

The value of CD9 as a prognostic predictor for various human malignant neoplasms: a meta-analysis

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Abstract

Background

Recent studies indicate that the correlation in the expression level of CD9 and the prognosis differs from tumor types. This meta-analysis was performed to illustrate the predictive value of CD9.

Methods

This analysis included 13 studies involving a total of 1231 patients. We used PubMed, Cochrane Library, Web of Science and Embase for comprehensive literature search and manual search for related bibliography. To probe into the relationships between CD9 expression and patient prognosis, pooled hazard ratios (HRs) with 95% confidence intervals (CIs) were used to describe patient survival and disease recurrence.

Results

This meta-analysis contained 13 eligible studies. High expression of CD9 indicate worse overall survival (OS) (the pooled HR = 1.14, 95%CI: 0.66–1.99), although the result was not statistically significant (P = 0.638). Moreover high expression of CD9 could not result in the worse disease-free survival (DFS) / recurrence-free survival (RFS) of the tumor patients (the pooled HR = 0.67, 95% CI: 0.24–1.87; P = 0.445).

Conclusions

The expression of CD9 could not be used to regard as an independent prognostic predictor.

Background

Tetraspanin is a kind of protein which has four transmembrane domains, a small extracellular loop (termed SEL), a large extracellular loop (known as LEL), and short intracellular N- and C-terminal tails (1). Tetraspanin acts as a cell surface organizer by recruiting particular partner proteins to tetraspanin-enriched micro domains to roll into the meditation of various of cellular activities (2), such as signal transduction, cell growth, cell adhesion, cell motility and differentiation, and sperm-egg fusion (3–5).

CD9, also known as tetraspanin 29, Motility-Related Protein-1 or Multidrug resistance protein-1 (MRP-1), is widely expressed and expressed in a variety of hematopoietic cells and non-hematopoietic cells (6–8). SFQ, a site in the LEL of CD9, is essential for CD9 functions in sperm-egg fusion. CD9 is widely expressed in various normal and cancer tissues (9). Some studies have found that CD9 expression is a common pathogenic switch that directly leads to chronic glomerulonephritis and focal segmental glomerular sclerosis in humans and mice (10). CD9 can also participate in the MDR mechanism by binding to ATP binding cassette (ABC) transporters such as p-glycoprotein (P-gp/MDR1) (11). Through the hydrolysis of ATP, CD9 actively removes substrates from cytoplasm and lead to cell drug resistance (12, 13). CD9 has been found remarkably up regulated in some kinds of tumor tissues. There are various researches indicate that the high level of CD9 expression is associate with poor prognosis (14–17). However, other researchers hold the opposite opinion(18–21). some studies even revealed that there was no significant relationship between CD9 expression and patient's prognosis (22–24). Results of these studies are inconsistent. It is necessary to combine these researches and conduct this analysis to evaluate the ability of CD9 predicting the prognosis of human malignant neoplasms.

The purpose of this study was to assess the overall survival risk of CD9 for survival in cancer patients. The possibility to regard CD9 as a prognosis predictor for clinical treatment and statistics was also discussed.

Methods

search strategy

Original articles to discuss the correlation between the CD9 expression and patient's prognosis were searched in PubMed, Cochrane Library, Web of Science, Embase and other databases. Articles were searched through different combinations of the following keyword: 'CD9', 'MRP-1', 'TSPAN29', 'tetraspanin 29', 'cancer', 'carcinoma', 'tumor', 'incidence', 'death', 'mortality' and 'survival'.

Criteria of inclusion and exclusion

The study meets following criteria: (1) trials were for human cancers; (2) the relationship between CD9 and overall survival has been evaluated; (3) the measurement of the CD9 expression in tumor tissue was done; (4) OS and the hazard ratios (HR) could be directly extracted or indirectly calculated. Articles that met the following criteria were excluded: (1) researches were not for human cancer; (2) reviews, comments, economic analyses, conference abstracts, case reports, animal studies, and laboratory studies; (3) critical information about survival outcomes were missing; (4) HR and 95% CI could not be obtained according to the available data.

