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Chapter 12

Transcriptional Regulation of the Intestinal Cancer Stem Cell Phenotype

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Abstract

Colorectal cancer (CRC) is one of the most frequent cancers worldwide. Current treatments include surgery and chemotherapy, but disease recurrence occurs frequently. The continuous renewal of intestinal epithelium relies on the presence of intestinal stem cells that are also at the origin of CRC and contribute to therapy resistance and metastatic dissemination. Several nuclear signaling pathways and transcription factors regulate both intestinal cell homeostasis and tumorigenesis. However, the transcriptional events that govern the emergence of aggressive therapy-resistant cancer stem cells are still poorly defined. This review summarizes the relevance of transcription factors in intestinal stem cell biology and their involvement in colon cancer development and drug resistance.

Keywords: transcription factors, intestinal cancer stem cells, colon cancer, chemotherapy

1. Introduction

Colorectal cancer (CRC) is one of the most frequent cancers worldwide. The current standard-of-care management includes surgery, radiotherapy and chemotherapy, sometimes in association with targeted agents to block tyrosine kinase receptors or their ligands. However, cancer recurs in 30–50% of patients [1].

The intestinal epithelium is continuously renewing, thanks to the presence of multipotent stem cells (SCs) within the intestinal crypts that give rise to all the differentiated cell types [2]. Different signaling pathways, including Wnt and Notch, and transcription factors are involved in intestinal development, homeostasis and maintenance of the intestinal SC properties [3]. These signaling cascades must be finely controlled because their deregulation is involved in gut tumorigenesis. Importantly, recent studies suggest that tumor-initiating cells or cancer stem cells (CSCs) can regenerate a tumor and might be at the origin of CRC [4, 5]. Thus, a better understanding of CSC function in tumor initiation, progression and resistance to treatment is necessary to improve the screening, prevention and clinical management of patients with CRC.

In this review, we propose an overview of key transcriptional regulations that are involved in intestinal SC/CSC biology. We present the major signaling pathways and the main transcription factors involved in intestinal homeostasis as well as their roles in the transcriptional regulation of intestinal CSCs.

2. The intestinal epithelium and the stem cell compartment

The main functions of the small intestine are food digestion and absorption and production of gastrointestinal hormones. It is subdivided in duodenum, jejunum and ileum, and is one of the most rapidly self-renewing tissues [6]. It is characterized by the presence of villi and Lieberkühn crypts. The large intestine (cecum, colon and rectum) is specialized in compacting stool for rapid excretion, and is arranged in multiple crypts associated with a flat luminal surface. It shows slower renewal capacities than the small intestine [7].

The intestinal epithelium develops from the embryonic endoderm [8] and its cellular composition is quite similar along the entire intestinal tract. The intestine incredible self-renewal capacity is supported by the SC compartment located at the bottom of the crypts. Specifically, transit-amplifying (TA) cells undergo four to five rounds of rapid cell division and then move out of the crypt and terminally differentiate into enterocytes, goblet cells, Tuft cells and entero-endocrine cells (**Figure 1**). These differentiated cells continue to move up along the villus and die by anoikis 2 or 3 days after having reached the villus tip. Paneth cells also derive from intestinal SC, but migrate downwards and settle at the crypt base where they live for 6–8 weeks [9]. Two other cell types have been detected in the intestinal epithelium: M cells that are associated with Peyer's patches and Cup cells that are located in the ileum.

To date, two SC populations have been identified in the crypts, highlighting the high plasticity of the intestinal epithelial SC compartment. The first one corresponds to crypt-based columnar (CBC) cells that express the leucine-rich receptor, LGR5 and are interspersed between Paneth cells (**Figure 1**). CBC cells are required for the long-term maintenance of the self-renewing epithelium. Indeed, they cycle steadily to produce the rapidly proliferating TA cells that can differentiate into all lineages [6]. In the colon, LGR5+ cells are considered to be SCs because they are pluripotent and can maintain epithelial cell self-renewal over long periods of time. However, LGR5+ cells in the small intestine seem to divide more actively than in the colon, possibly due to differences in the epithelial turnover rates [6].

The second crucial SC population corresponds to 'reserve' SCs that can be rapidly recruited to maintain epithelial homeostasis following injury [7]. They are located at position four from the crypt base (hence, the name of +4 SCs) and are generally considered to be relatively quiescent and resistant to acute injury (**Figure 1**). This population was discovered by Potten et al.

