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TOPIC HIGHLIGHT

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Intestinal stem cells and the colorectal cancer microenvironment

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Abstract

Colorectal cancer (CRC) remains a highly fatal condition in part due to its resilience to treatment and its propensity to spread beyond the site of primary occurrence. One possible avenue for cancer to escape eradication is via stem-like cancer cells that, through phenotypic heterogeneity, are more resilient than other tumor constituents and are key contributors to cancer growth and metastasis. These proliferative tumor cells are theorized to possess many properties akin to normal intestinal stem cells. Not only do these CRC "stem" cells demonstrate similar restorative ability, they also share many cell pathways and surface markers in common, as well as respond to the same key niche stimuli. With the improvement of techniques for epithelial stem cell identification, our understanding of CRC behavior is also evolving. Emerging evidence about cellular plasticity and epithelial mesenchymal transition are shedding light onto metastatic CRC processes and are also challenging fundamental concepts about unidirectional epithelial proliferation. This review aims to reappraise evidence supporting the existence and behavior of CRC stem cells, their relationship to normal stem cells, and their possible dependence on the stem cell niche.

Key words: Colon cancer stem cells; DCLK1 protein; Stem cell niche; Cell dedifferentiation

Core tip: Colorectal (CRC) cancer stem cells are a theorized but poorly characterized cell population believed to be crucial for tumor growth, spread, and tenacity. CRC stem cells share many similar characteristics of normal intestinal stem cells and are hypothesized to originate directly from them. It appears, however, that both the regulation of normal intestinal stem cells and the development of CRC are far more complex than previously imagined. Likely pivotal to the success of both are plasticity pathways able to reverse cellular fate, and stem cell niche signals, ultimately leading to self-replenishment and sometimes also unwanted dissemination.

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INTRODUCTION

Colorectal cancer (CRC) remains a highly morbid and fatal disease among both developed nations and globally[1-3]. Based on 2008 world data, CRC is the fourth leading cause of cancer-related mortality behind lung, stomach, and liver cancer, respectively[4,5]. Since Fearon *et al*[6] introduced a model for colorectal tumorigenesis in 1990, the study of the molecular basis of CRC has

been rapidly evolving. While a handful of tumor suppressors and oncogenes (*e.g.*, APC, KRAS, and P53) are commonly found among CRCs, a vast number of low-frequency somatic mutations have since been discovered that are believed to contribute to CRC heterogeneity[7,8]. Given the expanded number of potentially functional mutations, that no CRC therapy is completely curative should come as no surprise[9].

More importantly, individual colorectal cancers can themselves demonstrate phenotypic variability via sub-delegation of constituent cells. Core to this notion are cancer "stem" cells which act as ringleaders that drive CRC proliferation and metastasis[10]. Like normal stem cells, they self-perpetuate and expand in accordance with stem cell hierarchy[10]. Much remains unknown about the origins and regulation of CRC stem cells, though implicated in CRC inception are the signals expressed within the normal intestinal stem cell niche. New light has also been shed onto plasticity pathways that may perhaps be pivotal to CRC metastasis and treatment. The aim of this review is to reappraise current evidence supporting the existence and behavior of CRC stem cells, their relationship to normal stem cells, and their possible dependence on the stem cell microenvironment.

FEARON AND VOGELSTEIN'S MODEL FOR COLORECTAL CARCINOGENESIS

Fearon and Vogelstein's model for colorectal carcinogenesis illustrates how genetic alterations may allow colorectal cells to escape defined behaviors of the normal intestinal epithelium. By the early 1990s, Fearon *et al*[11] established three key features about colorectal cancer. First, cells within a colorectal cancer are monoclonal in nature, suggesting that CRC arises from clonal expansion of a small number of cells. Second, Fearon *et al*[6] surmised that key genetic alterations found commonly among CRC (*e.g.*, RAS, P53, APC) confer functional traits advantageous to the development and expansion of sporadic cancer and are acquired in a sequentially preferred order. For instance, APC mutations often occurred early prior to adenoma formation, whereas P53 mutations frequented tumor phases during the transition of adenomas to overt carcinomas[6]. Finally, based on their own observations and those of others, Fearon *et al*[6] concluded that the number of accumulated mutations in a tumor was the most consistent feature associated with the clinical and histopathological manifestation of CRC[12].

Fearon and Vogelstein's original CRC model has since been greatly expounded upon. Numerous low-frequency candidate mutations have been identified among candidate CRC genes, likely contributing to CRC phenotypic heterogeneity[7,8]. Also, carcinogenesis might not rely strictly on Fearon and Vogelstein's hypothesized mutational gateways. For example, one study found no genetic change between genome-sequenced primary colorectal cancers and their respective metastases, suggesting that insufficient time passed to allow either primary or metastatic lesions to acquire distinguishing mutations[7].

NORMAL INTESTINAL STEM CELLS

Two functionally distinct populations of putative normal epithelial stem cells have been identified in intestinal crypts of humans and mice: Lgr5$^+$ crypt base columnar stem cells and quiescent label-retaining cells[13-17]. These two cell types replenish and maintain the intestinal epithelium[13].

