

Current understanding concerning intestinal stem cells

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Abstract

In mammals, the intestinal epithelium is a tissue

that contains two distinct pools of stem cells: active intestinal stem cells and reserve intestinal stem cells. The former are located in the crypt basement membrane and are responsible for maintaining epithelial homeostasis under intact conditions, whereas the latter exhibit the capacity to facilitate epithelial regeneration after injury. These two pools of cells can convert into each other, maintaining their quantitative balance. In terms of the active intestinal stem cells, their development into functional epithelium is precisely controlled by the following signaling pathways: Wnt/ β -catenin, Ras/Raf/Mek/Erk/MAPK, Notch and BMP/Smad. However, mutations in some of the key regulator genes associated with these signaling pathways, such as *APC*, *Kras* and *Smad4*, are also highly associated with gut malformations. At this point, clarifying the biological characteristics of intestinal stem cells will increase the feasibility of preventing or treating some intestinal diseases, such as colorectal cancer. Moreover, as preclinical data demonstrate the therapeutic effects of colon stem cells on murine models of experimental colitis, the prospects of stem cell-based regenerative treatments for ulcerous lesions in the gastrointestinal tract will be improved all the same.

Key words: Intestinal stem cell; Cell development; Colorectal cancer

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Core tip: Although the specific roles of intestinal stem cells in epithelial homeostasis and regeneration have been explored, the specific markers for identifying intestinal stem cells (ISCs) remain unclear. The reserve pool of intestinal stem cells is located at the 4+ position of crypts, and their biological characteristics are distinct from the intestinal stem cells at the crypt basement membrane. Intestinal stem cells are important cellular sources for initiating colorectal cancers. Managing murine models of ulcerous colitis using colon organoids indicates the therapeutic effects of ISCs.

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INTRODUCTION

The intestinal epithelium in mammals turns over every 4-5 d^[1]. The fast-cycling cells located at the crypt basement membrane, termed intestinal stem cells (ISCs), are responsible for maintaining epithelial homeostasis under intact circumstances^[1]. In the homeostatic process, ISCs produce daughter cells, the transit-amplifying (TA) progenitors, who undergo 4-5 divisions and migrate along the villus-crypt axis to differentiate into functional cells within the epithelium. In the small intestine for example, TA progenitors are committed into absorptive cells, endocrine cells, goblet cells and Paneth cells^[2]. Herein, the former three cell types constitute the villus-domain of the intestinal epithelium, and the Paneth cells move towards the crypt bottom because of active Wnt signals within this domain regulating their maturation^[3] (Figure 1). However, ISCs within the crypts are not uniform because a reserve pool of ISCs at the 4+ position of the crypts exhibit special roles in epithelial regeneration^[4]. Relative to the reserve ISCs, ISCs located at the crypt basement membrane, which are involved in epithelial homeostasis, are classified into a more active pool. In recent years, great effort has been made to compare the differences between these two pools of ISCs in their biological characteristics and in investigating their relative origins. Moreover, as the growth signals for active ISCs *in vivo* are clarified, emerging protocols for culturing these ISCs have been established and are constantly being optimized. In addition, colon-derived ISCs have exhibited therapeutic potential in experimental models of colitis^[5,6]. Based on these advancements, this review will first introduce the mechanisms by which niche-signals regulate the development of active ISCs into functional epithelial cells under intact conditions. Then, issues concerning the locations of ISCs and their diverse populations will be presented. Subsequently, advancements involved in identifying and expanding ISCs will be summarized in this review. For ISC-related malformations of the gut, sequential mutations of the *APC*, *p53*, *Smad4* and *Kras* genes are exclusively associated with the transformation of ISCs into colorectal cancer stem cells (CSCs), which are regarded as the primary sources for initiating colorectal cancers (CRCs)^[1]. Additionally, the most important event for mediating cancer progression, namely, cross-talk between colorectal CSCs and their niche cells, will be summarized in this review in relation to recently published findings. In reviewing the topics above, the prospects for the

clinical use of ISCs for managing some epithelial injuries will be analyzed along with presenting our insights on the transplantation of ISCs.

DEVELOPMENT OF THE ACTIVE ISC POOL

Within crypt domains, robust self-renewing active ISCs enable constitutive epithelial turnover, and the development of active ISCs into functional epithelial cells is generally mediated by the following signaling pathways: Wnt/ β -catenin, Ras/Raf/Mek/Erk/MAPK, Notch and BMP/Smad^[1,4,7]. In this process, Paneth cells are capable of secreting niche signals for ISCs, including Wnt3 (an agonist of Wnt/ β -catenin), epidermal growth factor (EGF), and Delta-like ligand1/4 (Dll1/4, ligands of Notch receptors)^[8]. Another population of niche cells include the myofibroblasts located around the crypts^[9,10]. These cells can produce some bioactive proteins for ISCs, such as R-spondin1 (an amplifier of Wnt3-activated signals) and Noggin (an antagonist of BMP/Smad)^[10,11]. All these proteins are essential for maintaining the proliferative status in ISCs (Table 1).