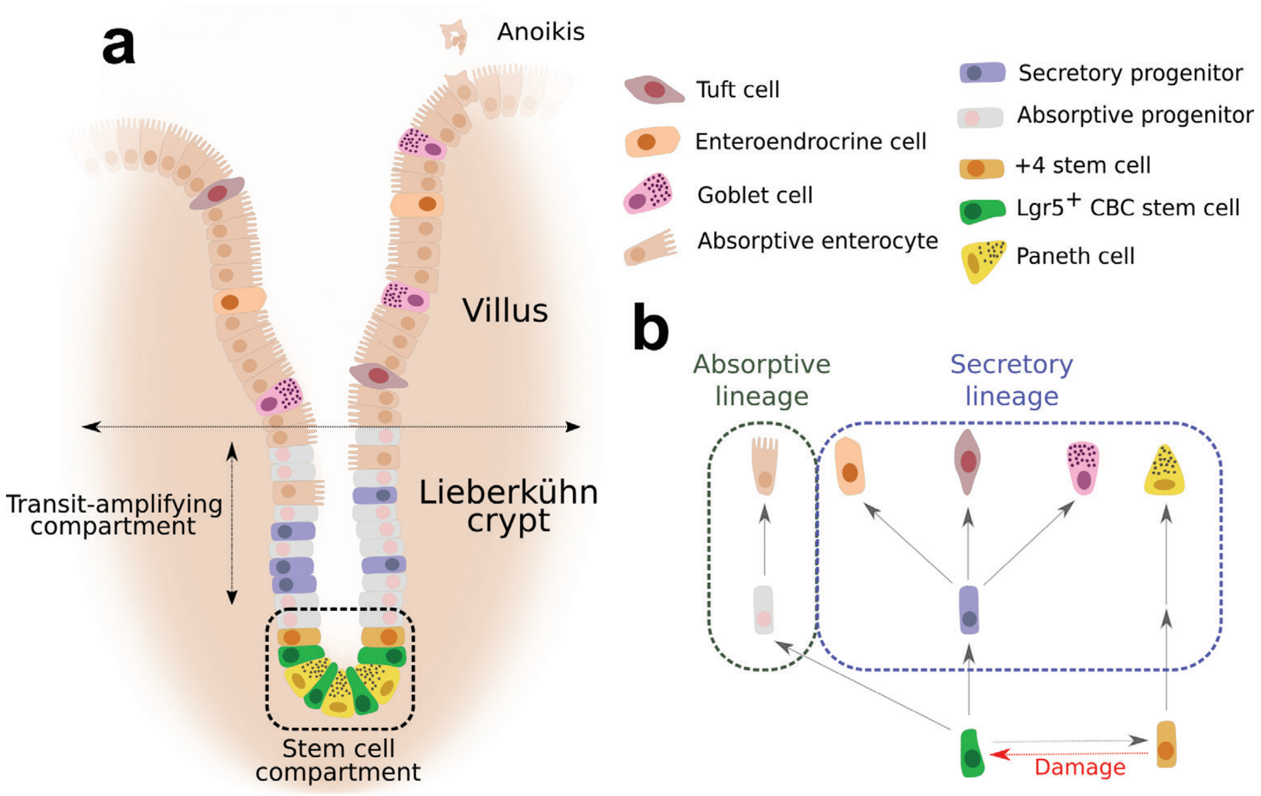

Figure 1. Schematic representation of the intestinal epithelium and the hierarchy of intestinal lineages. Self-renewal of the intestinal epithelium is fueled by small intestinal stem cells (at the bottom of the crypt) that give rise to progenitor cells. These can subsequently differentiate into the mature cell types required for normal gut function.

and was described as the only one responsible for the maintenance of intestinal homeostasis, but without counterpart in the colon [10]. They can retain DNA labels (a surrogate SC marker), possibly due to their infrequent replication or selective retention of labeled DNA during division. Their relative quiescence also explains their resistance to radiation. This SC population was identified thanks to its strong and localized expression of the *BMI1* gene that encodes a component of the Polycomb repressor complex [7]. Lineage tracing of these cells revealed strict terminal differentiation toward the Paneth cell lineage. However, following injury, this population can start cycling and show typical intestinal SC activity and multipotency [11]. These features are typical of SCs, despite the fact that, differently from CBC cells, they do not generate all epithelial lineages.

3. Colorectal cancer and intestinal cancer stem cells

3.1. Colorectal cancer

Genetic or epigenetic changes can lead to deregulated cell proliferation, resulting in tumor growth [12]. In the intestine, tumors start with the formation of small lesions called aberrant crypt foci (ACF). ACF expansion gives rise to an adenoma that can progress to *in situ* carcinoma and finally to invasive adenocarcinoma [12]. Studies in humans and in animal models suggest that intestinal tumor development is a process where each successive genetic change confers growth advantage to tumor cells. Collectively, these genetic changes in cancer cells allow tumor progression through different stages [12]. Indeed, CRC development is considered as a paradigm of stepwise tumorigenesis with subsequent histopathological stages that precede invasive neoplastic growth and are associated with a progressively increasing number of specific genetic aberrations [11].

3.2. Intestinal cancer stem cells

Intriguingly, the biology of intestinal SCs and CRCs is highly interconnected. In many intestinal malignancies, it is assumed that the 'cell of origin' is a SC that acquired the initial mutation(s) necessary for malignant conversion [11]. These genetic alterations promote self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, programmed cell death evasion, limitless replicative potential, sustained angiogenesis, tissue invasion and ultimately metastasis formation [13]. Additionally, heterogeneously differentiated cell types are found in individual CRC specimens, contributing to the idea that such tumors are 'caricatures' of the normal intestinal tissue. This notion is further strengthened by the discovery of SC-like cancer cells that express intestinal SC markers and display multipotency and self-renewal capacities.

It is thought that intestinal CSCs are the cells that drive tumor growth and progression [11]. Indeed, intestinal CSCs, but not intestinal SCs, can regenerate tumors upon transplantation in animals [14]. CSCs are defined by four main characteristics: (i) they can be serially transplanted for multiple generations because of their self-renewal capacity; (ii) CSCs can generate bulk populations of non-tumorigenic cells by asymmetrical division, which is consistent with the hierarchical model of tumor development. Conversely, symmetrical division allows CSC maintenance within the tumor; (iii) CSCs retain their tumorigenic potential when transplanted into animals and (iv) CSCs can be separated from non-SCs using specific surface markers [14, 15]. In the last decades, the concept of CSC hierarchical arrangement has changed our understanding of tumor cell heterogeneity. The current CSC model postulates that CSCs reside at the top of the tumor hierarchy and differentiate unidirectionally into highly proliferative non-CSCs [12].

4. Nuclear signaling pathways that control intestinal CSCs

In this part, we will focus on the major signaling pathways and transcription factors that are involved in the transcriptional regulation of intestinal SC/CSCs (**Figure 2**) and that could consequently be associated with tumor development/progression and/or cancer cell resistance to therapy.

