

# Prognostic significance of NANOG expression in solid tumors: a meta-analysis

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**Purpose:** NANOG is a tumor marker and indicates poor prognosis in various neoplasms; however, the evidence is controversial. This meta-analysis investigated the association of NANOG expression and clinicopathological features, and its impact on survival of patients with malignant tumors.

**Methods:** Studies published through May 31, 2018 were retrieved from PubMed, Web of Science, Embase, and the China National Knowledge Infrastructure. Two researchers independently screened the content and quality of studies and extracted data. Correlations of NANOG expression, clinicopathological variables, and survival were analyzed and the combined odds ratios (ORs) and hazard ratios (HRs) with 95% confidence intervals (95% CIs) were calculated.

**Results:** Thirty-three articles including 35 data sets of 3,959 patients were analyzed. Overall, elevated NANOG expression was associated with poor overall survival (HR = 2.19; 95% CI: 1.87–2.58,  $P < 0.001$ ) and poor disease-free survival (HR = 2.21, 95% CI: 1.54–3.18,  $P < 0.001$ ). Subgroup analysis found that NANOG expression was associated with worse overall survival in non-small cell lung (HR = 1.87; 95% CI: 1.26–2.76,  $P = 0.002$ ), head and neck (HR = 2.29; 95% CI: 1.75–3.02,  $P < 0.001$ ), and digestive system (HR = 2.38; 95% CI: 1.95–2.91,  $P < 0.001$ ) cancers. Moreover, we found that high NANOG expression was associated with poor tumor differentiation (OR = 2.63; 95% CI: 1.59–4.55,  $P = 0.001$ ), lymph node metastasis (OR = 2.59; 95% CI: 1.50–4.47,  $P = 0.001$ ), advanced TNM stage (OR = 2.22; 95% CI: 1.42–3.45,  $P < 0.001$ ), and T stage (OR = 0.44; 95% CI: 0.20–0.93,  $P = 0.031$ ).

**Conclusion:** The evidence supports NANOG as a tumor biomarker to guide clinical management and indicate prognosis. Additional studies are needed to further validate these results.

**Keywords:** NANOG, cancer, prognosis, meta-analysis

## Introduction

NANOG is a transcription factor that contains a DNA-binding domain, and acts to maintain pluripotency, self-renewal, and the undifferentiated state of embryonic stem cells.<sup>1</sup> It belongs to the *NK-2* gene of the ANTP superfamily, which is primarily expressed in the blastocyst inner cell mass.<sup>2</sup> However, cancer stem cells (CSCs) involved in tumor recurrence and metastasis express NANOG as a surface marker in addition to CD133, CD90, EpCAM, and CD44.<sup>3,4</sup> NANOG protein expression is a biomarker that indicates poor clinical outcome in lung, breast, gastric, colorectal, pancreatic, and ovarian cancer as well as in hepatocellular, oral squamous cell, esophageal, and nasopharyngeal carcinoma.<sup>5–14</sup> Ravindran et al reported that elevated NANOG expression was associated with poor overall survival (OS) and disease-free survival (DFS), lymph node metastasis, tumor stage, and differentiation of oral squamous cell carcinoma (OSCC),<sup>15</sup> but Hwang et al found no association of NANOG expression with clinical stage or OS.<sup>10</sup> Vaz et al found that NANOG expression was not related

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to prognosis in rectal cancer.<sup>16</sup> Therefore, the prognostic value of NANOG expression in solid tumors is controversial. This meta-analysis was conducted to overcome design limitations and sample size limitations of previous studies to further evaluate the potential prognostic and clinical values of NANOG in patients with malignant cancers.

## Materials and methods

### Search strategy

Articles published through May 31, 2018 were retrieved from PubMed, the Web of Science, Embase, and the China National Knowledge Infrastructure (CNKI). Combinations of the MeSH headings and keywords “NANOG or NANOG homeobox protein or NANOGP8”, “cancer or malignancy or neoplasm or tumor or carcinoma”, and “prognosis or outcome or survival” were used in the searches. The reference lists of the retrieved articles were searched manually to supplement the literature retrieval.

### Selection criteria

Studies with a pathologically confirmed solid tumor diagnosis, immunohistochemical (IHC) assay of NANOG

expression in primary and tumor tissue, OS and/or DFS as primary outcomes, and reporting HRs with 95% CIs for OS and DFS or with the possibility of calculating them from survival curves were eligible. Moreover, the inclusion criteria included stratification of patients into NANOG-positive and -negative or high and low expression groups for the survival analysis. A sample size of  $\geq 40$  cancer patients was required, and the publications were limited to those in English and Chinese. Articles reporting overlapping or duplicate results, lacking information on survival outcomes, reviews, letters, expert opinions, conference abstracts, case reports, and animal studies were excluded. A flow diagram of article selection is shown in Figure 1.

### Data extraction and quality assessment

Two investigators (LZ and JL) independently undertook data extraction and data quality evaluation. Disagreements were resolved by consultation with a third investigator (SC). The study information included the first author, country, language, publication year, cancer type, sample size, follow-up duration, assay methods, cutoff scores, and outcome measures. Patient characteristics included age, sex ratio, tumor