Active Wnt signaling is believed to be the main driving force leading to ISC proliferation^[1]. In this process, acting *via* a co-receptor binding approach, Wnt3 couples with LRP5/6 and Frizzled receptors, leading to the cytoplasmic accumulation of β -catenin, which up-regulates *c-Myc* expression through β -catenin/TCF4-mediated transcriptional activation^[7]. R-spondin1 is capable of protecting LRP6 against Dkk1/Kremen-mediated internalization by binding to its receptors (Lgr4/5), resulting in an increase in LRP6 on the cell surface^[12-14]. As a result of the actions of R-spondin1, ISCs become more sensitive to Wnt3. Moreover, the inactivation of *Lgr4* gene function results in a significant reduction of Paneth cells in the crypts^[15]. Likewise, a loss of *TCF4* gene function hampers the maturation of Paneth cells^[3]. All these results suggest that Wnt signals are not only essential for driving the proliferation of ISCs but also for their commitment into mature Paneth cells.

The other driving force for ISC proliferation relies on the EGF-mediated activation of the Ras/Raf/Mek/Erk/MAPK signaling pathway. Previous data suggest that more than 50% of mitosis in ISCs and TA progenitors relies on high levels of EGF within the crypt-domains^[16]. In addition, Dll1/4-mediated activation of the Notch pathway also contributes to the proliferative potential of ISCs^[17]. This is supported by evidence showing that the proliferative potential of ISCs from *Dll1/4* knock-out mice are decreased, but this depletion of *Dll1/4* expression *in vivo* increases the potential for ISCs to differentiate into secretory cell lineages, including goblet cells, endocrine cells and Paneth cells. In contrast, ISCs from *Dll1/4* over-expressing mice show accelerated proliferation, leading to a decreased number of secretory cells within the

Table 1 Bioactive proteins from niche cells maintain the proliferative status in intestinal stem cells

Niche cell	Bioactive protein	Receptor	Target pathway	ISC proliferation	ISC differentiation
Paneth cell	Wnt 3	LRP5/6 and Frizzled	Wnt/ β -catenin	↑	/
Paneth cell	Dll1/4	Notch 1/2	Notch	↑	↓
Paneth cell	EGF	EGFR	Ras/Raf/Mek/Erk/MAPK	↑	/
Myofibroblast	R-spondin1	Lgr4/5	Wnt/ β -catenin	↑	/
Myofibroblast	Noggin	BMP4	BMP/Smad	/	↓

ISCs: Intestinal stem cells. ↑: Increase; ↓: Decrease; /: No alterations.

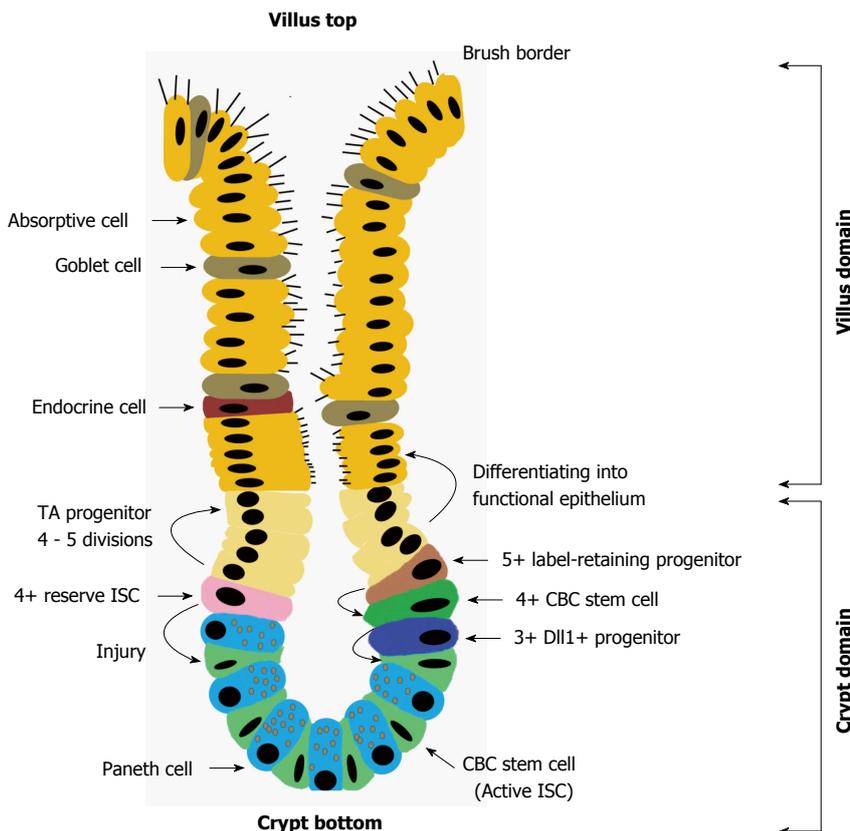


Figure 1 Structure of villus-crypt axis. There are two pools of stem cells within crypts, the CBC stem cells and 4+ reserve ISCs. The former ones maintain homeostasis of intestinal epithelium under intact condition through producing TA progenitors, while the latter ones are responsible for epithelial regeneration after injuries by converting themselves into CBC stem cells. Besides, some progenitors can reprogram themselves into active ISCs upon tissue injuries. ISC: Intestinal stem cell; TA: Transit-amplifying.

