Kunshan Hospital of Traditional Chinese Medicine, Kunshan 215300, China*
[3]*Department of Radiotherapy and Oncology, Kunshan First People's Hospital Affiliated to Jiangsu University, Kunshan 215300, China*

Summary: This meta-analysis was carried out to evaluate the relationship between NM23 expression and the prognosis of patients with colorectal cancer. We searched PubMed, EMBASE and Web of Science for relevant articles. The pooled odd ratios (ORs) and corresponding 95%CI were calculated to evaluate the prognostic value of NM23 expression in patients with colorectal cancer, and the association between NM23 expression and clinicopathological factors. In total, 2289 patients were pooled from 24 available studies. The incorporative OR combined by 16 studies with overall survival showed that high NM23 expression was associated with better overall survival (OR=0.67, 95%CI: 0.49–0.93, P=0.02, I^2=56%, Ph=0.004). And a new estimate without heterogeneity was produced when only combining high-quality studies (OR=0.70, 95%CI: 0.56–0.86, P=0.0007, I^2=46%). In disease free survival (DFS), we also obtained a good prognosis (OR=0.30, 95%CI: 0.14–0.68, P=0.004). Although we failed to find any significance in N status (P=0.10), elevated NM23 expression was related to well tumor differentiation (OR=0.60, 95%CI: 0.44–0.820, P=0.001) and Dukes' A&B (OR=0.55, 95%CI: 0.32–0.95, P=0.03). These results indicated that over-expressed NM23 might be an indicator of good prognosis, well tumor differentiation and Dukes' A&B of patients with colorectal cancer, but no significance was found in N status.
Key words: NM23, colorectal cancer, prognosis, survival and meta-analysis.

Colorectal cancer, with high propensity for metastasis, has become one of the most terrible diseases[1]. In spite of numbers of biomarkers involved in colorectal cancer emerging ceaselessly, prognosis remains to be dismal mainly due to local recurrence, lymphatic vessel invasion and distant metastasis[2]. Furthermore, patients at the same status, such as with the same differentiation, lymph node invasion and tumor staging, may have varied clinical outcomes[3]. Hence, it is urgent to explore reliable and valid markers to predict the prognosis and monitor the management of colorectal cancer.

Non-metastatic clone 23 (NM23) has been found associated with the development and progression of various neoplasms[4, 5]. Gao *et al* discovered that the level of NM23 mRNA and protein was inversely related to metastasis in experimental animals, through implanting ovarian cancer cells into 160 nude mice subcutaneously[6]. After transfecting NM23 into melanoma cells, biological behaviors, such as invasion, motility, colonization, etc., were also suppressed, compared with control group[7]. Boissan *et al*[8] also detected that, in hepatocellular and colon carcinomas, NM23 could inhibit cell adhesion and migration at early stages of invasion. Moreover, NM23 expression in cytoplasm was more strictly related to age, tumor location, and tumor histological structure than that in nucleus in gastric cancer patients[9]. In colorectal cancer, NM23 also plays a critical role in many respects. Kim *et al*[10] confirmed, both by Western blotting and immunohistochemistry, that NM23 was over-expressed in tumor cell cytoplasm. There was a negative correlation between NM23 expression and stage or lymph node metastasis, while no significant difference of NM23 expression level was found among the different grades of colorectal cancer[11]. Also, *in vitro*, after silencing NM23 expression in human colon carcinoma cells, cellular scattering, motility, and extracellular matrix invasion were all promoted[8].

Whether the prognostic and clinicopathological value of NM23 can become a promising prognostic indicator has stirred up strong reactions and extensive discussion. Many studies reported that it was associated with a better overall survival of various cancers, but some showed that NM23 was not a metastasis suppressor gene and not correlated with metastasis, including a previous meta-analysis which showed that the level of NM23 expression didn't influence survival in colorectal cancer patients[12, 13]. As a result of this heated debate, we performed this systematic review of the literatures with meta-analysis to clarify this question and explore its value in patients with colorectal cancer.

1 MATERIALS AND METHODS

1.1 Database Search Strategy

Three databases, PubMed, EMBASE and Web of

Wei HAN, E-mail: hans64@126.com; Jun MA, E-mail: 531681644@qq,com
†These authors contributed equally to this work.
#Corresponding author, E-mail: dinghouzhong@163.com

Science, were searched from their incipiency to December, 2015. The retrieval strategy was used as follows: [NM23 or (non-metastasis 23) or (nucleoside diphosphate kinase) or NDPK or NM23-H1 or (non-metastasis 23-H1) or (nucleoside diphosphate kinase A) or NDPK-A or NME1 or NM23-H2 or (non-metastasis 23-H2) or (nucleoside diphosphate kinase B) or NDPK-B or NME2 and (colorectal or colonic or rectal) and (neoplasms or cancer or carcinoma or tumor or tumour or adenocarcinoma or malignant) and (prognosis or prognostic or predict or survival or outcome or prognos*) or (clinical variables) or clinicopatholog* or (clinical pathology) or (clinic pathology)]. Reference lists of articles and reviews were hand-searched for additional studies. Manuscripts were also manually scanned to obtain potential articles most relevant to this review. Only studies published in peer reviewed journals were included and the language was limited to English. All the initially identified articles were scrutinized independently by two investigators (Wei HAN and Jun MA). For more details and for information, please see our protocol with the registration number: CRD42015029488[14].