Date extraction

According to the needs, we extracted the following data from qualified research: (1) the Author's name, the publication year; (2) the characteristics of the patients, including age, gender, country, ethnicity, tumor type, pathologic type, and sample type; (3) follow-up time; (4) Sample detection method and the expression levels of CD9; (5)HRs of elevated CD9 expression on overall survival (OS), recurrencefree survival (RFS) and diseasefree survival (DFS) with 95% confidence intervals (CIs) and *P* values. If the data were not provided intuitively but only in the form of a Kaplan-Meier curve, the data was extracted from the survival curves and the HRs were estimated using the method described earlier (25).

statistical methods

*I*² statistics was used to measure the heterogeneity of these studies. The evaluation criteria were as follows: *I*² value between 50% and 100% suggested high heterogeneity, 25%-50% suggested low heterogeneity, and 0–25% suggested no heterogeneity. A fixed-effect model would be used in combination with HRs and 95% CI when *I*² < 50% and *P* > 0.10; A random-effect model would be chosen if *I*² > 50% and *P* < 0.10. Then subgroup analysis would be conducting.

The Egger's and Begg's bias indicator test would be performed to evaluate the publication bias. A two-sided *P* value < 0.05 was considered statistically significant. STATA Statistical Software Version 12.0 (Stata Corp, College Station, TX, USA) and Excel software 2019 were used to conduct all calculation.

Results

Study selection

580 studies were obtained from PubMed, Cochrane Library, Embase, Web of science and other databases. There were 469 remaining after removing duplicate researches. By reading the title and abstract, 40 researches were chosen. Due to lack of HR, confidence intervals, survival curves, or not the original data, we finally included 13 eligible studies. Figure 1 showed the flow chart of the study selection process.

Characteristics of the included studies

Table 1 and Table 2 summarized the main characteristics of the 13 eligible studies. The meta-analysis included 13 studies with a total of 1231 patients. The malignant tumors assessed in this meta-analysis included lung adenocarcinoma, non-small cell lung cancer (NSCLC), breast cancer, pancreatic cancer, diffuse non-Hodgkin's lymphoma, acute lymphoblastic leukemia, adult T-cell leukemia, head and neck squamous cell carcinoma, oral squamous cell carcinoma, gastric cancer and malignant pleural mesothelioma. 8 studies used Immunohistochemistry (IHC) staining to measure CD9 expression. Real time polymerase chain reaction (RTPCR) was used in 4 studies, slot blot analysis and flow cytometry were used to in the left 2 studies respectively. The pathologic types involved in this meta-analysis included squamous cell carcinoma (SCC), adenocarcinoma (Ad), invasive ductal carcinoma, Non-Hodgkin's lymphoma (NHL), stromal tumor and Mesothelioma. These studies were all retrospective in design.

Table 1
Main characteristics of studies included in the meta-analysis

First author, Publication year	Dominant case nationality	Dominant ethnicity	Malignant Disease	Main type of pathology	Detected sample	Survival analysis	Source of HR	Maximum months of follow-up
Masahiko,1995	Japan	Asian	NSCLC	Ad/SCC/La	Tissue	OS	Reported	55
Masayuki,1996	Japan	Asian	Breast cancer	Invasive ductal carcinoma	Tissue	OS/DFS	Reported	61
Masahiko,1997	Japan	Asian	Lung cancer	Adenocarcinoma	Tissue	OS/DFS	SC	83
Masayuki,1998	Japan	Asian	Pancreatic cancer	Adenocarcinoma	Tissue	OS	Reported	62
Huang,1998	Japan	Asian	Breast cancer	Invasive ductal carcinoma	Tissue	OS/DFS	Reported	NM
Jonathan,1998	Canada	Caucasian	Non-Hodgkin's lymphoma	NHL	Tissue	OS	SC	NM
Nobuhito,2001	Japan	Asian	Adult T-cell Leukemia	NHL	Cells	OS	Reported	NM
Boban,2003	Austria	Caucasian	HNSCC	SCC	Tissue	OS/DFS	Reported	NM
Ge,2009	China	Asian	Gastric cancer	Adenocarcinoma	Tissue	OS	Reported	NM
Serdar,2010	Turkey	Caucasian	Gastric cancer	Adenocarcinoma	Tissue	OS/DFS	SC	NM
Vishwa,2013	Japan	Asian	Malignant pleural mesothelioma	Mesothelioma	Tissue	OS	Reported	79
Liang,2017	China	Asian	ALL	NHL	Cells	OS	Reported	NM
Marcilei,2020	Brazil	Caucasian	OSCC	SCC	Tissue	OS/DFS	SC	NM

OS, overall survival; DFS, disease-free survival; NSCLC, non-small cell lung cancer; HNSCC, head and neck squamous cell carcinoma; ALL, acute lymphoblastic leukemia; OSCC, oral squamous cell carcinoma; Ad, adenocarcinoma; SCC, squamous cell carcinoma; LA, large cell carcinoma; NHL, non-Hodgkin's lymphoma; SC, survival curve; NM, not mentioned.

