Int J Clin Exp Med 2016;9(11):20674-20683
www.ijcem.com /ISSN:1940-5901/IJCEM0032947

Review Article

The prognostic role of CD44v6 in hepatocellular carcinoma: a meta-analysis

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Received May 29, 2016; Accepted September 8, 2016; Epub November 15, 2016; Published November 30, 2016

Abstract: Objective: This study aimed to investigate the correlation between CD44v6-positive hepatocellular carcinoma (HCC) and clinicopathological features and its effect on survival. A meta-analysis based on published studies was conducted to accurately evaluate the association between the presence of cancer stem cells (CSCs) in clinical samples and clinical outcome. Methods: A search in the PubMed, EMBASE, and Wanfang databases (up to September 30, 2015) was conducted. Publications assessing the clinical or prognostic significance of CD44v6 expression in HCC were identified and reviewed until September 30, 2015. A meta-analysis was performed to clarify the association between CD44v6 expression and clinical outcomes. Results: A total of 13 publications satisfied the criteria and comprised 1202 cases. Analysis of these data showed that CD44v6 expression was not significantly associated with tumor capsular invasion (OR = 1.80, 95% confidence interval [CI]: 1.00-3.22, P = 0.05), HBsAg status (OR = 1.04, 95% CI 0.63-1.71, P = 0.89), vascular invasion (OR = 2.65, 95% CI 0.88-7.94, P = 0.08), tumor size (OR = 1.20, 95% CI 0.80-1.78, P = 0.38), AFP level (OR = 0.90, 95% CI 0.62-1.30, P = 0.58) or tumor differentiation degree (OR = 1.47, 95% CI 0.99-2.17, P = 0.06). However, in the identified studies, CD44v6 expression was highly correlated with extrahepatic metastasis of HCC (OR = 4.13, 95% CI 2.62-6.52, P < 0.00001), increased TNM stage (pooled OR = 2.83, 95% CI 1.73-4.64, P < 0.0001) and reduced overall survival (relative risk [RR]: 1.51, 95% CI: 1.11-2.05, P = 0.008). Conclusion: CD44v6-positive HCC patients had worse prognosis, which was associated with common clinicopathological features and poor prognostic factors.

Keywords: Hepatocellular carcinoma, cancer stem cells, CD44v6, outcome

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the most common malignant primary tumor in the liver [1]. Despite improvements in treatment modalities during the past few decades, the prognosis of HCC is still very poor because of frequent intrahepatic metastasis and tumor recurrence [2].

A rare subpopulation of cells within cancer cells, termed cancer stem cells (CSCs), have been reported to be responsible for the initiation, progression, metastasis, and recurrence of cancer, as these CSCs have a distinct self-renewal property and could generate all the heterogeneous lineages of cancer cells that eventually constitute tumor bulk [3]. In recent years, these CSCs have been reported to be responsible for the poor outcome of HCC [4, 5]. Furthermore, CSC markers, such as CD44, CD90 and CD133, are potential indicators of HCC prognosis [6]. Among these CSC markers, CD44 is the most frequently reported in HCC. Several studies similarly utilised CD44 positivity to isolate cells with stem cell-like and cancer-initiating properties from other cancer cells [6, 7].

CD44 is the major hyaluronan (HA) receptor, and CD44 bound to HA has been proven to participate in various tumor biological activities, including tumor progression, metastasis and proliferation [8, 9]. CD44 plays a critical role in cell migration, with involvement in multiple steps. Once activated, the cytoplasmic tail of CD44 interacts with the actin cytoskeleton, and CD44 is induced to the leading edge of migrating cells [10]. Up to date, CD44v6 was the most reported isoform in HCC. It has been reported that CD44v6 can contribute to

