

## Original Article

# Overexpression of CD133 confers poor prognosis in colorectal cancer: a systematic review and meta-analysis

Rui Li<sup>1</sup>, Hongli Dong<sup>2</sup>, Jiabin Zhu<sup>1</sup>, Hongkui Yi<sup>1</sup>, Shengyu Liu<sup>1</sup>

<sup>1</sup>Department of Hepatobiliary Surgery, The People's Hospital of China Medical University, Shenyang, China;

<sup>2</sup>Department of Social Medical Service, Shengjing Hospital of China Medical University, Shenyang, China

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**Abstract:** Objective: CD133 is considered a useful marker to identify the so-called cancer stem cells in colorectal cancers (CRCs) and its expression has been shown to have prognostic significance in CRC patients. However, previous research studies related to CD133 expression and CRC has inconsistent results. Thus, we comprehensively reviewed the observational studies on the role of CD133 expression in patients with CRC. Methods: A systematic literature search for relevant articles published from 2010 to 2015 was conducted in PUBMED and EMBASE digital databases. A random effects model was used to quantify effect sizes, subgroup analysis for identifying potential moderating variables and Egger's test for publication bias. Results: A total of twenty-eight studies were included in this study. The results of the study demonstrated that CRC patients with high level of CD133 expression suffered the poor overall survival (RR = 0.72, 95% CI 0.58 to 0.86) and disease free survival (RR = 0.68, 95% CI 0.58 to 0.79). In subgroup analyses, different ethnicities, sample size, research technique and adjunctive therapy confirmed the stability of the relationships, patients with high level of CD133 expression got a significant poor prognosis. Besides, the survival benefit receiving adjuvant therapy appeared to be confined to those patients with low level of CD133 expression. Conclusions: Our results indicate that CD133 may have a potential predictive role of poor prognosis, and be a promising tool in the selection of adjuvant therapy for CRC patients. In consideration of the limitations and flaws of included studies, better designed RCTs are still in need to comprehensively evaluate the role of CD133.

**Keywords:** Cancer stem cell, CD133, colorectal cancer, meta-analysis

## Introduction

Cancer stem cells (CSCs) are cancer cells that possess characteristics associated with normal stem cells, specifically the ability to give rise to all cell types found in a particular cancer sample. CSCs were first identified by John Dick in acute myeloid leukemia in the late 1990s. Since the early 2000s they have been an intense cancer research focus, the CSC hypothesis has fundamental implications for cancer biology, in addition to clinical implications for cancer risk assessment, early detection, prognostication, and prevention. The hypothesis suggests that upon CSC elimination, cancer could regress due to differentiation and cell death, which has greatly changed the concept of cancer therapy. They are also believed to play a pivotal key role in resistance to chemotherapy and

radiotherapy [1, 2]. Thus, identification and characterization of CSCs could lead to development of directed and more effective treatments for cancer.

Reliable markers that identify CSCs will pave the way to better understanding of signaling pathways. CSCs represent a small subpopulation of cells within a tumor that express cell surface markers including CD133, CD44 and CD24 [3]. Among these markers, CD133 is one of the most important stem cell markers in many solid cancers such as brain tumors [4], colon cancer [5], lung cancer [6], liver cancer [7] and prostate cancer [8]. Furthermore, CD133 was expressed exclusively by stem-like cells within tumors, but was rapidly down-regulated in their progeny, illuminating that CD133+ tumor cells could be regarded as CSCs [9, 10].

At present, colorectal cancer (CRC) is among the most common malignant disease in the western world, whereas cancers of the upper gastrointestinal tract and liver are more predominant in the East. Moreover, many Asian countries have experienced a two to four fold increase in the frequency of CRC during the past few decades [11, 12]. Recently, O'Brien CA, et al. [13] reported that CD133-positive cells separated from colorectal cancer exhibited the C-IC properties of self-renewal and high tumorigenic potential. Despite a variety of basic and clinical studies on CD133 expression and CRC, using CD133 as a positive marker for CSCs generated conflicting results. Therefore, it is of virtual importance to update these findings, analyzing the association between CD133 expression and CRC. Our study may provide further insight into the anticancer mechanisms of therapeutic resistance and tumor regrowth.

### Materials and methods

#### *Literature search*

The protocol for this systematic review was based on the PRISMA statement [14]. We performed systematic literature searches of PubMed, Embase and Cochrane databases for possible publications. Reports cited the references identified in this systematic review and relevant reviews were also searched to include potentially missed studies. The following terms were used in the search procedure: ('colorectal cancer' or 'colon cancer' or 'rectal cancer' or 'colorectal adenocarcinoma' or 'colon adenocarcinoma' or 'rectal adenocarcinoma') AND ('cancer stem cell' or 'neoplastic Stem Cells') AND ('CD133' or 'prominin-1' or 'AC133'). The retrieved studies were carefully examined to exclude potential duplicates or overlapping data. Titles and abstracts of articles selected from the initial search were first scanned, and then full papers of potential eligible studies were reviewed.

#### *Study selection*

Eligibility of studies for inclusion was assessed independently by two investigators. Studies were eligible for inclusion if all the following criteria were fulfilled: (1) The study evaluated the correlation between CD133 expression and clinical outcomes of CRC. (2) Diagnosis of CRC was proven by histopathologic analysis. (3) Studies of CD133 overexpression based on CRC

tissue (via either biopsy or surgical), rather than serum or any other kinds of specimen were included. (4) The data provided must be sufficient to estimate either disease free survival (DFS) or overall survival (OS). If the data sets overlapped or were duplicated, we only extracted the most detailed or recent information. Only studies published in English were included.

