

The Prognostic Roles and Clinical Features of CD44v9 in Human Solid Cancers—A Meta-Analysis

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Abstract

Background: CD44 is the primary receptor for hyaluronic acid and serves as a marker for cancer stem cells. CD44v9 is one of CD44's variants and takes part in cancer's growth and metastasis. However, the prognostic roles and clinical features of CD44v9 in cancers remain unclear. Therefore, we conducted this meta-analysis to summarize the prognostic significance and clinical features of CD44v9 in human solid cancers.

Methods: we systematically searched all of related studies in PubMed, the Web of Science, Embase and Cochrane library up to June 2020. We analyzed the pooled hazard ratios (HRs) and odds ratios (ORs) with corresponding 95% confidence intervals (CIs) to assess the prognostic functions and clinical features of CD44v9 in various human solid cancers.

Results: In this meta-analysis, we included 1705 cancer patients among 12 studies. Results indicated that high expression of CD44v9 was significantly related to poorer overall survival (OS) (HR=1.60, 95%CI 1.28-1.99, P<0.0001), recurrence-free survival/progression-free survival/disease-free survival (RFS/PFS/DFS). (HR=1.81, 95%CI 1.16-2.84, P=0.009) and disease-specific survival/cancer-specific survival (DSS/CSS) (HR=2.93, 95%CI 1.69-5.10, P<0.001). At the same time, we also found that high expression of CD44v9 increased the possibility of lymphoid infiltrates (OR=1.59, 95%CI 1.16-2.20, P=0.005), vascular invasion (OR=1.57, 95%CI 1.11-2.22, P=0.010) and higher TNM stage (OR=1.63, 95%CI 1.19-2.23, P=0.002).

Conclusion: Our results demonstrate that CD44v9 overexpression is associated with worse OS, RFS/PFS/CFS and DSS/CSS in patients with solid cancers, which might be a biomarker in the diagnosis and prognosis of cancers in the future.

1. Background

Cancer has long been one of the most difficult problems in global health. Statistically, there were 18.1 million people diagnosed cancer and caused 9.6 million deaths worldwide in 2018[1]. The median cancer-related cost of state-level is estimated at \$3.7 billion in America[2]. So, it is urgent to strengthen the early diagnosis and treatment of cancer.

Recently, more and more evidences indicate that there is heterogeneity in cancer cells [3, 4]. That is, the cells in the same cancer tissue are not completely the same, most of the cells do not have the ability to differentiate, and only a small number of cells have the self-renewal ability similar to the stem cells in normal tissues, such as embryos and bone marrow, and can differentiate into mature cancer cells. This group of cells is regarded as cancer stem cells (CSCs)[5]. CSCs can produce specific markers in different cancers, such as the CD34 + HLA-DR- surface marker phenotype in leukemic stem cells, CD44 + ESA + surface marker phenotype in breast stem cells and so on[6]. Therefore, these surface markers may provide better evidences for the diagnosis and treatment of cancers.

CD44 is a member of the single-stranded transmembrane glycoprotein family and is overexpressed in some CSCs [7, 8]. As an adhesion molecule, CD44 binding HA is involved in tumor proliferation, adhesion, migration, and invasion [9]. CD44 contains both standard (CD44s) and variant (CD44v) types. The CD44 standard form is produced by exons 1–5 and 16–20, and the CD44v1-10 by exons 6–15[10]. Many studies have shown that CD44 is closely related to the occurrence and development of cancers [11–14]. CD44v9 is one of CD44 variants. Although it has been reported that overexpression of CD44v9 is associated with poor cancer survival and may be a therapeutic target for cancers [15–18], it's still controversial [19]. Therefore, we conducted this systemic meta-analysis in order to reveal the relationship between CD44v9 and human solid cancers.

2. Methods

2.1 Search strategy:

We searched primary literatures by keyword plus free words from electronic databases such as PubMed, Web of science, Embase and Cochrane library until June 30, 2020. The specific PubMed search strategy as following: ("Neoplasms" OR "Neoplasia" OR "Neoplasias" OR "Neoplasm" OR "Tumors" OR "Tumor" OR "Cancer" OR "Cancers" OR "Malignancy" OR "Malignancies" OR "malignant neoplasms" OR "malignant neoplasm" OR "neoplasm malignant" OR "neoplasms malignant" OR "benign neoplasms" OR "neoplasms benign" OR "benign neoplasm" OR "neoplasm benign" OR) and ("CD44v9 antigen" OR "CD44(v9)" OR "hyaluronan receptors" OR "CD44v9" OR "CD44 variant 9"). All the searched literatures and related references were evaluated for inclusion in our meta-analysis. If studies continue on the same batch samples, we included the largest one.