Lgr5$^+$ crypt base columnar cells

Lgr5$^+$ crypt base columnar cells (CBCs) are multipotent stem cells located in crypts of the small intestine and colon[14]. Lgr5 is an orphan G protein-coupled receptor expressed during embryogenesis and among epithelial stem cell populations in the adult intestine, hair follicles, stomach, mammary glands, and taste buds[18]. CBCs were first characterized in 1974 when an electron microscopy study identified a population of crypt cells that shared common secretory components with all differentiated epithelial cell lineages in the mouse intestine[19]. More recently, Barker *et al*[14] demonstrated that Lgr5-mediated activation of a permanent cell-labeling gene identified a line of cells originating from the intestinal crypt that yielded three differentiated cell types. The authors surmised that enteroendocrine cells were too rare to be detected among labeled cells[14]. A subsequent *in vitro* study demonstrated that organoids derived from single Lgr5$^+$ cells form crypt domains containing all lineages of the adult intestinal epithelium including enteroendocrine and crypt paneth cells[20]. Taken together, these findings strongly suggest that multipotent Lgr5$^+$ CBCs are true intestinal epithelial stem cells.

Quite contrary to expected stem cell behavior, evidence suggests that the expansion of Lgr5$^+$ CBCs follows stochastic principles in which cells are equipotent and segregate chromosomes randomly[18,21,22]. Lgr5$^+$ cells are also mitotically-active and demonstrate little asymmetric division[13,21]. Proliferation of these stem cells can at times approximate a square root growth curve, suggesting that they contain potential for rapid, yet very random clonal expansion[13,21,23]. As a likely consequence of their stochastic properties, Lgr5$^+$ stem cells are subject to neutral drift, often resulting in monoclonal or oligoclonal populations in the intestinal crypt[21].

It seems dangerous for a stem cell to propagate in a manner dictated largely by chance. Random chromosomal segregation risks the introduction of genomic errors that can subsequently be passed to both daughters and self-perpetuating clones. Lgr5$^+$ cells also seem to have little control over cell fate, suggesting that they are likely critically regulated by the surrounding milieu.

Quiescent label-retaining cells

Quiescent DNA label-retaining intestinal stem cells (LRCs) have remained controversial since the 1970s when these mitotically-inactive cells were found at and around the

+4 crypt position[24-26]. Although intestinal LRCs express a number of stem cell markers including Hopx, Tert, Lrig1, and Dclk1, they are widely identified by their expression of Bmi1, a member of chromatin-silencing polycomb-repressing complex 1[13,15,27]. Like Lgr5$^+$ CBCs, Bmi1$^+$ LRCs can form spheroids *in vitro* containing all differentiated epithelial cell types[13,20]. The multipotency of Bmi1$^+$ LRCs has also been confirmed *in vivo* through lineage experiments[15]. In contrast to early reports of the radiation sensitivity of +4 position crypt cells, recent evidence suggests that quiescent stem cells are both resistant to and activated by moderate levels of radiation damage, thus suggesting a crucial role in recovery following intestinal injury[13,28]. Notably, Bmi1$^+$ LRCs can single-handedly restore radiation-ablated mouse intestinal epithelium in the total absence of Lgr5$^+$ stem cells[13].

Whether +4 quiescent LRCs are actually stem cells remains a matter of debate. Quiescent stem cells have only been found in the proximal small intestine and to date no presence has yet been found of a corresponding population in the colon[15,29]. Moreover, one study has identified quiescent LRCs not as stem cells, but rather as partially-differentiated secretory precursors[30]. Quiescent stem cell markers (including Bmi1, Tert, Hopx, and Lrig1) have also been found among Lgr5$^+$ stem cells thereby questioning the validity of using such markers to identify a uniquely separate stem cell population[31].

An evolving model of normal intestinal stem cell behavior

In contrast to current single-lineage stem cell theories, the coexistence of two putative intestinal stem cell types may suggest a more complex pathway for the development of the intestinal epithelium (Figure 1)[10,32]. On one hand, evidence exists supporting the subordinancy of LRCs to LGR5$^+$ cells: LRCs have been characterized as secretory precursors and may not share markers unique from Lgr5$^+$ cells[30,31,33]. On the other hand, evidence also exists conversely that Lgr5$^+$ cells may be subordinate to LRCs: Bmi1$^+$ LRCs restore radiation-ablated Lgr5$^+$ cell populations[13,29]. These findings when taken together suggest that LRCs likely interconvert with Lgr5$^+$ CBCs, regardless of whether LRCs are actually stem cells. Such findings suggest that intestinal epithelial development is neither as hierarchical nor as unidirectional as once thought, though the extent of which is not yet known.

Based on the discussion thus far, perhaps the actions of the stem cell pool as we currently understand it are comprised of the combined properties of Lgr5$^+$ and quiescent stem cells in the crypt (Figure 1). Under normal conditions, Lgr5$^+$ stem cells could function to self-sufficiently maintain epithelial homeostasis through high-output cell production in response to trophic niche signals (*e.g.*, Wnt)[34,35]. However, Lgr5$^+$ CBCs are likely as sensitive to genetic damage as they are to injury. In these situations, the quiescent LRC population may assist with recovery from intestinal injury, either directly or by restoring Lgr5$^+$ stem cells.

