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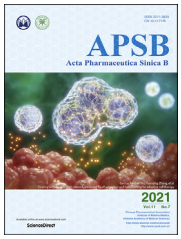

REVIEW

3D organoids derived from the small intestine: An emerging tool for drug transport research

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KEYWORDS

3D organoid;
Small intestine;
Drug transporter;
Caco-2 cell monolayer;
P-glycoprotein

Abstract Small intestine *in vitro* models play a crucial role in drug transport research. Although conventional 2D cell culture models, such as Caco-2 monolayer, possess many advantages, they should be interpreted with caution because they have relatively poor physiologically reproducible phenotypes and functions. With the development of 3D culture technology, pluripotent stem cells (PSCs) and adult somatic stem cells (ASCs) show remarkable self-organization characteristics, which leads to the development of intestinal organoids. Based on previous studies, this paper reviews the application of intestinal 3D organoids in drug transport mediated by P-glycoprotein (P-gp), breast cancer resistance protein (BCRP) and multidrug resistance protein 2 (MRP2). The advantages and limitations of this model are also discussed. Although there are still many challenges, intestinal 3D organoid model has the potential to be an excellent tool for drug transport research.

Abbreviations: ASCs, adult somatic stem cells; BCRP, breast cancer resistance protein; BMP, bone morphogenetic protein; CDF, 5(6)-carboxy-2′,7′-dichlorofluorescein; cMOAT, canalicular multispecific organic anion transporter; DDI, drug−drug interactions; EGF, epidermal growth factor; ER, efflux ratio; ESCs, embryonic stem cells; FGF, fibroblast growth factor; iPSCs, induced pluripotent stem cells; Lgr5^{+}, leucine-rich-repeat-containing G-protein-coupled receptor 5 positive; MCT, monocarboxylate transporter protein; MRP2, multidrug resistance protein 2; NBD, nucleotide-binding domain; OATP, organic anion transporting polypeptide; OCT, organic cation transporter; OCTN, carnitine/organic cation transporter; P_{app}, apparent permeability coefficient; PEPT, peptide transporter protein; P-gp, P-glycoprotein; PMAT, plasma membrane monoamine transporter; PSCs, pluripotent stem cells; Rh123, rhodamine 123; SLC, solute carrier; TEER, transepithelial electrical resistance; TMDs, transmembrane domains.

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1. Introduction

Drug absorption, distribution and elimination are closely related to drug transport, which plays an important role in drug discovery and development[1,2]. It has been generally accepted that small intestine is the main site of drug absorption because of its more permeable epithelium and larger surface area. Therefore, the establishment of an *in vitro* model that can highly simulate the physiological structure and function of the small intestine will provide a reliable tool for the evaluation of drug absorption and excretion.

Up to now, various cell models have been used to imitate the intestinal epithelium for drug transport study *in vitro*. In particular, the Caco-2 cell monolayer model has been widely used as a standard tool for high-throughput screening of drug permeability and identification of substrates and/or inhibitors of drug transporters due to its advantageous differentiation similar to the intestinal enterocytes[3,4]. However, it should be noted that the immortalized cell monolayer could not recapitulate the tissue architecture of the intestinal mucus layer realistically. In addition, the interaction between different types of cells of intestine (including the enterocytes, Paneth cell, enteroendocrine cell, and goblet cell, etc.) does not exist. Thus, Caco-2 monolayer model has its own limitations in drug transport research.

With the development of 3D cell culture technology, 3D organoids are emerging as a burgeoning *in vitro* culture model which can be used in disease model construction[5−7], human organ development[8], drug screening and toxicity testing[9,10], and personalized medicine research[11]. Organoids are 3D self-organizing structure derived from pluripotent stem cells (PSCs) or adult somatic stem cells (ASCs), which can recapitulate the structure and function of organs or tissues *in vivo*[5,12]. Different from traditional 2D cell models, organoid is composed of various cell types with regenerative ability, which perform their own functions and are arranged into three-dimensional tissues. Therefore, the main characteristics of organoid are self-organized, multicellular and functional[13].

Since the first intestinal organoid model was cultured from ASCs of mouse small intestine in 2009[14], more and more organoid culturing systems have been adapted to generate different kinds of mouse and human organoids. As the first generation of organoid, small intestinal 3D organoid may be the most representative example of organoid characteristics. The origins of intestinal organoids are the purified intestinal crypts or single leucine-rich-repeat-containing G-protein coupled receptor 5 positive ($Lgr5^+$) stem cells, which have the capability of long-term growth and expansion of intestinal epithelia cells in Matrigel with a series of essential components of intestinal stem cell niche, including R-spondin-1, epidermal growth factor (EGF) and bone morphogenetic protein (BMP) inhibitor Noggin. Intestinal organoids develop crypt-villus structures with stratified epithelium, which consist of all the major different types of cell lineages in the gut, including columnar epithelial enterocytes with a brush border of apical microvilli (Fig. 1). These organoids show the biological functions of the gut, including absorption and secretion activities[14,15].

As one of the determinants of pharmacokinetics, drug transporters, more specifically, membrane transporters have a great impact on the safety and efficacy of drugs[16]. After oral administration, the drug must pass through the intestinal mucosa to the portal vein. And the membrane transporters expressed in the small intestine play a decisive role in drug absorption. Until now, there are few reports on drug transport mediated by membrane transporters with usage of 3D organoids. In recent years, we have studied the expression and function of ATP-binding-cassette transporters, containing P-glycoprotein (P-gp), breast cancer resistance protein (BCRP) and multidrug resistance associated protein 2 (MRP2) in 3D organoids for drug efflux transport research.

In this paper, we reviewed the methods of culturing mouse and human small intestinal 3D organoids in ASCs and PSCs, and summarized the application of intestinal 3D organoids in transporter-mediated drug transport based on previous research.

2. Intestinal 3D organoids culture

Intestinal organoid is stem cell-derived and self-organizing 3D culture model, which simulates the phenotypic structure, cell composition and partial function of the small intestine[17]. A special media composition can recapitulate the stem cell niche signaling pathway *in vivo*, which is essential for the growth and development of intestinal organoid. The media composition has the ability to maintain the function of stem cells and drive their expansion, and eventually their differentiation[17,18]. Organoids can be initiated from two main types of stem cells: (1) PSCs, including embryonic stem cells (ESCs) and the synthetic induced pluripotent stem cells (iPSCs), which are responsible for organ embryonic development; and (2) ASCs, organ-restricted stem cells, which are crucial for mature organ regeneration and homeostasis[12,17]. For ESCs and iPSCs, the infinite expansion potential is a necessary prerequisite for their discovery and development. On the contrary, once cultured in the proper matrix and the right growth factors, ASCs have shown the ability to grow into organoids, even though they were not thought to proliferate *in vitro*.

