Between cancer and therapy: Studies of the colon
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CHAPTER 1

Colonic stem cells in homeostasis and cancer

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Chapter 1

BETWEEN HOMEOSTASIS AND CANCER
Basic signaling pathways involved in the development and homeostasis of the adult tissue are often conserved during malignant transformation. Understanding these mechanisms in health is therefore instrumental to gain insights into the biology of the malignant transformation. For example, Wnt signaling acts as the main driver of stem cell proliferation in the healthy intestine. A truncating mutation in Wnt negative regulator APC causes hyperactivated Wnt signaling resulting in aberrant stem cell proliferation, adenoma formation and predisposition to colorectal cancer (CRC) development. Conversely, activation of the unfolded protein response (UPR) forces intestinal stem cells into differentiation, a finding that in this thesis is described to be conserved in colorectal cancer. Importantly, UPR induced differentiation renders therapy resistant colon cancer stem cells more sensitive to chemotherapy, further demonstrating that findings in fundamental stem cell biology can be of significant clinical impact.

The tight link between stem cells in homeostasis and cancer development has recently been underlined by Tomasetti et al. They found that the lifetime risk of developing a tumor is tightly correlated to the number of divisions of the stem cells that maintain homeostasis in the healthy tissue. In this introduction the basics of colonic embryogenesis and homeostasis of the adult tissue will first be discussed before entering the field of cancer stem cells and therapy response.

FUNCTION AND ANATOMY OF THE COLON
The large intestine or colon is the distal part of the intestinal tube between the ileum and the anal canal and has a length of around 1.5 m. The main function of the colon is the absorption of water and salts from the luminal content after most nutrients have been digested and absorbed in the stomach and small intestine. The colonic epithelium has little digestive activity but its lumen contains a very rich microbiome that is able to ferment potential nutrients such as complex carbohydrates that may not have been hydrolyzed in the small intestine. This not only reduces the osmotic effect of unabsorbed carbohydrates which otherwise may hinder the formation of firm stool but it is also an important source of short-chain fatty acids that maintain growth of colonic epithelial cells.

The proximal end of the colon or caecum contains two important landmarks; the vermiform appendix and ileocaecal valve. The ileocaecal valve or Bauhin’s valve forms the transition zone between small intestine and colon. The valve is formed by two lips that protrude into the colonic lumen that prevent reflux of colonic content into the small intestine. The vermiform appendix is a worm-shaped tube that is connected to the caecum and is around 8 cm in length in humans. The function of the appendix is incompletely understood. It may serve specific immune functions, as it contains part of the gut associated lymphoid tissue.

Histologically, the lumen of colon is covered by a single layer of polarized epithelial cells that cover the lamina propria. The lamina propria contains a variety of mesenchymal cells such as myofibroblast-like cells and a large variety of both adaptive and innate immune cells. The epithelium and lamina propria
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are surrounded by a thin muscle layer, the muscularis mucosae. The muscularis mucosae is separated from the muscularis propria by the submucosa, a layer of connective tissue. The epithelium of the colon is a rapidly renewing tissue in which the dividing cells lie in tube-like invaginations called crypts. Stem cells at the base of the crypts generate transit amplifying cells that cycle a number of times and then differentiate into one of the epithelial lineages of the colon. The major epithelial cell types in the colon are the colonocytes that absorb water and salt, goblet cells that produce mucus and endocrine cells that secrete gut hormones.

MORPHOLOGICAL PATTERNING OF THE COLON

As most research on signaling pathways with a role in colon development has been performed on mice, both human and mouse embryology will be discussed. The primitive gut tube consists of three separate regions: the foregut, the midgut, and the hindgut. The gut tube derives its blood supply from the mesenteric arteries and gut vascularization clearly marks the transitions between midgut and hindgut. The midgut is vascularised by the superior mesenteric artery and the hindgut is vascularised by the inferior mesenteric artery\(^*\). The adult caecum, ascending colon and proximal two-thirds of the transverse colon will be formed from the last part of the midgut and is vascularized by the superior mesenteric artery, while the last third of the distal transverse colon, descending colon, sigmoid and anorectum are derived from hindgut and vascularized by the inferior mesenteric artery (Figure 1).

