**Name of Journal: World Journal of Gastroenterology**

**Manuscript NO: 25986**

**Manuscript Type: MINIREVIEWS**

**Current understanding concerning intestinal stem cells**

Cui S *et al*. Current understanding concerning ISCs

Shuang Cui, Peng-Yu Chang

**Shuang Cui, Peng-Yu Chang,** Department of Radiation Oncology, The First Bethune Hospital of Jilin University, Changchun 130021, Jilin Province, China

**Author contributions:** Cui S prepared the majority of the writing; and Chang PY formed the figures and tables, and designed the outline and coordinated the writing of the manuscript.

**supported by** National Natural Science Foundation of China, No. 81502751 and No. 81372929.

**Conflict-of-interest statement:** All authors claim that there are no conflicts of interest.

**Open Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

**Manuscript source:** Invited manuscript

**Correspondence to:** **Peng-Yu Chang, MD,** Department of Radiation Oncology, The First Bethune Hospital of Jilin University, Changchun 130021, Jilin Province, China. edvence@163.com

**Telephone:** +86-431-88783840

**Fax:** +86-431-88783840

**Received:** March 27, 2016

**Peer-review started:** March 28, 2016

**First decision:** May 12, 2016

**Revised:** May 21, 2016

**Accepted:** June 15, 2016

**Article in press:**

**Published online:**

**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 *BMP4*, 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 word:** Intestinal stem cell; Cell development; Colorectal cancer

**© The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**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.

Cui S, Chang PY. Current understanding concerning intestinal stem cells. *World J Gastroenterol* 2016; In press

**INTRODUCTION**

The intestinal epithelium in mammals turns over every 4-5 d[1]. The fast-cycling cells located the at 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 will 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 in intestinal stem cells 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), epithermal 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 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 indeed dependent on essential signals from Paneth cells[3]. In 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. After the recent clarification that the *Lgr5* gene is a target of the Wnt/β-catenin signaling pathway, a strain of transgenic (Tg) mice carrying the *eGFP*, *IRES* and *CreERT2* genes in separate loci of its *Lgr5* gene (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, 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]*, Cd24I*[32]*, Cd44 variant 4-10 (Cd44v4-10)*[33]*, Cd133*[34] and *Cd166*[35]. Hence, these genes are referred as ISC-related genes.

However, Potten *et al*[36] believed that the cells locating 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 replenish 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 heterogenicity 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 proliferative: 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 reverse ISCs and CBC stem cells, Tian *et al*[45] firstly reported 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 tracing 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 a relative 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 wide-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 had 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 understating the biological characteristics of ISCs, a novel culturing system for ISCs was established by Clevers, H. and colleagues[23]. These ISCs are enwrapped by Matrigel containing laminin-α1/α2 and supplemented with Wnt3, EGF, Noggin, R-spondin 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 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-κB 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 division[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 cancer stem cells (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 heterogenicity 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-3β 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 cytolysis), 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, 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 that 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 heterogenicity 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, M. has described a strategy for the autologous transplantation of colonic stem cells into patients with IBD[105]. 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, allogenic 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 hopeful 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.

**Acknowledgments**

We thank Bo Chen for his kind English language editing.

**References**

1 **van der Flier LG**, Clevers H. Stem cells, self-renewal, and differentiation in the intestinal epithelium. *Annu Rev Physiol* 2009; **71**: 241-260 [PMID: 18808327 DOI: 10.1146/annurev.physiol.010908.163145]

2 **Umar S**. Intestinal stem cells. *Curr Gastroenterol Rep* 2010; **12**: 340-348 [PMID: 20683682 DOI: 10.1007/s11894-010-0130-3]

3 **van Es JH**, Jay P, Gregorieff A, van Gijn ME, Jonkheer S, Hatzis P, Thiele A, van den Born M, Begthel H, Brabletz T, Taketo MM, Clevers H. Wnt signalling induces maturation of Paneth cells in intestinal crypts. *Nat Cell Biol* 2005; **7**: 381-386 [PMID: 15778706 DOI: 10.1038/ncb1240]

4 **Li L**, Clevers H. Coexistence of quiescent and active adult stem cells in mammals. *Science* 2010; **327**: 542-545 [PMID: 20110496 DOI: 10.1126/science.1180794]

5 **Jung P**, Sato T, Merlos-Suárez A, Barriga FM, Iglesias M, Rossell D, Auer H, Gallardo M, Blasco MA, Sancho E, Clevers H, Batlle E. Isolation and in vitro expansion of human colonic stem cells. *Nat Med* 2011; **17**: 1225-1227 [PMID: 21892181 DOI: 10.1038/nm.2470]

6 **Yui S**, Nakamura T, Sato T, Nemoto Y, Mizutani T, Zheng X, Ichinose S, Nagaishi T, Okamoto R, Tsuchiya K, Clevers H, Watanabe M. Functional engraftment of colon epithelium expanded in vitro from a single adult Lgr5⁺ stem cell. *Nat Med* 2012; **18**: 618-623 [PMID: 22406745 DOI: 10.1038/nm.2695]

7 **Sato T**, Clevers H. Growing self-organizing mini-guts from a single intestinal stem cell: mechanism and applications. *Science* 2013; **340**: 1190-1194 [PMID: 23744940 DOI: 10.1126/science.1234852]

8 **Sato T**, van Es JH, Snippert HJ, Stange DE, Vries RG, van den Born M, Barker N, Shroyer NF, van de Wetering M, Clevers H. Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. *Nature* 2011; **469**: 415-418 [PMID: 21113151 DOI: 10.1038/nature09637]