epithelium^[17]. Therefore, Dll1/4 appears to maintain the proliferative status of ISCs within the crypts, preventing ISCs from differentiating into secretory cell lineages. Similar effects have also been observed in relation to Noggin expression. Noggin binds to and inactivates the BMP4 protein, resulting in a blockade of the BMP/Smad signaling pathway, which helps ISCs maintain their proliferative status^[18].

Thus, the fast turnover of the intestinal epithelium not only relies on ISC proliferation but also on the differentiation of ISCs into functional cells. As described above, TA progenitors become mature when they migrate along the villus-crypt axis. In this process, the levels of Wnt3, EGF and R-spondin1 progressively decrease from the crypt basement membrane to the upper regions of crypt, whereas BMP4 levels are obviously increased^[7]. Combined with

the expressions of some lineage-devoted genes, such as *Math1* (secretory lineage)^[19] and *Hes1* (absorptive lineage)^[20], these TA progenitors stop dividing and ultimately differentiate into functional epithelial cells. In all, the spatial differences in the expressions of niche cell-derived signals along the villus-crypt axis maintain the proliferative status of ISCs and prevent these cells from differentiating within the crypt-domains.

LOCATION OF ISCS IN CRYPTS

Within the crypt-domains of the small intestine, three types of cells are arranged, including ISCs, TA progenitor cells and Paneth cells^[2]. ISCs are exclusively located within the lower domain of the crypts because the development of active ISCs is dependent on essential signals from Paneth cells^[3]. In

Table 2 Debate on intestinal stem cells' locations and their relevant characteristics

Location	Representative research by	Number per crypt	Sensitivity to IR	Cell-cycle	Cell-division	DNA segregation
CBC	Clevers H's team	14-16	Less than mature cells	21.5 h	Symmetry	Random
4+ position	Potten CS's team	4-6	Apoptosis upon receiving 1 cGy	Approximately 24 h	Asymmetry	Hierarchy

CBC: Crypt basement column cells.

1974, Cheng and Leblond pointed out that the column cells in the crypt basement membrane, located next to the Paneth cells, seemed to be the progenitors of intestinal epithelial cells because they observed that these column cells were rapidly cycling^[21]. However, due to a lack of available markers for identifying these epithelial progenitors at that time, the "stemness" of these cells was difficult to define. As recent clarification that the *Lgr5* gene is a target of the Wnt/ β -catenin signaling pathway, the transgenic (Tg) mouse (Strain name: *Lgr5-eGFP-IRES-CreERT2*) was established by Clevers, H.'s group^[22]. Based on this model, this team first demonstrated, both *in vitro* and *in vivo*, that these crypt basement column cells (CBC), which highly express *Lgr5*, were ISCs (also called CBC stem cells)^[22,23]. Since then, various promising data concerning the characteristics of these *Lgr5*⁺ ISCs have been published, such as their numbers in the crypts (14-16 per crypt)^[22], their resistance to foreign stimuli (less radiosensitive than mature epithelial cells)^[24], their cell-cycle duration (about 21.5 h), their mitotic process (symmetrical division) and their DNA segregation pattern (random distribution to offspring)^[25] (Table 2). Apart from these points, several genes have been reported to be highly expressed by *Lgr5*⁺ ISCs, such as *Musashi-1*^[26], *Sox9*^[27], *Ascl2*^[28], *Smoc2*^[25], *Rnf43*^[29], *Znrf3*^[30], *Olfm4*^[31], *Cd241*^[32], *Cd44 variant 4-10 (Cd44v4-10)*^[33], *Cd133*^[34] and *Cd166*^[35]. Hence, these genes are referred to as ISC-related genes.