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**Figure 1.** Components of the adult colon
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In humans, by 5 weeks the primitive gut tube increases more than the length of the embryo, causing the midgut to bulge out into the body stalk. Hereby a hairpin-like structure is formed, called the midgut loop. The cranial limb of the midgut loop will contribute to the adult ileum, the caudal limb will contribute to the adult colon. The mid- and hindgut assume their adult positions by a series of rotations. First the caudal limb of the midgut loop rotates 270° clockwise (as seen from the embryo’s perspective) around the cranial limb, causing the future colon to move across the future small intestine.

At 9 weeks of human gestation, the abdominal cavity has enlarged sufficiently to accommodate the intestinal tract and the herniated intestinal loops begin to move back into the abdominal cavity. After the small intestines move into the peritoneal cavity, the colon also returns. At the twelfth week, the caecal primordium on the right starts to move downwards, causing the colon to assume its definitive C-shaped position. The main events during human and mouse colon development are summarized in Table 1.

Table 1. Events during colon development

<table>
<thead>
<tr>
<th>Developmental Events</th>
<th>Human (weeks)</th>
<th>Mouse (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrulation</td>
<td>2</td>
<td>6,25</td>
</tr>
<tr>
<td>Formation of tubular gut tube</td>
<td>3</td>
<td>9,5</td>
</tr>
<tr>
<td>Midgut loop herniates into yolk stalk</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>Formation of the caecum</td>
<td>6</td>
<td>11,5</td>
</tr>
<tr>
<td>Herniation of intestinal loop</td>
<td>7</td>
<td>12,5</td>
</tr>
<tr>
<td>Return of gut tube into body cavity</td>
<td>9</td>
<td>15,5</td>
</tr>
<tr>
<td>Formation of allantois</td>
<td>6</td>
<td>11,5</td>
</tr>
<tr>
<td>Division of cloaca into rectum and urogenital sinus by urorectal septum</td>
<td>6</td>
<td>11,5</td>
</tr>
<tr>
<td>Complete separation of intestinal and urinary outflow tract by perineum</td>
<td>8</td>
<td>13,5</td>
</tr>
<tr>
<td>Colonization of the gut by neural crest cells</td>
<td>8</td>
<td>15</td>
</tr>
</tbody>
</table>

HISTOGENESIS OF THE COLON

In humans, gastrulation occurs in the second week of gestation. Endodermal differentiation starts in week 6 and is completed in week 9 when a columnar epithelial monolayer is formed. Right after the onset of endodermal differentiation, at week 7, the mesoderm will start to differentiate into mesenchyme and muscle layers which are completely formed and positioned at week 12 of development.¹⁰
In mice, gastrulation occurs at E6.25 and the primitive gut tube is formed during a complex folding process which is completed at E9.5\(^1\). Just after gastrulation, the endodermal layer is a histologically uniform layer of columnar epithelial cells. Despite its morphological uniformity, the endodermal layer is already patterned along the anterior-posterior (AP) axis at this point in development\(^12,13\). This patterning information is encoded by regional expression of the transcription factors *Sox2*, *Hhex*, *Foxa2* and *Cdx2*. The endodermal cells that were formed first and have moved most anteriorly express *Sox2*\(^14\) and the homeobox gene *Hhex* (or *Hex*)\(^15\), whereas later endodermal cells express *Foxa2* (or *Hnf3β*), and the last endodermal cells that form the posterior endoderm and will therefore form the colon express *Cdx2*\(^16\). Elegant explant experiments in which endoderm and mesoderm were cultured separately or in various combinations have shown that the next phase of patterning of the posterior endoderm around E7.5, such as the induction of the expression of intestinal fatty acid binding protein (iFabp), is controlled by soluble factors derived from the posterior mesoderm\(^17\); however the nature of these mesodermal factors has not been resolved.

