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# Association Between Expression of Cancer Stem Cell Markers and Poor Differentiation of Hepatocellular Carcinoma

## A Meta-Analysis (PRISMA)

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**Abstract:** The role of cancer stem cell (CSC) markers in differentiation of hepatocellular carcinoma (HCC) remains uncertain. We conducted a meta-analysis to first investigate the association between expression of CSC markers (CD133, CD90, CD44, and EpCAM) and poor differentiation of HCC, and second, to determine if these CSC markers can be classified as biomarkers for patient classification and HCC differentiated therapy.

The relevant literature was searched using PubMed, EMBASE, Elsevier, and Chinese Biological Medicine databases for association between CSC markers and HCC from January 1, 2000 to June 30, 2014. Data were synthesized using random-effect or fixed-effect models. The effect sizes were estimated by measuring odds ratios (OR) with 95% confidence interval (CI).

The meta-analysis included 27 studies consisting of 2897 patients with HCC. The positive expression of CSC markers was associated with poor differentiation (OR = 2.37, 95% CI = 2.03–2.77, $P <$ 0.00001). Similarly, the positive expression of CSC markers was only associated with HCC tissues compared with noncancerous liver tissues (OR = 9.26, 95% CI = 3.10–27.65, $P <$ 0.0001). CD90 has a specificity of 91.9% for HCC and a sensitivity of 48.22% in predicting poor differentiation.

The positive expression of CSC markers is associated with poor differentiation and aggressive phenotype of patients with HCC. The CD90 marker might be a promising target for patient with HCC classification and differentiation therapy.

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**Abbreviations**: AFP = a-fetoprotein, CI = confidence interval, CSCs = cancer stem cells, EGFR = epidermal growth factor receptor, HBV = hepatitis C virus, HCC = hepatocellular carcinoma, HCV = hepatitis C virus, HER2 = human epidermal growth factor receptor 2, HNF4α = hepatocyte nuclear factor-4α, MeSH = Medical Subject Heading, OR = odds ratio, OSM = oncostatin M, PVTT = portal vein tumor thrombus, STAT3 = signal transducer and activator of transcription 3.

## INTRODUCTION

Hepatocellular carcinoma (HCC) is the 6th most prevalent cancer in the world and the third leading cause of cancer-related mortalities.[1] Only 10% to 20% of the HCCs can be surgically excised, although attended with a high frequency of recurrence.[2] Further, as HCC is chemoresistant and the current drug therapies are associated with limited efficacy, the prognosis of these patients is generally poor.[3] Currently, there is a lack of not only predictive biomarkers that are linked to prognosis of patients with HCC but also effective therapeutic targets.

Cancer classification is expected to establish prognosis, provide adequate treatment options, and aid researchers to design controlled clinical trials. Edmondson Grading is a widely accepted histological classification method for HCC and has been endorsed by clinical management guidelines.[4] However, this method does not predict the biological behavior of HCC accurately. Therefore, molecular biomarkers can be helpful in classifying patient population based on cellular lineages within tumors and therapy response. For example, the human epidermal growth factor receptor 2 (HER2) status in breast cancer is used to distinguish subgroups of patients with different outcomes and treatment responses to trastuzumab.[5] Similarly, epidermal growth factor receptor (EGFR) mutation status in nonsmall cell lung cancer is helpful in determining the efficacy of erlotinib treatment.[6] However, no such molecular data are available to predict HCC outcomes in combination with histological classification.

Cancer stem cells (CSCs) are a small subpopulation of cells within tumors endowed with the potential for self-renewal, differentiation, and tumorigenicity.[7] The existence of CSCs in HCC partially explains its heterogeneity, metastasis, recurrence after resection, and chemoresistance.[8] Recent studies have started exploring the potential of hepatic CSC markers in HCC diagnosis, prognosis prediction, and development of novel therapeutics. Several cell surface markers, including CD133, CD44, CD90, and EpCAM are often used to identify and enrich HCC CSCs.[9] According to the CSC theory, multistep dedifferentiation induced by the CSCs is considered important for

multicentric carcinogenesis and aggressive phenotype. However, the relation between the expression of CSC markers and poor differentiation of HCC remains uncertain.

Therefore, this meta-analysis was carried out to determine the association between the expression of CSC markers and poor differentiation of HCC. These results may help us understand the role of CSC markers in differentiation of HCC and provide more reliable molecular markers for patient classification and potential targets for differentiation therapy.

## MATERIALS AND METHODS

### Search Strategy and Selection Criteria

We searched PubMed, EMBASE, Elsevier, and Chinese Biological Medicine databases (January 1, 2000 to June 30, 2014) using the Medical Subject Heading keywords "CD133," "Prominin," "CD44," "CD90," "Thy-1," "EpCAM," "HCC," "liver cancer," "liver tumor," "differentiation," "tumor grade," "Edmondson Grading," and the individual corresponding free terms. Furthermore, we reviewed citations in the retrieved articles to search for additional relevant studies. Searches were limited to papers published in English and Chinese language only. The study was approved by the Conduct of Human Ethics Committee of the First Affiliated Hospital, College of Medicine, Xi'an Jiaotong University.

Studies were included in the meta-analysis, if they included patients with distinct HCC diagnosis by 2 independent pathologists according to the American Association guidelines; data on CD133 (Prominin), CD44, CD90 (Thy-1), and EpCAM expression and are full-length papers; information about differentiation of HCC; and data about odds ratios (ORs) with 95% confidence intervals (CI), or at least adequate data to calculate 95% CIs. The following studies were excluded; overlapping articles or duplicate data; articles about cell lines or animals; review articles and conference records without original data and full text; studies lacking information on differentiation; and studies with fewer than 10 participants. In cases where the study population was overlapped by more than 30% in 2 or more papers published by the same authors, we only included the study with the larger number of participants.

To investigate the specific expression of CSC markers in HCC tissues and their sensitivity in predicting poor differentiation of patients with HCC, the data from HCC tissues were compared with noncancerous liver tissues. Moreover, the comparison of CSC marker sensitivity and specificity was also accomplished by comparing the data between poor HCC tissues and nonpoor (well or moderate) HCC tissues from the included studies.