1.2 Inclusion Criteria and Exclusion Criteria

Inclusion criteria were: (a) clinical studies researched patients with colorectal cancer; (b) NM23 expression in cytoplasm of tissue specimens of patients with colorectal cancer, who received neither chemotherapy nor radiation therapy before surgery, was measured with immunohistochemistry (IHC); (c) studies reported the association between NM23 expression and survival outcome or clinicopathological information; (d) only the most recent or the most complete report would be enrolled, if the study population was duplicated or overlapping. Disagreement was resolved by discussion between the two reviewers or consultation with a third reviewer (Min-bin CHEN).

Exclusion criteria were: (a) literature published as letters, editorials, abstracts, reviews, case reports and expert opinions; (b) experiment *in vitro* or *in vivo* but not based on patients; (c) articles with neither the ORs with 95%CI about clinicopathological information, nor the Kaplan-Meier survival curves; (d) repeated and similar studies.

1.3 Data Extraction

The following information was extracted from each article: (a) basic information from papers, including first author, publication year, country (area) of origin, age and gender of the study patients, sample size and the follow-up duration; (b) clinicopathological characteristics, including differential grade, lymph node metastasis/N status and tumor staging (TMN staging or Dukes stage); (c) method to determine NM23 expression and number of patients stratified by NM23 expression; (d) survival outcomes, including overall survival (OS), disease-free survival (DFS), metastasis-free survival (MFS) and recurrence-free survival (RFS). Correlative ORs with 95%CI of these outcomes were estimated from Kaplan-Meier curves. All eligible articles were reviewed independently by two investigators and disparity was resolved by discussion or consultation.

1.4 Quality Assessment

Two reviewers (Wei HAN and Jun MA) independently assessed the quality of each study with the Newcastle-Ottawa Quality Assessment Scale (NOS)[15]. A study with NOS>5 was regarded as a high-quality study[16]. Disagreements were resolved by consensus.

1.5 Data Synthesis and Analysis

Survival data, including OS, DFS, MFS and RFS, associated with NM23 expression in patients with colorectal cancer, were the primary outcome. Relationship between NM23 and clinicopathological variables was the secondary outcome. OR with its 95%CI was used to be the effect measure of interest. Estimates of ORs were weighted and pooled using the Mantel-Haenszel method. A combined OR<1, with its 95% CI not overlapping 1, indicated a better survival for the group with NM23 expression.

A test of heterogeneity of combined ORs was performed by Q test and I^2 statistic. A random or Fixed model was used according the heterogeneity analysis. A random effect model was applied if $I^2 \geq 50\%$; the fixed effect model was selected if $I^2<50\%$. $I^2>50\%$ or Ph<0.05 was considered to have high heterogeneity, and then subgroup analyses were conducted to find the source of heterogeneity. The publication bias was assessed by a funnel plot and Egger's linear regression test, and a value <0.05 indicated an inevitable significant publication bias[17].

All statistical tests were two-tailed and $P<0.05$ was considered statistically significant. All the analyses were conducted by Review Manager software version 5.3 and STATA statistical software package version 12.0.

2 RESULTS

2.1 Search Results

In the initial search, a total of 432 records were retrieved, including 13 yielded by manual searching. After removing 185 duplicates, we read the titles and abstracts of the 247 studies left. Among them, 166 citations were excluded from analysis based upon abstracts or titles, and 81 studies were kept for further full-text review. After meticulous reading, 57 studies were excluded: 48 studies, including reviews or letters, were excluded for no or insufficient survival data; three[18–20] were removed for they investigated the expression of NM23 mRNA, using PCR; five[21–25] were only about allelic imbalance of NM23; and the left one[26] had a duplicated population. As a result, 24 eligible studies[27–50] with 2289 patients in total were enrolled in this meta-analysis (fig. 1).

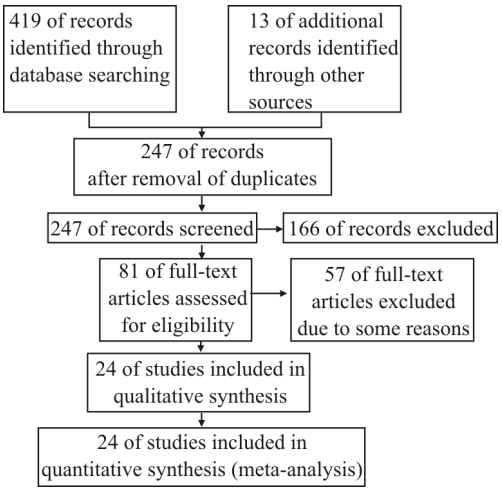

Fig. 1 Flow chart for selection of records to include

2.2 Study Characteristics

The basic characteristics of the 24 studies[27–50], published ranging from 1993 to 2013, are summarized in table 1. Briefly, study sample sizes ranged from 30 to 202; 14 studies were conducted in Caucasian populations, while the remaining ten used Asian populations; all studies measured the expression of NM23 in cytoplasm of tissue specimens with IHC, and all patients didn't receive any preoperative chemotherapy or radiation therapy, as we had written before; all of the primary antibodies were anti-NM23 antibodies, including polyclonal and monoclonal antibodies. Except two articles[27, 44], all studies reported their cut-off of NM23 expression, most of which identified more than about 30%–50% staining cancer cells as high expression. One study[40] used matched normal tissue as positive control, and another one[36] identified the cut-off based on the relative visual intensity of chromogenic label. Although the cut-offs of these two studies were different from those of other studies, the effect, to some extent, is similar in more than 30%. However, the cut-off of another one[48] might be too low as compared with others.

