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Prognostic and Clinical Value of CD44 and CD133 in Esophageal Cancer: A Systematic Review and Meta-analysis

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ABSTRACT

Despite the importance of CD44 and CD133 in various cancers, the clinicopathological and prognostic values of these biomarkers in esophageal cancer remain debated. Hence, in this study, we did a meta-analysis to explore the correlation between overexpression of these markers and some clinicopathological features and their influence on the survival of esophageal cancer patients.

A search in PubMed and Web of Science (among all articles published up to January 16, 2018) was done using the following keywords: esophageal cancer, CD44, CD133, prominin-1, AC133. Suitable studies, that were selected based on the criteria listed in the Materials and Methods section, were chosen and hazard ratios with 95% confidence intervals were estimated if available. Heterogeneity and sensitivity were also analyzed. Furthermore, publication bias was assessed using funnel plots, Egger, and Begg tests.

The study included 1346 patients from 13 related studies. The median rates of marker expressions by immunohistochemistry were 35.7% (30%-76.6%) from 9 studies for CD44 and 31.9% (21%-44.2%) from 5 studies for CD133. The accumulative 5-year overall survival rates of CD44-positive and CD133-positive were 1.59% (1.22-2.06) and 1.27% (0.93-1.73), respectively. Meta-analysis showed that CD44 expression had a significant correlation with 5-year overall survival.

CD44 overexpression showed a correlation with some clinicopathological features such as lymph node metastasis, vascular invasion, and recurrence of the disease, while it was not the case for coexpression of CD44 and CD133. In conclusion, CD44 overexpression was associated with a 5-year overall survival rate and thus this biomarker can be a suitable prognostic tool in esophageal cancer.

Keywords: CD133; CD44; Clinicopathological features; Esophageal cancer; Meta-analysis; Prognosis

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INTRODUCTION

Cancer is one of the most frequent causes of death especially in developing countries, and malignancies of

gastrointestinal (GI) tract such as esophageal, stomach and colorectal cancers are among the most common cancers in these countries.¹ Esophageal cancer (EC) is the 8th most common cancer and the 6th most important reason for mortalities related to malignancies worldwide.² EC is very aggressive and is generally diagnosed at a locally advanced stage with a poor prognosis and 5-year survival rate (SR) of $\cong 20\%$.^{2,3} Esophageal cancers have two key histologic subtypes including esophageal adenocarcinomas (EACs) and esophageal squamous cell carcinomas (ESCCs), which combine to represent the majority of these cancers.³

Studies in the last two decades indicated the involvement of a specific cell population termed cancer stem cells (CSCs) in cancer initiation and progression. It is noted that CSCs with unique properties such as malignant potential and self-renewal, could be important and are considered as the main cause for drug resistance, recurrence, and metastasis of cancers.^{1,4} However, despite many studies on CSCs with GI tract origin, the exact identification of esophageal CSC markers have remained elusive.⁵ Several studies have shown that among stem cell surface markers of various cancers, CD44 and CD133 have significant prognostic values in gastrointestinal cancers.⁶⁻⁸

CD44 is a transmembrane glycoprotein involved in several pathological and physiological conditions.⁹ Studies have proposed that CD44 family proteins can mediate epithelial-mesenchymal transition (EMT).¹⁰ CD44 has a major role in remodeling and degradation of hyaluronan that leads to cancer invasion, cell migration, and metastasis.¹¹ Furthermore, in several solid tumors, CD44 is one of the most important markers to identify a subpopulation of cells with CSC properties and it is broadly known as a marker for poor prognosis in different cancers such as EC.^{5,7,12-16}

CD133 is a transmembrane glycoprotein which is also known as Prominin-1 and AC133. It is noted that the expression of this marker down-regulates quickly following cell differentiation.¹⁷ Furthermore, CD133 is another key biomarker of CSCs in various cancers such as the brain,¹⁸ colons,¹⁹ prostate,²⁰ liver,²¹ lung,²² kidneys,²³ ovaries,²⁴ and skin.²⁵ This stem cell marker is also identified as another marker for poor prognosis in many cancers.^{17,26}

The high incidence of EC in the developing world and the increase in its related death rate necessitate more studies to provide specific biomarkers that may

predict response or resistance to therapy and have prognostic values. In this study, to clarify the relationships between CSC markers CD44 and CD133 and clinicopathological features and also their prognostic values in EC based on current pieces of evidence, we performed a systematic review and meta-analysis on related published literature.

