Special Issue "Epithelial regeneration in inflammatory diseases"

Mini Review

Epithelial regeneration in inflammatory bowel diseases

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Inflammatory bowel diseases (IBD), including ulcerative colitis and Crohn's disease, are characterized not only by the sub-mucosal accumulation of inflammatory cells, but also by the severe damage of the epithelial layer. Recent clinical studies have featured "mucosal healing" as the most significant prognostic factor for long-term remission in IBD patients, suggesting that accomplishment of epithelial regeneration is critically required to improve the treatment for IBD. From series of recent studies, we now know that several key molecular pathways dominantly regulate not only the homeostasis, but also the repair process of the intestinal epithelium. Future studies may allow us to facilitate regeneration of the damaged intestinal epithelia, either by molecular-based manipulation of endogenous regenerative signals, or by a tissue-engineering based strategy utilizing ex-vivo expansion of primary intestinal epithelial cells.

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Introduction

Inflammatory bowel disease (IBD) is a gastrointestinal disease featured by spontaneous inflammation of the intestinal mucosa. Although its precise pathophysiology still remains to be elucidated, studies have shown that IBD develops upon co-existence of several risk actors, including genetic susceptibility, changes in luminal bacterial flora, environmental triggers including foods, and impaired regulation of the immune response [1-5]. Thus, major interest of research upon IBD has been highlighted to the immunological mechanism of the disease. Success of such immunological studies has provided great advance in therapy of IBD, which has dramatically shifted to antibody-based targeted therapy, against molecules that are responsible for the persistence of intestinal inflammation. Among such molecular-targeted therapy, anti-TNF-α therapy has been widely used both in Japan and in western countries, and proved outstanding therapeutic effect upon otherwise refractory patients[6].

However, the great success of anti-TNF-α therapy has told us that treatment of IBD should consider both immune and epithelial cells. In active phase of the disease, the epithelial layer is damaged by the inflammatory environment, which results in development of multiple refractory ulcers. Despite good control of inflammation can be achieved by current immuno-modulating therapies, including anti-TNF-α therapy, we occasionally experience refractory ulcers that show poor response of regeneration. Also, in a much long term, patients of IBD have significantly high risk of developing colorectal cancer, which is referred to as colitis-associated cancer[7]. These findings strongly suggest that epithelial cells play a certain and un-ignorable role in pathophysiology of IBD. Thus, along with sub-mucosal inflammation, it is clear that damage and repair of the epithelial tissue is another important aspect of IBD.

Clinical importance of "mucosal healing"

Based on the idea that IBD is initiated and maintained by spontaneous development of mucosal inflammation, current therapies for IBD are designed to manage and take control of the immune response within the intestinal mucosa. Along with corticosteroids and immune-modulators, anti-TNF-α therapy has become one of highly effective and established treatment, to control persisting inflammation of the intestinal mucosa. However, as a result of powerful immune-modulating therapy, subsequent achievement of epithelial repair following reduction of sub-mucosal inflammation has acquired high interest, as several cohort studies have suggested that long-term remission of IBD is determined by "mucosal healing" [8),9)].

Mucosal healing has been defined as "complete repair of the epithelial layer, at both endoscopic and microscopic level" [9)]. The importance of "mucosal healing" was first suggested by a cohort study in Norway, showing that mucosal healing serve as the most significant prognostic factor for maintaining long term remission and low risk of surgical treatment in Crohn's disease patients[10)]. Following such study, series of studies showed that "mucosal healing" serve as a common prognostic factor for long-term remission, among various IBDs[8),9)]. Thus, current goal of IBD therapy has been set to "mucosal healing", instead of improvement in clinical symptoms[8),9)].

Although we now know that epithelial repair is important for IBD treatment, so far we have no therapeutic procedure for IBD patients suffering from refractory ulcers, which is specifically directed to promotion of epithelial tissue regeneration. Thus, an established and effective approach to promote epithelial repair in IBD patients needs to be developed.

Molecular mechanism supporting maintenance and repair of the intestinal epithelium

The intestinal epithelium is renewed every 2-3days throughout human lifetime[11), 12)]. Such a rapid turnover of the entire tissue is supported by the "intestinal crypt", which includes intestinal stem cells, serving as origin of all the intestinal epithelial cells. Within the crypt, position of cells is strictly determined upon degree of differentiation; stem cells reside at the lowest part, whereas progenitor cells reside at the upper part of the crypt, respectively (Fig. 1). Under normal condition, number of functional epithelial cells is maintained by the balance between shedding of old cells from the tip of the villi, and generation of newborn cells from the crypt by proliferation of progenitor cells. Newborn cells migrate from the crypt along the crypt-villus axis, and acquire specific functions as they differentiate into one of the intestinal epithelial cell lineages (Fig. 2).