Table 1 Characteristics of included studies

First author	Year	NOS	Study country or region	N. of P.	Tumor stage	Cut-off of NM23-H1 high expression	Primary antibody	Follow-up time	Survival analysis
Abad[27]	1996	5	Austria	62	NR	NR	Monoclonal antibody NCL-nm23-2	6–10 years	OS, DFS
Ayhan[28]	1993	6	Japan	84	UICC: I–IV	"++" or "+++"	Anti-nm23 antibody	NR	NR
Berney[29]	1998	8	Australia	58	Dukes' A–C	Score=3 or 4 (3=strong staining of 25% to 50% or moderate staining of >80%; 4=strong staining of >50% tumor cells)	A monoclonal antibody (0.08 mg/mL) that recognized both nm23-H1 and nm23-H2 was used (Clone 56; Transduction Laboratories, USA).	10 years	OS, MFS
Bertucci[30]	2004	8	France	191	AJCC: 1–4	Cutoff was the median value.	Anti-NM23 rabbit polyclonal antibody was purchased from Dako (Dako, Trappes, France) and used at 1:100 dilution, which detects both NME1 and NME2 proteins.	Median 74 (2–133) months	OS, MFS
Cheah[31]	1998	7	Singapore	141	Dukes' A-D	Moderate and strong staining	Monoclonal antibody (NM23 Ab-1, clone NM301 from Oncogene Science)	>5 years	OS, DFS
Chen[32]	2007	6	Mainland China	103	Dukes' A–D	Moderate and marked staining	Mouse anti-human monoclonal antibodies to nm23-h1 (1:50 dilution; Shanghai Chang-DoBiotechnology Co. Ltd)	NR	NR
Dursun[33]	2001	8	Turkey	185	Dukes' A–D	> 60%	Prediluted primary polyclonal antibody (NDPKinase/nm23-h1Ab-1, NeoMarkers, US)	Median 36 months (2–95) months	OS, DFS
Heys[34]	1998	7	UK	92	Dukes' A–D	>45%	The monoclonal antibody to NM23 (diluted 1 in 50; Novocastra, UK)	5 years	OS
Ichikawa[35]	1994	8	Japan	56	Dukes' A–C	Staining intensity of grade 2 and 3	Anti-NM23 monoclonal antibody	5.9–71.3 months	OS, RFS
Kapitanovic[36]	2004	7	Croatia	73	Dukes' A–C	On the basis of the relative visual intensity of chromogenic label	Mouse monoclonal antibody to human nm23-H1 (NM301 monoclonal antibody; Molecular Oncology Inc, Gaithersburg, Maryland, USA)	about 300 weeks	OS
Lee[37]	2001	7	Taiwan, China	146	Dukes' A–D	More than 50% or "++"	Monoclonal anti-nm23-H1 antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA)	54 months (3–91) months	OS
Lindmark[38]	1996	7	Sweden	202	Dukes' A–D	Strong and moderate homogeneous intensity	Mouse monoclonal anti-nm23-H1 antibody, cloned NM301, from Becton and Dickinson (San Jose, CA, USA)	>90 months	OS
Madbouly[39]	2007	8	USA	153	UICC: I, II	More than 25% of cells were positively stained.	NM23 antibodies	Mean 65.12±32.41 months	OS, RFS
Martinez[40]	1995	6	France	35	Astler-Coller: A-D	Signal more intense than in matched normal tissue	Anti-NDP kinase A monoclonal antibody (HA-37.6) raised by Hybridolab, Pasteur Institute, France	NR	NR
Messinetti[41]	2003	8	Italy	41	Dukes' A, B	+2,+3 intensity and >10% cells stained	A murine monoclonal anti Nm23-H1 antibody	Median 73 months (1–154 months)	OS, DFS
Oliveira[42]	2010	8	Brazil	130	UICC: I–IV	>50%	A monoclonal anti-NM23 antibody (Neomarkers, USA), at 1:1000 dilution	Mean 25.4 months (1–47 months)	OS, DFS
Pasz-Walczak[43]	2010	7	Poland	102	Astler-Coller: BCD	>60%	The primary monoclonal antibody anti-Nm23-H1 (clone 37.6, 1:500 dilution, Leica Biosystems, UK)	>100 months	OS