MATERIALS AND METHODS

Literature Search Strategy

A broad literature search of electronic databases PubMed and Web of Science was performed on articles published up to January 16th, 2018. Search strings were ("CD44" [Title/Abstract]) AND ("esophageal neoplasms" [MeSH Terms]) AND ("carcinoma" [MeSH Terms]) OR ("esophageal cancer" [Title/Abstract]) and ("CD133" [Title/Abstract]) OR ("AC133" [Title/Abstract]) OR ("Prominin 1" [Title/Abstract]) AND ("esophageal neoplasms" [MeSH Terms]) AND ("carcinoma" [MeSH Terms]) OR ("esophageal cancer" [Title/Abstract]), respectively for CD44 and CD133. Furthermore, the reference lists of articles were screened to find more related studies.

Study Selection

Two independent observers selected the suitable studies, and differences were decided by conversation. Titles and abstracts were assessed to recognize related literature, and the full texts of candidate publications were more assessed when required. The principles for inclusion were as follows: diagnosis of esophageal cancer was confirmed by tissue processing and histopathological methods and the study could be case-control or cohort studies, as randomized controlled or observational studies; expressions of CD44 and CD133 were investigated by immunohistochemistry (IHC) method; papers evaluating primary EC tissue (via either biopsy or surgical sampling) but not based on serum or any other types of specimen were included. All studies related to the correlation of CD44 and CD133 overexpression with clinicopathological features and disease-free and overall survival (OS) of EC were included. There was a restriction on the English language but without any limitation on the origin and minimum patient number of each unique study. If there were several articles on the same population and using equal recognition procedures, only the most recent or

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the largest publication was included. Moreover, some article types such as reviews, comments, and case reports were excluded.

Data Extraction

Two independent observers carried out the data extraction separately, and dissimilarities were resolved by a 2nd expert observer. Fact tables were made to extract all important data from texts, figures and tables of all involved studies, including the name of the first author, publication year and country, number of cases, study method, CSC markers, cutoff value, positive percentage, clinicopathological features, and related survival. In some articles that prognosis was only plotted as Kaplan-Meier curve, to digitize and extract the data, the software GetData Graph Digitizer 2.24 (<http://getdata-graph-digitizer.com/>) was used.

CD44 and CD133 Expression Status Stratification

All studied samples were derived from esophageal cancer tissues by biopsy and/or surgical resection. IHC was employed for screening the overexpression levels

of CD44 and CD133. In details, IHC on CD44 protein level was investigated in 8 included studies, IHC on CD133 protein level was investigated in 4 studies and both markers were considered in the remaining one study. The median rates of marker expressions were 35.7% (30%-76.6%) from 9 studies for CD44 and 31.9% (21%-44.2%) from 5 studies for CD133 (Table 1).

Statistical Analysis

STATA version 12.0 (StataCorp LP, Texas, USA) was used to conduct statistical calculations and hazard ratio (HR) was used as a common relationship index for all studies. To assess multivariate-adjusted HRs and its 95% confidence interval (CI), a forest-plot was drawn.

In the present meta-analysis, HR's logarithms with their standard error were utilized. Summary of HR estimates and its corresponding confidence interval were calculated using the method of DerSimonian and Laird³⁶ as well as fixed effects model and the random-effects model.

