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Intestinal Epithelial Organoids: A Platform for Discovering Mucosal Healing Drug Candidates for the Treatment of Inflammatory Bowel Diseases

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Abstract

Inflammatory bowel diseases (IBDs) are chronic, remitting inflammatory disorders of the digestive system. Intestinal epithelial dysfunction constitutes an integral part of the pathogenesis of IBD. Epithelial regeneration is a complex process, and the healing of injured tissue requires the resolution of inflammation followed by the proper and rapid proliferation of epithelial cell groups involving stem cell activation and mobilization. In recent years, an *in vitro* culture method for intestinal epithelial stem cells has been established. Known as "organoid culture", this novel culture system regenerates differentiated crypt lineages and reflects the architecture of intestinal mucosa. The mechanisms for controlling the growth and differentiation of intestinal epithelium have been investigated at the molecular level. Furthermore, this approach has been used for studying various intestinal diseases including cancer, based on the fact that functional abnormality of intestinal epithelium contributes to the development of infectious diseases. Therefore, using organoid culture to investigate the mucosal healing of IBD by identifying the molecule that controls the regenerative response induced by intestinal epithelial stem cells may lead to potent therapies for IBD patients.

Keywords: Organoid; Mucosal healing; Stem cell; Inflammatory bowel diseases

Abbreviations: IBD: Inflammatory bowel disease; IL-6: Interleukin-6; IL-22: Interleukin-22; IL-33: Interleukin-33; LncRNA: Long non-coding RNA; DIC: Disseminated Intravascular Coagulation

Introduction

Inflammatory bowel diseases (IBDs), comprised of Crohn's disease and ulcerative colitis, are intractable diseases in which inflammation of uncertain origin occurs in the digestive tract [1]. Symptoms include diarrhea, bloody stools, and abdominal pain, and a subset of patients with IBD are at increased risk to develop colorectal cancer [2]. The occurrence of IBD worldwide has been continuously increasing both in terms of prevalence and incidence for the last few decades [3]. Although the etiology is still unknown, growing evidence indicates that intestinal epithelium integrity is involved in the pathogenesis of IBD [4]. For a long time, the goal of treatment was to alleviate symptoms. Recently, however, the advent of targeted therapeutic agents such as biological agents (anti-TNF antibody, anti-α4 or α4β7 integrin antibody, anti-IL-12/IL-23 antibody), have greatly improved the clinical outcomes of IBD patients [5]. With these now classes of therapeutic agents, achieving long-term remission has become the treatment goal in IBD. However, despite considerable advances in the management of IBD, there are still a significant proportion of patients who remain unable to reach or maintain long-term remission. A therapeutic strategy called "mucosal healing", aimed at the structure and functional recovery of damaged tissues, was developed. Initial clinical studies suggest that "mucosal healing" is not only a marker of long-term remission, but also a predictor of long-term life quality in IBD patients [6-8]. Consequently, research aimed at intestinal mucosal regeneration has garnered a great deal of attention. In the meantime, a major advance has been made utilizing intestinal stem cells as part of three-dimensional (3D) culture system, also known as organoid culture. This system could give rise to all differentiated cell types of the intestinal epithelium and facilitate the replication of physiological structures of tissue *in vitro* [9,10]. Recent research on the mechanism underlying intestinal tract regeneration using intestinal epithelial stem cell-derived organoids and the development of therapeutic agents based on it have sharply increased.

The development of intestinal epithelial organoids

The intestinal epithelium is a dynamic organ that is continuously renewed every 3 to 5 days through a process of cell proliferation, differentiation and shedding. Proliferative cells (stem cells) that reside at the bottom of the intestinal crypt are the source of all intestinal epithelial cells. Until 13 years ago, *in vitro* culture of normal intestinal epithelium had been technically impossible, but in 2007, Barker et al. identified LGR5, one of the target molecules of Wnt signaling, as a specific marker for stem cells [11]. Two years later, an *in vitro* culture method for mouse intestinal epithelial stem cells was established by Sato's et al. [12]. In addition, in 2012, it became possible to culture normal human intestinal tissue on a culture dish [13].

The intestinal epithelial stem cells produced by this culturing method and the mass of epithelial cells differentiated from stem cells are called "intestinal epithelial organoids". To cultivate organoid, primarily intestinal epithelial stem cells are isolated from the intestinal tract of a living body. These are embedded into a specific basement membrane matrix (extracellular matrix) known as a 3D tissue culture, such as Matrigel, in order to maintain stem cell replication and differentiation [14,15]. Embedded crypts were then cultured in a

culture medium containing various molecules (R-spondin, EGF, Noggin-like peptide, etc.) (Figure 1). Thus, when a single isolated epithelial stem cell self-renews, it migrates to the villus region, where it differentiates into Paneth cells required for stem cell niche formation in the crypt region, and for mature functional intestinal epithelial cell formation (absorbing epithelial cells, goblet cells, enteroendocrine cells) (Figure 2). Therefore, the intestinal epithelial organoid has a crypt-villus structure similar to that of the intestinal epithelium of a living body. At the same time, is smaller than a living organ and is more suitable for experimental research.