#### *Excluding criteria*

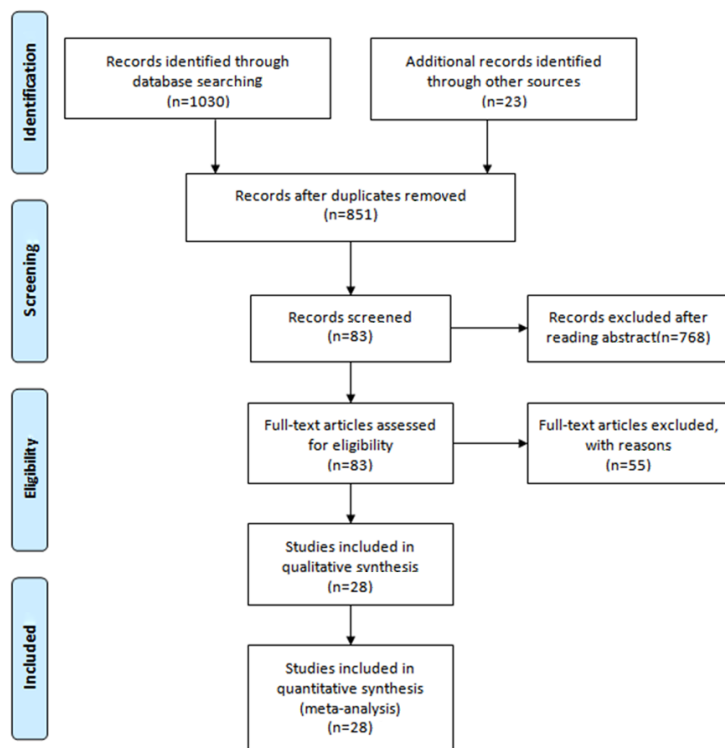
Abstracts, letters, editorials, expert opinions, reviews without original data, case reports, and studies lacking control groups were excluded. The following studies or data were also excluded: (1) Outcomes and parameters of patients were not clearly reported; (2) It was impossible to extract the appropriate data from the published results; and (3) There was an overlap between authors or centers in the published literature.

#### *Data extraction and quality assessment*

Data was extracted by two of the authors independently using the same standardized form. The fields extracted included first author, year of publication, area of research, number of patients, research techniques, and level of CD133 expression. For the articles with the same population resources or overlapping data sets, the paper which included the largest population or contained more useful information was included. In accordance with the Newcastle-Ottawa Quality Assessment Scale (NOS), the quality assessment of all included studies were performed by 2 reviewers independently. Any disagreement was resolved by a third reviewer. The scores of each study ranged between 1 and 9, and studies with the scores > 6 were recognized as of high quality. All studies in this study are higher than 6 scores.

#### *Statistical analysis*

All statistical tests were two-sided, and all statistical analyses were carried out with SPSS 16.0 and Stata Statistical Software 13.0. A random effects model was used to estimate pooled RRs in order to take into account the heterogeneity of the risk estimates and to provide more conservative estimates compared with the fixed effects model. Statistical heterogeneity between studies was assessed with the chi-square statistic and quantified by  $I^2$ , a statistic that represents the percentage of total varia-



**Figure 1.** Flow chart for included articles.

tion contributed by between-study variation [15, 16]. A significant heterogeneity was defined as a  $P$  value  $< 0.10$ . To investigate potential sources of between studies heterogeneity, subgroup analyses was conducted. Also, sensitivity analyses were carried out to assess whether the summary estimates are robust to inclusion of studies. Bias was assessed using the tests by Egger [17], and Begg, and the contour enhanced funnel plots.

## Results

### *Study selection and characteristics*

**Figure 1** summarizes the process of study identification, exclusion, and inclusion. After the removal of all studies that did not meet our criteria, 28 studies [18-45] from 1053 publications including 4546 CRC cases were finally included in our meta-analysis. Individual study characteristics are outlined in **Table 1**. Included articles were published in the period 2007-2015. The majority of the studies ( $n = 20$ ) were conducted in Asi an population. Eight studies were performed in a western population. All the 4546 CRC cases received surgical treatment. Based on the treatment method, those studies

were divided into two groups: patients with further postoperative adjuvant therapy, including radiotherapy, chemotherapy and the combination of radiotherapy and chemotherapy, and patients with no postoperative adjuvant therapy. Additionally, based on CD133 expression cut-off value, those studies were divided into two groups: patients with high level, and patients with low level. In the analyses of overall survival (OS), 23 studies were comprised with 1157 high level patients and 2344 low level patients. While in the analyses of disease free survival (DFS), 15 studies were included with 533 high level patients and 1087 low level patients. CD133 expression was measured by two methods PCR (4 studies) and immunohistochemistry (IHC) (24 studies).

### *Quantitative data synthesis*

The results of the quantitative synthesis of the data were summarized in **Table 2**. Individuals with high level of CD133 expression were significantly associated with increased risk of CRC, the relative risk values for OS and DFS were 0.72 (95% CI: 0.58-0.86) and 0.68 (95% CI: 0.58-0.79), respectively, compared with low level (**Figures 2, 3**).

The results of subgroup analyses for the association between CD133 expression and OS or DFS are demonstrated in **Table 2**. In subgroup analyses, different ethnicities, sample size, research technique and adjunctive therapy confirmed the stability of the relationship (**Supplementary Figures 1, 2, 3, 4, 5 and 6**), patients with high level of CD133 expression got a significant poor prognosis.

Additionally, CD133 Low patients could benefit from adjuvant treatments, while CD133 High patients should be given more aggressive treatments besides adjuvant therapy (**Figures 4, 5**).

