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REVIEW

Advancing Intestinal Organoid Technology Toward Regenerative Medicine

Tetsuya Nakamura[1] and Toshiro Sato[2]

[1]Department of Advanced Therapeutics for GI Diseases, Tokyo Medical and Dental University; and [2]Department of Gastroenterology, Keio University School of Medicine, Tokyo, Japan

SUMMARY

Based on the progress in organoid technology, researchers have explored the use of intestinal organoids for regenerative medicine. This article showcases recent progress in different strategies of organoid-based regenerative medicine, such as the generation of highly functional organoids in vitro, or direct transplantation of various forms of currently available organoids.

With the emergence of technologies to culture intestinal epithelial cells in vitro as various forms of intestinal organoids, there is growing interest in using such cultured intestinal cells as a source for tissue engineering and regenerative medicine. One such approach would be to combine the organoid technology with methodologies to engineer the culture environment, particularly the three-dimensional scaffold materials, to generate intestines that exquisitely recapitulate their original structures and functions. Another approach to use organoids for regenerative medicine would be to urge them to mature into functional intestines by implanting them into hosts. This process includes the tissue-engineered small intestine that uses synthetic scaffolds for tissue regeneration and direct organoid transplantation that takes advantage of submucosal tissues in the native intestines as a scaffold. Further study in these subfields could lead to the development of therapeutic options to use different types of organoids with various cell types in regenerative medicine for intestinal diseases in humans. *(Cell Mol Gastroenterol Hepatol 2018;5:51–60; https://doi.org/10.1016/j.jcmgh.2017.10.006)*

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The intestinal epithelium constitutes a single-layered lining of cells that permits nutrient absorption in the body. It also acts as a protective barrier to restrict microbes and noxious substances in the intestinal lumen. Thus it is clear that the impairment of the intestinal epithelium leads to decreased nutrient absorption and increased ability of microorganisms and toxins to access the body, which may cause a variety of clinical manifestations in humans. Indeed, patients with severe forms of short bowel syndrome (SBS), which occurs as a result of extensive resection of the intestine,[1–3] or those with congenital disorders[4] suffer from malnutrition because of reduced or dysregulated absorptive function of the intestine. Also, disruption of the intestinal epithelial barrier function has been increasingly recognized as an important mechanism in the development and progression of human inflammatory bowel disease (IBD).[5,6] Many efforts have been made to restore the intestinal epithelial functions in severe forms of those diseases, such as an attempt to maximize the adaptive epithelial response to intestinal resections in SBS patients[7] or to regain the intact barrier function of the intestinal epithelium in IBD patients.[8] However, no standardized approach has been established.

In the last decade, there have been breakthroughs in culture technologies to maintain intestinal epithelial cells (IECs) in vitro. Studies have shown that when protein factors and extracellular matrix (ECM) scaffolds are adequately supplied, epithelial cells of the small intestine,[9–14] colon,[10,13,15–17] and fetal intestine[18,19] of mice, humans, and many other species are organized into unique three-dimensional (3D) structures with efficient expansion of their stem cell populations. Also, there have been advances in maintaining IECs with mesenchymal cells[20,21] or inducing directed differentiation of the intestine from pluripotent cells,[22,23] both of which allow for mixed 3D culture of intestinal epithelial/non-epithelial cells in vitro. Although there have been various nomenclatures for those diverse 3D structures,[24] the term *organoid* is now broadly accepted to describe a structure consisting of organ-specific cell types that self-organizes tissue-like structures in vitro.[25–28] We thus use organoid in this article to describe intestinal tissue–derived structures, irrespective of whether they contain mesenchyme or not, and also pluripotent stem cell–derived intestinal structures. On the basis of these methodologies to culture various types of intestinal organoids, there is a growing interest in using those cultured intestinal cells as a source for regenerative medicine. One such approach would be to place organoid cells in a highly engineered environment with various types of 3D scaffolds to

Abbreviations used in this paper: **2D, two-dimensional; 3D, three-dimensional; ECM, extracellular matrix; HIO, human intestinal organoid; IBD, inflammatory bowel disease; IEC, intestinal epithelial cell; ISC, intestinal stem cell; SBS, short bowel syndrome; TESI, tissue-engineered small intestine.**

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assemble fully functional tissues that precisely recapitulate their original structures. Although this biofabrication approach is primarily aimed at generating biological systems to use for testing a variety of tissue functions, it can also be applied to the use of organoid cells in replacement therapy. Another approach would be to place intestinal organoids back into the body and urge them to undergo functional maturation for their use in regenerative medicine. In this article, we review recent studies that use organoid-based approaches for intestinal tissue engineering and regenerative medicine.