2.2 Inclusion and exclusion criteria.

The included studies need to conform to the following criteria: 1) Study needs to assess prognosis about the expression of CD44v9 in cancers. So, overall survival (OS), progression-free survival (PFS), recurrence-free survival (RFS), disease-free survival (DFS), cancer specific survival (CSS) or disease specific survival (DSS) should be reported. 2) Related hazard ratios (HRs) with 95% confidence intervals (CIs) or Kaplan–Meier survival curves should be included. 3) The included studies analyze the relationship between the positive and negative expression of CD44v9 and disease characteristics.

The following studies should be excluded: 1) Sample size less than 50. 2) Reviews, case reports, letters, editorials, meeting abstracts and animal trial. 3) No related survival analysis. 4) Not published in English. 5) Not available the full text.

2.3 Data extraction and quality assessment:

Literature screening, data extraction and analysis were performed by two researchers (Yuanxiu Deng and Jie Wang) independently. Any disagreement would be solved by other two researchers (Shenhui Ji and Lu Huang). The following information was extracted from each study: first author, published year, original country, included period, cancer type, average age, sample size, follow-up time, assay method, cut-off

value, clinical features, NOS scale, survival outcome and HR with corresponding 95% CI. The software Getdate Graph Ditizer 2.26 was used to extract relevant data to calculate HR with corresponding 95% CI for survival analysis presented by Kaplan-Meier curve. If the article used univariate and multivariate analyses to report the HR value, we choose the univariate analysis. We used STATA15.1 software to calculate ORs with corresponding 95% CI from the extracted data of positive and negative expression number of CD44v9 and disease characteristics. We used the Newcastle-Ottawa scale (NOS) to evaluate the quality of eligible studies. The NOS score ranges from 0 to 9, and any score no less than 6 can be regarded as high quality.

2.4 Statistical Analysis Methods

We pooled the HRs with 95% CI to assess the prognostic role of overexpressed CD44v9 in human solid cancers. The ORs with 95% CI was combined to evaluate clinicopathological characteristics. We used the Higgins I^2 statistics and Chi-squared test to evaluate heterogeneity among the included literatures. When the P value is more than 0.10 and the I^2 value is less than 50%, it is thought to be no inter-study heterogeneity in the eligible articles. Then a fixed-effects model was applied for pooling the data. Otherwise, the random-effects model would be used. If there were heterogeneity, subgroup analysis was used to explore the sources of heterogeneity. We used Begg funnel plot test to quantify the existence of publication bias. Obvious publication bias should be considered when P value is less than 0.1. Sensitivity analysis was used to evaluate the stability of the results from the included articles. All data analysis was analyzed using STATA15.1 software. When P-value is less than 0.05, the results are regarded as statistically significant.

3. Results

3.1: Literature screening and characteristic:

3.1.1 Literature screening

We preliminarily searched related 418 literatures about CD44v9 and human solid cancers through electronic databases up to June 30, 2020. After excluding 100 duplicate references, 318 studies remained. Then, by reading the literature titles and abstracts, 280 unqualified articles were eliminated. We then evaluated the remaining 38 articles by reading the full text. We excluded articles that did not have prognostic analysis and clinical characteristics analysis. In the end, 12 articles with composite requirements were included in this meta-analysis. The specific screening flow chart is shown in Fig 1.

3.1.2 Characteristics of the included literatures

In the 12 articles, there were 1705 subjects with eight kinds of cancers included. Eight articles were published in Japan, the remaining published in Korea, China, Netherland and Germany, respectively. Among these articles, three are for gastric cancer[20-22], two for breast cancers[19, 23], one for

esophageal squamous cell carcinoma[24], one for upper tract urothelial carcinoma [25], one for Hepatocellular carcinoma [26], one for pancreatic carcinoma[27], two for bladder cancer[28, 29], one for Colorectal Cancer[30]. The sample sizes range from 62 to 333. All the included literatures performed survival analysis among positive and negative expression of CD44v9 in patients with cancers, using OS, PFS, RFS, DFS DSS, CSS. Since there were few references that contain RFS, PFS, DFS, DSS and CSS, we combined RFS, PFS and DFS together, DSS and CSS together for analysis. All the included literatures showed high quality because NOS score was no less than 6. The main information of the twelve articles is presented in Table 1.