Table 1. Characteristics of included studies

No. of study	Reference	Year	Country	Cases (n)	Method	CSC marker/s	Positive percentage	Quality score
1	11	2016	Japan	47	IHC	CD44/CD133	CD44: 34% CD133: 31.9%	7
2	27	2016	Japan	56	IHC	CD44	31%	ND
3	28	2016	Korea	127	IHC	CD44	30%	8
4	29	2014	Netherlands	94	IHC	CD44	76.6%	9
5	12	2011	China	171	IHC	CD44	69.59%	ND
6	6	2004	Japan	81	IHC	CD44	46.9%	7
7	30	2000	USA	67	IHC	CD44	35.7%	ND
8	31	2000	Japan	233	IHC	CD44	30.1%	9
9	32	1997	France	66	IHC	CD44	70%	ND
10	33	2013	Japan	86	IHC	CD133	44.2%	8
11	34	2015	China	154	IHC	CD133	21%	8
12	35	2012	Japan	54	IHC	CD133	33.8%	7
13	4	2012	China	110	IHC	CD133	27.27%	ND
Summary	-	Year	Continent	Cases (n)	Method	CSC marker	Median rates	
		1997-	Asia: 10	CD44:	IHC	CD44	CD44: 35.7%	
		2016	Europe: 2	942		CD133	CD133: 31.9%	
			America: 1	CD133:				
			Africa: 0	451				