2.1. *Intestinal organoids derived from pluripotent stem cells*

Since the establishment of PSC cell lines, biologists have established several types of differentiated cells with the support of developmental biology[12,19,20]. So far, it has been reported that human intestinal organoids come from ESC and iPSC, while mouse intestinal organoids are generated from ESC[5]. WNT and fibroblast growth factor (FGF) signals are known to promote the transformation of endoderm into the middle/hind gut. By adding WNT3A and FGF4, human PSCs treated with activin were cultured into 3D middle/hind gut spheroids from the 2D monolayer epithelium[21−23]. These 3D spheroids were further cultured in Matrigel along with R-spondin-1, EGF and noggin, and eventually differentiated into intestinal organoids. After 1−3 months of

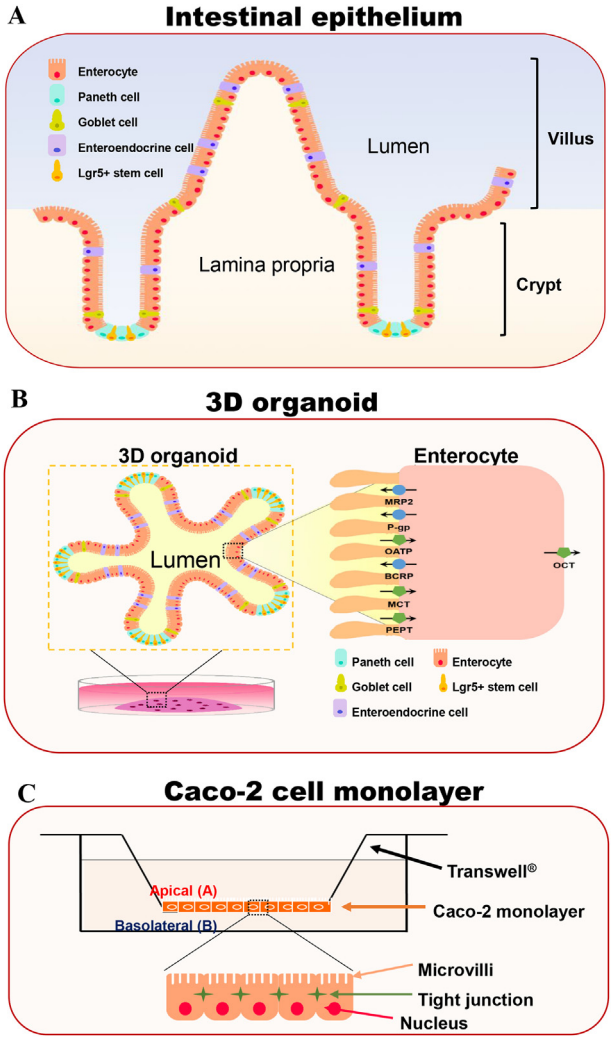


Figure 1 Schematic representation of intestinal epithelium (A), intestinal 3D organoid (B) and Caco-2 cell monolayer (C). Small intestinal epithelial cells are mainly divided into two segments: villus and crypt. The villus part is mainly involved in drug transport, and the crypt part is responsible for the renewal of small intestinal epithelium. The 3D organoids are embedded in laminin-rich Matrigel and cultured in serum-free medium with a defined set of niche factors including R-spondin-1, EGF and the BMP inhibitor Noggin, which contain Paneth cells, enterocytes, goblet cells, Lgr5$^+$ stem cells and enteroendocrine cells. Different kinds of absorption and efflux transporters are expressed on the cytomembrane of enterocytes. While the Caco-2 cells are cultured on the transwell membrane with DMEM medium, and they will form a well-polarized cell monolayer joined by tight junctions after 21 days cultivation.

expansion, the organoids form a polarized intestinal epithelium with villus-like structures and crypt-like regions, containing all known intestinal cell types[24].

2.2. *Intestinal organoids derived from adult stem cells*

Different from the organoids derived from PSCs, ASCs can be induced to form 3D organoids by providing suitable conditions to simulate the niche environment of intestinal stem cells in the process of tissue self-renewal. In 2009, a culture system was established, which allowed the long-term growth and expansion of villus-like epithelial domain from a single sorted Lgr5$^+$ stem cell or purified intestinal crypts[14,24,25]. The single Lgr5$^+$ stem cell or whole crypts were embedded in laminin-rich Matrigel and cultured in serum-free medium, including R-spondin-1, EGF and BMP inhibitor Noggin[14,26]. Within a few days, cystic single cell epithelial structures and central lumen were formed, known as "crypt-like budding". Then the single crypt-like structure changed into multiple discrete budding crypts, in which all types of cells were distributed at normal numbers[14,27]. The 3D organoids showed simple high polarization, the enterocyte brush border of intestinal epithelial cells formed the luminal surface, and the basolateral side faced outward[28]. The organoids are remarkably stable, and can grow every 5−7 days continuously for years, without genetic and phenotypic changes[29].

The growth and differentiation of crypt stem cells are mainly regulated by four signaling pathways[12,30]. First of all, WNT is the key pathway to promote the proliferation and maintain the survival of stem cells. Secondly, the NOTCH pathway helps to maintain the undifferentiated state of proliferating cells. Thirdly, the EGF signals play an important role in promoting the mitosis of stem cells. Finally, the BMP signaling pathway takes active part in the process of 3D organoids development. Therefore, inhibiting BMP signal is a key measure to create a suitable environment for crypt differentiation.

3. Application of intestinal 3D organoids in drug transport research

In recent years, the cultivation technology of 3D organoids is becoming more and more mature and has been widely used in various fields. However, although 3D organoids are a good model, there are few studies on their applications in drug metabolism and pharmacokinetics. In the past few years, we have studied and developed this model, especially in the field of drug delivery. In the next section, we will summarize the applications of 3D organoids in transporter-mediated drug transport research in combination with the published studies and knowledge of this model (Fig. 2).

3.1. *P-gp mediated drug transport in intestinal 3D organoids*

P-gp, an ATP-dependent membrane transport protein, is a 170-kDa single polypeptide with 1280 residues. It consists of two homologous half parts, each containing one nucleotide-binding domain (NBD) and six transmembrane domains (TMDs)[31]. P-gp is present in various tissues and organs, extremely in the small intestine, colon, liver, kidney and brain−blood barrier, which is involved in the pharmacokinetics of drugs[32]. It acts as an efflux pump to affect the absorption of endogenous and exogenous substances, and to protect the body from toxins and xenobiotics[33]. However, the efflux effect can also inhibit drug absorption, promote drug clearance and reduce the bioavailability of oral drugs. In addition, overexpression of P-gp is associated with many diseases, such as Alzheimers disease[34], HIV[35], inflammatory bowel disease[36] and various cancers.