However, Potten^[36] believed that the cells located at the 4+ position of the crypts were active ISCs. It has been shown that ISCs at this position expand their numbers every 24 h and that they are very sensitive to ionizing irradiation, with only 1 cGy of ionizing irradiation being enough to initiate apoptosis among these ISCs^[36]. Thereafter, through the double labeling of DNA using 3HTdR and BrdU, Potten *et al.*^[37] found that when these ISCs divided, parental DNA strands were allocated to their larger daughter cells, while the newly synthesized strands were allocated to the smaller progeny^[37]. Because of this action, the larger cells keep their stem-cell identities and genomic stability, and the smaller cells become TA progenitors for replacing dead cells within the epithelium^[37]. Although evidence for the hierarchical distribution of parental DNA strands is well supported, there has been an inconclusive debate concerning this issue. Data from Clevers H's team recently demonstrated that the dividing *Lgr5*⁺ ISCs randomly segregate the parental DNA stands into equal-

sized offspring^[25]. Thereafter, the daughters adhering to Paneth cells remain at the crypt basement membrane and continue to function as ISCs, whereas the cells not receiving resources from Paneth cells develop into TA progenitors^[38,39]. In spite of such discrepancies in the characteristics of ISCs at different locations, recent evidence indeed supports the idea that the ISCs at the 4+ position are more sensitive to foreign stimuli-induced cell-death than are ISCs at the crypt basement membrane^[40]. A study on this was carried out by Zhu *et al.*^[40], and they found that tamoxifen-preconditioning for *Cre*-expression in *Lgr5-eGFP-IRES-CreERT2* Tg mice induced apoptosis in a small portion of ISCs. Herein, the dead ISCs were fast-cycling cells, positive for *Lgr5*, and mainly located at the 4+ position of crypts. Additionally, ionizing irradiation (1 cGy) also caused the death of ISCs at the 4+ position^[40]. *Lgr5*⁺ ISCs at the crypt basement membrane replenished such cell loss^[21]. Accordingly, nearly 10% of *Lgr5*⁺ ISCs were confirmed to be located around the same sites in crypts^[21]. To a certain extent, these findings indicate the heterogeneity among *Lgr5*⁺ ISCs.

ISC POPULATIONS

The ISCs mentioned above are referred to as an active pool for maintaining epithelial homeostasis in intact circumstances. Thus, the niches of the ISCs manipulate their fates for tissue regeneration^[4]. Recently, a distinct population of ISCs located at the 4+ position of crypts, has been extensively investigated, and the relevant factors that identify these cells are as follows: (1) Marker genes: *Bmi1*, *HopX*, *mTERT* and *Lrig1*^[41-44]; (2) Status under intact conditions: No more than 2% of these ISCs are proliferative (termed label-retaining cells, LRC)^[41]; (3) Response to foreign stimuli: They activate their cell-cycles to replenish dead cells through direct differentiation into functional epithelial cells or by converting themselves into CBC stem cells^[41,45-47]; and (4) Forces driving proliferation: Independent of the Wnt/ β -catenin signaling pathway^[41] (Table 3). Because of such characteristics, these ISCs are classified as a reserve population. In mammals, some tissues or organs also share the same pattern of maintaining their homeostasis using a system of diverse stem cells, such as hematopoietic stem cells in bone marrow and hair follicle stem cells in skin^[4].

In terms of the inter-conversion between reserve ISCs and CBC stem cells, Tian *et al.*^[45] firstly reported

Table 3 Characteristics of crypt basement column cells stem cells and reserve intestinal stem cells

ISC type	Main location	Cell-cycle status under intact conditions	Differentiating capability	Foreign stimuli-induced response	Leading pathway	Typical marker genes
CBC stem cell	Crypt basement	Active	Yes	Apoptosis	Wnt/ β -catenin	<i>Lgr5</i>
Reserve ISC	4+ position	Slow	Yes	Proliferation	Unknown but independent of Wnt/ β -catenin	<i>Bmi1</i> , <i>HopX</i> , <i>Lrig1</i> , <i>mTERT</i>

CBC: Crypt basement column cells; ISC: Intestinal stem cell.

their findings through the use of a Tg mouse strain whose *Lgr5* gene contained the sequence encoding the human diphtherotoxin receptor (*DTR*). When treated with diphtherotoxin, the *Lgr5*⁺ cells were depleted in the crypts, while numbers of *Bmi1*⁺ cells increased. However, *Lgr5*⁺ cells emerged within the crypts 48 hours later. Thereafter, the number of *Lgr5*⁺ compartments increased and they migrated progressively from the 4+ position to the crypt basement membrane, indicating a revival of CBC stem cells. Based on these findings, the offspring of Tg mice (Strain: *Bmi-CreER*) crossed with an *Lgr5-DTR*/+ mouse strain were then crossed with different Tg mice (Strain: *Rosa26-LacZ*), and these offspring was used to trace the lineage of the restored CBC stem cells. The results of this analysis demonstrate that the CBC stem cells were derived from *Bmi1*⁺ ISCs. Similar results were also reported in studies using murine models of radiation-induced ISC apoptosis^[46,47] Moreover, when affected by foreign stimuli, some TA progenitors are also capable of converting themselves into ISCs, such as the *Dll1*⁺ secretory progenitors at the 3+ position^[48] and the label-retaining progenitors at the 5+ position^[49]. Apart from the issues mentioned above, the *Lgr5*⁺ ISCs were shown to be able to convert into *HopX*⁺ ISCs both *in vitro* and *in vivo*^[42]. All these findings suggest niche conversion between distinct stem cell populations, but the relevant mechanisms remained unclear until now. Thus, it can be confirmed that the expressions of the above mentioned marker genes of reserve ISCs overlap in active ISCs but at relatively low levels, meaning that ISCs still lack specific definable markers^[21]. Hence, it is only feasible to discriminate the distinct populations of ISCs based on their locations and the genes that are highly expressed^[50].

