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COMMENTARY

Cancer stem cells: here, there, and everywhere

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ABSTRACT

By using marker-free lineage tracing in combination with quantitative analysis, we recently revealed cancer stem cell functionality in established human colon cancer is not intrinsically defined, but fully spatiotemporally regulated.

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Author's comment

Colorectal cancers (CRC) are heterogeneous tissues that harbor cells with various degrees of differentiation. The cancer stem cell (CSC) model posits that cells with an immature phenotype, identified by markers that are also found on normal stem cells such as *LGR5* (leucine-rich-repeat-containing G-protein-coupled receptor 5, also known as *GPR49*) and CD133/Prominin1, have the unique ability to drive tumor growth and initiate metastasis[1]. This notion has predominantly been based on transplantation assays of single-cell suspensions in immune compromised mice, and the ability to form tumors is interpreted as stem cell functionality[1].

Critically, by disrupting the original tumor organization, these assays test stem cell properties in an artificial way, and the capacity to initiate a tumor does not equal stem cell functionality during tumor growth. Previously it was resolved that 5–7 cells in the crypt bottom function as stem cell[1,2], however a much larger number of cells in the crypt bottom express putative stem cell markers such as *Lgr5*[1]. Therefore, the cells that function as stem cells in the normal gut and those that express stem cell markers do not fully coincide. Moreover, it was demonstrated that following damage to the intestinal monolayer the pool of cells with stem cell potential is even larger, as also *Lgr5*-low progenitor cells were found to adopt a stem cell phenotype and function as *bona fide* stem cells during regeneration[3]. Therefore, stem cell *phenotype, activity, functionality* and *potential* are distinct qualities that all require dedicated assays to be evaluated[1]. We argue that previous research has predominantly investigated CSC phenotype, activity and potential in CRC: *CSC phenotype* is assessed by studying stem cell marker expression; *CSC activity* is tested by transplantation assays; and *CSC potential* is tested by ablation of specific cell populations (summarized in Figure 1). Importantly, until now it has remained unclear which cells drive expansion of unperturbed CRC tissue, or in other words; what are the functional CSCs during tumor growth, and how do these relate to cells with stem cell phenotype, activity and potential?

To answer these fundamental questions, we have recently used a marker-free lineage tracing system in combination with quantitative analysis in established colon cancer to define the growth mode of CRC[4]. With this system we were able to study the clonal behavior of sporadically labelled cells in their native environment during tumor growth and treatment. Using these data in conjunction with a mathematical model we inferred the underlying clonal dynamics and found that cells that drive tumor expansion were mainly located at the tumor edges. This finding is in line with a recently published study from Lamprecht and colleagues that proposed that the clonogenic outgrowth takes place from the tumor edge to the center, while we interpreted our clonal distribution, with the larger clones located at the tumor edges, as radial outgrowth from the center to the edge[5]. In addition, from the experimental data and the mathematical model for tumor growth, we concluded that stem cell functionality is fully defined by the microenvironment rather than by cell-intrinsic properties.

Next, we compared the distribution of functionally active stem cells with the expression patterns of putative stem cell markers, and were unable to find a positive correlation. Cells that are functionally active were located mainly at the tumor edges while cells positive for CSC markers were distributed equally throughout the tumor. These findings are in contrast with the results of a recent study in which CRISPR-Cas9 was used to visualize *LGR5*$^{+}$ and cyto-keratin 20 positive (*KRT20*) cells in xenotransplanted human colon cancer organoids[6].

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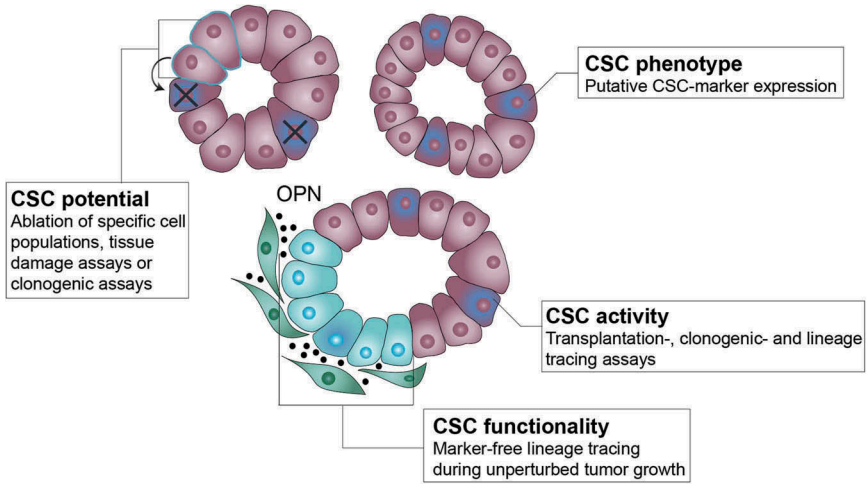

Figure 1. Cancer stem cell functionality in colon cancer. Cancer stem cells (CSCs) have distinct characteristics that all require appropriate assays to be studied. Here, we define CSC *activity* as a feature of cancer cells that are temporarily capable to generate tumors or clones. These CSCs can express putative CSC-markers, which also define the CSC *phenotype*. CSC *potential* is assessed by studying clonal dynamics following a certain intervention such as the ablation of cell populations. By using a non-biased lineage tracing method in situ, we recently showed that CSC *functionality* is driven by the microenvironment and does not necessarily coincide with CSC *phenotype* (functional CSCs are depicted as light blue cells; OPN, Osteopontin). Figure is adapted from reference [1].

Here it was found that *LGR5* expressing cells were mainly situated at the tumor edges while cells expressing the differentiation marker *KRT20* were located in the center. Possible explanations for this discrepancy could be a difference in tumor size or differentiation grade, as we studied small tumors with almost no ischemia or central necrosis. Furthermore, Shimokawa and colleagues selected for cell lines with a clear differentiation gradient related to *LGR5* and *KRT20* expression. In this study it was also demonstrated that selective ablation of $LGR5^+$ cells resulted in tumor regression. After regrowth of the tumors, $LGR5^+$ cells reappeared as $KRT20^+$ cells gave rise to $LGR5^+$ cells. This implies that, under the extreme situation of full ablation of the $LGR5^+$ population, CSC marker-negative cells have the potential to drive tumor growth. At the same time this phenomenon has also been shown in a murine model, in which $Lgr5^-$ cells rapidly repopulated the primary tumor after specific ablation of the $Lgr5^+$ cells, preventing tumor regression[7]. Both of these findings are in close agreement with our conclusion that CSC functionality is a highly plastic characteristic defined by the microenvironment. An important difference to consider between these studies and ours is that CSC activity and potential were tested after ablation of the *LGR5*/*Lgr5*-positive cell population, while in our model we have been able to evaluate CSC functionality during unperturbed tumor growth[4].

Having defined the critical role of the microenvironment on CSC functionality, we queried different environmental factors and identified Osteopontin (gene name *Spp1*), a protein secreted by cancer-associated fibroblasts (CAFs), to be a key regulator of *in vivo* clonogenicity. Although the influence of the stroma on therapy resistance, prognosis and the dedifferentiation process has been described before[8-10], we could now show that CAF secreted factors are key in defining tumor growth and therapy resistance in a marker-independent fashion and in unperturbed tissue.

We propose that targeting the crosstalk between the microenvironment and cancer cells in CRC is likely to be more effective than specifically targeting CSCs based on their phenotype, given that neighboring cells continuously take over CSC functionality after CSC ablation and even in non-treated cancers. However, to effectively implement this, a full understanding of the interplay between the functional CSCs and the microenvironment is warranted.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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