Table 2
HRs and 95% CIs for patient survival or disease progression in association with CD9 expression in enrolled studies

First author, publication year	Main assay method	Case number		Total	OS		DFS/RFS		PFS/MFS	
		High Expression	low expression		HR (95%CI)	P Value	HR (95%CI)	P Value	HR (95%CI)	P Value
Masahiko, 1995	RT-PCR	67	42	109	0.302(0.155–0.587)	0.0004	NM	NM	NM	NM
Masayuki, 1996	IHC	97	46	143	0.245(0.086–0.696)	0.0084	0.228(0.109–0.479)	0.0010	NM	NM
Masahiko, 1997	IHC	88	44	132	0.220(0.050–0.940)	0.0690	0.330(0.120–0.920)	0.0290	NM	NM
Masayuki, 1998	RT-PCR	18	22	40	3.440(1.290–9.120)	0.0100	NM	NM	NM	NM
Huang, 1998	RT-PCR	73	36	109	3.092(0.732–13.052)	0.1245	3.332(1.579–7.032)	0.0016	NM	NM
Jonathan, 1998	IHC	14	37	51	1.240(0.250–6.220)	0.256	NM	NM	NM	NM
Nobuhito, 2001	Slot Blot	8	26	48	2.72(1.139–6.471)	0.0240	NM	NM	NM	NM
Boban, 2003	IHC	13	21	34	0.450(0.200–1.000)	0.0490	0.200(0.060–0.600)	0.0057	NM	NM
Ge, 2009	IHC	78	35	113	2.550(1.780–3.640)	0.0100	NM	NM	NM	NM
Serdar, 2010	IHC	11	38	49	4.780(0.690–33.150)	0.0017	5.220(1.540–17.690)	0.0120	NM	NM
Vishwa, 2013	IHC	76	36	112	1.990(1.080–3.820)	0.0261	NM	NM	NM	NM
Liang, 2017	Flow Cytometry	68	44	112	2.165(1.171–4.002)	0.0140	NM	NM	NM	NM
Marcilei, 2020	IHC	104	75	179	0.590(0.360–0.960)	0.0710	0.390(0.240–0.650)	0.0180	NM	NM

OS, overall survival; DFS, disease-free survival; PFS, progression-free survival; PFS, progression-free survival; MFS, metastasis-free survival; HR, hazard ratio; CI, confidence interval; IHC, immunohistochemistry; NM, not mentioned.

OS associated with the expression of CD9

Due to the high remarkably heterogeneity ($P = 0.000$, $I^2 = 84.7\%$), the OS analysis of the 13 studies used a random-effects model (Fig. 2A). The pooled HR is 1.14 (95%CI: 0.66–1.99), which indicated that high level of CD9 may suggest worse OS, but the effect was not statistically significant ($P = 0.638$).

Also because of high heterogeneity, subgroup analysis was conducted by assorting studies into subgroups of tumor location, pathological type, case nationality, test method, simple type, major race and data source. When conducted by different tumor location, significant relationship were observed between the high expression of CD9 in hematological tumor and worse OS (random-effects model: HR = 2.21; 95% CI: 1.37–3.56; $P = 0.001$), the digestive system tumor showed similar results (random-effects model: HR = 2.69; 95% CI: 1.93–3.74; $P = 0.000$). However, the other type tumor (HNSCC and OSCC) displayed opposite results (random-effects model: HR = 0.55; 95% CI: 0.36–0.83; $P = 0.005$), which indicate high level of CD9 may be a favorable factor. The outcome of reproductive system tumor (random-effects model: HR = 0.55; 95% CI: 0.12–2.39; $P = 0.954$) and respiratory system tumor (random-effects model: HR = 0.55; 95% CI: 0.36–0.83; $P = 0.422$) indicated no statistically significant effect (Fig. 3A). When it came to pathological type, a worse OS was found in the Non-Hodgkin's lymphoma (random-effects model: HR = 2.21; 95% CI: 1.37–3.56; $P = 0.001$), and a better OS was found in the squamous cell cancer (random-effects model: HR = 0.55; 95% CI: 0.36–0.83; $P = 0.005$) (Fig. 3B). When subgroup analysis was conducted according to case nationality, China seemed to be linked up with tragic OS (random-effects model: HR = 2.45; 95% CI: 1.80–3.33; $P = 0.000$), Austria (random-effects model: HR = 0.20; 95% CI: 0.06–0.63; $P = 0.006$) and Brazil (random-effects model: HR = 0.59; 95% CI: 0.39–0.96; $P = 0.035$) played a protective factor here (Fig. 3C). When came to test method, the more commonly used immunohistochemistry (IHC) and rt-PCR did not show significant effect, but

other method (Slot Blot and Flow Cytometry) did (random-effects model: HR = 2.34; 95% CI: 1.41–3.86; P = 0.001) (Fig. 3D). In subtotal analyses of sample types, cells could predict worse OS (random-effects model: HR = 2.16; 95% CI: 1.17–4.00; P = 0.014), tissues did not show significant correlation (random-effects model: HR = 1.07; 95% CI: 0.58–1.97; P = 0.818) (Fig. 3E). There was no significant effect in subgroup analysis of ethnicities and data source (Fig. 3F and Fig. 3G).

