肠道干细胞和潘氏细胞在肠道稳态和疾病中的作用

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摘要 肠道是最复杂的器官之一, 负责营养的吸收和消化。肠道具有多层结构保护整个肠道免受病原体的侵害。 肠道上皮是由单层柱状上皮细胞组成, 是抵抗病原体的第一道屏障。因此, 肠上皮必须保持完整性以保护肠免受感染和毒性剂的侵害。上皮细胞分为两个谱系(吸收型与分泌型), 并且每隔3~4天脱落至肠腔中。细胞的快速更替是由于肠道干细胞的存在, 肠道干细胞排列在隐窝底部终极分化的潘氏细胞之间并沿隐窝绒毛轴分化成不同的上皮细胞。一旦肠道干细胞受到损伤, 潘氏细胞将通过提供WNT配体和Notch刺激来补充肠道干细胞。因此, 潘氏细胞充当辅助细胞以维持干细胞微环境, 即生态位。该综述探讨了干细胞和潘氏细胞之间的相互作用, 进一步探讨了维持肠道稳态的信号通路。

关键词 肠道上皮; 干细胞; 潘氏细胞; 再生

The Role of Intestinal Stem Cells Paneth Cells in Intestinal Homeostasis and Disease

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Abstract The intestine is one of the most complex organs in the human body responsible for the absorption and digestion of nutrients. The intestine is composed of multiple layers in which the epithelium is a monolayer of columnar epithelial cells and is the first barrier against pathogens. Thus, the intestinal epithelium must maintain the integrity to protect intestine from infection and toxin. Epithelial cells can be separated into two lineages (absorptive and secretory) and then shed into the intestinal lumen every 3-4 days which reside between terminally differentiated Paneth cells at the bottom of the intestinal crypt, differentiating to different epithelial cells along the crypt-villus axis. Once stem cells are injured, Paneth cells will replenish intestinal stem cells rapidly by providing WNT ligands ang Notch stimuli. Therefore, Paneth cells act as helper cells to maintain the stem cells microenvironment, which is called a niche. This review will discuss the interaction between stem cells and Paneth cells further investigate the signaling pathways that maintain intestinal homeostasis.

Keywords intestinal epithelium; stem cells; Paneth cells; regeneration

The intestinal epithelium consists of different cell types, such as stem cells, Paneth cells, goblet cells, enteroendocrine, tuft cells, enterocytes, M cells. This article focuses on stem cells, Paneth cells and its secreted

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substance (mucus). Intestinal epithelium is exposed to multiple factors that can lead to disorders of the intestine (Fig.1). Therefore, mucus plays an important role in protecting the intestine from foreign pathogens [1]. ISCs are located at the bottom of the epithelium divided into different intestinal cell types and repair injured epithelium. Due to the proliferation ability of stem cells, epithelial cells turnover rapidly maintaining the homeostasis of the intestine [2]. Additionally, Paneth cells are known for secreting AMPs (antimicrobial peptides), such as defensins and lysozyme. They are also located at the bottom of the crypt and interspersed between stem cells, acting as bodyguard. In addition to ISCs regeneration, there are also secretory cells at the top of the villi that secrete mucus against foreign pathogens to maintain homeostasis. ZAREPOUR [3] showed that the mucus barrier provided partial protection against several enteric bacterial pathogens, including *Yersinia enterocolitica*, *Shigella flexneri*, and *Citrobacter rodentium*. In this review, we discuss how Paneth cells protect stem cells to maintain intestinal homeostasis and how signaling pathways influence stem cells and further cause diseases.

1 Crypt-villus structure

The intestine consists of epithelial cells, crypts, villi, mucus, lymphocytes, enteric neurons, muscle layers, etc. Firstly, we start from crypt-villus structure. The intestine is described as a complex tissue that contains different cell types and there are millions of crypt-villus units in the intestine. Cell differentiation migrates upward towards the crypt-villus axis. The crypt is located at the base of the intestine and consists of ISCs (intestinal stem cells), Paneth cells and +4 label-retaining cells, stem cells further divide into secretory cells and absorptive cells that enhance the origin of all the epithelial components [4-6] (Fig.2). At the bottom of the crypt, cell division occurs rapidly [7].