9 **Hirokawa Y**, Yip KH, Tan CW, Burgess AW. Colonic myofibroblast cell line stimulates colonoid formation. *Am J Physiol Gastrointest Liver Physiol* 2014; **306**: G547-G556 [PMID: 24481605 DOI: 10.1152/ajpgi.00267.2013]

10 **Lei NY**, Jabaji Z, Wang J, Joshi VS, Brinkley GJ, Khalil H, Wang F, Jaroszewicz A, Pellegrini M, Li L, Lewis M, Stelzner M, Dunn JC, Martín MG. Intestinal subepithelial myofibroblasts support the growth of intestinal epithelial stem cells. *PLoS One* 2014; **9**: e84651 [PMID: 24400106 DOI: 10.1371/journal.pone.0084651]

11 **Peiris D**, Pacheco I, Spencer C, MacLeod RJ. The extracellular calcium-sensing receptor reciprocally regulates the secretion of BMP-2 and the BMP antagonist Noggin in colonic myofibroblasts. *Am J Physiol Gastrointest Liver Physiol* 2007; **292**: G753-G766 [PMID: 17138967 DOI: 10.1152/ajpgi.00225.2006]

12 **Roth S**, Fodde R. The nature of intestinal stem cells' nurture. *EMBO Rep* 2011; **12**: 483-484 [PMID: 21566649 DOI: 10.1038/embor.2011.97]

13 **Glinka A**, Dolde C, Kirsch N, Huang YL, Kazanskaya O, Ingelfinger D, Boutros M, Cruciat CM, Niehrs C. LGR4 and LGR5 are R-spondin receptors mediating Wnt/β-catenin and Wnt/PCP signalling. *EMBO Rep* 2011; **12**: 1055-1061 [PMID: 21909076 DOI: 10.1038/embor.2011.175]

14 **Binnerts ME**, Kim KA, Bright JM, Patel SM, Tran K, Zhou M, Leung JM, Liu Y, Lomas WE, Dixon M, Hazell SA, Wagle M, Nie WS, Tomasevic N, Williams J, Zhan X, Levy MD, Funk WD, Abo A. R-Spondin1 regulates Wnt signaling by inhibiting internalization of LRP6. *Proc Natl Acad Sci USA* 2007; **104**: 14700-14705 [PMID: 17804805 DOI: 10.1073/pnas.0702305104]

15 **Mustata RC**, Van Loy T, Lefort A, Libert F, Strollo S, Vassart G, Garcia MI. Lgr4 is required for Paneth cell differentiation and maintenance of intestinal stem cells ex vivo. *EMBO Rep* 2011; **12**: 558-564 [PMID: 21508962 DOI: 10.1038/embor.2011.52]

16 **Holmberg J**, Genander M, Halford MM, Annerén C, Sondell M, Chumley MJ, Silvany RE, Henkemeyer M, Frisén J. EphB receptors coordinate migration and proliferation in the intestinal stem cell niche. *Cell* 2006; **125**: 1151-1163 [PMID: 16777604 DOI: 10.1016/j.cell.2006.04.030]

17 **Pellegrinet L**, Rodilla V, Liu Z, Chen S, Koch U, Espinosa L, Kaestner KH, Kopan R, Lewis J, Radtke F. Dll1- and dll4-mediated notch signaling are required for homeostasis of intestinal stem cells. *Gastroenterology* 2011; **140**: 1230-1240.e1-7 [PMID: 21238454 DOI: 10.1053/j.gastro.2011.01.005]

18 **He XC**, Zhang J, Tong WG, Tawfik O, Ross J, Scoville DH, Tian Q, Zeng X, He X, Wiedemann LM, Mishina Y, Li L. BMP signaling inhibits intestinal stem cell self-renewal through suppression of Wnt-beta-catenin signaling. *Nat Genet* 2004; **36**: 1117-1121 [PMID: 15378062 DOI: 10.1038/ng1430]

19 **Yang Q**, Bermingham NA, Finegold MJ, Zoghbi HY. Requirement of Math1 for secretory cell lineage commitment in the mouse intestine. *Science* 2001; **294**: 2155-2158 [PMID: 11739954 DOI: 10.1126/science.1065718]

20 **Suzuki K**, Fukui H, Kayahara T, Sawada M, Seno H, Hiai H, Kageyama R, Okano H, Chiba T. Hes1-deficient mice show precocious differentiation of Paneth cells in the small intestine. *Biochem Biophys Res Commun* 2005; **328**: 348-352 [PMID: 15670790 DOI: 10.1016/j.bbrc.2004.12.174]

21 **Barker N**, van Oudenaarden A, Clevers H. Identifying the stem cell of the intestinal crypt: strategies and pitfalls. *Cell Stem Cell* 2012; **11**: 452-460 [PMID: 23040474 DOI: 10.1016/j.stem.2012.09.009]

22 **Barker N**, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, Haegebarth A, Korving J, Begthel H, Peters PJ, Clevers H. Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature* 2007; **449**: 1003-1007 [PMID: 17934449 DOI: 10.1038/nature06196]

23 **Sato T**, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE, van Es JH, Abo A, Kujala P, Peters PJ, Clevers H. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature* 2009; **459**: 262-265 [PMID: 19329995 DOI: 10.1038/nature07935]

24 **Hua G**, Thin TH, Feldman R, Haimovitz-Friedman A, Clevers H, Fuks Z, Kolesnick R. Crypt base columnar stem cells in small intestines of mice are radioresistant. *Gastroenterology* 2012; **143**: 1266-1276 [PMID: 22841781 DOI: 10.1053/j.gastro.2012.07.106]

