Minireview

Convergence of human pluripotent stem cell, organoid, and genome editing technologies

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Impact statement

Physiologically relevant experimental models are essential in human developmental biology studies, disease modeling, and therapeutic development. Recent technical advances that have been explored to significantly improve the models include generating iPSCs from patient-specific somatic cells, and establishing three-dimensional cell/tissue culture conditions recapitulating physiological features of human tissues. Meanwhile, the new generation of genome editing technologies based on designer nucleases like CRISPR-Cas has been revolutionizing almost all areas of biomedical research. These technologies have come together to improve our ability to address some of the biggest challenges in life science. This review aspires to stimulate discussions on improving functional evaluation of genome editing. While genome editing methods are being developed at an unprecedented pace, there have been limited models for their safety or efficacy evaluation. Given the wide adaptation of iPSCs and their functional progeny in biological research, it is beneficial to discuss their potential and limitations in genome editing evaluation.

Abstract

The last decade has seen many exciting technological breakthroughs that greatly expanded the toolboxes for biological and biomedical research, yet few have had more impact than induced pluripotent stem cells and modern-day genome editing. These technologies are providing unprecedented opportunities to improve physiological relevance of experimental models, further our understanding of developmental processes, and develop novel therapies. One of the research areas that benefit greatly from these technological advances is the three-dimensional human organoid culture systems that resemble human tissues morphologically and physiologically. Here we summarize the development of human pluripotent stem cells and their differentiation through organoid formation. We further discuss how genetic modifications, genome editing in particular, were applied to answer basic biological and biomedical questions using organoid cultures of both somatic and pluripotent stem cell origins. Finally, we discuss the potential challenges of applying human pluripotent stem cell and organoid technologies for safety and efficiency evaluation of emerging genome editing tools.

Keywords: Pluripotent stem cells, organoid, genome editing, CRISPR, disease models

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Introduction

Stem cells are defined by their capacity to either self-renew or differentiate into functionally specialized cell types depending on signals in their microenvironments. Because they play fundamental roles in normal physiology and under many pathological conditions, stem cells have been actively studied in laboratories to facilitate greater understanding of biological processes and to develop regenerative medicines.[1] Derivation of the first human embryonic stem cell (ESC) lines brought unprecedented interest in and attention to stem cell research.[2] Unleashing the seemingly unlimited potential of stem cells then faced three major challenges: (1)The limited availability and genetic diversity of stem cell lines; (2) A lack of understanding of their differentiation processes; and (3) Difficulty in achieving precise genetic modifications.

Adult stem cells have restricted developmental potential, exist in very low-abundance in tissues, and are generally difficult to maintain in culture conditions. In contrast, ESCs can be maintained in defined culture conditions for a prolonged time without losing their pluripotency, the potential to give rise to all types of cells that make up the body. However, only very limited human ESC lines are available because they are derived from the inner cell mass of blastocyst stage embryos. For basic research, this means that investigators would have limited access to these cells and even more limited genetic diversity to work with. For therapeutic development purposes, overcoming immunogenicity would be a significant challenge because of HLA-mismatch between ESC-derived cellular products and potential recipients.[3,4] The distinct epigenetic state of ESCs also means that extra differentiation steps, as compared to differentiating adult stem cells, are often needed to convert them to specialized cell types. Productive differentiation of ESCs requires extensive knowledge in developmental processes, which is often fragmented. Lastly, the inability to efficiently and precisely modify genomic sequences of ESCs also posed a technical obstacle in their practical use for a broader range of research and for therapeutic development.

Major breakthroughs have since been made to address these challenges, notably two Nobel Prize-winning groundbreaking discoveries in induced pluripotent stem cell (iPSC) and designer nuclease-mediated genome editing technologies.[5–7] While efficient iPSC differentiation currently remains the most significant challenge, technical improvements have also been made, including the application of three-dimensional organoid culture systems in iPSC differentiation.

Induced pluripotent stem cells

The creation of mouse ESCs and development of homologous recombination (HR) technology in 1980s have revolutionized mammalian genetics research and developmental biology.[8–10] Creating knock-out or knock-in mouse models by HR in mouse ESCs has long been a standard technique for assessing gene functions. ESCs are capable of long-term proliferation in cultures while preserving relative genomic stability. They also retain the ability to differentiate into cell types representing all three germ layers. The prospect of being able to create functional cells, tissues, or even organs in culture dishes for human developmental studies and regenerative therapies has fueled the research that resulted in the derivation of first human ESC lines.[2] Human ESCs can be generated from culturing dissociated blastocysts, through somatic-cell nuclear transfer or the process of parthenogenesis;[11–14] however, these procedures require donation of oocytes or pre-implantation embryos, which are not only of limited resources but also associated with controversial ethical issues.[15] The groundbreaking discovery that somatic cells can be reprogrammed into pluripotency by transient expression of transcriptional factors Oct3/4, Sox2, Klf4, and c-Myc (often collectively referred to as "Yamanaka factors") was a game changer.[5,6] It was demonstrated, soon after these landmark studies, that iPSCs can be generated from a wide range of human somatic cells including not only healthy donor-derived skin fibroblasts, blood cells, keratinocytes, primary hepatocytes, neural stem cells, but also cells from malignancies, as well as banked immortalized lymphoblastoid cell lines.[16–30] These discoveries greatly enhanced the feasibility of using iPSCs for studies on human genetics, disease modeling, and personalized medicine, although the impact of iPSC genetic and epigenetic variations on their differentiation capacity and other applications remains to be fully understood.[31,32] With robust reprogramming technologies in place, the remaining technical challenges in iPSC research are the ability to differentiate them into functional cell types and to genetically modify them with high efficiency.

iPSCs and the development of three-dimensional organoid culture systems

One of the major applications of human ESCs or iPSCs considered to be essential is facilitating human developmental biology studies. Much of our knowledge on molecular and cellular mechanisms underlying human development came from studies of animal models, many of which are still indispensable to biomedical research but also evolutionarily distant from humans. It is also impossible for animal models to capture the vast genetic diversity of human populations. Human iPSCs, being created from somatic cells, appear to be ideally suited to address these challenges and provide an ideal bridge to translate the knowledge from animal studies into human genetics.