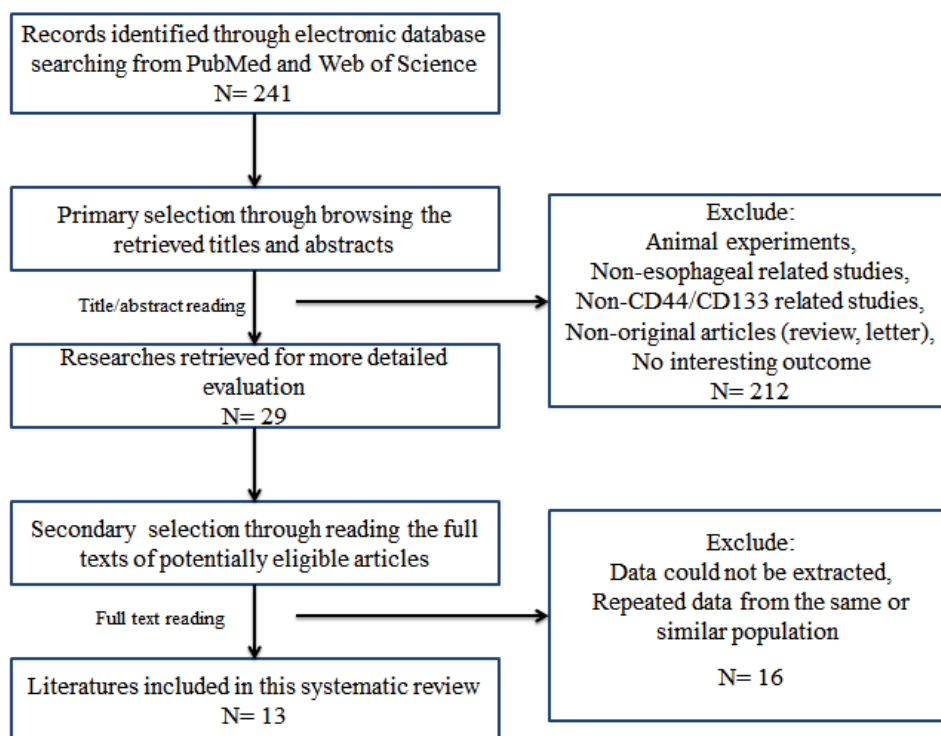


Figure 1. Flowchart for selection of articles

Statistical heterogeneity of HR between the studies was investigated using Cochran's Q test and I^2 statistic.³⁷ In case $I^2 \geq 50\%$ and $p \leq 0.05$ heterogeneity were considered statistically significant. The random-effects model was used for pooling the results of the study,³⁸ since this model takes into account the study's sample size and between studies variations.³⁶ Otherwise, a fixed-effect model was applied when there was no significant heterogeneity. Potential publication bias was assessed by visual inspection of the funnel plot. In the funnel plot, HRs were plotted against the inverse of the square of the standard error (a measure of precision). Besides, Egger's test and Begg's test were also used to evaluate publication bias. Sensitivity analysis was introduced to evaluate the influence of a single study on the overall estimate. Above all, the effects of CD44 or CD133 expression on pathological features and survival were considered as statistically significant if the pooled estimates of HR with 95% CI did not overlap the value of 1. $p < 0.05$ was considered as statistically significant.

RESULTS

Search Outcomes and Characteristics of Included Studies

Detailed search phases are shown in a flowchart (Figure 1). In the first step, 241 articles were selected according to the search strategy as mentioned before. In the next step, 212 articles were excluded owing to non-esophageal cancer types, review and letter articles, animal investigations, duplicate papers, non-CD44 and non-CD133-related studies, and not testing tumor tissues and also non-immunohistochemical research through reading titles and abstracts by two independent observers. The full texts of the remaining 29 articles were carefully considered by two observers, another 16 articles were disqualified because of insufficient or no interested outcome, repeated data from the same or similar population or the language of the publication. Eventually, 13 eligible articles were examined (Figure. 1). In general, the 13 final included studies were based on different populations, including 1 from Korea,²⁸ 1 from the Netherlands,²⁹ 1 from the USA,³⁰ 1 from France,³² 3 from China^{4,12,34} and the remaining 6 from

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Japan.^{6,11,27,31,33,35} A total of 1393 patients (942 patients for CD44 and 451 patients for CD133) with a median of 83.5 (ranged from 47 to 233) were included, most of which were male patients (68.5%) (Table 1 shows the characteristics of included studies).

CD44 and CD133 Overexpression and 5-year Overall Survival

5-year overall SR was extracted from 5 studies for CD44 and from 4 studies for CD133, all of which focused on the IHC method. The accumulative 5-year overall SR of CD44-positive and CD133-positive EC patients were 1.59 (1.22-2.06) and 1.27 (0.93-1.73), respectively (Figure 2).

CD44 and CD133 Overexpression and Clinicopathological Features

All 13 studies, 9 studies related to CD44 and 5 related to CD133 (one of them is related to both markers), presented data on clinicopathological features. However, some differences were observed in the scale of various involved studies, i.e., for tumor size, Yang et al took 4 cm as borderline while Hang et al used 5 cm, thus, a systematic review was conducted in a narrative way instead of meta-analysis. In EC patients, there were no clear associations between both CD44 and CD133 overexpression and age (11

out of 11 studies), sex (11 out of 11 studies), tumor size (4 out of 4 studies), tumor depth (5 out of 5 studies), tumor grade (1 out of 9 studies for CD44 and 1 out of 5 studies for CD133) and lymph node metastasis (1 out of 5 studies for CD44 and 3 out of 3 studies for CD44). However, overexpression of CD markers was slightly associated with TNM stage (3 out of 7 studies for CD44 and 1 out of 4 studies for CD133) and vascular invasion (1 out of 4 studies for CD44). Furthermore, only one involved study investigating the association between CD44 and recurrence showed that CD44 overexpression was significantly related to this parameter (1 out of 1 study for CD44) (Table 2).

Publication Bias and Sensitivity Analysis

A funnel plot of every 2 groups was conducted with log (HR) as the x-axis and standard error of log (HR) as the y-axis. All plots are symmetric, indicating that publication bias is low (Figure 3). The Egger and Begg's tests were also applied to examine potential publication bias. Following the results of funnel plots, little publication bias is identified ($p < 0.05$).