25 **Schepers AG**, Vries R, van den Born M, van de Wetering M, Clevers H. Lgr5 intestinal stem cells have high telomerase activity and randomly segregate their chromosomes. *EMBO J* 2011; **30**: 1104-1109 [PMID: 21297579 DOI: 10.1038/emboj.2011.26]

26 **Maria Cambuli F**, Rezza A, Nadjar J, Plateroti M. Brief report: musashi1-eGFP mice, a new tool for differential isolation of the intestinal stem cell populations. *Stem Cells* 2013; **31**: 2273-2278 [PMID: 23712573 DOI: 10.1002/stem.1428]

27 **Formeister EJ**, Sionas AL, Lorance DK, Barkley CL, Lee GH, Magness ST. Distinct SOX9 levels differentially mark stem/progenitor populations and enteroendocrine cells of the small intestine epithelium. *Am J Physiol Gastrointest Liver Physiol* 2009; **296**: G1108-G1118 [PMID: 19228882 DOI: 10.1152/ajpgi.00004.2009]

28 **van der Flier LG**, van Gijn ME, Hatzis P, Kujala P, Haegebarth A, Stange DE, Begthel H, van den Born M, Guryev V, Oving I, van Es JH, Barker N, Peters PJ, van de Wetering M, Clevers H. Transcription factor achaete scute-like 2 controls intestinal stem cell fate. *Cell* 2009; **136**: 903-912 [PMID: 19269367 DOI: 10.1016/j.cell.2009.01.031]

29 **Koo BK**, Spit M, Jordens I, Low TY, Stange DE, van de Wetering M, van Es JH, Mohammed S, Heck AJ, Maurice MM, Clevers H. Tumour suppressor RNF43 is a stem-cell E3 ligase that induces endocytosis of Wnt receptors. *Nature* 2012; **488**: 665-669 [PMID: 22895187 DOI: 10.1038/nature11308]

30 **Koo BK**, van Es JH, van den Born M, Clevers H. Porcupine inhibitor suppresses paracrine Wnt-driven growth of Rnf43; Znrf3-mutant neoplasia. *Proc Natl Acad Sci USA* 2015; **112**: 7548-7550 [PMID: 26023187 DOI: 10.1073/pnas.1508113112]

31 **VanDussen KL**, Carulli AJ, Keeley TM, Patel SR, Puthoff BJ, Magness ST, Tran IT, Maillard I, Siebel C, Kolterud Å, Grosse AS, Gumucio DL, Ernst SA, Tsai YH, Dempsey PJ, Samuelson LC. Notch signaling modulates proliferation and differentiation of intestinal crypt base columnar stem cells. *Development* 2012; **139**: 488-497 [PMID: 22190634 DOI: 10.1242/dev.070763]

32 **Van Limbergen J**, Geddes K, Henderson P, Russell RK, Drummond HE, Satsangi J, Griffiths AM, Philpott DJ, Wilson DC. Paneth cell marker CD24 in NOD2 knockout organoids and in inflammatory bowel disease (IBD). *Gut* 2015; **64**: 353-354 [PMID: 23704317 DOI: 10.1136/gutjnl-2013-305077]

33 **Zeilstra J**, Joosten SP, van Andel H, Tolg C, Berns A, Snoek M, van de Wetering M, Spaargaren M, Clevers H, Pals ST. Stem cell CD44v isoforms promote intestinal cancer formation in Apc(min) mice downstream of Wnt signaling. *Oncogene* 2014; **33**: 665-670 [PMID: 23318432 DOI: 10.1038/onc.2012.611]

34 **Snippert HJ**, van Es JH, van den Born M, Begthel H, Stange DE, Barker N, Clevers H. Prominin-1/CD133 marks stem cells and early progenitors in mouse small intestine. *Gastroenterology* 2009; **136**: 2187-2194.e1 [PMID: 19324043 DOI: 10.1053/j.gastro.2009.03.002]

35 **Levin TG**, Powell AE, Davies PS, Silk AD, Dismuke AD, Anderson EC, Swain JR, Wong MH. Characterization of the intestinal cancer stem cell marker CD166 in the human and mouse gastrointestinal tract. *Gastroenterology* 2010; **139**: 2072-2082.e5 [PMID: 20826154 DOI: 10.1053/j.gastro.2010.08.053]

36 **Potten CS**. Stem cells in gastrointestinal epithelium: numbers, characteristics and death. *Philos Trans R Soc Lond B Biol Sci* 1998; **353**: 821-830 [PMID: 9684279 DOI: 10.1098/rstb.1998.0246]

37 **Potten CS**, Owen G, Booth D. Intestinal stem cells protect their genome by selective segregation of template DNA strands. *J Cell Sci* 2002; **115**: 2381-2388 [PMID: 12006622]

38 **Snippert HJ**, van der Flier LG, Sato T, van Es JH, van den Born M, Kroon-Veenboer C, Barker N, Klein AM, van Rheenen J, Simons BD, Clevers H. Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells. *Cell* 2010; **143**: 134-144 [PMID: 20887898 DOI: 10.1016/j.cell.2010.09.016]

39 **Lopez-Garcia C**, Klein AM, Simons BD, Winton DJ. Intestinal stem cell replacement follows a pattern of neutral drift. *Science* 2010; **330**: 822-825 [PMID: 20929733 DOI: 10.1126/science.1196236]