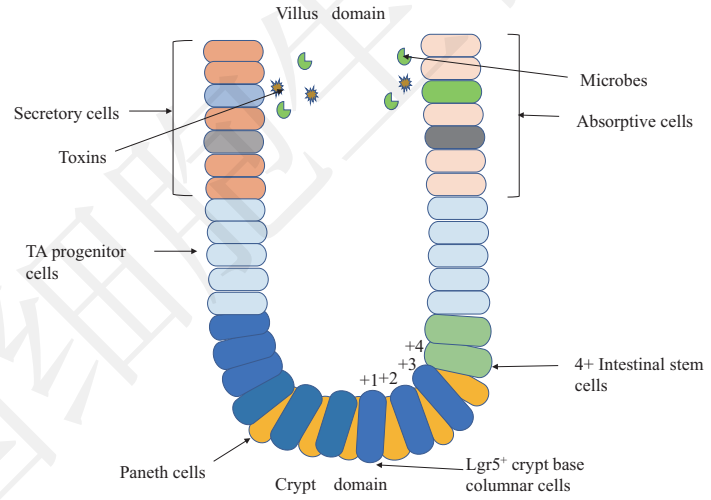

图1 肠道上皮细胞受多种因素的影响

Fig.1 Many factors influence intestine epithelial cells

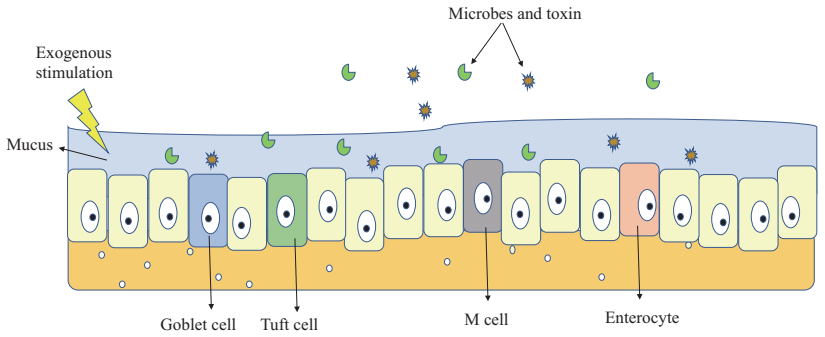

图2 肠道隐窝–绒毛结构

Fig.2 Intestine crypt-villus structure

These cells move towards villi and finally shed into the lumen. The villi protrude into the lumen and take part in absorption and digestion of nutrients. At the tip of villus, cells shed into lumen every 3-4 days for homeostasis. A higher number of villi can enhance nutrient absorption. This rapid cellular turnover significantly promotes epithelial regeneration. BARKER [8] demonstrated that this renewal was primarily due to ISCs proliferation and differentiation [9]. This crypt-villus structure enables the intestine with better absorptive and protective effect. Therefore, it is meaningful to study the ISCs, Paneth cells and interaction between them. It's also necessary to know which signaling pathway or mechanisms control stem cells proliferation and further induce diseases.