Su[44]	2004	5	Mainland China	30	Dukes' A–D	NR	Anti-NM23-H1 antibody	NR	NR
Tabuchi[45]	1999	6	Japan	52	UICC: I–IV	Positive reactivity for strong staining	Mouse monoclonal antihuman nm23-H1 antibody (H1-229, 2 μg/mL, Seikagaku, Japan)	>5 years	OS
Tannapfel[46]	1995	6	Germany	100	UICC: I–III	>60%	A 1:200 dilution of nm23-h1Ab-1, Clone NM301, obtained from Oncogene Science Cambridge, MA, USA	NR	NR
Tripkovic[47]	2012	7	Croatia	100	Dukes' A–D	Score 3–6	Monoclonal antibodies for nm23 protein (DAKO, rabbit anti human, AO 485 diluted 1:200)	5 years	OS
Wu[48]	2013	7	Mainland China	87	UICC: I–III	with >1% cell staining	Mouse anti-human monoclonal antibody nm23	5 years	DFS
Yamaguchi[49]	1993	6	Japan	36	UICC: I–IV*	strongly stained	The primary antibody to nm23-H1 (mAb HI-229)	NR	NR
Yang[50]	2008	6	Mainland China	30	UICC: I–IV	Excel function to compute the value of positive unit (PU)	Anti-nm23H1 antibody	NR	NR

NOS: Newcastle-Ottawa Quality Assessment Scale; N. of P.: number of patients; UICC: Union for International Cancer Control; AJCC: American Joint Committee on Cancer. *: The study didn't report the stage, and the result was produced by discussion.

2.3 Quality Assessment

The study quality scores based on the NOS, ranged from 5 to 8, with a mean of 6.83. Except two studies[27,44] gaining a NOS=5, all studies had high levels of methodological quality (NOS≥6) in this meta-analysis (table 2).

Table 2 Quality assessment with NOS

First author	Year	NOS	Selection	Comparability	Outcome
Abad[27]	1996	5	★★*	★	★★*
Ayhan[28]	1993	6	★★★	★*	★★
Berney[29]	1998	8	★★★	★★	★★★*
Bertucci[30]	2004	8	★★★*	★★*	★★★
Cheah[31]	1998	7	★★★*	★★	★★*
Chen[32]	2007	6	★★★	★	★★
Dursun[33]	2001	8	★★★*	★★*	★★★
Heys[34]	1998	7	★★★	★★	★★
Ichikawa[35]	1994	8	★★★	★★*	★★★*
Kapitanovic[36]	2004	7	★★★	★★	★★
Lee[37]	2001	7	★★★*	★★	★★*
Lindmark[38]	1996	7	★★★	★★	★★
Madbouly[39]	2007	8	★★★*	★★	★★★
Martinez[40]	1995	6	★★★	★	★★*
Messinetti[41]	2003	8	★★★*	★★	★★★*
Oliveira[42]	2010	8	★★★*	★★*	★★★*
Pasz-Walczak[43]	2010	7	★★	★★	★★★
Su[44]	2004	5	★★★*	★*	★*
Tabuchi[45]	1999	6	★★★	★*	★★
Tannapfel[46]	1995	6	★★★	★	★★
Tripkovic[47]	2012	7	★★★	★★	★★
Wu[48]	2013	7	★★*	★★	★★★
Yamaguchi[49]	1993	6	★★	★★	★★*
Yang[50]	2008	6	★★★*	★*	★★

*: The score was produced by the joint discussion; others were assessed by Wei HAN and Jun MA, individually.

2.4 NM23 and Survival Data

In this meta-analysis, 16 studies reported the data concerning the association between NM23 expression and overall survival (OS) of the patients. In these studies, three reported that NM23 was a good prognostic indicator[27, 30, 33], only one showed that NM23 was a poor biomarker[29], and others concluded that there was no relationship between NM23 and OS. With heterogeneity (I^2=56%, Ph=0.004), the pooled OR being 0.67 (95%CI: 0.49–0.93, P=0.02. fig. 2A) showed that over-expression of NM23 was associated with better OS. Then, we deleted the study "Abad 1996"[27], which had an NOS=5. And we obtained a new estimate without heterogeneity (OR=0.70, 95%CI: 0.56–0.86, P=0.0007, I^2=46%. fig. 2B). Since NM23 was reported as an unfavourable marker in only one study, we deleted this OR and pooled a new one also without heterogeneity (OR=0.62, 95%CI: 0.46–0.84, P=0.002, I^2=47%).

We also gained sufficient information from six cohorts, which reported the association between NM23 expression and DFS. However, no significance was found (P=0.27, fig. 2C). Fortunately, when we deleted the study "Abad 1996" as above, the new OR was 0.30 (95% CI: 0.14–0.68, P=0.004, I^2=61%, Ph=0.04, fig. 2D).

Both MFS and RFS had two studies, and the pooling ORs both had no significance (OR=2.00, 95%CI: 0.03–127.52, P=0.74, and OR=0.98, 95% CI: 0.41–2.34, P=0.97, respectively).