*Cdx2* plays a central role in gut morphogenesis and drives differentiation towards an intestinal phenotype. *Cdx2*\(^{-/-}\) heterozygous mice develop colonic polyps in which *Cdx2* expression is lowest in the center and gradually rises towards the outside of the polyp\(^18,19\). The center of these polyps consists of esophageal tissue, whereas towards the outside the tissue transforms into gastric, small intestinal and finally colonic origin. Therefore *Cdx2* directs endodermal differentiation towards a more caudal phenotype\(^18\).

In *Foxa3Cre;Cdx2fl/fl* mice in which *Cdx2* was specifically deleted in the early endoderm, the entire intestinal mucosa was converted into a fore-stomach phenotype and the distal colon was completely absent\(^20\), indicating that *Cdx2* is essential to suppress foregut fate. In these mice, multiple signaling pathways that are involved in epithelial-mesenchymal interactions were severely affected, suggesting that *Cdx2* may carry out its role by governing epithelial-mesenchymal interactions. Conversely, overexpression of *Cdx2* in the stomach endoderm results in incomplete intestinal metaplasia in the distal stomach, reflecting its ability to direct the intestinal program and repress foregut identity\(^21\). *Cdx2* expression persists throughout life in the entire intestinal mucosa where it appears to regulate the production of many gut enzymes\(^22\). Another important action of *Cdx2* is its tumor-suppressor function in the distal colon\(^23\).

In addition to *Cdx2*, Wnt signaling plays a key role in the specification of the intestinal epithelial phenotype. Simultaneous deletion of Tcf1 and Tcf4 leads to severe anomalies of the hindgut\(^24\). Furthermore, these mice showed anterior transformation at the stomach-duodenal junction. Expression analysis using specific markers for gastric epithelium and intestinal epithelium revealed duplications of the stomach, suggesting that Tcf1 and Tcf4 promote an “intestinal” fate within the primitive gut. This was further shown when a β-catenin/Lef1 fusion protein is misexpressed in lung endoderm, these cells turn on genes, normally restricted to the intestine\(^25\). This implies that in the colon Wnt signals instruct endodermal cells to become intestine as opposed to other endodermal lineages.
Once the basic structure of the intestinal tract is laid out, differentiation along the radial axis can take place. This occurs through instructive and permissive interactions between endoderm and mesoderm. At E9.5, the endodermal layer is a uniform layer of cuboidal epithelial cells overlying a thin layer of mesodermal cells. Gut tube length and circumference increase by expansion of the mesenchyme, epithelium and the lumen. Around E14, the epithelium reorganizes to form a polarized columnar epithelial monolayer. At the same time, mesenchymal condensation and subepithelial expansion induce the colon to develop epithelial folds at E16.5. These folds resemble small intestinal villi; however whereas villi in the small intestine are thin and elongated, the folds in the colon are wider and flattened. In the first two postnatal weeks, crypts are formed between the epithelial folds. In the third week after birth, the number of crypts rapidly increases through crypt fission. This process of crypt multiplication results from symmetric stem cell division. After four weeks, crypt morphogenesis and multiplication is complete.

Formation of the stem cell pool
The colonic epithelium is characterized by rapid and continuous renewal throughout life. Epithelial renewal occurs in the crypts through a coordinated series of events involving proliferation, differentiation and migration towards the colonic lumen. Simultaneous with the formation of crypts, the epithelium compartmentalizes into rapidly proliferating progenitor cells at the bottom of the crypt and terminally differentiated cells that localize to the upper part of the crypts. Intestinal epithelial stem cells are able to self-renew and give rise to all differentiated cell lineages. Stem cells form transit-amplifying progenitors that occupy the lower third of the crypt. These progenitor cells differentiate
into one of the functional cell types of the colon, including absorptive colonocytes, mucin-secreting goblet cells and hormone-releasing enteroendocrine cells (Figure 3). At the luminal surface, cells undergo apoptosis and/or are shed into the lumen. This entire process takes approximately 3-5 days.