40 **Zhu Y**, Huang YF, Kek C, Bulavin DV. Apoptosis differently affects lineage tracing of Lgr5 and Bmi1 intestinal stem cell populations. *Cell Stem Cell* 2013; **12**: 298-303 [PMID: 23415913 DOI: 10.1016/j.stem.2013.01.003]

41 **Sangiorgi E**, Capecchi MR. Bmi1 is expressed in vivo in intestinal stem cells. *Nat Genet* 2008; **40**: 915-920 [PMID: 18536716 DOI: 10.1038/ng.165]

42 **Takeda N**, Jain R, LeBoeuf MR, Wang Q, Lu MM, Epstein JA. Interconversion between intestinal stem cell populations in distinct niches. *Science* 2011; **334**: 1420-1424 [PMID: 22075725 DOI: 10.1126/science.1213214]

43 **Powell AE**, Wang Y, Li Y, Poulin EJ, Means AL, Washington MK, Higginbotham JN, Juchheim A, Prasad N, Levy SE, Guo Y, Shyr Y, Aronow BJ, Haigis KM, Franklin JL, Coffey RJ. The pan-ErbB negative regulator Lrig1 is an intestinal stem cell marker that functions as a tumor suppressor. *Cell* 2012; **149**: 146-158 [PMID: 22464327 DOI: 10.1016/j.cell.2012.02.042]

44 **Montgomery RK**, Carlone DL, Richmond CA, Farilla L, Kranendonk ME, Henderson DE, Baffour-Awuah NY, Ambruzs DM, Fogli LK, Algra S, Breault DT. Mouse telomerase reverse transcriptase (mTert) expression marks slowly cycling intestinal stem cells. *Proc Natl Acad Sci USA* 2011; **108**: 179-184 [PMID: 21173232 DOI: 10.1073/pnas.1013004108]

45 **Tian H**, Biehs B, Warming S, Leong KG, Rangell L, Klein OD, de Sauvage FJ. A reserve stem cell population in small intestine renders Lgr5-positive cells dispensable. *Nature* 2011; **478**: 255-259 [PMID: 21927002 DOI: 10.1038/nature10408]

46 **Yan KS**, Chia LA, Li X, Ootani A, Su J, Lee JY, Su N, Luo Y, Heilshorn SC, Amieva MR, Sangiorgi E, Capecchi MR, Kuo CJ. The intestinal stem cell markers Bmi1 and Lgr5 identify two functionally distinct populations. *Proc Natl Acad Sci USA* 2012; **109**: 466-471 [PMID: 22190486 DOI: 10.1073/pnas.1118857109]

47 **Metcalfe C**, Kljavin NM, Ybarra R, de Sauvage FJ. Lgr5+ stem cells are indispensable for radiation-induced intestinal regeneration. *Cell Stem Cell* 2014; **14**: 149-159 [PMID: 24332836 DOI: 10.1016/j.stem.2013.11.008]

48 **van Es JH**, Sato T, van de Wetering M, Lyubimova A, Nee AN, Gregorieff A, Sasaki N, Zeinstra L, van den Born M, Korving J, Martens AC, Barker N, van Oudenaarden A, Clevers H. Dll1+ secretory progenitor cells revert to stem cells upon crypt damage. *Nat Cell Biol* 2012; **14**: 1099-1104 [PMID: 23000963 DOI: 10.1038/ncb2581]

49 **Buczacki SJ**, Zecchini HI, Nicholson AM, Russell R, Vermeulen L, Kemp R, Winton DJ. Intestinal label-retaining cells are secretory precursors expressing Lgr5. *Nature* 2013; **495**: 65-69 [PMID: 23446353 DOI: 10.1038/nature11965]

50 **Clevers H**. Stem Cells: A unifying theory for the crypt. *Nature* 2013; **495**: 53-54 [PMID: 23446347 DOI: 10.1038/nature11958]

51 **von Furstenberg RJ**, Gulati AS, Baxi A, Doherty JM, Stappenbeck TS, Gracz AD, Magness ST, Henning SJ. Sorting mouse jejunal epithelial cells with CD24 yields a population with characteristics of intestinal stem cells. *Am J Physiol Gastrointest Liver Physiol* 2011; **300**: G409-G417 [PMID: 21183658 DOI: 10.1152/ajpgi.00453.2010]

52 **Chang PY**, Jin X, Jiang YY, Wang LX, Liu YJ, Wang J. Mensenchymal stem cells can delay radiation-induced crypt death: impact on intestinal CD44(+) fragments. *Cell Tissue Res* 2016; **364**: 331-344 [PMID: 26613604 DOI: 10.1007/s00441-015-2313-6]

53 **Sato T**, Stange DE, Ferrante M, Vries RG, Van Es JH, Van den Brink S, Van Houdt WJ, Pronk A, Van Gorp J, Siersema PD, Clevers H. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. *Gastroenterology* 2011; **141**: 1762-1772 [PMID: 21889923 DOI: 10.1053/j.gastro.2011.07.050]

54 **Gracz AD**, Ramalingam S, Magness ST. Sox9 expression marks a subset of CD24-expressing small intestine epithelial stem cells that form organoids in vitro. *Am J Physiol Gastrointest Liver Physiol* 2010; **298**: G590-G600 [PMID: 20185687 DOI: 10.1152/ajpgi.00470.2009]

55 **Gracz AD**, Fuller MK, Wang F, Li L, Stelzner M, Dunn JC, Martin MG, Magness ST. Brief report: CD24 and CD44 mark human intestinal epithelial cell populations with characteristics of active and facultative stem cells. *Stem Cells* 2013; **31**: 2024-2030 [PMID: 23553902 DOI: 10.1002/stem.1391]

