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Mini Review

Paneth cells and stem cells in the intestinal stem cell niche and their association with inflammatory bowel disease

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The intestinal epithelial cells are replaced in every three to four days. This system is maintained by self-reproduction, differentiation of the intestinal stem cells. The continuous monolayer of intestinal epithelial cells functions in innate immunity as the primary barrier against microbial colonization. Paneth cells secrete α-defensins, and they are actively involved in the innate immunity and maintain the intestinal homeostasis by controlling intestinal microbiota. In addition, recent analyses of epithelial renewal in the intestine illustrate that Paneth cells provide survival signals to crypt intestinal stem cells. Two types of intestinal stem cells, crypt base columnar (CBC) stem cell and +4 stem cell, have been identified. Lgr5 positive CBC stem cells, Bmi1 positive +4 stem cells and Paneth cells create the stem cell niche in the small intestine. In this review, recent advances in understanding the roles of Paneth cells and stem cells which involve stem cell niche, and their association of inflammatory bowel disease were discussed and summarized.

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Introduction

Paneth cells which reside at the base of the small intestinal crypt of Lieberkuhn produce antimicrobial peptides and other bactericidal molecules and play a crucial role in the intestinal innate immunity[1-6]. It has been widely recognized that Paneth cells are the guardians of the crypt stem cells by secreting microbicidal α-defensins as well as by their physical location residing beside the stem cells[1]. Recently, in addition to killing pathogens in the intestine, Paneth cells have been known to play a key role in maintaining homeostasis by controlling composition of the commensal microbiota in the small intestine[7, 8]. Molecular mechanisms on regeneration and differentiation of the intestinal epithelial cells have been vigorously studied and several lines of

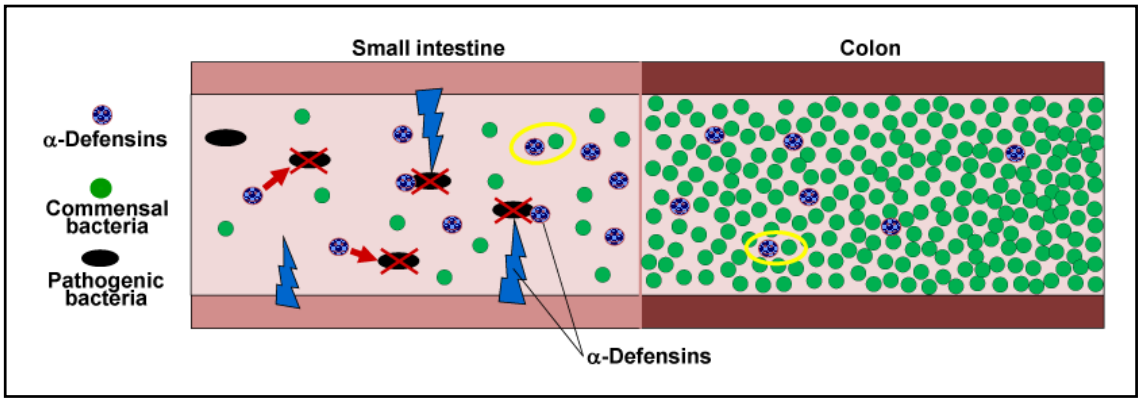

Fig.1 Paneth cell α-defensins controlling intestinal microbiota
A mouse α-defensin, cryptdin-4 elicits potent microbicidal activities against pathogenic bacteria whereas shows no or weak killing activities against certain commensal bacteria. Paneth cell α-defensins contribute to maintain the intestinal homeostasis by the selective bactericidal activities.

important progress have been revealed. The crypt stem cells which produce all the small intestinal epithelial cells are closely in contact with Paneth cells, and these cells appeared to create the stem cell niche[6]. Paneth cells elicit a critical role in regeneration and differentiation of the small intestinal epithelial cells. Abnormalities that occur in the stem cell niche as a result of Paneth cell dysbiosis due to adverse stimuli such as ER stress or α-defensin disruption are associated with inflammatory bowel disease. In this review, we summarize recent progress in the intestinal regeneration and further discuss relations to inflammatory bowel disease, especially Crohn's disease.

