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REVIEW ARTICLE

Organoid models of gastrointestinal Neoplasms: Origin, current status and future applications in personalized medicine

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Abstract The *in vitro* organoid model is a major technological breakthrough that has been established as an important tool in many basic biological and clinical applications. This near-physiological 3D culture system accurately models various biological processes, including tissue renewal, stem cell/niche functions and tissue responses to drugs, mutations or damage. Organoids have the potential value of being an accurate model for disease predictions or drug screening applications and to identify the ideal treatment for that patient. Carcinogenesis can be modeled by mutating specific cancer genes in wild-type organoids; and patient-derived organoids provide an important resource in the development of personalized cancer treatment. Organoids from cancer patients could be used to identify the ideal treatment for a specific patient by growing matched healthy and diseased organoids from human cancer patients which additionally enables clinical screens for drug combinations. Organoids could also provide autologous cells or—in the future—tissue for transplantation. In this review, we discuss the current advances, challenges and potential applications of this technique in gastrointestinal neoplasms.

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Introduction

Organoids are three-dimensional (3D) *in vitro* culture systems derived from self-organizing stem cells. They can recapitulate the *in vivo* architecture, functionality, and genetic signature of primary tissues. Under specific growth conditions, stem cells proliferate and form complex structures that represent an ideal tool to study developmental processes and tissue maintenance mechanisms due to the ease of genetically manipulating the cells. This technique is widely used within the research community, mainly because of the potential of organoids to model many complex *in vivo* processes *in vitro*. In addition, although stem cells are somatic cells, they can be cultivated far beyond the Hayflick limit[1] while maintaining their genomic stability.[2] (see Table 1, Figs. 1 and 2)

Development of organoid culture for gastrointestinal cells

The term "organoid" was initially used in oncology as a term synonymous with teratomas (for example, dermoid cysts) and was reported as early as 1946.[3] From the 1960s onward, the term was applied to organotypic cultures that self-organized during cell sorting and reaggregation experiments conducted by developmental biologists.[4,5] With renewed interest in organoids manifesting within the past decade, the definition of organoids was refined to 3D structures that were grown *in vitro and* derived from pluripotent stem cells (PSCs) or adult stem cells (ASCs) that self-organize into a near-native microanatomy with organ-specific differentiated cell types and tissue compartmentalization. Requisite for the initial development of the culture system of murine small intestinal epithelial organoids were several advancements in the understanding of the intestinal stem cell niche. First, leucine-rich repeat containing G-protein-coupled receptor 5 (Lgr5) was identified as an intestinal stem cell-specific marker gene, allowing the characterization and purification of these stem cells.[6] Subsequently, the understanding that adult intestinal stem cells can be both proliferative and long lived *in vivo*[6] led to the use of isolated Lgr5+ stem cells as a putative source for organoid cultures. Following the discovery of Wnt signaling as an essential cellular signaling pathway for stem cell maintenance

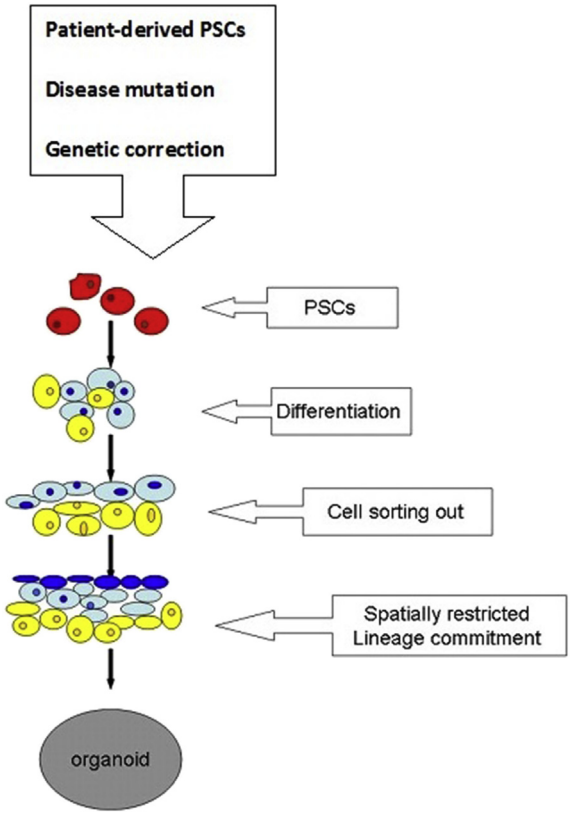

Figure 1 Organoid generation: Organoids derived from human pluripotent stem cells (PSCs) can be produced into a number of organs. Like organogenesis in vivo, organoids self-organize through both cell sorting out and spatially restricted lineage commitment of precursor cells.

Table 1 Organoid culture conditions for gastrointestinal organ epithelium.