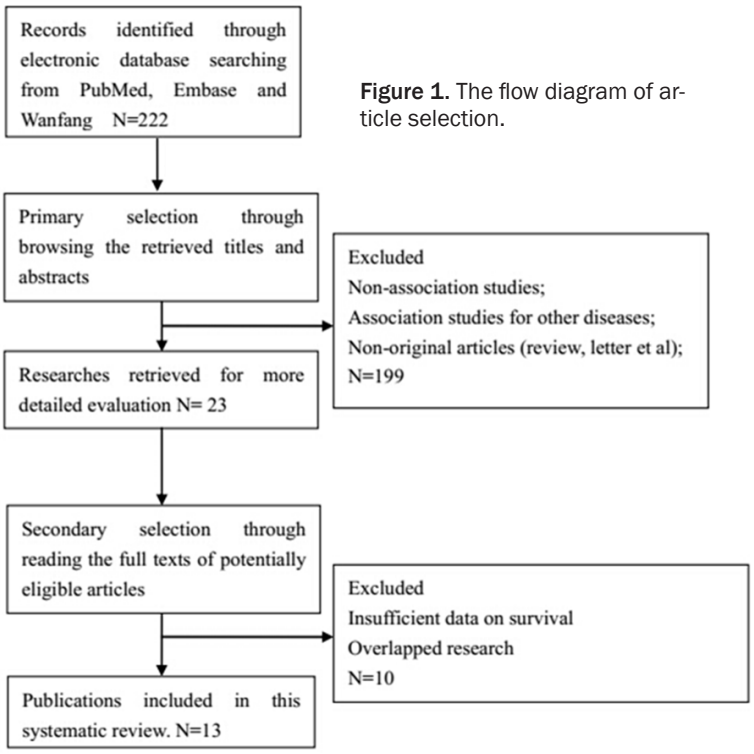


Figure 1. The flow diagram of article selection.

both PI3K/Akt and MAPK activation, which can regulate the extracellular matrix, promote cell motility, and suppress cancer apoptosis [11]. The prognostic value of CD44v6 expression in patients with HCC has been evaluated in many studies [12-24], however, the correlation between CD44v6 and the clinicopathological features of HCC and their prognostic values is still a subject of considerable discussion. Some researchers concluded that CD44v6 expression had no influence on survival [12-14]. Other researchers reported that CD44v6 expression was predictive of decreased survival outcome for HCC [15-24]. To evaluate this question, we conducted a systematic review and meta-analysis and determined the association between the expression of CD44v6 and common clinicopathological features of HCC. Our results may enable prognostic stratification of HCC patients with adjuvant therapy and provide new insights into the potential cellular origin of HCC and its activated molecular pathways.

Material and methods

Search strategy

The electronic database of Pubmed, Embase, and Wanfang were searched for studies that investigated the association of clinicopathological parameters and prognosis with CD44v6 expression in HCC to be included in the present meta-analysis. Studies were examined, and an updated search was conducted on September 30, 2015. The following search terms and combinations were used: “CD44v6” as well as “hepatocellular carcinoma” or “HCC” or “liver cancer” or “liver tumor” or “liver neoplasms” or “hepatocellular carcinoma”. The citation lists from all the retrieved studies were used to identify other relevant publications. Review articles were also scanned to identify additional eligible studies. The title and abstract of each identified study were scanned to exclude any irrelevant publications. The remaining articles were reviewed to determine whether they contained information on the topic of interest.

Selection criteria

Diagnosis of HCC was proven by histopathological methods. Studies of CD44v6 expression based on HCC tissue (after either surgical excision or biopsy sampling), rather than serum or any other kind of specimen were included. All studies on the correlation of CD44v6 expression with clinicopathological markers and the association of CD44v6 expression on overall survival (OS) of HCC patients were included. For inclusion into the analysis, there was no limitation on the minimum number of patients of every single study. When there were multiple articles by the same group based on similar patients and using same detection methods, only the largest or the most recent article was included.

