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REVIEW

CD133: A cancer stem cells marker, is used in colorectal cancers

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Abstract

Colorectal cancer is one of the most common malignant tumors worldwide. A model of cancer development involving cancer stem cells has been put forward because it provides a possible explanation of tumor hierarchy. Cancer stem cells are characterized by their proliferation, tumorigenesis, differentiation, and self-renewal capacities, and chemoradiotherapy resistance. Due to the role of cancer stem cells in tumor initiation and treatment failure, studies of cancer stem cell markers, such as CD133, have been of great interest. CD133, a five-transmembrane glycoprotein, is widely used as a marker to identify and isolate colorectal cancer stem cells. This marker has been investigated to better understand the characteristics and functions of cancer stem cells. Moreover, it can also be used to predict tumor progression, patient survival, chemoradiotherapy resistance and other clinical parameters. In this review, we discuss the use of CD133 in the identification of colorectal cancer stem cell, which is currently controversial. Although the function of CD133 is as yet unclear, we have discussed several possible functions and associated mechanisms that may partially explain the role of CD133 in colorectal cancers. In addition, we focus on the prognostic value of CD133 in colorectal cancers. Finally, we predict that CD133 may be used as a possible target for colorectal cancer treatment.

Key words: CD133; Colorectal cancer; Cancer stem cells; Prognosis; Chemoradiotherapy resistance

Core tip: CD133 is not a reliable marker to identify the entire population of cancer stem cells (CSCs). However, the abundance of CD133 may be a good indicator of CSC identity and consistent with the biological characteristics of CSCs; The expression of CD133 is correlated to the poor survival; CD133(+) cells exhibit more chemoresistant behavior than CD133(-) cells; Whether CD133-targeting therapies can be a specific or efficient treatment for colorectal cancer has not been confirmed.

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INTRODUCTION

Colorectal cancer is regarded as one of the most common cancers in the world, and a main cause of cancer-related death in western countries. In spite of progressing treatments, a large percentage of advanced tumors have poor prognosis. Recent studies have shown that a small population of tumor cells, known as cancer stem cells (CSCs), may be considered the main initiators of recurrence and metastasis. It is critical to find specific bio-

markers to identify and isolate CSCs as well as to predict patient prognosis. CD133 is one of the best-characterized markers of CSCs. However, its role in colorectal cancer needs further study. Here, we have attempted to elucidate the relationship between CD133 and colorectal cancer based on the results of previous studies.

INTRODUCTION OF CD133

CD133, a five-transmembrane glycoprotein, was first found to be expressed in hematopoietic stem and progenitor cells by Yin *et al*[1]. The protein has a molecular weight of 120 kDa[1] and localizes to membrane protrusions. The protein may be expressed as one of two isoforms, CD133-1 and CD133-2. CD133-1 was the first to be discovered, by Yin *et al*[1], and it is mainly expressed in human fetal liver, bone marrow and blood. CD133-2, first cloned and identified by Yu *et al*[2], is another cell surface antigen that is recognized by anti-AC133 monoclonal antibodies. Relative to AC133-1 cDNA, a small exon of 27 nucleotides is deleted in AC133-2 by alternative mRNA splicing[2]. CD133-2 mRNA is prominently expressed in human fetal tissue, adult tissues and several carcinomas[2]. It has also been suggested that CD133-2 is expressed in multiple stem cell niches[2]. Based on these biological characteristics, CD133 is widely used to identify and isolate stem cells and cancer stem cells. However, its function is still unclear. It is hypothesized to be associated with the cell-cell interaction or signal transduction.

CANCER STEM CELLS AND CD133

Tumor cells show heterogeneity in their morphology, inheritance, functions and other characteristics. However, some tumor cells present not only heterogeneity but also hierarchy. The increasing CSCs model represents a breakthrough in explaining this phenomenon. In this model, CSCs, despite being only a small subset of cancer cells, have the capability to self-renew and sustain the tumor. These CSCs also have the ability to proliferate, resulting in expansion of the CSC pool, and to differentiate into the heterogeneous cancer cell subgroups that may not themselves be tumorigenic but usually constitute the majority of the tumor[3].