Paneth cells in innate immunity and in intestinal homeostasis

The intestinal epithelium is the largest surface exposed to the various microbes, and mucosal immunity plays a crucial role in host defense in the intestine. Paneth cells, one of four major epithelial cell lineages in the small intestine, reside at the base of the crypts and have apically-oriented secretory granules[9]. Paneth cell granules contain high levels of antimicrobial peptides of the α-defensin family and additional host defense molecules. For example, the secretory granules contain a variety of antimicrobial agents such as α-defensins, lysozyme, secretory phospholipase A_2, CRS-1C, CRS-4C, RegIIIγ and angiogenin[10]. Among them, α-defensins, termed cryptdins in mouse and HD5 and HD6 in humans, are the major constituents and vital effectors for the host defense. In mice, six cryptdin isoforms, cryptdin-1 to cryptdin-6 are the microbicidal constituents of granules of Paneth cells. Paneth cells secrete microbicidal granules containing activated α-defensins when exposed *ex vivo* to bacteria or their antigens. Thus, Paneth cells contribute to the mucosal immunity by sensing microbes and releasing microbicidal activities mostly in the form of activated α-defensins at effective concentrations[1].

In the intestinal lumen, huge numbers of commensal bacteria colonize and elicit beneficial effects in the host. Masuda et al.[8] reported that native cryptdin-4 showed only minimal or no bactericidal activity against 8 out of 12 commensal bacterial species, including *Bifidobacterium bifidum* and *Lactobacillus casei*, whereas folded cryptdin-4 elicited potent bactericidal activities against all 11 of the non-commensal bacterial species tested, such as *Salmonella* enterica serovar Typhimurium. Furthermore, reduced cryptdin-4 demonstrated significantly greater bactericidal activities against 7 of 12 commensal bacteria than did oxidized cryptdin-4. These findings suggested that cryptdins have selective bactericidal activities against intestinal microbiota and that the activities are dependent on the disulfide bonds. A recent study of ileal microbiota in MMP-7-deficient mice that lacked active forms of cryptdins in the small bowel lumen showed that a significantly higher percentage of *Firmicutes* and a significantly lower percentage of *Bacteroides* were detected in the small bowel of MMP-7-deficient mice compared to wild type mice[7]. The total bacterial numbers in both types of mouse were not changed, so that it appeared that cryptdins regulate the composition of the intestinal bacteria. This is consistent with the results

that demonstrate the selective bactericidal activity of cryptdins *in vitro*[8]. These findings provide evidence that Paneth cells are important for intestinal homeostasis via α-defensin secretion (Fig.1).

Small intestinal epithelia are known as most rapidly migrating cells in adult tissues having three to four days of cell transit from the crypt base to the villus tip. Early pioneering studies revealed that the epithelial cells leave the crypt base, migrate onto villi and finally exfoliate into the intestinal lumen[11-14]. Cell kinetic studies determined a function of cellular position within the crypt structure. A concept of stem cell niche, the microenvironment that maintains intestinal stem cells, was first described by cell marking experiments demonstrating that differentiated cells have to leave stem cell zone prior to differentiation. Paneth cells are the only differentiated cells in the intestine which migrate downward from the proliferative cell zone, and localize at the crypts base interspersed within the stem cell zone. Paneth cells are long-lived compared to the other epithelial lineages in the intestine, and they play key roles in innate immunity and maintaining the intestinal homeostasis. Paneth cell produce many factors which regulate cell proliferation, differentiation, metabolism, and inflammation, in addition to α-defensins. Therefore, much attention has been focused on the Paneth cells and their relation to the stem cell niche. A lack of both quality stem cell markers and adequate culture systems for stem cells in the intestine had been major limiting factors in the stem cell biology in small intestine and colon. However, recent advances revealed that Paneth cells create stem cell niche by providing surviving signals to the intestinal stem cells[15]. This progress came from studies that solved both aspects that had limited intestinal stem cell research, the markers and the culture systems.

Two types of stem cells in the intestine

Tissue stem cells move to restricted sites where thay remain during ontogeny. The gastrointestinal tract is composed of three germ layers during ontogeny, and the four lineages, columnar cells, goblet cells, enteroendocrine cells and Paneth cells of the small intestinal epithelium are derived from intestinal stem cells. The small intestinal epithelial cells are replaced in every three or five days. This system is maintained by self-reproduction, differentiation of the intestinal stem cells. In addition, certain cells derived from bone marrow have been reported to differentiate into intestinal epithelium in certain conditions[16]. The stem cells and the microenvironment necessary for stem cell function, in other words, a stem cell niche, work together to insure the constant supply of functional, differentiated cells.