4.1. The Wnt pathway and its effectors

4.1.1. The Wnt pathway

The Wnt pathway is involved in many biological processes and is essential for epithelial intestinal homeostasis (**Figure 2**) [16]. Accumulation and translocation of β-catenin into the

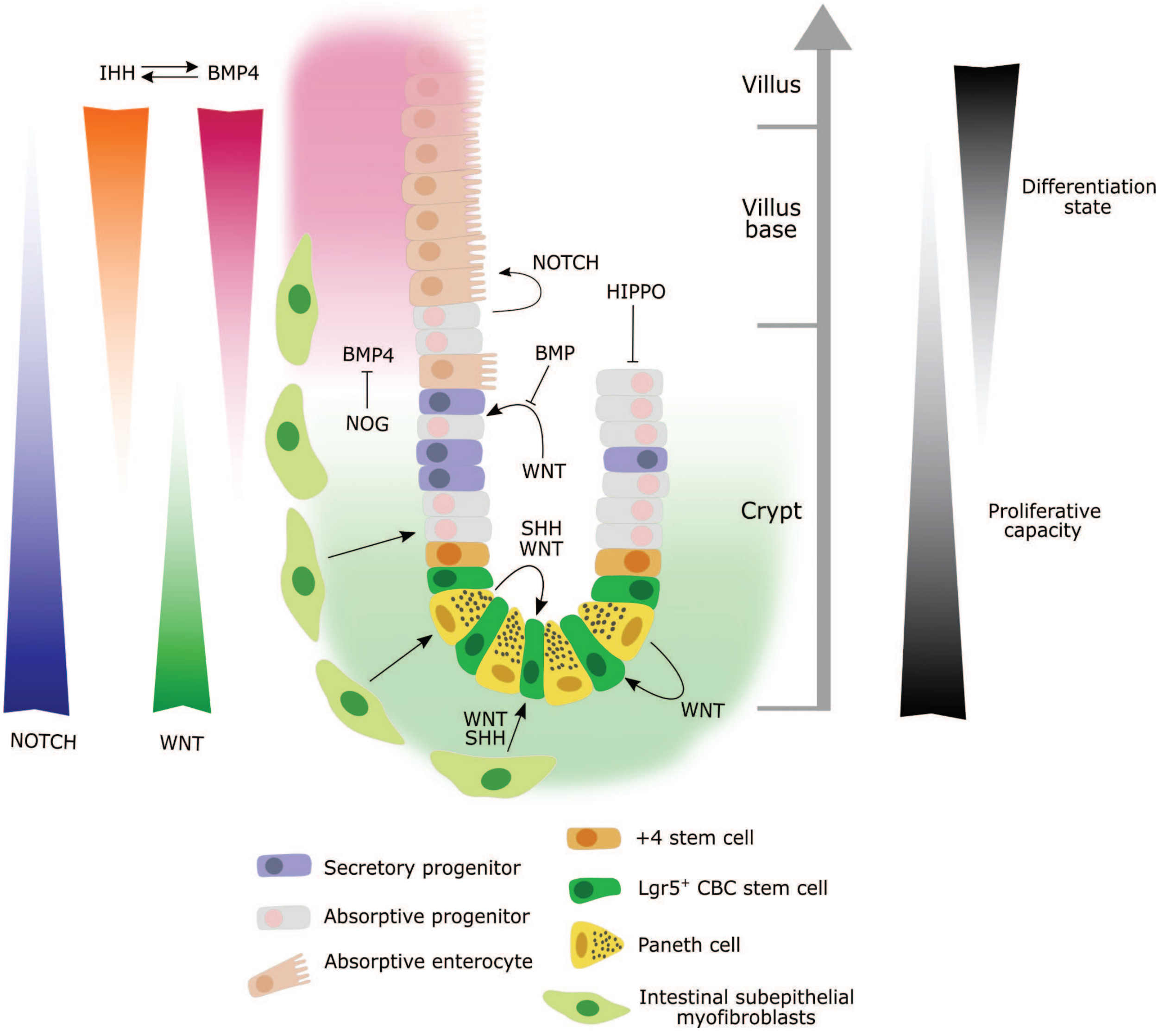

Figure 2. Schematic representation of the major signaling pathways involved in cancer stem cell biology. A gradient of BMP and Hh signaling, with relatively high activity in the villus and less activity within the crypt, regulates cell renewal and lineage specification. Wnt and Notch signaling gradients in the opposite direction (highest expression at the crypt base) play an important role in maintaining the stem cell compartment.

nucleus are the hallmark of the canonical Wnt pathway activity. In the absence of Wnt ligands, β-catenin is phosphorylated, ubiquitinylated and degraded by the proteasomal machinery. Binding of Wnt ligands to their receptors results in the cytoplasmic accumulation of β-catenin that then translocates into the nucleus where it functions as a transcriptional co-activator of Wnt-target genes. The best characterized binding partners of β-catenin in the nucleus are the members of the lymphoid enhancer factor (LEF)/T cell factor (TCF) DNA-binding transcription factors [17]. Some of the downstream targets of the Wnt signaling pathway, such as SOX9 and KLF4/5, are involved in the control of the intestinal CSC phenotype and in CRC development and will be described below (see Sections 4.1.2 and 4.1.3).

In up to 80% of colorectal carcinomas, mutations in molecules that are part of the Wnt/β-catenin pathway (notably truncating mutations in the *Apc* gene) lead to the formation of constitutive nuclear TCF/β-catenin complexes and to uncontrolled transcription of TCF-4 target genes [18]. In the mouse, specific deletion of the *Apc* gene in LGR5+ SCs triggers the formation of many LGR5+ adenomas in the small and large intestine [5]. Similarly, lack of β-catenin repression in intestinal +4 SCs promotes the formation of BMI1+ adenomas [19]. Moreover, loss of APC negative control induces constitutive nuclear β-catenin/TCF complex activation and hyper-proliferation of the SC compartment [19].

The Wnt signaling pathway has a role also in human intestinal CSCs. In spheroid cultures of CSCs isolated from biopsies of patients with CRC, Wnt expression is heterogeneous. Injection of $\mathrm{Wnt}^{\mathrm{high}}$ cells in mice results in more effective tumor formation compared with $\mathrm{Wnt}^{\mathrm{low}}$ cells. The heterogeneous Wnt expression pattern is maintained in the tumors and is related to the expression of several intestinal SC markers, such as LGR5 and ASCL2 [20]. Additionally, colonospheres developed from human CSCs show increased β-catenin expression, associated with transcriptional activation of TCF/LEF [21]. Hence, activation of Wnt/β-catenin signaling can convert intestinal SCs into CSCs, which corresponds to the first step of malignant transformation [19].