### *Sensitivity analyses*

Sensitivity analysis was performed by excluding studies and the rest was analyzed sequentially

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**Table 1.** Characteristics of studies

First author	Country	Year	Cases	Age	Tumor site	Histology	Technique	Antibody used	Cut-off standard
Choi [18]	South Korea	2009	523	59.0 (17-87)	Cecum (18); Colon (255); Rectum (250)	Well (23); Mod (393); Poor (100); Un (7)	IHC	Polyclonal anti-CD133 Ab (Santa Cruz)	Cytoplasmic positivity
Kojima [19]	Japan	2008	189	62.1 ± 9.7	Colon (66); Rectum (83)	Well/Mod (160); Poor (29)	IHC	Anti-CD133 Ab (AC133, Miltenyi Biotec.)	The percentage of CD133-positive cells ≥ 10%
Li [20]	China	2009	104	ND	Colon (104)	Well (5); Mod (80); Poor (19)	IHC	Monoclonal anti-CD133 Ab (Abcam)	The percentage of CD133-positive cells ≥ 5%
Lin [21]	USA	2007	66	61.3 ± 13.5	Colon (66)	Well (4); Mod (55); Poor (7)	PCR		CD133 mRNA levels ≥ 4.79
Horst [22]	Germany	2009	110	ND	ND	G2 (99); G3 (11)	IHC	Monoclonal antiCD133 Ab (Cell Signaling Technology)	The percentage of CD133-positive cells ≥ 50%
Wang [23]	China	2009	73	50.2 ± 14.1	Rectum (73)	Well (5); Mod (39); Poor (29)	IHC	Polyclonal Ab (Abcam)	The percentage of CD133-positive cells ≥ 10%
Artells [24]	Spain	2010	64	70 (39-88)	Colon (64)	A (9); B (55)	PCR		Not known
Huh [25]	Korea	2010	61	64 (30-78)	Colon (30); Rectum (31)	Well/Mod (53); Poor (8)	PCR		Not known
Kojima [26]	Japan	2010	102	55.9 ± 11.4 57.8 ± 9.7#	Rectum (102)	Well/Mod (160); Poor (29)	IHC	Anti-CD133 Ab (AC133; Miltenyi Biotec)	The percentage of CD133-positive cells ≥ 10%
Ong [27]	Singapore	2010	501	ND	ND	ND	IHC		The percentage of CD133-positive cells ≥ 10%
Takahashi [28]	Japan	2010	151	67.1 (3-89)	Colon (99); Rectum (52)	Well (59); Mod (92)	IHC	Polyclonal anti-CD133 Ab (Abcam)	The percentage of CD133-positive cells ≥ 50%
García [29]	Spain	2011	88	66 (34-84)	Rectum (88)	ND	IHC	Polyclonal anti-CD133 Ab [AC133, Miltenyi Biotec]	The percentage of CD133-positive cells > 10%
Nagata [30]	Japan	2011	58	ND	Rectum (58)	ND	IHC	Polyclonal anti-CD133 Ab (AC133; ABGENT)	Not known
Xi [31]	China	2011	201	20-81	ND	Well (24); Mod (110); Poor (67)	IHC	Polyclonal anti-CD133 Ab (Abcam)	Final scores (multiplying the intensity of positivity and the extent of positivity scores) ≥ 5
Bonetti [32]	Italy	2012	95	69.4 ± 10.5	CRC	Well (26); Mod/Poor (69)	IHC	Polyclonal anti-CD133 Ab (Santa Cruz)	The percentage of CD133-positive cells ≥ 50%
Coco [33]	Italy	2012	137	66.8 (31-86)	Colon (137)	Well/Mod (95); Poor (42)	IHC	Polyclonal anti-CD133 Ab (Santa Cruz) monoclonal AC133 Ab (Miltenyi Biotec)	The percentage of CD133-positive cells ≥ 5%
Hongo [34]	Japan	2012	303	61.2 ± 10.1 63.4 ± 10.9#	Cecum (11); Colon (234); Rectum (58)	Well (224); Mod (69); Poor (7); Mucinous (3)	IHC	Primary anti-CD133 Ab (AC133; Miltenyi Biotec)	The percentage of CD133-positive cells ≥ 5%
Jao [35]	China	2012	233	57.11 ± 5.85 (≤ 64); 83.63 ± 5.86 (≥ 64)	Colon (157); Rectum (76)	Well (38); Mod/ Poor (195)	IHC	Monoclonal antiCD133 Ab (Clone C24B9, Cell Signaling Technology)	Immunoreactivity scores (the percentage of CD133-positive cells at each level multiplied by the corresponding intensity) > 150
Li [36]	China	2012	200	58.1 (18-85)	CRC	Well (61); Mod (93); Poor (46)	IHC	Polyclonal anti-CD133 Ab (Abcam)	Final scores (multiplying the intensity of positivity and the extent of positivity scores) ≥ 4

