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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

POINT-COUNTERPOINT

In Vivo Studies Should Take Priority When Defining Mechanisms of Intestinal Crypt Morphogenesis

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Studies of the intestinal biology have been greatly assisted by organoid culture systems, in which isolated stem cells can generate 3-dimensional structures that recapitulate the functional and cellular organization of the epithelium. It has been proposed that the emergence of these 3-dimensional structures in vitro recapitulates features of intestinal epithelial morphogenesis. It is, however, clear that any extrapolation from organoid studies to a physiological context requires in vivo validation.

In humans, intestinal morphogenesis proceeds through the first and second trimester, whereas the same process in mice occurs from embryonic day 12.5 until the 2 first weeks after birth. During morphogenesis, the intestine forms as a tube with a smooth inner surface covered by epithelial cells. The surface subsequently begins folding into rudimentary villi surrounded by a continuum of intervillus space.[1] The epithelial cells in this space express markers associated with adult stem cells. Following a phase of intestinal growth and increase in the number of villi, crypts begin to form from the intervillus space.[2] As crypts enlarge, Paneth cells emerge at the crypt top and subsequently relocalize to the bottom, where they intercalate between adult stem cells (Figure 1A).[3] Because of the difficulty of studying tissue morphogenesis in humans beyond the analysis of histologic material, most of the knowledge regarding the formation of the intestine comes from studies in mice and other experimental animals.

A breakthrough in the field of intestinal biology came with the development of cell culture systems, where single intestinal epithelial stem cells from human and mouse could be cultured as 3-dimensional organoid structures with distinct crypt-like and villus-like domains.[4,5] This pioneering technology provided a tractable model system for studies focusing on developmental biology; regenerative medicine; and pathologies, such as cancer and inflammatory bowel disease. Although the organoid models are attractive in vitro models, it is important to keep in mind that they represent reductionistic cell culture models and consequently they do not necessarily recapitulate in vivo cell behavior. In the discussion next, we exemplify this with the process of crypt and crypt-like formation, which is governed by different rules in mice and organoids, respectively. Moreover, we describe several research areas in which organoid models are currently too simplistic to provide comprehensive results.

Organoid growth from the mouse small intestine follows an almost invariant pattern. Single cells or crypts seeded in tumor-derived matrices initially grow as seemingly homogenous spheroids. These spheroids go on to form crypt-like domains, where stem cells are intercalated between Paneth cells.[4] For small intestinal organoids, the initial crypt-like domain formation has been linked with the first symmetry-breaking event and establishment of the secretory lineage around which the crypt-like domain is subsequently formed.[6] The emerging Paneth cell constitutes a source of Wnt3a, and a signaling gradient emanating from the bud of the domain leads to the further patterning of the organoid into domains of stem cells and differentiated cells (Figure 1B). Given the emergence of crypt-like domains during early organoid formation, organoids have been used to study crypt formation; however, there are striking differences between crypt-like domain formation in vitro and crypt formation in vivo.

In vivo Paneth cells appear late in crypt formation and is preceded by stem cell marker expression. In contrast, the appearance of Paneth cells in organoids represents the earliest patterning event followed by the appearance of cells expressing stem cell markers.[6] Moreover, organoid studies identify Paneth cells as an essential source of canonical Wnt signaling molecules.[7] However, in vivo Paneth cell loss and elimination of epithelial Wnt secretion do not compromise the integrity or the maintenance of the epithelium.[8,9] Taking this into account, one should be very careful extrapolating findings directly from organoid models to physiological conditions, and keep in mind that the reductionistic approach used for culturing intestinal organoids might not recapitulate in vivo processes. In fact, the physiological counterpart for what is modelled during crypt-like formation using organoids remains to be elucidated given that Paneth cells in vivo are dispensable.