Several studies tried to correlate β-catenin activation/expression level with the outcome of patients with CRC. For instance, in 2007, Lugli et al. analyzed tissue microarray data on more than 1400 CRC biopsies and found that high level of β-catenin nuclear expression is an independent adverse prognostic factor [22].

4.1.2. SOX9

The SRY-related high-mobility group box 9 (*SOX9*) gene is a physiological target of the TCF/β-catenin complex that promotes cell proliferation. This key terminal effector of the Wnt pathway is required for +4 SC differentiation into Paneth cells [23]. In the intestinal epithelium, SOX9 expression pattern in the SC compartment almost perfectly overlaps with that of the proliferative marker Ki-67. Interestingly, SOX9 positively regulates its own expression in many cell types and exerts a negative feedback-loop on TCF/β-catenin activity, leading to restriction of intestinal SC proliferation [23, 24].

SOX9-deficient mice exhibit higher cell proliferation, extensive colon hyperplasia with numerous enlarged crypts. However, SOX9 deletion is not sufficient to induce malignancy [25]. Moreover, SOX9 overexpression in human CRC cells results in cell cycle progression and apoptosis bypass, due to increased *BMI1* gene expression [26]. Additionally, in colon epithelial cells, high SOX9 expression is associated with undifferentiated states, SC-like properties and high LGR5 mRNA level *in vitro* [27]. SOX9 has several pro-oncogenic properties, including the ability to promote cell proliferation, to inhibit senescence and to collaborate with other oncogenes in neoplastic transformation [26]. However, recent *in vitro* and *in vivo* studies have described SOX9 tumor suppressor activities in CRC cells. Specifically, SOX9 inhibits β-catenin activity by interacting physically with this protein and removing it from chromatin. It also decreases expression of the c-Myc oncogene, a target of the Wnt/β-catenin pathway [28].

The strong expression of SOX9 in CRC cells due to the constitutive activity of the Wnt pathway can contribute to cancer progression and/or influence tumor differentiation. *SOX9* displays missense or frameshift mutations in almost 10% of CRC [29]. SOX9 mutation rate is higher in more advanced tumors and is correlated with activated KRAS, an oncogene frequently mutated during

CRC development, thus facilitating transformation and tumor progression [29]. Furthermore, a SOX9 splice variant (MiniSOX9) that contains the HMG domain responsible for binding to DNA but devoid of the trans-activating domain has been discovered [30]. MiniSOX9 inhibits SOX9 activity by a dominant-negative effect *in vitro* and can promote the Wnt/β-catenin pathway, resulting in β-catenin over-activation. In addition, strong MiniSOX9 expression is observed in CRC tumor tissue, while it is undetectable in the adjacent normal tissue [30]. Wild type and many SOX9 mutants regulate tumor proliferation capacity, notably through regulation of the CSC pool. Nevertheless, SOX9 protein level could not be clearly associated with patient prognosis [31].

4.1.3. Krüppel-like factors (KLF)

4.1.3.1. KLF4

KLF4 was originally identified as a gut-enriched transcription factor in the intestine and is expressed in terminally differentiated columnar intestinal epithelial cells [32]. KLF4 regulates intestinal epithelial homeostasis and has a critical role in the development and terminal differentiation of goblet cells [32]. In human HT-29 CRC cells, KLF4 inhibits cell proliferation by blocking progression from the G1 to S phase of the cell cycle through inhibition of cyclin D1 expression [33].

Moreover, mutations in the Wnt/β-catenin pathway are associated with KLF4 downregulation in human CRC cell lines. Indeed, KLF4 is an indirect APC target and is considered to be a repressor of BMI1 transcriptional activity [34, 35]. Furthermore, using a KLF4 inducible system in CRC cell lines, it was demonstrated that KLF4 reduces colony formation, cell migration and invasion [34]. Additionally, KLF4 overexpression in human adenocarcinoma cells leads to reduced [3H]-thymidine uptake, whereas inhibition of KFL4 expression increases DNA synthesis, confirming that KLF4 plays an essential role in colon cell growth arrest [36]. Surprisingly, despite its tumor suppressor activity, KLF4 is overexpressed in colon CSC-enriched spheroids compared with the parental CRC cells from which the spheroids were derived [37]. Moreover, KLF4 knockdown affects the stemness phenotype and decreases the malignant profile of these CSC-enriched spheroid cells, in line with its role in reprogramming murine fibroblasts into stem cells [37, 38].

In agreement with its tumor suppressor activity, KLF4 expression is frequently lost in CRC and its downregulation is strongly associated with tumor development. Moreover, loss of heterozygosity on chromosome 9q31, where the *KLF4* gene is localized, is frequently found in human CRC, and could lead to uncontrolled cell proliferation and to a SC-like phenotype of differentiated cells [33]. Low KLF4 expression levels are also found in colon adenomas and metastases [33]. Lee et al. confirmed that *KLF4* mRNA expression levels are lower in CRC tumor tissue compared with normal tissue [39]. However and surprisingly, they observed that high KLF4 level in normal tissue is correlated with high KLF4 expression in tumors and is associated with poor patient survival [39].

The conflicting results between clinical studies concerning KLF4 prognostic value could be explained by the differential regulation of KLF4 mRNA and protein expression in CRC or by the presence also of KLF4+ stromal cells in the tumor samples. Additional investigations are needed to elucidate these data; nevertheless, KLF4 expression levels in normal and tumor tissues are prognostic markers for CRC.

4.1.3.2. KLF5

The transcription factor KLF5 can interact with several components of different signaling pathways (e.g., the Wnt, Hippo, TGF-β and Notch signaling cascades) and mediate their activity [40]. In physiological conditions, KLF5 is strongly expressed by intestinal progenitor and stem cells, suggesting a role in cell proliferation control [41].

Stable KLF5 overexpression in HT-29 CRC cells promotes spheroid formation [40]. Conversely, deletion of the *KLF5* gene in mouse LGR5+ SCs promotes β-catenin nuclear localization and the appearance of abnormal apoptotic cells in the intestinal crypts, due to inhibition of their proliferation and survival capacities [41]. In agreement, KLF5 is required for the tumor-initiating activity of β-catenin during intestinal tumorigenesis in Apc^{Min} mice [41]. Inhibition of *KLF5* gene expression in CRC cell lines reduces cell proliferation and transformation as well as anchorage-independent growth [42].