Table 1

Main characteristics of the eligible studies

Author	Year	Country	Included period	Disease	Age	Sample size (high/low)	Follow-up	Assay method	Cut-off value	NOS score	Endpoints	HR 95%CI
Taniguchi, D	2018	Japan	2002-2012	ESCC	NA	133(59/74)	NA	IHM	≥3+	6	OS/RFS	R/R
Yamakawa Y	2017	Japan	2011-2012	GC	NA	103(42/61)	61m	IHM	≥4+	7	RFS	R
Kodama, H	2017	Japan	2007-2009	GC	NA	123(47/76)	68m	IHM	≥75%	6	DSS	R
Hagiwara, M	2016	Japan	1990-2007	UTUC	69	110(82/28)	57m	IHM	>10	8	RFS/CSS	SC/SC
Kakehashi, A	2016	Japan	2006-2011	HCC	72	90(64/26)	96m	IHM	≥3	8	OS/RFS	R/SC
Go, S. I	2015	Korea	1999-2007	GC	64.5	333(164/169)	77.7m	IHM	>0	7	OS	SC
Li.J	2014	China	2008-2019	Pca	NA	101(51/50)	12m	qPCR	M	8	OS	SC
Kobayashi, K	2016	Japan	2001-2012	NMIBC	71	62(24/38)	50m	IHM	>170	8	PFS/CSS	SC/SC
Kato, S	2015	Japan	2003-2011	CTC	NA	150(60/90)	NA	RT-PCR	NA	6	OS	R
Foekens, J. A	1999	Netherland	1980-1991	BC	56	210(101/109)	93m	IHM	≥3	7	RFS	SC
Friedrichs.K	1995	Germany	1981-1990	BC	52.8	227(165/62)	56.9m	IHM	NA	8	OS/DFS	SC/SC
Ogihara, K	2019	Japan	1997-2007	MIBC	69	63(36/27)	58m	IHM	≥5%	8	RFS/CSS	R/R

ESCC: esophageal squamous cell carcinoma; GC: gastric cancer; UTUC: upper tract urothelial cancer; HCC: Hepatocellular cancer; Pca: pancreatic carcinoma; NMIBC: non-muscle invasive bladder cancer; CTC: colorectal cancer cell; BC: breast cancer. MIBC: muscle invasive bladder cancer. NA: not available; IHM: Immunohistochemistry; M: median cut-off point; OS: overall survival; RFS: recurrence-free survival; PFS: progression-free survival; CFS: cancer-free survival; DSS: disease-specific survival; CSS: cancer-specific survival.

3.2 Meta-analysis

3.2.1 The correlation between CD44v9 expression and prognosis of cancers

Totally, there were 6 studies with 1034 patients included information related to OS. By pooled HRs analysis, we found that overexpression of CD44v9 tended to have poor OS (HR=1.60, 95%CI=1.28-1.99, P<0.001 Fig2-A). Because of slightly heterogeneity ($I^2=47.6\%$, $P=0.089$), we used fixed-effects model to pool the data. And eight studies with 998 patients provided RFS/PFS/DFS ($n=6$, $n=1$, $n=1$). A random-effect model was used to calculate the pooled HRs and 95% CIs because of the significant heterogeneity ($I^2 = 79.3\%$, $P<0.0001$). The combined results indicated that highly expressed CD44v9 had shorter RFS/PFS/DFS (HR=1.81, 95%CI 1.16-2.84, $P=0.009$. Fig2-B) in patients with solid cancers. Four studies

with 358 patients included DSS/CSS analysis (n=1, n=3). The pooled HR values and 95% CIs revealed that high expression of CD44v9 tended to be significantly associated with worse DSS/CSS (HR=2.93, 95%CI 1.69-5.10, P<0.001. Fig2-C) by using fixed-effects model because of little heterogeneity ($I^2 = 24.9%$, P=0.262).

We used subgroup analysis to explore the source of heterogeneity for OS, RFS/PFS/DFS and DSS/CSS respectively. We performed this analysis from NOS score, sample size and HR value acquisition method. NOS scores no less than 8 were assigned to the high group, while the rest were assigned to the low group. We defined small sample events below 110, large sample events no less than 110. As shown in Fig3.1 A-C, for OS, when HR value extraction was used for subgroup analysis, indirect extraction results showed no statistical significance (HR=1.28, 95%CI 0.97-1.68, P=0.0080. Fig3.1B). As for RFS/PFS/DFS (Fig3.2 A-C), when subgroup analysis was performed, the results were not statistically significant except for small sample events (HR=3.73, 95%CI 1.87-7.42, P=0.0001. Fig3.2.C). It can be inferred from the above results that there were not enough large sample events. Three subgroup analyses showed a negative correlation between the high expression of CD44v9 and the DSS/CSS (Fig3.3A-C).

3.2.2 The correlation between CD44v9 expression and clinicopathological traits of cancers

We summarized the clinical and pathological parameters from sex, age, differentiation, tumor size, lymphoid invasion, vascular invasion and TNM stage in table2. Age and tumor size were defined according to the original literatures. The pooled results between CD44v9 overexpression and the clinicopathological features were summarized in table2. The high expression of CD44v9 was not associated with age, sex, tumor size and tumor differentiation (P \geq 0.05). However, the high expression of CD44v9 was positively associated with lymphatic infiltration (OR=1.59, 95%CI 1.16-2.20, P=0.005), vascular infiltration (OR=1.57, 95%CI 1.11-2.22, P=0.010) and high TNM stage (OR=1.63, 95%CI 1.19-2.23, P=0.002) (Fig 4A-C).