It is clear that the organoid system in its current form is very simplistic and that processes, such as morphogenesis, homeostasis, and tissue regeneration, are most likely too complex to model using this approach. Here the environment including the fibroblasts, enteric neurons, blood and lymphatic vessels, immune cells, and

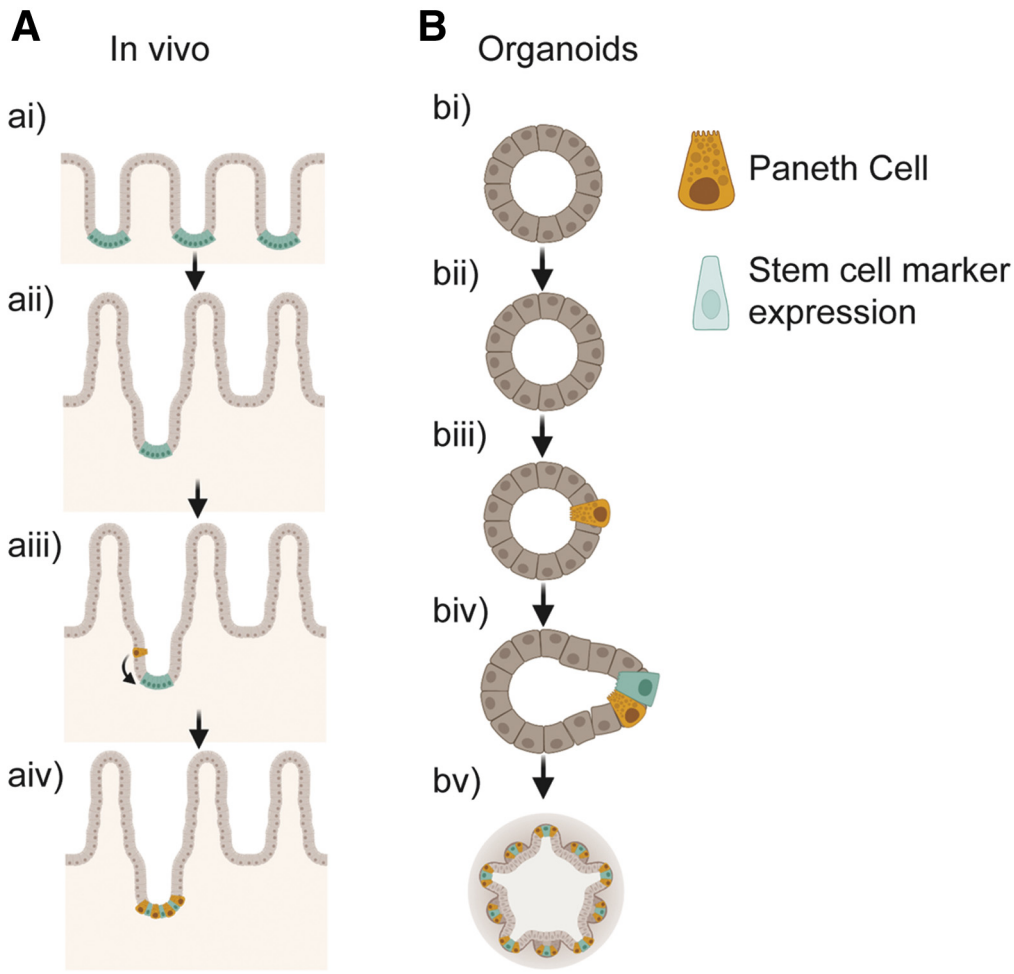

Figure 1. Process of crypt formation in vivo (*A*) and in organoids (*B*). In vivo intervillus cells express stem cell markers in the absence of Paneth cells (*ai*). After birth crypts are formed independently of Paneth cell (*aii*). Once crypts are formed Paneth cells emerge in the crypts (*aiii*). Subsequently, Paneth cells are located at the bottom of the crypts intercalated between the stem cells (*aiv*). Organoids are initially spherical (*bi*). The formation of Paneth cells (*bii*) constitutes a symmetry-breaking event (*biii*) that leads to bud formation (*biv*) to finally produce branching organoids (*bv*). Figure Created with BioRender.com

commensal microbes generates a permissive environment for the epithelium supporting growth, maintenance, or tissue remodeling. Parameters including growth factors and extracellular matrix components, and biomechanical properties, such as shape and stiffness of the environment, will simultaneously influence cell behavior, and cannot be accounted for using the traditional static culture methods for organoid growth. Here, it is also important to point out that changing the matrix supporting organoids from a tumor-derived matrix rich in laminins to type I collagen, the prevalent extracellular matrix component observed during tissue regeneration, is sufficient to severely impact cell behavior in vitro.[10] Modelling of biologic processes using organoids therefore requires substantial prior knowledge and insight into the physiological context. Yet, the number of parameters that can be assessed are limited, and it is a tall order to simulate all the possible interactions with immune cells, enteric neurons, fibroblast, and microbiome.