Two types of stem cells with different characteristics and different markers, the crypt base columnar (CBC) stem cell and the +4 stem cell have been identified recently. The CBC stem cell located between Paneth cells at the base of mouse small intestinal crypts is rapid in cell division, and expresses leucine-rich-repeat-containing G-protein-coupled receptor 5 (Lgr5) gene as well as Lgr4 gene as the marker[17]. On the other hand, the +4 stem cell which is located above the Paneth cell and corresponds to the cell of approximately fourth cell from the bottom of the crypt is slower in its rate of cell division, and +4 stem cells also express the Bmi1 gene, homeodomain only protein x (Hopx) gene and mTert gene[18, 19]. These two stem cell populations have been shown to supply all lineages of intestinal epithelial cells by recent studies. Such capability for Lgr5 positive cells in the base of crypts to generate entire crypt-villus structures was revealed by in organoid culture experiments. Recent studies using targeted lineage tracing technique revealed that the CBC stem cell located between Paneth cells is the intestinal stem cell. Sato et al. reported a close relationship between Lgr5 positive cells and Paneth cells by conducting organoid culture studies as well as other *in vivo* studies[15, 20]. They showed that Paneth cells express all factors essential to maintain stem cells in culture, and the organoid formation is dramatically enhanced when a single Lgr5 positive stem cell is co-cultured with individual Paneth cells in organoid culture, relative to growth without the Paneth cell. Moreover, the genetic deletion of Paneth cells resulted in the loss of Lgr5 stem cells. These findings suggest that Paneth cells are indispensable for intestinal stem cell self-renewal and differentiation of all intestinal epithelial cell lineages. It was also reported that Lgr5 positive stem cells have a close physical association with Paneth cells which express all essential signals, EGF, TGF-α, Wnt3 and Dll4, for stem cell maintenance in culture. Lgr5 positive stem cells and Paneth cells create the stem cell niche in the small intestine. Importantly, Paneth cells provide surviving signals to the stem cell, so if Paneth cell dysbiosis is induced such as under conditions of ER stress, these cells no longer support stem cells. (Fig.2)

Wnt signaling and Notch signaling are the two major pathways associated with the intestinal homeostasis[21-26].

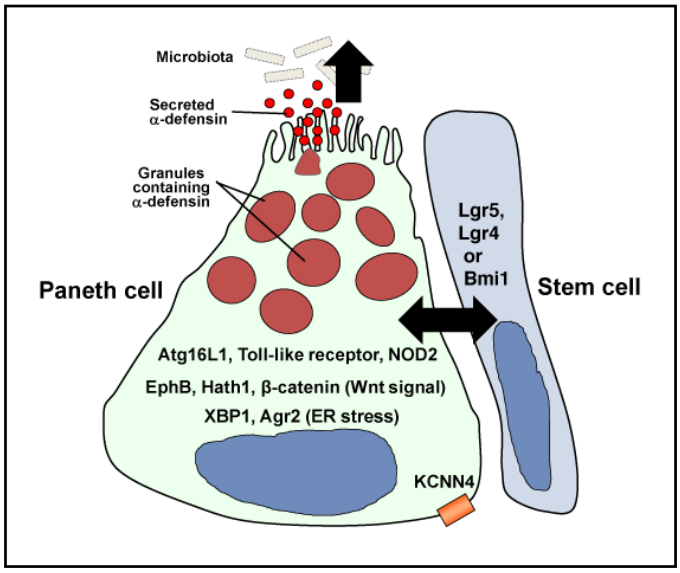

Fig.2 Paneth cell and stem cell in the small intestine

Paneth cells produce key molecules in the innate immunity called α-defensins and contribute to the intestinal homeostasis. Paneth cells also express IBD related genes such as NOD2, Atg16L1, XBP1 and KCNN4. Two types of stem cells, CBC stem cell expressing Lgr5 and +4 stem cell expressing Bmi1, contact with Paneth cells and create the stem cell niche which is important for intestinal regeneration.