56 **Hou NY**, Yang K, Chen T, Chen XZ, Zhang B, Mo XM, Hu JK. CD133+ CD44+ subgroups may be human small intestinal stem cells. *Mol Biol Rep* 2011; **38**: 997-1004 [PMID: 20526812 DOI: 10.1007/s11033-010-0195-y]

57 **Wang F**, Scoville D, He XC, Mahe MM, Box A, Perry JM, Smith NR, Lei NY, Davies PS, Fuller MK, Haug JS, McClain M, Gracz AD, Ding S, Stelzner M, Dunn JC, Magness ST, Wong MH, Martin MG, Helmrath M, Li L. Isolation and characterization of intestinal stem cells based on surface marker combinations and colony-formation assay. *Gastroenterology* 2013; **145**: 383-95.e1-21 [PMID: 23644405 DOI: 10.1053/j.gastro.2013.04.050]

58 **Li VS**, Clevers H. In vitro expansion and transplantation of intestinal crypt stem cells. *Gastroenterology* 2012; **143**: 30-34 [PMID: 22613623 DOI: 10.1053/j.gastro.2012.05.017]

59 **Huch M**, Dorrell C, Boj SF, van Es JH, Li VS, van de Wetering M, Sato T, Hamer K, Sasaki N, Finegold MJ, Haft A, Vries RG, Grompe M, Clevers H. In vitro expansion of single Lgr5+ liver stem cells induced by Wnt-driven regeneration. *Nature* 2013; **494**: 247-250 [PMID: 23354049 DOI: 10.1038/nature11826]

60 **Huch M**, Bonfanti P, Boj SF, Sato T, Loomans CJ, van de Wetering M, Sojoodi M, Li VS, Schuijers J, Gracanin A, Ringnalda F, Begthel H, Hamer K, Mulder J, van Es JH, de Koning E, Vries RG, Heimberg H, Clevers H. Unlimited in vitro expansion of adult bi-potent pancreas progenitors through the Lgr5/R-spondin axis. *EMBO J* 2013; **32**: 2708-2721 [PMID: 24045232 DOI: 10.1038/emboj.2013.204]

61 **Barker N**, Huch M, Kujala P, van de Wetering M, Snippert HJ, van Es JH, Sato T, Stange DE, Begthel H, van den Born M, Danenberg E, van den Brink S, Korving J, Abo A, Peters PJ, Wright N, Poulsom R, Clevers H. Lgr5(+ve) stem cells drive self-renewal in the stomach and build long-lived gastric units in vitro. *Cell Stem Cell* 2010; **6**: 25-36 [PMID: 20085740 DOI: 10.1016/j.stem.2009.11.013]

62 **Ootani A**, Li X, Sangiorgi E, Ho QT, Ueno H, Toda S, Sugihara H, Fujimoto K, Weissman IL, Capecchi MR, Kuo CJ. Sustained in vitro intestinal epithelial culture within a Wnt-dependent stem cell niche. *Nat Med* 2009; **15**: 701-706 [PMID: 19398967 DOI: 10.1038/nm.1951]

63 **Yin X**, Farin HF, van Es JH, Clevers H, Langer R, Karp JM. Niche-independent high-purity cultures of Lgr5+ intestinal stem cells and their progeny. *Nat Methods* 2014; **11**: 106-112 [PMID: 24292484 DOI: 10.1038/nmeth.2737]

64 **Clevers H**. At the crossroads of inflammation and cancer. *Cell* 2004; **118**: 671-674 [PMID: 15369667 DOI: 10.1016/j.cell.2004.09.005]

65 **Zeuner A**, Todaro M, Stassi G, De Maria R. Colorectal cancer stem cells: from the crypt to the clinic. *Cell Stem Cell* 2014; **15**: 692-705 [PMID: 25479747 DOI: 10.1016/j.stem.2014.11.012]

66 **Schwitalla S**, Fingerle AA, Cammareri P, Nebelsiek T, Göktuna SI, Ziegler PK, Canli O, Heijmans J, Huels DJ, Moreaux G, Rupec RA, Gerhard M, Schmid R, Barker N, Clevers H, Lang R, Neumann J, Kirchner T, Taketo MM, van den Brink GR, Sansom OJ, Arkan MC, Greten FR. Intestinal tumorigenesis initiated by dedifferentiation and acquisition of stem-cell-like properties. *Cell* 2013; **152**: 25-38 [PMID: 23273993 DOI: 10.1016/j.cell.2012.12.012]

67 **Westphalen CB**, Asfaha S, Hayakawa Y, Takemoto Y, Lukin DJ, Nuber AH, Brandtner A, Setlik W, Remotti H, Muley A, Chen X, May R, Houchen CW, Fox JG, Gershon MD, Quante M, Wang TC. Long-lived intestinal tuft cells serve as colon cancer-initiating cells. *J Clin Invest* 2014; **124**: 1283-1295 [PMID: 24487592 DOI: 10.1172/JCI73434]

68 **Barker N**, Ridgway RA, van Es JH, van de Wetering M, Begthel H, van den Born M, Danenberg E, Clarke AR, Sansom OJ, Clevers H. Crypt stem cells as the cells-of-origin of intestinal cancer. *Nature* 2009; **457**: 608-611 [PMID: 19092804 DOI: 10.1038/nature07602]

69 **Adams PD**, Jasper H, Rudolph KL. Aging-Induced Stem Cell Mutations as Drivers for Disease and Cancer. *Cell Stem Cell* 2015; **16**: 601-612 [PMID: 26046760 DOI: 10.1016/j.stem.2015.05.002]