INTESTINAL STEM CELL NICHE

Like other tissues among higher organisms, all intestinal cells reside within a carefully defined construct of chemical signals that directs genetically identical cell populations towards divergent behaviors[36]. Contained in and around the intestinal crypt are a multitude of molecular and cellular effectors that define a unique microenvironment - a "niche"- that directs the optimal function of stem cells[10]. Components of the niche include the subepithelial stroma, adjacent epithelial cells, natural enteric flora, and soluble epithelium-derived factors. Alteration of niche effectors can also lead to aberrant and dysregulated crypt behavior, which in turn may foster neoplasia.

Wnt signaling pathway

A multitude of signals in the intestinal crypt affect the function and growth of intestinal stem cells (Figure 2A and B)[37]. Of these, Wnt proteins are one of the most crucial for maintaining stem cell homeostasis[34,35,37,38]. Wnt promotes both cellular dedifferentiation and proliferation during embryogenesis and in many adult animal tissues[39-42]. Inhibition of the Wnt pathway results in crypt loss and a marked reduction in epithelial proliferation[43]. Among mice with inducible APC-knockouts, Wnt results in intestinal mucosa populated by undifferentiated cells[44]. Wnt activity is also among the essential signals for the formation of crypt structures from single stem cell cultures as well as for the reprogramming of somatic cells into induced pluripotent stem cells (iPSCs)[20,34,39,41]. Cell-proliferative genes are activated by Wnt *via* nuclear β-catenin intermediaries and include cell migration controllers (EPH), proliferative signals (c-myc, cyclin D1), and stem and cancer cell markers (Lgr5, Bmi1)[10,14,35,45-47].

The Wnt pathway is also a highly influential mediator of cancer (Figure 2C). APC mutations facilitate Wnt activity by dysregulating β-catenin-mediated gene expression[45,48]. APC mutations are common, occurring in over 80% of sporadic colorectal cancer[48]. Vermeulen *et al*[49] showed that primary spheroidal cultures derived from human CRCs are regulated by Wnt signals in the surrounding microenvironment, such as those secreted by intestinal myofibroblasts. They also demonstrated that extrinsic Wnt pathway activation was an important determinant in the cellular acquisition of cancer stem cell features (*e.g.*, formation of tumors when injected into immune-deficient mice and *in vitro* recapitulation of xenograft isolate behavior to that of the original tumor)[49].

Intestinal subepithelial myofibroblasts

Intestinal subepithelial myofibroblasts (ISEMFs), located underneath the basement membrane in the crypt, are stromal cells widely known to promote stem cell self-renewal and differentiation (Figure 2A and B)[20,34,35]. ISEMFs originate from regional intestinal fibroblasts and possibly trans-differentiated bone marrow cells[50]. Intestinal myofibroblasts function as anchors for cell adhesion and provide trophic signals to stem cells *via* cell-