Table 2

Meta-analysis of CD44v9 and clinicopathological features in cancer patients

Categories	Trials	OR (95%CI)	P-value	I ²	Ph
Sex(M/F)	9	0.93(0.72,1.21)	0.599	0.0%	0.979
Age (old/young)	7	0.78(0.49,1.24)	0.297	50.1%	0.061
Differentiation (bad/good)	4	0.78(0.35,1.70)	0.527	75.6%	0.006
Tumor size (big/small)	8	1.13(0.77,1.65)	0.544	50.2%	0.05
LI (yes/no)	7	1.59(1.16,2.20)	0.005	0.0%	0.548
VI (yes/no)	6	1.57(1.11,2.22)	0.010	0.0%	0.864
TNM stage (III IV / I II)	6	1.63(1.19,2.23)	0.002	34.6%	0.177

VI: Vascular invasion; LI: Lymphoid invasion; P-value: for statistical significance based on Z test; Ph: P value for heterogeneity based on Q test.

3.3 Publication bias and sensitivity analysis among eligible studies

We used Begg’s test for possible publication bias in quantitative analysis for survival analysis. As shown in Fig 5A-C, no publication bias exists ($P \geq 0.1$). Furthermore, sensitivity analysis was used to verify the stability of the meta-analysis for CD44v9 in the OS, RFS/PFS/DFS and DSS/CSS (Fig 6A-C). The results showed that each of the included articles had no effect on the overall stability, that is, the meta-analysis results were stable.

4. Discussion

Within cancer cells, CD44v9 interacts with the subunit cystine/glutamate antiporter (xCT) to promote intracellular synthesis of the antioxidant glutathione (GSH)[31]. This response can enhance the defense against reactive oxygen species (ROS) and promote tumor growth [32, 33]. Some anti-tumor drugs targeting this mechanism may be more effective in controlling tumor progression [29, 31, 34].

Many scholars have studied the relationship between CD44v9 and human cancer prognosis. For example, Suwannakul’s study reveals that CD44v9 is a novel marker of human cholangiocarcinoma stem cells and is closely associated with the development of inflammation-related cancers [15]. Their subsequent researches confirm that CD44v9 overexpression induces a stem-like phenotype that promotes proliferation, migration, invasion, and redox balance of cancer cells in cholangiocarcinoma [35]. However, the effect of CD44v9 in human solid cancers remains unclear. Takaki’s study shows that CD44v9 is highly expressed in tumors with EGFR mutations, especially in early-stage lung adenocarcinoma, but not significantly associated with advanced lung adenocarcinoma [36]. Hirata reports that among patients with early gastric cancer, the recurrence rate in those with CD44v9 overexpression is significantly higher than those with low expression [37]. But a meta-analysis suggests that CD44v9 overexpression is closely related to 5-year CSS and might be a therapeutic target for advanced tumors[10]. Sato observes that

patients with low expression of CD44v9 have a poorer five-year survival rate than those with high expression and have a higher risk of lymphatic metastasis among primary squamous cell carcinoma of the tongue. This may be related to the adhesion of CD44v9. When the expression of CD44v9 decreased, the adhesion weakened, which promoted the metastasis of tumor cells through lymphatics[38].

In this meta-analysis, our results reveal that overexpression of CD44v9 is closely related to the poor OS, RFS/PFS/DFS and DSS/CSS. However, when NOS score, HR extraction method and sample size were used for subgroup analysis, there was no statistical significance between CD44v9 and RFS/PFS/DFS. The main reason is that in these studies, HR values were analyzed by single factor analysis or extracted from the survival curve, ignoring the influence of confounding factors. And the fact that only a few of the literature contain this information is another major factor. Furthermore, according to statistical data, high expression of CD44v9 was positively correlated with lymphatic infiltration, vascular infiltration and high TNM stage, with no or little heterogeneity. There was no publication bias and the results were stable.

There still exist several limitations in this meta-analysis. Firstly, when OS, RFS/PFS/DFS, and DSS/CSS were analyzed respectively, only a few articles provided survival analysis. More studies are needed to provide relevant data to make the results more convincing in the future. Secondly, the CD44v9 immunohistochemistry cut-off value was defined differently. Thus, more specific studies are needed to define CD44v9 positive expression criteria. Thirdly, most of the studies were from Japan. And extensive research is needed to see whether the results are applicable to other regions. Finally, some HR values were obtained indirectly from Kaplan-Meier survival curves, with unavoidable statistical bias.

5. Conclusion

To our knowledge, this is the first meta-analysis to systematically analyze the clinical characteristics and prognostic roles of CD44v9 in human solid cancers. Our results suggest that elevated expression of CD44v9 may be a significant predictor of poor prognosis in these patients. CD44v9 might be a targeted molecular marker for the diagnosis and treatment of cancers in the future.