Tumor Species	Expansion culture conditions	Differentiated culture conditions
Stomach organoids	(h, m)Wnt3a, R-spondin, ECF, Noggin, FGF-10, nicotinamide,gastrin	(m)EGF, R-sponding, Noggin
Small intestinal organoids	(m)Wnt3a, R-spondin, EGF, Noggin nicotinamide, gastrin	(m)R-spondin, EGF, Noggin
	(h)(m) + TGF-βi, p38i	(h)EGF, Noggin, TGF-βi,
Colon organoids	(m)Wnt,R-spondin,ECF, Noggin gastrin	(m)R-spondin, EGF, Noggin
	(h)(m) + TGF-βi,p38i	(h) EGF, Noggin, TGF-βi
Pancreatic organoids	(m)R-spondin, ECF, Noggin, FGF-10, nicotinamide, gastrin	(m)Matrigel, 0.2%FBS, Activin A (3 days)
	(h)(m) + Wnt3a, TGF-βi	ILV, FGF-10, 2%FBS(4—5 days) 1%B27, Noggin,RA, Cyclo (6 days) 1%B27, NOTCHi (2—4 days)

Abbreviations. (h) indicates human organoids (m) indicates mouse organoids,; TGF-βi, TGF-β inhibitor; p38i, p38 inhibitor; NOTCHi, Notch inhibitor; ILV, indolatam V; RA, retinoic acid; Cyclo, cyclopamine; FBS, fetal bovine serum; (m)+ means in addition to mouse organoid culture conditions.

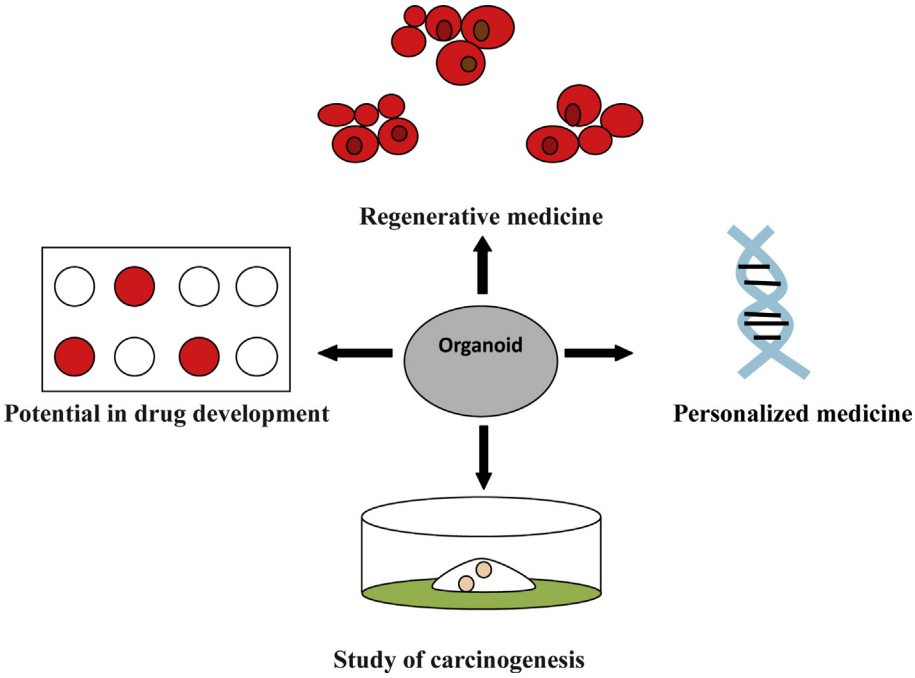

Figure 2 Applications of organoids: Applications of organoids provide the opportunity for drug testing, potentially allowing the development of personalized treatment strategies. The ability to establish and expand organoids derived from a single adult stem cell and subsequently transplant these organoids into experimental animal models raises the possibility of using organoids in regenerative medicine.