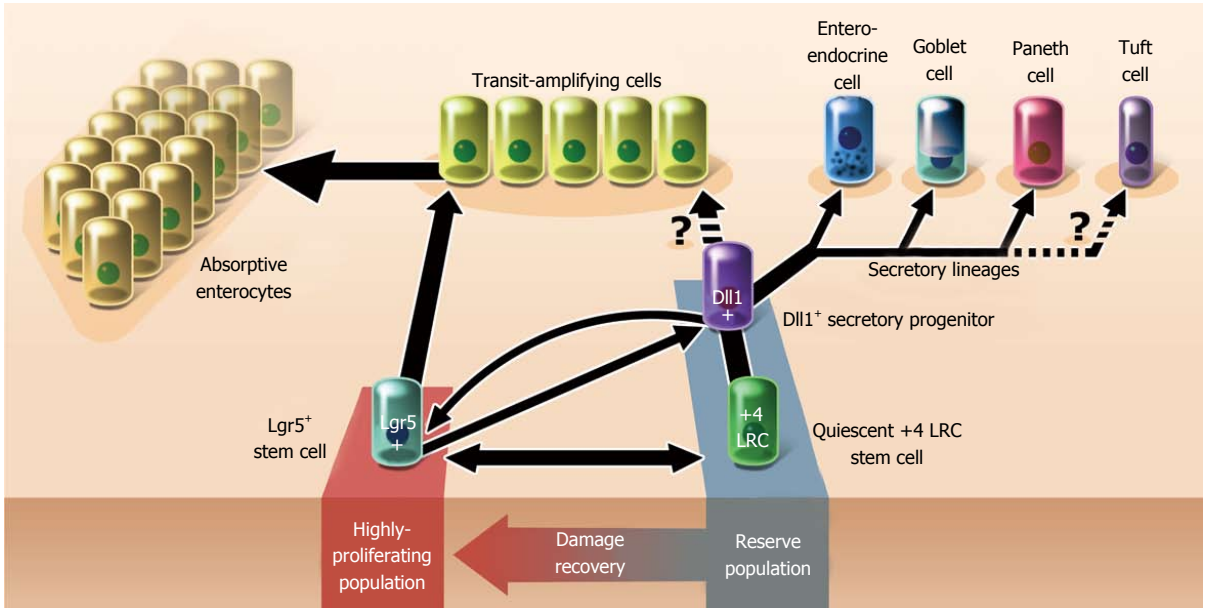

Figure 1 Origin and development of normal intestinal stem cells. Lgr5$^+$ CBCs and +4 LRCs coexist in the crypt. Each stem cell is fully multipotent. Lgr5$^+$ cells likely maintain intestinal homeostasis under normal conditions. Following intestinal injury, the reserve population comprised of +4 LRCs and Dll1$^+$ secretory progenitors restore both the epithelium and Lgr5$^+$ CBCs. Tuft cells are Bmi1$^+$ cells that may be synonymous with or descendants of +4 LRCs. CRC: Colorectal cancer; CBCs: Crypt base columnar cells; LRCs: Label-retaining intestinal stem cells.

cell interactions and secreted mediators[51]. ISEMFs also contribute to wound healing, mucosal protection, fluid and electrolyte transport, and growth of the basement membrane[50,52]. Secreted myofibroblast mediators are numerous: Wnt proteins, hepatocyte growth factor, fibroblast growth factor, TGF-β, keratinocyte growth factor, matrix metalloproteinases, stem cell factor, VEGF, and numerous interleukins, to name a few[52,53].

ISEMFs have long been implicated in promoting colorectal cancer growth and invasion (Figure 2C)[51]. Little clarity exists regarding whether peri-CRC myofibroblasts are derived from normal ISEMFs. Based on knowledge gleaned from other cancer systems, functional differences between normal and CRC fibroblasts do likely exist[54]. Still, even normal myofibroblasts are capable of facilitating CRC growth. Vermeulen *et al*[49] found that normal colonic myofibroblasts prevented both the morphological and molecular differentiation of co-cultured colorectal cancer cells. Furthermore, these myofibroblasts were shown to re-induce tumorigenic potential in subpopulations of CRC cells with low degree of proliferative activity[49].

Paneth cells

Paneth cells are terminally-differentiated secretory cells intermingled between Lgr5$^+$ CBCs at the base of crypts in the small intestinal mucosa[55]. Though unclear why no Paneth cells have been found elsewhere in the intestine, a population of c-kit$^+$/CD117$^+$ goblet cells in the colon may perhaps function analogously[33,56]. Co-culture of c-kit$^+$ cells with Lgr5$^+$ stem cells promotes the growth of organoids in similar fashion to those produced from Paneth/Lgr5$^+$ cell co-cultures[55,56].

Paneth cells contribute to the preservation of the stem cell compartment through the expression of Wnt proteins and other secreted signals such as epidermal growth factor and Notch ligands, all important in the maintenance of the Lgr5$^+$ CBC population[55]. Paneth cells also secrete antimicrobial peptides[57]. Furthermore, they facilitate epithelial repair by deactivating paneth-specific genes and converting to a phase that promotes Bmi1$^+$ cell proliferation[58].

Paneth cells seemingly serve a redundant role in the intestinal crypt. Wnt proteins released from Paneth cells are also derived from other sources in and around the intestinal crypt[59]. Notably, the complete removal of paneth cells in mouse model systems has not been shown to affect the proliferation of Lgr5$^+$ CBCs[60].

INTESTINAL TUMOR/CANCER STEM CELLS

Cells of origin

Is there a population of cells in the intestinal epithelium that reliably serves as the source for most, if not all of colorectal cancers? Intestinal stem cells are prime suspects due to their pre-existing proliferative and self-restorative behavior, making them perhaps more sensitive to overt carcinogenesis[10,35]. In support of this notion, Barker *et al*[61] demonstrated that APC deletions only among Lgr5$^+$ stem cells (6.5% of tumor mass) promoted the formation of adenomas, even in the setting of uniform tumor Wnt target gene activation. Barker and colleagues concluded that Lgr5$^+$ stem cell transformation-especially *via* loss of APC function-is a highly efficient pathway to neoplasia[61]. Multi-color reporter lineage