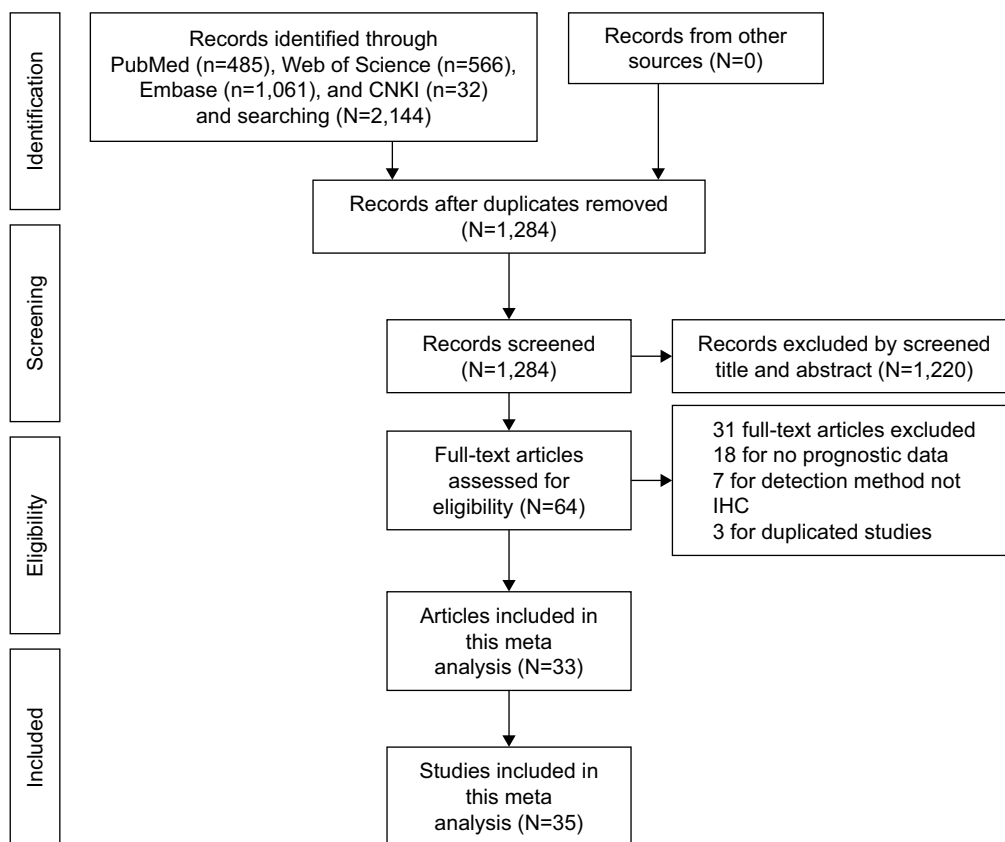


Figure 1 Flowchart of the steps of literature retrieval and selection.

Abbreviations: CNKI, China National Knowledge Infrastructure; IHC, immunohistochemical.

differentiation, T stage, tumor size, TNM stage, lymph node metastasis, lymphatic infiltration, and vascular infiltration. HRs and 95% CIs of survival outcome were directly retrieved from the study or were estimated from Kaplan–Meier survival curves. The Newcastle–Ottawa Scale (NOS) was used to evaluate quality of selected literature.<sup>17</sup> An NOS score  $\geq 5$  indicated high quality; low-quality studies were excluded. Discrepancies were resolved through discussion.

## Statistical analysis

STATA version 14.0 (Stata Corporation, College Station, TX, USA) was used for the meta-analysis, and Engauge Digitizer version 4.1 (<http://markumitchell.github.io/engauge-digitizer/>) was used to extract survival data from Kaplan–Meier curves as previously described by Tierney et al.<sup>18</sup> HRs and 95% CIs were pooled to estimate the impact of NANOG on OS and DFS. An HR  $> 1.0$  indicated a poor prognosis. ORs and 95% CIs were used to assess the relationship of NANOG expression and clinical pathological features. The chi-squared test and Cochran's  $I^2$  coefficient were calculated to assess heterogeneity in pooled studies. A chi-square  $P > 0.10$  or an  $I^2 < 50\%$  indicated low heterogeneity. If  $P$  was  $\geq 0.1$  and  $I^2 < 50\%$ , the fixed-effects model was used for analysis; otherwise ( $P < 0.1$  and/or  $I^2 \geq 50\%$ ), a random-effects model was used, and subgroup analysis was carried out to determine the origin of the heterogeneity. Sensitivity analysis was conducted by continuous omission of individual studies to evaluate the effectiveness and reliability of the meta-analysis. Publication bias was assessed in Begg's funnel plots and Egger's test.  $P < 0.05$  was considered as statistically significant.

## Results

### Study inclusion and characteristics

The study selection process is described in Figure 1. A total of 33 studies<sup>5–16,19–39</sup> published from 2008–2018 and including 35 data sets with 3,959 patients were selected to evaluate the relationship of NANOG expression and tumor prognosis. The average study population size was 113, ranging from 42 to 312 patients. Twenty studies were conducted in China, five were conducted in Korea, and three in Japan. Twenty-eight were published in English and five in Chinese. Four studies evaluated non-small cell lung cancer (NSCLC),<sup>5,32–34</sup> four evaluated gastric cancer (GC),<sup>9,26,27,38</sup> and five evaluated OSCC,<sup>8,15,28,29,36</sup> breast cancer,<sup>6,30,31</sup> hepatocellular carcinoma (HCC),<sup>7,25,39</sup> and ovarian cancer (OC)<sup>13,21,22</sup> were each evaluated in three studies. Esophageal squamous cell carcinoma (ESCC),<sup>10,23</sup> colorectal cancer (CRC),<sup>11,16</sup> and pancreatic

cancer (PC)<sup>12,24</sup> were each evaluated in two studies. Nasopharyngeal carcinoma (NPC),<sup>14</sup> astrocytoma,<sup>20</sup> cervical cancer (CC),<sup>37</sup> tongue squamous cell carcinoma (TSCC),<sup>35</sup> and mucoepidermoid carcinoma (MEC)<sup>19</sup> were each evaluated in one study. Thirty-three studies<sup>5–16,19–39</sup> reported OS and nine studies<sup>6,9,13,15,21,22,31,32,38</sup> reported DFS. NANOG expression was assayed in all tumor tissues by IHC and stratified by high and low expression. In most studies, the threshold of high NANOG expression included both staining percentage and intensity scores. Some studies reported the percentage of positively stained cells as the cutoff value. The quality scores of the selected studies ranged from 6 to 8, indicating that they were adequate for inclusion in the quantitative meta-analysis. Table 1 shows the characteristics of the included studies.

### High NANOG expression and OS

All 33 articles reported the relationship of the NANOG expression and prognosis. Because of heterogeneity ( $I^2 = 37\%$ ,  $P = 0.016$ ), a random-effects model was used to pool HRs and 95% CIs. High NANOG expression was significantly associated with worse OS (HR = 2.19; 95% CI: 1.87–2.58,  $P < 0.001$ , Figure 2). The effects of NANOG expression on OS in different solid tumors are shown in Figure 3. Elevated NANOG expression was significantly related to worse OS in NSCLC (HR = 1.87; 95% CI: 1.26–2.76,  $P = 0.002$ ), head and neck cancers (HR = 2.29; 95% CI: 1.75–3.02,  $P < 0.001$ ), and digestive system cancers (HR = 2.38; 95% CI: 1.95–2.91,  $P < 0.001$ ), which included liver, gastric, colorectal, esophageal, and pancreatic cancer. Table 2 shows the results of subgroup analysis of OS. The pooled HR for OS was 2.26 (95% CI: 1.95–2.62,  $P < 0.001$ ) in Asians and 1.87 (95% CI: 1.08–3.23,  $P = 0.025$ ) in Caucasians. The pooled HR estimate of OS was 2.09 (95% CI: 1.06–2.74,  $P < 0.001$ ) in studies with sample sizes  $> 100$  cases and 2.25 (95% CI: 1.86–2.72,  $P < 0.001$ ) for sample sizes of  $< 100$ . The association of high NANOG expression and poor OS was significant in both multivariate (HR = 2.16; 95% CI: 1.77–2.62,  $P < 0.001$ ) and non-multivariate (HR = 2.19; 95% CI: 1.73–2.77,  $P < 0.001$ ) analysis.