The progression and recurrence of disease associated with the expression of CD9

To better evaluate tumor progression and recurrence, the concepts of Disease-free survival (DFS) and Recurrence-free survival (RFS) were introduced. A total of 6 studies concentrated on DFS/RFS. Due to the high heterogeneity ($P = 0.000$, $I^2 = 89.3\%$), random-effect model was used to do the analysis (Fig. 2B). However, there were no significant effect of this analysis (random-effects model: HR = 0.67; 95% CI: 0.24–1.87; P = 0.445), which implied the high level of CD9 could not result in worse DFS/RFS of the tumor patient. Furthermore, because of the absence of the data of PFS/MFS in these studies, analysis of PFS/MFS could not be conducted.

Publication bias

Begg's funnel plot and Egger's test were used to detect the publication bias (Fig. 4). The Begg's funnel plot did not show conspicuous asymmetry, and the Egger's test shown the similar result ($P = 0.535$). Because the amount of studies of DFS/RFS was less than 10, only Egger's test was performed. Similarly, no publication bias was found ($P = 0.651$).

Sensitivity analyses

As shown in the Fig. 5A and 5B, no alterations in the results was found in the OS and DFS/RFS studies due to the inclusion of any individual study, which mean that no single study significantly impact the pooled HR or the 95% CI.

Discussion

As mentioned above, CD9 is a member of tetraspanin superfamily proteins. It seems that research on CD9 has never stopped, and results have not been consistent. Some studies reported that CD9 could inhibit cancer cells metastasis and invasion via modulating the function of integrins, it could be regarded as a suppressors of metastasis in solid tumors (25–27). Some researchers hold the different view, they think that CD9 inhibits cancer progression by maintaining normal cell adhesion and motility in malignant mesothelioma (28). However, some people hold completely opposite views on the function of CD9. They think CD9 is not a favorable factor to the prognosis of the cancer patients. CD9 could bind to ATP-binding cassette and lead to chemo resistance in gastric cancer (17). CD9 could also cooperate with heparin-binding epidermal growth factor-like growth factor (HB-EGF) and promote the progress of human gastric cancer. Up to now, more and more functions of CD9 have been discovered, but its physiologic functions and related regulatory mechanisms are still not particularly clear.

The relationship between the expression of CD9 and the OS of the various malignant cancer patients is the key point of this meta-analysis. The pooled HR of OS was 1.14, which means the high level of CD9 indicates a worse prognosis. But it was not statistically significant ($P = 0.664$). The pooled HR of DFS/RFS was 0.67, the high expression of CD9 turned out to be a protective factor, although it was not statistically significant too. This phenomenon maybe caused by the different function of CD9. Recent studies have reported that CD9 expression was negatively correlated with the high prevalence of lymph node metastasis. CD9 was also involved in cell growth, adhesion and movement, and the change of CD9 expression may be related to tumor invasion and metastasis (29). CD9 could also act as a metastasis suppressor by neutralizing Aggrus-mediated platelet aggregation as Nakazawa Y reported (30). What's more, the result of DFS/RFS analysis could not be persuasive enough because of the limited number of included researches.

Because of the high heterogeneity, we had to conduct subgroup analysis. The results suggested that high level of CD9 indicate poor prognosis in hematological tumor and digestive system tumor. On the other hand, CD9 could be favorable factor of the squamous cell cancer. As mentioned above, this phenomenon could be caused by different function of CD9. When it came to nationality, China was a risk factor for tumor prognosis. However, no significant effect was observed in ethnicities. This difference can be attributed to environmental differences.

We must admit there are still some restrictions that cannot be ignored in this meta-analysis. At first, there is no standard for the high level of CD9 expression. In some studies, more than 10% are considered positive (16), while in other studies the standard is 30% (31) or 50% (18), and because the method of determination is different, there must be some error between the research results and the real results. Secondly, the number of studies included in the analysis is still too small, especially for the DFS/RFS analysis, and may reduce the statistical power of the result. Thirdly, we had to calculate or extracted data from the survival curves because of the absence of some critical survival

information, which would bring some minor differences to the conclusion. At last, these studies did not mention the survival data of MFS/PFS, so we could not conduct research about this aspect. These factors should be taken into account in drawing a conclusion.