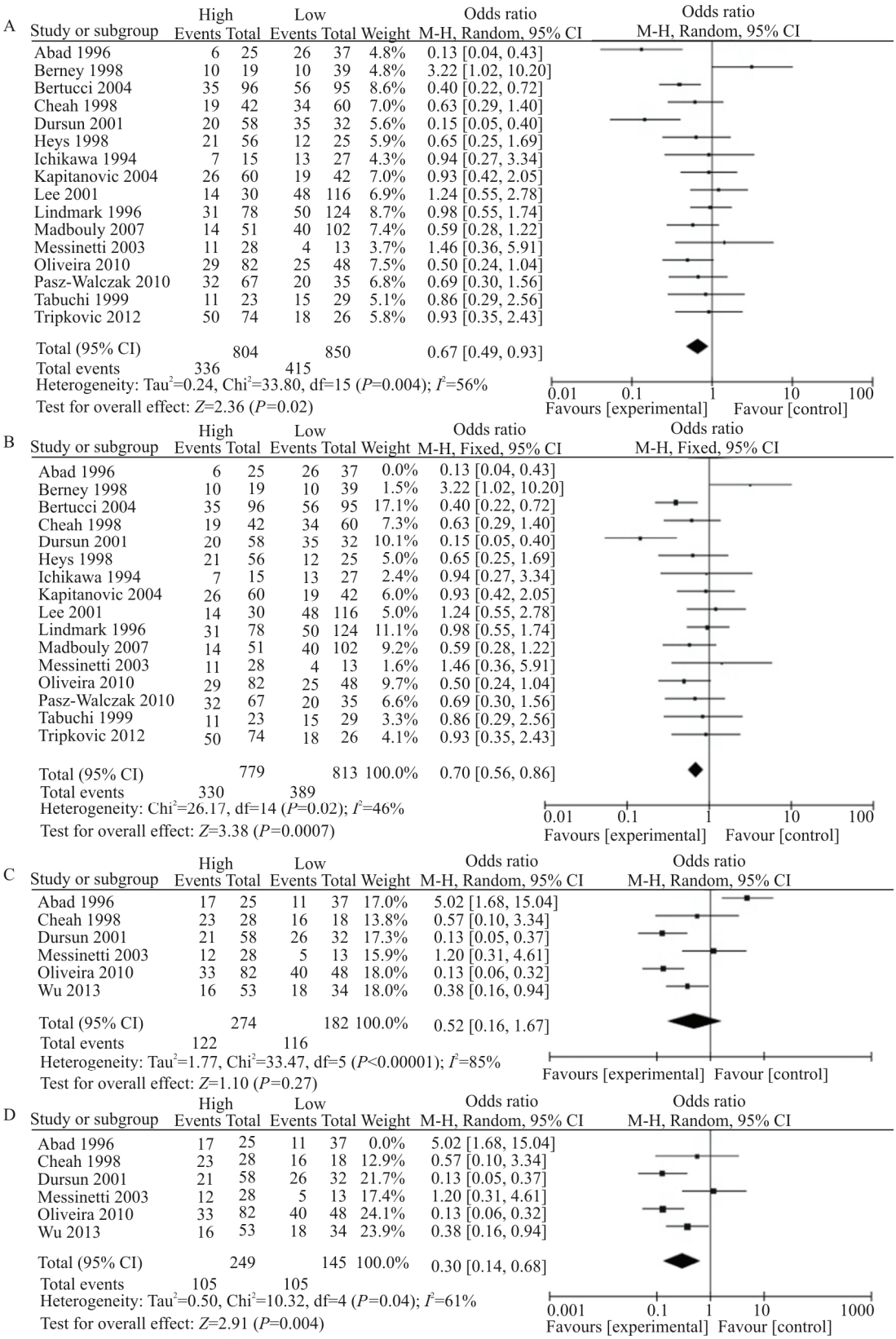

Fig. 2 Forest plot of odds ratio (OR) for the association between NM23 over-expression and survival data in patients with CRC
A: OS; B: OS without "Abad 1996"; C: DFS; D: DFS without "Abad 1996"

2.5 NM23 and Clinicopathology Features

Thirteen cohorts reported ORs for the relationship between NM23 over-expression and differential grades; 11 reported N status; 13 reported tumor staging. Without any heterogeneity (I^2=38%, Ph=0.08), we found a pooled estimate with statistical significance in different grades

(OR=0.60, 95%CI: 0.44–0.820, *P*=0.001, fig. 3A). However, we failed to gain any significance in statistics, when evaluating the pooled ORs of N status and tumor staging (*P*=0.11 and *P*=0.10, respectively, fig. 3B and 3C). In tumor staging, ten studies reported Dukes' stage, one reported histological stage, one reported TNM staging, and one used Astler-Coller stage. Thus, we combined these ten studies and produced a new OR being 0.55 (95%CI: 0.32–0.95, *P*=0.03, I^2=72%, Ph=0.0002. fig. 3D).