### High NANOG expression and DFS

Nine studies<sup>6,9,13,15,21,22,31,32,38</sup> with ten data sets reported the association of high NANOG expression and DFS. Because of heterogeneity ( $I^2 = 64.4\%$ ;  $P = 0.003$ ), a random-effects model was used to pool HRs and 95% CI. As shown in Figure 4, NANOG expression was associated with worse DFS (HR = 2.21, 95% CI: 1.54–3.18,  $P < 0.001$ ). A subgroup analysis was conducted to investigate the origin of heterogeneity depending

**Table 1** Main characteristics of 33 studies in the meta-analysis

Study	Year	Country	Language	No. of patients	Type of tumor	Follow-up (months)	Cutoff method and scores	Outcome measures	NOS scores
Park et al <sup>32</sup>	2016	Korea	English	142	NSCLC	NA	SP $\geq$ 100	OS (S)/DFS (S)	7
Luo et al <sup>33</sup>	2013	China	Chinese	62	NSCLC	36–60	NA	OS (S)	7
Xue et al <sup>7</sup>	2016	China	Chinese	89	HCC	36	NA	OS (S)	7
Park et al <sup>32</sup>	2016	Korea	English	226	NSCLC	NA	SP $\geq$ 100	OS (M)/DFS (M)	7
Li et al <sup>34</sup>	2013	China	English	309	NSCLC	52 (7–69.5)	PP $\geq$ 5%	OS (M)	7
Chang et al <sup>5</sup>	2017	Korea	English	112	NSCLC	NA	SP $\geq$ 180	OS (M)	7
Nagata et al <sup>6</sup>	2017	Japan	English	208	TNBC	80.7 (20–162)	SP $\geq$ 3	OS (S)/DFS (S)	7
Jin et al <sup>30</sup>	2016	China	English	312	BC	60	PP $\geq$ 1%	OS (M)	7
Nagata et al <sup>31</sup>	2014	Japan	English	100	BC	80.7 (20–162)	SP $\geq$ 3	OS (S)/DFS (S)	7
Li et al <sup>9</sup>	2015	China	English	69	GC	35 (6–50)	SP $\geq$ 5	OS (S)/DFS (S)	8
Li et al <sup>23</sup>	2014	China	Chinese	69	ESCC	65	PP $\geq$ 10%	OS (S)	8
Lu et al <sup>24</sup>	2013	China	English	43	PC	NA	SP $\geq$ 5	OS (S)	6
Yin et al <sup>25</sup>	2012	China	English	228	HCC	60 (1–83)	SP $\geq$ 1	OS (S)	7
Lin et al <sup>26</sup>	2012	China	English	105	GC	NA	SP $\geq$ 2	OS (S)	7
Matsuoka et al <sup>27</sup>	2012	Japan	English	253	GC	NA	SP $\geq$ 5	OS (S)	6
Hwang et al <sup>10</sup>	2014	China	English	41	ESCC	13 (0.3–57.4)	NA	OS (S)	6
Lee et al <sup>28</sup>	2015	Korea	English	57	OSCC	35.9 (3–127)	SP $\geq$ 4	OS (M)	7
Ravindran et al <sup>15</sup>	2015	India	English	60	OSCC	31.9 (14–48)	PP $\geq$ 16%	OS (M)/DFS (M)	7
Luo et al <sup>14</sup>	2013	China	English	122	NPC	60.1 (8–92)	SP $\geq$ 6	OS (S)	7
Chiou et al <sup>29</sup>	2008	China	English	52	OSCC	NA	NA	OS (S)	6
Wang et al <sup>8</sup>	2018	China	English	144	OSCC	62.97 (24–120)	PP $\geq$ 50%	OS (M)	6
Meng et al <sup>11</sup>	2010	China	English	175	CRC	NA	SP $\geq$ 4	OS (S)	6
Gao et al <sup>12</sup>	2016	China	English	47	PC	NA	SP $\geq$ 2	OS (S)	6
Kenda Šuster et al <sup>13</sup>	2017	Slovenia	English	106	OC	75 (65.8–84.1)	SP $\geq$ 3	OS (S)/DFS (S)	6
Elsir et al <sup>20</sup>	2014	Sweden	English	42	Astrocytoma	NA	PP $\geq$ 50%	OS (M)	8
Elsir et al <sup>20</sup>	2014	Sweden	English	71	Astrocytoma	NA	PP $\geq$ 50%	OS (M)	8
Vaz et al <sup>16</sup>	2014	America	English	100	CRC	69.6	PP $\geq$ 26%	OS (S)	6
Xu et al <sup>19</sup>	2017	China	English	75	MEC	11–124	PP $\geq$ 10%	OS (S)	6
Lee et al <sup>22</sup>	2012	Korea	English	74	OC	92 (7–183)	SP $\geq$ 4	OS (M)/DFS (S)	7
Siu et al <sup>21</sup>	2013	China	English	90	OC	63 (4–209)	SP At mean	OS (S)/DFS (S)	7
Wang et al <sup>37</sup>	2018	China	Chinese	78	CC	72 (12–144)	SP $>$ 3	OS (M)	7
Ouyang et al <sup>39</sup>	2016	China	Chinese	116	HCC	16.5 (3–36)	SP $\geq$ 2	OS (M)	8
Zhang et al <sup>38</sup>	2018	China	Chinese	60	GC	NA	SP $>$ 2	OS (S)/DFS (S)	6
Kim et al <sup>36</sup>	2017	Korea	English	62	OSCC	35.5	SP $\geq$ 4	OS (M)	7
Rodrigues et al <sup>35</sup>	2018	Brazil	English	60	TSCC	NA	SP $\geq$ 4	OS (S)	6

**Abbreviations:** BC, breast cancer; NSCLC, non-small cell lung cancer; TNBC, triple-negative breast cancer; ESCC, esophageal squamous cell carcinoma; OSCC, oral squamous cell carcinoma; HCC, hepatocellular carcinoma; NPC, nasopharyngeal carcinoma; GC, gastric cancer; OC, ovarian cancer; PC, pancreatic cancer; MEC, mucoepidermoid carcinoma; CRC, colorectal cancer; CC, cervical cancer; TSCC, tongue squamous cell carcinoma; PP, percentage of positive cells; SP, staining intensity score and percentage of positive cells; OS, overall survival; DFS, disease-free survival; M, multivariate; S, survival curves; NA, not available.

on the type of cancer (Figure 5). Elevated NANOG expression was significantly associated with worse DFS in ovarian (HR = 2.95; 95% CI: 1.65–5.27,  $P < 0.001$ ), and breast cancer (HR = 4.75; 95% CI: 2.70–8.34,  $P < 0.001$ ) but not NSCLC (HR = 1.23; 95% CI: 0.65–2.36,  $P = 0.524$ ). Additional studies with larger sample sizes are required to reach a consensus.