70 **Tomasetti C**, Vogelstein B. Cancer etiology. Variation in cancer risk among tissues can be explained by the number of stem cell divisions. *Science* 2015; **347**: 78-81 [PMID: 25554788 DOI: 10.1126/science.1260825]

71 **Biswas S**, Davis H, Irshad S, Sandberg T, Worthley D, Leedham S. Microenvironmental control of stem cell fate in intestinal homeostasis and disease. *J Pathol* 2015; **237**: 135-145 [PMID: 25974319 DOI: 10.1002/path.4563]

72 **Wielenga VJ**, Smits R, Korinek V, Smit L, Kielman M, Fodde R, Clevers H, Pals ST. Expression of CD44 in Apc and Tcf mutant mice implies regulation by the WNT pathway. *Am J Pathol* 1999; **154**: 515-523 [PMID: 10027409 DOI: 10.1016/S0002-9440(10)65297-2]

73 **Davidson LA**, Callaway ES, Kim E, Weeks BR, Fan YY, Allred CD, Chapkin RS. Targeted Deletion of p53 in Lgr5-Expressing Intestinal Stem Cells Promotes Colon Tumorigenesis in a Preclinical Model of Colitis-Associated Cancer. *Cancer Res* 2015; **75**: 5392-5397 [PMID: 26631266 DOI: 10.1158/0008-5472.CAN-15-1706]

74 **Drost J**, van Jaarsveld RH, Ponsioen B, Zimberlin C, van Boxtel R, Buijs A, Sachs N, Overmeer RM, Offerhaus GJ, Begthel H, Korving J, van de Wetering M, Schwank G, Logtenberg M, Cuppen E, Snippert HJ, Medema JP, Kops GJ, Clevers H. Sequential cancer mutations in cultured human intestinal stem cells. *Nature* 2015; **521**: 43-47 [PMID: 25924068 DOI: 10.1038/nature14415]

75 **Liu Z**, Dai W, Jiang L, Cheng Y. Over-expression of LGR5 correlates with poor survival of colon cancer in mice as well as in patients. *Neoplasma* 2014; **61**: 177-185 [PMID: 24063790 DOI: 10.4149/neo\_2014\_016]

76 **Demidov ON**, Timofeev O, Lwin HN, Kek C, Appella E, Bulavin DV. Wip1 phosphatase regulates p53-dependent apoptosis of stem cells and tumorigenesis in the mouse intestine. *Cell Stem Cell* 2007; **1**: 180-190 [PMID: 18371349 DOI: 10.1016/j.stem.2007.05.020]

77 **Barry ER**, Morikawa T, Butler BL, Shrestha K, de la Rosa R, Yan KS, Fuchs CS, Magness ST, Smits R, Ogino S, Kuo CJ, Camargo FD. Restriction of intestinal stem cell expansion and the regenerative response by YAP. *Nature* 2013; **493**: 106-110 [PMID: 23178811 DOI: 10.1038/nature11693]

78 **Gregorieff A**, Liu Y, Inanlou MR, Khomchuk Y, Wrana JL. Yap-dependent reprogramming of Lgr5(+) stem cells drives intestinal regeneration and cancer. *Nature* 2015; **526**: 715-718 [PMID: 26503053 DOI: 10.1038/nature15382]

79 **Merlos-Suárez A**, Barriga FM, Jung P, Iglesias M, Céspedes MV, Rossell D, Sevillano M, Hernando-Momblona X, da Silva-Diz V, Muñoz P, Clevers H, Sancho E, Mangues R, Batlle E. The intestinal stem cell signature identifies colorectal cancer stem cells and predicts disease relapse. *Cell Stem Cell* 2011; **8**: 511-524 [PMID: 21419747 DOI: 10.1016/j.stem.2011.02.020]

80 **Sagiv E**, Starr A, Rozovski U, Khosravi R, Altevogt P, Wang T, Arber N. Targeting CD24 for treatment of colorectal and pancreatic cancer by monoclonal antibodies or small interfering RNA. *Cancer Res* 2008; **68**: 2803-2812 [PMID: 18413748 DOI: 10.1158/0008-5472.CAN-07-6463]

81 **Todaro M**, Gaggianesi M, Catalano V, Benfante A, Iovino F, Biffoni M, Apuzzo T, Sperduti I, Volpe S, Cocorullo G, Gulotta G, Dieli F, De Maria R, Stassi G. CD44v6 is a marker of constitutive and reprogrammed cancer stem cells driving colon cancer metastasis. *Cell Stem Cell* 2014; **14**: 342-356 [PMID: 24607406 DOI: 10.1016/j.stem.2014.01.009]

82 **Ricci-Vitiani L**, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C, De Maria R. Identification and expansion of human colon-cancer-initiating cells. *Nature* 2007; **445**: 111-115 [PMID: 17122771 DOI: 10.1038/nature05384]

83 **Zhang SS**, Han ZP, Jing YY, Tao SF, Li TJ, Wang H, Wang Y, Li R, Yang Y, Zhao X, Xu XD, Yu ED, Rui YC, Liu HJ, Zhang L, Wei LX. CD133(+)CXCR4(+) colon cancer cells exhibit metastatic potential and predict poor prognosis of patients. *BMC Med* 2012; **10**: 85 [PMID: 22871210 DOI: 10.1186/1741-7015-10-85]

