Is CD133 a biomarker for cancer stem cells of colorectal cancer and brain tumors? A meta-analysis

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ABSTRACT

Background: CD133 has been used to identify normal and cancer stem cells from several different tissues. Nowadays some researchers have reported that CD133 expression was not restricted to cancer stem cells (CSCs) of colorectal cancer and brain tumors, and CD133-negative subsets could also initiate tumors. We therefore performed a meta-analysis to assess the value of CD133 as a biomarker of CSCs for colorectal cancer and brain tumors.

Methods: A Medline search was performed to identify relevant studies for the analysis. The meta-analysis was done using RevMan 5.0 software. Outcome measures were colony formation rate and xenotransplanted tumor formation rate.

Results: Fifteen identified studies were available for analysis. For in vitro tests, there were no significant differences in the colony formation rates between CD133-positive and CD133-negative cells for colorectal cancer and brain tumors. For in vivo tests, the xenotransplanted tumor formation rate showed a significant difference between CD133-positive cells and CD133-negative cells in colorectal cancer only, corresponding to a risk difference of 0.40 (95%CI: 0.07, 0.73). Samples (cell lines versus tissues), applied biomarkers (combined versus single), and injection site were included as factors in sensitivity analyses, but the results were very inconsistent.

Conclusions: CD133 may not be suitable as a universe biomarker in identifying CSCs of colorectal cancer and brain tumors. Additional studies are necessary to further delineate its role.

Key words: Cancer stem cells (CSCs), CD133, In vitro tests, In vivo tests, Statistics, Meta-analysis

INTRODUCTION

Cancer is a major health hazard worldwide. It has been estimated that in developed countries one-fourth of deaths are closely related to malignancies (1). The cancer stem cell theory first proposed by Hamburger and Salmon (2) states that cancer stem cells (CSCs), a small subset of cells in tumors, are responsible for cancer initiation and development (2, 3). It is believed that tumors are generated and maintained by CSCs, which are capable of self-renewal and differentiating into the bulk tumor population (4, 5). Therapies targeting cancer stem cells have the potential to become an effective treatment for cancer in the future. Recent advances in stem cell research have demonstrated the existence of CSCs in neoplasia of the breast, brain, prostate, blood, lung and pancreas (6-12). Due to the paucity of CSCs in the tissue of origin and a lack of specific markers, identification of CSCs is one of the forefront topics in tumor biology research. Nowadays, 2 different approaches are typically applied to CSC identification. One is an in vitro method termed spheroid colony formation. The other is the observation of tumor formation after in vivo xenotransplantation of candidate CSCs into immunodeficient mice (13).

CD133 (also called prominin-1), which is specifically expressed in hematopoietic stem and progenitor cells from fetal and adult cord blood, peripheral blood and bone marrow, is a 5-transmembrane domain glycoprotein with a molecular weight of 117 kDa. It is a marker for murine neuroepithelial cells and several other embryonic epithelia (14-17). Although the biological function of CD133 remains unknown, as a potential stem cell marker it has been used to identify normal stem cells and CSCs in different tissues such as colon, brain, liver, blood, kidney and prostate (18-25). Nowadays, some researchers have reported that CD133 expression was not restricted to CSCs of colorectal cancer and brain tumors, and CD133 subsets could also initiate tumors (26-34).
CD133 for CSCs

Therefore, the role of CD133 in identifying and purifying CSCs of colorectal cancer and brain tumors still needs addressing. This meta-analysis aims to determine the value of CD133 as a biomarker of CSCs of colorectal cancer and brain tumors based on the methodology of evidence-based medicine.

METHODS

Search strategy and study identification

We searched the electronic database of PubMed. The reference lists from relevant articles were then screened for eligibility for the study.

For a comprehensive search, the relevant articles were then screened for eligibility for the study.

Inclusion and exclusion criteria

Original articles evaluating the effectiveness of CD133 as a marker of CSCs of either colorectal cancer or brain tumors by in vitro tests or in vivo methods were considered eligible.

The CD133+ and CD133− subpopulations in the included articles were sorted out by magnetic bead or fluorescence-activated cell sorting (FACS) and the acceptable enrichment of CD133+ cells should be more than 80% (19). For inclusion into the meta-analysis, the identified articles had to provide at least one of the following: (i) the number of colony formation and the number of total seeded cells in the CD133+ and CD133− groups; (ii) the number of immunodeficient mice with transplanted tumor formation and the total number of mice injected with CD133+ and CD133− tumor cells. For in vitro tests, single–cell-derived colonies should be equal to or more than 32 cells. For in vivo tests, the injected cells should be equal in CD133+ and CD133− groups. Studies with more cells injected but less tumor formation could also be included (this could avoid bias caused by the cell number effect). Either primary xenotransplanted tumors or serial xenotransplanted tumors were included.