More and more studies have indicated that CD133 is a surface marker of CSCs. CD133 has been found in many tumors, including cancers of the brain[4], colon[5,6], liver[7], pancreas[8], kidney[9], lung[10], endometrium[11], ovary[12] and bone[13]. The exploration of CD133 as a surface marker of colon cancer stem cells (Co-CSCs) is still in progress. In 2007, both O'Brien *et al*[14] and Ricci *et al*[15] found that CD133(+) cells in colon cancers had the ability to initiate tumor growth. The colon cancer-initiating cells (CC-ICs) represented enrichment in CD133(+) populations. These two studies strongly support CD133 as a marker of Co-CSCs based on the evidence that CD133(+) cells could produce tumors with preserved self-renewal and differentiation capabilities and without phenotypic alterations after serial transplantation. The long-term tumorigenic potential of CD133(+) colon cancer cells has also been confirmed *in vitro*[15]. More importantly, CD133(-) colon cancer cells have no ability to form tumors. However, Shmelkov *et al*[16] discovered that CD133 was ubiquitously expressed in differentiated colonic epithelium rather than restricted to stem cells. Furthermore, *in vitro*, both CD133(+) and CD133(-) metastatic tumor subpopulations formed colonospheres. Both subpopulations maintained long-term tumorigenesis in a NOD/SCID serial xenotransplantation model. It should be noted that Shmelkov *et al*[16] used subpopulations from metastatic tumors. However, the distinction of CSCs and the function of CD133 in primary and metastatic tumors are still unknown. Despite this fact, the discovery challenged the view of CD133 as a marker of Co-CSCs, and further studies were performed to investigate the discrepancy.

Kawamoto *et al*[17] showed that although both CD133(+) and CD133(-) cells could form tumors after injection into NOD/SCID mice, the CD133(+) cells formed larger tumors. There remained a difference in these tumors. CD133(+) cells could not be found in tumors generated by the injection of CD133(-) cells but were observed in tumors from injections of CD133(+) cells, suggesting that CD133(+) cells had self-renewing capability whereas CD133(-) cells did not. The investigation is consistent with Shmelkov's finding that CD133(-) cells also have the ability to form tumors, although it seems that the CD133(+) cells were associated with stronger tumorigenesis than CD133(-) cells. Thus, the presence of CD133 may not be a reliable indicator of CSC *vs* non-CSC identity. A more appropriate distinction is the relative abundance of the CD133 protein[18]. Liao *et al*[19] attempted to confirm this hypothesis by sorting cancer cells according to the abundance of CD133. CD133 (High), CD133 (Mid), and CD133 (Low) subgroups of SW620 cells (a colorectal cancer cell line) were distinguished, and their biological characteristics were analyzed. The CD133 (High) subgroup exhibited a higher growth rate than the CD133 (Mid) and CD133 (Low) subgroups did. However, despite its much slower growth, the CD133 (Low) subgroup retained its tumorigenicity.

One likely explanation is that CD133 is expressed on not only CSCs but also differentiated tumor cells. However, during CSC differentiation, the specific epitopes recognized by AC133 are masked due to differential glycosylation[20]. The expression of CD133 could be modulated by factors in the microenvironment, such as energy supply[21]. In addition, the inactivation of CD133 during the progression of colorectal cancer can be considered a result of transcriptional repression, due to promoter hypermethylation of the CD133 CpG islands[22]. CD133(-) cells likely lack the AC133 epitopes, the expression of which is influenced by posttranslational modification under certain conditions. It appears that CD133 is not a reliable marker to identify the entire population of CSCs. However, the abundance of CD133 may be a good indicator of CSC identity.