in vivo,[7,8] the observation that R-spondins, later identified as Wnt agonists that bind to Lgr5,[9] are mitogens that cause stem cell hyperplasia,[10] and the study revealing the contrasting roles of tyrosine kinase receptor signaling, with epidermal growth factor (EGF) as another potent mitogen and the bone morphogenetic protein (BMP) inhibitor Noggin as crucial for the maintenance of stem cell niche[11–13] organoid culture medium was developed and contains EGF, Noggin, and R-spondin. In addition, a Rho kinase (ROCK) inhibitor was added to primary cultures to inhibit anoikis, which was previously observed in purified colonic epithelial cells.[14] Finally, embedding of purified Lgr5+ stem cells in Matrigel as an *ex vivo* substitute for the extracellular matrix (ECM) was performed based on previous experience with feeder layer cultures showing that stem cell clonogenicity is further enhanced by fibroblasts producing ECM to support stem cell growth and preincubation of tissue culture plastic with ECM proteins such as collagen or laminin.[15] Furthermore. ECM-based hydrogels such as Matrigel foster 3D aggregation and polarization of stem cells.[16,17] Stem cell proliferation first resulted in cystic spheroids, which then formed crypt-like buddings that further developed into "mini-guts" with distinct crypt-villus compartmentalization within 2 weeks as seen *in vivo*. The entire epithelium consisted of a monolayer of fully polarized epithelial cells in direct contact with the ECM provided by the Matrigel. The crypt-like structures were composed of Lgr5+ stem cells intermingled between Paneth cells and, above the crypt bottom, transit-amplifying progenitor cells, while the villus-like domain contained fully differentiated enterocytes. Scattered throughout the organoid epithelium were the other two mature intestinal cell types: goblet cells, and enteroendocrine cells. Oudenaarden and colleagues sequenced the mRNA transcripts purified from single cells of intestinal epithelial organoids and found a heterogeneous population of cells reflective of the *in vivo* epithelium in these cultures.[18] Furthermore, organoids could also be derived from individual intact crypts isolated from the intestinal epithelium by ethylenediaminetetraacetic acid (EDTA) digestion. Contrary to established studies on organoids, one study showed that self-organizing near-native intestinal epithelial structures can be built from single stem cells in the absence of a mesenchymal cellular niche.[19] Subsequently, the addition of Wnt3a allowed for the generation of organoids from mouse colon crypts as well as from purified Lgr5+ colonic stem cells.[20] Furthermore, the addition of the niche factors Wnt3a, EGF, Noggin, and R-spondin-1, as well as the addition of nicotinamide, A83-01 (a small-molecule inhibitor of transforming growth factor β (TGFβ) type I receptor kinase, also known as activin-like kinase 5 (ALK5)), and SB202190, a p38 mitogen-activated protein kinase (MAPK) inhibitor, were required for the eventual establishment of long-term organoid cultures derived from primary adult human small intestinal or colonic epithelial tissue.[20,21] Later, Jensen and colleagues succeeded in adapting this protocol to generate organoid cultures from both mouse and human fetal intestinal epithelium.[22] Modifications of the growth factors provided in the original intestinal organoid culture medium allowed the establishment of epithelial organoid cultures from several other murine and human gastrointestinal organs, including the gallbladder,[23] liver,[2,24] pancreas,[25,26] and stomach[27–29]

Intestinal organoids

The intestinal organoid culture system has been applied to various samples of digestive tissue epithelium and diseased epithelium, including mouse intestinal adenoma and human colorectal cancer (CRC) cells.[20,30] Because Wnt signaling is aberrantly activated in mouse adenoma and most human CRCs, organoids derived from tumor epithelium readily proliferate independent of Wnt and R-spondin. Presumably for similar reasons, CRC cells can often proliferate with fewer niche factors than their normal counterparts. Importantly, however, CRC cells often remain dependent on some niche factors required for normal intestinal stem cells (ISCs), suggesting that these factors may play a role in the maintenance of cancer stem cells (CSCs). In CRC organoid cultures, the success rate of establishing a culture is superior to that of previously reported culture systems with noncancerous cells. Furthermore, single CRC cells are immobilized in Matrigel, and their clonal CRC organoids can be tracked on a real-time basis, which may enable visualization of the self-renewal capacity of CSCs in a dish. Their clonal expansion capacity could be applied to various biomedical analyses, including deep sequencing, which normally requires genomic DNA on the order of micrograms. When combined with integrated molecular information, the establishment of "living biobanks" would be a useful resource for both basic research and clinical applications.

A striking advantage of CRC organoids is their expansion efficiency (approximately 1000 divisions per month), enabling the quick preparation of a large number of CRC cells in a short time. This scalability and rapid expandability make organoid culture suitable for drug testing and personalized medicine using patient-derived cancer samples. Although it is not possible to perfectly recapitulate patient-derived cancer using a certain experimental platform, the best option at this stage is to use a combination of two complimentary systems (depending on the applications): patient-derived xenografts (PDXs) and CRC spheroids/organoids.

There are some drawbacks in CRC organoid culture. Organoids are composed of pure epithelial cells, making it difficult to assess the effect of treatments targeting non-epithelial cells, such as endothelial cells or immune cells. Anti-VEGF therapy targeting tumor vascularization has been used clinically for CRC, but assessing this targeted therapy is more difficult in CRC organoids than in the PDX model, in which mouse-derived endothelial cells migrate into xenografted CRC tumors and form the tumor vasculature.[31,32] Although CRC organoids maintain glandular histologic structures and retain some differentiation capacity, it remains unknown to what extent these organoids could mimic the tumor in the patient's body.