P-gp expressed in intestinal 3D organoids has been detected at both mRNA and protein levels. At the mRNA level, the expression of P-gp in mouse and human intestinal organoids was similar to that in normal intestinal tissues and isolated intestinal crypts[37,38]. At the protein level, immunohistochemical results showed that P-gp maintained physiological expression and located in the apical

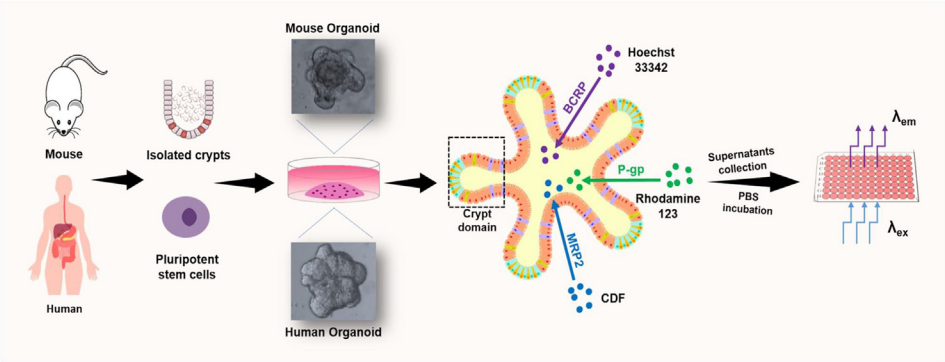


Figure 2 Flow chart of the intestinal 3D organoids cultivation and its application in drug transport evaluation. The intestinal 3D organoids are derived from isolated crypts and synthetic induced pluripotent stem cells. After cultivation in Matrigel with a series of essential niche factors, the stem cells will arrange themselves into cystic epithelial structures with a central lumen and can reflect the physiological architecture and function of the intestine. The fluorogenic probe substrates, Rh123, Hoechst 33342 and CDF, are selected as the substrates of P-gp, BCRP and MRP2 for drug transport study, respectively.

side of mouse and human 3D intestinal organoids, which was consistent with that in small intestine tissue.

The intestinal 3D organoids have been used in the study of drug efflux transport mediated by P-gp. Rhodamine 123 (Rh123), as a typical autofluorescence substrate of P-gp, has been widely used in the detection of drug efflux transport mediated by P-gp. Rh123 can be conducted active trans-epithelial transport by 3D organoids in the basolateral to apical direction after supplement in the medium[39]. In addition, P-gp inhibitors, including verapamil, quinidine and mitotane, were utilized to assess the inhibition of the Rh123 transporting across 3D organoids[37,38,40]. Apart from this, the intestinal 3D organoids were also used as a model to evaluate the inhibitory effect of cucurbitacin E on P-gp, which was consistent with the results of Caco-2 monolayer model[41].

3.2. *BCRP mediated drug transport in intestinal 3D organoids*

BCRP is a 72-kDa membrane transport protein with 655 amino acids, belonging to *ABCG* gene family. As an efflux transporter, BCRP is highly expressed in apical epithelium of small intestine and colon, which can restrict the absorption of its substrates[42]. Recent studies have shown the importance of BCRP in diabetes[43], rheumatoid arthritis[44] and various cancers[45,46].

Both mRNA and protein expression levels of BCRP in intestinal 3D organoids have been investigated[47]. Results in our previous study showed that the mRNA levels of *BCRP* in crypts and villus were different among proximal, middle, and distal small intestine, while no significant difference was found in the organoids cultured from three parts of small intestinal crypts. Therefore, in the study of BCRP-mediated drug transport *via* culturing organoids, it is advisable to select the part from the proximal to the middle of the small intestine. In addition, the mRNA expression of BCRP was not affected by the culture time. Hoechst 33342, the fluorescence probe substrate of BCRP, has been used in the study of drug transport in intestinal 3D organs. BCRP inhibitors Ko143 and YHO-13177 significantly reduced the fluorescence intensity of Hoechst 33342 in the 3D organoids[40,47], which indicated that 3D organoids were a sensitive tool for screening BCRP specific inhibitors.

3.3. *MRP2-mediated drug transport in intestinal 3D organoids*

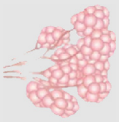
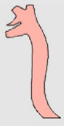
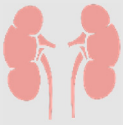
As an important efflux transporter involved in drug−drug interactions (DDI), MRP2 has attracted more and more attention. The transmembrane protein MRP2 contains 1545 amino acids, also known as canalicular multispecific organic anion transporter (cMOAT). MRP2 is expressed in the apical membrane of multiple normal tissues, such as small intestine, liver, kidney, brain and placenta. A large number of studies have shown the relationship between MRP2 and gastric cancer[48], colon cancer[49], breast cancer[50], lung cancer[51] and other tumors[52].

MRP2 was also expressed in mouse intestinal 3D organoids[53], which was located on the inner surface of organoids, the same as P-gp and BCRP. In addition, there were no significant differences in mRNA levels between the proximal, middle and distal parts of the small intestine. MK-571 and probenecid were selected as inhibitors, and 5(6)-carboxy-2′,7′-dichlorofluorescein (CDF) as the substrate of MRP2 for drug efflux transport study. MK-571 and probenecid significantly decreased the accumulation of CDF in 3D organoids. Results showed that 3D intestinal organoid model was a feasible model to evaluate MRP2-mediated drug transport[53].

4. Discussion

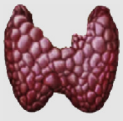
Recently, advanced 3D culture technology has made PSCs and ASCs exhibit their remarkable self-organizing properties[12]. Cultured in specific developmental medium, the brain, inner ear, intestine, kidney, liver, lung, pancreas, retina, stomach, thyroid organoids have been derived from the PSCs isolated from mouse and human tissues. PSCs have been cultured *in vitro* for a long time (murine in 1981 and human in 1998), while ASCs are initially thought to have limited proliferation *in vitro*[54,55]. Since the initial 3D organoids are generated from mouse intestinal isolated ASCs[14], there has been increasing organoid culturing systems from a broad range of mouse and human organs, including breast, colon, esophagus, intestine, lingual epithelium, liver, lung, ovary, pancreas, prostate, salivary gland and stomach. And most of these organoids have been used in the construction of disease and tumor models, human organ regeneration, host−pathogen

Table 1 The murine and human organoids derived from PSCs (including ESCs and iPSCs) and ASCs, and their application in different research and clinical aspects.

Tissue	Species source	Stem cell type	Application	Ref.
 Brain	Human Mouse	ESCs/iPSCs ESCs	Development model Disease model Tumor model Host-pathogen interaction	7,56–61
 Breast	Human	ASCs	Tumor model	62
 Colon	Human/mouse	ASCs	Disease model Tumor model Host-pathogen interaction	63–69
 Esophagus	Human/mouse	ASCs	Tumor model Development model	66,70
 Intestine	Human/mouse Human/mouse Human	ASCs ESCs iPSCs	Disease model Tumor model Gene correction Drug transporters model Host-pathogen interaction	6,9,14,37–41,47, 53,71–77
 Kidney	Human	ESCs/iPSCs	Disease model Tumor model Kidney regeneration	78, 79
 Lingual	Mouse	ASCs	Tumor model	80–82
 Liver	Human/Mouse Human	ASCs iPSC	Disease model Tumor model Liver regeneration Drug metabolism model	8, 83–90
 Lung	Human Human	ASCs ESCs/iPSCs	Development model Disease model Tumor model Host-pathogen interaction	88, 91–95
 Ovary	Human	ASCs	Tumor model	96

(*continued on next page*)

Table 1 (*continued*)

Tissue	Species source	Stem cell type	Application	Ref.
 Pancreas	Human/Mouse Human	ASCs iPSCs	Disease model Tumor model	97−101
 Prostate	Human/mouse	ASCs	Tumor model	102−104
 Retina	Mouse	ESCs	Retinal regeneration Gene correction	105−107
 Stomach	Human/mouse Human Mouse	ASCs iPSCs ESCs	Disease model Host-pathogen interaction	22,108−111
 Thyroid	Human	ASCs	Disease model Thyroid regeneration	112

ESCs, embryonic stem cells; iPSCs, induced pluripotent stem cells; ASCs, adult stem cells.

interaction, gene correction and drug testing. Various murine and human organoids and their application in different research and clinical aspects are summarized in Table 1.