Although some studies suggest that CD133(+) cells have characteristics consistent with those of CSCs, such as tumor initiation, proliferation, invasion, differentiation and self-renewing capacities[23-26], CD133 should be used as the sole marker of Co-CSCs with caution. Thus, additional markers for detecting CSCs and evaluating their clinical significance in colorectal cancers have been proposed. These markers include CD44[27-31], CD166[25,32], CD29[25,32,33], CD24[5,32,34], Lgr5[6,32], nuclear beta-catenin[32,35], EpCam[33,36], ALDH1[33,37], CDCP1[5], CXCR4[5] and CC188[38]. The use of the combination of these markers to identify CSCs in colorectal cancers will uncover more about the function of CSCs and will also play a significant role in clinical usage.

Some pathways, including the wingless related (Wnt), transforming growth factor-beta (TGF-β), Notch and Hedgehog signaling pathways[39], and other mechanisms have been found to be associated with CSCs and CD133 expression in colorectal cancers. The Wnt pathway plays an essential role in the growth and maintenance of CSCs[40]. This pathway is regulated at the level of β-catenin, which is degraded by adenomatous polyposis coli (APC). Mutations in the *APC* gene are found in most colorectal tumors[41]. As a result, β-catenin is accumulated in the nucleus, where it activates target genes with important functions in colorectal cancer development[42]. Some studies have confirmed the activation of the Wnt pathway in CD133(+) cells[43,44]. The TGF-β pathway acts as a tumor suppressor pathway in healthy tissues but as a promoter in colorectal cancers[45]. Mutations in the type Ⅱ receptor gene[46], type I receptor gene[45], Smad family member 4[47] and other Smads are observed in colorectal cancer specimens. Notch signaling is active in CC-ICs and is essential for the intrinsic maintenance of CC-ICs self-renewal and the repression of secretory cell lineage differentiation gene[48]. It has also been reported that the Hedgehog signaling, which is active in both colon cancer epithelial cells and, strikingly, CD133(+) cancer stem cells, promotes colon cancer growth, stem cell self-renewal and metastatic behavior in advanced cancers[49,50]. In addition, the CD133(+) CSCs may be relevant to the Ras-Raf[51,52], STAT3[53], Akt, mitogen-activated protein kinase[54], hypoxia-inducible factor-1α[55] and microRNAs[56]. Although CD133 was observed to be associated with actively proliferating cells, few studies have investigated the role of CD133 in the cell cycle. However, these studies could not explain the function of CD133 directly.

In colorectal cancer tissues, CD133 is localized to apical/endoluminal surfaces, the cytoplasm and to luminal contents[57-60]. CD133 is concentrated in plasma membrane protrusions[61], suggesting that CD133 may play a role in cell-cell and cell-matrix contact formation. CD133(+) cells have an enhanced ability to interact with adjacent carcinoma-associated fibroblasts[26,62], indicating that CD133(+) cells are more interactive with the stromal microenvironment, and thus more tumorigenic and invasive, than CD133(-) cells. Furthermore, CD133 contains a ganglioside-binding domain at its N-terminus. Through this epitope, certain gangliosides could modulate the accessibility of CD133 and regulate cell-cell contacts[63].

However, knocking down the CD133 did not affect the biological characteristics of the colon cancers, which indicated that CD133 has no obvious functions in tumor malignancy[64]. Whether CD133 has biological function remains a question, but the use of CD133 as one of the CSC markers in colorectal cancers is widely accepted.