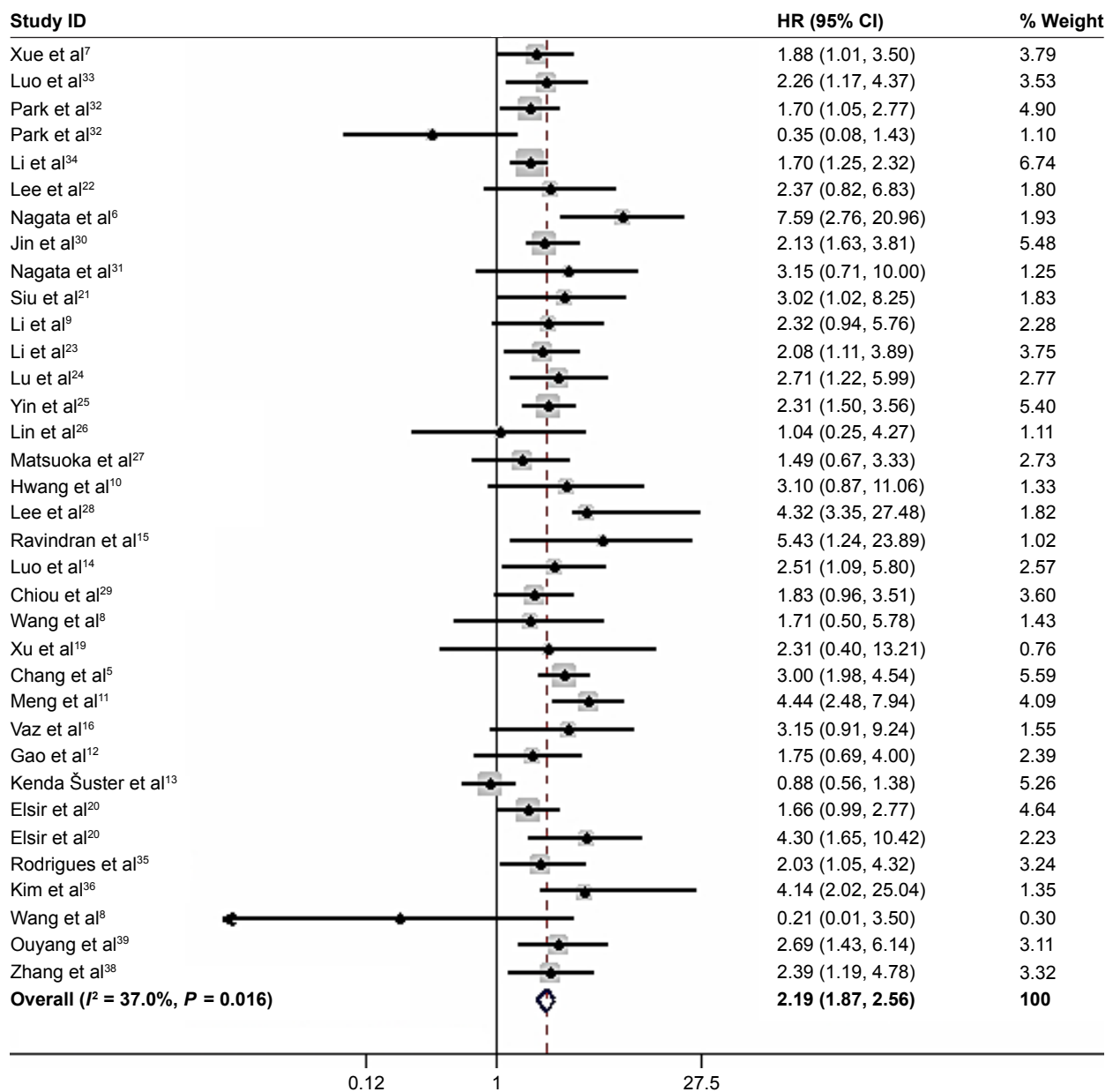
### High NANOG expression: clinical, and pathological characteristics

High NANOG expression was associated with poor tumor differentiation (OR = 2.63; 95% CI: 1.52–4.55,  $P = 0.001$ ), lymph node metastasis (OR = 2.59; 95% CI: 1.50–4.47,  $P = 0.001$ ), more advanced TNM stage (OR = 2.22;

95% CI: 1.42–3.45,  $P < 0.001$ ), and more advanced T stage (OR = 0.44; 95% CI: 0.20–0.93,  $P = 0.031$ ). NANOG expression was not significantly correlated with age, sex, tumor size, lymphatic infiltration, and vascular infiltration. Because of the lack of data, the relationships of NANOG expression and other clinicopathological variables were not determined. The results are shown in Table 3.

### Sensitivity analysis and publication bias

The sensitivity analysis conducted by sequential deletion of each study to assess the credibility of the pooled results found that no individual study influenced the relationship of NANOG expression and survival outcome (Figures 6 and 7).