Conclusion

To sum up, our meta-analysis result suggested that CD9 could not be regarded as prognostic predictor for multiple human malignant neoplasms. However, in hematopoietic tumors (especially NHL) and digestive system tumors, high CD9 expression can be considered as a risk factor for poor prognosis for OS and DFS/RFS, while for squamous cell carcinoma, high CD9 expression is a protective factor that heralds higher OS and DFS/RFS. As for cancer patients in China, a high level of CD9 is also a risk factor that requires sufficient attention.

Abbreviations

HR: the pooled hazard ratios; CIs: confidence intervals; OS: overall survival; DFS: disease-free survival; RFS: recurrence-free survival; PFS: progression-free survival; MFS: metastasis-free survival; SEL: small extracellular loop; LEL: large extracellular loop; MRP-1: multidrug resistance protein-1; ABC: ATP binding cassette; P-gp: p-glycoprotein; NSCLC: non-small cell lung cancer; HNSCC: head and neck squamous cell carcinoma; OSCC: oral squamous cell carcinoma; SCC: squamous cell carcinoma; IHC: immunohistochemistry; RT-PCR: real time polymerase chain reaction; Ad: adenocarcinoma; NHL: Non-Hodgkin's lymphoma; HB-EGF: heparin-binding epidermal growth factor-like growth factor.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this manuscript.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Study design: LP and LBJ. Acquisition, analysis and interpretation of data: YT, ZSB, DHY, LC and LJC. Statistical analysis: ZSB, YT, SSJ and LJC. Writing manuscript: YT, ZSB and DHY. Revision of manuscript: LP, LBJ and LJ. All authors have read and approved the final version of manuscript.