84 **Dalerba P**, Dylla SJ, Park IK, Liu R, Wang X, Cho RW, Hoey T, Gurney A, Huang EH, Simeone DM, Shelton AA, Parmiani G, Castelli C, Clarke MF. Phenotypic characterization of human colorectal cancer stem cells. *Proc Natl Acad Sci USA* 2007; **104**: 10158-10163 [PMID: 17548814 DOI: 10.1073/pnas.0703478104]

85 **Pang R**, Law WL, Chu AC, Poon JT, Lam CS, Chow AK, Ng L, Cheung LW, Lan XR, Lan HY, Tan VP, Yau TC, Poon RT, Wong BC. A subpopulation of CD26+ cancer stem cells with metastatic capacity in human colorectal cancer. *Cell Stem Cell* 2010; **6**: 603-615 [PMID: 20569697 DOI: 10.1016/j.stem.2010.04.001]

86 **Gao W**, Chen L, Ma Z, Du Z, Zhao Z, Hu Z, Li Q. Isolation and phenotypic characterization of colorectal cancer stem cells with organ-specific metastatic potential. *Gastroenterology* 2013; **145**: 636-46.e5 [PMID: 23747337 DOI: 10.1053/j.gastro.2013.05.049]

87 **Nakanishi Y**, Seno H, Fukuoka A, Ueo T, Yamaga Y, Maruno T, Nakanishi N, Kanda K, Komekado H, Kawada M, Isomura A, Kawada K, Sakai Y, Yanagita M, Kageyama R, Kawaguchi Y, Taketo MM, Yonehara S, Chiba T. Dclk1 distinguishes between tumor and normal stem cells in the intestine. *Nat Genet* 2013; **45**: 98-103 [PMID: 23202126 DOI: 10.1038/ng.2481]

88 **Goessling W**, North TE, Loewer S, Lord AM, Lee S, Stoick-Cooper CL, Weidinger G, Puder M, Daley GQ, Moon RT, Zon LI. Genetic interaction of PGE2 and Wnt signaling regulates developmental specification of stem cells and regeneration. *Cell* 2009; **136**: 1136-1147 [PMID: 19303855 DOI: 10.1016/j.cell.2009.01.015]

89 **Wang D**, Fu L, Sun H, Guo L, DuBois RN. Prostaglandin E2 Promotes Colorectal Cancer Stem Cell Expansion and Metastasis in Mice. *Gastroenterology* 2015; **149**: 1884-1895.e4 [PMID: 26261008 DOI: 10.1053/j.gastro.2015.07.064]

90 **Chang PY**, Qu YQ, Wang J, Dong LH. The potential of mesenchymal stem cells in the management of radiation enteropathy. *Cell Death Dis* 2015; **6**: e1840 [PMID: 26247725 DOI: 10.1038/cddis.2015.189]

91 **Calon A**, Lonardo E, Berenguer-Llergo A, Espinet E, Hernando-Momblona X, Iglesias M, Sevillano M, Palomo-Ponce S, Tauriello DV, Byrom D, Cortina C, Morral C, Barceló C, Tosi S, Riera A, Attolini CS, Rossell D, Sancho E, Batlle E. Stromal gene expression defines poor-prognosis subtypes in colorectal cancer. *Nat Genet* 2015; **47**: 320-329 [PMID: 25706628 DOI: 10.1038/ng.3225]

92 **Huynh PT**, Beswick EJ, Coronado YA, Johnson P, O'Connell MR, Watts T, Singh P, Qiu S, Morris K, Powell DW, Pinchuk IV. CD90(+) stromal cells are the major source of IL-6, which supports cancer stem-like cells and inflammation in colorectal cancer. *Int J Cancer* 2016; **138**: 1971-1981 [PMID: 26595254 DOI: 10.1002/ijc.29939]

93 **Lotti F**, Jarrar AM, Pai RK, Hitomi M, Lathia J, Mace A, Gantt GA, Sukhdeo K, DeVecchio J, Vasanji A, Leahy P, Hjelmeland AB, Kalady MF, Rich JN. Chemotherapy activates cancer-associated fibroblasts to maintain colorectal cancer-initiating cells by IL-17A. *J Exp Med* 2013; **210**: 2851-2872 [PMID: 24323355 DOI: 10.1084/jem.20131195]

94 **Zubeldia IG**, Bleau AM, Redrado M, Serrano D, Agliano A, Gil-Puig C, Vidal-Vanaclocha F, Lecanda J, Calvo A. Epithelial to mesenchymal transition and cancer stem cell phenotypes leading to liver metastasis are abrogated by the novel TGFβ1-targeting peptides P17 and P144. *Exp Cell Res* 2013; **319**: 12-22 [PMID: 23153552 DOI: 10.1016/j.yexcr.2012.11.004]

95 **Jung T**, Gross W, Zöller M. CD44v6 coordinates tumor matrix-triggered motility and apoptosis resistance. *J Biol Chem* 2011; **286**: 15862-15874 [PMID: 21372142 DOI: 10.1074/jbc.M110.208421]

96 **Zhang C**, Zhou C, Wu XJ, Yang M, Yang ZH, Xiong HZ, Zhou CP, Lu YX, Li Y, Li XN. Human CD133-positive hematopoietic progenitor cells initiate growth and metastasis of colorectal cancer cells. *Carcinogenesis* 2014; **35**: 2771-2777 [PMID: 25269803 DOI: 10.1093/carcin/bgu192]

97 **Varol U**, Yildiz I, Salman T, Karabulut B, Uslu R. Markers to predict the efficacy of bevacizumab in the treatment of metastatic colorectal cancer. *Tumori* 2014; **100**: 370-376 [PMID: 25296585 DOI: 10.1700/1636.17888]