Among four differentiated epithelial cell lineages in the intestine, only Paneth cells express β-catenin. It was reported that Paneth cell differentiation is reduced by the inhibition of β-catenin activity[27]. When Lgr4, a potent stimulator of Wnt signaling, is deleted, the morphology of Paneth cells was severely impaired[28]. Finally, Paneth cells produce ligands for Wnt signaling, Notch signaling, and EGF receptors. Thus, Paneth cells both regulate intestinal stem cells and their niche and contribute to normal epithelial cell differentiation via Wnt signals. Wnt signals associate with various cell functions throughout development and in adult, such as maintenance of homeostasis in varieties of tissues including the intestine and bone marrow. Especially, Wnt signals play critical roles in the stem cell biology. Wnts signal via β-catenin. In addition, the role of Wnt signaling participates in carcinogenesis has become more clear, and the possibility of therapeutics such as the use of Frizzled-related proteins (sfrp1-5) to inhibit Wnt signaling selectively have been considered. Moreover, the nuclear hormone receptor PPARβ inhibits Paneth cell differentiation via Wnt signals. Taken together, the participation of Wnt signals in Paneth cell differentiation and the stem cell niche became clear recently. Notch signals also have a crucial role in regulating differentiation of intestinal epithelial cells and also play a role in maintenance of the intestinal stem cells[25, 26]. Outcomes of Notch signals depend on other signaling pathways within the stem cell niche such as Wnt and Hedgehog signaling. It has been known that the enhancement of Notch signal inhibits differentiation of secretory cells, whereas loss of Notch signal elicits enrichment of secretory cell numbers in the intestinal crypts. Zheng et al. reported that a loss of Hes-1 which is a transcription factor down-stream of Notch led to the increase of secretory cells in mice[29]. Notch signaling also regulates intestinal stem cells.

The relation of Lgr5 stem cells to the +4 stem cell population was shown by a cell-lineage chase study, which showed they could convert each other. In mice whose Lgr5 positive cells were specifically deleted by selective expression of diphtheria toxin, villus-crypt structure was almost normal, and the CBC stem cells were replaced by cells derived from the +4 stem cell, which replaced cells that expressed the Lgr5 gene[30]. Lgr5 expression is found in the bottom of crypts not only in the small intestine but also in the colon. Isolation of human colonic stem cells was reported by Jung et al. They found that the highest ephrin type-B receptor 2 (EphB2) surface levels correspond to colonic epithelial stem cells and these cells have multipotent differentiation capacity in terms of the ability to expand colonic epithelial lineages *in vitro*[31]. Knowing underlying mechanisms of the stem cell niche will reveal many important fundamental questions such as the difference of small intestine and the colon in health and diseases.

Disruption of homeostasis in the stem cell niche relating inflammatory bowel disease

Inflammatory bowel diseases (IBD), Crohn's disease

(CD) and ulcerative colitis (UC), are intractable chronic inflammatory diseases of the gastrointestinal tract, and patients with IBD have been continuously increasing. Although the etiology of inflammatory bowel disease (IBD) is yet to be clarified fully, the certain genetic involvement and the association with altered intestinal microbiota due to a failure of the immune system were considered to play a central role[32].