EXPANDING ISCS *IN VITRO*

Precisely discriminating *Lgr5* expressing levels using FACS-sorting techniques facilitates the isolation of ISCs from Tg mice (Strain: *Lgr5-eGFP-IRES-CreERT2*)^[23]. For the wild-type hosts, recent works have also reported that several cell-surface markers may serve as potential candidates for sorting ISCs, including *CD24*^[51], *CD44*^[52], *EphB2*^[5,53], *CD133*^[34] and *CD166*^[35]. However, the main obstacle to this lies in the fact that not all sorted cells are ISCs because all these markers can be found not only among ISCs but also among TA progenitors and mature cells, which results in a low purity for the target cells. To solve this problem,

several novel strategies that use combinations of several surface makers, such as *CD24/Sox9*^[54], *CD24/CD44*^[55], *CD44/CD133*^[56] and *CD24/CD44/CD166/GRP78/c-Kit*^[57], enable the more precise identification of ISCs than is possible using one single marker for FACS analyses.

For culturing ISCs, extensive studies have been performed during the last two decades. At the beginning of this period, due to a profound lack of knowledge about the biological characteristics of ISCs, human colon cancer cell lines or an epithelial cell line (IEC6 from the small intestine of rats) were used as substitutes for ISCs^[58]. Thereafter, as advances were made in understanding the biological characteristics of ISCs, a novel culturing system for ISCs was established by Clevers, H. and colleagues^[23]. These ISCs are wrapped by Matrigel containing laminin- $\alpha1/\alpha2$ and supplemented with Wnt3, EGF, Noggin, R-spondin1 and Jagged-1 in a serum-free medium to enable their growth, together with Y-27632 dihydrochloride to protect ISCs against ROCK pathway-induced anoikis. In this three-dimensional (3D) system, one single isolated small intestine-derived ISC is capable of expanding in number, while also differentiating into functional epithelial cells for up to 1 year^[23]. The ISC-derived structures are termed intestinal organoids. Moreover, through the use of similar models, *Lgr5*⁺ stem cells from various organs or tissues, such as the liver^[59], pancreas^[60], stomach^[61] and colon^[5], can also be cultured and induced to form organoids. Another successful system was established by Ootani *et al.*^[62], which was based on a fetal calf serum-containing system for the long-term expansion of ISC-derived organoids. A remarkable difference from the system established by Clevers H's team lies in the fact that pieces of the small intestine were directly used *in vitro*. Details are shown in Table 4. Moreover, intestinal myofibroblasts were obviously expanded in this system. As we mentioned above, the myofibroblasts around the crypts form the niches for ISCs. To some extent, we propose that increased myofibroblast numbers will facilitate the development of ISCs into organoids based on previous findings that intestinal myofibroblasts are capable of increasing the organoid-forming potential of crypt fragments *in vitro*^[10]. Although these 3D-culture systems allow for ISC expansion, the commitment of ISCs into functional cells is hard to be prevented. Moreover, the colony-forming efficacy (CFE) of the ISCs is only about 10%^[23]. To solve this problem, Yin *et al.*^[63] used

Table 4 Two systems for expanding intestinal stem cells

Established by	Published year	System type	Substrate	Supplemental factor	Medium	Cultured content	Expanding duration of cultured content
Sato <i>et al.</i> ^[23]	2009	3D	Matrigel containing laminin $\alpha 1$ and $\alpha 2$	N2, B27, Wnt3, EGF, Noggin, R-spondin1, Jagged-1 and Y-27632 dihydrochloride	Serum-free	ISCs	Up to 1 yr
Ootani <i>et al.</i> ^[62]	2009	3D	Collagen gel containing collagen I and III	/	Containing 20% of FCS in medium	Small pieces of intestinal tissue	Up to 1 yr

EGF: Epithelial growth factor; ISCs: Intestinal stem cells.

two small molecules, CHIR99021 and valproic acid (VPA), which were found to synergistically improve the expansion of ISCs *in vitro*, showing a CFE that was about 100-fold greater than that of ISCs without these two molecules. In this case, CHIR99021 mainly targets the activation of the Wnt/ β -catenin signaling pathway, inhibiting absorptive cell differentiation, and VPA is capable of preventing ISCs from differentiating into secretory-lineage cells^[63].