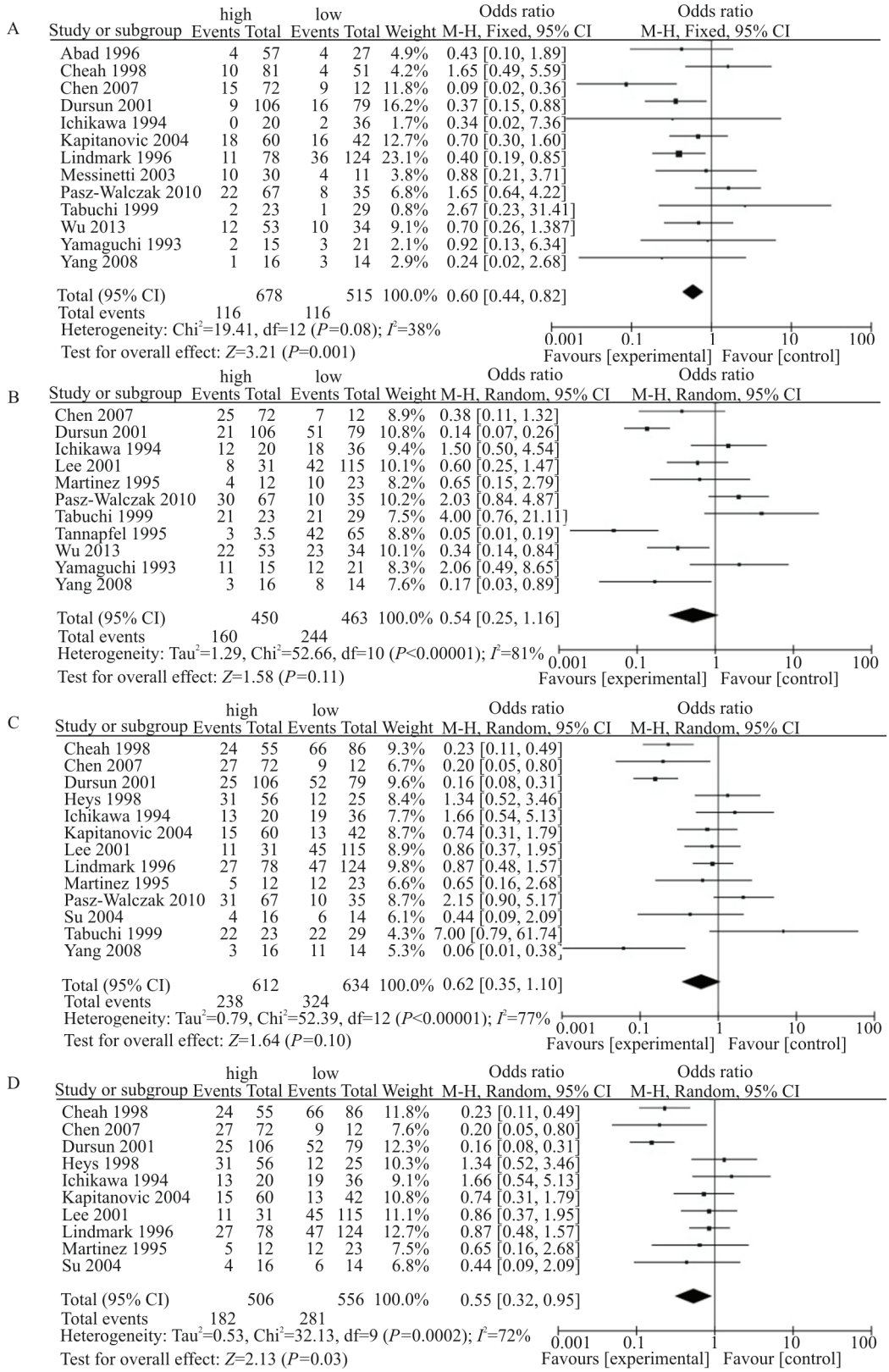

Fig. 3 Forest plot of OR for the association between NM23 over-expression and clinicopathological features in patients with colorectal cancer

A: different grades; B: lymph node metastasis/N status; C: tumor staging; D: Dukes' stage

2.6 NM23 and Subgroup-analyses

In view of the heterogeneity, we conducted subgroup analyses by stratifying the pooled data according to study region (Asian *vs*. Caucasian), sample size (<100 *vs*. ≥100), years (≤2000 *vs*. >2000), antibody type (NM23 *vs*. NM23-H1) and cut-off (Low *vs*. Moderate *vs*. High) (table 3). Because of too few cohorts in DFS, and no heterogeneity in different grades and OS with high-quality studies, we carried out the subgroup analyses only in N status and Dukes' stage. In cut-off, we identified "<30%" as "low", "30%–50%" or "moderate" as "moderate", and ">50%" or "strong" as "high". In addition, we classified these three studies, "Kapitanovic 2004", "Martinez 1995" and "Yang 2008", as "moderate". Despite "<100" and "NM23" in Dukes' stage had an I^2=0, we failed to obtain any potential source of heterogeneity.