**Figure 3.** Colonic stem cells in homeostasis

It is well established that renewal of the small intestinal epithelium is entirely dependent on continual stimulation of the Wnt pathway. In the colon, this has not been as well investigated, however, several lines of *in vivo* evidence show similar dependency on Wnt signaling of the colonic epithelium. First, rapidly proliferating progenitor cells at the bottom of the crypt are characterized by accumulation of nuclear β-catenin and expression of *Tcf4*, implying that these cells respond to Wnt stimulation.

Second, mutations that activate the Wnt β-catenin pathway can lead to colorectal cancer in humans. Third, adenoviral expression of Wnt inhibitor *Dickkopf-1* (*Dkk-1*) in the colonic epithelium inhibited proliferation in the colon accompanied by progressive architectural degeneration with the loss of crypts by 7 days. Fourth, ex vivo colonic crypt cultures are dependent on continuous Wnt stimulation. Finally, the Wnt target gene Lgr5 marks stem cells at the base of the crypt.

The differentiated compartment is marked by expression of P21, an important mediator of cell cycle arrest and differentiation. C-myc, one of the downstream targets of Wnt signaling, acts to inhibit P21, thereby preventing crypt progenitor cells from differentiation and going into cell cycle arrest. The majority of colorectal cancers display hyperactivation of the Wnt-β-catenin most display inactivating mutations in the negative regulator of Wnt signaling, the APC gene. However, other Wnt-activating mutations, such as in β-catenin and AXIN2, may ultimately lead to colorectal cancer.

Together these data show that Wnt signaling regulates colonic epithelial precursor cell fate and proliferation. Homeostatic systems however, exist by virtue of negative feedback loops. If the number
of stem cells is not tightly regulated this would result in abnormal growth. The most important 
negative feedback regulator of Wnt signaling in the colonic crypt that has been identified to date 
is Indian Hedgehog (Ihh). Hedgehog signals released by differentiated epithelial cells stimulate the 
mesenchymal cells to release Activins and Bone Morphogenetic Proteins (BMPs). BMP signaling 
in turn inhibits colonic stem cell self-renewal through suppression of Wnt-signaling. It was recently 
shown that activation of the so-called Unfolded Protein Response (UPR) forces intestinal stem cells 
into differentiation. The differentiation of intestinal stem cells upon UPR activation may act as a 
mechanism to guarantee integrity of the stem cell pool (Figure 3).

**Between clonal evolution and strict hierarchy**

Cancer originates from cells that have acquired genetic mutations which give them a survival advantage 
over the other surrounding cells. These ‘driver’ mutations may include activating mutations in genes 
that promote tumor growth (proto-oncogenes) or inactivating mutations in genes that slow down cell 
division, repair DNA mistakes, or mediate apoptosis (tumor suppressor genes). The acquisition of 
multiple additional driver mutations is necessary for the development of a carcinoma. This is called 
“clonal evolution.” The sequence of occurring mutations ultimately leading to CRC has been 
mapped by Fearon and Vogelstein in 1990 and starts with a Wnt activating mutation caused by either 
inactivation of Wnt negative regulator APC or activation of β-catenin (CTNNB1). Both mutations result 
in increased proliferation and formation of non-invasive adenomas. Similarly, patients with familial 
adegiated polyposis (FAP) carry a germline mutation in one copy of the APC gene and develop 
hundreds of intestinal adenomas early in life and their life time risk of developing CRC is almost 100%.
Transformation of a benign adenoma into an invasive adenocarcinoma only occurs when 
subsequent mutations occur in genes such as K-RAS, SMAD4, and/or p53. Although recent reports 
have identified alternative routes that may also lead to CRC, the route Vogelstein has proposed 
is still considered the classical and most predominant adenoma-to-carcinoma sequence (Figure 4).

The model of ‘clonal evolution’ may erroneously leave the impression that a tumor is nothing but 
a random pile of mutated, hyper-proliferating tumor cells. Similarly, the World Health Organization 
describes cancer as ‘the uncontrolled growth and spread of cells’. Increasing evidence is actually 
pointing the opposite direction towards the tumor as a highly organized tissue with a strict hierarchy 
amongst tumor cells. Similar to normal tissue homeostasis, tumors are believed to consist of stem 
cells (cancer stem cells) that fuel tumor growth as well as differentiated cancer cells that may not 
drive tumor growth themselves but are still indispensable for the maintenance of a tumor as a whole. 
Although the hypothesis that only a small subpopulation of cells is responsible for tumor growth 
(Figure 5) is much older; it was not until 1997 when the cancer stem cell hypothesis was functionally 
proven.