ISC AND CRC

CRC is the third leading cause of cancer-related death in developed countries, and several factors, such as a high fat diet, chronic inflammation and genomic instability, are highly associated with its occurrence^[64,65]. For example, the constitutive activation of NF- κ B in mature epithelial cells will initiate the formation of CRCs^[66]. Likewise, chronic inflammation also induces the generation of CRCs from APC-mutant DCLK1⁺ Tuft cells^[67]. Apart from these mature cells, ISCs are very important cellular sources for initiating CRCs^[68]. Recent evidence suggested that factors including aging^[69] and the total number of stem cell divisions^[70], increase the frequency of gene mutations in ISCs in addition to the inherent mutations from the germline. Accordingly, CRCs can be identified and typed based on their genetic or epigenetic profiles^[28,65,71].

As mentioned above, four main pathways precisely control the proper development of one single ISC. However, aberrant Wnt signals overwhelmingly drive the pathogenesis of CRCs in most cases^[65]. For example, mutant APC-induced robust activation of the Wnt/ β -catenin signaling pathway is regarded as the first step in ISC-initiated gut malformation^[72]. Along with the loss of function in *p53*, aberrant ISCs will wildly expand their numbers and show poor differentiating capabilities^[73]. In addition, when mutations in *Smad4* and *Kras* occur, invasive characteristics are conferred to these cells^[74]. As a result of these actions, normal ISCs are converted into CSCs (Figure 2), and several ISC-related genes are expressed by CSCs, such as *Lgr5*^[75], *Wip1*^[76], *Yap*^[77,78], *EphB2*^[79], *Cd24*^[80], *Cd44 variant 6*^[33,81], *Cd133*^[82,83] and *Cd166*^[35,84], which indicate a poor prognosis for CRC patients when highly expressed due to the fact that they encode products that play

roles in CSC expansion and invasion. However, not all CSCs share the same genetic profile. Recent data suggest that some subpopulations of colorectal CSCs have their own markers, such as CD26^[85], CD110 and CUB-domain-containing protein-1(CDCP1)^[86], which confer metastatic potential to CSCs. In addition, DCLK1 can be a specific marker of colorectal CSCs, which makes a distinction between colorectal CSCs and ISCs possible^[87]. These results suggest that colorectal CSCs possess diverse genetic profiles, a paradigm reflecting the heterogeneity of CRCs^[64,65,71].

As in ISCs, the development of colorectal CSCs also relies on their niches, which are composed of several types of cells, including endothelial cells, immune cells, myofibroblasts, cancer-associated fibroblasts (CAFs) and a small portion of mesenchymal cells^[65]. Under such circumstances, colorectal CSCs progressively expand their numbers and produce daughter cells by using the resources from the niche-cells. Moreover, CSCs communicate with their niche-cells, obtaining invasive phenotypes to promote tumor progression, although the relevant signals are quite complicated. For example, within the tumor microenvironment, high levels of prostaglandin E2 can decrease the activity of GSK-3 β through activating a cAMP/PKA cascade, leading to the cytoplasmic accumulation of β -catenin and facilitating the expansion and metastasis of colorectal CSCs^[88,89]. In addition, prostaglandin E2 has an immunosuppressive capacity, which acts on several types of immune cells, including NK cells (decreased proliferation and cytotoxicity), dendritic cells (increased IL-10 production) and regulatory T cells (increased proliferation)^[90]. Additionally, some CAF-derived cytokines, including HGF, osteopontin (OPN), SDF-1^[81], TGF- β ^[91], IL-6^[92] and IL-17A^[93], also exhibit effects that increase the frequencies of colorectal CSC-induced malformations. For example, the TGF- β -induced epithelial-mesenchymal transition (EMT) in colonic CSCs increases their potential for liver metastasis^[91,94], and HGF, OPN and SDF-1 are capable of up-regulating the expression of *Cd44v6* in colorectal CSCs by activating the Wnt/ β -catenin signaling pathway. In addition, colorectal CSCs show improved survival *via* the activation of the PI3K/Akt cascade, which is regulated by the interaction between HGF and the CD44 receptor^[81]. Similarly,