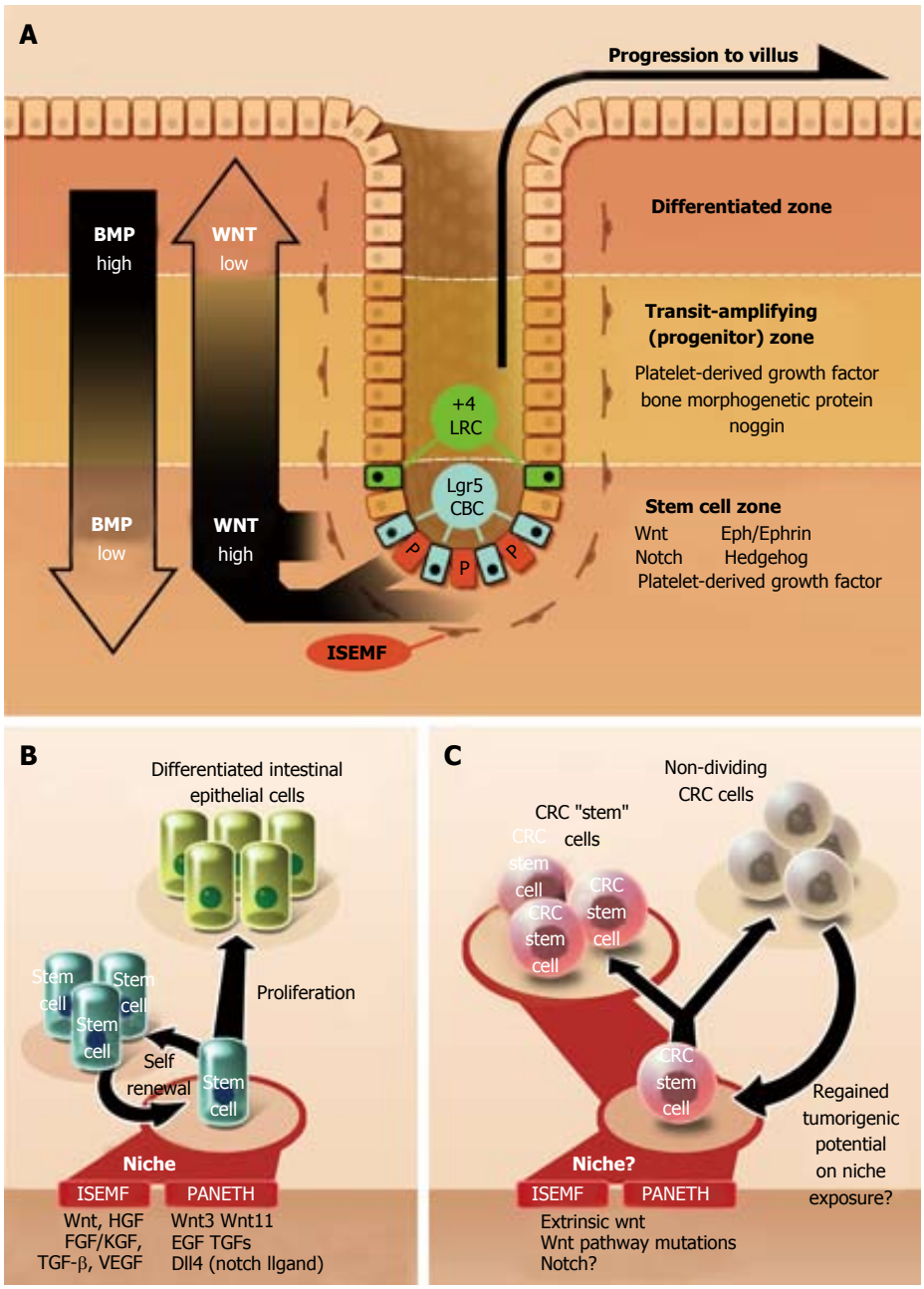

Figure 2 Niche regulation of the normal intestinal epithelium and colorectal cancer. A: Intestinal subepithelial myofibroblasts (ISEMFs) surround the crypt. Along with paneth cells (P), they supply the stem cell niche with trophic signals. Developing intestinal cells migrate upwards towards the villus apex, during which time they are subject to niches among the various strata in the crypt. B: Redundant mediators expressed by ISEMFs and Paneth cells contribute to the preservation of the stem cell compartment and normal intestinal proliferation. C: The local niche immediately around CRC likely fosters tumor growth by activating stem cell pathways. CRC cells lacking proliferative ability may re-awaken upon re-entry into the niche. CRC: Colorectal cancer; BMP: Basic metabolic panel.

retracing experiments by Schepers *et al*[62] have also confirmed that early adenomas are mostly of monoclonal origin, though occasionally oligoclonal. Schepers *et al*[62] also identified stem-like Lgr5$^+$ tumor origin cells at the base of adenomas that shared organizational resemblances to normal stem cells and were 20-fold more efficient at forming cell colonies *in vitro* than Lgr5-poor cells derived from the same population.

Still, evidence suggests that colorectal cancer may also arise from non-stem cells, supporting the idea that ultimately any cell harbors the potential to foster neoplasia. Early observations by Cole *et al*[63] reveal that early adenomatous polyps are positioned at the top of colonic crypts without contact with the stem cell compartment. Schwitalla *et al*[64] have also demonstrated that Wnt-constitutive intestinal cells can re-acquire stem cell properties in an NF-κB dependent manner and lead to tumor formation. These findings are congruent with iPSC research through which differentiated somatic cells have been reprogrammed back to proliferative stem-like states on account of key genetic alterations[41]. As with other non-intestinal cancers, no clear distinction yet

Table 1 Putative colorectal cancer stem cell markers

Marker	Function
ALDH1A1	Enzyme
ALDH1B1	Enzyme
β-catenin	Protein (nuclear)
Bmi-1	Protein (nuclear)
CD24	Cell surface glycoprotein
CD26	Cell surface glycoprotein
CD29	Cell surface glycoprotein
CD44	Cell surface glycoprotein
CD133	Cell surface glycoprotein
CD166 (ALCAM)	Cell surface glycoprotein
CDX-2	Transcription factor
c-myc	Transcription factor
Dclk-1	Serine-threonine kinase (?)
EpCAM	Cell surface glycoprotein
Klf-4	Transcription factor
Lgr-5	Cell surface receptor
Lin-28	Transcription factor
Msi-1	Protein (nuclear)
Nanog	Transcription factor
4-Oct	Transcription factor
Sox-2	Transcription factor

ALDH: Aldehyde dehydrogenase; Bmi-1: B lymphoma Mo-MLV insertion region 1 homolog; CD: Cluster of differentiation; CDX-2: Caudal type homeobox 2; Dclk-1: Doublecortin-like kinase-1; EpCAM: Epithelial cell adhesion molecule; Lgr-5: Leucine-rich repeat-containing G protein coupled receptor 5; Msi-1: Musashi-1.

exists identifying which CRCs, if any, are derived from non-stem cells[65].