Data extraction

The following information was extracted from the retrieved papers: author, country of the patient, ethnicity, publication year, time of collection, tumor pathological stage, number of patients, research technique used, the ages of

Table 1. Main characteristics and results of the eligible studies

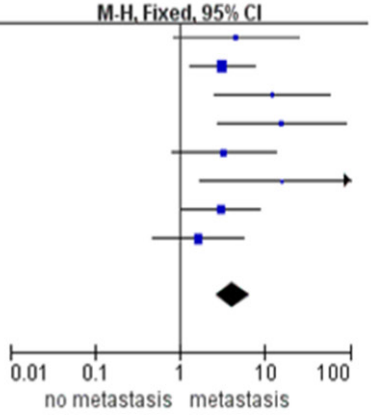
Study	Patient's country	Ethnicity	Year	Time of collection	Pathological stage	Method	Number of patients	Age in years	Follow-up months	Cut-off for CD44 positive	Survival analysis
Endo et al.	Japan	Asian	2000	ND	ND	IHC	107	17-80	80	> 0% staining	OS
Huang et al.	China	Asian	2001	1995-1999	I-IV	IHC	51	36-72	48	> 0% staining	OS
Zhang et al.	China	Asian	2005	1998-2003	ND	IHC	40	29-70	ND	> 10% staining	ND
Zheng et al.	China	Asian	2007	2000-2005	I-III	IHC	87	35-72	60	> 50% staining	OS
Zhang et al.	China	Asian	2008	2001-2004	I-IV	IHC	50	21-68	ND	> 20% staining	ND
Gao et al.	China	Asian	2008	2004-2006	I-IV	IHC	40	33-75	36	> 5% staining	OS
Jha et al.	China	Asian	2009	2001-2003	I-III	IHC	40	26-65	ND	> 50% staining	ND
Zhao et al.	China	Asian	2009	2000-2008	ND	IHC	42	28-75	ND	> 50% staining	ND
Liu et al.	China	Asian	2009	1999-2006	ND	IHC	33	38-85	ND	> 50% staining	ND
Peng et al.	China	Asian	2010	2005-2006	ND	IHC	76	19-69	36	> 25% staining	OS
Deng et al.	China	Asian	2011	2003-2007	I-IV	IHC	78	21-78	48	> 5% staining	ND
Mima et al.	Japan	Asian	2012	2004-2007	I-IV	IHC	235	ND	60	> 50% staining	OS
Zhou et al.	China	Asian	2012	2007-2010	I-III	IHC	323	ND	74	Median	OS

IHC: Immunohistochemistry; OS: Overall survival; ND: Not document.

A

Study or Subgroup	Case Events	Case Total	Control Events	Control Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Zhang et al 2005	16	30	2	10	7.4%	4.57 [0.83, 25.21]
Zheng et al 2007	25	45	12	42	29.0%	3.13 [1.28, 7.62]
Gao et al 2008	20	23	6	17	4.7%	12.22 [2.55, 58.69]
Jha et al 2009	11	13	7	27	3.7%	15.71 [2.77, 89.10]
Zhao et al 2009	6	12	7	30	10.5%	3.29 [0.80, 13.50]
Liu et al 2009	16	24	1	9	2.6%	16.00 [1.69, 151.11]
Peng et al 2010	11	20	16	56	19.9%	3.06 [1.06, 8.77]
Deng et al 2011	18	59	4	19	22.1%	1.65 [0.48, 5.66]
Total (95% CI)		**226**		**210**	**100.0%**	**4.13 [2.62, 6.52]**
Total events	123		55			

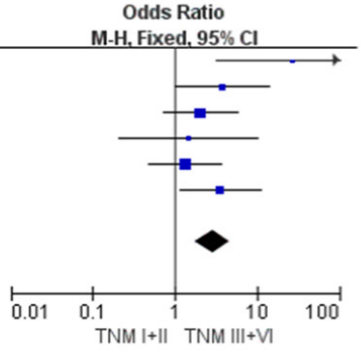
Heterogeneity: Chi² = 8.45, df = 7 (P = 0.29); I² = 17%
Test for overall effect: Z = 6.10 (P < 0.00001)