2 Origin of ISCs

Now, we know that the rapid cellular turnover of the intestinal epithelium is caused by crypt base columnar cells. However, it is not clear how the ISCs are discovered. CHENG [7] were the first to find the crypt base columnar cells, but there is no sufficient evidence showing that crypt base columnar cells are ISCs . With these questions, scholars have focused on the genetic tracking of crypt basal column cells [8-9]. GUIU [10] used lineage tracing and found that progeny of the LGR5-expressing population was maintained into adulthood and thereby contributed to the adult ISCs compartment. Based on these findings, crypt basal cells are considered to have stem cell properties. With the development of technology, crypt basal columnar cells are identified as ISCs. ISCs are considered to have multiple layers of potential and self-renewal [11-12]. The daughter cells of ISCs evolve into a specific type of intestinal epithelial cells through lineage differentiation [13]. To achieve this process, stem cells must differentiate into daughter cells, allowing for the daughter cells to further differentiate into epithelial cells with differentiation ability [14]. However, the stem cells can be systematically differentiated and form into two stem cells. There are two types of intestinal stem cells in the intestinal crypts: $Lgr5^+$ CBCs (columnar crypt base cells) and +4 label-retaining cells. $Lgr5^+$ and $Bmi1^+$ are potential markers of these stem cells while the $Bmi1^+$ marker is only detected in the proximal small intestine and is not found in the colon and rectum. Interestingly, the $Lgr5^+$ crypt base columnar cells divide rapidly into stem cells overnight during homeostasis. This rapid cycle process is critical for intestinal regeneration. The +4 label-retaining cell cycle is very slow and almost static. When the environment is stressed, these stem cells can protect the intestines: during injury or stress, +4 label-retaining cells are activated and produce intestinal progenitor cells in place of damaged crypt basal cells. In addition, if necessary, the $Lgr5^+$ crypt base columnar cells can regenerate +4 label-retaining cells. JIN [15] showed that partial ISCs depletion Drosophila ISCs failed to repopulate the gut.

3 Paneth cells

In the intestine, Paneth cells play a role in crypt development. Unhealthy intestine results in Paneth cell dysfunction. Thus, antimicrobial peptides secreted by Paneth cells in defending against foreign pathogens on the mucosa are vital for barrier homeostasis. Paneth cells are located at the bottom of the crypt, distributing between ISCs, secreting necessary factors that maintain normal ISCs function and provide a microenvironment for the stem cell niche [16]. In addition to the small intestine, these cells can also be found elsewhere in the gastrointestinal tract, including the stomach and colon, where they can respond to mucosal inflammation [17].

Paneth cells have ultrastructural features of specialized secretory cells, including extensive endoplasmic reticulum, Golgi networks and apical aggregation of secretory granules [18]. Many histological stains and lectins can easily divide these secretory granules, which are rich in alkali peptides, and some are modified with glycans. Although the content of the particles has been unclear for many years, they are the first line for host defence. Paneth cells appear when lysozyme is identified as a secreted product, as shown by experiment of OUELLETTE [19] that α-defensins are expressed in murine Paneth cells. This discovery leads to a large

number of investigations focusing on structure, function, and regulation of antibacterial molecules in Paneth cells. These studies provide a clearer understanding of the function of Paneth cells and the key role for these epithelial cells [20]. RIBA [21] showed that Paneth cell defect induced microbiota dysbiosis in mice and promoted visceral hypersensitivity. Therefore, Paneth cells also play a crucial role in maintaining intestinal homeostasis.

4 Paneth cells help to support stem cells

Although several signaling pathways for maintaining stem cell function and crypt homeostasis have been thoroughly studied, little is known about how metabolism promotes epithelial homeostasis. It has been reported that freshly isolated $Lgr5^+$ columnar crypt base cells and Paneth cells from mice show different metabolic processes [22]. Compared with Paneth cells, $Lgr5^+$ columnar crypt base cells show higher mitochondrial activity. Inhibition of mitochondrial activity of $Lgr5^+$ crypt base columnar cells or inhibition of Paneth cell glycolysis greatly affects stem cell function [23]. In addition, Paneth cells enhance mitochondrial oxidative phosphorylation of $Lgr5^+$ crypt base columnar cells by providing lactic acid to support stem cell function. RODRÍGUEZ-COLMAN [24] showed that oxidative phosphorylation activated p38/MAPK via a redox signaling pathway, and they also revealed the metabolic identity of Paneth cells and $Lgr5^+$ crypt base columnar cells in supporting stem cell function.

