Organoid-based regenerative medicine for inflammatory bowel disease

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Abstract

Inflammatory bowel disease (IBD) consists of two major idiopathic gastrointestinal diseases: ulcerative colitis and Crohn's disease. Although a significant advance has been achieved in the treatment of IBD, there remains a particular population of patients that are refractory to the conventional treatments, including the biologic agents. Studies have revealed the importance of "mucosal healing" in improving the prognosis of those difficult-to-treat patients, which indicates the proper and complete regeneration of the damaged intestinal tissue. In this regard, organoid-based regenerative medicine may have the potential to dramatically promote the achievement of mucosal healing in refractory IBD patients, and thereby improve their long-term prognosis as well. So far, studies have shown that hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs) may have some beneficial effect on IBD patients through their transplantation or transfusion. Recent advance in stem cell biology has added intestinal stem cells (ISCs) as a new player in this field. It has been shown that ISCs can be grown in vitro as organoids and that those ex-vivo cultured organoids can be employed as donor cells for transplantation studies. Further studies using mice colitis models have shown that ex-vivo cultured organoids can engraft onto the colitic ulcers and reconstruct the crypt-villus structures. Such transplantation of organoids may not only facilitate the regeneration of the refractory ulcers that may persist in IBD patients but may also reduce the risk of developing colitis-associated cancers. Endoscopy-assisted transplantation of organoids may, therefore, become one of the alternative therapies for refractory IBD patients.

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1. Introduction

Inflammatory bowel disease (IBD) is a disease that is characterized by idiopathic mucosal inflammation along the gastrointestinal tract [1]. Ulcerative colitis (UC) and Crohn's disease (CD) represent the two forms of IBD. Their incidence, as well as the prevalence, is continuously increasing at the global level, including the Asian, South American, and Middle Eastern countries [2]. The treatment of IBD has dramatically improved in the past decade, mainly by the outstanding clinical effect of biologic agents such as anti-TNF-α antibodies [3]. Those therapies were targeted mostly to control the inflammation that arises at the mucosa of IBD patients. However, less attention had been paid to the recovery of the tissue damage that may manifest as intestinal ulcers. Recent clinical studies have clearly shown that “mucosal healing” is an utmost requirement to achieve long-term remission in IBD patients [4]. “Mucosal healing” indicates complete restore of the mucosal structure and function. Therefore, a high demand exists for an alternative treatment that can promote tissue regeneration of refractory IBD patients.

The intestinal mucosa consists of three cell populations: lymphocytes, mesenchymal cells, and epithelial cells. In the past years, many studies have tried to use hematopoietic stem cells (HSCs) in mesenchymal stem cells (MSCs) for the treatment of IBD. Recent advance in culture methods has newly added ISCs as another candidate cell source for regenerative medicine in IBD patients. A more extensive choice of stem cell source may help to establish an effective stem cell-based alternative therapy for refractory IBD patients.

2. Old players of stem cell-based regenerative medicine in the treatment of IBD

The earliest challenge to establish stem cell-based therapy for IBD patient used HSC transplantation preceded by non-myeloablatve conditioning, to rebuild or reset the host immune system [5]. In the study, HSC transplantation appeared to have the ability to induce and maintain remission of refractory CD patients. In the following periods, a series of studies further suggested some clinical benefits in HSC transplantation [6–10]. However, studies raise concerns to the high incidence of serious adverse events [11], and also to the clinical benefit itself [12,13]. From a multi-center study conducted in Europe (ASTIC trial), it was concluded that autologous HSC transplantation might not be recommended to refractory CD patients [13]. However, the possible adverse effect of cyclophosphamide used for the transplantation group has been pointed out [14]. Conversely, the most recent retrospective study concluded that autologous HSC transplantation is relatively safe and appears to be effective for treatment-resistant Crohn’s disease [15]. Thus although a long time has been spent to examine the relevance of HSC transplantation to CD patients, it remains controversial whether it can be considered as a good alternative therapy for refractory CD patients.