Abbreviations

CD44v9: CD44 variant 9; CIs: confidence intervals; CSCs: cancer stem cells; CSS: cancer-specific survival; DFS: disease-free survival; DSS: disease-specific survival; GSH: glutathione; HRs: hazard ratios; LI: lymphatic infiltration; NOS: Newcastle-Ottawa Scale; ORs: odds ratios; OS: overall survival; PFS: progression-free survival; RFS: recurrence-free survival; ROS: reactive oxygen species, VI: vascular infiltration; xCT: cystine/glutamate antiporter

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data are included in this article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YD and JW collected and analyzed the data, YD wrote the paper; SJ and LH performed quality assessment and analyzed the data. MF conceived and designed this study, revised the manuscript. All authors reviewed the paper. All authors read and approved the final manuscript.

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Figures

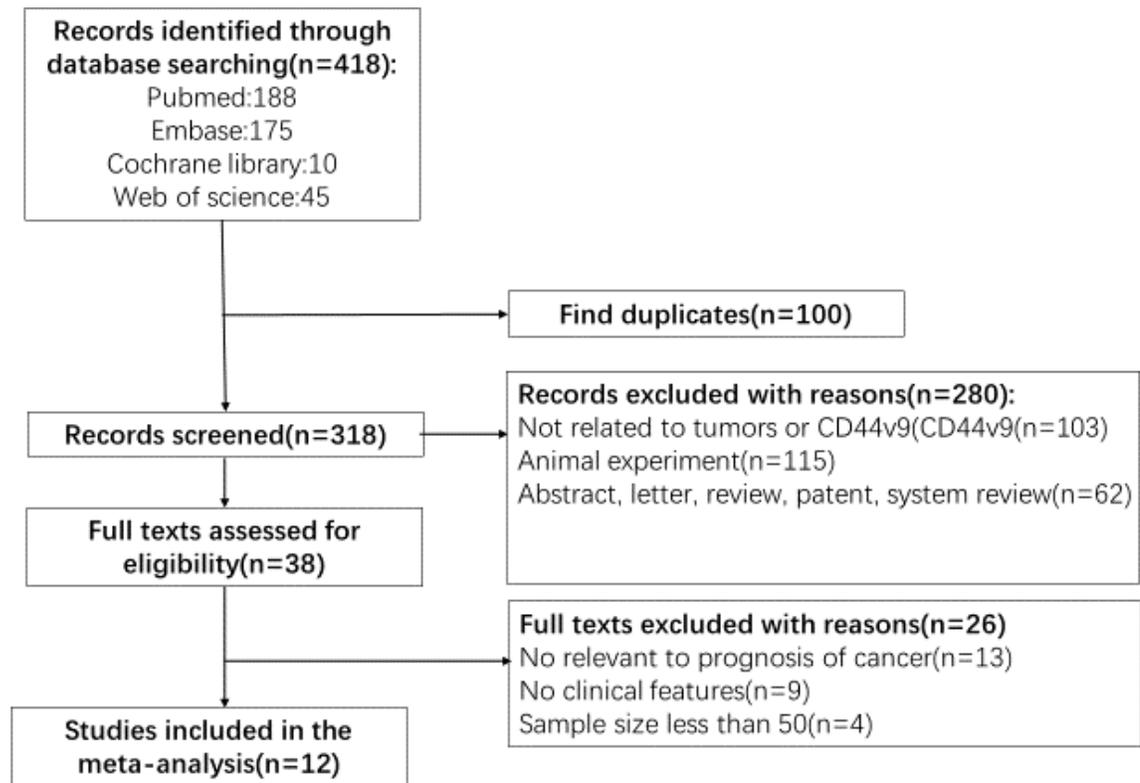
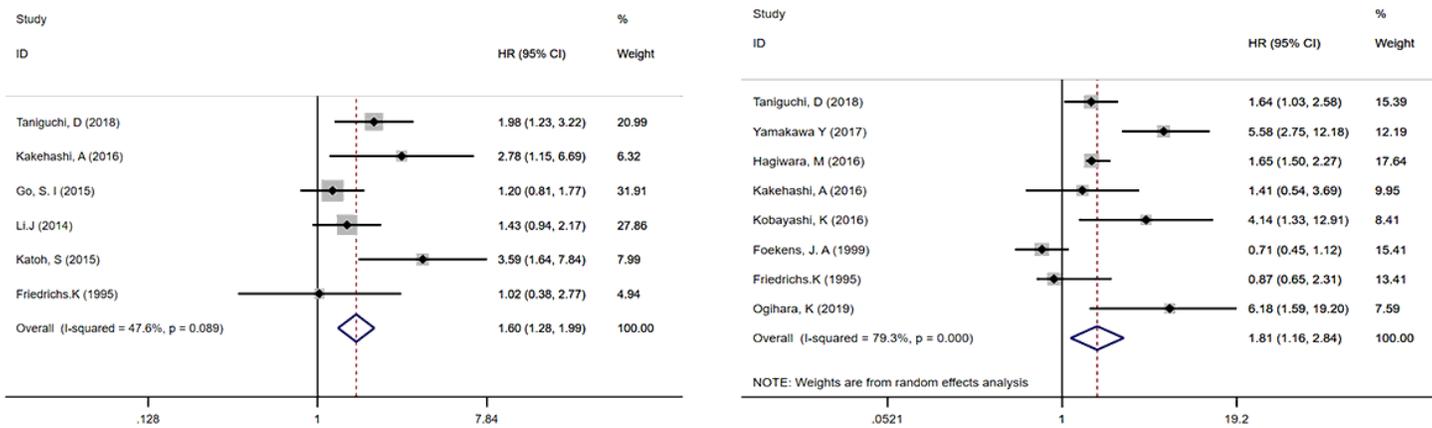