B

Study or Subgroup	Case Events	Case Total	Control Events	Control Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Huang et al 2001	13	25	1	26	2.5%	27.08 [3.16, 231.87]
Su et al 2006	10	16	8	26	11.9%	3.75 [1.01, 13.91]
Zheng et al 2007	13	45	7	42	26.8%	2.03 [0.72, 5.73]
Jha et al 2009	2	13	3	27	8.6%	1.45 [0.21, 9.98]
Peng et al 2010	10	20	24	56	32.9%	1.33 [0.48, 3.71]
Deng et al 2011	33	59	5	19	17.4%	3.55 [1.13, 11.15]
Total (95% CI)		**178**		**196**	**100.0%**	**2.83 [1.73, 4.64]**
Total events	81		48			

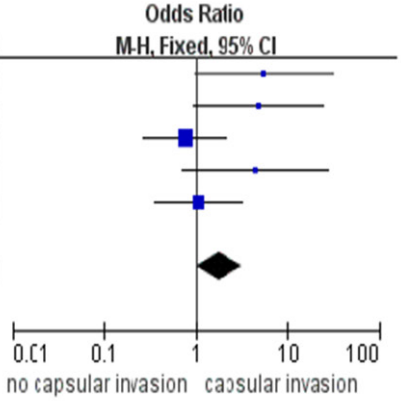
Heterogeneity: Chi² = 7.51, df = 5 (P = 0.19); I² = 33%
Test for overall effect: Z = 4.15 (P < 0.0001)



C

Study or Subgroup	Case Events	Case Total	Control Events	Control Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Su et al 2006	11	13	10	20	6.8%	5.50 [0.96, 31.43]
Zhang et al 2005	13	15	20	35	9.0%	4.88 [0.95, 24.94]
Peng et al 2010	12	20	37	56	43.6%	0.77 [0.27, 2.21]
Jha et al 2009	9	11	7	14	6.3%	4.50 [0.70, 28.79]
Mima et al 2012	41	46	92	104	34.4%	1.07 [0.35, 3.23]
Total (95% CI)		**105**		**229**	**100.0%**	**1.80 [1.00, 3.22]**
Total events	86		166			

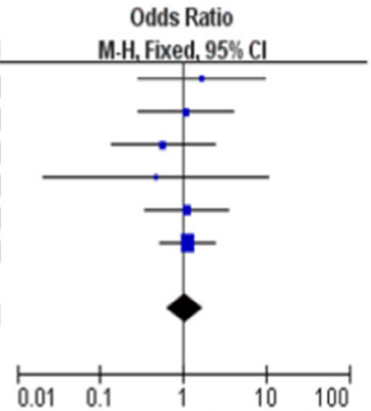
Heterogeneity: Chi² = 7.29, df = 4 (P = 0.12) I² = 45%
Test for overall effect: Z = 1.97 (P = 0.05)



D

Study or Subgroup	Case Events	Case Total	Control Events	Control Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Su et al 2006	14	16	21	26	6.7%	1.67 [0.28, 9.82]
Zheng et al 2007	40	45	37	42	14.2%	1.08 [0.29, 4.04]
Zhang et al 2005	11	15	29	35	15.5%	0.57 [0.13, 2.41]
Liu et al 2009	22	24	9	9	4.5%	0.47 [0.02, 10.83]
Peng et al 2010	15	20	41	56	18.0%	1.10 [0.34, 3.54]
Mima et al 2012	14	46	29	104	41.2%	1.13 [0.53, 2.42]
Total (95% CI)		**166**		**272**	**100.0%**	**1.04 [0.63, 1.71]**
Total events	116		166			

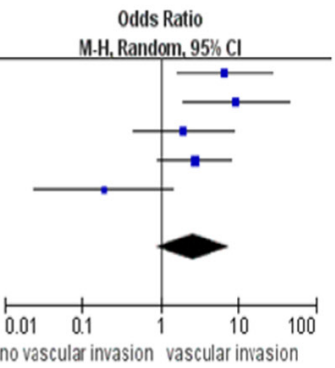
Heterogeneity: Chi² = 1.24, df = 5 (P = 0.94); I² = 0%
Test for overall effect: Z = 0.14 (P = 0.89)



