Special Issue: Inflammatory Bowel Diseases and Intestinal Epithelial Stem Cells

Brief Review

Inflammatory bowel diseases and intestinal epithelial stem cells

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Recent studies on human inflammatory bowel diseases (IBD) have brought our attention to continued epithelial injury and impaired epithelial regeneration as their key pathophysiological features. In order to develop a novel approach for the treatment of IBD, it is important to understand the ordered process of epithelial repair and the pivotal roles for the epithelial stem cells in this process. Recently, specific molecular markers for the intestinal stem cells have been identified, and this has led to characterization of the unique properties of this population of cells. In addition, advancement in technologies to isolate and culture intestinal stem cells enhances the prospect for the use of these cells as a diagnostic tool or the source of regenerative medicine. Here I review such recent progress in intestinal epithelial stem cell research and its possible application to IBD.

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Introduction

Current therapeutic strategies for treatment of human IBD are mostly aimed at modulating the immune system that is locally or systemically dysregulated in affected individuals. However, as continued epithelial injury and impaired epithelial regeneration are also regarded as key features of pathogenesis of the diseases[1], enhancement of intestinal epithelial repair mechanisms has received a growing attention as alternative approach for the treatment of IBD. Reports have shown that amelioration of persistent inflammation does not always lead to complete mucosal healing, suggesting the strategies for appropriate tissue repair to be needed in addition to the conventional immunotherapy[2, 3]. Along with this trend, our knowledge of intestinal epithelial regeneration has flourished in the last few years. In particular, advancement in intestinal stem cell biology and new technology to grow normal intestinal stem cells *in vitro* is expected to open up a wide array of possibilities to use these cells in clinical practice for IBD.

Mechanisms of intestinal epithelial repair

The homeostasis of intestinal epithelial tissues is maintained through the presence of stem cells residing near the base of crypts[4-6]. The stem cells are able to self-renew throughout life, continuously generating more committed precursor cells called transit-amplifying (TA) cells, which

actively divide within the lower part of the crypt. After several rounds of cell division, TA cells differentiate into one of the mature cell lineages, migrate upward to the top of the vertical axis and are exfoliated into the lumen, whereas Paneth cells in the small intestine migrate down to the crypt base. Once damage to the epithelial lining occurs, intestinal epithelium undergoes an injury-induced repair response to rapidly restore the structural and functional integrity[7, 8]. The efficient repair is accomplished by a process called epithelial restitution, which is then followed by more delayed mechanisms involving epithelial proliferation and differentiation.

Epithelial restitution is a process during which injured regions of the epithelial lining are covered by surrounding epithelial cells. The cells at the edge of the wound migrate over the denuded area, reform cell contacts, and reestablish barrier function, which is known to sequentially occur within minutes to hours[9-11]. The rapid and successful restitution after injury limits fluid and electrolyte losses, and prevents submucosal compartments from being directly exposed to foreign antigens in the intestinal lumen. A number of factors are known to play important roles in the restitution[10, 11]. These factors, or other substances that have potentials to promote epithelial restitution are considered to be attractive candidates for therapeutic options for treatment of IBD[12].

Once the injured areas are compensated for their loss of epithelium by restitution, epithelial cell proliferation and differentiation should follow to replenish the decreased cell pool and to restore proper epithelial functions, respectively. This requires the stem cell-based mechanism that involves several regulatory signals such as the Wnt, bone morphogenic protein (BMP), and Notch pathways. Recently, one of the Wnt target gene, a G protein-coupled receptor Lgr5, was shown to be a specific intestinal stem cell marker uniquely expressed at the base of the small intestinal and colonic crypts[13]. Further, lineage tracing experiments provided strong evidence that the Lgr5+ cells represent long-lived multipotent stem cells. Other intestinal stem cell markers have also been proposed, including Bmi1[14, 15] and Hopx[16], although the relationship between the cells positive for these different molecules is still under debate. What is important is that, the discovery of these markers has further boosted the research, exploring some unexpected features in intestinal stem cell properties. They are not quiescent[13], they do not divide asymmetrically[17], and they generate their own cellular niche within epithelial cell populations[18]. Whether these characters of the stem cell compartment are unique to intestinal epithelium or conserved among other mammalian tissues is an interesting and important question to be answered in the near future.