Drug transport is important in the process of drug discovery and development for assessing the DDI[113]. The most relevant organ responsible for drug transport is the small intestine. Therefore, the establishment of a reliable and physiological model of intestinal mucosa *in vitro* is the key to evaluate drug absorption and excretion. Intestinal 3D organoids have been used in gene research and therapy, disease modeling, intestinal organ transplantation, and intestinal tissue development[114]. Although a relatively perfect culture system has been established, until now, there are few studies on the evaluation of drug transport and metabolism by using 3D organoids. Based on previous studies, this paper reviews the application of intestinal 3D organoids in drug efflux transport mediated by P-gp, BCRP and MRP2.

As we all know, Caco-2 monolayer model has been widely recommended by regulatory authorities and pharmaceutical companies as a standard screening method for drug transport evaluation, mainly because it is similar to the innate differentiation of intestinal epithelial cells[115]. Caco-2 cells (from human colon cancer) were cultured in DMEM medium for 21 days to form a well polarized monolayer connected by tight junctions (Fig. 1C). However, it should be noted that the Caco-2 monolayer model is composed of a single cell type, which cannot really recapitulate the tissue architecture of the intestinal mucosa. Under normal physiological conditions, there are five main cell types in the intestinal epithelial layer, including enterocytes, goblet cells, enteroendocrine cells, Paneth cells and stem cells. Each of these cell lines plays an important role in cytoprotection of the intestinal mucosa[116]. Enterocytes, also known as absorptive cells, are responsible for most of the absorption in the small intestine. Goblet cells can release trefoil peptides and mucins, which are the key components of the protective barrier to protect mucosal surface, and also participate in the absorption[117]. Moreover, drug molecules absorbed into the bloodstream are considered to pass through protective barrier[118]. Mature enteroendocrine cells secrete chromaffin granules and cholecystokinin, which are thought to be involved in the regulation of absorption and metabolism[116]. Paneth cells can produce lysozymes and crypt defensins, involving in mucosal barrier. In addition, Paneth cells secrete growth factors and provide a niche for stem cells[116]. Stem cells are responsible for producing these four cell types described above. The 3D organoids are derived from crypts or single stem cells, and have all five different cell types. Therefore, compared with Caco-2 monolayer model, the 3D organoid model is closer to intestinal epithelium in physiology. In addition, it has been reported that the tight junction of Caco-2 cells is more stringent than that of human intestinal epithelial cells, resulting in a decrease of paracellular permeability[115,119]. At the same time, the expression of drug transporters, such as P-gp, in Caco-2 cells is higher than that in primary intestinal epithelial cells, which will enhance the transport activity during drug absorption[115,120]. Therefore, Caco-2 monolayer model has its own limitations in drug transport research. The key features, advantages and limitations between Caco-2 cell monolayer model and 3D organoid model are summarized in Table 2.

Table 2 The comparison between Caco-2 cell monolayer model and 3D organoid model.

Comparison item		Caco-2 cell monolayer model	3D organoid model
Key features	Origin	Derived from human colon cancer	Derived from PSCs or ASCs
	Stemness and multipotency	No	Yes
	In vivo-like complexity	No	Yes
	Easily passaged	Yes	Yes
Advantages		➢ Mature evaluation system ➢ Recommended as a standard screening method for permeability evaluation ➢ Low cost	➢ Various cell types with different functions ➢ Closer to intestinal epithelium in physiology ➢ Multiple applications
Limitations		➢ Single cell type ➢ More stringent tight junction than small intestine epithelial ➢ Higher expression of drug transporters ➢ Time consuming	➢ Unsound evaluation system for drug transport study ➢ High cost

Of course, 3D organoids are derived from normal small intestine cells, and their physiological structure and function are closer to the real situation. However, there are still many aspects of 3D organoids that need to be improved before being accepted by regulatory agencies and pharmaceutical companies for transporter-mediated drug transport evaluation. On the one hand, there is no standard evaluation system. For Caco-2 monolayer model, the robustness criteria of monolayer integrity and drug permeability have been widely recognized. In general, the transepithelial electrical resistance (TEER) values $>300\ \Omega/cm^2$ is usually used as a standard to evaluate the integrity of the monolayers[121]. Apart from this, the apparent permeability coefficient (P_{app}) value is used as a reference for permeability evaluation, and the efflux ratio (ER) value can be used as an indicator for assessing P-gp substrate or inhibitor[41,122]. On the other hand, the choice of transporter probe substrates is limited. According to current reports, only specific fluorescent substrates have been used in drug transport studies. In the future, the HPLC−MS instrument is expected to be applied for the detection of other common substrates. Therefore, further research is needed to support the improvement of the model.

In intestinal epithelium, the solute carrier (SLC) is another important classification of drug transporter, which is involved in drug absorption[123]. The SLC transporter family mainly includes organic anion transporting polypeptide (OATP), organic cation transporter (OCT), carnitine/organic cation transporter (OCTN), peptide transporter protein (PEPT), monocarboxylate transporter protein (MCT), and plasma membrane monoamine transporter (PMAT)[124]. Although the expression of different SLCs in human iPSCs derived organoids is explored[40], the research on drug transport mediated by SLCs still faces great challenges. Due to the valgus structure, SLCs are expressed in the lumen side of intestinal organoids, which makes it hard to directly monitor the transport process. Besides the intestinal tract, transporters expressed in other organs, especially in the liver, are also of great significance. Liver is the main organ of drug metabolism, and intrahepatic transporter-mediated drug evaluation is a hot spot in pharmaceutical research. The 3D organoids derived from the liver have been used in the development of organoid-based drug metabolism model[89] and drug-induced liver fibrosis test[90]. Further studies are needed to identify the expression and function of ABC and SLC transporters in liver.

5. Conclusions

The intestinal organoids, however, do not come without shortcomings, that is, they lack the natural microenvironment composed of innervation, blood vessels and immune cells. Despite of these limitations, the successful development of intestinal organoid culture provides useful knowledge for the study of intestinal epithelial cells and has become a physiological related drug transport model *in vitro*. In this review, we have summarized the application of 3D organoids derived from small intestine in the study of drug efflux transport mediated by P-gp, BCRP and MRP2. The advantages and limitations of the model are also discussed. Although there are still many challenges, intestinal 3D organoid model is becoming a powerful tool for drug transport evaluation. The application of this organ derived model system is expected to narrow the gap between traditional “cell” and “animal” in drug transport evaluation.