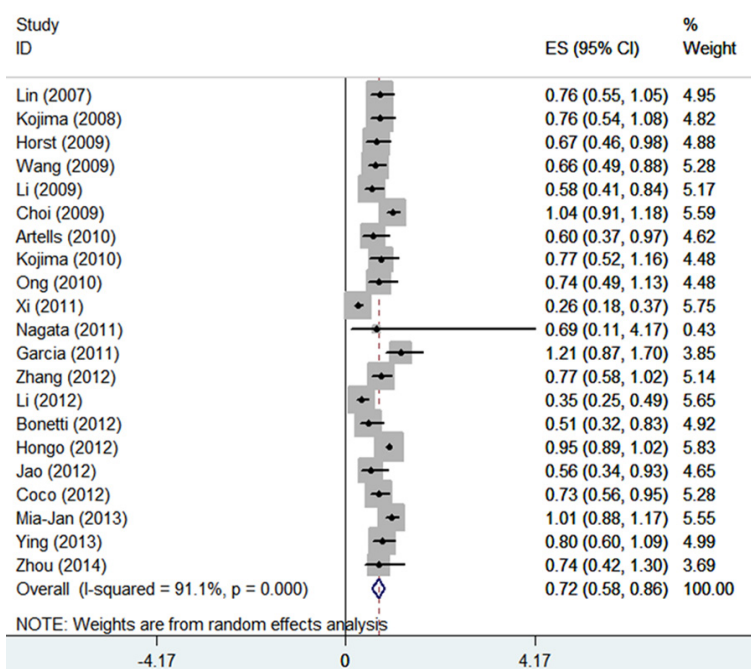
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Zhang [37]	China	2012	125	61.8	Colon (125)	Well (14); Mod (102); Poor (9)	IHC		Index sum (totaling the scores of intensity and percentages) $\geq 4$
Mia-Jan [38]	South Korea	2013	271	63.166 (27-101)	Colon (150); Rectum (121)	Well (16); Mod (225); Poor (30)	IHC	Anti-CD133 Ab (AC133, Miltenyi Biotec)	The percentage of CD133-positive cells $\geq 10\%$
Ying [39]	China	2013	176	54.9 $\pm$ 13.5	Colon (109); Rectum (67)	Well/Mod (138); Poor (38)	IHC	Monoclonal antiCD133 Ab (Cell Signaling Technology)	Using a ROC curve analysis
Antonio Oliver [40]	Spain	2014	123	71.73 $\pm$ 10.57	CRC	Well (37); Mod (59); Poor (21)	IHC	Anti-CD133 Ab (Miltenyi Biotec)	Not known
Shikina [41]	Japan	2014	234	ND	Colon (88); Rectum (61)	Well/Mod (129); Poor/Muc (20)	IHC	Anti-CD133 Ab (Clone AC133)	The percentage of CD133-positive cells $\geq 10\%$
Vaz [42]	Spain	2014	100	68 (45-92)	Colon (100)	ND	IHC	monoclonal antiCD133 Ab (Cell Signaling Technology)	The percentage of CD133-positive cells $\geq 10\%$
Zhou [43]	China	2014	60	51.6 (3268)	CRC	Well (20); Mod (20); Poor (20)	IHC	Anti-CD133 Ab (EarthOx, LLC)	The percentage of CD133-positive cells $\geq 20\%$
Hong [44]	Korea	2015	162	61 (29-85)	Colon (88); Rectum (74)	Well (19); Mod (123); Poor (20)	IHC	Anti-CD133 Ab (AC133, Miltenyi Biotec)	Scores of positive tumor cells $\geq 1$
Jing [45]	Korea	2015	36	66 (42-91)	Colon (21); Rectum (15)	Well/Mod (20); Poor (15)	PCR		CD133 mRNA levels 12675

Abbreviations: IHC, immunohistochemistry; PCR, polymerase chain reaction; CRC, colorectal cancer; ROC curve, receiver operator characteristic curve; mod, moderate.

**Table 2.** Subgroup analysis

Variables	Study number	RR	P value	Study number	RR	P value
Case number						
≥ 100	15	0.703 (0.586-0.842)	< 0.01	8	0.749 (0.615-0.913)	< 0.01
< 100	8	0.743 (0.606-0.911)	0.086	7	0.635 (0.483-0.834)	0.077
Ethnicity						
Asia	17	0.705 (0.591-0.840)	< 0.01	9	0.740 (0.586-0.933)	< 0.01
Western countries	6	0.741 (0.588-0.934)	0.034	6	0.669 (0.572-0.782)	0.531
Research technique						
IHC	21	0.716 (0.613-0.836)	< 0.01	12	0.697 (0.575-0.846)	< 0.01
PCR	2	0.704 (0.537-0.923)	0.430	3	0.721 (0.566-0.919)	0.550
Therapeutic strategy						
Adjuvant therapy	9	0.716 (0.554-0.926)	< 0.01	6	0.687 (0.554-0.852)	0.047
Non-adjuvant therapy	4	0.623 (0.481-0.807)	0.332	4	0.651 (0.519-0.817)	0.328
Not known	12	0.769 (0.631-0.937)	< 0.01	7	0.748 (0.550-1.018)	< 0.01



**Figure 2.** Forest plot of the association between CD133 expression and OS.

by meta-analysis. We performed leave-one-out sensitivity analysis by excluding a study at a time and recalculating RRs and 95% CIs.

When the studies [21-25, 29, 30, 32, 43] in which the number of cases below one hundred were excluded, sensitivity analysis showed that RR for OS was 0.73 (95% CI: 0.57-0.89), which also showed high level of CD133 expression got a significant poor prognosis. While when the studies [18, 27, 31, 34-36, 38, 41] in which the number of cases above two hundred were

excluded, sensitivity analysis showed that RR for OS was 0.71 (95% CI: 0.64-0.78), also demonstrating that high level of CD133 expression got a significant poor prognosis.

The stability of the relationship can also be observed in sensitivity analysis of DFS and other factors in the stratified analysis, suggesting the robust of our results.

*Publication bias*

Begg's funnel plot was used to check the existence of publication bias. The plot was symmetric, suggesting that the publication bias was little (Supplementary Figures 7, 8). There was no evidence of publication bias for asymmetrical shapes existed in either the OS or DFS

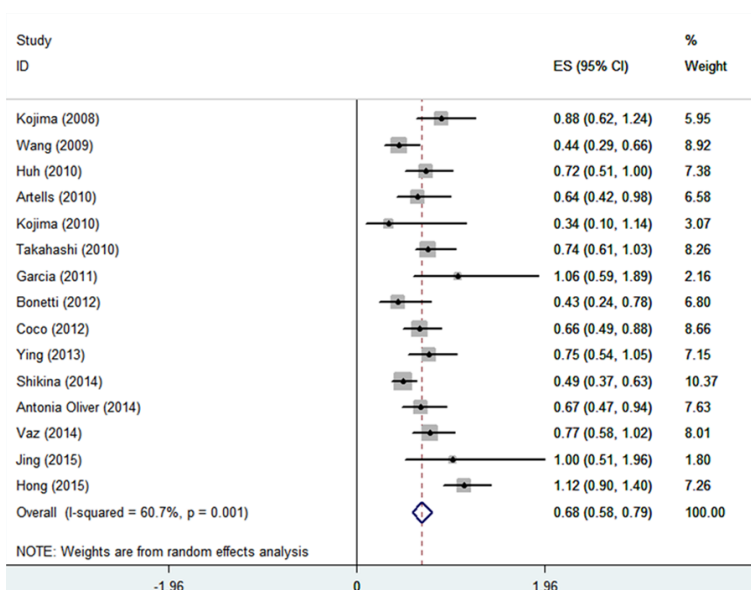
analyses (Begg's P values = 0.57 and 0.63, respectively). Thus, there was no obvious publication bias among including studies.