The range of results was between HR, 1.47 [95% CI, 1.09-1.007] and HR, 1.7 [95% CI, 1.27-2.28] for CD44 and HR, 1.15 [0.81-1.64]; HR, 1.45 [95% CI, 0.96-2.2] for CD133.

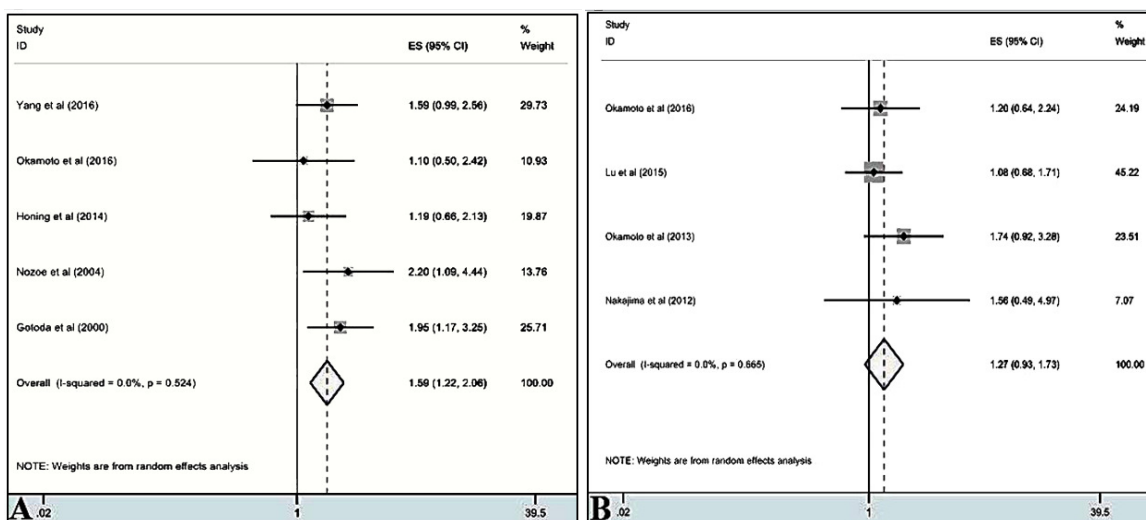


Figure 2. A meta-analysis of 5-year overall survival of CD44-positive (A) and CD133-positive (B) groups

Table 2. Description review of associations between clinicopathological features with CD44 and CD133 overexpression. Number of related references are shown in front of each CD marker.

Items	Significant correlation ($p < 0.05$)	Non-significant correlation ($p \geq 0.05$)
Age	-	CD44: 31, 29, 6, 11, 27, 28, 12 CD133: 4, 34, 33, 11
Sex	-	CD44: 31, 29, 6, 11, 27, 28, 12 CD133: 4, 34, 33, 11
Tumor size	-	CD44: 6, 28 CD133: 4, 34
Tumor Depth	-	CD44: 6, 11, 32 CD133: 34, 11
TNM stage	CD44: 6, 27, 28 CD133: 33	CD44: 29, 11, 32, 12 CD133: 4, 34, 11
Tumor grade	CD44: 12 CD133: 4	CD44: 31, 6, 11, 32, 30, 30, 27, 28 CD133: 34, 35, 33, 11
Lymph node metastasis	CD44: 6	CD44: 29, 11, 28, 12 CD133: 34, 33, 11
Vascular invasion	CD44: 6	CD44: 31, 33, 32
Recurrence	CD44: 29	-