In patients with CRC, intestinal tumor progression is associated with *KLF5* gene upregulation in the primary tumor and also in metastases, compared with healthy tissues [41]. Moreover, comparative genomic hybridization (CGH) array analysis of human CRC samples highlighted the frequent chromosomal amplification of the *KLF5* locus [41]. CRC samples with mutated KRAS also display KLF5 upregulation, associated with increased cell proliferation [42]. As activating KRAS mutations are found in more than 50% of CRC, KLF5 appears to be an important downstream mediator of activated KRAS during CRC development. These findings indicate that KLF5 is a major regulator of intestinal SC proliferation in normal and pathological conditions.

4.2. The Notch pathway and BMI1

4.2.1. The Notch pathway

The Notch signaling cascade is one of the major pathway involved in intestinal homeostasis and in the direct regulation of cell fate [43]. The initiating step of the Notch signaling cascade is the interaction between one of its five ligands (Delta-like1/3/4, Jagged1/2) and a Notch receptor (Notch1–4). Upon ligand binding, the receptor conformational change through proteolytic cleavage leads to nuclear translocation of cleaved Notch intracellular domain (NICD) and its association with the DNA-binding transcription factor CSL (also called RBP-Jκ). This turns the CSL complex from a transcriptional repressor into a transcriptional activator. The best known targets of the CSL/NICD complex are members of the *HES* gene family and their homologs, the *Hey* (also called HERP) gene family of basic helix-loop-helix transcription factors. This is known as the canonical Notch pathway [17, 43, 44].

In the colon, Notch signaling is an essential gatekeeper of intestinal progenitors and clearly plays an important role in the maintenance of the colon crypt compartment [45] (**Figure 2**). Using small-molecule inhibitors and short hairpin RNA-mediated knock-down, it has been demonstrated that Notch prevents apoptosis of colon cancer-initiating cells (CCICs) and is critical for self-renewal [46]. Moreover, the Notch pathway supports slow-cycling BMI1+ CCICs, by promoting their self-renewal, tumorigenicity and chemoresistance in tumor xenografts [47].

In CRC, the Notch pathway is strongly activated compared with normal tissue. Moreover, expression analysis of resection biopsies from patients with CRC showed that Notch1 expression level is correlated with poor prognosis and is a good predictive marker of cancer progression [48]. Intriguingly, the expression level of Notch2 is negatively correlated with that of Notch1 in CRC and Notch2 has anti-tumoral properties [48]. These opposite features could be used to develop a fine prognostic marker of CRC progression and recurrence.

4.2.2. BMI1

BMI1 is a downstream target of Notch signaling and a key component of the Polycomb group [49]. BMI1 is expressed in almost all tissue types and regulates a myriad of cellular processes that are critical for cell growth, cell fate decision, development, senescence, aging, DNA damage repair, apoptosis and SC self-renewal [49, 50]. BMI1 is highly expressed in intestinal SCs and isolated BMI1+ cells can generate epithelial organoids in culture [7]. Additionally, BMI1 loss decreases murine intestinal SC proliferation and promotes their differentiation into goblet cells [49]. BMI1 also contributes to the tumor-initiating and self-renewal abilities of human CRC cells because its downregulation inhibits tumor cell growth and is associated with reduction of tumor-initiating cells [51]. Moreover, BMI1 is involved in intestinal CSC invasion and migration. Indeed, a recent study demonstrated that BMI1 represses E-cadherin expression in colon CSCs, thus promoting metastasis formation via epithelial to mesenchymal transition [50].

Altogether, these data strongly support BMI1 role in the maintenance of the intestinal CSC phenotype. In agreement, clinical studies showed that BMI1 expression is a negative prognostic marker in CRC [52]. BMI1 mRNA and protein are overexpressed in colorectal adenomas and carcinomas compared with normal tissues [53]. A gradient of BMI1 expression has been reported in human colon precancerous and cancerous tissues and is correlated with the cancer stage, suggesting that BMI1 contributes to CRC progression [53].

Indeed, BMI1 is considered to be a negative CRC prognostic biomarker, and patients with BMI1-positive tumors are at higher risk of disease recurrence and/or metastases compared with those with BMI1-negative tumors. As BMI1 has a role in maintaining the intestinal CSC phenotype, high BMI1 expression could indicate the presence of a large CSC population in the tumor. Consequently, high proportion of CSCs in a tumor could be an indicator of poor prognosis [31, 54, 55].

4.3. Other signaling pathways

4.3.1. The Hedgehog pathway

The Hedgehog (Hh) signaling pathway is a key regulator of intestinal homeostasis. Hh proteins are part of a family of secreted proteins that are involved in the development and maintenance of the gastrointestinal tract [17]. Aberrant activation of the Hh signaling pathway is associated with tumorigenesis in various tissues. The roles of Hh signaling differ at each CRC stage, from adenoma to adenocarcinoma [56]. Moreover, Sonic Hedgehog (SHH), one of the Hh effectors, promotes CRC development, while Indian Hedgehog (IHH) inhibits CRC formation [56].

IHH regulates intestinal SC fates by interfering with the maturation and localization of the underlying stromal cells that in turn generate signaling molecules needed for the maintenance of the intestinal SC niche [56] (**Figure 2**). IHH, expressed by differentiated enterocytes, indirectly inhibits Wnt signaling at the crypt base and reduces the number of proliferating precursor cells [17, 57]. A decrease in Hh signaling is correlated with the expansion of the intestinal SC pool, with blunted enterocyte differentiation and activation of the Wnt pathway. Moreover, *IHH* gene knock-out leads to intestinal SC accumulation [57]. In addition, specific Hh activation in murine stromal cells induces complex transcriptional changes, leading to loss of colon SC-specific gene expression and upregulation of epithelial differentiation markers [58]. Most of the components of the Hh signaling pathway are upregulated (mRNA and protein) in CRC, with the exception of IHH that appears to be downregulated. Overexpression of members of the Hh signaling pathway is associated with poor survival and adverse clinical features [59]. However, in metastatic CRC, treatment with vismodegib, an Hh pathway inhibitor, in combination with standard chemotherapy, does not significantly improve patient survival [60].