Intestinal Stem Cells and Their Niche Factors In Vivo

The intestinal epithelium of both mouse and human is in an immature state at birth and undergoes further maturation during postnatal periods.[29–31] The mature tissue architecture in adult intestines (ie, crypt-villus structures in the small intestine and crypt structures in the colon, respectively) represents the functional unit of continuous self-renewal of the epithelial tissues.[32–35] Early studies revealed significant extracellular cues to regulate homeostasis of the IECs in vivo. Wnt signaling was demonstrated to be indispensable for their proliferation.[36,37] Moreover, animal studies showed that several other pathways such as tyrosine kinase receptor signaling[38,39] and Notch signaling[40,41] promote proliferation of IECs, whereas bone morphogenetic protein signaling has a negative effect on their growth.[42,43] Additional environmental cues that are provided by the ECM have also been suggested to be critical for survival of IECs in vivo, because the loss of attachment to the ECM induces a type of programmed cell death termed *anoikis* in IECs.[44,45]

On the basis of these previous findings, Barker et al[46] identified a gene encoding Lgr5, a member of the G-protein coupled receptor family of proteins, as the molecular marker of intestinal stem cells (ISCs). They demonstrated that *Lgr5* is one of the Wnt target genes in IECs and labels the cells at the crypt base of both small intestine and colon in adults, which were found to be ISCs by genetic lineage tracing. Several other genes have been subsequently identified as markers of ISCs by using genetic lineage tracing or in vitro clonogenic assay of sorted single cells.[47–49] Although a detailed description of the cells expressing these different markers is beyond the scope of this article, it is of note that identification of such ISC markers has stimulated the characterization of diverse ISC populations and their hierarchical and regulatory relationships. In addition, identification of ISC markers, as typified by Lgr5, has stimulated the cell-labeling technology that allows for the functional characterization of ISCs in various settings.[50–55]

Intestinal Organoids

Early studies attempted to culture normal IECs in vitro.[56,57] For example, Evans et al[56] developed a method to grow rat small intestinal cells seeded two-dimensionally in culture dishes and tested the effects of several growth factors and substrata in culture. However, such a protocol only allowed IECs to grow for around 10–14 days in the presence of contaminated subepithelial fibroblasts, indicating technical difficulties in maintaining long-term survival and proliferation of IECs in vitro. In the last decade, however, several technological breakthroughs have opened up new ways to grow normal, untransformed IECs containing ISC populations that unambiguously express stem cell markers with self-renewal and multi-differentiation capabilities. It was demonstrated that when crypt cells of the mouse small intestine are embedded in Matrigel, the pure epithelial cells grow for a long-term period with epidermal growth factor, noggin, and R-spondin in the culture medium.[9] Under these well-defined conditions that mimic the physiological ISC niche environment, cells organize into 3D tissue structures composed of a central spherical domain and numerous budding structures that protrude outward. The outer parts recapitulate the crypt structure of the small intestine, with *Lgr5*+ ISCs and Paneth cells residing at their apex, whereas the central sphere is composed mostly of differentiated cells.[9] Following this study, this type of organotypic epithelial culture was developed not only for small intestinal cells[9–14] but also for colonic cells[10,13,15–17] and fetal intestinal progenitor cells[18,19] of mouse and other species including humans. Interestingly, several region-specific and species-specific differences in epithelial culture methodologies were noted. For example, Wnt ligands are required for culturing murine and human colonic cells.[10,15–17] Small intestinal cells of human origin also require Wnt factors in culture.[14,15] Such phenomenon may be due to low production levels of Wnt ligands in those epithelial organoids, which clearly contrasts with the mouse small intestinal epithelial organoids that contain Wnt3-producing Paneth cells.[53] Intriguingly, when Wnt ligands are supplemented in mouse small intestinal epithelial organoid culture, the asymmetric architecture of organoids collapses due to the loss of local gradient of Wnt factors.[53] It is of note that 1 prominent feature of the epithelial organoid system is its capability to expand cells nearly infinitely, which allows us to initiate culture from a small number of cells or tiny pieces of tissue and then obtain a large-scale epithelial culture that can be used in a wide variety of applications.

Another breakthrough was the development of a composite culture of epithelial and non-epithelial elements of the intestine. Ootani et al[20] developed an air-liquid interface model to culture mouse intestinal spheres containing both epithelial and mesenchymal cell types in type I collagen gel. This study suggested that subepithelial myofibroblasts that lined the basal side of IECs might serve a role in supporting long-term culture and multi-lineage differentiation of ISCs in their system. Afterwards, Spence et al[22] reported an elegant method to generate intestinal tissues by inducing step-wise differentiation of human pluripotent stem cells (embryonic stem cells and induced pluripotent stem cells) into definitive endoderm, mid/hindgut spheroids, and then intestinal spheroids. Obtained structures, termed *human intestinal organoids* (HIOs), are composed of both epithelial and mesenchymal cell types, with the epithelial lining allocated to the luminal surface and the stratified mesenchyme to the outer parts. These multi-layered organoids consisting of cells derived from different germ layers have been shown to recapitulate the complex cellular diversity in original tissues and will become useful tools to study epithelium-mesenchyme interactions or developmental/morphogenetic processes in the intestine. For details of technical aspects and