Overall organoids represent an elegant model system that has transformed the field of intestinal biology. It gives the possibility to build on the descriptive studies of human development using cells isolated either directly from tissues or derived from pluripotent stem cells. Here, the organoid technology allows for manipulation of gene expression and reverse genetics using technologies such as CRISPR/Cas9 but in a human context. We can consequently now begin to model aspects of human development, which previously were not possible. However, we still need to keep in mind that the organoid technology has limitations, and that observations from the organoid system are not necessarily physiologically relevant. The process of crypt formation constitutes one example, that in vivo experiments are required for defining complex mechanisms directing the processes at the tissue level. In addition to this, there are plethora of biologic processes that currently cannot be modeled with organoid technology including the cross-talk between different cell types, such as immune cells and intestinal epithelium at organ and multiorgan level, the endocrine role of the intestine, and signaling of the gut-brain axis. These highly complex processes require in vivo models. Consequently, animal studies, which provide the physiological context, can be complemented with insights from organoid studies, but animal studies cannot be replaced by the organoid technology.

References

1. Guiu J, Jensen KB. From definitive endoderm to gut: a process

of growth and maturation. Stem Cells Dev 2015;24:1972–1983.
2. Guiu J, Hannezo E, Yui S, Demharter S, Ulyanchenko S, Maimets M, Jørgensen A, Signe P, Lundvall L, Salto Mamsen L, Larsen A, Olesen RH, Andersen CY, Thuesen LL, Hare KJ, Pers TH, Khodosevich K, Simons BD, Jensen KB. Tracing the origin of adult intestinal stem cells. Nature 2019;570:107–111.
3. Bjerknes M, Cheng H. The stem-cell zone of the small intestinal epithelium. II. Evidence from paneth cells in the newborn mouse. Am J Anat 1981;160:65–75.
4. Sato T, Clevers H. Growing self-organizing mini-guts from a single intestinal stem cell: mechanism and applications. Science 2013;340:1190–1194.
5. Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE, van Es JH, Abo A, Kujala P, Peters PJ, Clevers H. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature 2009;459:262–265.
6. Serra D, Mayr U, Boni A, Lukonin I, Rempfler M, Meylan LC, Stadler MB, Strnad P, Papasaikas P, Vischi D, Waldt A, Roma G, Liberali P. Self-organization and symmetry breaking in intestinal organoid development. Nature 2019;569:66–72.
7. Sato T, van Es JH, Snippert HJ, Stange DE, Vries RG, van den Born M, Barker N, Shroyer NF, van de Wetering M, Clevers H. Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. Nature 2011; 469:415–418.
8. Kim TH, Escudero S, Shivdasani RA. Intact function of Lgr5 receptor-expressing intestinal stem cells in the absence of Paneth cells. Proc Natl Acad Sci U S A 2012.
9. van Es JH, Wiebrands K, Lopez-Iglesias C, van de Wtering M, Zeinstra L, van den Born M, Korving J, Sasaki N, Peters PJ, van Oudenaarden A, Clevers H. Enteroendocrine and tuft cells support Lgr5 stem cells on Paneth cell depletion. Proc Natl Acad Sci U S A 2019.
10. Yui S, Azzolin L, Maimets M, Pedersen MT, Fordham RP, Hansen SL, Larsen HL, Guiu J, Alves MRP, Rundsten CF, Johansen JV, Li Y, Madsen CD, Nakamura T, Watanabe M, Nielesen OH, Schweiger PJ, Piccolo S, Jensen KB. YAP/TAZ-dependent reprogramming of colonic epithelium links ECM remodeling to tissue regeneration. Cell Stem Cell 2018; 22:35–49.

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