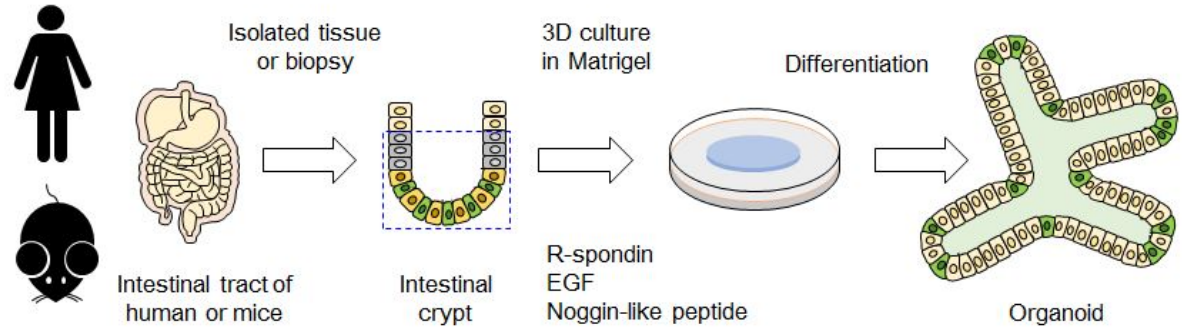

Figure 1: Schematic diagram of an organoid culture using intestinal crypts.

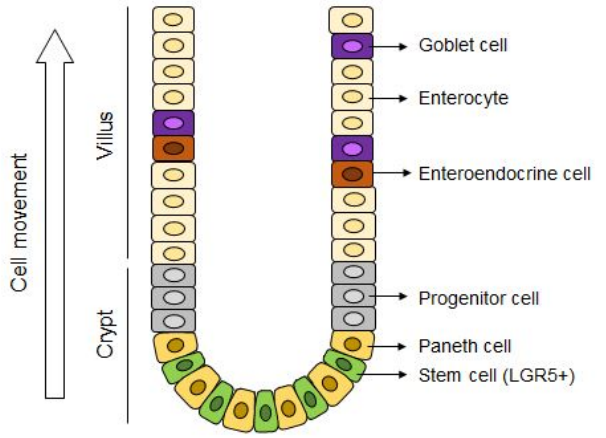

Figure 2: Composition of intestinal epithelial cells.

The use of intestinal epithelial organoids for studying mucosal healing

Increasing evidence indicates that dysregulation of the intestinal epithelium is one of the characteristics of IBD. During inflammation, proinflammatory cytokines such as IFN-γ and TNF-α, which promote apoptosis in epithelial cells, could increase intestinal permeability and substantially destroy the epithelium [16,17]. The structural basis of re-establishing mucosal homeostasis is an integrated gut epithelium; here, epithelial regeneration plays a critical role in intestine barrier maintenance and function following tissue damage [18]. By mimicking the tissue of origin, the intestine epithelial organoid is a very useful organ model that can be used as a substitute for the living body, elucidating the mechanisms underlying wound healing and leading to the development of therapeutic drugs for IBD patients.

By utilizing *in vitro* intestinal organoids, the experiment of Jeffery et al. [19]. demonstrated that interleukin (IL)-6 could induce the proliferation of stem cells by activating the phosphorylation of STAT3 in Paneth cells. Mahapatro M [20] et al. reported that overexpression of IL-33 induces the increased secretory lineage of the gut epithelium. In addition, a previous study suggested that IL-22 promotes re-establishment of intestinal epithelium by the stem cell mediated IL-22R/STAT3 signaling pathway [21]. Moreover, levels of IL-22 were found to be elevated at the outset and then gradually decreased with the onset of inflammation. Although IL-22 mediates organoid growth by activating cell proliferation, such as occurs with transit-amplifying cells, it inhibits the differentiation of intestinal epithelial cells and potentially reduces the survival of LGR5-positive stem cells [22,23]. Thus, the intestinal organoid culture system could also provide a platform for clarifying the roles played by other cytokines in the mucosal healing of inflamed intestinal tissue. The study of Geng et al. provided evidence that long non-coding RNA (lncRNA) H19 plays a crucial role in IL-22-dependent epithelial regeneration, showing that the lack of H19 impedes re-establishment of the intestinal epithelium [24]. In addition, lncRNA uc.173 has been reported to function as an essential regulator of mucosal renewal, stimulating gut mucosal growth by downregulating miR195 in intestinal epithelial cells [25].

Taken together, intestinal organoid representing a promising platform for novel drug discovery in IBD. Our laboratory is currently conducting basic research aimed at methods for regenerating of intestinal epithelial cells by elucidating of their regenerative control mechanism using small intestinal epithelial organoids. Our preliminary data have shown that recombinant thrombomodulin (Recomodulin™, ART-123), a therapeutic biologic agent approved for the treatment of disseminated intravascular coagulation (DIC) in sepsis, positively regulates stem cell function. Recomodulin™ may promote stem cell proliferation and differentiation using the established mouse intestinal epithelial organoid culture method.

Based on these studies, the utilization of intestinal organoid cultures may offer a sound basis for exploring novel treatment strategies for mucosal healing in clinical practice. In addition, intestinal epithelial

organoid transplantation has been used to treat mucosal injuries in mice under pathological conditions resulting from failed regeneration processes such as occurs in severe IBD and radioactive enteritis [26]. If these effects on regenerative treatment can be confirmed, their clinical application is envisioned in the near future.

Conclusion

As described above, we have realized a microenvironment that reproduces the stem cell niche of an intestinal epithelium crypt *in vitro*, maintaining the original replication and differentiation functions of LGR5-positive stem cells, and enabling a long-term culture system. These cultured tissues can subsequently be used as an effective tool not only for functional analyses of the small and large intestines under normal state, such as drug evaluation and gene expression analysis, but also for elucidating the pathological conditions of IBD. Furthermore, there is a possibility that intestinal-like cultured tissue transplantation could be applied to organ function regeneration. Therefore, elucidating the pathophysiology and treatment paradigms of gastrointestinal tract diseases by using intestinal epithelial organoids holds tremendous promise in the quest to realize mucosal healing of IBD.

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