Some studies have described that the homeostasis of the self-renewing intestinal crypt is caused by competition between $Lgr5^+$ crypt base columnar cells, which are located at the bottom of the crypt[25]. $Lgr5^+$ stem cells are interspersed between the differentiated Paneth cells that produce bactericidal products such as lysozyme, tube toxin, cryptins and defensins, which can culture single-expressed $Lgr5^+$ stem cells without a non-epithelial niche. Studies have shown that $Lgr5^+$ stem cells and Paneth cells have a close physical association *in vitro* and *in vivo*. $CD24^+$ Paneth cells express EGF, TGFα, WNT3 and Notch-ligand Dll4, all of which are required for maintenance of stem cells culture. Co-culture of stem cells with Paneth cells significantly increases organ formation. The demand for Paneth cells can be replaced by pulses of exogenous WNT. Gene removal from Paneth cells *in vivo* results in the loss of $Lgr5^+$ stem cells *in vivo*. In the colonic crypt, $CD24^+$ cells are located between $Lgr5^+$ stem cells and Paneth cells. We can conclude that the necessary signals required for $Lgr5^+$ stem cells are provided by specific daughter cells and Paneth cells.

5 Related signaling pathways to stem cells and Paneth cells

However, intestinal homeostasis is maintained by continuous cell proliferation and differentiation. The proliferation and differentiation of ISCs and Paneth cells are controlled by multiple signaling pathways[26] (Fig.3). Therefore, several signaling pathways related to homeostasis are mentioned and termed Notch, WNT and BMP. Notch signaling is famous for its regulation of the proliferation and differentiation of ISCs and progenitor cells, whereas cellular targets and specific functions of the Notch signal have not been directly identified [27]. Another marker is termed Olfm4. Studies have shown that Olfm4 has an important role in the immune response. Olfm4 expressed by the $Lgr5^+$ crypt base columnar cells are directly reliant on Notch signaling, and its transcriptional activation is via the RBPJ binding site. Inhibition of Notch signaling also leads to premature differentiation of epithelial progenitor cells into secretory cell types [28]. Notch signaling controls stem cells by inhibiting Atoh1. Notch signaling inhibits the transcription factor Atoh1 (asexual homologue 1), which provides a critical mechanism for regulating cell fate selection, and Atoh1 expression appears to be essential and sufficient for programmed differentiation of secretory cells. In general, damage to Notch signaling leads to increased expression of Atoh1 and further reduces secretory cell proliferation and overactivation of Notch signaling leads to decreased expression of Atoh1 and expansion of the proliferative zone, resulting in increased absorption. Notch functional analysis of the

