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.

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. 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.

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, however a much larger number of cells in the crypt bottom express putative stem cell markers such as Lgr5. 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. Therefore, stem cell phenotype, activity, functionality and potential are distinct qualities that all require dedicated assays to be evaluated. 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).

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. 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. 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.
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. 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.

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, 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.

**Funding**
This work is supported by the Academic Medical Center [Amsterdam], The New York Stem Cell Foundation, Cancer Research UK, and grants from KWF Kankerbestrijding [UVA2011-4969, UVA2014-7245 and 10529], the Mauritius en Anna de Kock Stichting [2013-2], Worldwide Cancer Research [14-1164], the Maag Lever Darm Stichting [MLDS-CGD 14-03], the European Research Council [ERC-StG 638193] and ZonMw [Vidi 016.156.308] to L.V.. L.V. is a New York Stem Cell Foundation – Robertson Investigator.

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