What are the triggers that stimulate a cell to progress to cancer? Based on the discussion thus far, the neoplastic potential of a cell might be directly correlated with the combined disruptive impact of affected genes. However, One might imagine a situation in which a cell lacking sufficient functional derangement can be driven to cancer in response to external stimuli. Signals may come from cell placement in a Wnt-rich intestinal crypt, or in response to inflammation in light of concurrent genetic Wnt derangements as Schwitalla *et al*[64] have explored.

Tumor stem cell markers

Not surprisingly, many normal stem markers such as Lgr5, DCLK1, CD133, CD44, CD24, and ALDH1 have also been found among highly proliferating fractions of colorectal cancers[10,52,66,67]. Given the apparent genetic heterogeneity among CRC[7,8], very few, if any, markers are both specific to CRC stem cells and ubiquitous among all CRCs[9]. Table 1 lists putative CRC stem cell markers as previously covered by other authors[10,68-71]. What remains unclear is whether such markers reflect carry-over from intestinal stem cell precursors as with other cancers (*e.g.*, leukemia)[35] or else a re-activation of stem cell pathways. Regardless of the underlying reason, that CRC and normal intestinal epithelial stem cells express many of the same cell surface markers poses a challenge to the isolation of tumor stem cells.

One putative stem cell marker, Doublecortin-like kinase 1 (Dclk1), may be a useful marker for both normal and neoplastic intestinal stem cells. Dclk1 is a complex multi-splicoform transmembrane serine-threonine kinase involved in embryonic neuronal migration through intracellular signaling pathways[72,73]. In the digestive tract, Dclk1$^+$ cells have been found in the stomach and at the +4 position of the intestinal crypt[74,75]. Intestinal Dclk1$^+$ cells are functionally akin to quiescent stem cells via their label retention and radiation-induced activity[74,76]. Some studies contend that Dclk1$^+$ cells are not intestinal stem cells at all. Dclk1 expression may be shared not only by stem cells but also among the enteroendocrine lineage[77]. Alternatively, Gerbe *et al*[78] propose that Dclk1$^+$ cells are actually novel differentiated tuft cells with unidentified function.

Interestingly, cells aberrantly expressing Dclk1 have been found among both mouse intestinal adenomas and human colorectal cancers, suggesting a potential role for Dclk1 to identify neoplastic stem-like intestinal cells[74,79]. Nakanishi *et al*[80] recently demonstrated that Dclk1 specifically identifies abnormal intestinal mucosa found among tumors in the small intestine of APC$^{min/+}$ mice. Not only did Dclk1$^+$ tumor cells co-express Lgr5, they also demonstrated higher expression of other cancer stem cell markers *versus* non-tumor cells[80]. Furthermore, ablation of Dclk1$^+$ cells led to regression of the containing polyps without apparent effect to normal intestine[80]. These results concur with findings from our group showing that siRNA-based Dclk1 interference leads to growth arrest of xenoplanted CRC[81,82]. Also notable is a recent study by Li *et al*[67] demonstrating increased Dclk1$^+$ expression among cell fractions with a higher percentage stem-like HCT116 human CRC cells. Taken together, these findings support the notion that Dclk1$^+$ cells can identify colorectal cancer stem cells and that Dclk1 is critical for tumor growth.

Identifying CRC tumor stem cells

Despite the strong evidence suggesting that only a small fraction of colorectal tumor cells is responsible for maintaining tumor growth, the isolation of "pure" colorectal cancer stem cells has remained an ongoing challenge due to numerous theoretical and practical reasons. In fact, the term "cancer stem cell" may be somewhat of a misnomer. There is no expectation that a dysregulated colorectal cancer cell follows the exact biochemical principles of a normal intestinal epithelial stem cell, even if they share common signaling pathways. So long as the phrase "cancer stem cell" is used loosely to refer to cells in control of the proliferative hierarchy demonstrated by CRC, there is no perceived problem.

The first studies documenting a tumor-initiating CRC subfraction came in 2007 with the identification of CD133$^+$ cells comprising 2.5% of tumor mass[83,84]. However, the significance of CD133 as a specific CRC marker has subsequently been debated[52]. Other markers have further assisted in the enrichment of CRC stem cell fractions (Table 1). Kemper *et al*[66] found that Lgr5$^+$ cells comprised only 1.9%-11.1% of putative stem cells already marked by

Epcam, although admittedly the Lgr5$^+$ fraction was more highly clonogenic. Isolation of DCLK1 among tumor stem cells has been previously discussed, but even Nakanishi *et al*[80] did not find DCLK1 universally among all tumors in their mouse experiments.

