POINT-COUNTERPOINT

Organoid vs In Vivo Mouse Model: Which is Better Research Tool to Understand the Biologic Mechanisms of Intestinal Epithelium?

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The mammalian intestinal epithelium consists of the glandular histologic unit, crypts. Crypts have a remarkable self-renewing capacity, and the entire unit is constantly regenerated within several days. The self-renewing capacity is instrumental for homeostasis and critical after intestinal injury, when new crypts must be formed from a landscape of denuded mucosa. Mouse models have provided an understanding of how crypts regenerate and which factors regulate the self-renewal of crypts. In the 1970s, Leblond, Cheng, and Bjerknes revealed the self-renewing dynamics of mouse intestinal crypts and proposed intestinal stem cells (ISCs) responsible for crypt self-renewal.[1,2] However, ISCs were not formally identified until the emergence of a lineage tracing system using mouse genetic models, when Barker and Clevers demonstrated the long-term self-renewing potential and multidifferentiation capacity of Lgr5+ ISCs.[3] The Lgr5 expression indicated the critical role of Wnt signaling in the maintenance of ISCs. Moreover, this finding underscored that microenvironmental factors, referred to as niche factors, could be used to support ISCs. Using a combination of niche factors including EGF (growth factor), Noggin (Bmp inhibitor), and R-spondin (Wnt signaling activator), Sato and Clevers developed an organoid culture system for mouse small intestinal epithelium.[4] Ever since the establishment of the organoid culture system, mouse genetic models and organoids have been 2 major tools to study ISCs and crypt development. Here, we discuss the advantages of the organoid culture system over mouse genetic models (Table 1).

The first, and most obvious, advantage of organoids is the applicability to the human intestinal epithelium. Although mouse intestinal epithelium shares major biologic and histologic traits with humans, recent studies highlighted several differences. Human and mouse organoids differ in their dependence on niche factors, suggesting fundamental differences in the molecular mechanisms of self-renewal in mouse versus human ISCs.[5] There are unique epithelial cell types found in humans but not in mouse, such as motilin+ enteroendocrine cells and BEST4+/OTOP2+ cells,[6,7] which may suggest different constraints during crypt development and regeneration. Organoids regenerated these cell types and might be able to reveal their as-yet-unknown function. Moreover, the effects of environmental signals, such as certain human microbes or the human diet, can be species-specific; to understand their roles in crypt development it is essential to use species-matched host cells, something that is readily achieved with organoids.

The second advantage is the throughput of the experimental platform. Both mouse genetic models and organoids provide causal evidence of pharmaceutical and genetic targeting. However, the throughput of organoid-based assays outweighs that of mouse models. Organoids can be plated in large multiplex arrays for drug screening or CRISPR-Cas9 editing. Such an increase of throughput in mouse models would raise miscellaneous issues, such as animal ethics, labor-intensive procedures, and mouse housing costs. Imaging-based screening of organoids has led to the discovery of key molecules regulating crypt development.[8] The advance of organoid-based imaging analysis by overcoming phototoxicity will drive further understanding of the crypt self-renewing mechanism. In addition, the potential differences between homeostatic state and regeneration phase should be taken into consideration because it is generally believed that early organoid growth primarily models regeneration.

Third, organoids provide a tractable technical platform to understand spatiotemporal regulation of crypt morphogenesis. Neonatal intestines start to form a crypt-villus axis along with the initiation of Paneth cell differentiation. After the initial maturation, adult mouse intestines undergo homeostatic regeneration, in which not only are individual cells constantly replaced, but also the total number of crypt units are maintained through a fluid combination of whole-crypt fission or fusion events. Although the dynamics of crypt development could, in principle, be captured by a high-end intravital imaging system, the detailed mechanisms remain elusive because of a lack of a manipulatable experimental platform. Organoid studies will help to bridge this gap. Budding events in organoids are broadly similar to crypt fission, an important mechanism through which crypts regenerate in vivo. Because budding occurs frequently and rapidly in organoids, this process can be interrogated using traditional microscopy. In addition to specific changes in the local density of stem and Paneth cells,[9] factors that drive budding include mechanical force.[10] Thus, organoid-based studies

Table 1. Characteristics of Organoid vs Mouse Models

	Organoids	In vivo mouse models
Species	Human, mouse, rat, pig, and so forth	Mouse
Derivation	Healthy/diseased tissue–derived or pluripotent-stem cell–derived	Wild-type or genetic engineering mouse model
In vitro	+++	++[a]
In vivo	- ~ +[b]	+++
Cost	+ ~ ++	++
Technical difficulty	In vitro: + In vivo: +++	+ ~ ++
Throughput	+++	- ~ +
Genetic manipulation	<1 mo	>3–6 mo
Human genetic relevance	+	-
Relevant microenvironment	- ~ +[c]	+++
Regulation	Ethical committee approval	Animal committee approval

[a]Mouse organoids can also be established from mouse models.
[b]Transplantation enables in vivo assessment.
[c]Coculture system or transplantation recapitulate the microenvironment.

enable high spatiotemporal resolution and perturbation of crypt-villus development.

Although intestinal organoids derived from adult human tissues emulate the diversity of in vivo intestinal epithelial cell types, it is true that they lack vascularization, neural innervation, fluid flow, interactions with other cells (eg, endothelial, mesenchymal, and immune cells), and intestinal luminal contents. To understand these complicated interactions between epithelium and nonepithelial cells, mouse models are currently the best experimental platform. However, in this specific regard, the gap between mouse models and organoids may be quickly closing. There has been some success in the coculture of organoids with other cell types (eg, endothelial cells[11]). However, it remains undetermined to what extent these coculture systems recapitulate the heterocellular interactions in vivo, and further optimization is required to tackle this challenge through the development of advanced culture platforms. As an alternative to the coculture system, organoid transplantation could provide an opportunity for the assessment of heterocellular interactions. We and others have established an organoid transplantation system to investigate epithelium-stromal interaction in vivo.[12] On the heterotopic interaction between small intestinal epithelium and colonic stroma, the engineered colon formed crypt-villi structures and lacteals, unique histologic features found in the small intestine, and rescued short bowel syndrome model in rats.[13] This result suggested that the heterocellular interaction instructs intricate tissue morphogenesis, findings that could be translated to regenerative medicine. The transplantation system can also be used to investigate the human intestinal epithelium in a tissue context. Interestingly, orthotopically transplanted human colonic organoids formed crypts that more closely resembled human colonic crypts than the surrounding mouse crypts.[12] Furthermore, CRISPR-Cas9-mediated genome editing enabled genetic studies of human intestinal epithelium in vivo, such as genetic lineage tracing of human LGR5^{+} ISCs and precise disease modeling of human colon tumorigenesis.[12,14] Therefore, the transplantation system offers a complementary approach to understand stem cell function and crypt morphogenesis of organoids in a tissue context.

In conclusion, organoids are the best research tool to study the biology of human intestinal epithelium and provide a complementary and alternative approach to the understanding of mouse intestinal physiology.

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