The main reasons for exclusion of studies were (i) the required data could not be extracted; (ii) CD133+ and CD133− cells were not separated in the studies.

Selection, assessment and data extraction

In order to select studies for further assessment, 2 independent reviewers screened the title, abstract and keywords of every record retrieved. Full articles were assessed if the information given suggested that the study conformed to our criteria. The literature search and article selection were carried out by Yang K and Chen XZ. Any disagreements in data collection and data analysis were discussed and solved by a third reviewer as the referee.

Data were extracted independently by 2 reviewers. Details of study samples (the sources of the samples, the species of the samples, the tumor types, the applied biomarkers, and the test methods used) and outcomes (colony formation rate and xenotransplanted tumor formation rate) were extracted. Additionally, the year and country of study and the reported results of the included studies were retrieved.

Outcome of interest

The primary end points were colony formation rate or xenotransplanted tumor formation rate. One or more outcome measures should be extracted in the study; if not, the study was excluded.

Statistical analysis

Outcomes of eligible studies were statistically analyzed by Review Manager (RevMan) version 5.0 provided by the Cochrane Collaboration (35). For dichotomous variables, the pooled statistics were calculated using a fixed effects model first. The effect size (risk ratio, RR) was estimated due to the low incidence of events in either group, the risk difference (RD) was calculated instead. The Mantel-Haenszel test was used to test significance, with p<0.05 being considered statistically significant.

Heterogeneities of treatment effects between studies were tested using a chi-squared statistic with significance being set at p<0.10, and I-square was used to estimate the total variation across studies that was due to heterogeneity rather than chance; <25% was considered low-level heterogeneity, 25% to 50% moderate-level heterogeneity, and >50% high-level heterogeneity (36).

If heterogeneity existed, one of the following techniques was used to attempt to explain it: 1) random effect model for meta-analysis; 2) subgroup analysis stratified by tumor type; 3) sensitivity analysis.
RESULTS

Included literature

There were 640 identified studies in total and the selection was performed according to the inclusion/exclusion criteria stated above. Only 135 identified studies concerning colorectal cancer and brain tumor CSCs were further evaluated. Ninety-five studies were excluded in the primary selection and 25 identified studies were excluded in the secondary selection (7, 26-30, 37-55). The study selection procedure and the reasons for exclusion are summarized in Figure 1.

Only 15 identified studies comparing the colony formation rates or xenotransplanted tumor formation rates between CD133+ and CD133- cells met the inclusion criteria (18, 19, 31-34, 56-64). The detailed characteristics of these studies are listed in Table I. The CD133- subgroup was considered as the control group in the meta-analyses.

The studies were carried out in Japan, Italy, China, Germany, Canada, USA, Korea and France.

Pooled estimates of in vitro tests

Although the difference was marginal, our results showed significantly higher xenotransplanted tumor formation rates by CD133+ cells in colorectal cancer, corresponding to an RD of 0.40. This indicated that in the xenotransplantation models of colorectal cancer, CD133+ cells could form more transplanted tumors in immunodeficient mice than CD133- cells. However, xenotransplant-