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Author contributions

Xin Wang was responsible for the conception and design of the review. Yuanjin Zhang, Shengbo Huang, Wenxia Chen and Bingyi Yao collected literatures. Yuanjin Zhang, Shengbo Huang, Weiguo Zhong and Xin Wang analyzed literatures and summarized results. Yuanjin Zhang and Shengbo Huang drafted the manuscript. Xin Wang and Weiguo Zhong revised the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

References

1. Li AP. Screening for human ADME/Tox drug properties in drug discovery. *Drug Discov Today* 2001;**6**:357−66.
2. Li Y, Meng Q, Yang M, Liu D, Hou X, Tang L, et al. Current trends in drug metabolism and pharmacokinetics. *Acta Pharm Sin B* 2019;**9**:1113−44.
3. Sarmento B, Andrade F, da Silva SB, Rodrigues F, das Neves J, Ferreira D. Cell-based *in vitro* models for predicting drug permeability. *Expet Opin Drug Metabol Toxicol* 2012;**8**:607−21.
4. Antunes F, Andrade F, Ferreira D, Nielsen HM, Sarmento B. Models to predict intestinal absorption of therapeutic peptides and proteins. *Curr Drug Metabol* 2013;**14**:4−20.
5. Dutta D, Heo I, Clevers H. Disease modeling in stem cell-derived 3D organoid systems. *Trends Mol Med* 2017;**23**:393−410.
6. Dekkers JF, Wiegerinck CL, de Jonge HR, Bronsveld I, Janssens HM, de Winter-de Groot KM, et al. A functional CFTR assay using primary cystic fibrosis intestinal organoids. *Nat Med* 2013;**19**:939−45.
7. Garcez PP, Loiola EC, Madeiro da Costa R, Higa LM, Trindade P, Delvecchio R, et al. Zika virus impairs growth in human neurospheres and brain organoids. *Science* 2016;**352**:816−8.
8. Prior N, Inacio P, Huch M. Liver organoids: from basic research to therapeutic applications. *Gut* 2019;**68**:2228−37.
9. Leslie JL, Huang S, Opp JS, Nagy MS, Kobayashi M, Young VB, et al. Persistence and toxin production by *Clostridium difficile* within human intestinal organoids result in disruption of epithelial paracellular barrier function. *Infect Immun* 2015;**83**:138−45.
10. Mittal R, Woo FW, Castro CS, Cohen MA, Karanxha J, Mittal J, et al. Organ-on-chip models: implications in drug discovery and clinical applications. *J Cell Physiol* 2019;**234**:8352−80.
11. Drost J, Clevers H. Organoids in cancer research. *Nat Rev Cancer* 2018;**18**:407−18.
12. Clevers H. Modeling development and disease with organoids. *Cell* 2016;**165**:1586−97.
13. Bartfeld S, Clevers H. Stem cell-derived organoids and their application for medical research and patient treatment. *J Mol Med (Berl)* 2017;**95**:729−38.
14. Sato T, Vries RG, Snippert HJ, 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* 2009;**459**:262−5.
15. Lancaster MA, Knoblich JA. Organogenesis in a dish: modeling development and disease using organoid technologies. *Science* 2014;**345**:1247125.
16. Keogh JP. Membrane transporters in drug development. *Adv Pharmacol* 2012;**63**:1−42.
17. Artegiani B, Clevers H. Use and application of 3D-organoid technology. *Hum Mol Genet* 2018;**27**:R99−107.
18. McCauley HA, Wells JM. Pluripotent stem cell-derived organoids: using principles of developmental biology to grow human tissues in a dish. *Development* 2017;**144**:958−62.
19. Chen KG, Mallon BS, McKay RD, Robey PG. Human pluripotent stem cell culture: considerations for maintenance, expansion, and therapeutics. *Cell Stem Cell* 2014;**14**:13−26.
20. Cherry AB, Daley GQ. Reprogramming cellular identity for regenerative medicine. *Cell* 2012;**148**:1110−22.
21. Munera JO, Wells JM. Generation of gastrointestinal organoids from human pluripotent stem cells. *Methods Mol Biol* 2017;**1597**:167−77.
22. McCracken KW, Cata EM, Crawford CM, Sinagoga KL, Schumacher M, Rockich BE, et al. Modelling human development and disease in pluripotent stem-cell-derived gastric organoids. *Nature* 2014;**516**:400−4.
23. Spence JR, Mayhew CN, Rankin SA, Kuhar MF, Vallance JE, Tolle K, et al. Directed differentiation of human pluripotent stem cells into intestinal tissue *in vitro*. *Nature* 2011;**470**:105−9.
24. Tsakmaki A, Fonseca Pedro P, Bewick GA. 3D intestinal organoids in metabolic research: virtual reality in a dish. *Curr Opin Pharmacol* 2017;**37**:51−8.
25. Sugimoto S, Sato T. Establishment of 3D intestinal organoid cultures from intestinal stem cells. *Methods Mol Biol* 2017;**1612**:97−105.
26. 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* 2011;**469**:415−8.
27. Grun D, Lyubimova A, Kester L, Wiebrands K, Basak O, Sasaki N, et al. Single-cell messenger RNA sequencing reveals rare intestinal cell types. *Nature* 2015;**525**:251−5.
28. Bigorgne AE, Farin HF, Lemoine R, Mahlaoui N, Lambert N, Gil M, et al. *TTC7A* mutations disrupt intestinal epithelial apicobasal polarity. *J Clin Invest* 2014;**124**:328−37.
29. Sato T, Clevers H. Growing self-organizing mini-guts from a single intestinal stem cell: mechanism and applications. *Science* 2013;**340**:1190−4.
30. Clevers H. The intestinal crypt, a prototype stem cell compartment. *Cell* 2013;**154**:274−84.
31. Zhou SF. Structure, function and regulation of P-glycoprotein and its clinical relevance in drug disposition. *Xenobiotica* 2008;**38**:802−32.
32. Dewanjee S, Dua TK, Bhattacharjee N, Das A, Gangopadhyay M, Khanra R, et al. Natural products as alternative choices for P-glycoprotein (P-gp) inhibition. *Molecules* 2017;**22**:871.
33. MacLean C, Moenning U, Reichel A, Fricker G. Closing the gaps: a full scan of the intestinal expression of p-glycoprotein, breast cancer resistance protein, and multidrug resistance-associated protein 2 in male and female rats. *Drug Metab Dispos* 2008;**36**:1249−54.
34. Pereira CD, Martins F, Wiltfang J, da Cruz ESOAB, Rebelo S. ABC transporters are key players in Alzheimer's disease. *J Alzheimers Dis* 2018;**61**:463−85.
35. Miller DS, Bauer B, Hartz AM. Modulation of P-glycoprotein at the blood−brain barrier: opportunities to improve central nervous system pharmacotherapy. *Pharmacol Rev* 2008;**60**:196−209.
36. Panwala CM, Jones JC, Viney JL. A novel model of inflammatory bowel disease: mice deficient for the multiple drug resistance gene, mdr1a, spontaneously develop colitis. *J Immunol* 1998;**161**:5733−44.
37. Zhang Y, Zeng Z, Zhao J, Li D, Liu M, Wang X. Measurement of Rhodamine 123 in three-dimensional organoids: a novel model for P-glycoprotein inhibitor screening. *Basic Clin Pharmacol Toxicol* 2016;**119**:349−52.
38. Zhao J, Zeng Z, Sun J, Zhang Y, Li D, Zhang X, et al. A novel model of P-glycoprotein inhibitor screening using human small intestinal organoids. *Basic Clin Pharmacol Toxicol* 2017;**120**:250−5.
39. Mizutani T, Nakamura T, Morikawa R, Fukuda M, Mochizuki W, Yamauchi Y, et al. Real-time analysis of P-glycoprotein-mediated drug transport across primary intestinal epithelium three-dimensionally cultured *in vitro*. *Biochem Biophys Res Commun* 2012;**419**:238−43.
40. Onozato D, Yamashita M, Nakanishi A, Akagawa T, Kida Y, Ogawa I, et al. Generation of intestinal organoids suitable for pharmacokinetic studies from human induced pluripotent stem cells. *Drug Metab Dispos* 2018;**46**:1572−80.
41. Lu J, Zhang Y, Sun M, Liu M, Wang X. Comprehensive assessment of cucurbitacin E related hepatotoxicity and drug−drug interactions involving CYP3A and P-glycoprotein. *Phytomedicine* 2017;**26**:1−10.
42. Maliepaard M, Scheffer GL, Faneyte IF, van Gastelen MA, Pijnenborg AC, Schinkel AH, et al. Subcellular localization and