4.3.2. The BMP pathway

The BMP pathway regulates many cellular mechanisms, including apoptosis and cell growth, depending on the specific cellular context. BMP ligands are secreted in their active form and homodimerize before binding to their cognate BMP receptors (BMPR). SMAD transcription factors are the main downstream effectors of BMP signaling that plays key roles in adult gut homeostasis, inflammation and cancer.

Specific inhibition of BMP signaling in intestinal epithelial cell does not lead to initiation of colon tumors *in vivo*, while suppression in mesenchymal myofibroblasts is associated with spontaneous tumor formation. This suggests that inhibition of BMP signaling in the mesenchymal cells surrounding the intestinal epithelium acts as a trigger of gastrointestinal tumorigenesis [61]. Moreover, BMP4 expression is lost in intestinal CSCs, leading to deregulation of the proliferative compartment [62].

Nevertheless, it is still unclear whether BMP limits expansion of intestinal epithelial cells by repressing LGR5+ intestinal SC self-renewal or by inhibiting epithelial cell proliferation. In addition, BMP type Ia receptor (*Bmpr1a*) conditional knock-out in the intestinal epithelium leads to intestine hyperplasia with multiple intestinal polyps due to hyperactive SCs [63]. Moreover, in these mice, the LGR5+ SC pool is enlarged due to increased survival, allowing better intestinal regeneration [63]. Among the BMP family members, BMP2 and BMP4 are specifically involved in intestinal CSC regulation by promoting their differentiation and antagonizing Wnt/β-catenin signaling [64]. Furthermore, a recent study showed that the transcription factor GATA6 is a key regulator of CSC expansion and self-renewal through downregulation of BMP genes [65].

Finally, mutations that affect BMP signaling are frequently observed in patients with juvenile polyposis syndrome that is characterized by non-cancerous polyps, as well as in patients with progressing CRC. Analysis of SMAD4 expression levels in patients with CRC showed that it is downregulated in CRC and associated with poor prognosis [66].

4.3.3. The Hippo pathway

The Hippo pathway regulates various cellular processes, including cell survival, proliferation and differentiation, but has been involved only recently in SC biology [67]. Yes-associated protein (YAP) and transcriptional co-activator with PDZ binding motif (TAZ) are the central effector molecules of this signaling cascade and are abundantly expressed in the cytoplasm of both proliferating and post-mitotic cells [17, 68].

In cancer, the Hippo pathway inhibits cell proliferation, promotes apoptosis and regulates stem/progenitor cell expansion. In cancer cells, YAP and TAZ are localized mainly in the nucleus and promotes cell and tumor growth. There is considerable evidence that abnormal Hippo signaling is associated with tumor progression and YAP/TAZ overexpression is frequently observed in CRC [67]. This overexpression could be linked to Wnt/β-catenin over-activation because YAP is a specific target of this pathway. Furthermore, the major components of the Hippo pathway (i.e., MST1/2 and MOBKL1A/B) that control YAP/TAZ activity display low expression levels in colon carcinomas [69]. YAP deletion in *Apc*^{min} mice prevents polyp formation and blocks the differentiation of *Apc*$^{-/-}$ organoids. Moreover, using a mosaic model of *Yap* and *Apc* gene deletion in intestinal SCs, YAP appears to be dispensable for tumor initiation, but crucial for progression of tumor-initiating cells to adenoma [70].

Hippo pathway dysregulation, leading to loss of YAP repression, has been observed in different cancer types [71]. In patients with CRC, YAP over-activation is closely related to β-catenin over-activation. Moreover, the tyrosine kinase c-Yes is hyper-phosphorylated in 5-fluorouracil-resistant cells with CSC features, thus preventing YAP nuclear translocation [72]. Finally, *YES1* and *YAP* levels are correlated with worse prognosis in chemotherapy-treated patients with CRC, suggesting that chemotherapy favors the selection of intestinal CSCs with deregulated c-Yes and YAP [72].

4.4. Other intestinal CSC-related transcription factors

4.4.1. PXR

Pregnane X Receptor (PXR, NR1I2), a member of the nuclear receptor superfamily, is highly expressed in the colon. PXR targets are genes that encode phase I and II metabolic enzymes and phase III drug transporters. Members of the nuclear receptor superfamily function as ligand-activated transcription factors and play critical roles in nearly every aspect of development and adult physiology [73]. Interestingly, it has been reported that the Wnt/β-catenin signaling pathway is crucial for PXR activity and notably that β-catenin is required for PXR-mediated induction of target gene expression [74].

Planque et al. have recently demonstrated that PXR is a potent intestinal CSC phenotype driver by regulating a network of downstream genes involved in self-renewal and chemoresistance [75]. PXR expression is associated with CSC enrichment, after cell sorting of cancer cells using ALDH activity to identify CSCs and after spheroid passaging. In addition, expression of CSC markers and self-renewal are increased in CRC cells with enhanced PXR transcriptional activity [75]. PXR expression in intestinal CSCs is also associated with tumor aggressiveness and chemoresistance [76]. Specifically, PXR increases the oxaliplatin efflux capacity of cancer cells, thus reducing the cell drug concentration and preventing its effects on cell proliferation and apoptosis [76]. Another study demonstrated that PXR is a master regulator of chemoresistance by regulating genes involved in drug resistance, such as cytochrome P450, multidrug resistance 1 and multidrug resistance-associated protein 2 [77]. Furthermore, PXR is associated with poor survival, particularly after drug treatment. Indeed, in patients with CRC, it allows clonal selection after treatment, leading to the emergence of resistant and more aggressive clones with molecular signatures of poor prognosis [75, 77].