TABLE I - CHARACTERISTICS OF STUDIES INCLUDED IN THE META-ANALYSIS

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Samples</th>
<th>Species</th>
<th>Cancer</th>
<th>Biomarkers</th>
<th>In vitro tests</th>
<th>In vivo tests</th>
<th>Favor CD133</th>
</tr>
</thead>
<tbody>
<tr>
<td>O'Brien 2007 (18)</td>
<td>Canada</td>
<td>Tissues</td>
<td>Human</td>
<td>Colorectal cancer</td>
<td>CD133</td>
<td>No</td>
<td>Yes</td>
<td>+</td>
</tr>
<tr>
<td>Ricci-Vitiani 2007 (19)</td>
<td>Italy</td>
<td>Tissues</td>
<td>Human</td>
<td>Colorectal cancer</td>
<td>CD133</td>
<td>Yes</td>
<td>Yes</td>
<td>+</td>
</tr>
<tr>
<td>Beier 2007 (31)</td>
<td>Germany</td>
<td>Tissues</td>
<td>Human</td>
<td>Glioblastoma</td>
<td>CD133</td>
<td>Yes</td>
<td>Yes</td>
<td>-</td>
</tr>
<tr>
<td>Ogden 2008 (32)</td>
<td>USA</td>
<td>Tissues</td>
<td>Human</td>
<td>Brain tumors</td>
<td>A2B5/CD133</td>
<td>No</td>
<td>Yes</td>
<td>-</td>
</tr>
<tr>
<td>Du 2008 (33)</td>
<td>China</td>
<td>Tissues</td>
<td>Human</td>
<td>Colorectal cancer</td>
<td>CD133/CD44</td>
<td>Yes</td>
<td>Yes</td>
<td>-</td>
</tr>
<tr>
<td>Joo 2008 (34)</td>
<td>South Korea</td>
<td>Tissues</td>
<td>Human</td>
<td>Glioblastoma</td>
<td>CD133</td>
<td>Yes</td>
<td>Yes</td>
<td>-</td>
</tr>
<tr>
<td>Qiang 2009 (56)</td>
<td>China</td>
<td>Cell lines</td>
<td>Human</td>
<td>Glioblastoma</td>
<td>CD133</td>
<td>Yes</td>
<td>Yes</td>
<td>+</td>
</tr>
<tr>
<td>Todaro 2007 (57)</td>
<td>Italy</td>
<td>Tissues</td>
<td>Human</td>
<td>Colorectal cancer</td>
<td>CD133</td>
<td>Yes</td>
<td>Yes</td>
<td>+</td>
</tr>
<tr>
<td>Haraguchi 2008 (58)</td>
<td>Japan</td>
<td>Cell lines</td>
<td>Human</td>
<td>Colorectal cancer</td>
<td>CD133/CD44</td>
<td>No</td>
<td>Yes</td>
<td>-</td>
</tr>
<tr>
<td>Beier 2008 (59)</td>
<td>Germany</td>
<td>Tissues</td>
<td>Human</td>
<td>Oligodendrogial tumors</td>
<td>CD133</td>
<td>Yes</td>
<td>Yes</td>
<td>+</td>
</tr>
<tr>
<td>Ieta 2007 (60)</td>
<td>Japan</td>
<td>Cell lines</td>
<td>Human</td>
<td>Colorectal cancer</td>
<td>CD133</td>
<td>Yes</td>
<td>Yes</td>
<td>+</td>
</tr>
<tr>
<td>Ping 2007 (61)</td>
<td>China</td>
<td>Cell lines</td>
<td>Human</td>
<td>Glioma</td>
<td>CD133</td>
<td>Yes</td>
<td>Yes</td>
<td>+</td>
</tr>
<tr>
<td>Dittfeld 2009 (62)</td>
<td>Germany</td>
<td>Cell lines</td>
<td>Human</td>
<td>Colorectal cancer</td>
<td>CD133</td>
<td>Yes</td>
<td>Yes</td>
<td>-</td>
</tr>
<tr>
<td>Chen 2010 (63)</td>
<td>USA</td>
<td>Tissues</td>
<td>Human</td>
<td>Glioblastoma</td>
<td>CD133/PTEN</td>
<td>Yes</td>
<td>Yes</td>
<td>-</td>
</tr>
<tr>
<td>Tchoghandjian 2010 (64)</td>
<td>France</td>
<td>Tissues</td>
<td>Human</td>
<td>Glioblastoma</td>
<td>A2B5/CD133</td>
<td>Yes</td>
<td>Yes</td>
<td>-</td>
</tr>
</tbody>
</table>
pared to the primary results, whether single biomarker or combined biomarkers were used. However, when combined biomarkers were used, the results of in vitro tests and in vivo tests did not favor CD133 as a marker of colorectal CSCs. Injecting CD133\(^+\) colorectal cancer cells into the renal capsule seemed to induce more xenotransplanted tumor formation than CD133\(^-\) cell injection. Nevertheless, the xenotransplanted tumor formation rate did not show any significant difference between the CD133\(^+\) and CD133\(^-\) subgroups when the cells were injected subcutaneously.

**DISCUSSION**

There is increasing evidence that cancers contain a...
small subset of CSCs. Whilst there are similarities between normal stem cells and CSCs, it remains unclear to what extent the properties of self-renewal and multipotency are being shared by the 2 types of stem cells.