**Stem cells in cancer**

Cancer stem cells (CSCs) are believed to drive tumor initiation, growth, and metastasis. As CSCs are 
more resistant to chemo-, radiation therapy than differentiated cancer cells, they are considered
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Figure 4. Adenoma to carcinoma sequence

Figure 5. The cancer stem cell hypothesis
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to play an important role in post-therapeutic tumor relapse. Correct identification of CSCs would therefore greatly facilitate the development of prognostic and therapeutic tools. The golden standard for identification of CSC in various hematologic and solid tumors has long been by xenotransplantation (Figure 6, left). Xenotransplantation involves subcutaneous injection of a subpopulation of human cancer cells, characterized by the expression of certain cell surface markers, in immune deficient mice. In contrast to non-stem cells or ‘differentiated’ cancer cells, CSCs are able to expand and propagate the disease subcutaneously, characterized by the production of new CSCs as well as differentiated cancer cells. Upon injection CSCs are thus capable of both self-renewal and multi-lineage differentiation, the two hallmarks of stemness (Figure 3). These newly made CSCs can be re-isolated from the xenografts and re-injected to form secondary, tertiary (and further) tumors, a procedure also known as ‘serial transplantation’ (Figure 6, right).

Figure 6. Identification of cancer stem cells by xenotransplantation

The existence of cancer stem cells was proven for the first time in acute myeloid leukemia (AML) in 1997. By performing xenotransplantations Bonnet & Dick showed that only a very small population of human AML cells, characterized by the expression of CD34 and the absence of expression of CD38, were capable of reproducing disease in immune deficient mice. Since then cancer stem cells have been identified in several solid tumors as well. For the colon, this happened in 2007 when two independent research groups identified colon cancer stem cells (colon-CSCs) by the expression of CD133. Both groups demonstrated that CD133+ cells (about 0.1-10% of all tumor cells) were able to induce tumors in mice that resembled the original malignancy. Since the first identification of colon-CSCs by CD133, several other markers have been suggested to identify colon-CSCs, including CD166, CD44 and LGR5 (Table 2). Most CSC surface markers identified thus far however, are expressed also by normal ISCs, preventing their potential use as therapeutic targets.
Distinction between cancer stem cells and differentiated cancer cells has not only been made by cell surface markers, but also on functional levels. As such cancer stem cells have been characterized by high activity in aldehyde dehydrogenase 55 and Wnt signaling54 (Table 2). Vermeulen et al established spheroid cultures with a TCF/LEF driven GFP reporter for Wnt signaling activity that allow comparing the effects of treatment on cancer stem cells (Wnthigh) to more differentiated cells (Wntlow) within the same experiment54. This reporter system was also used in experiments described in chapter 7 of this thesis.

Table 2. Proposed markers for colon cancer stem cells

<table>
<thead>
<tr>
<th>Cell surface markers</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESA+/CD44+/CD166(ALCAM)</td>
<td>Dalerba et al, PNAS, 2007</td>
</tr>
<tr>
<td>CD24/CD29</td>
<td>Vermeulen, PNAS, 2008</td>
</tr>
<tr>
<td>Bmi1</td>
<td>Sangorgi, Nat. Gen, 2008</td>
</tr>
<tr>
<td>Lgr5</td>
<td>Barker, Nature, 2009</td>
</tr>
<tr>
<td></td>
<td>Schepers, Science, 2012</td>
</tr>
<tr>
<td></td>
<td>Kemper, Stem Cells, 2012</td>
</tr>
<tr>
<td>Dclk1</td>
<td>Nakanishi, Nat. Gen, 2013</td>
</tr>
<tr>
<td></td>
<td>Westphalen, JCI, 2014</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Functional activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldehyde dehydrogenase</td>
<td>Huang, Cancer Res, 2009</td>
</tr>
<tr>
<td></td>
<td>Chu, Int. J. Cancer, 2009</td>
</tr>
</tbody>
</table>