### Review of the Studies

The datasets were independently extracted by 2 investigators (RL and YS) with a concordance rate of 94.1% and subsequently verified by the other authors. Discrepancies were resolved by consensus. The patients from all the studies were divided into positive and negative groups for each marker. Data were extracted only for participants whose differentiation status was known. The quality of the studies was evaluated by 2 investigators (HG and YS). Table 1 shows the data profile of each article included in this study.

### Statistical Analysis

The association of CSC markers (CD133, CD90, CD44, and EpCAM) with differentiation (well + moderate vs poor) and other clinicopathological conditions of HCC (such as tumor size [≤5 cm vs >5 cm], tumor stage [I + II vs III + IV], tumor capsule [positive vs negative], metastasis [positive vs negative], microvascular invasion [positive vs negative], portal vein tumor thrombus [PVTT] [positive vs negative], alpha-fetoprotein [AFP] level [≤200 ng/mL vs >200 ng/mL], hepatitis [positive vs negative], cirrhosis vs noncirrhosis, and liver function of Child-Pugh [A + B vs C]) were estimated by calculating ORs with 95% CI. Statistical heterogeneity among studies was evaluated using the chi-squared test, $P$ values, and $I^2$ statistics. A random-effect model or fixed-effect model was used to obtain pooled OR. To calculate the sensitivity and specificity of each CSC marker for HCC, $2 \times 2$ tables were generated by using the pooled data from cancerous and noncancerous liver tissues and poorly and nonpoorly differentiated HCC tissues. The sensitivity in HCC tissues was calculated as the ratio of HCC tissues with the specific CSC marker(s) to the HCC tissues with and without the expression; the specificity in HCC tissues was calculated as the ratio of the non-HCC tissues without the specific CSC marker(s) to the non-HCC tissues with and without the expression. Similarly, the sensitivity and specificity for poorly differentiated HCC tissues were also calculated. Publication bias was estimated by using funnel plots and Egger's test. $P$ value of <0.1 was indicative of statistically significant publication bias. Sensitivity analysis was conducted by removing 1 study each time to evaluate its contribution on the overall analysis. The meta-analysis was performed using Review Manager version 5.3 (The Nordic Cochrane Centre, the Cochrane Collaboration, Copenhagen, Denmark) and statistical analysis was done by STATA version 10.0 (Stata Corporation, College Station, TX) software. All statistical tests were 2-sided and a $P$ value of <0.05 was considered statistically significant.

## RESULTS

### Description of Studies

Based on the search criteria, a total of 1292 articles were retrieved. Among these, 1265 records were excluded for various reasons as shown in Figure 1. Twenty-seven studies (10–36) published as full papers were finally analyzed retrospectively. The sample size of the included studies ranged from 12 to 387, and data from a total of 2897 patients were analyzed. The patients from all the studies were divided into positive and negative groups based on the marker expression. The 2 patients' cohorts of Yamashita et al were divided into 4 subgroups on the basis of EpCAM and AFP expression. Different studies have analyzed different CSC markers; for example, 15 studies used CD133 as CSC marker,[10,11,17,18,20,22–27,30–33] 7 studies tested CD90 as CSC marker,[12,17,20,21,31,32,36] 9 studies considered CD44-positive tumor cells as CSCs,[11,13,15,16,18,19,28,34,35] and 7 studies used EpCAM as the CSC marker.[10,14,17,22,28,29,32] In 2 studies co-expression of CD133 and CD44 together was considered as a CSC marker.[11,23] The main characteristics of the studies are shown in Table 1.

### Association of CSC Markers With HCC Differentiation

The overall analysis by a fixed-effect model showed that expression of CSC markers was associated with poor differentiation of HCC tissues (pooled OR = 2.37, 95% CI = 2.03–2.77, $P < 0.00001$). Further, the subgroup analyses suggested statistically significant association of poorly differentiated HCC tissues with expression of CD133 (pooled OR = 2.75, 95% CI = 2.12–3.57, $P < 0.00001$); CD90 (pooled OR = 1.69,