distribution of the breast cancer resistance protein transporter in normal human tissues. *Cancer Res* 2001;**61**:3458−64.
43. Liu H, Liu L, Li J, Mei D, Duan R, Hu N, et al. Combined contributions of impaired hepatic CYP2C11 and intestinal breast cancer resistance protein activities and expression to increased oral glibenclamide exposure in rats with streptozotocin-induced diabetes mellitus. *Drug Metab Dispos* 2012;**40**:1104−12.
44. Atisha-Fregoso Y, Lima G, Pascual-Ramos V, Banos-Pelaez M, Fragoso-Loyo H, Jakez-Ocampo J, et al. Rheumatoid arthritis disease activity is determinant for ABCB1 and ABCG2 drug-efflux transporters function. *PLoS One* 2016;**11**:e0159556.
45. Brackman DJ, Giacomini KM. Reverse translational research of ABCG2 (BCRP) in human disease and drug response. *Clin Pharmacol Ther* 2018;**103**:233−42.
46. Chen L, Manautou JE, Rasmussen TP, Zhong XB. Development of precision medicine approaches based on inter-individual variability of BCRP/ABCG2. *Acta Pharm Sin B* 2019;**9**:659−74.
47. Zhang L, Zhao J, Liang C, Liu M, Xu F, Wang X. A novel biosensor based on intestinal 3D organoids for detecting the function of BCRP. *Drug Deliv* 2017;**24**:1453−9.
48. Kamata S, Kishimoto T, Kobayashi S, Miyazaki M. Expression and localization of ATP binding cassette (ABC) family of drug transporters in gastric hepatoid adenocarcinomas. *Histopathology* 2008;**52**:747−54.
49. Durmus S, van der Valk M, Teunissen SF, Song JY, Wagenaar E, Beijnen JH, et al. ABC transporters Mdr1a/1b, Bcrp1, Mrp2 and Mrp3 determine the sensitivity to PhIP/DSS-induced colon carcinogenesis and inflammation. *Arch Toxicol* 2019;**93**:775−90.
50. Hjorth CF, Nielsen AS, Sorensen HT, Lash TL, Damkier P, Hamilton-Dutoit S, et al. Multi-drug resistance protein 2 (MRP2) expression, adjuvant tamoxifen therapy, and risk of breast cancer recurrence: a Danish population-based nested case-control study. *Acta Oncol* 2019;**58**:168−74.
51. Sun N, Sun X, Chen B, Cheng H, Feng J, Cheng L, et al. MRP2 and GSTP1 polymorphisms and chemotherapy response in advanced non-small cell lung cancer. *Cancer Chemother Pharmacol* 2010;**65**:437−46.
52. Sandusky GE, Mintze KS, Pratt SE, Dantzig AH. Expression of multidrug resistance-associated protein 2 (MRP2) in normal human tissues and carcinomas using tissue microarrays. *Histopathology* 2002;**41**:65−74.
53. Zhang L, Liang C, Xu P, Liu M, Xu F, Wang X. Characterization of *in vitro* Mrp2 transporter model based on intestinal organoids. *Regul Toxicol Pharmacol* 2019;**108**:104449.
54. Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. *Nature* 1981;**292**:154−6.
55. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, et al. Embryonic stem cell lines derived from human blastocysts. *Science* 1998;**282**:1145−7.
56. Wells MF, Salick MR, Wiskow O, Ho DJ, Worringer KA, Ihry RJ, et al. Genetic ablation of AXL does not protect human neural progenitor cells and cerebral organoids from Zika virus infection. *Cell Stem Cell* 2016;**19**:703−8.
57. Qian X, Nguyen HN, Song MM, Hadiono C, Ogden SC, Hammack C, et al. Brain-region-specific organoids using mini-bioreactors for modeling ZIKV exposure. *Cell* 2016;**165**:1238−54.
58. Lancaster MA, Renner M, Martin CA, Wenzel D, Bicknell LS, Hurles ME, et al. Cerebral organoids model human brain development and microcephaly. *Nature* 2013;**501**:373−9.
59. Jo J, Xiao Y, Sun AX, Cukuroglu E, Tran HD, Goke J, et al. Midbrain-like organoids from human pluripotent stem cells contain functional dopaminergic and neuromelanin-producing neurons. *Cell Stem Cell* 2016;**19**:248−57.
60. Hubert CG, Rivera M, Spangler LC, Wu Q, Mack SC, Prager BC, et al. A three-dimensional organoid culture system derived from human glioblastomas recapitulates the hypoxic gradients and cancer stem cell heterogeneity of tumors found *in vivo*. *Cancer Res* 2016;**76**:2465−77.
61. Camp JG, Badsha F, Florio M, Kanton S, Gerber T, Wilsch-Brauninger M, et al. Human cerebral organoids recapitulate gene expression programs of fetal neocortex development. *Proc Natl Acad Sci U S A* 2015;**112**:15672−7.
62. Williams KE, Lemieux GA, Hassis ME, Olshen AB, Fisher SJ, Werb Z. Quantitative proteomic analyses of mammary organoids reveals distinct signatures after exposure to environmental chemicals. *Proc Natl Acad Sci U S A* 2016;**113**:E1343−51.
63. 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.
64. van de Wetering M, Francies HE, Francis JM, Bounova G, Iorio F, Pronk A, et al. Prospective derivation of a living organoid biobank of colorectal cancer patients. *Cell* 2015;**161**:933−45.
65. Tsuruta T, Saito S, Osaki Y, Hamada A, Aoki-Yoshida A, Sonoyama K. Organoids as an *ex vivo* model for studying the serotonin system in the murine small intestine and colon epithelium. *Biochem Biophys Res Commun* 2016;**474**:161−7.
66. Sato T, Stange DE, Ferrante M, Vries RG, van Es JH, van 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.
67. Hibiya S, Tsuchiya K, Hayashi R, Fukushima K, Horita N, Watanabe S, et al. Long-term inflammation transforms intestinal epithelial cells of colonic organoids. *J Crohns Colitis* 2017;**11**:621−30.
68. Fujii M, Shimokawa M, Date S, Takano A, Matano M, Nanki K, et al. A colorectal tumor organoid library demonstrates progressive loss of niche factor requirements during tumorigenesis. *Cell Stem Cell* 2016;**18**:827−38.
69. Michels BE, Mosa MH, Grebbin BM, Yepes D, Darvishi T, Hausmann J, et al. Human colon organoids reveal distinct physiologic and oncogenic WNT responses. *J Exp Med* 2019;**216**:704−20.
70. Trisno SL, Philo KED, McCracken KW, Cata EM, Ruiz-Torres S, Rankin SA, et al. Esophageal organoids from human pluripotent stem cells delineate Sox2 functions during esophageal specification. *Cell Stem Cell* 2018;**23**:501−515 e7.
71. Zhang YG, Wu S, Xia Y, Sun J. Salmonella-infected crypt-derived intestinal organoid culture system for host-bacterial interactions. *Phys Rep* 2014;**2**:e12147.
72. Yin Y, Bijvelds M, Dang W, Xu L, van der Eijk AA, Knipping K, et al. Modeling rotavirus infection and antiviral therapy using primary intestinal organoids. *Antivir Res* 2015;**123**:120−31.
73. Schwank G, Koo BK, Sasselli V, Dekkers JF, Heo I, Demircan T, et al. Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. *Cell Stem Cell* 2013;**13**:653−8.
74. Lukovac S, Belzer C, Pellis L, Keijser BJ, de Vos WM, Montijn RC, et al. Differential modulation by *Akkermansia muciniphila* and *Faecalibacterium prausnitzii* of host peripheral lipid metabolism and histone acetylation in mouse gut organoids. *mBio* 2014;**5**. e01438-14.
75. Forbester JL, Goulding D, Vallier L, Hannan N, Hale C, Pickard D, et al. Interaction of *Salmonella enterica* Serovar Typhimurium with intestinal organoids derived from human induced pluripotent stem cells. *Infect Immun* 2015;**83**:2926−34.
76. Ettayebi K, Crawford SE, Murakami K, Broughman JR, Karandikar U, Tenge VR, et al. Replication of human noroviruses in stem cell-derived human enteroids. *Science* 2016;**353**:1387−93.
77. Drost J, van Jaarsveld RH, Ponsioen B, Zimberlin C, van Boxtel R, Buijs A, et al. Sequential cancer mutations in cultured human intestinal stem cells. *Nature* 2015;**521**:43−7.
78. Takasato M, Er PX, Chiu HS, Maier B, Baillie GJ, Ferguson C, et al. Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis. *Nature* 2015;**526**:564−8.
79. Freedman BS, Brooks CR, Lam AQ, Fu H, Morizane R, Agrawal V, et al. Modelling kidney disease with *CRISPR*-mutant kidney organoids derived from human pluripotent epiblast spheroids. *Nat Commun* 2015;**6**:8715.