**Figure 2** Forest plot of the HR for the relationship between high NANOG expression and OS.

**Note:** Weights are from random-effects analysis.

**Abbreviation:** OS, poor overall survival.

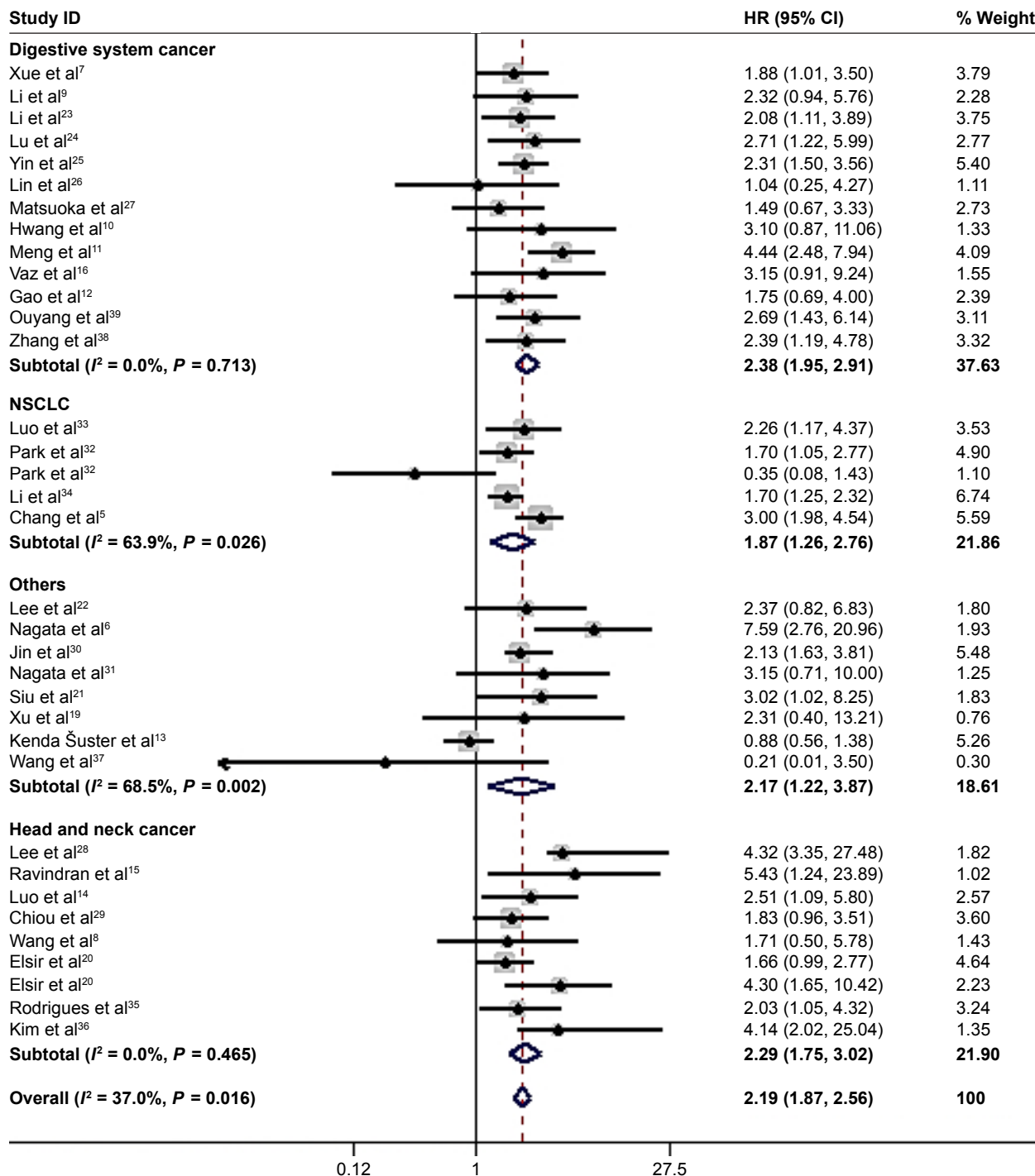
This confirmed the credibility of this meta-analysis. Publication bias was assessed by Begg's funnel plots and Egger's test. The Begg's funnel plots in Figures 8 and 9 show that there was no significant publication bias in the estimates of OS and DFS. The Egger's test  $P$ -values of 0.286 for OS and 0.103 for DFS confirmed the lack of significant publication bias.

## Discussion

Cancer is a public health problem and the second leading cause of death worldwide, with 1,735,350 new cancer cases and 609,640 cancer deaths projected in the United States

in 2018.<sup>40</sup> Improved understanding of cancer mechanisms and prognosis will help to improve patient survival. *Nanog* gene expression decreases with cell differentiation, and is not detectable in terminally differentiated cells.<sup>41</sup> However, NANOG protein is overexpressed in germ cell tumors and in many solid tumor types, where it is expressed in CSCs.<sup>42</sup> The *Nanog* gene is active during the malignant conversion of normal cells; maintains self-renewal of tumor stem cells; regulates the proliferation, migration, and invasion of tumor cells; and promotes tumor immune escape.<sup>1,43,44</sup>

Recent preclinical studies have investigated the effects of targeting NANOG expression in CSCs on treatment



**Figure 3** Subgroup analysis of OS and solid tumor type.  
**Note:** Weights are from random-effects analysis.  
**Abbreviation:** OS, poor overall survival.

resistance, invasiveness, and tumorigenesis. Huang et al found that targeting NANOG significantly inhibited the tumorigenicity of CSCs in head and neck squamous cell carcinoma, and increased cisplatin sensitivity.<sup>45</sup> Tsai et al found that the OCT4 and NANOG expression increased with the development of cisplatin resistance in OSCC tumors.<sup>46</sup> Stable transfection of NANOG into EC-9706 esophageal cancer

cells increases drug resistance by upregulating expression of the multidrug resistance gene *MDR-1*.<sup>47</sup> Interfering with NANOG-mediated transcription by genome editing, small-molecule inhibitors, transcription factor bait, and small interfering RNA may prove effective for targeting CSCs.<sup>48</sup> Rad et al demonstrated that ODN decoys downregulated NANOG expression in P19 embryonal cancer cells.<sup>49</sup> Ding et al

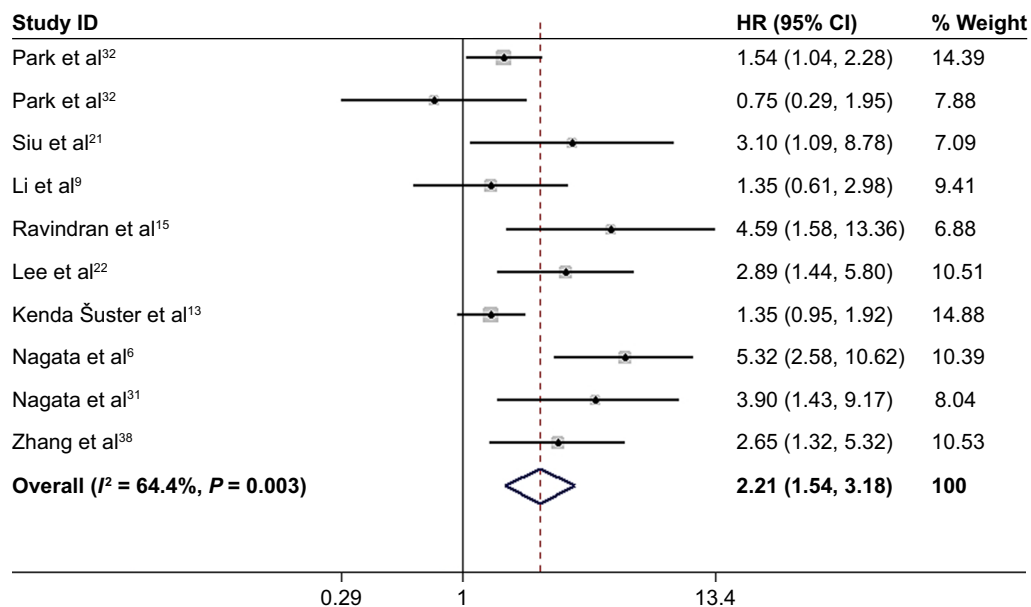
**Table 2** Pooled HRs for overall survival according to subgroup analyses