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Figures

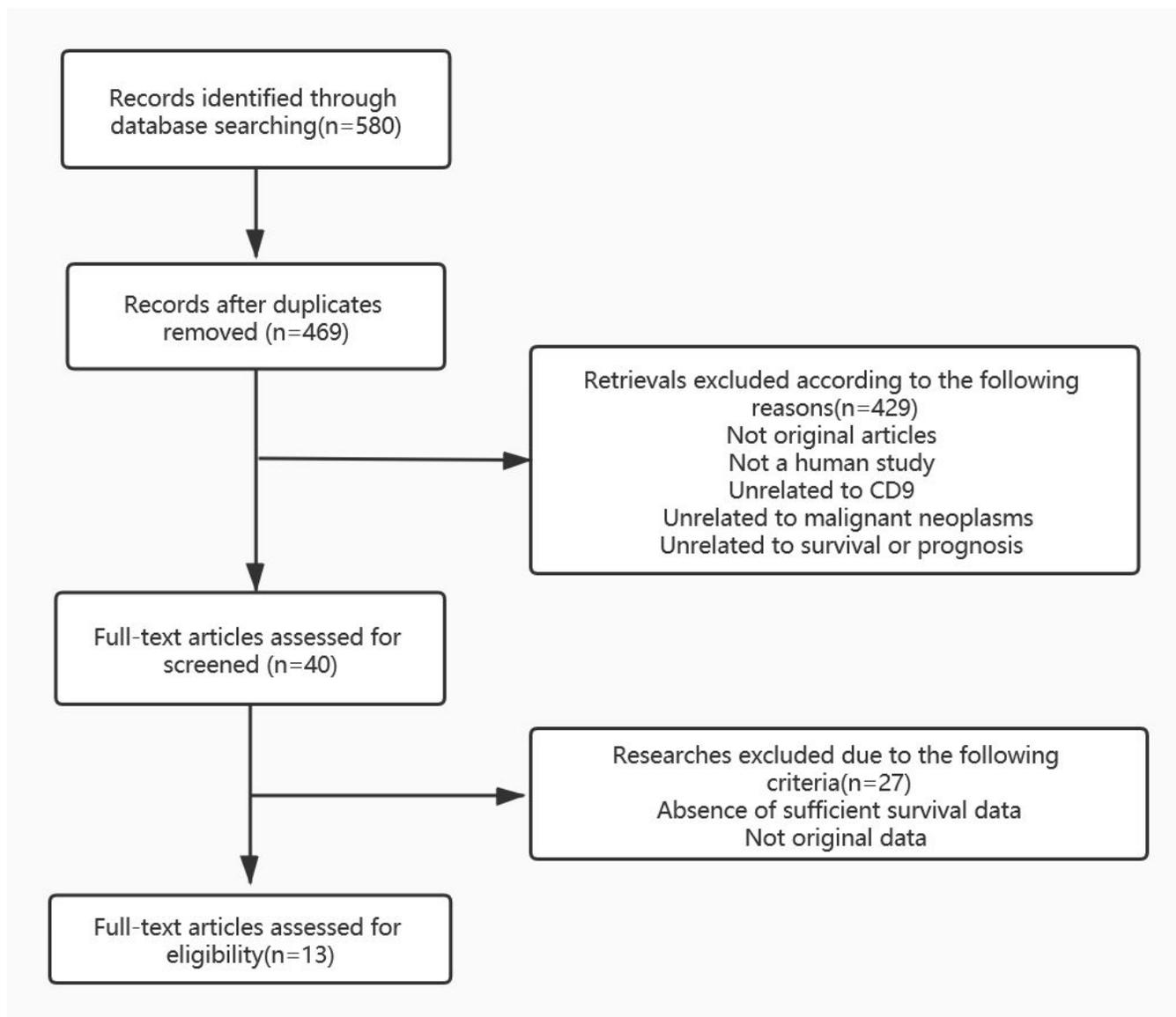
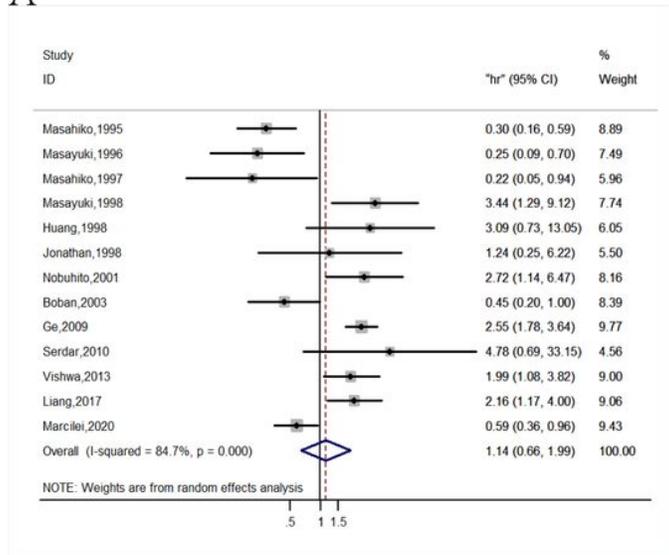


Figure 1

Selection process of studies for meta-analysis.

A



B

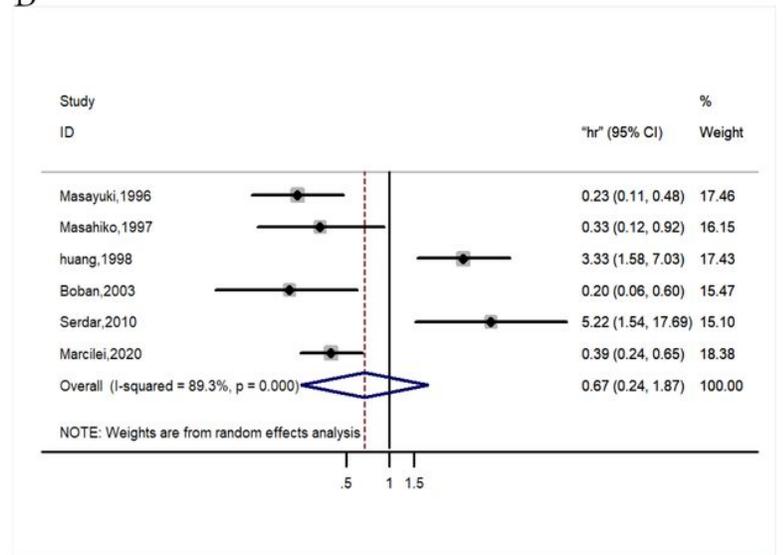


Figure 2

Forest plots of merged analyses for survival associated with CD9 expression. Notes: (A) Forest plot to assess the OS analysis; (B) Forest plots for the DFS/RFS analysis.