Once such balance is disrupted by sudden injury

of the epithelial layer, the repair program will be carried out sequentially over time (Fig. 3)[13), 14)]. The most acute and rapid response is called restitution, which is mediated by epithelial cells lining the margin of the damaged epithelial area (ulcer). Upon ulcer formation, these cells gain function to migrate and re-distribute to cover the damaged area, so as to restore the integrity of the epithelial layer as soon as possible. By such re-distribution and re-shaping of epithelial cells, the epithelial tissue makes it possible to reconstitute the barrier between the intestinal lumen and the submucosa without waiting for the increase of epithelial cell number.

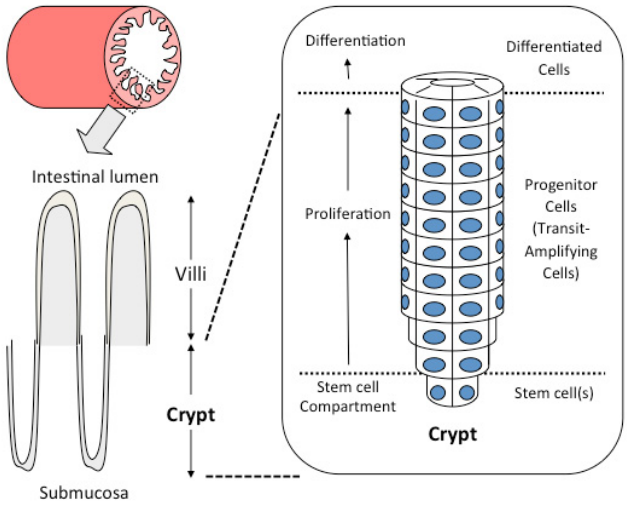

Fig. 1 Hierarchical structure of the intestinal crypt.

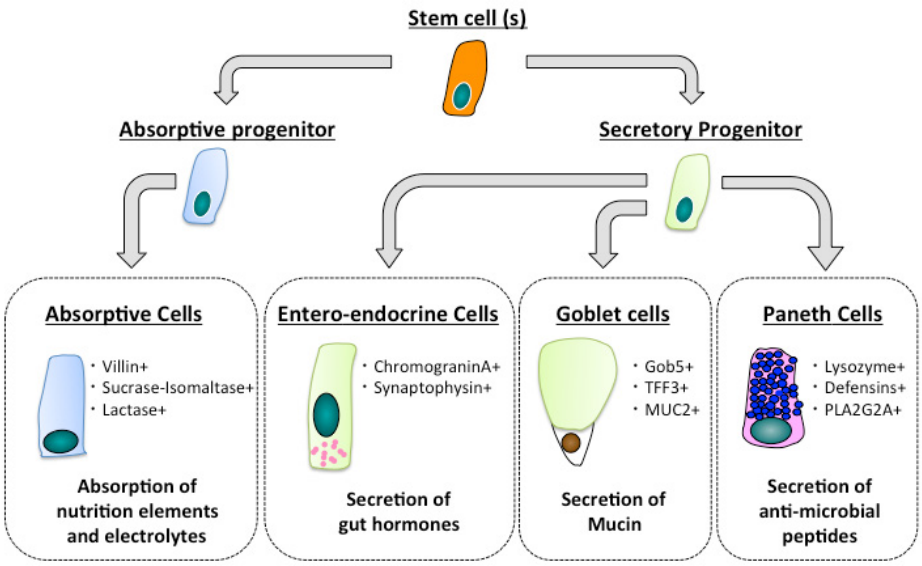

Fig. 2 Development of differentiated intestinal epithelial cells.

However, in a later phase, the depth of the remaining crypt becomes elongated, which is due to the increase in rapidly proliferating, progenitor cell population. By such response of the "intestinal crypt", the epithelial tissue restores number of epithelial cells to re-construct its structure. Following such response, crypt itself may go through division, which completes regeneration of the tissue structure. Such a regenerative response needs to be carried out sequentially, and failure in one of the sequential steps may lead to persistence of refractory ulcers.