THE PROGNOSTIC VALUE OF CD133 IN COLORECTAL CANCERS

As CD133 is a notable marker of CSC identity, it is thought to be a predictive indicator for colorectal cancer. A number of studies have demonstrated that CD133 expression was correlated with survival, recurrence, metastasis and chemotherapy resistance. Horst *et al*[60] analyzed tissues from 57 colorectal cancer patients (T2/T3, N0, and M0) using immunohistochemistry (IHC). The CD133-high patients had a worse 5- and 10-survival than CD133-low patients. Further investigations have been performed with larger sample sizes, specific tumor stage, different IHC evaluations, combinations with other markers, the use of polymerase chain reaction (PCR) and preoperative chemotherapy conditions. The majority of these results support the hypothesis that CD133 expression is predictive of survival[35,51,55,57,59,65-68]. However, Choi *et al*[34] investigated 523 colorectal cancer patients with various tumor stages using the IHC approach and reported that survival was not significantly related to CD133 expression. In addition, Kijima *et al*[69] analyzed samples from 189 patients with different stages of colorectal cancer by IHC and found that patients with and without CD133 overexpression exhibited no differences in recurrence-free survival but had significantly poorer overall survival. A summary of related studies published is presented in Table 1. Different patient patterns, study designs and the use of commercial antibodies for IHC, which may lead to high background noise, could cause the discrepancies among these studies. In studies involving IHC, different researchers used different valuation criteria. Some studies evaluated IHC results as positive or negative. Others evaluated the results according to the positivity extent. As discussed above, the abundance of CD133 may be a better indicator of CSCs than the presence *vs* absence of CD133. Despite a large sample size, Choi evaluated the presence or absence of CD133, which might not reliably indicate CSC identity. In addition, that study included patients with various stages of colorectal cancers. Those who had poorly-differentiated tumors or higher stage, especially stage Ⅳ, but were CD133 negative, could introduce significant statistical confounding. Because no biologically relevant IHC cut-off point has been established to date, most studies set the cut-off arbitrarily. The use of a receiver operating characteristic curve to determine the cut-off point has been proposed to improve the clinical utility of IHC findings[70]. All studies[51,55,59,65,67] that used PCR to measure CD133 expres-

Table 1 Studies of the relationship between CD133 expression and survival

Ref.	*n*	Cancer category	Tumor stage	Method	Did CD133 expression predict poor survival?
Choi *et al*[34]	523	Colorectal adenocarcinomas	Stages Ⅰ-Ⅳ	IHC	No: overall survival
Kemper *et al*[51]	90	Colorectal cancers	Stage Ⅱ	RT-PCR	Yes: relapse-free survival
Saigusa *et al*[55]	52	Rectal cancers (post-CRT)	Unclear	RT-PCR	Yes: recurrence-free survival
Jao *et al*[57]	233	Colorectal adenocarcinomas (post-CRT)	Stages Ⅰ-Ⅳ	IHC	Yes: overall survival
Saigusa *et al*[59]	33	Rectal cancers (post-CRT)	Stage Ⅱ/Ⅲ	RT-PCR	Yes: disease-free survival
Horst *et al*[60]	77	Colorectal adenocarcinomas	T2/T3, N0, M0	IHC	Yes: cancer-specific survival
Yasuda *et al*[65]	40	Rectal cancers (post-CRT)	Advanced	RT-PCR	Yes: disease-free survival
Li *et al*[66]	104	Colon carcinomas	Stage ⅢB	IHC	Yes: overall survival
Artells *et al*[67]	60	Colorectal cancers	Stages Ⅰ-Ⅲ	RT-PCR	Yes: overall survival
Kojima *et al*[69]	160	Colorectal cancers	Stages Ⅰ-Ⅳ (well- and moderately-differentiated)	IHC	Yes: overall survival
Kojima *et al*[69]	140	Colorectal cancers	Stages Ⅰ-Ⅳ (well- and moderately-differentiated)	IHC	No: recurrence-free survival

IHC: Immunohistochemistry; RT-PCR: Reverse-transcription polymerase chain reaction; CRT: Chemoradiotherapy.

sion at the mRNA level, concluded that CD133 expression is inversely correlated with survival. The majority of these studies included post-chemoradiotherapy patients. However, all of these studies used small sample sizes. Although some studies had deficiency in their design, majority of them came to the similar conclusion that the expression of CD133 was correlated to the poor survival, and more uniform studies should be performed to demonstrate this confusion.