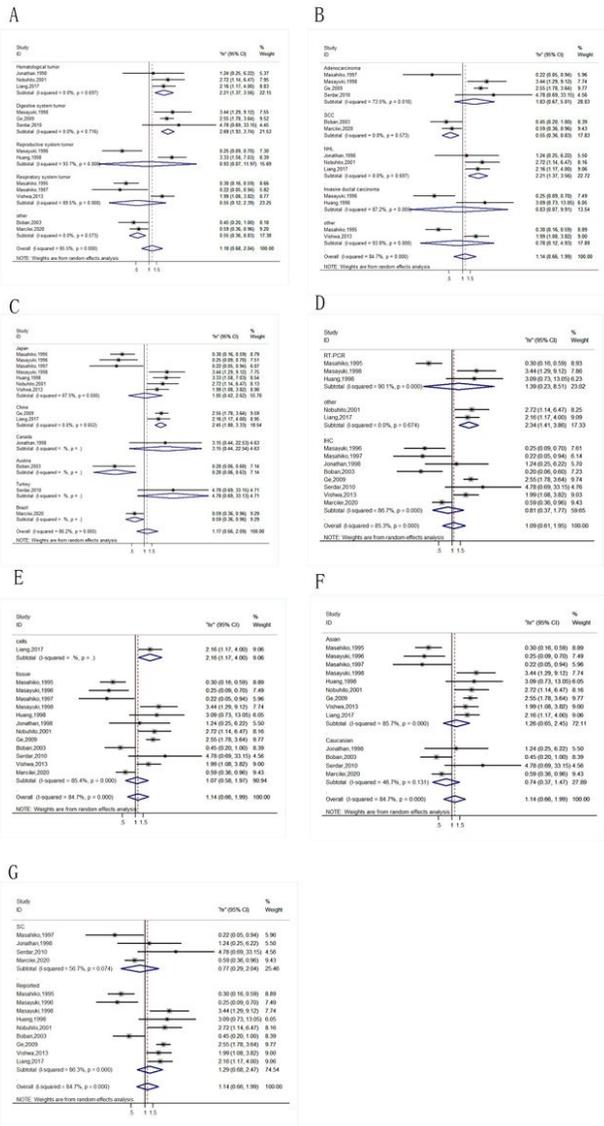


Figure 3

Forest plots of subgroup analyses for OS associated with CD9 expression. Notes: (A) Forest plots for the subgroup analysis in tumor location; (B) Forest plots for the subgroup analysis in different pathological type; (C) Forest plots for the subgroup analysis in different case nationalities; (D) Forest plots for the subgroup analysis in different test methods; (E) Forest plots for the subgroup analysis in different sample types; (F) Forest plots for the subgroup analysis in different ethnicities; (G) Forest plots for the subgroup analysis in different data sources.