E

Study or Subgroup	Case Events	Case Total	Control Events	Control Total	Weight	Odds Ratio M-H, Random, 95% CI
Su et al 2006	12	16	8	26	21.1%	6.75 [1.66, 27.51]
Zhang et al 2008	7	15	3	35	19.5%	9.33 [1.96, 44.36]
Zhao et al 2009	4	12	6	30	20.1%	2.00 [0.45, 8.94]
Deng et al 2011	33	59	6	19	24.4%	2.75 [0.92, 8.22]
Mima et al 2012	1	46	11	104	14.9%	0.19 [0.02, 1.50]
Total (95% CI)		**148**		**214**	**100.0%**	**2.65 [0.88, 7.94]**
Total events	57		34			

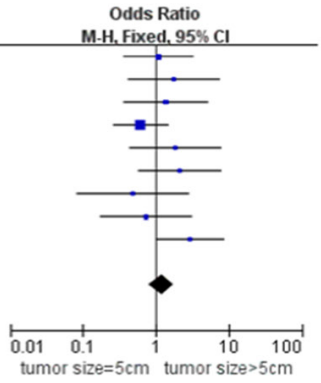
Heterogeneity: Tau² = 0.98; Chi² = 10.98, df = 4 (P = 0.03); I² = 64%
Test for overall effect: Z = 1.74 (P = 0.08)



F

Study or Subgroup	Case Events	Case Total	Control Events	Control Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Huang et al 2001	12	25	12	26	13.8%	1.08 [0.36, 3.24]
Zhang et al 2005	19	30	5	10	6.2%	1.73 [0.41, 7.33]
Su et al 2006	11	16	16	26	8.6%	1.38 [0.37, 5.15]
Zheng et al 2007	18	45	22	42	30.8%	0.61 [0.26, 1.42]
Zhang et al 2008	12	15	24	35	6.5%	1.83 [0.43, 7.84]
Gao et al 2008	15	23	8	17	7.2%	2.11 [0.59, 7.60]
Liu et al 2009	15	24	7	9	8.6%	0.48 [0.08, 2.81]
Zhao et al 2009	8	12	22	30	9.5%	0.73 [0.17, 3.09]
Deng et al 2011	40	59	8	19	8.8%	2.89 [1.00, 8.37]
Total (95% CI)		**249**		**214**	**100.0%**	**1.20 [0.80, 1.78]**
Total events	150		124			

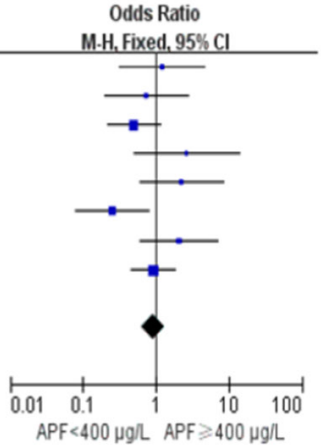
Heterogeneity: Chi² = 8.02, df = 8 (P = 0.43); I² = 0%
Test for overall effect: Z = 0.88 (P = 0.38)



G

Study or Subgroup	Case Events	Case Total	Control Events	Control Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Huang et al 2001	20	25	20	26	6.6%	1.20 [0.31, 4.58]
Su et al 2006	10	16	18	26	8.6%	0.74 [0.20, 2.75]
Zheng et al 2007	18	45	24	42	25.0%	0.50 [0.21, 1.17]
Zhang et al 2008	13	15	25	35	3.4%	2.60 [0.49, 13.67]
Jha et al 2009	9	15	10	25	5.0%	2.25 [0.61, 8.31]
Peng et al 2010	12	20	48	56	17.0%	0.25 [0.08, 0.80]
Deng et al 2011	21	59	4	19	6.5%	2.07 [0.61, 7.05]
Mima et al 2012	22	46	52	104	27.9%	0.92 [0.46, 1.84]
Total (95% CI)		**241**		**333**	**100.0%**	**0.90 [0.62, 1.30]**
Total events	125		201			