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An important pitfall in xenograft-based identification of CSC markers is the artificiality of the procedure which involves the establishment of a single cell suspension, FACS-sorting and subcutaneous injections. Sceptics claim that the proposed markers may identify cells capable of surviving these harsh experimental manipulations rather than identifying which cells are responsible for tumor growth in vivo. To circumvent this issue, Schepers et al. performed in vivo lineage tracing and subsequent re-tracing within adenomas using a multi-color reporter mouse55. They confirmed that Lgr5+ cells do not only drive tissue growth under homeostatic conditions52 but also drive tumor growth in well-established adenomas. Furthermore, these cells were capable of producing both other Lgr5+ stem cells as well as Lgr5− differentiated cells, further proving their stem cell capacities. Although this elegant approach was the first (and thus far the only) report to prove stem cell function within established adenoma’s, two points of critique must be mentioned. First, the experiments were
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only performed in mouse adenomas and did not involve human derived cancer cells. Second, in contrast to xenografts experiments where Lgr5- cancer cells were not able to induce tumor growth, the specificity of Lgr5 as an exclusive CSC-marker was not investigated in this research article, leaving the option open that other cells not expressing Lgr5 may also contribute to tumor growth.

Cancer cell or origin

Although xenograft studies and lineage tracings may effectively prove hierarchical organization and identify stem cells within a tumor, they do not necessarily prove that the tumor originated in normal-tissue stem cells. Alternatively, differentiated cells could have acquired the appropriate mutations that transformed them into cancer stem cells. To address the issue of “cell-of-origin” Zhu et al. and Sangiorgi & Capechi activated β-catenin specifically in intestinal stem cells expressing Cd133 or Bmi1. In both cases, β-catenin activation led to the development of intestinal adenomas, proving that transformed intestinal stem cells can give rise to intestinal adenomas. The specificity of Cd133 and Bmi1 however was not investigated in these studies as β-catenin was not activated in cells devoid of these markers. More specific proof for the normal intestinal stem cells as the origin of cancer came from Barker et al. They conditionally deleted tumor-suppressor gene Apc in Lgr5+ stem cells or in short lived transit amplifying cells and found that only Lgr5+ cells were capable of generating intestinal adenomas. In contrast, mutated transit amplifying cells generated microadenomas or cystic structures that failed to develop into macroscopic adenomas.

The whole tumorigenic process however, develops from multiple DNA mutations and Schwitalla et al. showed that simultaneous deletion of Apc and IkBα (resulting in hyperactivated NFkB signaling) caused malignant transformation in de-differentiating epithelial cells. Also chemical induced inflammation conferred non stem cells (rare Dclk1+ tuft cells) susceptible to malignant transformation. Taken together, these studies show that generally tumors may arise in mutated normal-tissue stem cells, but under specific conditions such as inflammation and subsequent NFkB activation, also non-stem cells may act as the cell-of-origin in cancer.

Plasticity in cancer stem cell dynamics

CSCs were initially considered as a population with well-defined phenotypic and molecular features. Accumulating evidence however suggests that CSCs are a dynamic population continuously shaped by genetic, epigenetic and microenvironmental factors. In contrast to stem cells in normal tissue homeostasis, the process of maintaining stemness versus differentiation in tumors is subject to much higher levels of plasticity and often referred to as “the dynamic cancer stem cell model” (Figure 7). In the normal colon for example, colonocytes are considered to be ‘terminally’ differentiated. Only under very specific conditions such as damage or inflammation are these colonocytes capable of reverting into stem cells. Cancer in contrast shows much more plasticity as non-stem cells can spontaneously revert into a stem cell-like state. Furthermore several stroma derived factors including HGF, IL17A, OPN, SDF1 and even chemotherapy treatment can trigger non-stem cells to revert into CSCs.
Several other cell types within the CSC microenvironment contribute to stemness of colon-CSCs. First, endothelial cells were shown to promote Notch signaling by the production of Notch ligand Dll1 and thereby promote self-renewal of colon-CSCs. Secondly, prostaglandin E2 and cytokines produced by mesenchymal cells were shown to induce β-catenin activation and CSC formation. Lastly, CD4+ T cells were shown to secrete IL-22, which acted on cancer cells to promote activation of the transcription factor STAT3 and expression of methyltransferase DOT1L responsible for the transcription of stem cell-associated genes.