**Discussion**

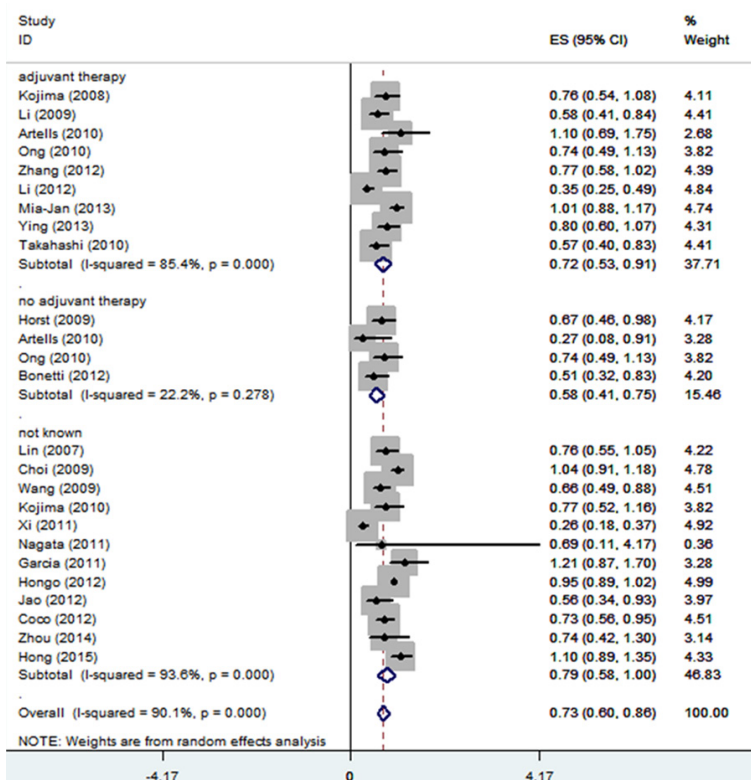
*Summary*

Nowadays, CSCs are tumorigenic (tumor-forming), perhaps in contrast to other non-tumorigenic cancer cells. CSCs may generate tumors through the stem cell processes of self-rene-

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**Figure 3.** Forest plot of the association between CD133 expression and DFS.



**Figure 4.** Subgroup meta-analysis based on adjuvant therapy between CD133 expression and OS.

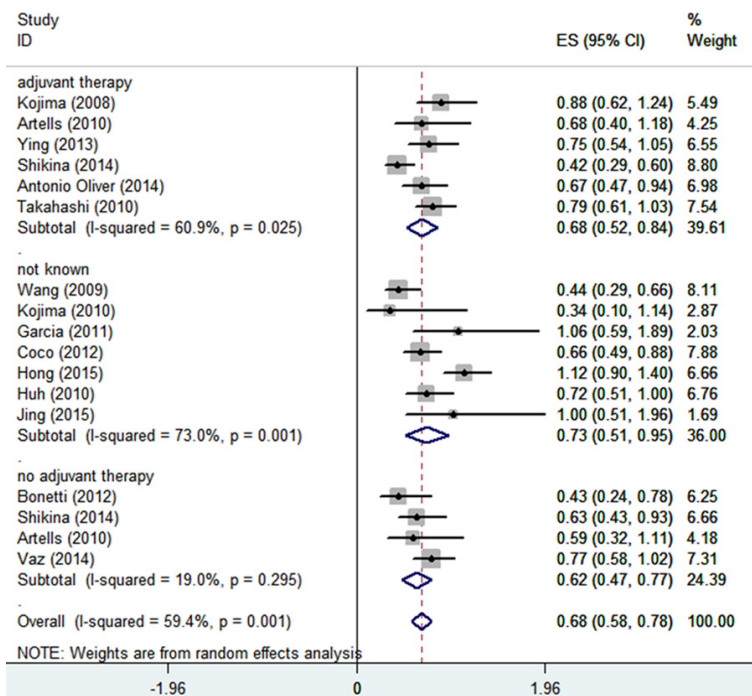
wal and differentiation into multiple cell types. Such cells are hypothesized to persist in tumors as a distinct population and cause relapse and

metastasis by giving rise to new tumors. Therefore, development of specific therapies targeted at CSCs holds hope for improvement of survival and quality of life of CRC patients. CD133 is also considered a useful marker to identify the CSCs and its expression has been shown to have prognostic significance in CRC patients.

### Relevant clinical studies

Previous studies have attempted to evaluate the role of CD133 expression and CRC histological parameters, including lymph node metastases, vascular invasion, and tumor recurrence. A study from Italy [32] discovered that a positive staining for CD133 was detected in 52% of the cases with poor prognosis and only in 9% of the group with good prognosis, and disease-free survival and cancer-specific survival of CD133 negative tumors were significantly longer compared to positive cases. These findings demonstrate that CD133 is a useful predictor of high risk progression in stage I CRC patients. Subsequently, the results of one study from China [39] revealed that CD133 expression was significantly correlated with preoperative serum carcinoembryonic antigen level and tumor differentiation grade. And high CD133 expression was identified as a significant predictor for poor disease-free survival and overall survival. But these results contradictory to another study from China [43], Zhou f et al. confirmed that no significant difference was identified between CD133-positive and -negative cases in terms of survival time. More recently, Hong l et al. [44] proposed that CD133 expression tend-

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**Figure 5.** Subgroup meta-analysis based on adjuvant therapy between CD133 expression and DFS.

ed to be stronger in primary tumor than in metastatic lymph nodes, and low CD133 expression was associated with advanced tumor stage. According to such conflicting findings, we could not reach the real relationship of CD133 expression and CRC patients prognosis.