80. Hisha H, Tanaka T, Ueno H. Lingual epithelial stem cells and organoid culture of them. *Int J Mol Sci* 2016;**17**:168.
81. Hisha H, Tanaka T, Kanno S, Tokuyama Y, Komai Y, Ohe S, et al. Establishment of a novel lingual organoid culture system: generation of organoids having mature keratinized epithelium from adult epithelial stem cells. *Sci Rep* 2013;**3**:3224.
82. Aihara E, Mahe MM, Schumacher MA, Matthis AL, Feng R, Ren W, et al. Characterization of stem/progenitor cell cycle using murine circumvallate papilla taste bud organoid. *Sci Rep* 2015;**5**:17185.
83. Takebe T, Sekine K, Enomura M, Koike H, Kimura M, Ogaeri T, et al. Vascularized and functional human liver from an iPSC-derived organ bud transplant. *Nature* 2013;**499**:481−4.
84. Saheli M, Sepantafar M, Pournasr B, Farzaneh Z, Vosough M, Piryaei A, et al. Three-dimensional liver-derived extracellular matrix hydrogel promotes liver organoids function. *J Cell Biochem* 2018;**119**:4320−33.
85. Huch M, Gehart H, van Boxtel R, Hamer K, Blokzijl F, Verstegen MM, et al. Long-term culture of genome-stable bipotent stem cells from adult human liver. *Cell* 2015;**160**:299−312.
86. Hindley CJ, Cordero-Espinoza L, Huch M. Organoids from adult liver and pancreas: stem cell biology and biomedical utility. *Dev Biol* 2016;**420**:251−61.
87. Broutier L, Andersson-Rolf A, Hindley CJ, Boj SF, Clevers H, Koo BK, et al. Culture and establishment of self-renewing human and mouse adult liver and pancreas 3D organoids and their genetic manipulation. *Nat Protoc* 2016;**11**:1724−43.
88. Dye BR, Hill DR, Ferguson MA, Tsai YH, Nagy MS, Dyal R, et al. *In vitro* generation of human pluripotent stem cell derived lung organoids. *Elife* 2015;**4**:e05098.
89. Park E, Kim HK, Jee J, Hahn S, Jeong S, Yoo J. Development of organoid-based drug metabolism model. *Toxicol Appl Pharmacol* 2019;**385**:114790.
90. Leite SB, Roosens T, El Taghdouini A, Mannaerts I, Smout AJ, Najimi M, et al. Novel human hepatic organoid model enables testing of drug-induced liver fibrosis *in vitro*. *Biomaterials* 2016;**78**:1−10.
91. Rock JR, Onaitis MW, Rawlins EL, Lu Y, Clark CP, Xue Y, et al. Basal cells as stem cells of the mouse trachea and human airway epithelium. *Proc Natl Acad Sci U S A* 2009;**106**:12771−5.
92. Nadkarni RR, Abed S, Draper JS. Organoids as a model system for studying human lung development and disease. *Biochem Biophys Res Commun* 2016;**473**:675−82.
93. Huang SX, Islam MN, O'Neill J, Hu Z, Yang YG, Chen YW, et al. Efficient generation of lung and airway epithelial cells from human pluripotent stem cells. *Nat Biotechnol* 2014;**32**:84−91.
94. Firth AL, Menon T, Parker GS, Qualls SJ, Lewis BM, Ke E, et al. Functional gene correction for cystic fibrosis in lung epithelial cells generated from patient iPSCs. *Cell Rep* 2015;**12**:1385−90.
95. Barkauskas CE, Chung MI, Fioret B, Gao X, Katsura H, Hogan BL. Lung organoids: current uses and future promise. *Development* 2017;**144**:986−97.
96. Kessler M, Hoffmann K, Brinkmann V, Thieck O, Jackisch S, Toelle B, et al. The Notch and Wnt pathways regulate stemness and differentiation in human fallopian tube organoids. *Nat Commun* 2015;**6**:8989.
97. Wills ES, Drenth JP. Building pancreatic organoids to aid drug development. *Gut* 2017;**66**:393−4.
98. Huch M, Bonfanti P, Boj SF, Sato T, Loomans CJ, van de Wetering M, et al. Unlimited *in vitro* expansion of adult bi-potent pancreas progenitors through the Lgr5/R-spondin axis. *EMBO J* 2013;**32**:2708−21.
99. Huang L, Holtzinger A, Jagan I, BeGora M, Lohse I, Ngai N, et al. Ductal pancreatic cancer modeling and drug screening using human pluripotent stem cell- and patient-derived tumor organoids. *Nat Med* 2015;**21**:1364−71.
100. Boj SF, Hwang CI, Baker LA, Engle DD, Tuveson DA, Clevers H. Model organoids provide new research opportunities for ductal pancreatic cancer. *Mol Cell Oncol* 2016;**3**:e1014757.
101. Boj SF, Hwang CI, Baker LA, Chio II , Engle DD, Corbo V, et al. Organoid models of human and mouse ductal pancreatic cancer. *Cell* 2015;**160**:324−38.