**TABLE 1.** Characteristics of the Studies Included in the Meta-Analysis

| References | Markers | Technology | Cutoff, % | Number of Patients | Age, Mean/Median | Male, % | Treatment | Cases (Marker +) | Cases (Marker−) | Significant | Control (Marker +) | Control (Marker−) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chan et al[10] | CD133 | IHC | NS | 282 | 55.4 | 84 | LR | 38 | 244 | No | | |
| | EpCAM | IHC | NS | | | | | 56 | 226 | Yes | | |
| Chen et al[11] | CD133 | IHC | 0 | 387 | NS | 88 | LR | 216 | 171 | Yes | | |
| | CD44 | | 10 | | | | | 234 | 153 | Yes | 20 | 30 |
| | CD133/CD44 | | NS | | | | | 161 | 226 | Yes | 0 | 10 |
| Cheng et al[12] | CD90 | IHC | NS | 50 | NS | NS | LR/LT | 36 | 14 | Yes | 0 | 47 |
| Gao et al[13] | CD44 | IHC | NS | 40 | 49 | 75 | LR | 23 | 17 | Yes | 0 | 13 |
| Govaere et al[14] | EpCAM | IHC | 5 | 167 | 60.3 | 73 | LR | 25 | 141 | No | 0 | 10 |
| Guan et al[15] | CD44 | IHC | NS | 67 | NS | NS | LR | 18 | 49 | Yes | 0 | 10 |
| Guo et al[16] | CD44 | IHC | 0 | 49 | NS | NS | LR | 19 | 30 | No | 0 | 10 |
| Guo et al[17] | CD133 | IHC | 5 | 50 | NS | NS | LR | 42 | 8 | No | 5 | 3 |
| | CD90 | IHC | 5 | | | | | 32 | 18 | Yes | 3 | 5 |
| | EpCAM | IHC | 5 | | | | | 21 | 29 | No | 0 | 10 |
| Lingala et al[18] | CD133 | IHC | 5 | 23 | 56.4 | 80 | NS | 10 | 13 | No | 56 | 26 |
| | CD44 | IHC | 5 | | | | | 15 | 8 | No | 0 | 82 |
| Liu et al[19] | CD44 | IHC | 0 | 39 | NS | NS | LR | 27 | 12 | Yes | 19 | 40 |
| Liu et al[20] | CD133 | IHC | NS | 245 | 48 | 84 | LR | 45 | 200 | Yes | 0 | 70 |
| | CD90 | IHC | NS | | | | | 91 | 154 | No | 21 | 49 |
| Lu et al[21] | CD90 | IHC | NS | 59 | 55 | 83 | LR | 43 | 16 | Yes | | |
| Pan et al[22] | CD133 | IHC | 0 | 70 | 55 | 57 | LT | 49 | 21 | No | | |
| | EpCAM | IHC | 0 | | | | | 49 | 21 | Yes | | |
| Salnikov et al[23] | CD133 | IF | NS | 12 | 73 | 58 | TACE/LR/LT | 6 | 6 | No | | |
| | CD133/CD44 | IF | NS | 12 | 73 | 58 | | 5 | 7 | No | | |
| Sasaki et al[24] | CD133 | IHC | 0 | 136 | 61 | 112 | LR | 30 | 106 | Yes | | |
| Song et al[25] | CD133 | IHC | 1.32 | 63 | 50 | 79 | LR | 26 | 27 | Yes | | |
| Tsuchiya et al[26] | CD133 | IHC | NS | 31 | 69 | 81 | LR | 9 | 22 | No | | |
| Wu et al[27] | CD133 | IHC | 0 | 190 | 58.7 | 93 | LR | 42 | 148 | Yes | | |
| Xu et al[28] | EpCAM | IHC | NS | 106 | NS | 83 | LR | 52 | 54 | Yes | | |
| | CD44 | IHC | NS | | | | | 15 | 8 | | | |
| Yamashita et al[29] (cohort 2) | EpCAM | TMA | NS | 238 | 51 | 87 | LR | 95 | 143 | Yes | | |
| Yamashita et al[29] (cohort 3) | EpCAM | IHC | 5 | 101 | 49 | 92 | LR | 39 | 62 | Yes | | |
| Yeh et al[30] | CD133 | IHC | NS | 154 | 56 | 74 | LR | 24 | 130 | Yes | | |
| Yilmaz et al[31] | CD133 | IHC | 0 | 35 | 64 | 86 | LR/biopsy | 24 | 11 | Yes | 32 | 31 |
| | CD90 | IHC | 0 | | | | | 9 | 26 | Yes | 0 | 63 |
| Yu et al[32] | CD133 | IHC | NS | 20 | 51 | 85 | LR/LT | 17 | 3 | Yes | | |
| | EpCAM | IHC | NS | | | | | 17 | 3 | No | | |
| | CD90 | IHC | NS | | | | | 18 | 2 | Yes | | |
| Zeng et al[33] | CD133 | IHC | 10 | 109 | 58 | 94 | LT | 44 | 65 | Yes | 0 | 41 |

| References | Markers | Technology | Cutoff, % | Number of Patients | Age, Mean/ Median | Male, % | Treatment | Cases (Marker +) | Cases (Marker−) | Significant | Control (Marker +) | Control (Marker−) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Zhang et al[34] | CD44 | IHC | 0 | 51 | 51 | 63 | LR | 23 | 34 | No | 7 | 39 |
| Zheng et al[35] | CD44 | IHC | NS | 87 | 55 | 86 | LR | 45 | 42 | No | 4 | 16 |
| Zheng et al[36] | CD90 | IHC | 10 | 36 | 55 | 69 | LR | 23 | 13 | Yes | 0 | 20 |

EpCAM, epithelial cell adhesion molecule; IHC, immunohistochemistry; IF, immunofluorescence; LR, liver resection; LT, liver transplantation; NS, not specified; TACE, transcatheter arterial chemoembolization; TMA, tissue microarray.
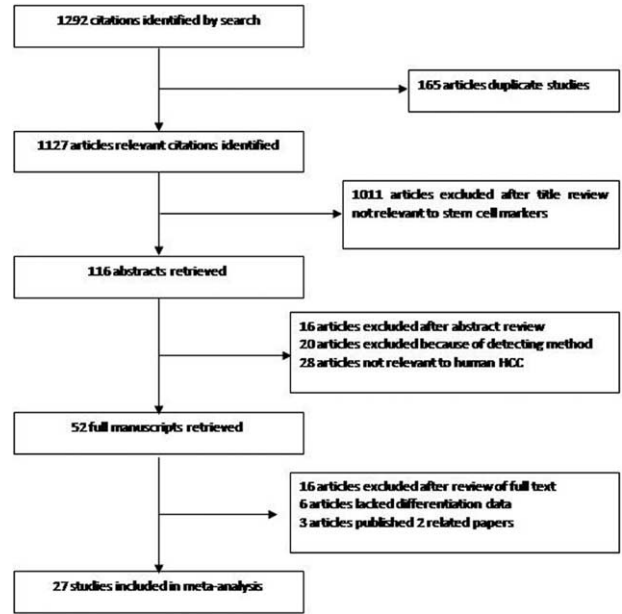


**FIGURE 1.** Flow chart of article selection. HCC, hepatocellular carcinoma.

95% CI = 1.14–2.49, P = 0.009); CD44 (pooled OR = 1.95, 95% CI = 1.38–2.78, P = 0.002); and EpCAM (pooled OR = 2.40, 95% CI = 1.64–3.51, P < 0.00001). Moreover, the double-positive expression of CD133 and CD44 was also associated with poorly differentiated HCC (pooled OR = 3.44, 95% CI = 2.11–5.61, P < 0.00001) (Figure 2).