In vitro culture technology for intestinal stem cells

In the last few years, the technologies to culture intestinal cells *in vitro* have also been advanced. Ootani et al. have reported a system that allows intestinal cells containing stem cell populations to grow *in vitro*[19] in the presence of non-epithelial stromal cells. Another culture system was also reported for the cells of mouse small intestine[20]. By this method, mouse small intestinal crypts were shown to grow as organoids that resemble the physiological epithelial architecture with properly situated stem cells. This condition requires Rspo1 (Wnt agonist), Noggin (BMP inhibitor) and EGF, but no support of non-epithelial cells. This observation indicates that, with the appropriately supplied factors, intestinal stem cells can be maintained even in the absence of myofibroblasts or other non-epithelial cell types that had been thought to be critical components of stem cell niche *in vivo*.

Importantly, these technologies have now been adapted to human intestinal epithelial tissues. Jung et al. have shown that human colon cells can grow as closed spheroids *in vitro*, especially when the cells expressing high EPHB2, a receptor tyrosine kinase, are used as a starting population[21]. Another report has described the method for the culture of not only small intestine and colon epithelium, but also colorectal cancer cells and metaplastic cells of the Barrett's esophagus, all of which are of human origin[22]. These culture systems will undoubtedly serve as important tools, for example, to study regulatory mechanisms for proliferation and differentiation of stem/progenitor cells *in vitro*, and provide us with a novel insight into intestinal epithelial biology.

Intestinal stem cell research for IBD clinic

As described previously, currently available therapies for IBD aim to a reduction in mucosal inflammation, and most of them do not target the other key pathophysiological feature of the disease, i.e., impaired repair of damaged epithelium. Therefore, new therapy to facilitate epithelial repair and aid appropriate mucosal healing will broaden treat-

ment strategies for IBD. In parallel with the rapid progress in the basic biology of intestinal stem cells, new technology to maintain intestinal epithelial stem cells *in vitro* would be directed toward to many clinical applications. For example, the human cell culture would be a useful system to investigate the response of physiological epithelial cells to a variety of substances such as growth factors, proinflammatory cytokines, bacterial components, and chemical compounds, and this will provide an excellent screening system by which we can assess the action of novel candidates for restitution- and/or repair-promoting therapies. Moreover, as the technologies now allow us to expand intestinal epithelial cells *in vitro*, we will soon be in a situation where we are able to perform multiple assays, such as the molecular diagnosis by using epithelial genes or proteins, and prediction of efficacy/toxicity of drugs in intestinal epithelium, by using a small piece of endoscopic biopsies obtained from individual IBD patients.

In addition to the potential application described above, the cultured cells could be used as a source of therapeutic transplants of intestinal epithelium. Although a few animal studies in the literature showed that intestinal epithelium, which were transplanted to recipients immediately after isolation, underwent temporal growth when placed to surgically manipulated intestine[23, 24], it remains completely unknown whether cultured epithelial cells could repair the tissue at the site of injury on gastrointestinal tract. Given that the intestinal stem cells can now be expanded in vitro, it would be of great importance to answer this question. Further advancement in such technology would be a major step forward in the stem cell therapy for the damaged epithelium in IBD patients.

Conclusion

The current understanding of IBD pathogenesis is driving researchers to develop novel approaches to IBD treatment, i.e., promotion of epithelial repair. Further progress in research on the intestinal stem cells and the mechanisms of intestinal tissue repair after injury is expected to revolutionize the clinical practice for IBD.

In this issue of Inflammation and Regeneration, I have asked four leading researcher working on this field to review the recent progress in more detail. I wish to express my thanks and gratitude to all the authors who made contribution. I hope that the information contained in these articles would be useful to researchers, clinicians, and students who are eager to develop innovative treatment for IBD.