Clinical impact of cancer stem cells
As CSCs are responsible for tumor expansion and disease progression, they have to be eradicated specifically in order to achieve a sustained response to therapy. The necessity of targeting CSCs is further underlined by the finding that CSC profiles are highly prognostic for patients with CRC. Unfortunately, CSCs are in fact more resistant to chemo- and radiation therapy than differentiated cancer cells. Conventional therapies may therefore effectively reduce the bulk of a tumor, mainly consisting of differentiated cells, but leave behind the small population of CSCs that subsequently drive tumor regrowth after therapy withdrawal (Figure 8, upper panel). Even when tumors seem to have been completely eradicated based on latest imaging techniques, relapses may occur. This implies that a very small population of CSCs, invisible to radiologic imaging, is capable of withstanding the toxic effects of the therapy. Consistent with this model, clinical data show that the relative proportion of CSCs in a tumor is massively increased after chemo-, radiation therapy.

Several pathways have been suggested to influence therapy resistance. For example, colon-CSCs express high levels ATP-binding cassettes member 5 (ABCBS). This transporter is responsible for the efflux of chemotherapy across the cell membrane and was shown to mediate resistance to 5-FU.
Recently, decreased mitochondrial priming was shown to contribute to chemoresistance due to selective production of anti-apoptotic molecules by colon-CSCs such as BCLXL. Todaro et al. have shown that auto- and paracrine signaling of IL4/IL4-R by colon-CSCs maintains high levels of BCLXL and thereby protects against chemotherapy. Several compounds including ABT-737 and WEHI-539 are capable of inhibiting these anti-apoptotic molecules and may selectively...
target the CSC pool within a tumor. Interference with pathways that confer resistance to CSCs may thus serve as an attractive target for therapy and is currently under clinical investigation.

Since colon-CSCs are greatly dependent on signal transduction pathways that regulate healthy stem cell dynamics, targeting these pathways may provide a novel window of opportunities. Specific targeting of the Wnt\textsuperscript{83,84} and Notch pathway\textsuperscript{85,86} was shown to suppress cancer stemness and decrease tumor-initiating cell frequency. Compounds targeting Wnt and Notch are currently under investigation in clinical trials\textsuperscript{87,88}. By contrast, activation of pathways that oppose Wnt and Notch, such as bone morphogenetic protein (BMP) signaling was shown to induce differentiation of both intestinal stem cells as well as colon-CSCs by the inhibition of Wnt signaling\textsuperscript{89}.

Although specific ablation of the CSC pool may seem an attractive and feasible avenue, two factors may complicate this approach. First, the suggested pathways to be targeted are also indispensable normal stem cells and homeostasis of the healthy tissue and may therefore cause severe side effects. Second, when only the CSC compartment of a tumor is eradicated, de-differentiating non-stem cells may cause regeneration of the CSC pool and disease recurrence (Figure 8, middle panel). In an even worse scenario, therapies ablating only the CSC pool may even elicit a reactive response resulting in hyper-proliferation of the surviving tumor cells and recurrence of the tumor in a more aggressive form. In a clinical setting, these drugs should therefore always be combined with conventional chemotherapies that target the differentiated cells.

An alternative and promising approach to achieve a sustained response to therapy is by sensitizing the therapy resistant CSCs to conventional therapy. As differentiated cells are more sensitive to chemo-, radiation therapy, forced differentiation of colon-CSCs is a very attractive target which could render them more sensitive to conventional chemotherapy (Figure 8, lower panel). This approach is further investigated in chapter 7 of this thesis.
REFERENCES


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