By the recent genome-wide association studies worldwide, many susceptibility genes to CD and UC have been identified[33]. These include innate pattern recognition molecules such as NOD2/CARD15 and CARD9, autophagy such as ATG16L1 and IRGM, ER stress such as XBP1 and AGR2, and Th17 reaction such as IL23R and STAT3. In addition, the expression of the intestinal antimicrobial peptide, α-defensin, which plays a pivotal role in the regulation of the intestinal microbiota was reported to associate with the progression of enteritis in a CD model mouse, IL-10 null mouse as well as CD patients[34-36]. Furthermore, it was reported that the proform of a human Paneth cell α-defensin, HD5, was reduced in some patients with CD[37]. The reduced pro-HD5 was degraded by trypsin, a processing enzyme of HD5 *in vivo* and the bactericidal activity was diminished. Links to innate immunity is extremely important in IBD, especially in CD. Paneth cells as well as phagocytic leukocytes carry mutated NOD2 in certain CD patients in western populations, and that causes a failure of innate immunity in intestinal homeostasis[38, 39]. Another important example is the autophagy related molecule. Autophagy is one of the major intracellular degradation systems. It serves not only elimination of pathogens, but also metabolism, regeneration and differentiation. Autophagy is known to contribute to the pathogenesis of many diseases including IBD, especially CD. A role for autophagy in the pathology of CD was reported. A single-nucleotide polymorphism (SNP) of Atg16L1, an autophagy-related gene, has been identified in patients with CD and is considered as the disease-sensitive gene[40]. ATG16L1-deficient Paneth cells show significant failure in the granule formation and secretion and the genetically modified mice got severe ileitis. CD patients with the mutation of ATG16L1 gene have abnormalities in Paneth cell granules similar to those seen in ATG16L1-deficient mice[41]. These findings strongly suggest that impaired autophagy increases a risk of CD by defective defense against intracellular bacteria as well as impaired Paneth cell granule constituents such as α-defensins and regulation of their secretions related with a potassium channel, KCNN4[42, 43]. Several transcription factors have been known to play critical roles in the villus-crypt epithelial cell structure. It was reported that *Hath1* gene expression was suppressed in the undifferentiated state of human intestinal epithelial cells, and the suppression of Hath1 is associated with goblet cell depletion in UC[29]. A transcription factor named X-box binding protein 1, XBP1 plays a critical role in ER stress and in mediating unfolded protein responses. XBP1 deletion in intestinal epithelial cells leads to spontaneous enteritis with associated Paneth cell abnormalities. Furthermore, XBP1 is considered as susceptibility gene related to both CD and UC[44, 45]. SNPs inducting ER stress also disrupts intestinal homeostasis by targeting Paneth cells and associates with IBD. Genetic defects in the protein disulfide isomerase, Agr2 that works in protein folding and the unfolded protein response cause Paneth cell defects in number and the morphology, and these abnormalities associate with chronic ileitis and colitis[46].

In many tissues, chronic inflammation causes fibrosis, and the fibrosis disturbs regeneration and differentiation in the tissues. Intestinal stenosis often becomes a big problem in patients with CD. Many humoral factors including HGF, EGF, FGF, TGF-β may be involved in such processes. In the process of the wound healing, many signal transduction systems are activated and cell proliferation occurs. However, excessive cell proliferation in the wound healing process often causes chronic inflammation and fibrosis as a result. Because wound healing often acts as a double-edged sword for regeneration and differentiation in the intestine, discovering the entire intestinal regeneration process will likely provide great contributions to many patients with IBD.

The homeostasis of stem cell niche which is created by the Paneth cell and the stem cell is disrupted in IBD. Paneth cell failure due to various ER stresses or others directly affects stem cells. Such disrupted Paneth cells can no longer create a normal stem cell niche to support the stem cells, resulting serious stem cell failure. As described in this review, Paneth cells maintain intestinal homeostasis by controlling intestinal microbiota via secretion of α-defensins and also by creating the stem cell niche. This recent evidence strongly suggests further roles for Paneth cells, intestinal stem cells and the stem cell niche in health and diseases. However, the precise details of cross talk among the two different

intestinal stem cells and Paneth cells[47] remain unknown as do the molecular determinants that regulate direct contact of stem cells and Paneth cells.

There are many difficulties in the use human embryonic stem (ES) cells for clinical applications. An induced pluripotent stem (iPS) cell appeared as a new technique to enable regenerative medicine without having serious ethical problems that are unavoidable in the ES cell study. However, even in bone marrow transplantation, perspectives of the molecular mechanisms of reproduction and differentiation have not yet become totally clear. To provide fundamental therapeutic modalities in regenerative medicine to patients suffering from intractable gastrointestinal diseases, including IBD, deeper understanding of the molecular mechanisms that regulate the intestinal stem cell system are necessary.

Conclusion

Recent rapid progresses on Paneth cells, intestinal stem cells and the stem cell niche influence have had great impact on the entire field of gastroenterology, including IBD. Important findings in both innate immunity and regenerative medicine allow us to focus on Paneth cell biology and their stem cell interactions. Mechanisms regulating the "stem/Paneth" niche remain to be determined.

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