Stomach organoids

The first gastric organoid system developed was a direct advancement of the above mentioned original intestinal culture system from the Clevers group.[19] Meritxell Huch isolated murine antral gastric glands, embedded them into ECM and identified WNT, FGF-10 and GAST as stomach-specific niche factors required in addition to the intestinal factors RSPO, EGF and NOG to allow growth of the glands into organoids.[27] Murine antral organoids formed more cystic structures than their intestinal counterparts but also have small buds that harbor Lgr5+ stem cells. Cells in the organoids differentiate into Mucin 6 (MUC6)+ neck cells, MUC5AC + pit cells, pepsinogen C (PGC)+ chief cells and enteroendocrine cells. After the glands are seeded, they expand within approximately 1 week to full size and can be split at a ratio of 1:5 every week, and cultures can be expanded long term (9 months at initial publication).[27] The same conditions also allow culture of organoids from the murine corpus[29,33] and isolated single Tnfrsf19/TROY + chief cells.[20] Corpus organoids also harbor neck, pit, chief and enteroendocrine cells.[20] In addition, parietal cells are present in the initial seeding, but their numbers decrease over time.[33] To enable research on human stem cells, this culture system was adapted for human intestine, which, in addition to WNT, EGF, NOG, and RSPO, requires inhibition of transforming growth factor β (TGF-β) signaling, inhibition of p38 signaling and the addition of nicotinamide to inhibit sirtuin activity. A ROCK inhibitor is also added after seeding to inhibit anoikis.[20] The conditions used to grow mouse gastric organoids plus the addition of nicotinamide allow the initial growth of human organoids[34] but not their long-term expansion.[28] The culture conditions used to grow human intestinal organoids also support the growth of gastric glands into spheres.[35] The minimal and optimized culture for human gastric stem cells (including only EGF, NOG, RSPO, WNT, FGF10, and GAST as well as TGF-β inhibition) allows the growth of gastric glands or single cells into complex organoids with a cystic body comprising mostly pit cells and glandular buddings containing neck cells, chief cells and an extremely small number of enteroendocrine cells.[28] By manipulating the culture conditions, researchers could differentiate organoids into three different types distinguished by differences in the number of mucus-producing cells. The first one consists of all four gastric lineages and is termed the "complete type", the second has only the gland domain and is termed the "gland type", and the third contains mainly pit cells and is termed the "pit type". Although these cultures lack parietal cells, they contain the other four different cell types and can be maintained for more than a year without the loss of expansion or differentiation capacity.[28] ASC-derived organoids are probably the most practical because they can self-renew and thus can be cultured long term. After the organoids are initially established, they have a short maturation time of only one (for mice) or two (for humans) weeks. An interesting feature of this system is that it allows the expansion of healthy tissue stem cells, as well as cells from gastric metaplastic diseases, such as Barrett's esophagus[20] or gastric cancer cells [28]. Thus, both cancer and healthy organoids can be established from the same patient, rendering this an ideal model for drug testing.[28,36,37]

GS-GCs could accumulate functional genetic mutations through copy neutral loss of heterozygosity (CN-LOH) without any evident sign of aneuploidy. Indeed, copy number analysis identified an additional CN-LOH on chromosome 20. Although such precise genetic analyses of GS-GCs have been challenging owing to their low tumor purity, organoid-based cancer cell expansion enabled the

detection and characterization of minor genetic events occurring in GS-GC tissues.[38]

Pancreas organoids

A group led by Muthuswamy[38] developed pancreatic exocrine progenitor organoids from human PSCs with global gene expression similar to the human pancreas. The authors also developed another efficient model to generate 3D organoids from human pancreatic adenocarcinoma. They cultured the cells as an overlay in media on top of a Matrigel bed. This culture medium was supplemented mainly with B27, ascorbic acid, insulin, hydrocortisone, fibroblast growth factor 2, all-trans retinoic acid, and Y267632. Interestingly, the organoid culture does not require stimulation of Wnt signaling. The group also demonstrated similar drug sensitivities (using epigenetic regulators, specifically EZH2 inhibitors) between 3D organoids and the matched primary tumor. A similar approach, namely, inducing pluripotency in pancreatic cancer patient-derived cells and differentiating these cells into the pancreatic ductal lineage to recapitulate carcinogenesis, has been reported.[39] Remarkably, this study led to the use of thrombospondin-2 in combination with the established serum marker CA19-9 to detect early-stage pancreatic cancer, thus underscoring the translational potential of organoids.[40] The Skala group published another methodology for pancreatic tumor organoids that allows the propagation of fibroblasts and tumor cells within a 1:2 mixture of Matrigel and supplemented media.[41] The culture media contains 10% fetal bovine serum, 1% penicillin-streptomycin, and 10 ng/mL EGF receptor. These conditions allow the formation of tumor cells with fibroblasts from human and murine pancreatic ductal adenocarcinoma (PDAC). Finally, the Kuo group published a methodology based on an air-liquid interface that consists of a Transwell (Corning) with collagen gel coating on the inner chamber that is directly exposed to air; therefore, cells seeded on this surface are in contact with high levels of oxygen. This approach uses a matrix comprising type I collagen instead of Matrigel, which permits 3D organoid growth from neonatal or adult murine tissues without the need for exogenous growth factor supplementation.[42,43]

Liver organoids

Although quiescent in healthy adult liver, Lgr5+ cells can be induced following liver damage in bile ducts. Lineage tracing reveals that Lgr5+ cells can generate both hepatocytes and biliary duct cells *in vivo*, indicating that Lgr5 is also a marker for hepatic stem cells. The gene expression profile of these ductal Lgr5+ cells is similar to that of Lgr5+ ISCs, which is consistent with their similar biological properties. Unsurprisingly, biliary duct fragments or single Lgr5+ cells could be successfully expanded in a previously established culture system of intestinal organoids. Biliary duct-derived organoids possess bipotentiality of differentiation, which indicates that they could be biased toward hepatocyte maturation when cultured with differentiation medium.[24] In addition to their expression of hepatocyte markers, differentiated organoids can also exhibit hepatocyte function, including low-density lipoprotein uptake, glycogen accumulation, albumin secretion and cytochrome function. Recently, Huch et al.[20] successfully cultured human liver organoids from healthy donor liver biopsy specimens and suggested that the organoids exhibited hepatocyte functions comparable to those of freshly isolated hepatocytes.[2]