MSCs are another stem cell population that has long been studied for its use in IBD treatment. Studies have shown that MSCs have the potential to adjust the host immune response, and at the same time promote repair of the damaged tissue. Such an effect of MSCs may come from their paracrine effect, and also from their direct engraftment to the target lesions [16,17]. Another distinct feature of MSCs is its low immunogenicity [18]. Therefore, MSCs has been used for autologous use as well as allogeneic transplantation, in various disease models [19,20]. Based on such a function of MSCs, they have gone through a series of trials to treat the luminal and fistulizing type of CD. In the initial trials for luminal CD, both autologous and allogeneic transgene of MSCs improved the disease activity of refractory CD patients, with low risk of adverse events [21,22]. Also, it has been shown that local injection of autologous MSCs to fistulating CD can induce closure of the fistula, also with low risk of adverse events [23–26]. A recent double-blind dose-finding study concluded that allogeneic bone-marrow-derived MSC therapy for CD associated perianal fistulas is safe and effective in terms of long-term outcome [27]. Thus, infusion or injection of MSC appears to be a safe and effective treatment for a particular population of CD patients. However, several issues remain unclear and need further studies. For example, it is not known whether the difference in the origin of MSCs may determine the clinical outcomes when they were used for the treatment of refractory CD. Also, it is not clear whether a transfusion or a local injection is better for the treatment of CD. The effect of a single MSC treatment is mostly transient and may not persist in the long term. So the frequency or the duration of the treatment may also have to be optimized.

3. Intestinal stem cells: new player in the field of stem cell-based IBD treatment

Intestinal stem cells (ISCs) reside at the bottom of the intestinal crypt and play indispensable roles in maintaining the homeostasis and the rapid renewal of the intestinal epithelium [28–30]. They are characterized by the expression of ISC-specific genes, such as LGR5 [31]. Also, studies have shown that lost of stem cell-specific properties may retard or disrupt the regeneration of the damaged intestinal epithelium [32,33]. Thus, it may be easy to think that transplantation of ex vivo cultured ISCs may help promote the regeneration of the damaged intestinal epithelium in IBD patients. However, the question of how we could efficiently culture and expand donor ISCs in vitro has remained an unsolved problem for an extended period.

Series of studies by Sato et al. has provided an apparent breakthrough in this area, by their establishment of a novel culture method for ISCs [34]. They succeeded in long-term culture of ISCs by maintaining them in a 3D-structure, which was named as “organoids” [35]. The culture method required at least four growth factors, which were Wnt3a, R-Spondin-1, EGF, and Noggin. In their later studies, those factors turned out to be the indispensable components of the stem cell niche, which is supplied by the Paneth cells in vivo [36]. Therefore, the success was based on the careful in vitro reconstitution of the stem cell niche microenvironment. The culture method can be applied to grow both mice as well as human organoids [37], which can be continued infinitely for over the years. Other groups have reported that endoscopic biopsies can be used as a starting material to establish patient-derived organoids [38] and that those organoids retain the specific properties of their site-of-origin within the gastrointestinal tract [39].

Yui et al. further developed an original culture method using collagen instead of Matrigel [40]. Proving that collagen can be used as an extracellular matrix for the culture of intestinal organoids is essential for the development of organoid-based regenerative therapy, as Matrigel is not allowed for clinical use. Their recent study further showed that extracellular collagen could induce “fetalization” of organoids, which indicates a partial acquisition of the fetal intestine-specific phenotype by adult-derived intestinal organoids [41]. Such a “fetalization” is also observed in the regenerating epithelia of UC patients, thus providing the validity of using organoid cultured in collagen gels for the treatment of those patients.

Another breakthrough that has been acquired in this area was the proof that those ex vivo cultured ISCs could engraft orthotopically, and thereby contribute to the reconstruction of the damaged mucosa. A study using a DSS–colitis model showed that organoids...
could engraft onto the surface of the rectal ulcer when they were delivered through an intraluminal route [40]. Those donor-derived cells formed a clear crypt structure that was integrated into the recipient epithelial crypts and remained there for over months. These observations provided the evidence that ex vivo cultured ISCs can engraft and contribute to the regeneration of the damaged intestinal epithelium. Further studies showed that organoids derived from the fetal intestine or the adult small intestine are also able to engraft onto the damaged epithelium of the colon, but shows the difference in their ability to adapt to the surrounding environment through a mechanism of cell plasticity [42]. These two breakthroughs provided a sound basis to apply ex-vivo cultured ISCs for the treatment of refractory IBD. A recent study by Sugimoto et al. further confirmed that human intestinal organoids could also reconstruct the damaged mucosa of immunodeficient mice [43].

4. Expected advantages and requirements of ISC transplantation for IBD patients

Based on those previous studies, intestinal organoids can now be considered as one of the candidate sources to repair the ulcers that may appear in refractory IBD patients. One of the strategies that may be taken for such treatment is autologous, endoscopic transplantation of ISCs (Fig. 1). ISCs can be collected from the intact lesion of a patient through the endoscopic biopsy, and then expanded in vitro by the established organoid culture method. After growing them to a desired number of cells, they can be transplanted onto the target site through an endoscopic delivery method. Presumably, it would be better to reduce or achieve good control of the mucosal inflammation before the organoid transplantation, to guarantee high engraftment efficiency.