The current methods employed to identify CRC stem cells are derived from non-exclusive properties shared by all intestinal stem cells. These methods include: DNA label retention, *in vitro* and *in vivo* proliferation assessments, and detection of cell surface markers[10]. Consequently, the isolation of CRC stem cells is fraught with as much, controversy as normal intestinal stem cells. Not the least of which, subtle differences between humans and animal models may consequently make experimental findings difficult to generalize. The apparent genetic heterogeneity of CRC lends further worry that finding a universal identification standard for CRC stem cells may long remain a daunting task[7,8].

Plasticity

It is becoming increasingly apparent that both the normal intestine and colorectal cancer are subject to "plasticity" processes that convert cells back to less-differentiated forms. Conventional stem cell theory holds that cellular development follows a unidirectional and irreversible hierarchy through semi-differentiated intermediates and concludes with terminal differentiation[85]. The implied goal of such a model is to produce cells capable of specialized organ functions[86]. In the intestine, recent evidence has revealed that short-lived Dll1$^+$ secretory progenitors can readily revert to Lgr5$^+$ stem cells following radiation injury (Figure 1)[87,88]. The apparent conversion of quiescent Bmi1$^+$ LRCs to Lgr5$^+$ stem cells is another clear demonstration of cellular plasticity[30,31]. That differentiated somatic cells, too, can fate-reprogram into iPSCs carries profound implications regarding the exclusivity of stem cell traits and the potential for any cell in an organism to participate in tissue regeneration[41].

Cellular plasticity processes may also depend largely on the cellular microenvironment. For example, extrinsically-derived Wnt signals can sufficiently replace Myc gene mutations during iPSC creation[39]. Also, non-proliferating CRC cells possessing low Wnt activity have been shown to regain proliferative tumorigenic potential when co-cultured with colonic myofibroblasts or the conditioned medium derived from myofibroblast cultures[49]. These results indicate that extrinsic signals -notably activators of the Wnt pathway- are perhaps sufficient to induce behavioral reprogramming, especially in CRC[49].

That fate-reversal occurs in CRC suggests that CRC expansion adheres to a proliferative pattern somewhere in between the classical hierarchical and stochastic growth models[49,85]. Admittedly, however, it is not known to what degree cellular plasticity plays a role in the proliferation of colorectal cancer. Perhaps even among different CRCs there is variation in functional dependence on extrinsic signals, ultimately affecting the growth patterns and behavior of the neoplastic phenotype. In this way, perhaps an extreme disturbance of either genetic derangement or environmental signals alone would also be a sufficient trigger for carcinogenesis[36].

EPITHELIAL MESENCHYMAL TRANSITION: PREVAILING METASTATIC PROGRAM?

The presence of cancer cells in the lymphatic and systemic circulation have long been known to correlate with poor prognosis, even despite the resection of primary lesions and/or chemotherapy[89-95]. With the apparent monoclonality of colorectal cancer[11], one might infer that circulating cancer stem cells originate from a primary colorectal tumor. Because cell migration brings with it certain constraints on adhesion and cellular interactions, circulating cancer stem cells may be functionally divergent from primary tumor cells.

Epithelial-mesenchymal transition (EMT) is a critical extension of cellular plasticity that is believed to govern not only the development of normal tissues but also the growth and spread of colorectal cancer. EMT is defined as the process by which epithelial cells convert to a mesenchymal-like phenotype. *Via* EMT, a cell relinquishes its native cell-cell interactions, loses tissue-specific polarity, and acquires migratory mesenchymal traits[96]. Important aspects of the EMT process such as the loss of E-cadherin (a hallmark of EMT) is mediated by the Wnt pathway[97]. This process is reversible and plays a key role in normal embryonic development as well as normal wound healing and fibrosis in the adult animal. The opposing process of mesenchymal-epithelial transition (MET) likely occurs through inverse regulation of EMT and is critical for final organ formation once embryonic cells have sufficiently migrated via mesenchymal intermediates[96]. Boundaries demarcating the degree of lineage reprogramming during the EMT process remain vastly gray territory. In fact, cells undergoing EMT may not necessarily have re-written fates, for such changes might only involve alterations to cell mobility.

EMT is likely a dominant mechanism driving colorectal cancer metastasis (Figure 3). In fact, CRC cells that display EMT characteristics have been shown to also possess traits of stem cells[98,99]. Critical to both CRC stem cell formation and EMT induction are Wnt mediators (*e.g.*, nuclear β-catenin), most markedly active at the invasive front of colorectal tumors[97]. Microarray analysis has demonstrated up-regulation of EMT-mediating genes among human CRC (*e.g.*, VIM, TWIST 1 + 2, SNAIL, and FOXC 1 + 2)[100]. EMT is also controlled *via* the microRNA miR-200 family[100,101]. MicroRNAs are small, non-coding RNAs that regulate post-transcriptional gene expression and serve to activate oncogenes and silence tumor suppressors. The presence of miR-200 family members (notably miR-200c and miR-141) is associated with a gain of epithelial cell characteristics[101]. In contrast, down-regulation of miR-200 family members