98 **Colombo M**, Raposo G, Théry C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. *Annu Rev Cell Dev Biol* 2014; **30**: 255-289 [PMID: 25288114 DOI: 10.1146/annurev-cellbio-101512-122326]

99 **Hu Y**, Yan C, Mu L, Huang K, Li X, Tao D, Wu Y, Qin J. Fibroblast-Derived Exosomes Contribute to Chemoresistance through Priming Cancer Stem Cells in Colorectal Cancer. *PLoS One* 2015; **10**: e0125625 [PMID: 25938772 DOI: 10.1371/journal.pone.0125625]

100 **Hong BS**, Cho JH, Kim H, Choi EJ, Rho S, Kim J, Kim JH, Choi DS, Kim YK, Hwang D, Gho YS. Colorectal cancer cell-derived microvesicles are enriched in cell cycle-related mRNAs that promote proliferation of endothelial cells. *BMC Genomics* 2009; **10**: 556 [PMID: 19930720 DOI: 10.1186/1471-2164-10-556]

101 . The exosomes in tumor immunity. *Oncoimmunology* 2015; **4**: e1027472 [PMID: 26405598 DOI: 10.1080/2162402X.2015.1027472]

102 **Cha DJ**, Franklin JL, Dou Y, Liu Q, Higginbotham JN, Demory Beckler M, Weaver AM, Vickers K, Prasad N, Levy S, Zhang B, Coffey RJ, Patton JG. KRAS-dependent sorting of miRNA to exosomes. *Elife* 2015; **4**: e07197 [PMID: 26132860 DOI: 10.7554/eLife.07197]

103 **Matsumura T**, Sugimachi K, Iinuma H, Takahashi Y, Kurashige J, Sawada G, Ueda M, Uchi R, Ueo H, Takano Y, Shinden Y, Eguchi H, Yamamoto H, Doki Y, Mori M, Ochiya T, Mimori K. Exosomal microRNA in serum is a novel biomarker of recurrence in human colorectal cancer. *Br J Cancer* 2015; **113**: 275-281 [PMID: 26057451 DOI: 10.1038/bjc.2015.201]

104 **Ogata-Kawata H**, Izumiya M, Kurioka D, Honma Y, Yamada Y, Furuta K, Gunji T, Ohta H, Okamoto H, Sonoda H, Watanabe M, Nakagama H, Yokota J, Kohno T, Tsuchiya N. Circulating exosomal microRNAs as biomarkers of colon cancer. *PLoS One* 2014; **9**: e92921 [PMID: 24705249 DOI: 10.1371/journal.pone.0092921]

105 **Watanabe M**. Adult tissue stem cell therapy for gastrointestinal diseases. *J Gastroenterol Hepatol* 2014; Epub ahead of print [PMID: 24547965 DOI: 10.1111/jgh.12555]

106 **Quetglas EG**, Mujagic Z, Wigge S, Keszthelyi D, Wachten S, Masclee A, Reinisch W. Update on pathogenesis and predictors of response of therapeutic strategies used in inflammatory bowel disease. *World J Gastroenterol* 2015; **21**: 12519-12543 [PMID: 26640330 DOI: 10.3748/wjg.v21.i44.12519]

107 **Zhong XY**, Yu T, Zhong W, Li JY, Xia ZS, Yuan YH, Yu Z, Chen QK. Lgr5 positive stem cells sorted from small intestines of diabetic mice differentiate into higher proportion of absorptive cells and Paneth cells in vitro. *Dev Growth Differ* 2015; **57**: 453-465 [PMID: 26122164 DOI: 10.1111/dgd.12226]

108 **Cong L**, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, Zhang F. Multiplex genome engineering using CRISPR/Cas systems. *Science* 2013; **339**: 819-823 [PMID: 23287718 DOI: 10.1126/science.1231143]

109 **Bhattacharjee T**, Zehnder SM, Rowe KG, Jain S, Nixon RM, Sawyer WG, Angelini TE. Writing in the granular gel medium. *Sci Adv* 2015; **1**: e1500655 [PMID: 26601274 DOI: 10.1126/sciadv.1500655]

**P-Reviewer:** Manguso F **S-Editor:** Ma YJ **L-Editor:** **E-Editor:**

**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-spondin | Lgr4/5 | Wnt/β-catenin | **↑** | **/** |
| Myofibroblast | Noggin | BMP4 | BMP/Smad | **/** | **↓** |

ISCs: intestinal stem cells.

**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, C.S.’s team | 4-6 | Apoptosis upon receiving 1cGy | ~ 24 H | Asymmetry | Hierarchy |

**Table 3 characteristic comparing between CBC stem cells and reverse intestinal stem cells**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **ISC type** | **Main location** | **Cell cycle** | **Differentiating capability** | **Foreign stimuli-induced response** | **Leading pathway** | **Typical marker genes** |
| CBC stem cell | Crypt basement | Active | Yes | Apoptosis | Wnt/β-catenin | Lgr5 |
| Reverse ISC | 4+ position | Quiescent | Yes | Proliferation | Unknown but independent of Wnt/β-catenin | Bmi1, HopX, Lrig1,  mTERT |

**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 *et al*[23] | 2009 | 3D | Matrigel containing laminin α1 and α2 | N2, B27, Wnt3, EGF, Noggin, R-spondin, Jagged-1  and Y-27632 dihydrochloride | Serum-free | ISCs | Up to 1 yr |
| Ootani *et al*[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 |