To test the genetic stability of human liver organoids during expansion, Huch et al performed two cloning steps followed 3 months of culture and then whole genome sequencing (WGS) analysis to determine the accumulation of genomic variation in a single cell. They showed that compared with induced pluripotent stem (iPS) cell reprogramming, expansion of adult liver organoids introduced 10-fold fewer base substitutions. The genetic stability of human liver organoids after long-term culture also reflected no gross chromosomal aberrations or copy number variants (CNVs).[20] Embryonic stem (ES) cells and iPS cells are two potent alternatives in organoid culture when attempting to create epithelial-based organoids. However, recent studies have revealed that during the processes of derivation and reprogramming, genetic and epigenetic aberrations occur,[2,24,25] including chromosomal abnormalities, CNVs and somatic coding mutations.[26–28] These aberrations complicate their use in regenerative medicine.[44]

Applications of organoid technology

The capability to grow near-physiological, self-renewing organoids in culture provides us with an excellent model system for a wide range of both basic research and translational applications. A major advantage of this system is the ability to greatly expand both tissue-specific stem cells and their differentiated progeny from highly limited amounts of starting material (such as biopsies); this expansion ability facilitates in-depth analyses of stem cell behavior, drug screening, disease modeling and genetic screening. The success of this process has spurred efforts to create cryopreserved biobanks of healthy and diseased human organoids as a renewable resource that is accessible to researchers worldwide.[24]

Organoids in the study of carcinogenesis

Carcinogenesis can be modeled by mutating specific cancer genes in wild-type organoids. The first study used lentiviral-based ectopic expression or silencing of cancer genes such as KRAS or TP53 in hiPSC-derived pancreatic organoids.[2] However, the advent of CRISPR/Cas9 genome editing has greatly enhanced the capabilities of organoids as a cancer model. Specifically, two research groups have shown that sequential mutation of APC, TP53, KRAS and SMAD4 by CRISPR in intestinal organoids reproduces colorectal cancer progression *in vitro*.[36,44] Orthotopic transplantations of intestinal organoids carrying different combinations of those gene mutations allowed us to evaluate the specific weight that each individual gene contributes to the metastatic process.[38] Similarly, mutations were introduced in genes responsible for DNA repair, such as MLH1 and NHTL1.[45] After the initial editing and single cell cloning of the

mutated organoids, the mutant organoids were subcloned after a fixed period of 2—3 months. The subclones were expanded, and WGS of the clones and subclones revealed the accumulation of mutational changes over time. This allowed us to determine the consequent gene mutation rate and signatures.[45] Therefore, in addition to its contribution to tumor modeling, targeted gene editing can potentially dissect specific gene roles in the carcinogenic process. In conclusion, organoid culture has revealed itself as a valuable resource for cancer research.[46] Recent works also aimed to integrate 3D cultures of epithelial organoids with nonepithelial cells, such as stromal and immune cells, to obtain a cancer model that incorporates the tumor microenvironment.[47,48] These studies, however, are still at an early stage but could constitute interesting future developments of the field.[49]

Potential of organoids in drug development

Many existing 2D cell lines harbor multiple culture-induced mutations or contamination from other cell lines that limit their value as an accurate model for disease predictions or drug screening applications.[50,51] Indeed, overreliance on such inherently nonphysiological models is likely to have contributed to the high failure rate of many drug discovery programs over the past two decades. This setback has fueled efforts to develop high-throughput screening methods that incorporating the far more stable and more physiological patient-derived organoids for use in early drug discovery programs and toxicity screens.[52,53] Patient-derived organoids represent an important resource in the development of personalized treatment regimens. *In vitro* amplification of patient organoids from disease-site biopsies can deliver sufficient material for deep sequencing to reveal causal mutations or for in-depth phenotypic profiling, which can facilitate more tailored treatment regimens. The ability to grow matched healthy and diseased organoids from human patients additionally enables clinical screens for drug combinations that selectively target the diseased tissue, helping to identify more effective treatments with minimal side effects. Many of the side effects of anticancer drugs can be attributed to acute liver toxicity. One could therefore envision the use of hepatic organoids to predict the *in vivo* liver toxicity of experimental drug combinations before commencing expensive clinical trials.[54] Other clinical applications include the use of disease-derived organoids to predict the acquisition of drug resistance and to develop drugs that effectively target candidate CSCs. Furthermore, organoids can be tracked over time via 4D microscopy to assess cancer stem cell behavior and viability in response to active drugs to predict patient outcomes.