Table 3 Subgroup analyses of N status and Dukes' stage

	N status					Dukes' stage				
	n	OR (95%CI)	I^2	Ph	*P*	*n*	OR (95%CI)	I^2	Ph	*P*
Overall	11	0.54 [0.25, 1.16]	81%	<0.00001	0.11	10	0.55 [0.32, 0.95]	72%	0.0002	0.03
Study region										
Asian	7	0.73 [0.36, 1.49]	59%	0.02	0.39	5	0.50 [0.22, 1.13]	66%	0.02	0.10
Caucasian	4	0.31 [0.06, 1.61]	91%	<0.00001	0.16	5	0.60 [0.26, 1.38]	80%	0.0005	0.23
Sample size										
<100	6	0.85 [0.35, 2.06]	63%	0.02	0.72	5	0.95 [0.58, 1.56]	0	0.57	0.84
≥100	5	0.33 [0.10, 1.11]	88%	<0.00001	0.07	5	0.37 [0.17, 0.80]	81%	0.0004	0.01
Years										
≤2000	5	0.81 [0.18, 3.72]	84%	<0.0001	0.79	5	0.75 [0.36, 1.56]	70%	0.01	0.45
>2000	6	0.41 [0.17, 0.98]	80%	0.0001	0.05	5	0.39 [0.18, 0.88]	71%	0.009	0.02
Antibody type										
NM23	2	0.69 [0.16, 2.95]	76%	0.04	0.61	2	1.47 [0.71, 3.03]	0	0.78	0.30
NM23-H1	9	0.51 [0.20, 1.28]	83%	<0.00001	0.15	8	0.43 [0.24, 0.77]	69%	0.002	0.004
Cut-off										
Low	1	0.34 [0.14, 0.84]	–	–	0. 02	–	–	–	–	–
Moderate	5	0.59 [0.31, 1.10]	25%	0.25	0.10	8	0.68 [0.41, 1.12]	58%	0.02	0.13
High	5	0.62 [0.12, 3.17]	91%	<0.00001	0.57	1	0.16 [0.08, 0.31]	–	–	<0.00001

2.7 Sensitivity Analyses

To test the stabilization of our results, we deleted one individual cohort each time and calculated the pooled ORs of the studies left. In OS with high-quality studies, N status and different grades, no significant differences were observed between the corresponding results and the overall results (data not shown). In DFS with high-quality studies, a different result was produced when we deleted the study "Messinetti 2003", which investigated patients only at Dukes' A and B. And then, the new pooled OR, without this cohort, being 0.22 (95%CI: 0.11–0.43, *P*<0.00001), had no heterogeneity (I^2=37%, Ph=0.19). In Dukes' stage with high-quality studies, we gained two results different from the overall, when we deleted these two studies, "Heys 1998" and "Ichikawa 1994", respectively. And the new combined estimate had a *P*=0.008, but still had heterogeneity (I^2=74%, Ph=0.0009).

2.8 Publication Bias

We carried out the publication bias assessment of the 24 eligible studies. There was no obvious asymmetry in these funnel plots (fig. 4). Except RFS and MFS, which couldn't be calculated, no evident publication bias was found in all results, with the *P* value of Egger's test (*P*=0.724, *P*=0.433, *P*=0.978, *P*=0.366 and *P*=0.672 for OS, DFS, differentiation, N status and tumor stage, respectively).

3 DISCUSSION

This meta-analysis, with 24 articles and 2289 patients, evaluated the prognostic value of the expression of NM23 in patients with colorectal cancer. Among them, there were sixteen studies reporting the association between NM23 expression and OS, and the pooled estimate showed that high NM23 expression had a better OS (OR=0.67, 95%CI: 0.49–0.93, *P*=0.02). After deleting the low-quality study, we obtained a similar result (OR=0.70, 95%CI: 0.56–0.86, *P*=0.0007), but without any heterogeneity. This similar result without heterogeneity could also be found when deleting study "Berney 1998" (OR=0.62, 95%CI: 0.46–0.84, *P*=0.002, I^2=47%), which was the only one study showing NM23 to be a poor indicator. This study, "Berney 1998", with high NOS and detailed information, only included 58 patients. Despite some studies also had small sample size, deviation and bias could be enlarged exponentially at some point. Thus, elevated expression of NM23 in patients with colorectal cancer was associated with better survival. We also obtained a *P*<0.05, when pooling high-quality studies with DFS. However, we failed to gain any statistical significance, when pooling ORs of MFS and RFS (*P*>0.05). In the following analysis, we found that elevated NM23 expression was related to well differentiation and Dukes' A&B in patients with colorectal cancer, but we failed to reach any statistical significance, when

pooling ORs of N status (P>0.05). Hence, the association between NM23 overexpression and better clinicopathological outcome could be proven partly, through this meta-analysis.