## Sensitivity and Specificity of CSC Markers in HCC

To investigate the relationship between expression of CSC markers and HCC, we analyzed data pertaining to CSC markers from 1010 cancerous and 549 noncancerous liver tissues (Figure 3). Overall, a statistically significant association of CSC markers with cancerous compared with noncancerous liver tissues (pooled OR = 9.26, 95% CI = 3.1–27.65, P < 0.00001) was observed, as analyzed by a random-effect model. Subsequent subgroup analysis showed significant association between the expression of CD90 (pooled OR = 28.17, 95% CI = 5.20–152.59, P = 0.0001); CD44 (pooled OR = 6.78, 95% CI = 2.25–20.49, P = 0.0007); and EpCAM (pooled OR = 5.79, 95% CI = 2.87–11.67, P < 0.00001), whereas CD133 expression was not significantly associated (pooled OR = 5.55, 95% CI = 0.39–79.57, P = 0.21) with HCC tissues.

Further, we investigated the individual CSC markers for their specificity and sensitivity in HCC tissues compared with noncancerous liver tissues. Among all CSCs, CD90 had the highest specificity of 91.9% (95% CI = 88.3–95.5) whereas EpCAM had the highest sensitivity of 58.3% (95% CI = 46.7–69.9). The sensitivity and specificity of CD133 were 40.0% (95% CI = 33.4–46.6) and 65.9% (95% CI = 59.0–72.8), respectively, and for CD44 they were 47.8% (95% CI = 40.3–55.3) and 88.2% (95% CI = 82.8–93.6), respectively, as shown in Table 2.