Personalized medicine

As the miniaturized avatar of an organ from a specific patient, organoids have the potential to identify the ideal treatment for that patient. A prime example is cystic fibrosis. While CF is fairly common (incidence of approximately 1 in 3000 live births), some of the CFTR mutations are rare, and patients with rare mutations may not receive the ideal treatment. This was the case for the first CF patient treated on the basis of their organoid screening results: the one drug available in the Netherlands at that time was neither prescribed nor reimbursed for patients with this specific mutation because it was too rare to have been tested in a clinical trial. Researchers grew organoids from a rectal biopsy from the patient and, using the forskolin-induced swelling assay, identified a positive response to the drug Kalydeco. A second patient presented the same rare mutation. The treatment was given to the patients, who both showed significant improvements.[55] After this initial translational success, blinded follow-up studies with larger patient cohorts have now been initiated. Based on this approach, organoids from cancer patients could be used to identify the ideal treatment for a specific patient, but because cancer organoids retain the genetic heterogeneity of the primary tumor, it is likely that the same resistant clones may manifest under the application of a specific drug as occurs *in vivo*, thus predicting the acquisition of drug resistance during treatment. Ongoing studies will have to demonstrate the accuracy of these predictions.[56]

Regenerative medicine

Materials for transplantation are always scarce, and alternative sources are urgently needed. As organoids can be initiated from minuscule amounts of donor cells, expanded and differentiated *in vitro*, they could provide autologous cells or—in the future—tissue for transplantation. Organoids have already been transplanted into the murine colon, where they engrafted and retained typical organ features such as tissue architecture and cell differentiation status.[22,57] Similarly, human liver organoids have been engrafted into mouse liver, and kidney organoids transplanted under the kidney capsule have become vascularized.[2,58,59] Future studies need to show whether grafts can execute all functions of the native tissue. Autologous organoid transplantations would also allow CRISPR/Cas9-mediated gene correction of disease-causing mutations.[56]

Challenges of organoid modeling

The use of organoids, however, do not come without shortcomings. They are devoid of the native microenvironment consisting of stromal cells, muscle cells, blood vessels, and immune cells. The development of coculture conditions of organoids with immune cells or other cell types constitutes the next frontier. However, *in vivo* tumors harbor a wide range of genetic heterogeneity, and it remains unclear if tumoroids grown *in vitro* can capture whole heterogeneous populations stemming from the original tumors. One cannot exclude the possibility that established culture conditions may be suitable for the growth of particular subpopulations of tumor cells. In addition, the key components used to implement 3D culture (e.g., Matrigel and its relative basement membrane extract, BME) are animal-derived matrices that are poorly defined and are not mechanically pliable after plating. Clinical applications will warrant a transition to better defined and mechanically dynamic matrices to fully exploit

the potential of organoid technologies. Hybrid polyethylene glycol (PEG) hydrogels represent one example of such next-generation 'designer matrices' that could expand the applicability of organoids.[24] Nonetheless, it should be noted that, although unlikely, there is always a possibility that an organoid culture will not be successfully established, particularly if the source cells are derived from diseased tissue, and establishment may be limited by factors such as biopsy size and/or differential growth factor requirements.

Outlook and future prospects

Organoids are one of the most accessible and physiologically relevant models to study the dynamics of stem cells that can be derived from various sources and monitored in a controlled environment, as exemplified by epithelial organoids. In combination with genetic, transcriptome and proteomic profiling, both murine- and human-derived organoids have revealed crucial aspects of development, homeostasis and disease. The progress in generating organoids that faithfully recapitulate human tissue composition *in vivo* has extended organoid applications from just a basic research tool to a translational platform with a wide range of uses. The capacity to indefinitely culture organoids without introducing genetic variation makes these systems a sound model for conducting high-throughput preclinical screenings, designing targeted and personalized therapies, and providing a source of fully functional tissue for regenerative medicine applications.

As interest in organoid technology grows, the commercial development of more standardized, validated organoid culture media will also be valuable to ensure that the organoid system becomes accessible to various academic and clinical scientists, thereby helping to maximize the potential of organoid-based research. When coupled with a more defined ECM, a highly accurate, reproducible culture model could emerge, overcoming current limitations that hinder the technology's transition from bench to bedside. Organoid technology has synergized well with current methodologies and has engendered a wide range of downstream functions and applications, underscoring its broad applicability and potential for manipulation. These features, in conjunction with the physiological relevance of the system, make organoids one of the most exciting and promising technologies that have emerged in recent times to study human development, disease and therapy.

Conflict of interests

The author has no conflict of interest to disclose.