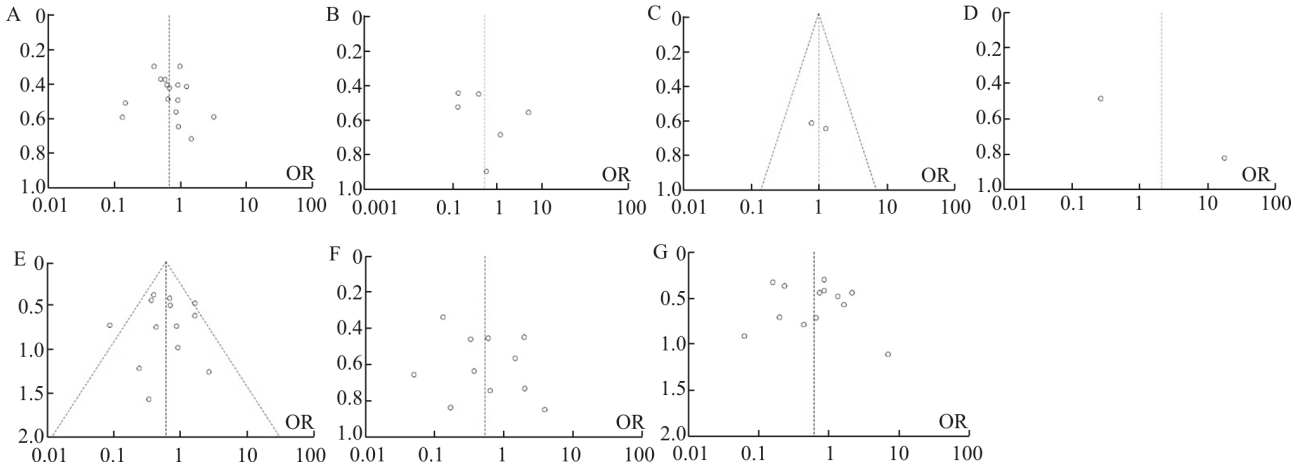

Fig. 4 Funnel plots for NM23 expression and OS, DFS, RFS, MFS, different grades, N status and tumor staging
A: OS; B: DFS; C: RFS; D: MFS; E: different grades; F: N status; G: tumor staging

In our subgroup analysis, to find the source of heterogeneity, we stratified the pooled data according to study region, sample size, years, antibody type and cut-off. However, we failed to find any possible source of heterogeneity in N status and Dukes' staging. Though there was heterogeneity in OS combined by all studies, including "Abad 1996", no heterogeneity was found in OS of high-quality studies (I^2<50%). So, we didn't carry out a subgroup analysis in OS. In the following sensitivity analysis, firstly in DFS with high-quality studies, we deleted study "Messinetti 2003" to produce a new OR with significance but without any heterogeneity, indicating that study "Messinetti 2003", in which the patients were only at Dukes' A and B, might be the source of heterogeneity; then, in Dukes' stage with high-quality studies, we deleted two studies, "Heys 1998" and "Ichikawa 1994", and the new combined estimate had a P=0.008, but still had heterogeneity (I^2=74%, Ph=0.0009). Finally, according to our funnel plots, without any obvious asymmetry and Egger's test, without any P<0.05, we could say, there was no obvious publication bias in our meta-analysis.

In the development and progression of colorectal cancer, NM23 was involved in two independent genetic pathways and crucial functions, loss of heterozygosity (LOH) and microsatellite instability (MSI). LOH of NM23, predicting the occurrence of highly aggressive tumors, mostly arose in the late stage of colon cancer[51], and its deletion also indicated a poor 5-year overall survival, in patients with colorectal cancer[21]. In lung carcinoma, the LOH rate of NM23 with metastasis was significantly higher than that without metastasis[52]. In addition, MSI of NM23 was an early stage molecule marker in gallbladder carcinoma[53]. Another key molecular mechanism of NM23 is regulating Ras-BRaf-MAPK pathway[54, 55]. Overexpressed NM23 reduced KSHV-induced cell invasiveness by suppressing the activation of Ras-BRaf-MAPK pathway[54]. However, few feasible and effective therapeutic targets had been developed from these known substrates of NM23[55].

Undeniably, there were some limitations in our meta-analysis. Firstly, in view of quality of available articles, we only adopted articles written in English from three authoritative databases, PubMed, Embase and Web of Science. The previous meta-analysis[13] including 19 cohort studies searched not only from these three databases, but also from China BioMedicine (CBM) and China National Knowledge Infrastructure (CNKI), both of which were Chinese databases, retrieved two more databases, but our study had five more enrolled articles than the previous study, which means that the quality of the previous meta-analysis seems suspicious. However, some unpublished studies or articles in other languages could be ignored inevitably. Secondly, some antibodies recognized both NM23-H1 and NM23-H2, but some only recognized NM23-H1. Despite NM23-H1 and NM23-H2 encoded two subunits of nucleoside diphosphate kinase (NDPK), with 88% identity in their amino acid sequences[56], these articles might cause some selection bias or else. Thirdly, due to lack of studies reporting HRs of OS, we estimated ORs from the Kaplan-Meier curves of these 24 studies in our meta-analysis, which could also produce some bias. Fourthly, all cohorts we included were investigated by IHC. Maybe other methods, such as RT-PCR, immunofluorescence, allelic deletion and so on, could also indicate the prognostic value of NM23 expression. Though a total of 24 cohorts reported the role of NM23 in colorectal cancer, further studies are still required to be carried out in the future.

In conclusion, our meta-analysis showed that high NM23 expression was significantly associated with better survival, tumor differentiation and Dukes' A&B of patients with colorectal cancer, but no significance was found in association between high NM23 and N status. More and further researches should be performed to reveal the role of NM23.

Conflict of Interest Statement

All of the authors declare no conflict of interest.

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(Received May 10, 2016; revised Nov. 16, 2016)