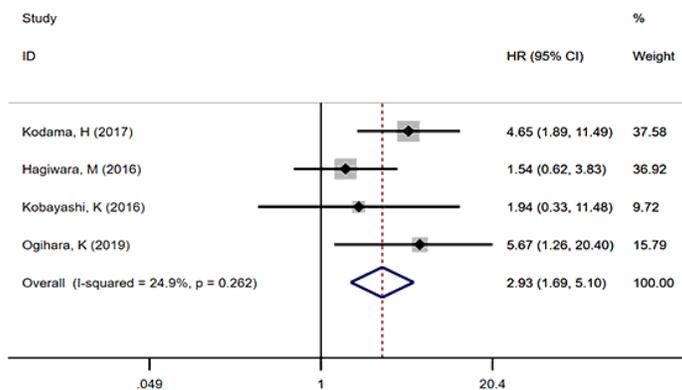
Figure 1

Flow diagram of the study selection process.



A

B



C

Figure 2

Forest plot for the association between the CD44v9 expression and the prognostic analysis: A): Forest plots for the relationship between the high expression of CD44v9 and OS; B): Forest plots for the relationship between the high expression of CD44v9 and RFS/PFS/DFS; C) Forest plots for the relationship between the high expression of CD44v9 and DSS/CSS.

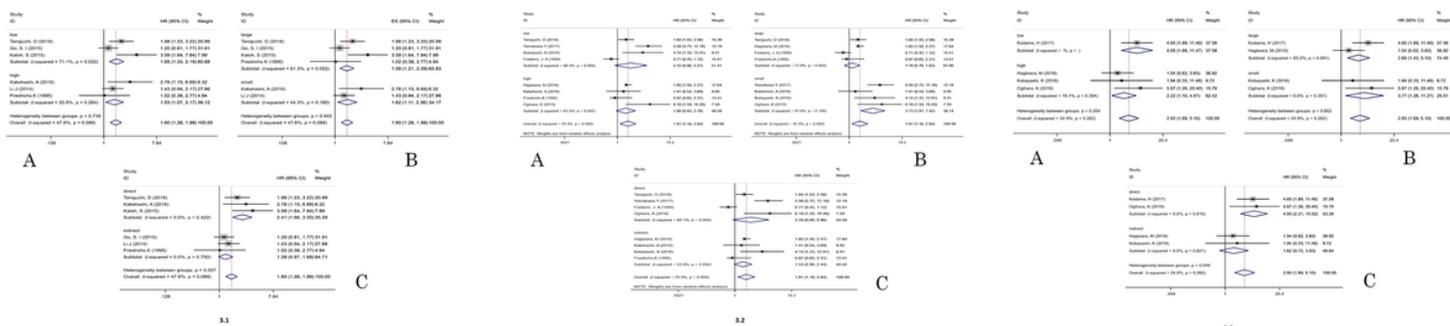


Figure 3

3.1: Forest plot for the subgroup analysis between the CD44v9 expression and OS (A-C): A): NOS score, B): sample size C): HR value acquisition method. 3.2: Forest plot for the subgroup analysis between the

CD44v9 expression and RFS/PFS/DFS (A-C): A): NOS score, B): sample size C): HR value acquisition method. Forest plot for the subgroup analysis between the CD44v9 expression and DSS/CSS (A-C): A): NOS score, B): sample size C): HR value acquisition method.