CD133 can be found in not only primary tumors but also metastatic tumors such as liver metastases. It was reported that the CD133(+)/CD44(+) subpopulation was responsible for this metastasis[30,71]. Neumann *et al*[72] reported that cases with MLH1(+), CD133 (high scores) and β-catenin (high scores) tumors were associated with a very high rate of distant metastases (94.4%). Thus, it seems that CD133, in combination with other markers, may be a good predictor of metastasis risk.

Several studies have investigated whether the CD133 mRNA level in peripheral blood is useful for prognosis in colorectal cancers. Lin *et al*[73] first described that an increased level of CD133 mRNA in the peripheral blood could predict colon cancer recurrence, independent of tumor-node-metastasis stage. Pilati *et al*[68] investigated patients with liver-confined hepatic metastasis from colorectal cancers. The level of CD133, used as a marker of circulating tumor cells, increased inversely with the survival. Iinuma *et al*[74] showed that in patients with Dukes' stage B and C cancer, CEA/CK/CD133 demonstrated significant prognostic value. In contrast, no significant differences were seen in patients with Dukes' stage A disease. These studies support the use of CD133 mRNA in peripheral blood as prognostic marker. However, Gazzaniga *et al*[75] reported that there was no correlation between the expression of CD133 in circulating tumor cells isolated from peripheral blood and outcomes in patients with metastatic colorectal cancers. All these studies used an reverse-transcription polymerase chain reaction approach, but this method may have low sensitivity and lead to a high rate of false positive results in detecting circulating tumor cells[76]. Due to the limitation of this method and the inclusion of early stage (stage Ⅰ) patients with better outcomes as well as advanced stage (stage Ⅳ) patients with worse outcomes, current studies may not accurately distinguish between the outcomes in patients with early and advanced stages of colorectal cancer. However, the prognostic value of CD133 mRNA in the peripheral blood of patients with middle-stage disease (stages Ⅱ and Ⅲ) has been confirmed by some studies.

Chemoradiotherapy (CRT) resistance is a major problem that affects the survival of patients with colorectal cancer. Furthermore, the acquired resistance has a long-term memory[77]. Conventional chemotherapy targets rapidly dividing cells, but CSCs divide slowly. Therefore, CSCs are likely to contribute to the resistance of cytotoxic systemic therapies[78]. Efforts have been made to demonstrate that the role of CD133(+) colorectal tumors are more resistant to 5-fluorouracil-based chemotherapy than CD133(-) tumors. Ong *et al*[79] conducted a clinical study containing 501 primary colorectal cancer cases that provided evidence supporting this hypothesis. Moreover, recent studies showed that the level of CD133 increased in post-CRT specimens[65] and that CD133 expression was detected in 27.5% of non-CRT and 70% of CRT specimens[80]. *In vitro*, CD133 was overexpressed in human colon cancer cell lines that were resistant to 5-fluorouracil and oxaliplatin, such as HT29/5FU-R and HT29/OxR[81]. These results suggest that CD133 is a good predictor of CRT resistance. However, Hongo *et al*[82] recently reported that CD133(-) cells are more resistant to 5-fluorouracil than CD133(+) cells, which challenged the previous view. However, these authors isolated CD133(+) and CD133(-) cells from a single colorectal cancer cell line, and the original characteristics may have been altered during long-term culture. Therefore, the clinical studies are more reliable than *in vitro* studies. Meanwhile, Ong's research was conducted in a large clinical sample size, and other clinical[57,80,83] and *in vitro* studies[81,84] supported their results. Considering that Hongo's study was based on a single cell line and that no further studies have drawn similar conclusions to date, we feel it is appropriate to conclude that CD133(+) cells exhibit more chemoresistant behavior than CD133(-) cells. However, more studies, especially

clinical studies, should be performed to clarify the role of CD133 in chemoresistance.