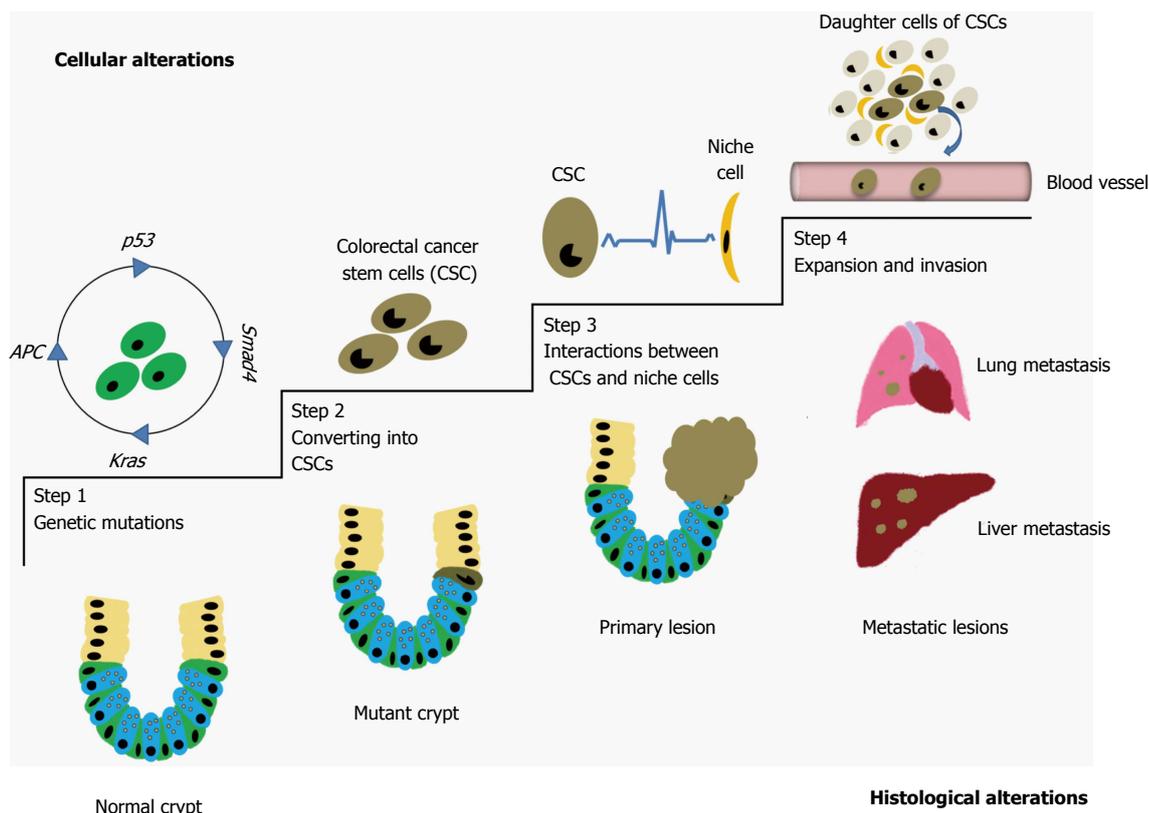


Figure 2 Relationship between intestinal stem cell genetic alterations and colorectal cancer development. Step1: Sequential mutations of *APC*, *p53*, *Smad4* and *Kras* in ISCs; Step2: Normal ISCs are converted into CSCs; Step 3: By using the feeding from niche cells, CSCs expand their numbers along with producing daughter cells to form a solid tumor; Step 4: Upon escaping from their primary sites, some CSCs will systematically migrate into other organs to form metastatic lesions. ISCs: Intestinal stem cells.

the survival and invasive capacities of CSCs can be increased through the interaction between the CD44 receptor and hyaluronan, a molecule mediating cell adhesion^[95]. Additionally, SDF-1 is an important attractant for some bone marrow progenitors, such as CD133⁺ hematopoietic progenitor cells and endothelial progenitor cells, to the tumor microenvironment, which facilitates tumor growth and metastasis^[96,97]. For the immune mediators mentioned above, it has been reported that preconditioning using IL-6 could increase the proportion of ALDH⁺ cancer stem-like cells among cultured colonic cancer cells and increase the expression of *Lgr5* by these cells. Moreover, another tumor-facilitating action of IL-6 lies in its capacity to increase the number of Th17 cells and increase their production of IL-17A, a cytokine favoring tumor growth through its support of the expansion of colorectal CSCs^[92,93]. CAFs have been reported to be capable of increasing IL-17A secretion after chemotherapy, and the increase in this immune mediator enables colorectal CSCs to acquire tolerance to anti-cancer drugs^[93].

In addition to the cytokines mentioned above, at the subcellular level, exosomes also participate in establishing the network mediating intercellular communications. Exosomes are bioactive nanoparticles originating from multiple cell types (including cancer cells), and can be endocytotically taken up

by adjacent or distant cells. Exosomes regulate the biological responses of target cells using their cargoes of proteins, lipids, miRNAs and mRNAs^[98]. Of the specific roles of exosomes in regulating the generation of CRCs, fibroblast-derived exosomes have been demonstrated to be capable of enhancing the expansion of CRCs and conferring a resistance to 5-fluorouracil^[99]. In providing feedback to niche-cells, exosomes from SW480 CRC cells have been shown to enhance the proliferation of endothelial cells *via* their cell cycle-facilitating mRNAs^[100]. Moreover, tumor exosomes have antagonistic effects on immune cells, decreasing the cytotoxicity of NK cells, impairing the anti-tumor response of CTL cells and increasing the numbers of regulatory T/B cells, which establishes an immunosuppressive microenvironment, favoring tumor growth *in vivo*^[101].