4.4.2. HOPX

The homeodomain-only protein homeobox (HOPX) is strongly expressed in normal colorectal mucosa, and is considered a marker of the +4 SC population in the intestine [78]. Conversely, HOPX-β (an isoform of HOPX) represses conversion to the CBC phenotype in +4 SCs in physiological contexts in mice [78].

HOPX shows tumor suppressor functions in CRC by regulating cell proliferation and inhibiting angiogenesis [79]. Microarray data analysis revealed that, in CRC samples, HOPX downregulates oncoproteins, such as c-FOS and EGR-1. Moreover, EphA2 (which increases tumor invasion and survival) is overexpressed in patients with *HOPX* gene hypermethylation. In addition, HOPX-β promoter is frequently hypermethylated in CRC cell lines and tissues. This methylation results in the downregulation of HOPX mRNA and protein levels. Importantly, in patients with stage III CRC, HOPX-β promoter hypermethylation is associated with worse prognosis [79]. Moreover, in patients with CRC, *HOPX* gene hypermethylation is accompanied by increased expression of Cyr61/CCN1, a critical downstream member of the Hh signaling pathway that affects the pro-angiogenic tumor microenvironment [80].

4.4.3. Sp1

Specificity protein 1 (Sp1) is a transcription factor ubiquitously expressed in mammalian cells that recruits the basal transcription machinery. Sp1 is active in all cell types, but it is also tightly regulated because Sp1 activity can alter the expression of genes involved in cell cycle and growth (including many tumor suppressor genes and oncogenes) in response to signaling pathways and specific cellular conditions [81].

Interestingly, Sp1 levels are higher in colon CSCs than in the parental tumor cells [82]. Moreover, siRNA-mediated *SP1* silencing suppresses the specific features of CSCs derived from CRC cells and promotes apoptosis of colon CSCs *in vitro* [82]. *SP1* silencing also decreases the expression of several CSC markers. Hence, colon CSC self-renewal ability, drug resistance and metastasis potential could be partially related to high Sp1 expression. In agreement, Sp1 overexpression correlates with tumor stage and poor prognosis [81].

5. Conclusion

Cancer management is one of the major issues in our society and therefore, much research is focused on improving our understanding of cancer development and progression. Here, we presented an overview of the transcriptional dysregulation that affect intestinal epithelium homeostasis and that can lead to tumor initiation and development. In the last decade, considerable progress has been made in understanding the molecular and cellular mechanisms linked to CRC development/progression and a major breakthrough was the identification of cells with CSC properties. Studies in mouse models have shown that CRC development is mainly supported by intestinal CSCs that can self-renew and generate tumor cell heterogeneity even after *in vitro* or *in vivo* passaging. However, CSCs do not cycle as fast as cancer cells. This means that the current therapies that target cycling cancer cells are not efficient against the relatively quiescent CSCs.

CSC fate and properties are regulated through a wide transcriptional network controlled by signaling cascades that often crosstalk and regulate each other (**Figure 3**).

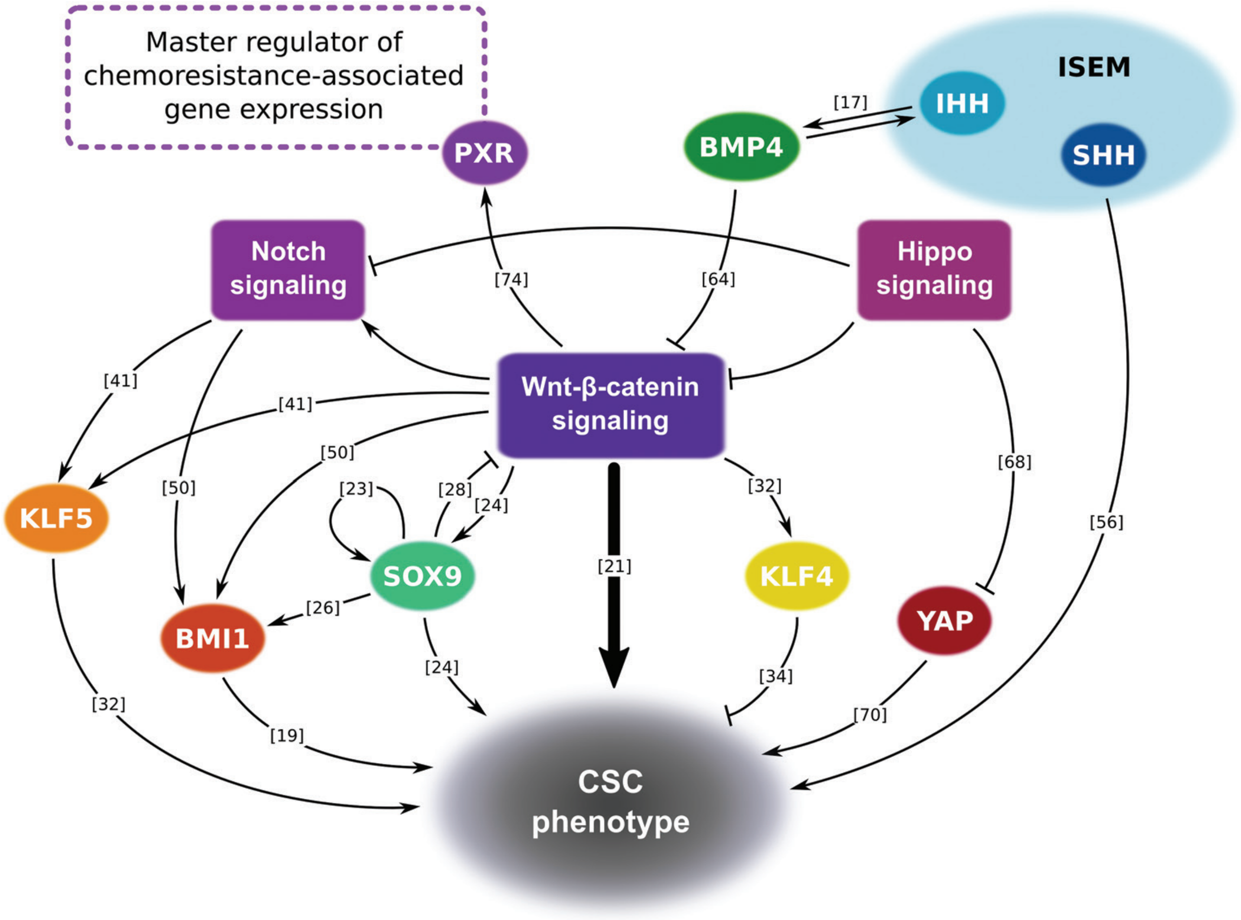

Figure 3. Transcriptional landscape associated with the CSC phenotype in CRC. Schematic representation of the positive (arrows) and negative (bar-ended arrows) regulations between transcription factors and signaling pathways and associated with the CSC phenotype within the tumor. ISEM = intestinal subepithelial myofibroblasts.