Categories	Studies (N)	No. of patients	Random-effects model		Heterogeneity	
			HR (95% CI) for OS	P-value	I <sup>2</sup> (%)	P-value
Overall survival	35	3,959	2.19 (1.87–2.58)	<0.001	37	<0.001
Nationality						
Caucasians	26	2,092	1.87 (1.08–3.23)	0.025	68.7	0.012
Asians	8	1,805	2.26 (1.95–2.62)	<0.001	16.8	0.212
Analysis type						
Multivariate	13	1,663	2.16 (1.77–2.62)	<0.001	16.6	0.276
Non-multivariate	22	2,296	2.19 (1.73–2.77)	<0.001	47	0.008
Sample size						
≥ 100	16	2,758	2.09 (1.06–2.74)	<0.001	63.8	<0.001
< 100	19	1,201	2.25 (1.86–2.72)	<0.001	0	0.866

reported that the invasiveness and chemoresistance of HeLa cells decreased when NANOG was destroyed by genomic editing with transcriptional activator like effect nuclease (TALEN),<sup>50</sup> and CRISPR/CAS9 knockout of NANOG or NANOP8 confirmed their involvement in the in vivo tumorigenicity of DU145 prostate cancer cells in an experimental mouse model.<sup>51</sup> Furthermore, inhibition of NANOG was found to enhance the cytotoxicity of BH3 mimetic targeting of Bcl-2 family members in CRC cells.<sup>52</sup>

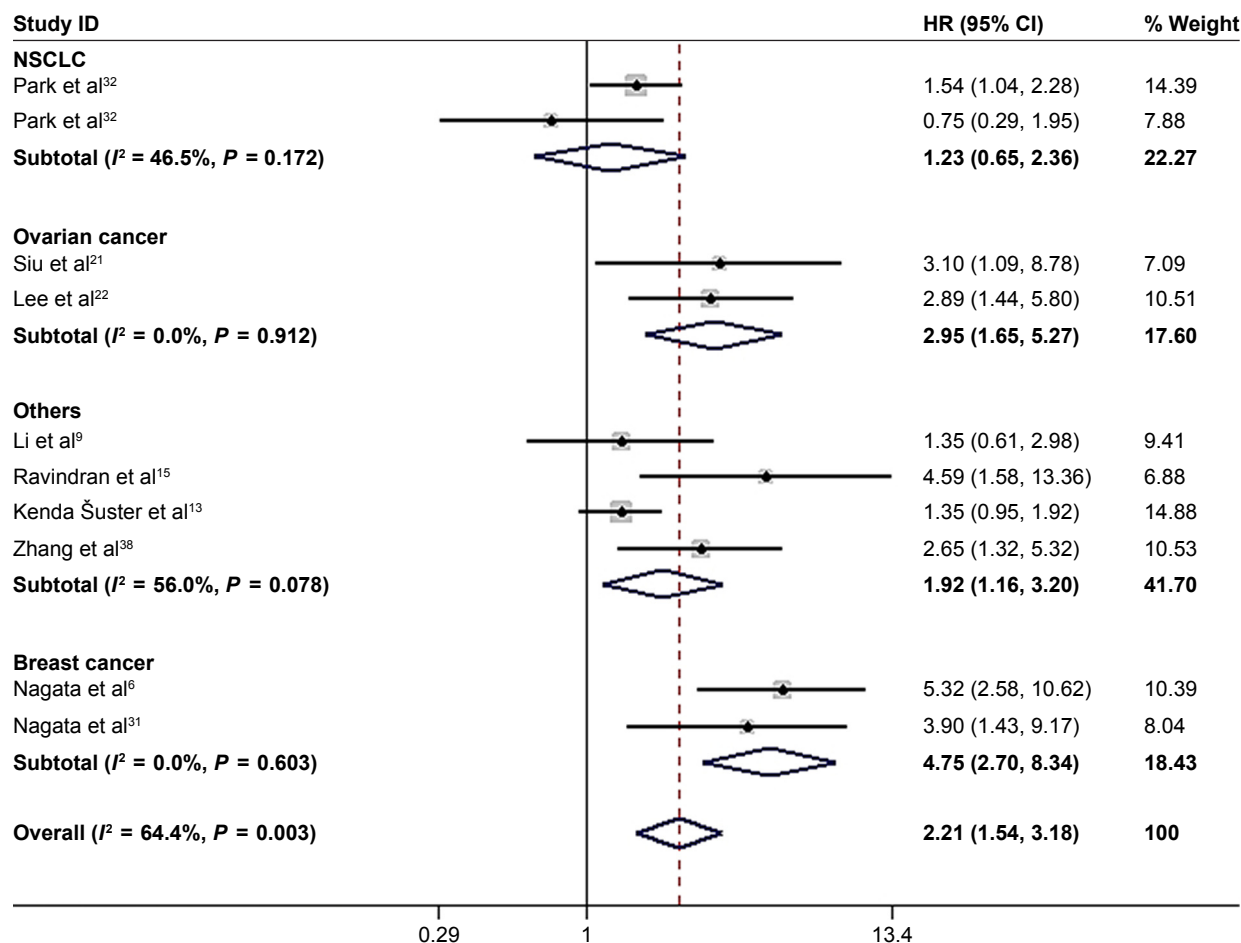
The evidence supports NANOG as a novel indicator of cancer prognosis. Studies of the relationship of clinicopathological variables, NANOG expression, and prognosis are listed in Table 1. The data of individual studies are inconclusive. This meta-analysis was conducted to clarify the prognostic influence of NANOG expression in solid tumors.