So based on the previous literatures, we systematically reviewed the correlation between levels of CD133 expression and CRC. Then we found that CD133 expression was associated with significant differences in DFS and OS. High level of CD133 expression was of lower 5-year overall survival (OS) and disease free survival (DFS). According to stratified analysis, high level of CD133 expression patients got significantly shorter survival time compared with low level patients with or without adjuvant therapy, demonstrating that postoperative adjuvant therapy was no use for CRC patients with high level of CD133 expression. Thus these patients must need other new therapies to achieve longer survival time besides surgery and postoperative adjuvant therapy. These results suggest that level of CD133 expression is correlated with a number of adverse parameters that are traditionally associated with poor prognosis

and may be useful as a novel independent prognostic factor.

### *Mechanism in chemotherapy*

As CSCs are considered to be the driving force behind tumor growth, therapies will have to focus on strategies that include targeting of CSCs.

As we know, radio- or chemotherapy of cancer often incompletely eradicates tumor cells and this is thought to be due to a selective survival advantage of CSCs, which could explain relapse of the tumor after many years. Researchers around the world are constantly scrambling to understand the biological and molecular mechanisms. CSCs produce DNA repair proteins, which could increase their resistance towards chemotherapy. The surviving

CSCs then repopulate the tumor, causing a relapse [46]. Selectively targeting CSCs may allow treatment of aggressive, non-resectable tumors, as well as prevent metastasis and relapse.

For example, studies have shown that colon CSCs are more resistant to treatment with 5-FU or oxaliplatin [47, 48]. In addition, when CRC cell lines were treated with 5-FU or oxaliplatin in vitro, an increase in CD133+CD44+ cells was observed [49], indicating that the CSC fraction was enriched and thus resistant to these therapeutics. Recurrence of colon cancer and appearance of distant metastasis many years after initial treatment are therefore hypothesized to be caused by residual CSCs. So, by targeting the CSCs specifically, it should be possible to obtain more complete degeneration of the tumor. Obviously, combination therapies that target both CSCs and more differentiated progeny will in the end be more efficient for use in the clinic. Especially as new studies have shown that factors produced by the microenvironment can revert differentiated cells back to a more stem cell-like state [50], indicating that killing the CSCs alone might not be sufficient to diminish tumor growth.



*Limitation*

First, the numbers of the studies and patients included in the current meta-analysis are relatively small. Secondly, twenty of the studies are based on Asian population, eight from western countries. Due to lack of statistics on other countries, further studies are needed to investigate the role of CSCs in other population. As is known, there are significant differences such as etiology, biology features, clinical types, and prognosis in the risk of CRC in different ethnic groups within a given geographical area. Although in the subgroup analysis, ethnicity, sample size, and research technique did not significantly influence the prognosis value of CD133. Third, the cutoff value was defined differently (0%, 5%, 10% or 50%) in these studies, leading to between-study heterogeneity. Thus we had adopted random effect model and subgroup sensitivity analyses to adjust for the shortcomings. Next, our study didn't examine the correlation between other putative CSC markers and the risk of CRC. Finally, no attempt was made to identify unpublished work and grey literature, for example university theses or conference proceedings. As a result, publication bias may have influenced the results [51, 52]. And only English literatures were included in this study, it was possible that our findings were biased for many non-English literatures were not included. Therefore, we should consider all factors that may affect bias when explaining the pooled analysis.

In conclusion, our study revealed that patients with high level of CD133 expression got significant poor prognosis, with poor OS and DFS. The current evidence for the aforementioned adverse effects, however, is weak. More carefully designed, conducted, adequately powered studies (both RCTs and observational studies) are warranted to examine the effect on the long-term patient important outcomes.

**Disclosure of conflict of interest**

None.

**Address correspondence to:** Rui Li, Department of Hepatobiliary Surgery, The People's Hospital of China Medical University, Shenyang 110016, China. E-mail: gjh@art.edu.lv