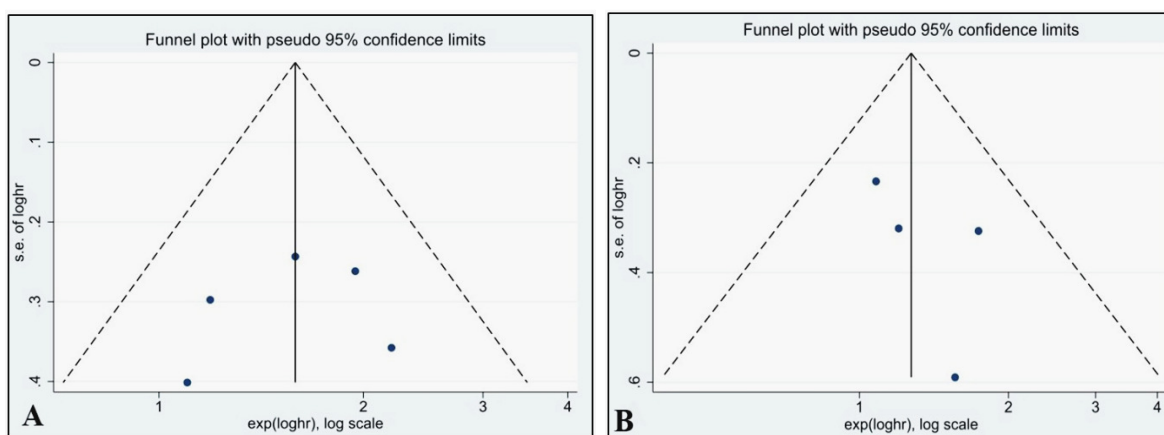


Figure 3. Funnel plot for publication bias test of CD44- (A) and CD133- (B) related studies and overall survival

DISCUSSION

In recent years, CSCs, as small subpopulations of cancerous cells that are responsible for tumor growth, invasion, metastasis, and recurrence of many kinds of solid tumors, have changed our previous understanding of cancer.⁸ According to CSC theory, tumor growth

starts with minor numbers of CSCs present in tumors. Furthermore, this theory describes some clinical facts such as drug resistance, metastasis and recurrence of cancers after primary effective chemotherapy and/or radiotherapy.³⁹ Moreover, other related studies showed that these cells may have many key properties such as distinct metabolic profile or specific surface markers

that distinguish them from other tumor cells.³⁹ Studies also revealed that the existence of stem-like cells in various cancers can be related to poor prognosis in cancer patients and associated with a high recurrence ratio.⁴⁰ However, one reliable method for identification and isolation of this CSCs is the use of particular surface markers, including epithelial cell adhesion molecule/epithelial-specific antigen (EpCAM/ESA), ABCB5, CD133, CD24, CD44, CD19, CD20, CD24, CD38 and CD90 for various tumors.⁴¹ Of course, it is of high prominence to note that there has been extensive dissimilarity over the utility of certain biomarkers for different tumor types. For example, CD133 was used in the primary investigations on colon and brain CSCs but was challenged in the following studies on these two cancers.⁴¹ Thus, in this study, we tried to overcome the conflicts and ambiguities about the importance of CD44 and CD133 as likely suitable biomarkers for EC.

Despite recent advancements in the diagnosis of gastrointestinal cancers and multimodal therapies, due to the high frequency of metastasis and therapeutic resistance, prognosis in patients with these cancers remains poor.⁴² The accurate clinical significance of CD44 and CD133, as the two most commonly used CSC markers for cancer, remains conflicting and indecisive. However, various studies showed that overexpression of these two markers is associated with certain clinicopathological features. For example, Okamoto et al showed that different expression statuses of CD44 and CD133 before therapy dictate the malignant potential of ESCC and might be considered as new prognostic markers for these patients after treatment.¹¹ On the other hand, Nakajima and colleagues reported that expression of these markers was not associated with histological properties of esophageal carcinoma, and also these expressions were not correlated to each other.³⁵ Moreover, our original article on KYSE30 cells, an esophageal squamous cell carcinoma cell line, by several experimental methods indicated that CD44 could serve as a reliable CSC marker for ESCC.⁵ On the other hand, several studies have shown that loss of CD44 expression is a prognostic factor for poor survival and also it has been related to malignant progression in EC patients.^{29,43,44} Additionally, Schizas et al revealed that lymphovascular invasion and positive lymph node ratio have a meaningful statistical association with CD44