102. Karthaus WR, Iaquinta PJ, Drost J, Gracanin A, van Boxtel R, Wongvipat J, et al. Identification of multipotent luminal progenitor cells in human prostate organoid cultures. *Cell* 2014;**159**:163−75.
103. Gao D, Vela I, Sboner A, Iaquinta PJ, Karthaus WR, Gopalan A, et al. Organoid cultures derived from patients with advanced prostate cancer. *Cell* 2014;**159**:176−87.
104. Drost J, Karthaus WR, Gao D, Driehuis E, Sawyers CL, Chen Y, et al. Organoid culture systems for prostate epithelial and cancer tissue. *Nat Protoc* 2016;**11**:347−58.
105. Volkner M, Zschatzsch M, Rostovskaya M, Overall RW, Busskamp V, Anastassiadis K, et al. Retinal organoids from pluripotent stem cells efficiently recapitulate retinogenesis. *Stem Cell Rep* 2016;**6**:525−38.
106. Assawachananont J, Mandai M, Okamoto S, Yamada C, Eiraku M, Yonemura S, et al. Transplantation of embryonic and induced pluripotent stem cell-derived 3D retinal sheets into retinal degenerative mice. *Stem Cell Rep* 2014;**2**:662−74.
107. Deng WL, Gao ML, Lei XL, Lv JN, Zhao H, He KW, et al. Gene correction reverses ciliopathy and photoreceptor loss in iPSC-derived retinal organoids from retinitis pigmentosa patients. *Stem Cell Rep* 2018;**10**:2005.
108. Huang JY, Sweeney EG, Sigal M, Zhang HC, Remington SJ, Cantrell MA, et al. Chemodetection and destruction of host urea allows *Helicobacter pylori* to locate the epithelium. *Cell Host Microbe* 2015;**18**:147−56.
109. Bartfeld S, Clevers H. Organoids as model for infectious diseases: culture of human and murine stomach organoids and microinjection of *Helicobacter pylori*. *JoVE* 2015;**105**:53359.
110. Bartfeld S, Bayram T, van de Wetering M, Huch M, Begthel H, Kujala P, et al. *In vitro* expansion of human gastric epithelial stem cells and their responses to bacterial infection. *Gastroenterology* 2015;**148**:126−136 e6.
111. Barker N, Huch M, Kujala P, van de Wetering M, Snippert HJ, van Es JH, et al. Lgr5^{+ve} stem cells drive self-renewal in the stomach and build long-lived gastric units *in vitro*. *Cell Stem Cell* 2010;**6**:25−36.
112. Saito Y, Onishi N, Takami H, Seishima R, Inoue H, Hirata Y, et al. Development of a functional thyroid model based on an organoid culture system. *Biochem Biophys Res Commun* 2018;**497**:783−9.
113. Elmeliegy M, Vourvahis M, Guo C, Wang DD. Effect of P-glycoprotein (P-gp) inducers on exposure of P-gp substrates: review of clinical drug−drug interaction studies. *Clin Pharmacokinet* 2020;**59**:699−714.
114. Chusilp S, Li B, Lee D, Lee C, Vejchapipat P, Pierro A. Intestinal organoids in infants and children. *Pediatr Surg Int* 2020;**36**:1−10.
115. Li N, Wang D, Sui Z, Qi X, Ji L, Wang X, et al. Development of an improved three-dimensional *in vitro* intestinal mucosa model for drug absorption evaluation. *Tissue Eng C Methods* 2013;**19**:708−19.
116. Yin YB, de Jonge HR, Wu X, Yin YL. Enteroids for nutritional studies. *Mol Nutr Food Res* 2019;**63**:e1801143.
117. Kong SE, Heel K, McCauley R, Hall J. The role of enterocytes in gut dysfunction. *Pathol Res Pract* 1998;**194**:741−51.
118. Le Ferrec E, Chesne C, Artusson P, Brayden D, Fabre G, Gires P, et al. *In vitro* models of the intestinal barrier. The report and recommendations of ECVAM Workshop 46. European Centre for the Validation of Alternative methods. *Altern Lab Anim* 2001;**29**:649−68.

119. Sun H, Chow EC, Liu S, Du Y, Pang KS. The Caco-2 cell monolayer: usefulness and limitations. *Expet Opin Drug Metabol Toxicol* 2008;**4**:395−411.
120. Press B, Di Grandi D. Permeability for intestinal absorption: Caco-2 assay and related issues. *Curr Drug Metabol* 2008;**9**:893−900.
121. van Breemen RB, Li Y. Caco-2 cell permeability assays to measure drug absorption. *Expet Opin Drug Metabol Toxicol* 2005;**1**:175−85.
122. Wessler JD, Grip LT, Mendell J, Giugliano RP. The P-glycoprotein transport system and cardiovascular drugs. *J Am Coll Cardiol* 2013;**61**:2495−502.
123. Yin J, Wang JN. Renal drug transporters and their significance in drug−drug interactions. *Acta Pharm Sin B* 2016;**6**:363−73.
124. Estudante M, Morais JG, Soveral G, Benet LZ. Intestinal drug transporters: An overview. *Adv Drug Deliv Rev* 2013;**65**:1340−56.