To our knowledge, this meta-analysis is the most thorough appraisal of the clinical studies that investigated the prognostic value of NANOG expression in human solid tumors. Thirty-three eligible studies comprising 35 data sets met the selection criteria. The evidence supports NANOG overexpression as an independent, predictive biomarker of poor OS and DFS in solid tumors. Elevated NANOG expression was associated with poor prognosis in most digestive system cancers ( $I^2 = 0\%$ ), NSCLC ( $I^2 = 63.9\%$ ), and head and neck cancer ( $I^2 = 0\%$ ). Moreover, subgroup analysis indicated that high NANOG expression was significantly correlated with OS regardless of sample size, nationality, or type of analysis, which further supported its prognostic value. Elevated NANOG expression was significantly associated with worse DFS in ovarian and breast cancers but not in

**Figure 4** Forest plot of HR for high NANOG expression and DFS.

**Note:** Weights are from random-effects analysis.

**Abbreviation:** DFS, disease-free survival.



**Figure 5** Subgroup analysis of solid tumor type and DFS.  
**Note:** Weights are from random-effects analysis.  
**Abbreviations:** NSCLC, non-small cell lung cancer; DFS, disease-free survival.

NSCLC. Because of the limited number of articles, it cannot be concluded that NANOG expression, when compared with the tumor type, had a greater impact on survival. The sensitivity analysis failed to find the cause of heterogeneity; therefore, the random-effects model was adopted for the combined results. Study heterogeneity may have resulted

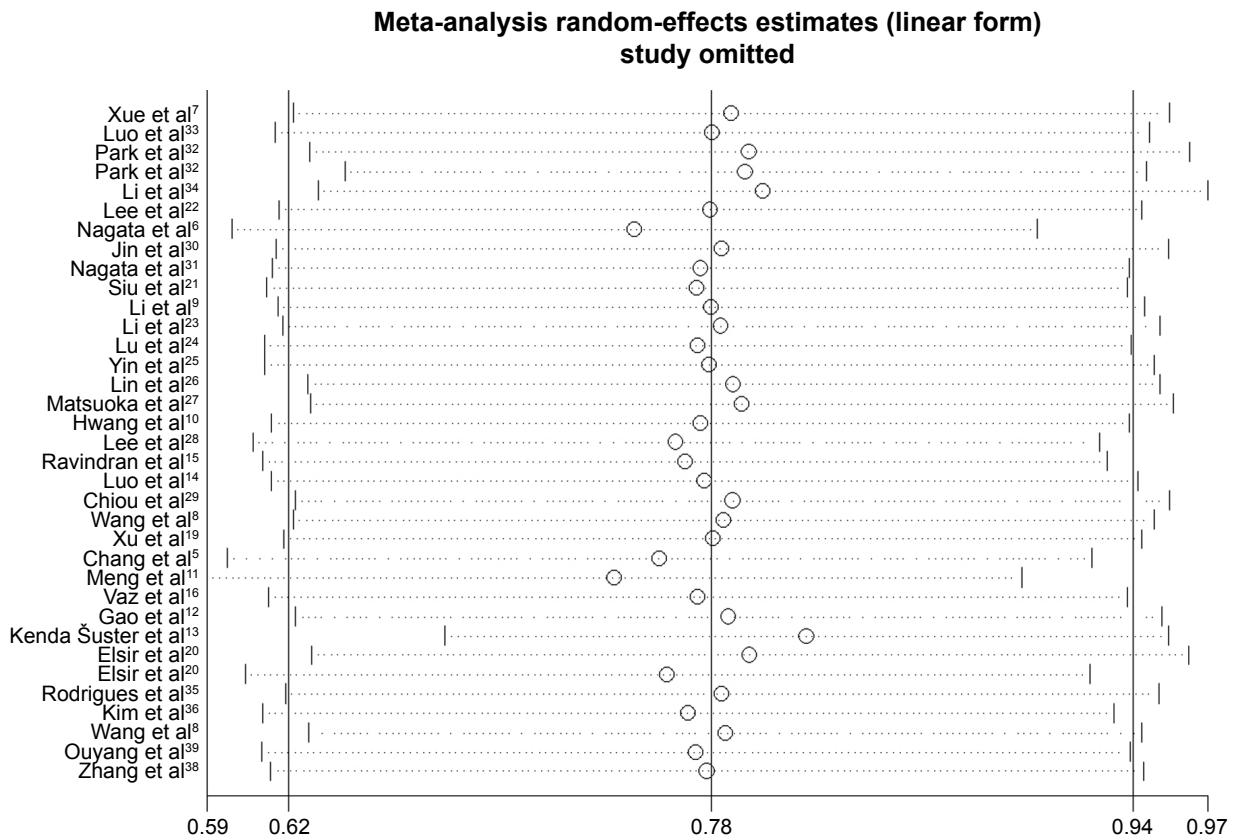
from differences in follow-up intervals, threshold values of high NANOG expression, and the types of solid tumors studied.

In this meta-analysis, NANOG expression was associated with age in eight, sex in 17, tumor size in four, lymphatic infiltration in four, and vascular infiltration in six studies. The