The mechanism of chemoresistance is still under investigation. Intrinsic factors such as antiapoptotic proteins and soluble microenvironmental molecules, including growth factors and cytokines, may cause the refractoriness of CSCs. Several studies have demonstrated that the interleukin 4 (IL-4) produced by cancer cells themselves negatively regulated apoptosis[85-87]. Todaro *et al*[88] reported that IL-4 protected CD133(+) cells from apoptosis in colon cancer. They later found that treatment with an IL-4 receptor alpha chain antagonist or anti-IL-4 neutralizing antibody strongly enhances the antitumor efficacy of standard chemotherapeutic drugs through selective sensitization of CD133(+) cells[89]. These studies highlight the importance of IL-4 in chemoresistance. In addition, the secretion of IL-4 induced an immunosuppressive state in the microenvironment of the tumor, which facilitates the tumor progression. These results indicate that IL-4 may be a good target of colorectal cancer treatment.

COLORECTAL CANCER TARGET THERAPIES

There are two major targets for advanced colorectal cancer therapy: epidermal growth factor receptor and vascular endothelial growth factor. The Wnt pathway is an additional potential target. Data has shown that Wnt pathway activity could be responsible for the chemoresistance of CD133(+) cells in colorectal cancer cells. Deng *et al*[43] demonstrated that 5-fluorouracil upregulated Wnt activity in CD133(+) colon cancer stem-like cells. Dickkopf-1, an inhibitor of Wnt signaling, decreased the expression of CD133 and Lgr5. It also reduced the proliferation, migration, and invasion of colon cancer cells[90]. This indicates that blocking the Wnt pathway may be one possible solution to the problem of chemoresistance. Furthermore, other pathways, such as the Notch and Hedgehog signaling pathways involved in maintaining CSC identity, and other regulators, such as STAT3[53,91,92] and microRNAs, could be conceivable targets.

CSCs can be eliminated by targeting membrane proteins such as CD133 and then delivering medicines that can specifically induce to death. Several efforts have been made to utilize CD133 in the treatment of cancers. Damek-Poprawa *et al*[93] conjugated a CD133 monoclonal antibody (MAb) to a genetically modified cytolethal distending toxin (Cdt). The proliferation of CD133(+) cells in cell lines derived from head and neck squamous cell carcinomas was preferentially inhibited in a rate- and dose-dependent manner by the Cdt-MAb complex. Moreover, Rappa *et al*[94] decreased the number of CD133 molecules using two different short hairpin RNAs in FEMX-I melanoma cells. The cell growth, cell motility and spheroids-forming capacity were inhibited as a result of downregulation of CD133. In addition, Wang *et al*[95] investigated glioblastoma (GBM) using single-walled carbon nanotubes (SWNTs) conjugated with CD133 monoclonal antibodies. GBM cells were exposed to these SWNTs and then irradiated with near-infrared laser light. They found that the CD133(+) cells were eliminated, and the tumorigenic and self-renewal characters of CD133(+) cells were also blocked. However, some have argued that CD133(-) cells can be reversed to CD133(+) cells through microenvironmental factors and that CD133 is not the only marker able to identify CSCs, indicating that targeting CSCs through CD133 alone is not sufficient to cure tumors. Whether CD133-targeting therapies can be a specific or efficient treatment for colorectal cancer requires further exploration.

CONCLUSION

Although CD133 has been used as a CSC marker, it cannot be the only marker used to characterize CSCs in colorectal cancers. It seems that a small population of CD133(-) cells also have the biological characteristics of CSCs. Other markers should be used in combination to identify CSCs. Which combination of these markers is the best to identify CSC or shows consistent biological characters with CSC remains a question. Moreover, little is known about the function of CD133. Whether CD133 participates in the biological behavior of CSC or merely acts as a marker of the CSC phenotype is not clear. A variety of studies have confirmed the prognostic value of CD133 in colorectal cancers. However, these studies lack uniform samples, clinical conditions, methods and evaluations. Although CD133 is believed to be a target of advanced colorectal cancer, few efforts have been made to confirm this hypothesis directly. Regardless, it is desirable to explore new strategies of colorectal cancers by the use of CD133.

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