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References

1. Hayflick L. The limited in vitro lifetime of human diploid cell strains. *Exp Cell Res*. 1965;37:614—636.
2. Huch M, Gehart H, van Boxtel R, et al. Long-term culture of genome-stable bipotent stem cells from adult human liver. *Cell*. 2015;160:299—312.
3. Smith E, Cochrane WJ. Cystic organoid teratoma: (report of a case). *Can Med Assoc J*. 1946;55:151—152.
4. Lancaster MA, Knoblich JA. Organogenesis in a dish: modeling development and disease using organoid technologies. *Science*. 2014;345:124—125.
5. Weiss P, Taylor AC. Reconstitution of complete organs from single-cell suspensions of chick embryos in advanced stages of differentiation. *Proc Natl Acad Sci USA*. 1960;46:1177—1185.
6. Barker N, van Es JH, Kuipers J, et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature*. 2007;449:1003—1007.
7. Korinek V, Barker N, Moerer P, et al. Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. *Nat Genet*. 1998;19:379—383.
8. Pinto D, Gregorieff A, Begthel H, Clevers H. Canonical Wnt signals are essential for homeostasis of the intestinal epithelium. *Genes Dev*. 2003;17:1709—1713.
9. de Lau W, Barker N, Low TY, et al. Lgr5 homologues associate with Wnt receptors and mediate R-spondin signalling. *Nature*. 2011;476:293—297.
10. Kim KA, Kakitani M, Zhao J, et al. Mitogenic influence of human R-spondin1 on the intestinal epithelium. *Science*. 2005;309:1256—1259.
11. Clevers H. The intestinal crypt, a prototype stem cell compartment. *Cell*. 2013;154:274—284.
12. Haramis AP, Begthel H, van den Born M, et al. De novo crypt formation and juvenile polyposis on BMP inhibition in mouse intestine. *Science*. 2004;303:1684—1686.
13. Wong VW, Stange DE, Page ME, et al. Lrig1 controls intestinal stem-cell homeostasis by negative regulation of ErbB signalling. *Nat Cell Biol*. 2012;14:401—408.
14. Hofmann C, Obermeier F, Artinger M, et al. Cell-cell contacts prevent anoikis in primary human colonic epithelial cells. *Gastroenterology*. 2007;132:587—600.
15. Jensen KB, Driskell RR, Watt FM. Assaying proliferation and differentiation capacity of stem cells using disaggregated adult mouse epidermis. *Nat Protoc*. 2010;5:898—911.
16. Montesano R, Schaller G, Orci L. Induction of epithelial tubular morphogenesis in vitro by fibroblast-derived soluble factors. *Cell*. 1991;66:697—711.
17. Xu C, Inokuma MS, Denham J, et al. Feeder-free growth of undifferentiated human embryonic stem cells. *Nat Biotechnol*. 2001;19:971—974.
18. Grun D, Lyubimova A, Kester L, et al. Single-cell messenger RNA sequencing reveals rare intestinal cell types. *Nature*. 2015;525:251—255.
19. Sato T, Vries RG, Snippert HJ, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature*. 2009;459:262—265.
20. Sato T, Stange DE, Ferrante M, et al. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and barrett's epithelium. *Gastroenterology*. 2011;141:1762—1772.
21. Jung P, Sato T, Merlos-Suarez A, et al. Batlle EIsolation and in vitro expansion of human colonic stem cells. *Nat Med*. 2011;17:1225—1227.
22. Fordham RP, Yui S, Hannan NR, et al. Transplantation of expanded fetal intestinal progenitors contributes to colon regeneration after injury. *Cell Stem Cell*. 2013;13:734—744.