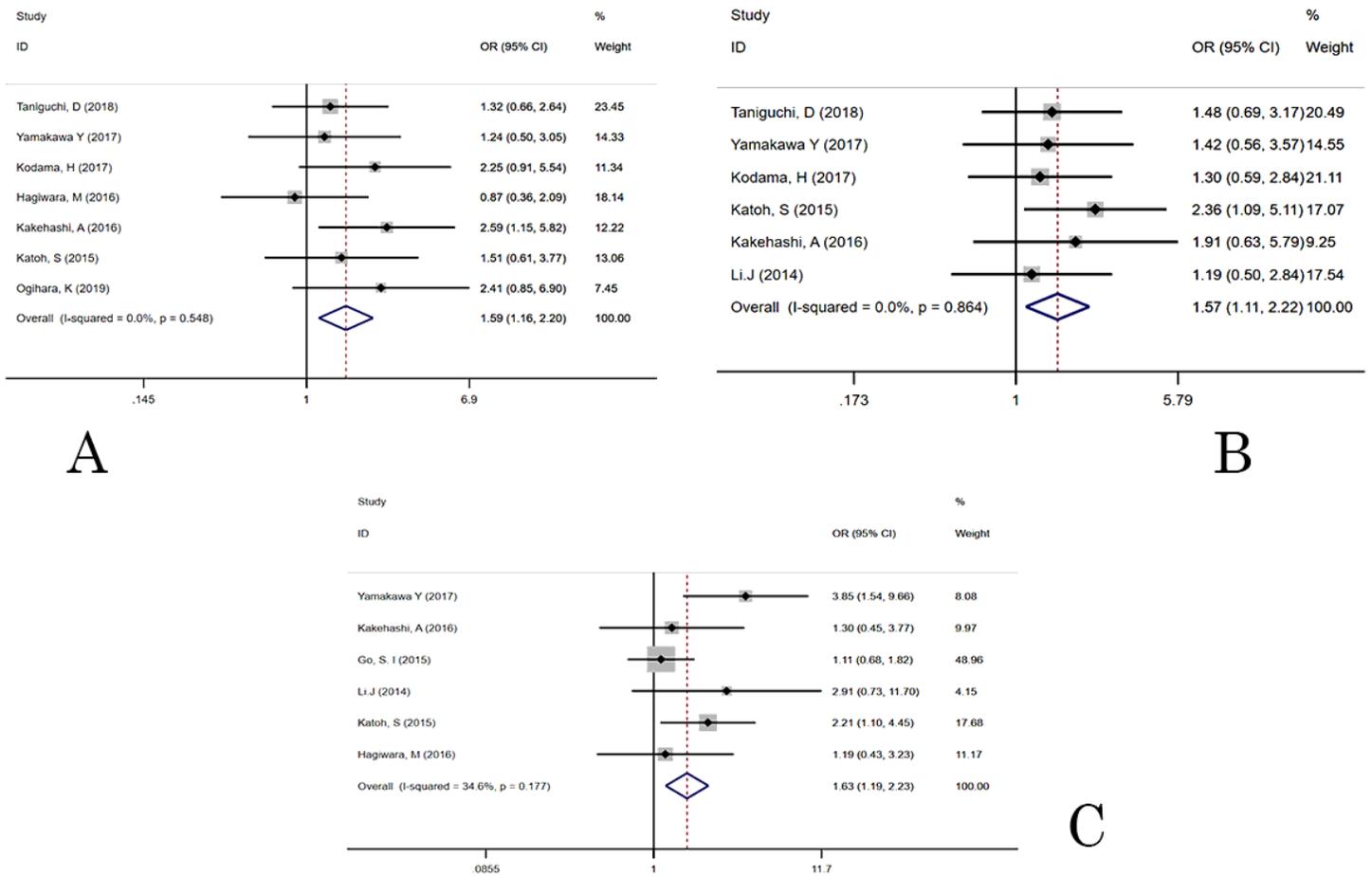
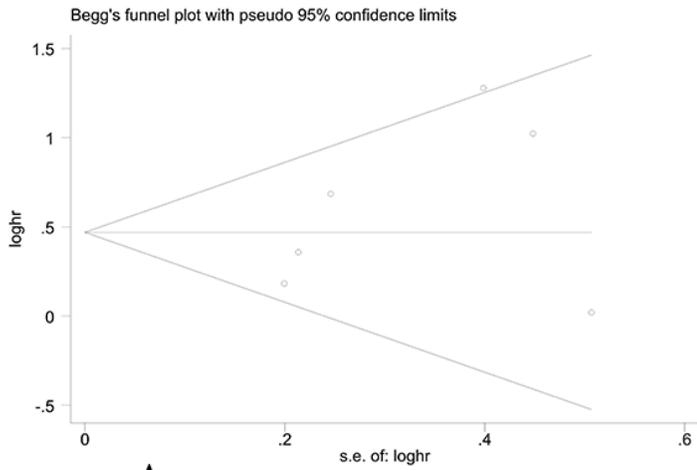
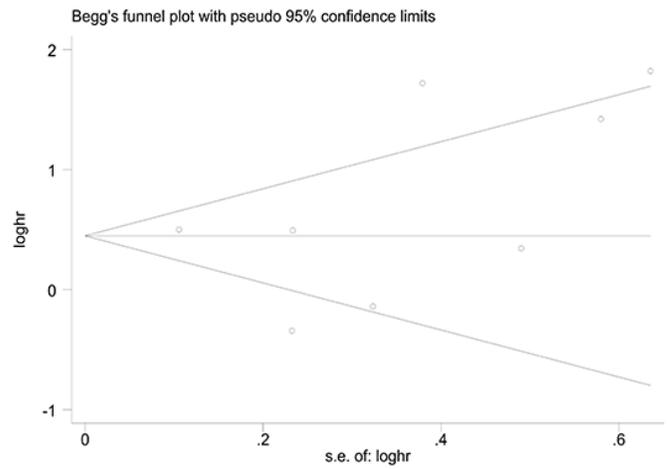