## Sensitivity and Specificity of CSC Markers in Poorly Differentiated HCC

In order to obtain potential biomarkers for prediction of poorly differentiated stages of HCC, we evaluated the
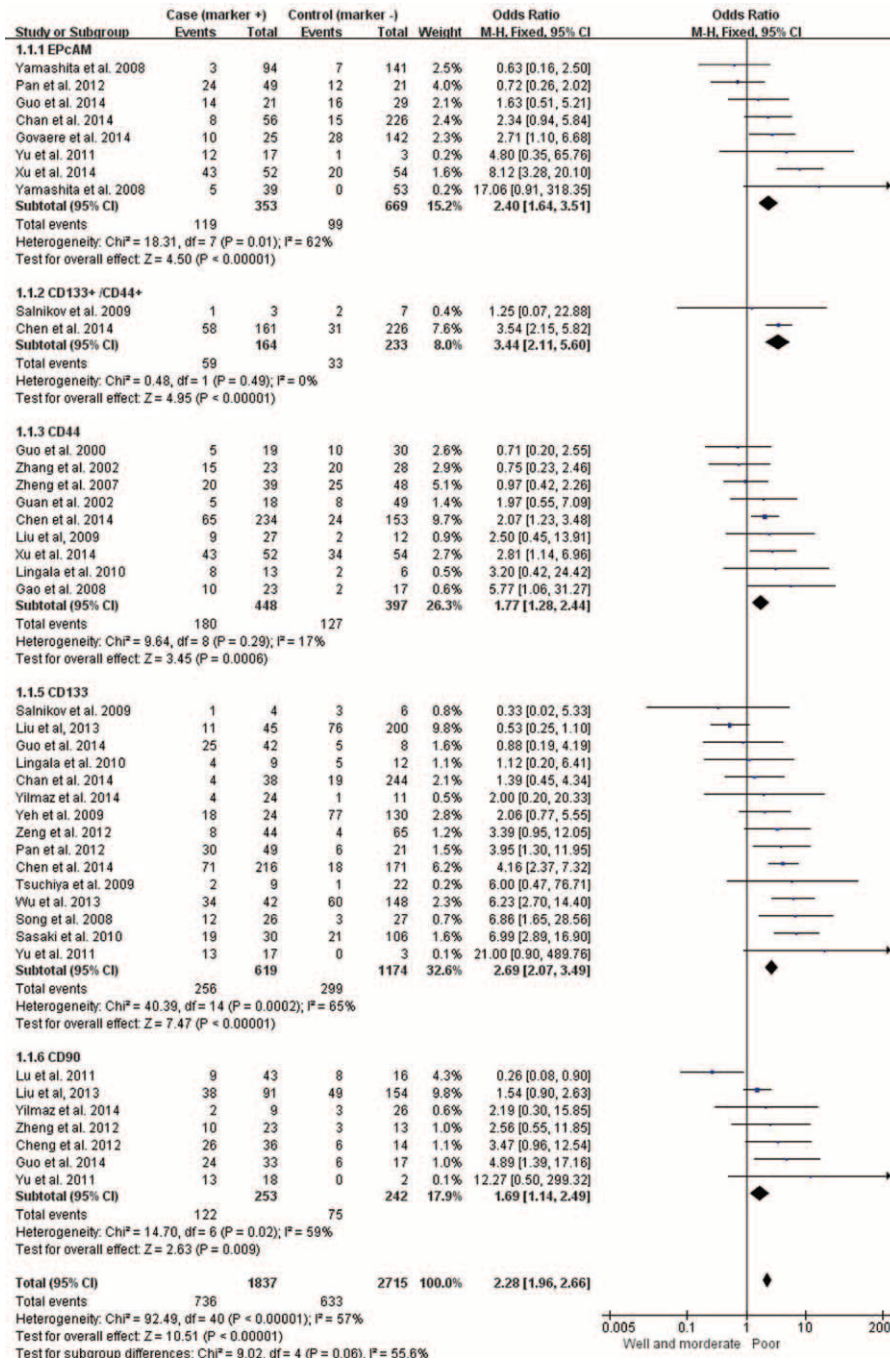
| Study or Subgroup | Case (marker +) Events | Total | Control (marker -) Events | Total | Weight | Odds Ratio M-H, Fixed, 95% CI |
|---|---|---|---|---|---|---|
| **1.1.1 EPcAM** | | | | | | |
| Yamashita et al. 2008 | 3 | 94 | 7 | 141 | 2.5% | 0.63 [0.16, 2.50] |
| Pan et al. 2012 | 24 | 49 | 12 | 21 | 4.0% | 0.72 [0.26, 2.02] |
| Guo et al. 2014 | 14 | 21 | 16 | 29 | 2.1% | 1.63 [0.51, 5.21] |
| Chan et al. 2014 | 8 | 56 | 15 | 226 | 2.4% | 2.34 [0.94, 5.84] |
| Govaere et al. 2014 | 10 | 25 | 28 | 142 | 2.3% | 2.71 [1.10, 6.68] |
| Yu et al. 2011 | 12 | 17 | 1 | 3 | 0.2% | 4.80 [0.35, 65.76] |
| Xu et al. 2014 | 43 | 52 | 20 | 54 | 1.6% | 8.12 [3.28, 20.10] |
| Yamashita et al. 2008 | 5 | 39 | 0 | 53 | 0.2% | 17.06 [0.91, 318.35] |
| **Subtotal (95% CI)** | | 353 | | 669 | 15.2% | 2.40 [1.64, 3.51] |
| Total events | 119 | | 99 | | | |
| Heterogeneity: Chi² = 18.31, df = 7 (P = 0.01); I² = 62% | | | | | | |
| Test for overall effect: Z = 4.50 (P < 0.00001) | | | | | | |
| **1.1.2 CD133+ /CD44+** | | | | | | |
| Salnikov et al. 2009 | 1 | 3 | 2 | 7 | 0.4% | 1.25 [0.07, 22.88] |
| Chen et al. 2014 | 58 | 161 | 31 | 226 | 7.6% | 3.54 [2.15, 5.82] |
| **Subtotal (95% CI)** | | 164 | | 233 | 8.0% | 3.44 [2.11, 5.60] |
| Total events | 59 | | 33 | | | |
| Heterogeneity: Chi² = 0.48, df = 1 (P = 0.49); I² = 0% | | | | | | |
| Test for overall effect: Z = 4.95 (P < 0.00001) | | | | | | |
| **1.1.3 CD44** | | | | | | |
| Guo et al. 2000 | 5 | 19 | 10 | 30 | 2.6% | 0.71 [0.20, 2.55] |
| Zhang et al. 2002 | 15 | 23 | 20 | 28 | 2.9% | 0.75 [0.23, 2.46] |
| Zheng et al. 2007 | 20 | 39 | 25 | 48 | 5.1% | 0.97 [0.42, 2.26] |
| Guan et al. 2002 | 5 | 18 | 8 | 49 | 1.4% | 1.97 [0.55, 7.09] |
| Chen et al. 2014 | 65 | 234 | 24 | 153 | 9.7% | 2.07 [1.23, 3.48] |
| Liu et al, 2009 | 9 | 27 | 2 | 12 | 0.9% | 2.50 [0.45, 13.91] |
| Xu et al. 2014 | 43 | 52 | 34 | 54 | 2.7% | 2.81 [1.14, 6.96] |
| Lingala et al. 2010 | 8 | 13 | 2 | 6 | 0.5% | 3.20 [0.42, 24.42] |
| Gao et al. 2008 | 10 | 23 | 2 | 17 | 0.6% | 5.77 [1.06, 31.27] |
| **Subtotal (95% CI)** | | 448 | | 397 | 26.3% | 1.77 [1.28, 2.44] |
| Total events | 180 | | 127 | | | |
| Heterogeneity: Chi² = 9.64, df = 8 (P = 0.29); I² = 17% | | | | | | |
| Test for overall effect: Z = 3.45 (P = 0.0006) | | | | | | |
| **1.1.5 CD133** | | | | | | |
| Salnikov et al. 2009 | 1 | 4 | 3 | 6 | 0.8% | 0.33 [0.02, 5.33] |
| Liu et al, 2013 | 11 | 45 | 76 | 200 | 9.8% | 0.53 [0.25, 1.10] |
| Guo et al. 2014 | 25 | 42 | 5 | 8 | 1.6% | 0.88 [0.19, 4.19] |
| Lingala et al. 2010 | 4 | 9 | 5 | 12 | 1.1% | 1.12 [0.20, 6.41] |
| Chan et al. 2014 | 4 | 38 | 19 | 244 | 2.1% | 1.39 [0.45, 4.34] |
| Yilmaz et al. 2014 | 4 | 24 | 1 | 11 | 0.5% | 2.00 [0.20, 20.33] |
| Yeh et al. 2009 | 18 | 24 | 77 | 130 | 2.8% | 2.06 [0.77, 5.55] |
| Zeng et al. 2012 | 8 | 44 | 4 | 65 | 1.2% | 3.39 [0.95, 12.05] |
| Pan et al. 2012 | 30 | 49 | 6 | 21 | 1.5% | 3.95 [1.30, 11.95] |
| Chen et al. 2014 | 71 | 216 | 18 | 171 | 6.2% | 4.16 [2.37, 7.32] |
| Tsuchiya et al. 2009 | 2 | 9 | 1 | 22 | 0.2% | 6.00 [0.47, 76.71] |
| Wu et al. 2013 | 34 | 42 | 60 | 148 | 2.3% | 6.23 [2.70, 14.40] |
| Song et al. 2008 | 12 | 26 | 3 | 27 | 0.7% | 6.86 [1.65, 28.56] |
| Sasaki et al. 2010 | 19 | 30 | 21 | 106 | 1.6% | 6.99 [2.89, 16.90] |
| Yu et al. 2011 | 13 | 17 | 0 | 3 | 0.1% | 21.00 [0.90, 489.76] |
| **Subtotal (95% CI)** | | 619 | | 1174 | 32.6% | 2.69 [2.07, 3.49] |
| Total events | 256 | | 299 | | | |
| Heterogeneity: Chi² = 40.39, df = 14 (P = 0.0002); I² = 65% | | | | | | |
| Test for overall effect: Z = 7.47 (P < 0.00001) | | | | | | |
| **1.1.6 CD90** | | | | | | |
| Lu et al. 2011 | 9 | 43 | 8 | 16 | 4.3% | 0.26 [0.08, 0.90] |
| Liu et al, 2013 | 38 | 91 | 49 | 154 | 9.8% | 1.54 [0.90, 2.63] |
| Yilmaz et al. 2014 | 2 | 9 | 3 | 26 | 0.6% | 2.19 [0.30, 15.85] |
| Zheng et al. 2012 | 10 | 23 | 3 | 13 | 1.0% | 2.56 [0.55, 11.85] |
| Cheng et al. 2012 | 26 | 36 | 6 | 14 | 1.1% | 3.47 [0.96, 12.54] |
| Guo et al. 2014 | 24 | 33 | 6 | 17 | 1.0% | 4.89 [1.39, 17.16] |
| Yu et al. 2011 | 13 | 18 | 0 | 2 | 0.1% | 12.27 [0.50, 299.32] |
| **Subtotal (95% CI)** | | 253 | | 242 | 17.9% | 1.69 [1.14, 2.49] |
| Total events | 122 | | 75 | | | |
| Heterogeneity: Chi² = 14.70, df = 6 (P = 0.02); I² = 59% | | | | | | |
| Test for overall effect: Z = 2.63 (P = 0.009) | | | | | | |
| **Total (95% CI)** | | 1837 | | 2715 | 100.0% | 2.28 [1.96, 2.66] |
| Total events | 736 | | 633 | | | |
| Heterogeneity: Chi² = 92.49, df = 40 (P < 0.00001); I² = 57% | | | | | | |
| Test for overall effect: Z = 10.51 (P < 0.00001) | | | | | | |
| Test for subgroup differences: Chi² = 9.02. df = 4 (P = 0.06). I² = 55.6% | | | | | | |