Several key molecules have been identified to have indispensable role in such regeneration response of intestinal epithelium. Trefoil factors, peptides secreted from ulcer-associated cell lineages (UACL) has been shown to play crucial role in the restitution phase, by promoting migration of the epithelial cells[15), 16)]. Also, TGF-β has been shown to play a major role in regulation of the regeneration program[17)]. However, the molecular mechanism supporting the rapid proliferation of epithelial cells during such regeneration process remains largely unknown.

Our recent study has shown that Notch signaling, a molecular pathway involved in development of various species[18)], plays indispensable role not only in maintenance of normal intestinal epithelium, but also in regeneration of damaged intestinal epithelia [19)]. Notch signaling has been shown as an indispensable molecular pathway for the maintenance of intestinal epithelium[20)], which cooperate and interacts with other molecular pathways such as Wnt and BMP[21)]. In our study, we found that Notch signaling may promote the rapid expansion of epithelial cells following restitution, by maintaining crypt cells at an undifferentiated state[19)]. Our results showed that activation of Notch signaling suppresses acquirement of goblet cell phenotype, the dominant cell phenotype of differentiated colonic epithelial cells. On the other hand, inhibition of Notch signaling by a gamma-secretase inhibitor significantly down-regulated intestinal cell proliferation, both in vitro and in vivo. Moreover, blockade of Notch activation in colitic mice had detrimental effect upon its clinical outcome, suggesting that activation of Notch signaling has indispensable role in recovery from colitis. As we also showed that Notch signaling is activated in increased number of intestinal epithelial cells in IBD patients[22)], activation of Notch signaling may also be required for epithelial repair in IBD.

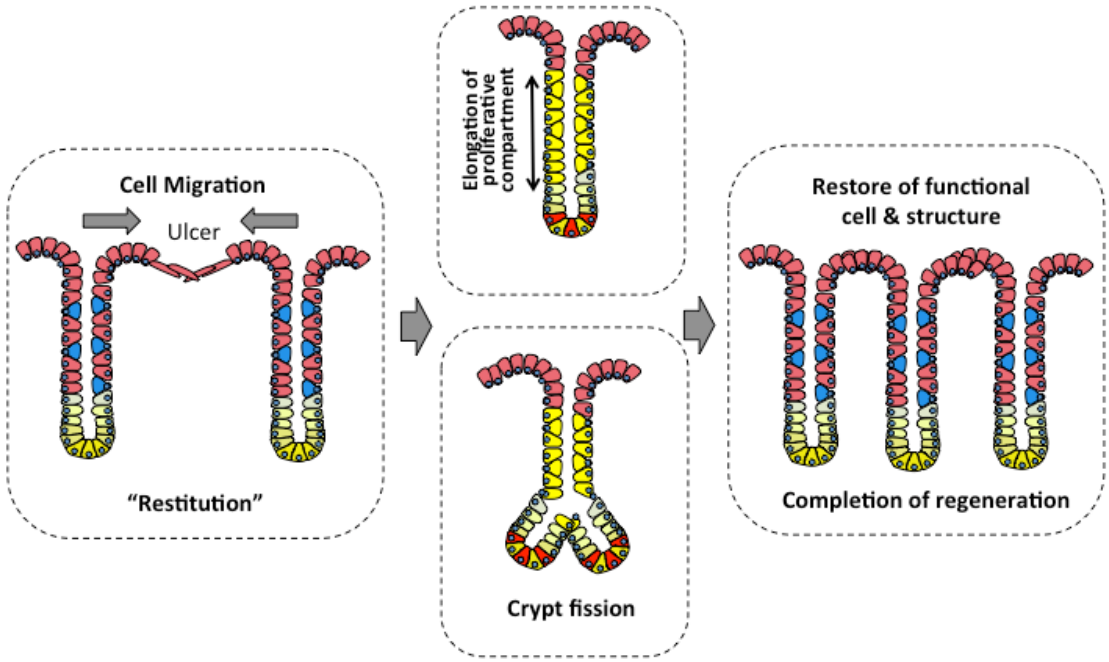

Fig. 3 Sequential steps of regeneration in the intestinal epithelium.

Mucosal environment of the inflamed gastrointestinal mucosa

In IBD, the environment of the epithelial layer dramatically changes between normal and inflamed condition (Fig. 4). Under normal condition, abundant luminal bacterial or environmental antigens are isolated from the sub-mucosa by continuous layer of epithelial cells. In contrast, in inflamed mucosa, such a continuity of the epithelial layer is lost, and consequently allows free access of luminal antigens to the sub-mucosa, until restitution is completed. These luminal antigens subsequently activates pro-inflammatory lymphocytes residing in the sub-mucosa, and promotes local inflammatory response. Epithelial cells remaining at the inflamed area are thus exposed to both luminal antigens and pro-inflammatory cytokines. Studies have suggested that inflammatory signals such as TNF-α, or IFN-γ, may interact and promote or enhance endogenous regeneration signals such as Notch[23)-25)]. However, precise molecular interactions between these two signals remains to be elucidated. Further studies regarding crosstalk between inflammation and regenerative signals may provide new insights to the regenerative response of intestinal epithelial cells.