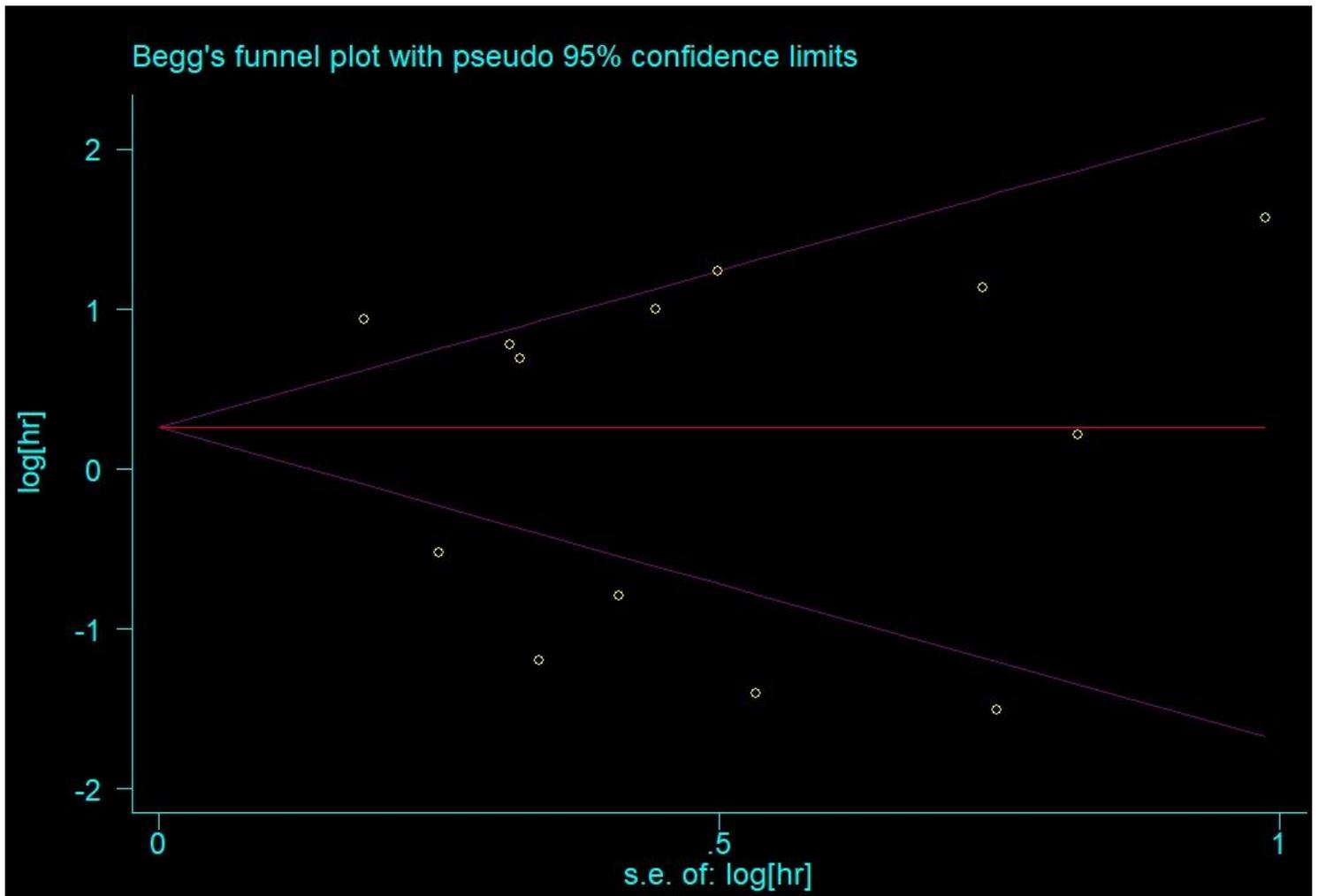


Figure 4

Begg's funnel plots of the publication bias. Notes: Begg's funnel plots of the publication bias for overall merged analysis of OS. Each point represents a separate study.

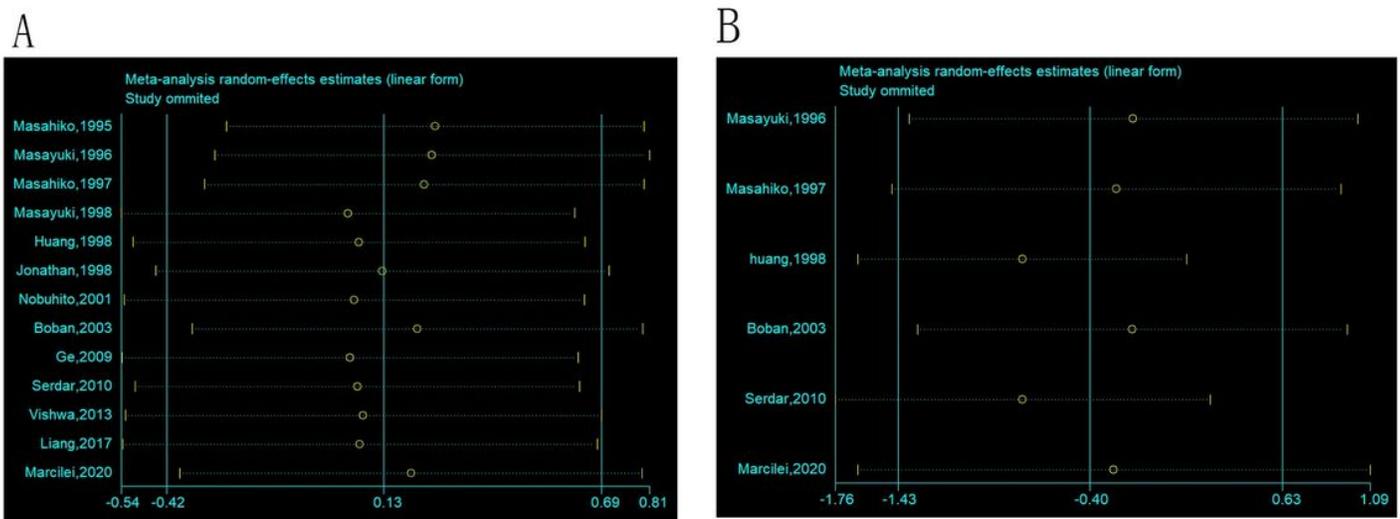


Figure 5

Sensitivity analysis of each included study. Notes: (A) Sensitivity analysis of OS for individual studies. (B) Sensitivity analysis of DFS/RFS for individual studies.

Supplementary Files

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- [renamedc5719.doc](#)