However, not all exosomes from CRC cells carry the same cargo profiles. For example, Cha *et al.*^[102] found that *miRNA-100* was the main type of exosomal miRNA in *Kras*-mutant CRC cells, whereas *miRNA-10b* is predominant in wild-type exosomes. Even when CRCs relapse, the main type of serum miRNA in patients with recurrent CRC are different from those in patients without recurrent CRC^[103]. It is possible that seven exosomal miRNAs, *let-7a*, *miRNA-1299*, *miRNA-1246*, *miRNA-150*, *miRNA-21*, *miRNA-223* and *miRNA-23a*, may be useful candidates for assessing

the progression of CRCs due to the high correlation between their serum levels and tumor burdens *in vivo*^[104]. In addition, exosomal miRNAs differ in type among different CRC cells, reflecting the epigenetic heterogeneity of CRCs.

Based on the findings presented above, we conclude that genomic mutations are prerequisites for the transformation from normal cells into CSCs, which will ultimately form solid tumors through interactions with cancer niche-cells.

ISCS AND THEIR APPLICATION

Clearly, the generation of Tg mice (Strain: *Lgr5-eGFP-IRES-CreERT2*) and establishment of 3D-culture systems for ISCs initiated the era of treating intestinal diseases using ISCs. Recently, data from two separate studies demonstrate that murine *Lgr5*⁺ colonic stem cells have the potential to healing epithelial injuries in immunodeficient mice with dextran sodium sulfate (DSS)-induced colitis^[5,6]. Moreover, these studies also suggest that engrafted clusters could survive within lesioned sites for more than 6 mo, undergoing crypt-fission and commitment into functional cells, indicating the effectiveness and feasibility of homogenous transplantation^[6]. Moreover, the protocols for expanding human ISCs from stomach, small intestine and colon tissues have been established, which holds great promise for treating some gastrointestinal diseases, such as inflammatory bowel disease (IBD), gastric ulcers (GCs) or microvillus inclusion disease^[5,53,58]. To this end, Watanabe^[105] has described a strategy for the autologous transplantation of colonic stem cells into patients with IBD. In brief, normal colonic tissues can be isolated through enteroscopy-guided biopsy. After expanding their number *in vitro*, the colonic stem cells can be transplanted into the lesioned sites^[105]. Based on this, we recommend that GCs can also be managed using a similar approach. Of note, we believe that the influence of the microenvironment within the site of the injury on the viability of the graft must be taken into consideration. For IBD, extended inflammation within intestinal lesions limits epithelial healing^[106]. To some extent, it would be beneficial to decrease apoptosis in engrafted stem cells *in vivo* through the use of immunosuppressive therapy. To date, it remains unclear whether stomach stem cells are acid-resistant. If so, this would help increase the viability of such grafts through the use of certain anti-acid drugs. Another issue concerning the application of autologous stem cells for treating IBD or GCs lies in the lack of evidence indicating whether the genomes of stem cells derived from such patients are stable. As mentioned above, chronic inflammation within the colon will initiate CRC formation. In addition, it is hard to determine whether freshly isolated samples contain mutant cells, especially when such cells are cultured with supplemental growth factors. Recent data suggest that ISCs from diabetic

mice are more inclined to differentiate into absorptive cells and Paneth cells than are ISCs from healthy mice^[107]. To avoid the above issues, we recommend that the genomic stability be determined before clinical transplantation. If instable genomes are detected, allogeneic transplantations could be used as substitutes. Li *et al*^[58] noted that a standardized strategy including HLA-matching between donors and recipients and the use of immunosuppressive drugs could be an option for dealing with graft rejection. However, the shortcomings of allogeneic transplantation lie in the long-term use of immunosuppressants and the limited availability of donor tissues.

For ISCs with gene mutations, it is hoped that they can be reprogrammed into normal cells using CRISPR/Cas9 technology, which is a powerful tool for editing multiple genes synchronously within an individual cell^[108]. Using this technology, sequential mutations of four key genes, *APC*, *p53*, *Smad4* and *Kras*, in human ISCs were first carried out *in vitro* to investigate the specific roles of these mutant ISCs in initiating CRCs *in vivo*^[74]. Apart from this, it is also possible that ISCs may be massively cultured within biocompatible frameworks to form artificial mucosa, which could be realized by using 3D-bioprinting technology^[109]. Therefore, any advancement concerning new theories in stem cell biology, new technologies for culturing stem cells or new applications of stem cells in treating diseases will push regenerative medicine forward.

CONCLUSION

Overall, the mechanisms by which ISCs maintain epithelial homeostasis are highly specific, and any stimuli altering the biological characteristics of ISCs will interrupt the homeostatic process, leading to lesions involving mucosal atrophy, hypertrophy or even malformation. Any information concerning biological alterations in ISCs will improve the public's awareness on how to prevent some intestinal diseases. Moreover, ISCs are valuable tools, and their potentials for healing ulcerous lesions in experimental models have been demonstrated. In spite of this, a detailed protocol, including quality control for ISC culturing, criteria for evaluating the effectiveness of grafts, and criteria for evaluating transplantation-related adverse effects, needs to be established before carrying out relevant clinical trials.

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