Odds Ratio M-H, Fixed, 95% CI

0.005   0.1   1   10   200

Well and morderate    Poor

**FIGURE 2.** Plot illustrates findings from meta-analysis of associations between the positive expression of cancer stem cell markers (CD133, CD90, CD44, and EpCAM) and differentiation of hepatocellular carcinoma (HCC). Squares represent study-specific estimates (size of the square reflects the study-specific statistical weight); horizontal lines represent 95% confidence intervals (CIs); diamonds represent pooled estimates with corresponding 95% CIs. All statistical tests were 2-sided.

frequencies of CSC markers in data extracted from included studies. CD90 had a higher sensitivity of 48.22% (95% CI = 39.3–57.1) but a lower specificity 69.0% (95% CI = 62.0–76.0), whereas CD133 had a sensitivity of 41.4% (95% CI = 35.4–47.4) and specificity of 75.4% (95% CI = 71.7–77.3). CD133/CD44 and EpCAM had high specificities of 85.8% (95% CI = 81.0–90.6) and 85.2% (95% CI = 82.1–88.3), respectively, but low sensitivities of 35.98% (95% CI = 23.8–48.2) and 33.71% (95% CI = 25.2–42.2), respectively, as shown in Table 3.

## Association of CSC Markers With Other Clinicopathological Parameters

Finally, the association of CSC markers with other clinicopathological parameters was assessed. We observed a
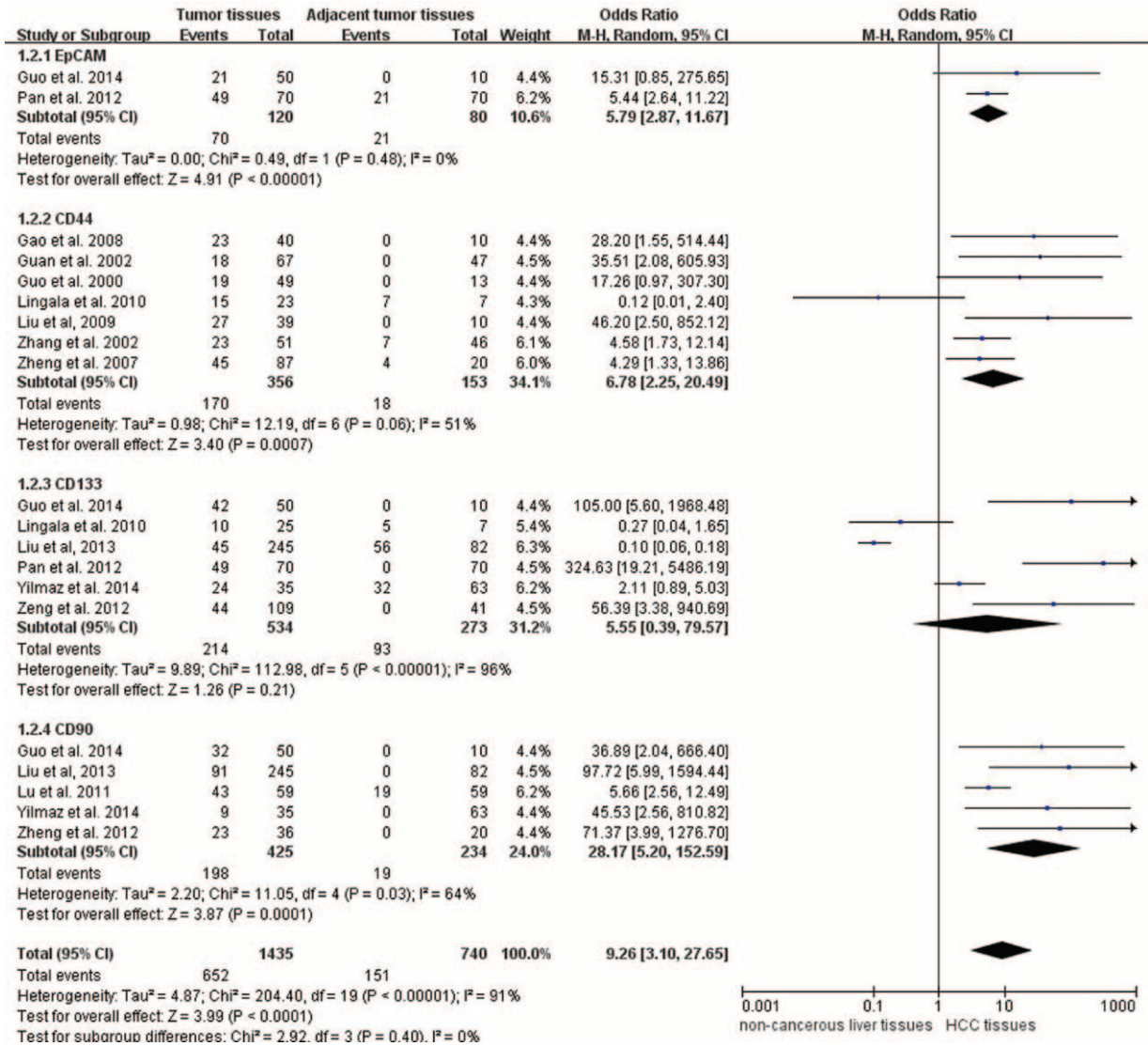
**FIGURE 3.** Plot illustrates findings from meta-analysis of the association between the positive expression of cancer stem cell markers (CD133, CD90, CD44, and EpCAM) and hepatocellular carcinoma (HCC) compared with noncancerous liver tissues.