Heterogeneity: Chi² = 11.96, df = 7 (P = 0.10); I² = 41%
Test for overall effect: Z = 0.55 (P = 0.58)



H

Study or Subgroup	Case Events	Case Total	Control Events	Control Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Huang et al 2001	5	25	4	26	7.6%	1.38 [0.32, 5.85]
Zhang et al 2005	7	30	1	10	2.8%	2.74 [0.29, 25.54]
Su et al 2006	10	16	8	26	5.6%	3.75 [1.01, 13.91]
Zheng et al 2007	20	45	19	42	26.6%	0.97 [0.42, 2.26]
Zhang et al 2008	9	15	18	35	10.5%	1.42 [0.42, 4.83]
Gao et al 2008	10	23	2	17	3.2%	5.77 [1.06, 31.27]
Jha et al 2009	5	13	6	27	5.8%	2.19 [0.52, 9.23]
Zhao et al 2009	2	12	5	30	5.8%	1.00 [0.17, 6.03]
Liu et al 2009	6	24	3	9	8.0%	0.67 [0.13, 3.53]
Mima et al 2012	9	46	20	104	24.0%	1.02 [0.43, 2.45]
Total (95% CI)		**249**		**326**	**100.0%**	**1.47 [0.99, 2.17]**
Total events	83		86			

Heterogeneity: Chi² = 7.71, df = 9 (P = 0.56); I² = 0%
Test for overall effect: Z = 1.91 (P = 0.06)

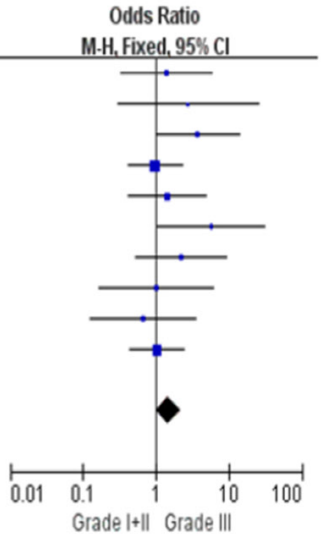


Figure 2. Forest plot of OR was assessed for association between stem cell markers and clinical pathologic features, such as extrahepatic metastasis of HCC (A), tumor TNM stage (B), capsular invasion (C), HBsAg status (D), vascular invasion (E), tumor size (F), AFP level(G), or tumor differentiation degree (H).

the patients, and the choice of cut-off scores for the definition of positive staining or staining intensity. Two major groups were established on the basis of the objective. One group clarified the association between the expression of CD44v6 and clinicopathological parameters, including capsular invasion, AFP level, HBsAg status, tumor TNM stage, vascular invasion, tumor size, differentiation degree and extrahepatic metastasis of HCC. Meanwhile, the other group investigated the association between the expression of CD44v6 and OS.

Statistical analysis

The meta-analysis was performed as previously described [25]. ORs with 95% CI were used to evaluate the association between CD44v6 and the clinicopathological features for HCC, including capsular invasion, AFP level, HBsAg status, tumor TNM stage, vascular invasion, tumor size, differentiation degree and extrahepatic metastasis of HCC. The RR was used for assessing the association of CD44v6 and OS combined over studies. For those RRs that were not given directly in the published articles, the published data and figures from original papers were used to assess the RR according to the methods described by Parmar et al. [26]. Heterogeneity across studies was evaluated with the Q test and *P* values. ORs and RRs were calculated by a random-effects model when the *P* value was less than 0.05. Otherwise, a fixed-effects model was used. The Begg and Egger funnel plot was used to assess publication bias. Statistical analyses were estimated using Review manager software. *P* values were two-sided, with significance at $P < 0.05$.