However, further researches and several technical developments are required to enable such a treatment (Table 1). At the cell culture level, we need to know whether the in vitro properties of ISCs that were derived from IBD patients is comparable to those derived from healthy donors. A recent study by our group has shown that organoids derived from active lesions of CD patients exhibit comparable growing potential compared to those from healthy donors and maintain mostly same ISC-specific gene expression profile at the single-cell level [44]. Besides, organoids can change their morphology or its ISC-content depending on the culture environment determined mainly by extracellular matrix and growth factors (Fig. 2). Further studies using transplantation model of colitis mice may reveal the optimized culture condition for transplantation therapy.

Also, to assure the safety of the treatment and avoid adverse events, validation in the quality of cultured ISCs must be established and performed. For example, the prevalence of infectious
pathogens in the donor tissues should be checked, and the amplification of those pathogens during the culture period should be carefully ruled out. Also, the frequency of genomic mutations in tumor-related genes might be checked in both donor-derived tissues and in cultured organoids to exclude an increase in tumorigenic potential by the ex-vivo culture. So far, the accumulation of mutation I ISCs does not seem to increase so much by the in vitro organoid culture [45]. Also, in vivo mucosal transplantation of organoids has not yet identified the formation of organoid derived tumors [40,43].

At the cell transplantation level, a new endoscopic cell delivery system may be required. At first, we need to know what kind of

**Table 1**

Remaining questions for the development of intestinal organoid transplantation therapy for IBD patients.

| Questions                                                                 | Suggested solution(s)                                                                 |
|--------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
| At the cell culture level                                                 | • May be possible depending on culture conditions and cell source (REF 44).           |
| Can we expand patient derived organoids in vitro at a proliferation efficiency comparable to those derived from healthy donors? | • Adaptation or modification of the collagen-based method may be suitable (REF 40).   |
| Can we expand patient derived organoids in a completely xeno-free culture condition? Or otherwise in a fully defined culture condition? | • Evaluation of stem cell function and/or content may be required.                    |
| What kind of tests should we apply for the quality assurance and quality control of donor organoids? | • Data of in vivo transplantation may be highly supportive (REF 43).                  |
| How could we exclude the tumorigenicity of the donor organoids?          | • Standard sterility tests, endotoxin tests should be confirmed. In addition, viruses or mycoplasmas should be examined following the standard methods for clinical grade products. |
| Can we expand patient derived organoids without enhancing the risk of infectious pathogen-related adverse events? |                                                                                       |
| At the cell transplantation level                                          | • Engraftment ability confirmed only for organoids.                                   |
| Is it better to deliver ISCs as an organoid, or otherwise as a cell sheet? | • Readily available endoscopic tools, or custom-made devices should be tested.        |
| What kind of device is suitable to efficiently deliver organoids through an endoscopic procedure? | • Additional procedure may be required to let the organoids stay at the desired region until they finish the initial engraftment process. |
| Is there any host mucosal condition that is beneficial or inversely unfavorable for the engraftment of the donor organoids? | • Needs to be evaluated in pre-clinical studies using colitis mice models, and further evaluated in initial-phase clinical trials. |
| At the clinical level                                                      | • Needs to be evaluated in early-phase clinical trials.                               |
| What will be the best index to evaluate the clinical effect of organoid transplantation? | • Identification of a reliable donor-cell marker, or clinically available method for donor-cell labeling should be developed. |
| What kind of patients is the best candidate of organoid transplantation? UC or CD? | • Pre-clinical evidence should be established using transplantation to the colitis model. |
| Can we identify the donor cell derived crypts within the recipient mucosa? |                                                                                       |
| Is it better to perform an allogenic organoid transplantation from a healthy donor instead of an autologous transplantation? |                                                                                       |

**Fig. 2.** Change in organoid morphology depending on culture condition. Human intestinal organoids show either round-shaped morphology, or otherwise a complex-shaped morphology depending on choice of extracellular matrix and on growth factor condition. Data acquired by confocal microscope system (FV3000, Olympus).
extracellular matrix could be used for the transplantation process. A recent study showed that collagen, as well as fibrin glue, can be used for intestinal organoid transplantation, without showing the deleterious effect on donor organoids [46]. Delivery of the organoids through an endoscopic tool may be feasible, but another choice may be manufacturing a sheet type donor epithelial cells, and delivering them by a formerly developed device [47]. Finally, at the clinical level, we currently do not know exactly which kind of IBD patients may most benefit from such a treatment. Our opinion is to start organoid transplantation studies in refractory UC patients, whose tissue damage is persistent and carries several refractory ulcers, although their inflammation is generally under reasonable control [48,49]. For the initiation of our first-in-human study, clinical-grade culture methods and endoscopic delivery methods are both under development [50].