positivity, whereas the association of tissue CD44 expression with OS and disease-free survival (DFS) was not significant.¹⁰ Liu et al revealed that reduced expression of KLF4 and higher expression of CD44 in ESCC can be associated with its incidence, prognosis and cancer development.⁴⁵ Moreover, it was suggested that CD44 and EpCAM are also reliable biomarkers to detect circulating tumor cells (CTCs) in ESCC.⁴⁶ In another study, by investigating the CD44v6 expression, as one of the CD44 variants, it was indicated that in ESCCs with overexpression of this marker, the stage of the tumor was significantly more advanced ($p=0.045$) and hence, CD44v6 overexpression can be a sign of malignant potential in EC.⁶ Moreover, it is shown that overexpression of CD44v6, as an independent prognostic indicator, is a suitable prognostic indicator of ESCC.⁴⁷ It is noted that CD44 expression can also be a good predictive biomarker for early recurrence in ESCC after chemoradiotherapy; a finding which suggests that probably CD44 is a suitable target for therapy-resistant EC cells leading to relapse.⁴⁸

On the other hand, there are currently many cancer-related studies on CD133, also termed prominin1 in a family of 5-transmembrane glycoproteins and its clinical benefits. In 2012, for the first time, Hang et al reported that CD133 expression was identified in 27.3% of ESCC patients. Furthermore, it was noted that the presence of CD133-positive cancer cells was significantly associated with tumor cell differentiation ($p=0.008$) but not related to the survival of ESCC patients ($p=0.085$) and thus not supporting its prognostic value and its importance as a CSC marker for this cancer.⁴ Mokrowiecka and coworkers proposed that CD133 positive stem cells could exist in premalignant lesions and these cells could be important for cancer progression in some types of EC.⁴⁹ By studying the samples from patients who received esophagectomy, it was shown that there is an association between the ratio of CD133-positive CSCs and 2-year recurrence.⁵⁰ Furthermore, Lu and colleagues demonstrated that no significant correlation was found between CD133 expression and prognosis and they indicated that patients with high CD133 expression had moderately lower 5-year DFS/OS. They proved that CD133 and CXCR4 may act together to facilitate the progression of ESCC and predict a poor prognosis in patients.³⁴ Another study indicated that CD133 might be important in the regulation of cancer

cell cycle through P16 and P27 and can be a good prognostic marker in ESCC patients.³³ Moreover, Wang et al showed that coexpression of CD133 and CD47 in EC cells was an independent and trusted prognostic factor for both progression-free survival and OS.⁵¹

Furthermore, so far several studies revealed that in addition to esophageal cancer, CD44 and CD133 are also detectable in other human gastrointestinal malignancies such as gastric cancer (GC) and colorectal cancer (CRC).⁵²⁻⁵⁶ For the first time, in 2009 Takaishi et al identified a subpopulation of gastric cancer cells that have CD44 expression as a specific CSC surface marker and they also showed that CD44-positive GC cells are resistant to chemoradiotherapy.⁵⁷ Jiang et al stated that CD44 and CD133 are independent biomarkers for poor survival in GC patients.⁵⁸ Furthermore, it is shown that CD44 and CD133 are overexpressed in gastric adenocarcinoma and had correlations with many clinicopathological parameters such as tumor size, cancer subtype, moderate differentiation and depth of invasion.¹⁵ However, several studies have confirmed that CD44 and its splice variants such as CD44v (v4-10, v6-10, v7-10, v8-10, v9) may play key roles in prognosis, diagnosis, and resistance or no resistance to chemoradiotherapy of GC cells and can also correlate with initiation, development, and progression of this malignancy.⁵⁹⁻⁶⁶