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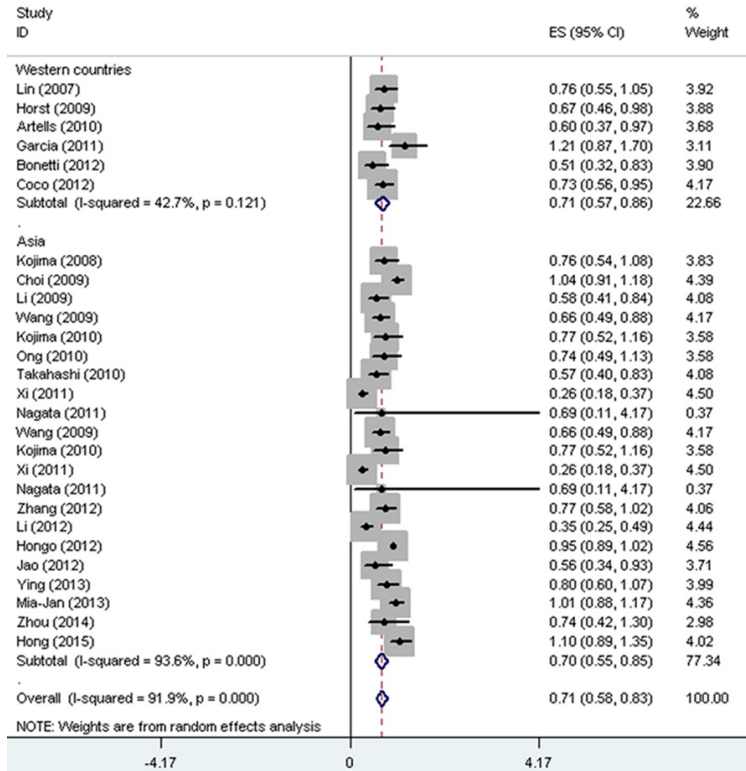
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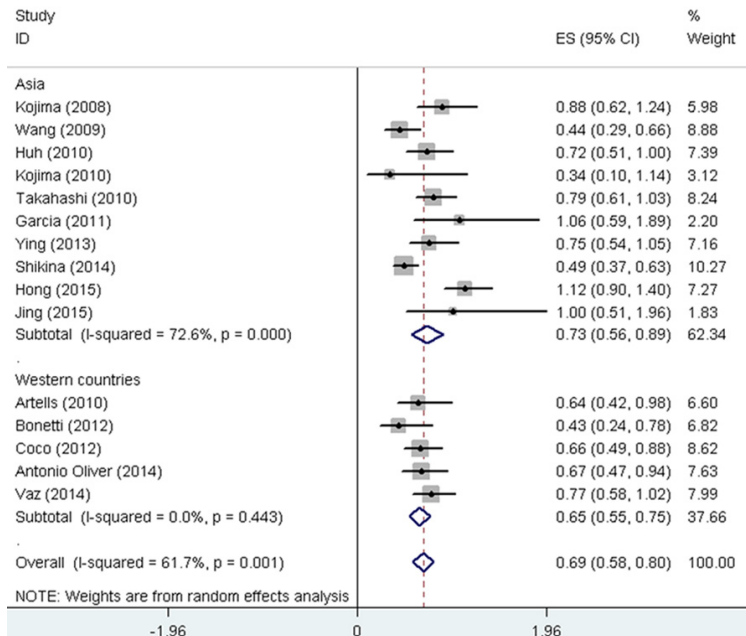
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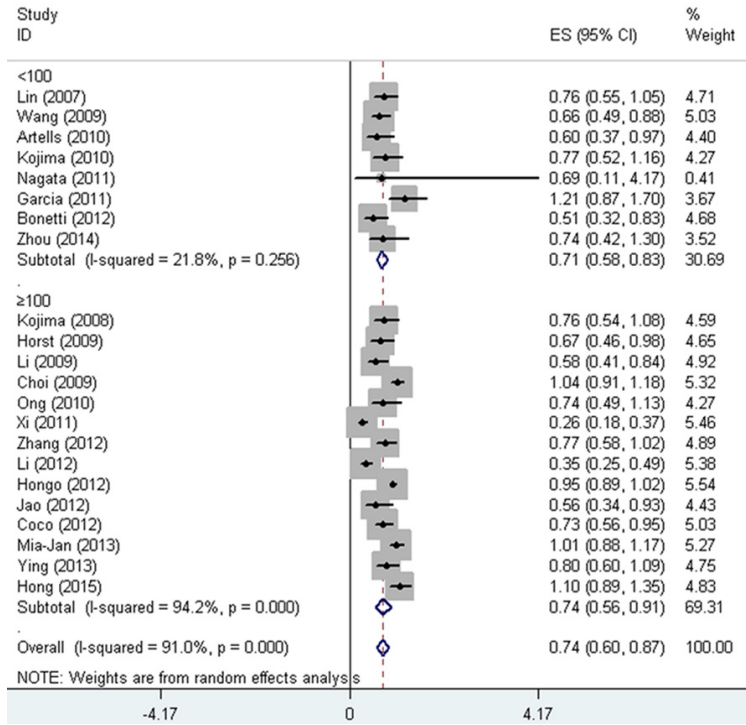