statistically significant association of CSC markers with advanced tumor stage (pooled OR = 2.31, 95% CI = 1.39–3.84, $P < 0.00001$, random effect) (Figure S1), positive tumor capsule (pooled OR = 0.48, 95% CI = 0.24–0.91, $P = 0.04$, random effect) (Figure S2), microvascular invasion (pooled OR = 2.74, 95% CI = 1.83–4.10, $P < 0.00001$, fixed effect) (Figure S3), positive hepatitis B virus (HBV) infection (pooled OR = 1.31, 95% CI = 1.04–1.65, $P = 0.02$, fixed effect) (Figure S4), higher level of AFP (pooled OR = 1.63, 95% CI = 1.36–1.95, $P < 0.00001$, fixed effect) (Figure S5), and the presence of PVTT (pooled OR = 1.72, 95% CI = 1.02–2.90, $P < 0.00001$, random effect) (Figure S6). However, no correlations existed between the expression of CSC markers and cirrhosis (pooled OR = 1.31, 95% CI = 0.84–2.03, $P = 0.23$, random effect), positive hepatitis C virus (HCV) infection (pooled OR = 0.69, 95% CI = 0.44–1.08, $P = 0.1$, fixed effect), bigger tumor size (pooled OR = 1.10, 95% CI = 0.78–1.56, $P = 0.59$, random effect), positive metastasis (pooled OR = 3.07, 95%

CI = 0.89–10.63, $P = 0.08$, random effect), or poor Child-pugh stage (pooled OR = 0.89, 95% CI = 0.55–1.44, $P = 0.65$, fixed effect) (data not shown).

## Publication Bias and Sensitivity Analysis

The studies on HCC differentiation showed no publication bias as analyzed by Egger's test ($t$ value = −0.89, 95% CI = −22.0 to 8.64, $P = 0.38$). The funnel plot also showed no publication bias (Figure 4). We conducted a sensitivity analysis to determine the influence of individual studies on the summary effect. The meta-analysis was not dominated by any single study, and exclusion of any study at a time made no difference (data not shown).

## DISCUSSION

CSCs can be distinguished by their properties of self-renewal and differentiation and subsequently generate cancer

**TABLE 2.** Sensitivity and Specificity of CSC Markers in HCC Tissues

| CSC Marker | HCC Tissues, N | Noncancerous Liver Tissues, N | Sensitivity, % (95% CI) | Specificity, % (95% CI) |
|---|---|---|---|---|
| CD133 | | | 40.0 (33.4–46.6) | 65.9 (59.0–72.8) |
|   Positive | 214 | 93 | | |
|   Negative | 320 | 180 | | |
| CD90 | | | 46.6 (39.6–53.6) | 91.9 (88.3–95.5) |
|   Positive | 198 | 19 | | |
|   Negative | 227 | 215 | | |
| CD44 | | | 47.8 (40.3–55.3) | 88.2 (82.8–93.6) |
|   Positive | 170 | 18 | | |
|   Negative | 186 | 135 | | |
| EpCAM | | | 58.3 (46.7–69.9) | 73.8 (62.5–85.0) |
|   Positive | 70 | 21 | | |
|   Negative | 50 | 59 | | |

CI = confidence interval; CSC = cancer stem cell; EpCAM = epithelial cell adhesion molecule; HCC = hepatocellular carcinoma.

cells with heterogeneity.[37] We have systematically evaluated the association between the expression of CSC markers and HCC differentiation. In the current meta-analysis, a total of 27 studies, consisting of 2897 patients with HCC, were included. The pooled results of the meta-analysis suggest that the positive expression of CSC markers was significantly correlated with poorly differentiated HCC. Subsequent subgroup analysis also confirmed that the expression of each of the CSC markers (CD133, CD90, CD44, and EpCAM) was markedly associated with poor differentiation of HCC. These results suggest a diagnostic or predictive value of CSC markers for patients with poorly differentiated HCC.

Typically, an ideal biomarker for diagnosis should have tumor-specific expression. Therefore, to investigate the sensitivity and specificity of CSC markers in HCC tissues, we first compared the frequencies of CSC marker expression between HCC and noncancerous liver tissues. We observed that the positive expression of CSC markers was significantly associated with only HCC tissues. Furthermore, CD90 had the highest specificity of 91.9% among all the analyzed CSC markers, suggesting that it might be a specific marker for HCC in. Based

on these data, we conclude that the positive expression of CSC markers, especially CD90, may play a key role in hepatocarcinogenesis.