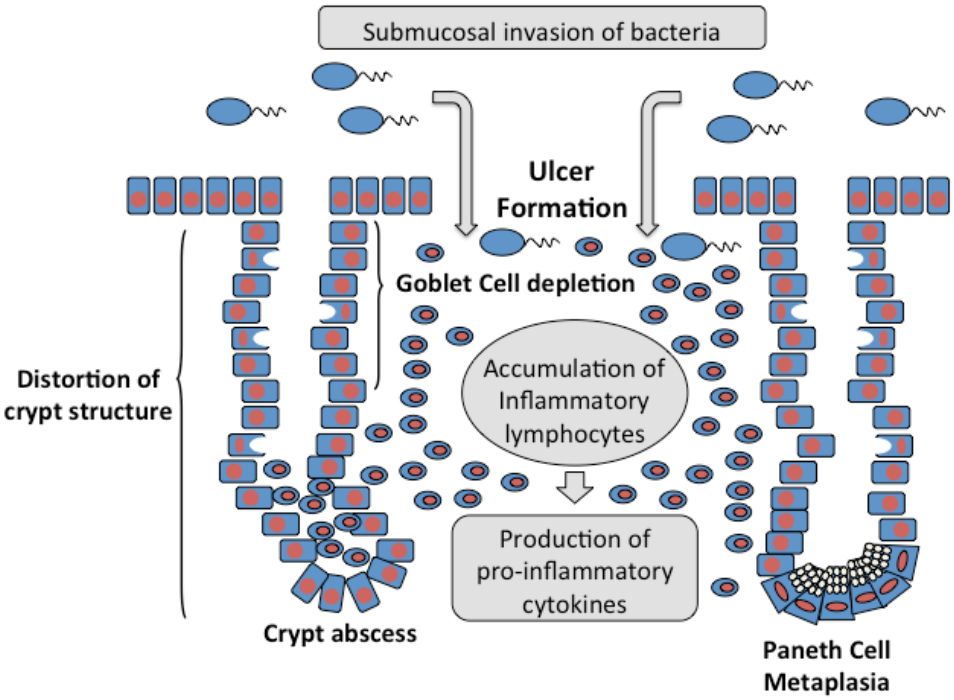

Fig. 4 Pathological features observed in the mucosa of inflammatory bowel disease.

Prospects for advance in therapeutic approach to promote tissue regeneration in inflammatory bowel disease patients.

Based on recent findings, two major approaches can be suggested to promote tissue regeneration in IBD. One is to accelerate the endogenous regeneration program by targeting key molecular pathways supporting its process. Based on such concept, various growth factors have gone through clinical trials so far[26)]. A pilot study using EGF was reported to promote epithelial regeneration in mild-to moderate ulcerative colitis patients[27)]. However, only a limited effect has been reported. From our recent study, manipulation of molecular signals such as Notch may be a good alternative candidate for such approach.

However, in severely damaged mucosa where large number of stem cell is lost, it may be difficult to expect rapid regenerative response from endogenous cell alone. Under such condition, transplantation of epithelial cells that were expanded ex-vivo, might be a much more successful strategy. Fortunately, re-

cent advance in tissue culture technique has finally allowed us to culture and expand primary cells of the intestinal epithelia in vitro. Methods established by Sato et al and Ohtani et al both require serum derived factors[28), 29)], and therefore are not applicable for clinical use in its present style. However, we now know that epithelial cells can surely be maintained and expanded in vitro for over years, and also know the minimal factors required for such culture. Thus, future studies may refine the methods into completely defined, serum free method, and provide basis for ex-vivo tissue engineering for intestinal epithelial repair therapy.

Concluding remarks

Establishment of therapy that can promote tissue regeneration might become a key approach to improve the clinical outcome of IBD, through achievement of "mucosal healing". Further analysis of crosstalk between the inflammatory and the regenerative signals may provide another novel molecular target that may be utilized for promoting epithelial regeneration. Also, improvement of tissue culture techniques may provide another novel approach such as refractory intestinal ulcer treatment through transplantation of ex-vivo expanded cells.

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