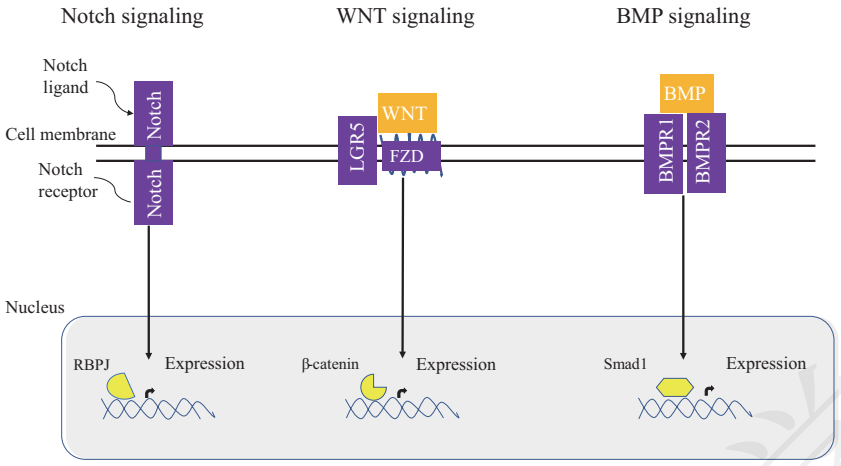

图3 多种信号调节干细胞途径

Fig.3 Several signals regulate stem cells fate

Atoh1-deficient intestinal tract showed that cell changes were reliant on Atoh1, whereas Notch regulated expression of the *Olfm4* gene independent of Atoh1 [29]. Studies have shown that Notch signaling maintained adult ISCs and regulates cell fate, thereby controled epithelial cell homeostasis by targeting different progenitor cell populations [30]. Notch signaling promotes differentiation of the digestive cell lineage, rather than the secretory cells lineage [31]. Thus, gene elimination of the Notch signaling component, including the key Notch DNA binding protein RBPJ, Notch1 and Notch2 receptors, results in reducing cells proliferation and the number of secretory cells in the intestinal crypts [32-33]. Notch signaling may target different stem and progenitor cell populations to regulate different aspects of intestinal homeostasis, although specific cellular targets have not been established. In the Notch signaling pathway, the key compositions are Notch1 and Notch2 receptors, Dll1 (Delta-like ligand1) and Dll4 (delta-like ligand). Importantly, lineage-tracking cells undergo active Notch signaling and long-lived progenitor cells that form all mature epithelial cell types that have been identified, indicating that Notch signaling is active in stem cells. More specifically, Notch signaling regulates CBCs by enriching the Notch1 receptor and modulating cell type of the stem cells.

WNT signaling also has an important role in the proliferation and expansion of stem cells. The WNT ligand secreted from the intestinal epithelium is required for the repair process. Extracellular WNT ligands binding the FZD (Frizzled) receptor on the WNT ligand-binding membrane initiates an intracellular translocation of the transcriptional coactivator β-catenin, which allows for the expression of target genes in the WNT classical pathway [34]. Studies by FARIN [35] have shown that intestinal epithelium is a redundant resource for WNT and is not essential for the stabilization of $Lgr5^+$ crypt base columnar cells. In contrast, a study by SATO [36] showed that WNT secreted by the intestinal epithelium is sufficient for the proliferation of stem cells. There are two kinds of WNT secretion, one secreted by the epithelium, and the other secreted by mesenchymal cells. Studies have shown that mesenchymal secretion is the main mechanism for regulating the proliferation response of the stem cell niche. It has been shown that in the process of virus-induced villous injury, the WNT ligand secreted by the epithelium is required for the expansion of $Lgr5^+$ stem cells [37]. Therefore, although epithelial WNTs are thought to have little effect in maintaining epithelium homeostasis, they are important component for the epithelial repair process [38].

BMP (bone morphogenetic proteins) is a member of TGF-β (transforming growth factor β) that binds to the serine/threonine kinase receptor and is regulated by Smad dependent or independent of signaling pathways. BMP signaling has the ability to regulate cell prolifera-

tion, differentiation, apoptosis and dysregulation in various diseases. Therefore, BMP signaling plays an important role in embryonic development and adult stem cell homeostasis [39]. BMP is a secreted protein that binds to BMPRs (BMP receptors) to form homodimers or heterodimers. The BMP signaling pathway in the gut plays a critical role in ISCs proliferation. However, it also requires the final differentiation of intestinal epithelial cells. Gremlin1, 2 and Noggin are specific antagonists in BMP signaling. A powerful BMP signaling pathway also drives the differentiation of cells into secretory progenitor cells. BMP and its antagonists have been shown to have an effect on the activation and proliferation of the +4 label-retained cells [40]. If BMP inhibits the WNT signaling pathway, it will lead to cell differentiation rather than proliferation.

BMP signaling pathways are important in mammals. BMP receptors are present in mesenchymal and epithelial cells of the intestine, suggesting that the signaling between mesenchymal and epithelial cells are bidirectional. As mentioned above, BMP antagonists regulate BMP activity. These antagonists include Noggin and Gremlin 1, which are mainly found in mesenchymal cells of surrounding crypts, and their abnormal expression may cause colonic tumors [41].

6 The role of stem cells and Paneth cells in diseases

The ability of the intestine to regenerate is important for intestinal homeostasis. The constant replacement of intestinal epithelial cells also ensures the integrity of the intestinal barrier. Once the intestinal barrier is infested by pathogens or stimulation, it will cause various diseases and affect human health.

6.1 IBD

IBD (inflammatory bowel disease) is known for continuous mucosal damage, epithelial structure reconstruction and repair. IBD has two phenotypes: CD (Crohn's disease) and UC (ulcerative colitis). CD is characterized by the formation of full-thickness inflammation and granuloma. With UC, on the other hand, the inflammation occurs on the mucosa of the colon and rectum. The main cause of IBD is still controversial, however, the common perception is an abnormal response of mucosal immunity to microbes. Numerous studies have shown that the use of antibiotics can reduce inflammation in UC, indicating the important role of intestinal microbes in IBD [42]. Genetic susceptibility may induce the occurrence of IBD, resulting in abnormal regulation of the mucosal immune response and reducing the diversity of the flora compared to that of healthy individuals [43].