**Supplementary Figure 1.** Subgroup meta-analysis based on ethnicities between CD133 expression and OS.

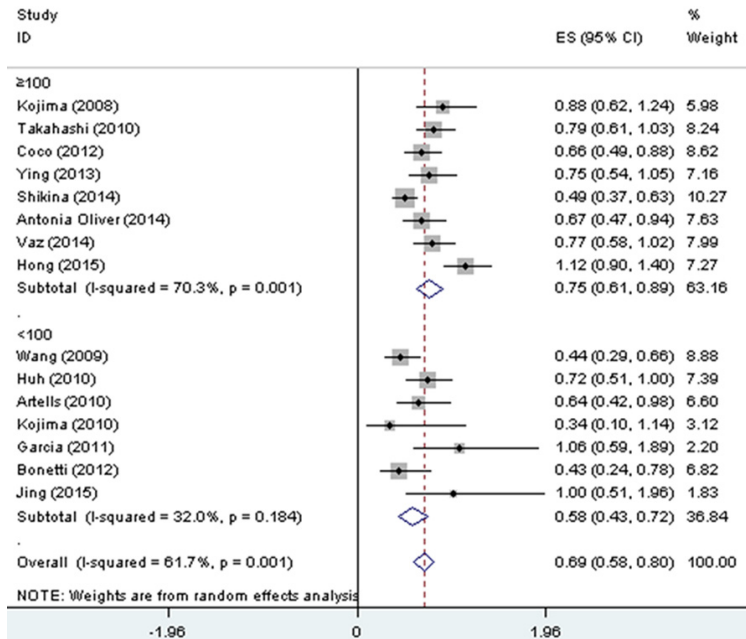


**Supplementary Figure 2.** Subgroup meta-analysis based on ethnicities between CD133 expression and DFS.

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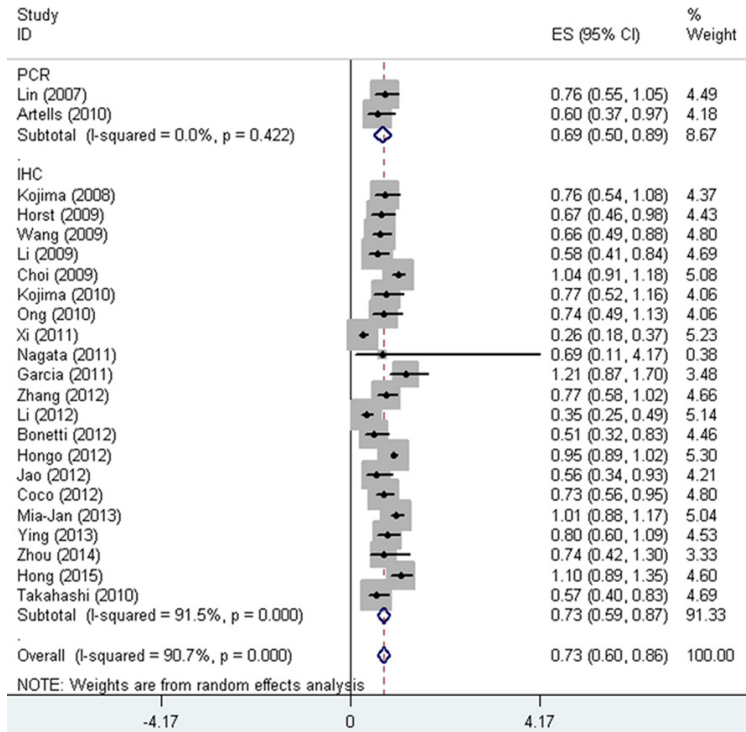


**Supplementary Figure 3.** Subgroup meta-analysis based on sample size between CD133 expression and OS.

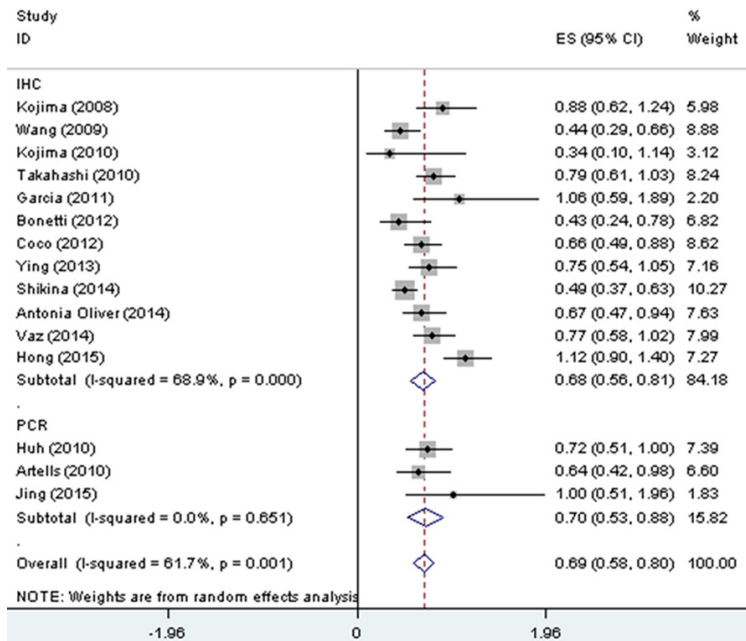


**Supplementary Figure 4.** Subgroup meta-analysis based on sample size between CD133 expression and DFS.

## CD133 and colorectal cancer

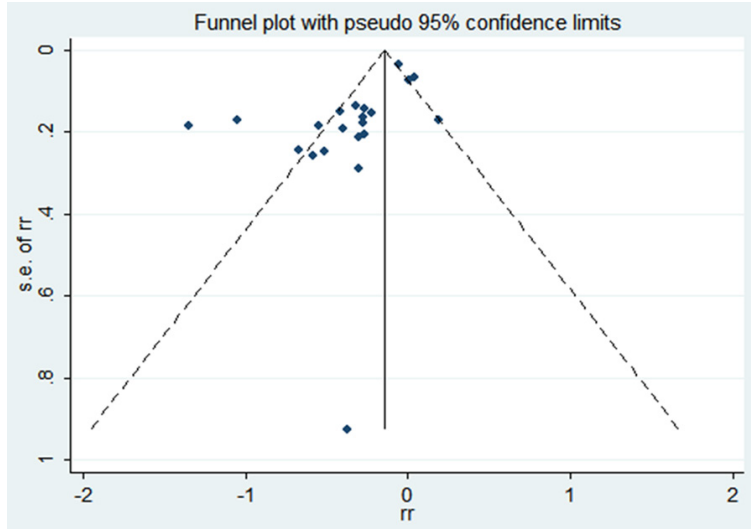


Supplementary Figure 5. Subgroup meta-analysis based on research technique between CD133 expression and OS.

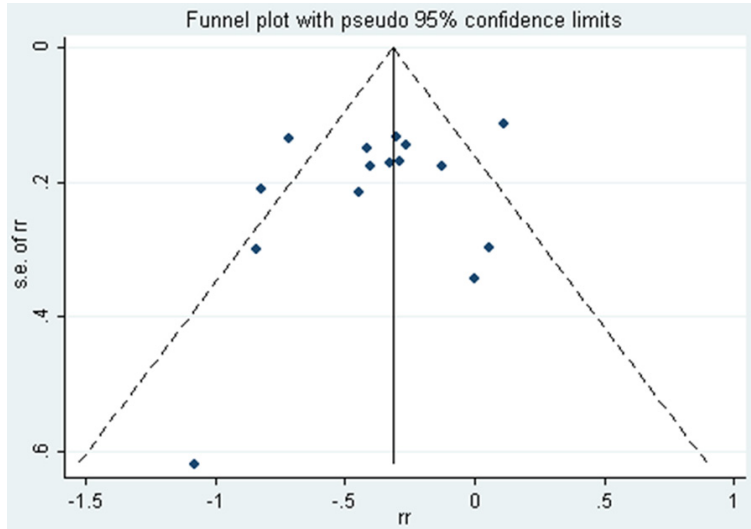


Supplementary Figure 6. Subgroup meta-analysis based on research technique between CD133 expression and DFS.

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Supplementary Figure 7. Funnel plot for publication bias test of OS.



Supplementary Figure 8. Funnel plot for publication bias test of DFS.