Results

Characteristics of the studies

A total of 222 articles were selected for the meta-analysis by browsing the databases PubMed, Embase, and Wanfang. Out of this total, 199 were excluded after the title and abstract were reviewed, and ten articles were excluded after the full publications were reviewed (**Figure 1**). The reasons for exclusion were: (a) studies were not associated with the topic of interest; (b) researchers of the article used neither histopathologic analysis nor close clinical and imaging follow-up for at least six months; (c) studies associated with other diseases (d); non-original articles; (e) data could not be extracted; and (f) repeated data from the same or similar population. Eventually, 13 publications met the criteria for the present analysis. The total number of patients was 1202, and each study had 33 to 323 patients. The main characteristics of the eligible studies are summarized in **Table 1**. A total of 13 articles dealt with clinicopathological factors. Moreover, the assessment of OS using Kaplan-Meier method was reported in 6 of these articles.

Correlation of CD44v6 expression with clinicopathological parameters

The association between CD44v6 and several clinicopathological parameters is illustrated in **Figure 2**. CD44v6 expression was significantly associated with extrahepatic metastasis of HCC (pooled OR = 4.13, 95% CI 2.62-6.52, $P < 0.00001$ fixed-effect) and tumor TNM stage (pooled OR = 2.83, 95% CI 1.73-4.64, $P < 0.0001$ fixed-effect) (**Figure 2A** and **2B**).

However, CD44v6 expression was not associated with capsular invasion (pooled OR = 1.80, 95% CI 1.00-3.22, P = 0.05 fixed-effect) (**Figure 2C**), HBsAg status (pooled OR = 1.04, 95% CI 0.63-1.71, P = 0.89 fixed-effect) (**Figure 2D**), vascular invasion (pooled OR = 2.65, 95% CI 0.88-7.94, P = 0.08 random-effect) (**Figure 2E**), tumor size (pooled OR = 1.20, 95% CI 0.80-1.78, P = 0.38 fixed-effect) (**Figure 2F**), AFP level(pooled OR = 0.90, 95% CI 0.62-1.30, P = 0.58 fixed-effect) (**Figure 2G**) or tumor differentiation degree (pooled OR = 1.47, 95% CI 0.99-2.17, P = 0.06 fixed-effect) (**Figure 2H**).

CD44v6 expression and prognosis of HCC

With the use of the methods described above, the OS of 757 patients in the 6 studies were analyzed. The main results of this meta-analysis are shown in **Figure 3**. The meta-analysis of the 6 studies for the prognostic value of CD44v6 expression showed that CD44v6 ex-

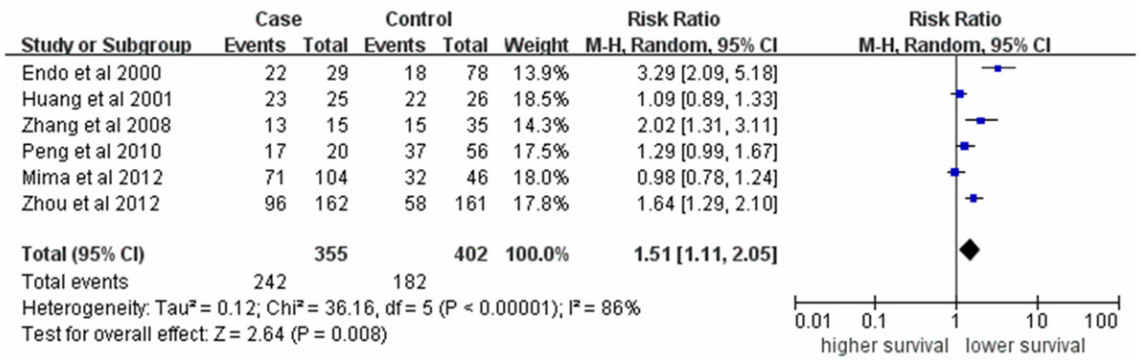


Figure 3. Analysis of CD44v6 expression and survival of HCC patients. Forest plot of RR for overall survival among included studies.