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References

1) Okamoto R, Watanabe M: Cellular and molecular mechanisms of the epithelial repair in IBD. Dig Dis Sci. 2005; 50 Suppl 1: S34-S38.
2) Lichtenstein GR, Rutgeerts P: Importance of mucosal healing in ulcerative colitis. Inflamm Bowel Dis. 2010; 16: 338-346.
3) Pineton de Chambrun G, Peyrin-Biroulet L, Lemann M, et al: Clinical implications of mucosal healing for the management of IBD. Nat Rev Gastroenterol Hepatol. 2010; 7: 15-29.
4) Potten CS, Booth C, Pritchard DM: The intestinal epithelial stem cell: the mucosal governor. Int J Exp Pathol. 1997; 78: 219-243.
5) Bjerknes M, Cheng H: Intestinal epithelial stem cells and progenitors. Methods Enzymol. 2006; 419: 337-383.
6) Barker N, van de Wetering M, Clevers H: The intestinal stem cell. Genes Dev. 2008; 22: 1856-1864.
7) Podolsky DK: Mucosal immunity and inflammation. V. Innate mechanisms of mucosal defense and repair: the best offense is a good defense. Am J Physiol. 1999; 277: G495-G499.
8) Booth D, Potten CS: Protection against mucosal injury by growth factors and cytokines. J Natl Cancer Inst Monogr. 2001: 16-20.
9) Basson MD: In vitro evidence for matrix regulation of intestinal epithelial biology during mucosal healing. Life Sci. 2001; 69: 3005-3018.
10) Dignass AU: Mechanisms and modulation of intestinal epithelial repair. Inflamm Bowel Dis. 2001; 7: 68-77.
11) Taupin D, Podolsky DK: Trefoil factors: initiators of mucosal healing. Nat Rev Mol Cell Biol. 2003; 4: 721-732.
12) Krishnan K, Arnone B, Buchman A: Intestinal growth factors: potential use in the treatment of inflammatory bowel disease and their role in mucosal healing. Inflamm Bowel Dis. 2011; 17: 410-422.
13) Barker N, van Es JH, Kuipers J, et al: Identification of stem cells in small intestine and colon by marker gene

Lgr5. Nature. 2007; 449: 1003-1007.
14) Sangiorgi E, Capecchi MR: Bmi1 is expressed in vivo in intestinal stem cells. Nat Genet. 2008; 40: 915-920.
15) Tian H, Biehs B, Warming S, et al: A reserve stem cell population in small intestine renders Lgr5-positive cells dispensable. Nature. 2011; 478: 255-259.
16) Takeda N, Jain R, Leboeuf MR, et al: Interconversion Between Intestinal Stem Cell Populations in Distinct Niches. Science. 2011; 334: 1420-1424.
17) Snippert HJ, van der Flier LG, Sato T, et al: Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells. Cell. 2010; 143: 134-144.
18) Sato T, van Es JH, Snippert HJ, et al: Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. Nature. 2010; 469: 415-418.
19) Ootani A, Li X, Sangiorgi E, et al: Sustained in vitro intestinal epithelial culture within a Wnt-dependent stem cell niche. Nat Med. 2009; 15: 701-706.
20) Sato T, Vries RG, Snippert HJ, et al: Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature. 2009; 459: 262-265.
21) Jung P, Sato T, Merlos-Suarez A, et al: Isolation and in vitro expansion of human colonic stem cells. Nat Med. 2011; 17: 1225-1227.
22) Sato T, Stange DE, Ferrante M, et al: Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. Gastroenterology. 2011; 141: 1762-1772.
23) Avansino JR, Chen DC, Woolman JD, et al: Engraftment of mucosal stem cells into murine jejunum is dependent on optimal dose of cells. J Surg Res. 2006; 132: 74-79.
24) Tait IS, Evans GS, Flint N, et al: Colonic mucosal replacement by syngeneic small intestinal stem cell transplantation. Am J Surg. 1994; 167: 67-72.