Colon cancer heterogeneity: Stem cells, signals and subtypes
De Sousa E Melo, F.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Outlook

In this thesis we have described our endeavor to characterize part of the molecular basis that determine the cause of heterogeneity within and across colon cancers. Below I summarize our main findings and discuss several important questions that remain unanswered or have been specifically raised by our work.

First of all, we have formally demonstrated the existence of cancer stem cells (CSCs) by demonstrating that at least a subpopulation of colon cancer cells possess self-renewal potential and multi-lineage differentiation capacity (chapter 2 [1]). Furthermore we have unraveled part of the molecular properties that define cancer stemness, in particular the presence of high Wnt signaling activity (chapter 3 [2]). We believe the use of a signaling pathway to define CSCs is a significant improvement over the plethora of markers that have been shown to enrich for CSC populations in xeno-transplantation assays [3]. We would argue it is a superior marker, as it does not rely on the isolation of cells based on cell surface markers that are usually associated with adhesion properties or proliferation rate [4], and which raises questions regarding their validity as bona-fide stem cell markers. Nevertheless, despite the improvements that transduction route provide over more traditional CSC markers, it remains to be established what is the best assay (i.e the one that most closely recapitulates a native tumor environment) to determine the presence and quantify the number of CSCs in a malignancy [5, 6]. In that respect, several groups have attempted to address these experimental limitations by looking into the CSC concept in mouse models where cells marked by known (cancer-) stem cell markers could be traced and their progeny followed [7-9] (discussed in Chapter 1). In the case of intestinal adenomas, this was done by tracing the lineages of Lgr5+ tumor cells using Cre-recombinase technology [7], demonstrating indeed that these cells could give rise to more-differentiated tumor cells and would qualify as adenoma stem cells in endogenously growing tumors. Similar tracing data were generated in papillomas in the skin. Altogether, these studies provide a glimpse of CSCs in their native habitat but do not resolve the CSC debate as these assays also have their own caveats: For instance, intestinal adenomas or skin-derived papillomas are benign tumors that only occasionally progress to full carcinomas. More importantly, in the Schepers’ study [7] only Lgr5+ cells have been subjected to lineage tracing while other populations remain untested. In other words Lgr5- cells could function as stem cells equivalently. Overall the same questions still remain: how many functional CSCs are present within a tumor and how do we more effectively quantify them? These questions are even more pressing as we have demonstrated that more differentiated cells can adopt a CSC phenotype upon exposure to signals present in particular microenvironments (Chapter 3 [2]). Clearly these questions are not easy to address, especially in unperturbed tumors, but they are key to our interpretation of the CSC concept for at least two main reasons: The first one is clinical; in simple terms, one would need to know the amount of CSCs -or clonogenic cells for that matter- prior and after treatment as this ultimately defines treatment efficacy (Chapter 4 [10, 11]). The second one is rather conceptual as it touches the heart of the CSC theory; by definition, it has been proposed CSCs are “relatively” rare and sit on top of the hierarchy within a tumor. It is relatively straightforward to define tumors that do not belong to that category when no CSCs can be prospectively isolated, but how do we define tumors that contain 10%, 50% or 80% of CSCs? At what frequency of CSCs should we stop...
de picting tumors as hierarchically organized? As mentioned, the lack of consensus in that area is even further complicated by the phenomenon of tumor cell plasticity and how impactful the microenvironment is on that process (described in Chapter 3 [2] and reviewed in Chapter 4 [10, 11]). Such plasticity or cell inter-conversion poses an evident clinical challenge as it immediately suggests that all tumor cells would have to be efficiently eliminated for a successful therapy to be achieved. How frequently does cell fate inter-conversion occur in a tumor? To our knowledge no data exists on whether inter-conversion is a rare or frequent event; the latter scenario will obviously shake the foundations of the CSC theory that posits that only a fraction of tumor cells are relevant for tumorigenesis and progression. Despite these open questions, it is evident that differences in CSC numbers, defined by any potential markers, exist among colorectal tumors; the clinical consequences of this remain more elusive. One prediction is that a relation might exist between the degree of self-renewal in a tumor and clinical outcome.

Several groups have tackled this issue in multiple tumor types by showing that (cancer)-stem cell derived signatures associate with patients that are at higher risk of developing relapses or distant metastasis [12-16]. Our results presented in chapter 5 [12] confirm the prognostic power of CSC derived signatures in colon cancer. However the underlying reason for this prognosis seems to be independent of CSC content in these tumors but rather relies on the identification of a different, more immature subset of tumors endowed with worse prognosis [12].

Stem cell derived profiles add up to a broad range of prognostic signatures in colon cancer [17, 18]. For example, the ColoPrint (a signature of 18 genes) [19] or the OncotypeDX [20] (a PCR-based assay) have demonstrated the ability to identify patients at high risk of relapse and can offer prognostication beyond traditional clinicopathological factors. The major drawback of most of these signatures is the lack of insights they provide on the biological processes that govern poor prognosis. Moreover they do not offer significant information on the heterogeneity of colorectal cancer (CRC).

To date, subtypes of CRC have mainly been described using classification based on (epi-) genetic defects. For instance, microsatellite instable (MSI) colon cancers are well defined clinically and geneti cally and often associated with mutations in the \textit{BRAF} gene and methylation at the \textit{MLH1} promoter [21-23]. MSI tumors are often opposed to chromosomal instable (CIN) cancers that are largely microsatellite stable (MSS) and present loss of heterozygosity (LOH) at specific loci [24-26]. Additional and more recent classifications have involved specific molecular features, such as the CpG island methylator phenotype (CIMP) [22, 27, 28]. Although this methodology has greatly contributed to our understanding of the disease, the attempts to define specific CRC subtypes based on unique mutations have not resulted in significant clinical relevance apart from the resistance of \textit{KRAS} mutated CRCs to EGFR targeted therapy [29].

An alternative and more recent approach involves the identification of clinically distinct subtypes of CRC through molecular profiling studies. This approach does not rely on any prior genetic or clinical information and therefore provides an unbiased separation of patients in distinct disease entities. Using this analysis over more than 1,000 patients we could identify three robust CRC subtypes that are each demarcated by distinct biological and clinical peculiarities (Chapter 6 [17]). The validity of our analysis is clearly illustrated by the fact that
two of the uncovered subtypes show a strong concordance with the more traditional subtypes of chromosomal instability (CIN) CRCs and microsatellite instability (MSI) CRCs, two distinct colon cancer entities markedly differing in their etiology and biological foundation. The strength of our methodology is further demonstrated by the discovery of a novel subtype that was previously unrecognized, despite the particularly unfavorable prognosis and poor clinical response to cetuximab treatment. Many research groups have made use of similar unbiased classification methods and not too surprisingly, independent studies identifying clinically distinct subtypes of CRC are starting to emerge [17, 24, 30, 31]. These different studies can cause confusion because no clear consensus exists on the extent of distinct subtypes one can describe in CRC. We have discussed this in the addendum to Chapter 6, where we show how our findings specifically relate to the ones that have been simultaneously published by the group of Douglas Hanahan [31]. Although both studies have identified subtypes that strongly relate to each other, the idiosyncrasies of each study stresses the need to generate a clear consensus on the nomenclature and the amount of subtypes present in CRCs (Addendum to Chapter 6). Ultimately this will require a translational effort to translate the rapid accrual of data in that area into a clinically applicable knowledge that would hopefully turn into benefit for patients.

Felipe De Sousa E Melo
Amsterdam, 2013

References
cells and predicts disease relapse. *Cell Stem Cell* **8**: S11-S24