23. Lugli N, Kamileri I, Keogh A, et al. R-spondin 1 and noggin facilitate expansion of resident stem cells from non-damaged gallbladders. *EMBO Rep*. 2016;17:769—779.
24. Huch M, Dorrell C, Boj SF, et al. In vitro expansion of single Lgr5+ liver stem cells induced by Wnt-driven regeneration. *Nature*. 2013;494:247—250.
25. Boj SF, Hwang CI, Baker LA, et al. Organoid models of human and mouse ductal pancreatic cancer. *Cell*. 2015;160:324—338.
26. Huch M, Bonfanti P, Boj SF, et al. Unlimited in vitro expansion of adult bi-potent pancreas progenitors through the Lgr5/R-spondin axis. *EMBO J*. 2013;32:2708—2721.
27. BarkerN, Huch M, Kujala P, 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.
28. Bartfeld S, Bayram T, van de Wetering M, et al. In vitro expansion of human gastric epithelial stem cells and their responses to bacterial infection. *Gastroenterology*. 2015;148:126—136. e126.
29. Stange DE, Koo BK, Huch M, et al. Differentiated Troy+ chief cells act as reserve stem cells to generate all lineages of the stomach epithelium. *Cell*. 2013;155:357—368.
30. Sato T, van Es JH, Snippert HJ, et al. Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. *Nature*. 2011;469:415—418.
31. Bi T, Lan H, Hu X, et al. Antitumor effect of FP3 on a patient-derived tumor tissue xenograft model of rectal carcinoma. *Hepatogastroenterology*. 2013;60:1950—1954.
32. Smith NR, Baker D, Farren M, et al. Tumor stromal architecture can define the intrinsic tumor response to VEGF-targeted therapy. *Clin Cancer Res*. 2013;19:6943—6956.
33. Schumacher MA, Aihara E, Feng R, et al. The use of murine-derived fundic organoids in studies of gastric physiology: the use of fundic organoids. *J Physiol*. 2015;593:1809—1827 [a].
34. Bertaux-Skeirik N, Feng R, Schumacher MA, et al. CD44 plays a functional role in Helicobacter pylori-induced epithelial cell proliferation. *PLoS Pathog*. 2015;11:e1004663.
35. Schlaermann P, Toelle B, Berger H, et al. A novel human gastric primary cell culture system for modelling Helicobacter pylori infection in vitro. *Gut*. 2016;65:202—213.
36. Van de Wetering M, Francies HE, Francis JM, et al. Prospective derivation of a living organoid biobank of colorectal cancer patients. *Cell*. 2015;161:933—945.
37. Pompaiah M, Bartfeld S. Gastric organoids: an emerging model system to study helicobacter pylori pathogenesis. *Curr Top Microbiol Immunol*. 2017;400:149—168.
38. Huang L, Holtzinger A, Jagan I, et al. Ductal pancreatic cancer modeling and drug screening using human pluripotent stem cell- and patient-derived tumor organoids. *Nat Med*. 2015;21:1364—1371.
39. Kim J, Hoffman JP, Alpaugh RK, et al. An IPSC line from human pancreatic ductal adenocarcinoma undergoes early to invasive stages of pancreatic cancer progression. *Cell Rep*. 2013;3:2088—2099.
40. Kim J, Bamlet WR, Oberg AL, et al. Detection of early pancreatic ductal adenocarcinoma with thrombospondin-2 and ca19-9 blood markers. *Sci Transl Med*. 2017;9:55—83.
41. Walsh AJ, Castellanos JA, Nagathihalli NS, Merchant NB, Skala MC. Optical imaging of drug-induced metabolism changes in murine and human pancreatic cancer organoids reveals heterogeneous drug response. *Pancreas*. 2016;45:863—869.
42. Li X, Nadauld L, Ootani A, et al. Oncogenic transformation of diverse gastrointestinal tissues in primary organoid culture. *Nat Med*. 2014;20:769—777.
43. Moreira L, Bakir B, Chatterji P, Dantes Z, Reichert M, Rustgi AK. Pancreas 3D organoids: current and future aspects as a research platform for personalized medicine in pancreatic cancer. *Cell Mol Gastroenterol Hepatol*. 2018;5:289—298.
44. Xu AT, Tong JL, Ran ZH. Organoids derived from digestive tract, liver, and pancreas. *J Dig Dis*. 2016;17:3—10.
45. Drost J, van Jaarsveld RH, Ponsioen B, et al. Sequential cancer mutations in cultured human intestinal stem cells. *Nature*. 2015;521:43—47.
46. Matano M, Date S, Shimokawa M, et al. Modeling colorectal cancer using CRISPR-Cas9-mediated engineering of human intestinal organoids. *Nat Med*. 2015;21:256—262.
47. Fumagalli A, Drost J, Suijkerbuijk SJE, et al. Genetic dissection of colorectal cancer progression by orthotopic transplantation of engineered cancer organoids. *Proc Natl Acad Sci USA*. 2017;114:E2357—E2364.
48. Drost J, van Boxtel R, Blokzijl F, et al. Use of CRISPR modified human stem cell organoids to study the origin of mutational signatures in cancer. *Science*. 2017;358:234—238.
49. Drost J, Clevers H. Organoids in cancer research. *Nat Rev Cancer*. 2018;18:407—418.
50. Tsai S, McOlash L, Palen K, et al. Development of primary human pancreatic cancer organoids, matched stromal and immune cells and 3D tumor microenvironment models. *BMC Cancer*. 2018;18:335.
51. Finnberg NK, Gokare P, Lev A, et al. Application of 3D tumoroid systems to define immune and cytotoxic therapeutic responses based on tumoroid and tissue slice culture molecular signatures. *Oncotarget*. 2017;8:66747—66757.
52. Marx V. Cell-line authentication demystified. *Nat Methods*. 2014;11:483—488.
53. Masters JR, Stacey GN. Changing medium and passaging cell lines. *Nat Protoc*. 2007;2:2276—2284.
54. Gracz AD. A high-throughput platform for stem cell niche co-cultures and downstream gene expression analysis. *Nat Cell Biol*. 2015;17:340—349.
55. Cao L. Development of intestinal organoids as tissue surrogates: cell composition and the epigenetic control of differentiation. *Mol Carcinog*. 2015;54:189—202.
56. Meng Q. Three-dimensional culture of hepatocytes for prediction of drug-induced hepatotoxicity. *Expet Opin Drug Metabol Toxicol*. 2010;6:733—746.
57. Dekkers JF, Berkers G, Kruisselbrink E, et al. Characterizing responses to CFTRmodulating drugs using rectal organoids derived from subjects with cystic fibrosis. *Sci Transl Med*. 2016;8:344384.
58. Yui S, Nakamura T, Sato T, et al. Functional engraftment of colon epithelium expanded in vitro from a single adult Lgr5+ stem cell. *Nat Med*. 2012;18:618—623.
59. Taguchi A, Kaku Y, Ohmori T, Sharmin S, Ogawa M, Sasaki H, Nishinakamura R. Redefining the in vivo origin of metanephric nephron progenitors enables generation of complex kidney structures from pluripotent stem cells. *Cell Stem Cell*. 2014;14:53—67.