Abstract. Background/Aim: The cancer stem cell model suggests that only a rare subpopulation, known as cancer stem cells (CSC) are responsible for tumor initiation. CSC from several human carcinomas are characterized by specific cell surface markers, such as CD133. The CD133 role in colon tumorigenesis remains controversial. Materials and Methods: CD133 was evaluated by immunohistochemistry in a mouse model of colitis-related colon tumorigenesis induced by a combined treatment with azoxymethane (AOM) and dextran sodium sulphate (DSS). Results: In normal tissue rare scattered positive cells were detectable at the bottom of the crypts. The percentage of positive cells significantly increased in dysplastic lesions and appeared to progressively decrease in the passage from dysplasia to adenoma and then to cancer, although always remaining greater in number than in the normal tissue. Conclusion: An increased CD133 expression occurs at early stages of colon tumorigenesis in the mouse. CD133-expressing cells might play an important role from the earlier phase and throughout the entire process of colon cancer development.

Short Report

The cancer stem cell hypothesis suggests that tumors are hierarchically organized like normal tissues and that only a rare subpopulation of undifferentiated cells (cancer stem cells, CSC) has the unique biological properties necessary for tumor initiation, maintenance and spreading (1). The CSC fraction within a variety of human carcinomas may be identified by the expression of specific cell surface markers, such as CD133. Indeed, normal as well as cancer stem cells in several human tissues, including the colon, have been reported to express CD133 (2). However, despite the enormous interest in this molecule, the significance of CD133 expression for the biology of colon normal and tumor cells remains unclear and controversies have arisen about its distribution in colon tissues and its role in colon tumorigenesis (3-5).

To gain insights on the role(s) played by CD133 in colon tumorigenesis, its expression in a widely used mouse model of colitis-related colon tumorigenesis induced by a combined treatment with azoxymethane (AOM) and dextran sodium sulphate (DSS) (6) in which cancer formation takes about two months to complete, was analyzed. Briefly, twelve mice were treated and four were sacrificed after five weeks, only when dysplastic lesions were evident. The remaining eight mice were sacrificed after 70 days treatment and all of them developed carcinomas with a mean of 1.5 tumors/mouse. The colons were removed, were routinely-fixed and paraffin-embedded. CD133 expression was evaluated by immunohistochemistry employing an avidin–biotin complex immunoperoxidase technique, as previously described (7). A rabbit monoclonal antibody (clone C24B9; Cell Signaling Technology, Danvers, MA, USA; 1:150) was used for the staining. Comparable results were obtained using a polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) as well as the monoclonal AC133 antibody (Miltenyi Biotec, Bergisch Gladbach, Germany).

As shown in Figure 1, staining was mainly cytoplasmic and in the normal tissue rare scattered positive cells were detectable at the bottom of the crypts, a pattern comparable to that previously reported for human colon (4). This distribution was consistent with the hypothesis that stem cells reside at the basis of the crypts and that CD133 expression is lost in more differentiated cells. The percentage of positive cells substantially increased, appearing to represent the majority of cells, in the dysplastic lesions compared to the normal tissue. The proportion of positive cells appeared then to decrease...
progressively in the passage from dysplasia to adenoma and then to cancer, where a dishomogeneous patchy distribution was evident, although always remaining greater in number than in the normal tissue. We believe these findings are of interest and might suggest an important involvement of the CD133 molecule in colon tumorigenesis and especially in the early phases of the process. This observation might support the hypothesis that an aberrant increase of the CD133 expressing cells, normally residing within adult colon tissue, might initiate and sustain the tumorigenesis (Table I). Over time, probably due to the fact that most of the cells retain the ability to undergo a differentiation-like process during the formation of the bulk of the tumor, the cells lose the expression of CD133 as well as other stemness features (i.e., the ability to initiate tumors), as occurs in normal tissues.

It would be of interest to determine the factor(s) responsible for the CD133+ cell population increase. Some possible hypotheses suggested that treatment might induce the conversion of normal CD133+ stem cells in CSC able to proliferate and initiate tumor development; since an increase in the percentage of CD133+ cells was detected very early in the mouse model, treatment could also simply induce an aberrantly increased proliferation of the normal CD133+ stem cells which might eventually favour the accumulation of mutation(s) responsible for their conversion to CSC and subsequent tumor initiation; treatment could target cells normally not expressing CD133 which might, eventually, acquire stemness properties, including the ability to initiate tumors and to express CD133. The hypothesized changes might occur, of course, through gene mutations, but it cannot be excluded that they might occur as a result of epigenetic dysregulation induced by the treatment. Although we do not necessarily favour the latter hypothesis, it is noteworthy that, as previously reported, several factors affect CD133 expression levels (8) and, regardless of the specific mechanism(s) involved, the microenvironment/cytokine milieu induced by inflammation seems most likely to play an important role in the process. In an attempt to investigate this hypothesis, we were unable to demonstrate a direct effect of inflammatory mediators (i.e., cytokines, such as TNF) on CD133 expression levels of colon cancer cells in vitro. However, it is noteworthy that a significant and reproducible increase of CD133 expression level (from about 47% to 70%, as assessed by flow cytometry analysis using the AC133 mouse monoclonal antibody) in HT29 human colon cancer cells exposed to fibroblast conditioned medium could be demonstrated, thus suggesting a potential role of surrounding (i.e., mesenchymal and inflammatory) cells in the regulation of CD133 expression on epithelial cells.

In conclusion, increased CD133 expression occurs at early stages of colon tumorigenesis in the mouse and the AOM/DSS-induced model of colon tumorigenesis could be useful to investigate the role(s) of this molecule in the entire process of colon cancer development.

Table I. Distribution of CD133* in various types of mouse colon tissues.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of mice</th>
<th>Dysplasia Total/CD133+ cases</th>
<th>Adenoma Total/CD133+ cases</th>
<th>Cancer Total/CD133+ cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 weeks</td>
<td>4</td>
<td>7/6</td>
<td>4/4</td>
<td>0</td>
</tr>
<tr>
<td>10 weeks</td>
<td>8</td>
<td>22/20</td>
<td>17/13</td>
<td>13/11</td>
</tr>
</tbody>
</table>

*CD133 expression was evaluated by immunohistochemistry. Lesions were considered positive (CD133+) when at least 10% of CD133 positive cells were detected.
References


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