Recent studies have suggested induction of targeted differentiation of CSC cells.[38] Induction of differentiation in hepatic CSCs abrogates their capacity for self-renewal. In recent years, several clinical investigators have attempted to explore the role of CSC markers in the treatment of solid cancers. CD133, CD90, CD44, and EpCAM are established stem cell markers in HCC. CD133 was the first target indicated for differentiation therapy. Hepatocyte nuclear factor-4α has been shown to suppress tumorigenesis and metastasis by inducing HCC differentiation to hepatocytes via decreasing ''stemness'' of gene expression and reducing CD90 and CD133 positive cell populations.[39] NSC74859 is a specific inhibitor of STAT3 (signal transducer and activator of transcription 3) activation and suppresses carcinogeneis by reducing CD133 positive HCC cells.[40] Oncostatin M (OSM) effectively induces differentiation and active cell division of dormant EpCAM positive hepatic CSC cells.[41] Arsenic trioxide also induced cell differentiation, and thereby increased the sensitivity of hepatic CSCs to

**TABLE 3.** Sensitivity and Specificity of Single and Combined Detection of CSC Markers in Poorly Differentiated HCC

| CSC Marker | Poor Differentiation, N | Nonpoor Differentiation, N | Sensitivity, % (95% CI) | Specificity, % (95% CI) |
|---|---|---|---|---|
| CD133 | | | 41.4 (35.4–47.4) | 74.5 (71.7–77.3) |
|   Positive | 256 | 299 | | |
|   Negative | 363 | 875 | | |
| CD90 | | | 48.2 (39.3–57.1) | 69.0 (62.0–76.0) |
|   Positive | 122 | 75 | | |
|   Negative | 131 | 167 | | |
| CD44 | | | 37.4 (30.1–44.7) | 67.0 (60.9–73.1) |
|   Positive | 170 | 115 | | |
|   Negative | 284 | 234 | | |
| EpCAM | | | 33.7 (25.2–42.2) | 85.2 (82.1–88.3) |
|   Positive | 119 | 99 | | |
|   Negative | 234 | 570 | | |
| CD133/CD44 | | | 36.0 (23.8–48.2) | 85.8 (81.0–90.6) |
|   Positive | 59 | 33 | | |
|   Negative | 105 | 200 | | |

CI = confidence interval; CSC = cancer stem cell; EpCAM = epithelial cell adhesion molecule; HCC = hepatocellular carcinoma.
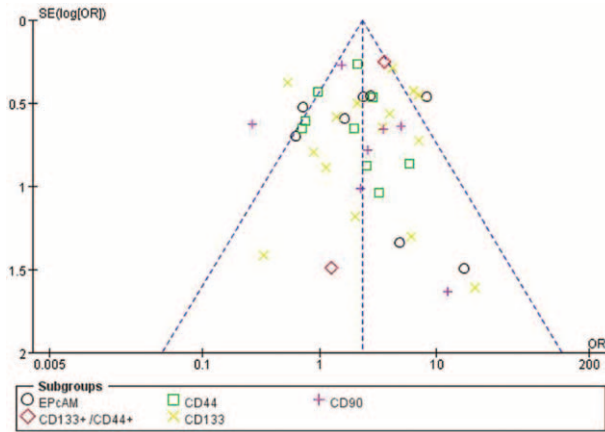
**FIGURE 4.** Funnel plot of the logarithm of the odds ratio (OR) for differentiation of hepatocellular carcinoma. The dashed line represents 95% CI. CI = confidence interval; SE, standard error.

conventional chemotherapy in HCC.[42] However, it was unclear which CSC markers were more sensitive in poorly differentiated HCC and more effective for differentiation inducing therapy. Therefore, we first elucidated the frequencies of CSC markers expressed in poorly and well-differentiated HCC tissues. The results showed that the sensitivities of CSC markers were <50% in poorly differentiated HCC tissues due to a limited number of CSC cells. However, we still believe that they have a clinical value based on their pivotal role in differentiation and progression of tumors. CD90 had the highest sensitivity of 48.22%, indicating that it might be a better candidate than other CSC markers in predicting poorly differentiated patients with HCC and for differentiation therapy.

It is well known that differentiation of HCC cells determines the pathophysiology of tumors. Based on the CSC dogma, cancer cell is differentiated from CSC and obtains the aggressive phenotype, which subsequently drives the progression and metastasis of tumor. The results of the meta-analysis suggest that CSC markers in HCC are positively associated with aggressive phenotypes, such as advanced tumor stage, positive tumor capsule, microvascular invasion, HBV infection, higher level of AFP, the presence of metastasis, and PVTT. Previous studies supported the notion that the presence of CSCs was associated with poor overall survival and disease-free survival.[43] Recently, Zhang et al indicated that CD133 overexpression is associated with poorer survival outcome in 2592 HCC patients.[44] Therefore, we speculate that positive expression of CSC markers might predict poor differentiation, aggressive phenotype and worse outcomes in patients with HCC. It is possible to distinguish and target the CSC markers in HCC to reverse the progression of disease. More evidence indicated targeted CSCs would be a promising method in cancer treatment.[45] Our results suggested that CD90 might be the more specific marker for HCC tissues and more sensitive in predicting poor differentiation in HCC, indicating that CD90 is a promising target for patient with HCC classification and differentiation therapy.

This study has several potential limitations. First, co-expression of a few CSC markers that associate significantly with HCC differentiation was not included since fewer patients manifested them. Second, only 4 CSC markers were included in this analysis as there were fewer patients available with other CSC markers. Third, noncancerous liver tissues were not further categorized into adjacent tumor tissues and normal liver tissues due to fewer numbers of studies involving normal liver tissues. Fourth, most studies included in this meta-analysis were conducted in Eastern Asia, where HCC is widely induced by HBV and HCV. Thus, our findings cannot be generalized across all populations of HCC because it can also be induced by alcohol, diabetes, and other factors. Fifth, our search strategy was restricted to articles only published in English or Chinese languages. Articles with potentially high-quality data published in other languages were not included due to anticipated difficulties in obtaining accurate medical translation.

In conclusion, we found that the positive expression of CSC markers is associated with poor differentiation of patients with HCC. CD90 might be a promising target for patient classification and differentiation therapy because of its specificity to HCC tissues and higher sensitivity in predicting poorly differentiated patient with HCC. In the future, similar to breast and lung cancer, the diagnosis and management of patients with HCC based on conventional differentiation criteria might be further classified into 2 subtypes based on the expression of CSC markers.

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