Table 2. Egger's test of funnel plot asymmetry

Clinicopathological parameters	t value	df	*P* value
Tumor differentiation	1.52	11	0.167
Extrahepatic metastasis	2.47	8	0.654
Tumor TNM stage	1.29	5	0.202
Capsular invasion	0.8	3	0.174
HBsAg carrier	0.21	5	0.846
Tumor size	0.4	10	0.839
Vascular invasion	1.35	4	0.269
AFP levels	1.11	8	0.311
Overall survival	1.85	7	0.123

df: Deflection; AFP: Alpha feto protein.

pression is associated with a poor OS. The combined RR was 1.51 (95% CI: 1.11-2.05, P = 0.008) (**Figure 3**).

Sensitivity analysis

In order to test for a bias introduced by the low numbers of available eligible publications we performed a sensitivity analysis. For this a single study involved in the meta-analysis was omitted for each round of analysis to investigate the influence of the individual data set of the particular study to the pooled ORs. We found that the corresponding pooled ORs were not essentially altered by substraction of any study (data not shown), indicating that our results were statistically robust.

Publication bias

The results of the meta-analyses of CD44v6 expression for the clinicopathological parameters and five-year OS did not show an evident asymmetrical shape in Begg's funnel plots. The results of Egger's test also did not show evidence of publication bias (**Table 2**).

Discussion

To the best of our knowledge, the present meta-analysis is the first to systematically estimate the association between CD44v6 and HCC survival. A quantitative aggregation of the survival results was conducted because of the presence of significant and non-significant studies addressing the importance of stem cells in HCC. The present results show that CD44v6 was significantly associated with tumor TNM stage and extrahepatic metastasis of HCC, as well as OS; this result also indicates that CD44v6 could be developed for clinical applications.

A previous study showed that CD44v6-positive tumor cells have CSC properties, such as self-renewal and tumorigenicity [27]. Expression of CD44v has been closely linked to tumor progression, metastasis, and treatment resistance processes in various cancers [28]. In particular, CD44v6 is closely associated with aggressive behavior and correlates with poor prognosis in a variety of human malignancies [29-31], and it has been shown to regulate malignant transformation by inducing tumor cell proliferation, adhesion, and migration [29, 32]. Furthermore, the prognostic value of CD44v6 expression in HCC is yet to be elucidated. The results of our study indicated that increased CD44v6 expression correlated with poor OS of HCC patients. However, contrasting results were also reported. For instance, Lipponen et al. reported that CD44v6 expression correlates

with favorable prognosis in transitional cell bladder cancer [33]. In addition, Ayhan et al. reported that endometrial cancer patients with higher expressions of CD44v6 survived longer and had lower recurrence rates [34]. These conflicting results suggest an elusive role of CD44v6 in cancer progression and metastasis. Thus, more prospective studies are needed to draw a definite conclusion.

For future studies, co-expression of HCC CSC markers associated with patient survival may be more meaningful for clinical application in HCC. Several studies have shown that CSC-related factors, including SOX2, OCT4, and LIN28 are associated with HCC progression [35-37]. In addition, CSCs have major phenotypic and functional heterogeneities, which may help distinguish them from cancer cells and may be of potential benefit in the development of anticancer therapies to improve clinical outcomes.

However, this meta-analysis has some limitations. First, the number of included studies, as well as the included HCC patients in each study, is relatively small. Thus, these factors might have reduced the power and accuracy of subcategory analysis. Second, the OS outcome was based on individual unadjusted RRs. Thus, a more precise assessment should be adjusted using other prognostic factors. Third, no clear guidelines are available as regards the methods used for the evaluation of the levels of CD44v6 in HCC patients. Such evaluation differs among all the studies. In the assessment of biomarkers, the use of a standard threshold has great importance. Although immunohistochemistry was the most commonly applied method, differences in the cut-off values for the positive applied method expression may have contributed to the observed heterogeneity. Thus, standardized methods and cut points that classify CD44v6 expression levels as “positive” or “negative” are urgently needed.

In conclusion, despite the limitations of this meta-analysis, our study suggests that CD44-v6 expression is significantly associated with worse OS in patients with HCC. Hopefully this analysis will stimulate further research with rigid criteria and large study populations to resolve any remaining controversy of the role of CD44v6 expression for the prognosis of HCC patients.

Disclosure of conflict of interest

None.

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