MSCs (mesenchymal stem cells) have become a research hotspot to cure IBD. MSCs were named by FRIEDENSTEIN [44] and famous for their regulation of immunity. Mucosal disorders are the cause of IBD, thus, regulating the immune response through inhibition of immune cells such as B cells and T-cells by MSCs could be a new aspect for treating IBD [45].

Paneth cells secrete several antimicrobial peptides that playing a vital role in defense against IBD. Once the epithelium undergoes serious threat, further protective properties are intestinal mucus and AMPs. The intestinal mucus is the first barrier for luminal bacteria. The intestinal mucus layer provides the intestinal epithelium three major factors: (1) hydration, (2) a physical barrier against luminal toxins, and (3) AMPs and IgA (immunoglobulin A). Goblet cells are found in the small and large intestines and produce protective intestinal mucus [46]. The intestinal mucus layer is divided into many layers that contain co-existing enterobacteria in a healthy colon [47]. Defensins and cathelicidins are two main barrier components in protecting intestine [48]. Defensins are antibiotics of the innate immune system that bind to phospholipids on the membrane and create pores that connect to the membrane of the bacterial surface, causing damage to the bilayer bacterial membrane [49]. They can be stimulated by Toll-like receptors and intracellular sensors, such as NLRs (NOD2 and NOD-like receptors), during pathological microbial invasion. Conversely, due to lack of defensins, NOD2 mutation may increase gene susceptibility to CD. Some studies on human tissue and animal models have shown that levels of α-defensins in Paneth cells are reduced,

thus decreasing antibacterial activity, which is considered to be a key cause of ileal CD [50]. Defensins have been divided into two types, α-defensins and β-defensins, which have broad antibacterial properties against gram-positive and gram-negative bacteria and yeast. The most common α-defensins (HD-5 and HD-6) are synthesized primarily by Paneth cells. In the colon, Paneth cells are usually absent, while in IBD, Paneth cells can be clearly found in the colon. In these cases, α-defensins can be found in the large intestine [51]. In addition, mouse studies have shown that active α-defensins are secreted into the small intestine and passed into the lumen of the colon. B-defensin can be found in intestinal epithelium among different epithelial tissues. hBD-1 (human β-defensin 1) exists in healthy colon tissue, while hBD-2, -3 and -4 are related to intestinal lumen responses [52]. Another important AMPs is lysozyme, which is produced primarily by Paneth cells. The function of lysozyme is to catalyze the hydrolysis of β (1,4)-glycosidic linkages between N-acetylmuramic acid and *N*-acetylglucosamine in the polysaccharide component in order to combat gram-positive bacteria.

Paneth cells, also play an important role in maintaining homeostasis by producing antibacterial substances and maintaining a host symbiotic bacterial balance. The latest data represent disorders of the intestinal mucosal barrier, especially Paneth cells, which are involved in the initiation and continuation of IBD [53]. Autophagy, a type of cellular stress response, encompasses a variety of physiological processes such as secretion of proteins, production of antimicrobial peptides and degradation of abnormal organs or proteins. In recent years, the role of autophagy in IBD caused by pathological conditions has been well studied. A recent study by WANG [54] showed that Paneth cells autophagy in IBD is similar to many autophagy-related protein mutations in Paneth cells.

In situation of multiple stresses, autophagy begins to cause a self-protective response that plays an important role in physiological processes, such as production of secreted antimicrobial peptides and degradation of abnormal organs. It also relieves over-activated inflammation and self-invasive responses. Abnormal function of autophagy is considered a major cause of IBD and may be related to the reduction of microbial death and the secretion of antibacterial substances. Based on the above evidence, an increasing number of researchers are beginning to focus on new therapeutic strategies for inflammation of autophagy and immune-related diseases.