Then what would be the expected benefit for those patients who underwent the ICC transplantation? The first may be the improvement in mucosal healing rate that would directly lead to an improved prognosis. The second will be the possible reduction of the risk in developing colitis-associated cancer [51]. As those cancers may arise from the accumulation of genetic as well as epigenetic changes within the ISC through their long-term exposure to the inflammatory Crohn’s environment, replacing such an exhausted ISC with those fresh and lively ISCs that were grown in the most ideal and suitable environment would possibly have the potential to reduce the initiation of colitis-associated cancers.

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References

[1] Kaser A, Zeissig S, Blumberg RS. Inflamm Bowel Dis 2010;16:338–41. https://doi.org/10.1097/MIB.0b013e3181e40f7d.
[2] Kaplan GG. The global burden of IBD: from 2015 to 2025. Nat Rev Gastroenterol Hepatol 2015;12:200–7. https://doi.org/10.1038/nrgastro.2014.206.
[3] Sandborn W, Hanauer S. Antitumor necrosis factor therapy for inflammatory bowel disease: a review of agents, pharmacology, clinical results, and safety. Inflamm Bowel Dis 1999;5:119–33. https://doi.org/10.1097/00011823-199903000-00010.
[4] Lichtenstein GR, Rutgeerts P. Importance of mucosal healing in ulcerative colitis. Inflamm Bowel Dis 2010;16:338–41. https://doi.org/10.1097/MIB.0b013e3181e40f7d.
[5] Burt BK, Crellin R, Trong CC, Qiu C, Crowe C, Moxham R, et al. Autologous adipose tissue-derived stem cells treatment demonstrated favorable and sustainable therapeutic effect for Crohn’s disease. Gut 2015;64:2575–81. https://doi.org/10.1136/gutjnl-2014-307377.
[6] Barnhoorn MC, Wasser MN, Roelofs H, Maljaars JP, Molendijk I, Bonsing BA, et al. Long-term evaluation of allogeneic bone marrow-derived mesenchymal stem cells treatment for refractory luminal Crohn’s disease: results of a phase 1 study. Gut 2015;64:1662–9. https://doi.org/10.1136/gutjnl-2014-307377.
[7] Lee W, Park K, Cho Y, Yoon S, Song K, Kim D, et al. Autologous bone marrow-derived mesenchymal stem cells in the treatment of fistulizing Crohn’s disease. Gut 2011;60:788–93. https://doi.org/10.1136/gut.2010.214841.
[8] Cho Y, Park K, Yoon S, Song K, Kim D, Jung S, et al. Long-term results of adipose-derived stem cell therapy for the treatment of Crohn’s fistula. Stem Cells Transl Med 2015;4:332–7. https://doi.org/10.5966/sctm.2014.0199.
[9] Crossier C, Stamatou B, Lewis J. Organizing cell renewal in the intestine: stem cells, signals and combinatorial control. Nat Rev Genet 2006;7:349–59. https://doi.org/10.1038/nrg1840.
[10] Okamoto R, Watanabe M. Cellular and molecular mechanisms of the epithelial repair in IBD. Dig Dis Sci 2005;50(Suppl 1). https://doi.org/10.1002/drr.20997.
[11] Okamoto R, Watanabe M. Role of epithelial cells in the pathogenesis and treatment of inflammatory bowel disease. J Gastroenterol 2016;51:11–21. https://doi.org/10.1002/jgc.20213.
[12] Barker N, van ES JT, Kuipers J, Kupala P, van den Born M, Cozijnsen M, et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature 2007;449:1003–7. https://doi.org/10.1038/nature06196.
[13] Okamoto R, Tsuchiya K, Nemoto Y, Akiyama J, Nakamura T, Kani T, et al. Requirement of Notch activation during regeneration of the intestinal epithelia. Am J Pathol-Gastr L 2009;174:595–604. https://doi.org/10.2353/ajpgi.2009.080753.
[14] Gregoriass A, Liu Y, Ianlou MA, Khochuk H, Wanl J. Yap-dependent reprogramming of Lgr5+ stem cells drives intestinal regeneration and cancer. Nature 2015;526:757–61. https://doi.org/10.1038/nature15182.
[15] Sato T, Vries RG, Snippen H, van de Wetering M, Barker N, Stange DE, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature 2008;459:262–7. https://doi.org/10.1038/ nature08193.
Sato T, Clevers H. Growing self-organizing mini-guts from a single intestinal stem cell: mechanism and applications. Science 2013;340:1190–4. https://doi.org/10.1126/science.1234852.