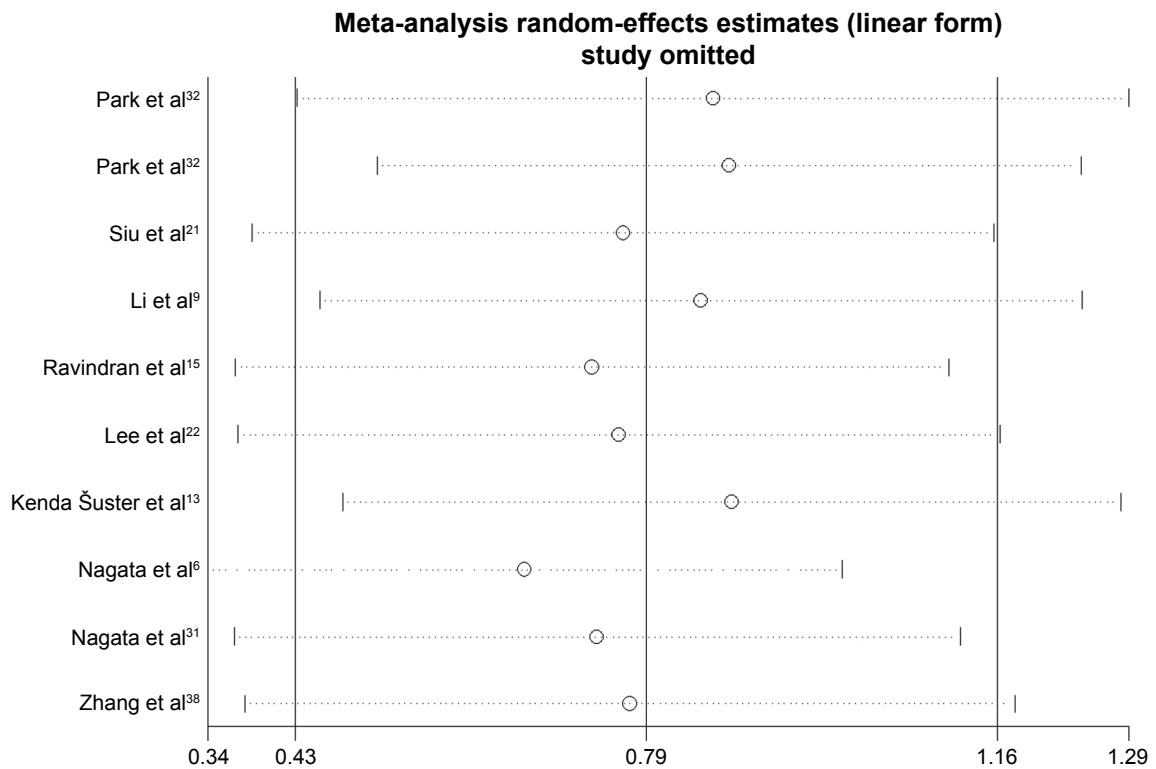
**Table 3** Meta-analytical results of the associations of high NANOG protein expression level with multiple clinicopathological parameters

Categories	Studies (N)	OR (95% CI)	P-value	Heterogeneity		
				I <sup>2</sup> (%)	P-value	Model
Age, in years (>50 vs <50)	8	1.0 (0.79–1.26)	1.00	5	0.39	Fixed effects
Sex (male vs female)	17	1.01 (0.82–1.25)	0.934	0	0.776	Fixed effects
Tumor differentiation (moderate/poor vs good)	18	2.63 (1.52–4.55)	0.001	79.9	<0.001	Random effects
T stage (T1–2 vs T3–4)	7	0.44 (0.20–0.93)	0.031	75.3	<0.001	Random effects
TNM stage (III/IV vs I/II)	16	2.22 (1.42–3.45)	<0.001	55.8	0.004	Random effects
Tumor size (>5 cm vs <5 cm)	4	1.28 (0.53–3.11)	0.13	78.6	0.003	Random effects
Lymph node metastasis (yes vs no)	16	2.59 (1.50–4.47)	0.001	77.6	<0.001	Random effects
Lymphatic infiltration (yes vs no)	4	1.22 (0.44–3.33)	0.703	79.1	0.002	Random effects
Vascular infiltration (yes vs no)	6	0.60 (0.38–1.09)	0.103	48.4	0.084	Fixed effects

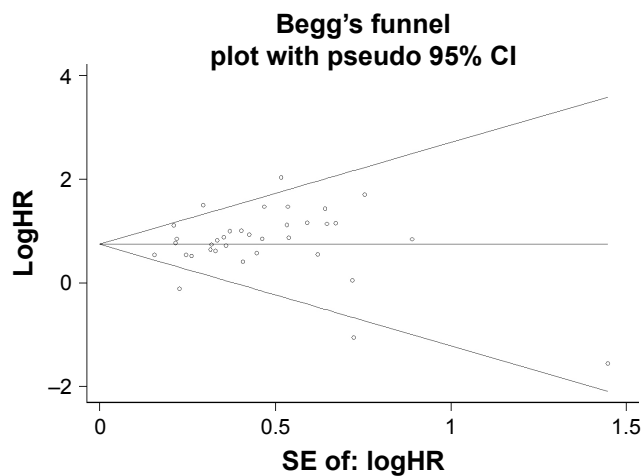




**Figure 6** Sensitivity analysis of OS.  
**Abbreviation:** OS, poor overall survival.



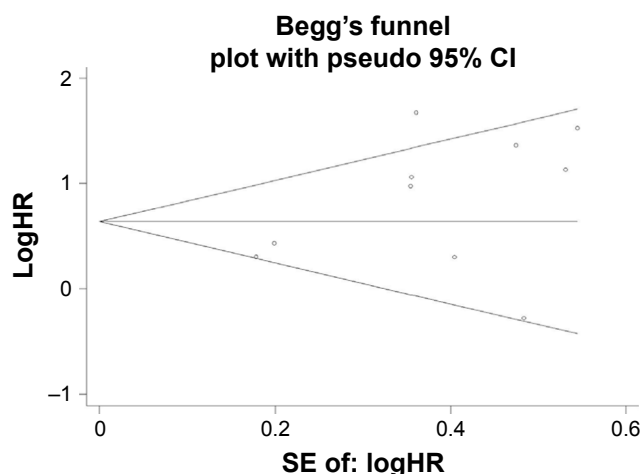
**Figure 7** Sensitive analysis of DFS.  
**Abbreviation:** DFS, disease-free survival.



**Figure 8** Begg's funnel plot of OS and publication bias.  
**Abbreviation:** OS, poor overall survival.

pooled results did not find statistically significant correlations of NANOG expression and those variables. NANOG expression was correlated with TNM stage in 16, tumor differentiation in 18, lymph node metastasis in 16, and T stage in seven studies. The pooled results found that NANOG expression was correlated with tumor differentiation, lymph node metastasis, T stage, and TNM stage.

This meta-analysis was intended to be comprehensive, but it has limitations. First, the threshold value of high NANOG expression was not the same in each study, and may have led to an increase in the heterogeneity. A common cutoff value should be defined. Second, HRs estimated from Kaplan–Meier curves as previously described by Tierney et al might not be as dependable as those extracted directly from the original text of the report, and may have affected the summary analysis. Third, many included studies did not report clinicopathological features, which may lead to bias. Finally,



**Figure 9** Begg's funnel plot of DFS and publication.  
**Abbreviation:** DFS, disease-free survival.

differences in analysis methods, sample sources, follow-up duration, and tumor types might have introduced statistical bias. Additional studies with larger samples and standard testing methods are required to reach a consensus.

## Conclusion

Increased NANOG protein expression in various human solid tumors was significantly correlated with poor OS and DFS. NANOG is a potential biomarker to guide clinical treatment and may have prognostic value in human solid tumors. The results of this meta-analysis warrant performance of additional clinical studies of NANOG in human solid tumors.

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## Disclosure

The authors report no conflicts of interest in this work.

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