Transcription factors	Physiological roles	Status in CRC	CRC-associated phenotype	Prognosis	References
SOX9	Differentiation of Paneth cells Promotion of SC proliferation	Overexpression	Cell cycle progression Apoptosis bypassing undifferentiated state	No correlation	[23–27, 31]
KLF4	Differentiation of Goblet cells	Low expression	Increased DNA synthesis Uncontrolled cell proliferation CSC-like phenotype	Poor	[32, 33, 36, 39]
KLF5	Promotion of cell proliferation	Overexpression	Promotion of cell proliferation Increase of cell survival capacities	No correlation	[41]
BMI1	Promotion of SC proliferation and renewal Prevention of senescence DNA damage repair	Overexpression	Tumor initiation Self-renewal of CRC cells Promotion of cell invasion and migration	Poor	[31, 49–51, 53–55]
IHH	Differentiation of enterocyte cells Inhibition of cell proliferation	Low expression	Expansion of the CSC pool Promotion of cell proliferation		[17, 56, 57]
SHH	Promotion of cell proliferation	Overexpression	Promotion of CRC development	Poor	[56, 59]
SMAD	Differentiation of enterocyte cells Inhibition of Lgr5$^+$ SC expansion	Low expression		Poor	[61, 66]
YAP/TAZ	Promotion of cell proliferation	Overexpression and over-activation	Tumor progression	Poor	[67, 70, 72]
PXR	Increase of cholesterol uptake Promotion of intestinal epithelial wound healing and repair		CSC self-renewal Drug resistance	Poor	[73, 75, 77]
HOPX	Maintenance of +4 SC identity	Low expression	Promotion of cell proliferation Promotion of angiogenesis	Poor	[78, 79]
SP1	Cell cycle and growth control	Overexpression	CSC renewal ability Drug resistance Metastasis potential	Poor	[81, 82]

Table 1. Phenotypic outcomes associated with the different transcription factors in normal and tumoral intestinal epithelium.

In this review, we focused on some of these transcription factors and major signaling pathways involved in the regulation of the intestinal CSC phenotype and in CRC development. The basis of CRC development is the over-activation of the Wnt/β-catenin signaling cascade. Then, the disruption of other signaling pathways potentiates the oncogenic process by maintaining or even amplifying these alterations. Similarly, mutations or altered expression of different transcription factors also contribute to the oncogenic network. All these mechanisms concur to promote tumor growth and aggressiveness due to CSC enrichment. Moreover, some of these pathways and transcription factors might confer chemoresistance to the CSC population and are involved in CRC relapse (**Table 1**). Therefore, they are considered poor prognostic markers. Consequently, effective CRC therapies should target not only the highly proliferative cancer cells but also colon CSCs, or sensitize them to therapies. These different signaling pathways and their downstream effectors could represent biomarkers of CRC progression and therapeutic targets.

To conclude, these data do not give the solution on how to cure CRC, but help understanding why its management is not simple. Several topics presented in this review are field of active research. Indeed, there are multiple and complex interactions between key signaling pathways known to control SC behavior. The knowledge on the transcriptional networks that control intestinal CSCs is not complete yet, and some findings are controversial. A better characterization and comprehension of these regulatory mechanisms, notably through network analysis, are needed to identify new therapeutic targets in order to improve patient care.

Abbreviations

ACF	Aberrant crypt foci
ALDH	Aldehyde dehydrogenase
APC	Adenomatous polyposis coli
ASCL2	Achaete-scute family bHLH transcription factor 2
BMI1	B lymphoma Mo-MLV insertion region 1 homolog
BMP4	Bone morphogenetic protein 4
BMPR	Bone morphogenetic protein receptor
CBC	Crypt-based columnar
CCICs	Colon cancer-initiating cells
CGH	Comparative growth hybridization
CRC	Colorectal cancer
CSCs	Cancer stem cells
Cyr61	Cysteine-rich angiogenic inducer 61
EGR1	Early growth factor response 1

EphA2	Ephrin receptor A2
GATA6	GATA binding protein 6
HES	Hairy and enhancer of split
HH	Hedgehog
HMG	High-mobility group
HOPX	Homeodomain-only protein homeobox
IHH	Indian hedgehog
ISEM	Intestinal subepithelial myofibroblasts
KLF	Kruppel-like factor
LEF	Lymphoid enhancer factor
LGR5	Leucine-rich repeat containing G protein-coupled receptor 5
MOBKL1A/B	Mps one binder kinase activator-like 1A and B
MST1/2	Mammalian Ste2-like kinases 1 and 2
MYC	Myelocytomatosis oncogene
NICD	Notch intracellular domain
NOG	Noggin
PXR	Pregnane X receptor
RBP-Jκ	Recombination signal binding protein for immunoglobulin kappa J region
SC	Stem cells
SHH	Sonic hedgehog
SMAD	Mother against Dpp
SOX9	SRY (sex-determining region Y)-related HMG box 9
SP1	Specificity protein 1
TAZ	Transcriptional co-activator with PDZ binding motif
TCF	T cell factor
TGF-β	Tumor growth factor-β
Wnt	Wingless-type MMTV (mouse mammary tumor virus) integration site family
YAP	Yes-associated protein

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