Sato T, van Es JH, Snippert HJ, Stange DE, Vries RG, van den Born M, et al. Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. Nature 2012;469:415–8. https://doi.org/10.1038/nature09637.

Sato T, Stange DE, Ferrante M, Vries RG, van Es JH, den Brink S, et al. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett’s epithelium. Gastroenterology 2011;141:1762–72. https://doi.org/10.1053/j.gastro.2011.07.050.

VanDussen KL, Marinshaw JM, Shaikh N, Miyoshi H, Moon C, Tarr PI, et al. Development of an enhanced human gastrointestinal epithelial culture system to facilitate patient-based assays. Gut 2015;64:911–20. https://doi.org/10.1136/gutjnl-2013-306651.

Middendorp S, Schneeberger K, Wiegerinck CL, Mokry M, Akkerman RD, van Wijngaarden S, et al. Adult stem cells in the small intestine are intrinsically programmed with their location-specific function. Stem Cells 2014;32:1083–91. https://doi.org/10.1002/stem.1655.

Yui S, Nakamura T, Sato T, Nemoto Y, Mizutani T, Zheng X, et al. Functional engraftment of colon epithelium expanded in vitro from a single adult Lgr5+ stem cell. Nat Med 2012;18:618–23. https://doi.org/10.1038/nm.2695.

Yui S, Azzolin I, Mainets M, Pedersen M, Fordham RP, Hansen SL, et al. YAP/TAZ-dependent reprogramming of colonic epithelium links ECM remodeling to tissue regeneration. Cell Stem Cell 2017;22:1–37. https://doi.org/10.1016/j.stem.2017.11.001.

Fukuda M, Mizutani T, Mochizuki W, Matsumoto T, Nozaki K, Sakamaki Y, et al. Small intestinal stem cell identity is maintained with functional Paneth cells in heterotopically grafted epithelium onto the colon. Genes Dev 2014;28:1752–7. https://doi.org/10.1101/gad.245233.114.

Sugimoto S, Ohta Y, Fujiy M, Matano M, Shimokawa M, Nanki K, et al. Reconstruction of the human colon epithelium in vivo. Cell Stem Cell 2017;22:1–17. https://doi.org/10.1016/j.stem.2017.11.012.

Suzuki K, Murano T, Shimizu H, Ito G, Nakata T, Fujiy S, et al. Single cell analysis of Crohn’s disease patient-derived small intestinal organoids reveals disease activity-dependent modification of stem cell properties. J Gastroenterol 2018;53:1–13. https://doi.org/10.1007/s00535-018-1437-3.

Blokzijl F, de Ligt J, Jager M, Sasselli V, Roerink S, Sasaki N, et al. Tissue-specific mutation accumulation in human adult stem cells during life. Nature 2016;538:260. https://doi.org/10.1038/nature19788.

Lee J, Jeong S, Kim H, Choi S, Jeong S, Lee J, et al. In vivo evaluation of scaffolds compatible for colonoid engraftments onto injured mouse colon epithelium. FASEB J 2019;33:201802692RR. https://doi.org/10.1096/fj.201802692rr.fj.

Maeda M, Kanai N, Kohbayashi S, Hosoi T, Takagi R, Ohki T, et al. Endoscopic cell sheet transplantation device developed by using a 3-dimensional printer and its feasibility evaluation in a porcine model. Gastrointest Endosc 2015;82:147–52. https://doi.org/10.1016/j.gie.2015.01.062.

Okamoto R, Watanabe M. Investigating cell therapy for inflammatory bowel disease. Expert Opin Biol Ther 2016;16:1–9. https://doi.org/10.1080/14712598.2016.1177019.

Okamoto R, Watanabe M. Perspectives for regenerative medicine in the treatment of inflammatory bowel diseases. Digestion 2015;92:73–7. https://doi.org/10.1159/000438963.

Takebe T, Wells JM, Helmrath MA, Zorn AM. Organoid center strategies for accelerating clinical translation. Cell Stem Cell 2018;22:806–9. https://doi.org/10.1016/j.stem.2018.06.008.

Grivennikov SI. Inflammation and colorectal cancer: colitis-associated neoplasia. Semin Immunopathol 2012;35:229–44. https://doi.org/10.1007/s00281-012-0352-6.