Furthermore, Ishigami et al revealed that even low-expression of CD133 in GC patients can be a suitable prognostic marker and this phenotype can also help to predict the risk of GC recurrence.⁶⁷ Similarly, in 2013 one systematic review published by Wen et al illustrated that GC patients with expression of CD133 had poorer prognosis, and this property was correlated with some clinicopathological parameters.¹⁷ Furthermore, Hashimoto et al showed that GC cells with cytoplasmic expression of CD133 have a high potential for malignancy, and this property was correlated with cancer relapse, progression, chemotherapy resistance, and poor prognosis.⁶⁸

In addition to EC and GC, it has been proven that the expression of CD44 and CD133 in colorectal cancer is also very important for prognosis, diagnosis and therapy of this cancer.⁶⁹ For example, Horst and colleagues examined the expression of CD44, CD166, and CD133 in CRC patients and revealed that CD133 is

a more reliable marker to predict low survival.⁵³ In another report, Choi et al studied the expression of CD24, CD44 and CD133 in 523 CRC human tissues by IHC and concluded that expression of CD44 and CD133 does not have a significant correlation with OS, but CD133 expression was correlated with T-stage and gender of patients⁵⁶. Interestingly, in this context, no relationship was found between the expression of CD133, CD44s, CD166, EpCAM, and ALDH1 and CRC patient clinicopathological parameters as reported by *Lugli* and colleagues.⁷⁰ Another study by Jing et al revealed that the expression of CD44, but not CD133, was associated with OS.⁷¹ Moreover, other studies showed that some CD44 variants such as CD44v6 and CD44v10 are detectable in colorectal adenocarcinoma patients and CD44v6 expression is correlated with poor prognosis.⁷²⁻⁷⁴ One meta-analysis which examined the association of CD44 expression and clinicopathological factors, also demonstrated that CD44 overexpression can be correlated with lymph node metastasis, distant metastasis, and poor differentiation and might be an unfavorable prognostic factor in CRC patients.⁷⁵ As for CD133, several studies revealed that its expression along with some other markers such as OCT4 or SOX2, can be suitable biomarkers for prognosis and might associate with low survival, decreased DFS, increased recurrence rate and response to chemoradiotherapy in CRC patients.⁷⁶⁻⁷⁹ Furthermore, the results of three meta-analyses also presented that CD133 overexpression is correlated with poorer 5 year OS and DFS in CRC patients and this factor can be a good predictive biomarker for poor prognosis.^{75,80,81}

Overall, this review has various restrictions. First, the number of involved studies and also the number of included esophageal cancer patients in each study are moderately small. Secondly, most clinicopathological data available in included studies were heterogeneous and for this reason, it was not suitable for meta-analysis. The third limitation is that the included studies had a large geographical dispersion so that most studies are based on Asian populations, including 6 from Japan, 3 from China, and 1 from Korea and the rest of the studies included 3 from Europe and 1 from the American continent. This is likely related to the frequency of the total number of patients identified from each continent. Of course, it should be noted that in each of these populations, in addition to abundance, many of the parameters associated with EC such as the

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site of tumor, etiology, biological features, and prognosis, are specific and different from others. Obviously, comprehensive and more detailed studies that have a larger population of patients worldwide, as well as increasing the knowledge about cancer biology and cancer stem cells, will give a more complete overview of the possible importance of CD44 and CD133 overexpression in EC related biomedical features.

Despite the mentioned restrictions, this study showed that CD44 and CD133 overexpression were associated with some clinicopathological parameters of esophageal carcinoma. Finally, CD44-, but not CD133-positive esophageal cancer patients had a worse prognosis, and CD44 overexpression was associated with poorer 5-year OS rates, and hence CD44 can be a good prognostic biomarker in EC patients. However, further studies on CD44 and CD133 and their relation to clinicopathological specifications and their potential as markers for EC prognosis in clinical practice are still essential to make a concrete conclusion.

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