The identification of CSCs is an exciting area of cancer research. Not only does it allow a better understanding of tumor formation, it also implies a potential of developing therapies that target cells causing the cancer. Once the CSCs are isolated, we can further study their properties, such as gene profile and drug resistance. Such information may become a key factor in the discovery of new drugs for treating cancer (65).

Despite the fact that rare cells which were distinct from the bulk of the tumor in driving tumor growth and maintenance have been isolated with a remarkable potential for self-renewal and multipotency by using CD133 in malignancies (8, 18-25), the functional aspects of CD133 and its efficacy as a marker for CSCs have been conjectural. An important notion of the current studies is that CD133 is of functional importance for cancer initiation and progression. Nevertheless, controversy exists in the aspects of CD133 as a marker for CSCs of colorectal cancer and brain tumors.

In this study, 2 different approaches identifying CSCs have been used to examine the efficacy of CD133 as a marker for CSCs of colorectal cancer and brain tumors. According to the pooled results of in vitro tests, CD133+ cells could not generate more colonies than CD133- cells in colorectal cancer and brain tumors, indicating that CD133 is unlikely to be an important marker of CSCs in colorectal cancer and brain tumors.

| TABLE III - RESULTS OF SENSITIVITY ANALYSIS |
| Number of | CD133+ n/N | CD133- n/N | RR/RD (95% CI) | P value for effect size | P value for heterogeneity | Effect model |
| In vitro tests | | | | | | |
| Cell lines | | | | | | |
| In vitro tests | | | | | | |
| Colorectal cancer | 1 | 175/1000 | 35/1000 | 5.00 (3.52, 7.11) | <0.00001 | Not applicable | Fixed |
| Brain tumors | 1 | 285/5000 | 115/5000 | 2.48 (2.00, 3.07) | <0.00001 | Not applicable | Fixed |
| In vivo tests | | | | | | |
| Colorectal cancer | 3 | 22/25 | 19/24 | 0.08 (-0.07, 0.24) | 0.30 | 0.13 | Fixed |
| Brain tumors | 1 | 5/5 | 2/5 | 0.60 (-0.06, 1.26) | 0.07 | Not applicable | Fixed |
| Tissues | | | | | | |
| In vitro tests | | | | | | |
| Colorectal cancer | 1 | 23/312 | 20/228 | 0.84 (0.47, 1.49) | 0.55 | Not applicable | Fixed |
| Brain tumors | 1 | 11/100 | 1/100 | 0.10 (0.04, 0.16) | 0.002 | Not applicable | Fixed |
| In vivo tests | | | | | | |
| Colorectal cancer | 4 | 77/137 | 4/135 | 0.58 (0.20, 0.96) | 0.003 | <0.00001 | Random |
| Brain tumors | 5 | 22/33 | 31/47 | 0.17 (-0.40, 0.73) | 0.56 | <0.00001 | Random |
| Combined biomarkers | | | | | | |
| In vitro tests | | | | | | |
| Colorectal cancer | 1 | 23/312 | 20/228 | 0.84 (0.47, 1.49) | 0.55 | Not applicable | Fixed |
| Brain tumors | 1 | 11/100 | 1/100 | 0.10 (0.04, 0.16) | 0.002 | Not applicable | Fixed |
| In vivo tests | | | | | | |
| Colorectal cancer | 2 | 4/33 | 3/33 | 0.03 (-0.13, 0.19) | 0.71 | 0.89 | Fixed |
| Brain tumors | 3 | 9/10 | 20/21 | -0.05 (-0.36, 0.27) | 0.77 | 0.62 | Fixed |
| Single biomarker | | | | | | |
| In vitro tests | | | | | | |
| Colorectal cancer | 1 | 175/1000 | 35/1000 | 5.00 (3.52, 7.11) | <0.00001 | Not applicable | Fixed |
| Brain tumors | 2 | 296/5100 | 116/5100 | 0.06 (-0.00, 0.12) | 0.07 | 0.05 | Random |
| In vivo tests | | | | | | |
| Colorectal cancer | 5 | 95/129 | 20/126 | 0.54 (0.13, 0.95) | 0.009 | <0.00001 | Random |
| Brain tumors | 3 | 18/28 | 13/31 | 0.49 (-0.38, 1.36) | 0.27 | <0.00001 | Random |
| Injection site: subcutaneous | | | | | | |
| In vivo tests: | | | | | | |
| Colorectal cancer | 6 | 74/112 | 23/109 | 0.38 (-0.02, 0.78) | 0.07 | <0.00001 | Random |
| Injection site: renal capsule | | | | | | |
| In vivo tests: | | | | | | |
| Colorectal cancer | 1 | 25/50 | 0/50 | 0.50 (0.36, 0.64) | <0.00001 | Not applicable | Fixed |