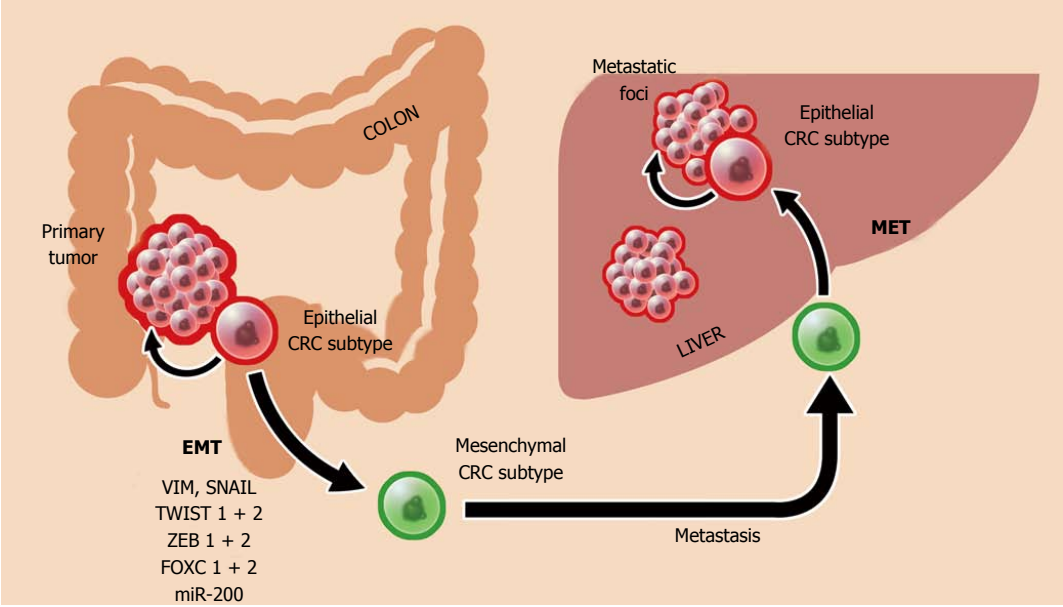

Figure 3 Epithelial-mesenchymal transition and mesenchymal-epithelial transition in colorectal cancer. In a primary tumor, CRC stem cells exist in a stationary phase that promotes growth. EMT transition to a migratory mesenchymal phase deactivates proliferative genes and cell adhesion molecules, generally allowing for metastatic dissemination to occur. Once at distant targets, mesenchymal cells transition back to the stationary phase *via* MET thereby resuming tumor expansion. EMT: Epithelial-mesenchymal transition; MET: Mesenchymal-epithelial transition; CRC: Colorectal cancer.

promotes an invasive mesenchymal phenotype, possibly through the activation of EMT mediators like ZEB1 and ZEB2[96,102,103]. In turn, epigenetic methylation pathways are in control of these miR-200 "switches" that altogether govern the shifting of CRC cells towards either mobile or stationary phases[96,101].

The combined effect of EMT/MET activity is metastatic advancement of a colorectal cancer: EMT enables primary tumor escape and spread by way of mesenchymal intermediates, and MET returns CRC to a highly-proliferative epithelial stem cell phenotype (Figure 3)[101]. In fact, these transitional phases may be the ultimate defining characteristic of CRC and may help direct future CRC therapy. Loboda *et al*[100] demonstrated that colorectal cancer, despite its vast mutational heterogeneity, can be organized principally as either epithelial or mesenchymal subtypes. Admittedly, the extent that EMT contributes to tumor spread remains unknown.

Interfering with EMT at critical phases of cancer growth is thus seemingly an attractive goal. For instance, anti-EMT therapy could be utilized to prevent primary tumor metastasis in early-stage CRC by forcing cells out of a mesenchymal phenotype or else preventing the entry into EMT (as is apparently the case with cetuximab administration)[96,104,105]. However, one concern regarding EMT/MET exploitation is that the two opposing processes may coexist inseparably. As such, unilaterally-directed therapy might lead to undesirable activity of cells in the opposite transitional phase. For instance, EMT processes are in part responsible for chronic resistance to oxaliplatin[106]. Difficulties in controlling mesenchymal processes may be further complicated by plasticity-mediated recruitment of additional CRC stem cells into the mesenchymal pool. Suffice it to say, our understanding of EMT is still in its infancy.

CONCLUSION

Much has been learned about the behavior of colorectal cancer stem cells owing to knowledge gained about normal intestinal stem cell behavior. The limitations inherent in our current isolation methods of pure stem cell fractions will likely bear heavily on how we observe and understand CRC as well. Newer developments in the field of stem cell research have provided insight into the vast potential for stem cells to not only be controlled by environmental factors but also be restored by its descendants. Also critical are core pathways such as Wnt that play an integral role in stem cell function, mesenchymal transition, and metastasis. Given the complexity of CRC "homeostasis", optimal CRC therapy will likely still remain a multi-pronged attack: first by control and/or alteration of trophic niche stimuli, second by the prevention of mesenchymal cell intermediates, and lastly by the elimination of stem cell ringleaders.

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