6.2 Diarrhea

Diarrhea is a common gastrointestinal disease that affects the health of the human body. Research reports that changes in intestinal barrier permeability was one of the causes of diarrhea. The intestinal barrier, as the key to maintaining intestinal health, protects epithelial cells from damage, against the infection of foreign pathogens, and enables intestinal epithelial cells to undergo normal differentiation. In the previous section, we mentioned that intestinal epithelial cells are proliferated and differentiated from stem cells at the bottom of the crypt, but the role of ISCs in diarrhea is not well understood. Interestingly, THIAGARAJAH [55] found a decrease in the number of intestinal epithelial cells in diarrhea, a decrease in the ratio of villus crypts, and an increase in the incidence of intestinal pathogens. At the same time, YE et al[56] found that the number of goblet cells and Paneth cells in the chronic diarrhea of infants and young children were reduced, and the secretion of mucous membranes was increased, which increased the susceptibility of pathogens. Based on the above experimental results, it can be concluded that ISCs, epithelial cells and the loss of their growth factors can cause intestinal disorders and chronic diarrhea. In chronic diarrhea and congenital diarrhea, Paneth cells and goblet cells also fail to exert normal functions to secrete antimicrobial peptides and mucus, increasing the susceptibility of intestinal pathogens and reducing the occurrence of serious diseases caused by intestinal immune function.

7 Colorectal cancer

As described above, inflammation is hallmark of cancer, inflammation responses are common in tumorigenesis. Tumor cells are very smart by using unique

mechanism to resist therapy. In recently, several studies using Lgr5 as marker for colon CSCs (cancer stem cells). Lgr5 is a known target of WNT signaling and plays an important role in CRC (colorectal cancer) development. WANG [57] showed that high Lgr5 expression level was significantly correlated with the occurrence of metastasis. JANG [58] showed that knockdown of Lgr5 resulted in a decline in the colony-forming and migration capacities in LoVo colorectal cancer cells.

In colorectal cancer, various levels of WNT signaling exist. Aberrant activation of this pathway is associated with cell proliferation, invasive behaviors, and cell resistance, suggesting its potential value as a therapeutic target in treatment of CRC. OHASHI [59] found that SKL2001, identified as an activator for WNT signaling by disrupting the Axin/β-Catenin complex, negatively regulated growth of colon cancer spheroids cultured in the 3D condition that simulates tumor microenvironment *in vivo*.

8 Conclusion

In general, the intestine has an intricate crypt-villus structure which plays an important role in the intestinal epithelial regeneration process. Stem cells located at the bottom of the crypt maintain intestinal homeostasis by proliferating and differentiating to different intestinal epithelial cells. Paneth cells alternately arranged with ISCs protect the stem cell niche by expressing factors such as EGF, TGFα, and WNT3. At the same time, Paneth cells ensure the normal function of stem cells by enhancing mitochondrial oxidative phosphorylation. In this review, we also describe how stem cell-associated signaling pathways Notch, WNT, and BMP regulate stem cell proliferation and differentiation. Paneth cells and stem cell-related signaling pathways jointly protect ISCs and prevent the occurrence of IBD, colon cancer and pancreatic cancer.

The ability of the intestinal epithelial cells to self-regenerate highlights the importance of stem cells in our body. Although the strong regenerative capacity of the intestine has been recognized for a long time, the exact mechanism of this adaptive response has only begun to be elucidated, and more research is needed in the future to reveal its molecular drivers. So, when the ISCs are missing, in what way does the reserve cells perceive this deficiency? In the previous section, we mentioned that Paneth cells regulated the microenvironment of ISCs through signaling factors, this concept gives us an inspiration to treat colon cancer by regulating the microenvironment associated with cancer. The signaling pathways involved in ISCs and the function of the intestinal barrier have been well studied, but how the intestinal microbiota and its metabolites, which are closely related to the intestinal barrier, affect the integrity of the intestinal barrier remains unclear. We hope that in the future, organoids can be used to study the effects of intestinal microbiota and metabolites on stem cell regeneration, proliferation and differentiation.

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