aThe effect measures were relative risk (RR) in case of a relatively high incidence, and otherwise relative difference (RD).
cancers and brain tumors. However, since the required data could not be extracted in most of these in vitro studies, only a limited number of studies of in vitro tests were included and our findings may have been biased. However, we could consult the results of in vivo tests for reference. In in vivo tests, there was no significant difference in xenotransplanted tumor formation rates between CD133⁺ cells and CD133⁻ cells for brain tumors. On the other hand, CD133⁺ cells were found to have formed more xenotransplanted tumors than CD133⁻ cells in the context of colorectal cancer. Nevertheless, the difference between CD133⁺ and CD133⁻ cells in colorectal cancer subgroups was only marginal. As mentioned above, most studies were excluded because the wanted data could not be extracted; some of these studies disfavored CD133⁺ cells as colorectal CSCs. In addition, with respect to the marginal difference between CD133⁺ cells and CD133⁻ cells in colorectal cancer for in vivo tests and the pooled results of colony formation of colorectal cancer, we believe that CD133 may not be a universal biomarker for colorectal CSCs. As a matter of fact, studies pointing against CD133 as a marker of CSCs were mainly focused on colorectal cancers and brain tumors after literature searches. From the results of this study and previous published reports, we also found CD133 not a suitable biomarker for identifying CSCs of brain tumors. However, due to the relatively small number of studies included in this meta-analysis, more well-designed experiments are needed to explore the effectiveness of CD133 as a biomarker of CSCs for colorectal cancer and brain tumors.

The results of our research turned out to be dispersive, which may be related to factors such as the injection site and/or the applied biomarkers. The differences may also be derived from the different biological features of the tumors. The antibodies routinely used for purification of CD133⁺ cells targeting poorly characterized glycosylated epitopes of uncertain specificity may be another reason (28). One study found recently that CD133 was expressed in both CSC and differentiated tumor cells, with only the AC133 epitope being lost upon CSC differentiation (55). This may be another clue to explain the inconsistent results. From this meta-analyses and previous research, we noticed that results disfavoring CD133 as a marker were usually reported when combined biomarkers were used. So if single CD133 marker was used to identify CSC which indeed had combined markers (such as CD133/CD44), other biomarkers might bias the outcomes as concealed confounding factors. In the present analysis, only studies with an equal amount of injected cells in the CD133⁺ and CD133⁻ groups and studies with more cells injected but less tumor formation were included, which could avoid bias caused by a difference in the number of injected cells between CD133⁺ and CD133 groups.

There are some limitations to this meta-analysis. First, the number of included studies was rather small, especially for in vitro tests. Second, there were considerable variations regarding tissue sources (primary tumors or metastases), choice of antibodies, cell sorting methods and cell purification techniques among the selected studies. Pooling data from these studies might lead to relatively high heterogeneities, as reflected in our results. However, these are inevitable in meta-analyses of experimental studies. We have adopted random effect model and subgroup analyses to adjust for the shortcomings. Although there was relatively high heterogeneity across the identified studies, the 2 subgroups (CD133⁺ and CD133⁻) were under the same conditions in each included study. So the results and data of each study were reliable. Putting these data into pooled analyses was still acceptable. Third, not only the number of implanted tumors is crucial as an end point, but tumor size and time until tumor formation are also important for reference. However, these data were largely unavailable from the selected studies. Fourth, as in most meta-analyses, these results should be interpreted with caution because the tumor samples used in these studies were not uniform and there were differences in biological properties, stage, patient ethnicity, etc. Finally and most importantly, some studies that disfavored CD133 as a marker of CSCs were excluded since the data could not be extracted successfully. Despite these limitations, by developing a detailed protocol before initiating the study, performing a cautious search for published studies, and using objective methods for study selection, data extraction, and data analyses, we have minimized the probability of bias as far as possible.

In conclusion, CD133 may not be suitable as a universal biomarker in identifying CSCs in colorectal cancers and brain tumors. Additional studies are warranted to further delineate its role.

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Abbreviations

CSCs: cancer stem cells
FACS: fluorescence-activated cell sorting
RR: risk ratio
RD: risk difference

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