Figure 4

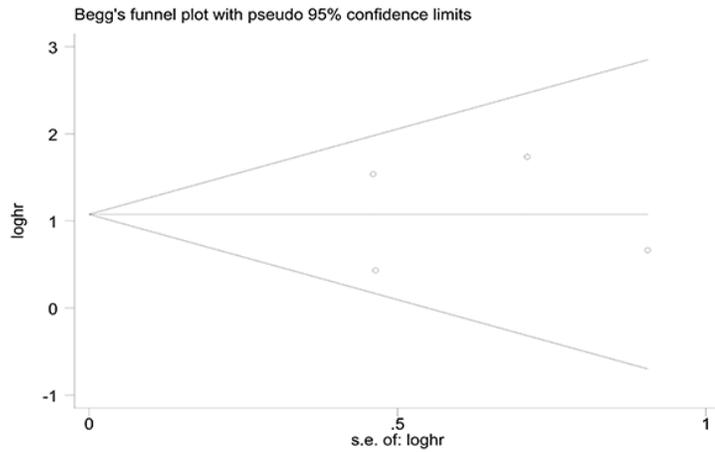
Forest plot for the association between the CD44v9 expression and the clinicopathological parameters (A-C): A): lymphatic infiltration, B): vascular infiltration, C): TNM stage.



A



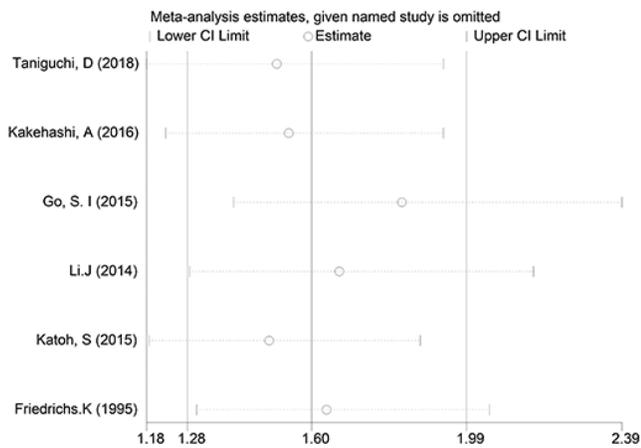
B



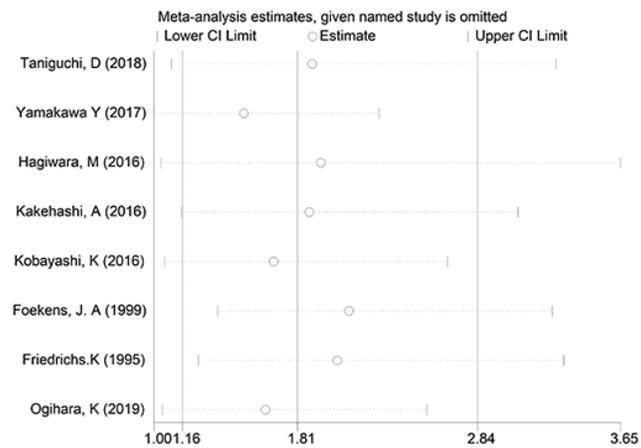
C

Figure 5

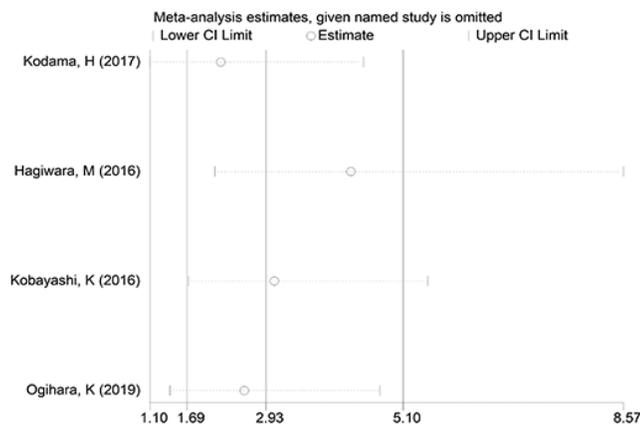
Begg's funnel plots for assessment of potential publication bias (A-C): A): Funnel plot of the CD44v9 expression and OS. B) Funnel plot of the the CD44v9 expression and RFS/PFS/DFS; C): Funnel plot of the CD44v9 expression and DSS/CSS.



A



B



C

Figure 6

Sensitivity analysis for the stability of the pooled HRs and its 95% CI (A-C) between the CD44v9 expression and the survival estimation (A-C): A): Sensitivity analysis of OS. B): Sensitivity analysis of RFS/PFS/DFS. C): Sensitivity analysis of DSS/CSS.

Supplementary Files

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- [PRISMA2009FlowDiagramMSWord.docx](#)
- [PRISMA2009ChecklistMSWord.docx](#)