To more accurately recapitulate human developmental processes, which are precisely orchestrated sequences of events involving various cell types responding to genetic and environmental cues, 3D *in vitro* models of tissues or organs have become increasingly necessary. Organoid culture systems have been created in response to such demands. Organoids are three-dimensional cell cultures self-organized in ways resembling, to various degrees, composition, and architecture of human tissues. Although organoid culture systems only gained popularity in recent years, using 3D cultures to differentiate stem cells is not new to stem cell investigators. The use of embryoid body (EB) formation, coupled with stimulation from exogenous growth factors, was one of the earliest and most robust methods to direct ESCs to exit pluripotent stage and initiate differentiation processes.[33] It takes advantage of ESC's intrinsic propensity to spontaneously establish complex cell adhesions and paracrine signaling cascades, after the three-dimensional spheroid microenvironment is formed by adhering to each other in suspension. In one of the first organoid studies that demonstrated the feasibility of 3D cultures in recapitulating spatial and temporal aspects of organogenesis, ESCs were used and cultured as EBs first to initiate self-aggregation and further differentiation towards neural lineages.[34]

Organoid culture systems have also been reported using adult stem cells and/or primary cells. In comparison, organoids initiated from ESCs or iPSCs have an advantage in that all cell types forming the desired tissue can be derived from the same cell lines, therefore have uniformed genetic

makeups. Importantly, iPSCs can be derived from patient samples, providing an exciting opportunity to establish more relevant disease models. Human iPSC-derived organoids may recapitulate some disease features more faithfully than 2D-cultured cell lines because they can simulate more closely certain tissue structures and functionalities. These organoids may also complement animal models, particularly in studying diseases that experimental animal models have not been established due to the intrinsic differences between human and nonhuman species in responses to biological or chemical agents.[35–37] To date, human ESC- or iPSC-derived organoid culture systems resembling tissues of brain/neural,[38–44] gut/intestinal,[45–48] lung [49–52] cardiac,[53,54] liver,[55–59] pancreatic/islet,[60,61] kidney,[62–64] as well as tumor[40] have been reported. In-depth discussion of the merits of using organoids as a human model, as well as descriptions of each organoid culture system are not provided here as there have been some insightful.

Recent literature reviews on the history, progress, mechanism, and limitations of current organoid systems.[65–72]

As this review is prepared during the COVID-19 pandemic, it is worth noting that human organoid systems have already contributed to our understanding of SARS-CoV-2 pathogenesis and tissue tropism in humans, an issue that cannot be fully addressed by animal models. Human iPSC-derived capillary organoids and kidney organoids were used to show that SARS-CoV-2 may be capable of infecting kidney as well as blood vessels, providing a plausible explanation to damage in kidney function observed in some patients, and a possible mechanism of virus spreading in the body.[73] The potential of these 3D models in COVID-19 therapeutic development is also demonstrated by observations that human recombinant soluble angiotensin-converting enzyme 2(ACE2) can block SARS-CoV-2 infection to these organoid structures.[73] Since gastrointestinal (GI) manifestations of COVID-19 have been reported in the literature, several studies were conducted to investigate SARS-CoV-2 infection and replication in organoids of GI tract and digestive system. These systems include human small intestinal organoids derived from primary gut epithelial cells[74,75] and cholangiocyte organoids derived from human iPSCs,[76] and provided evidence that SARS-CoV-2 can infect and replicate in various tissues of the digestive system. Human iPSC-derived brain organoids were also used to investigate central nervous system pathophysiology of COVID-19, including SARS-CoV-2 neurotropism and mechanisms of virus-induced brain dysfunction,[77–79] which may provide a platform for future investigations into neurological complications associated with COVID-19. Organoid systems have been applied to studies on viral infection before the pandemic,[80] and it is reasonable to anticipate an explosion in studies using human organoid system to study SARS-CoV-2 in the near future. Of particular interest will be investigations involving organoids representing human lung and airway system, given the fact that respiratory tract illnesses are the main clinical manifestations of COVID-19.

Overview of genome editing technology development

Derivation of mouse ESCs and development of homologous recombination-based genetic modifications have revolutionized mammalian genetics studies. Similarly, the creation of human iPSCs, as well as the recent progress in establishing physiologically-relevant human organoid tissue cultures, is expected to provide unprecedented opportunity to understand human biology and pathophysiology. To achieve this, effective ways of precise genetic modification are required. The traditional HR-based method, which works with sufficient efficiency in mouse ESCs, did not translate smoothly into human ESCs or iPSCs as low targeting efficiencies were observed in initial studies.[81,82] For the genetic modification efficiency to be sufficiently robust, methods that can improve HR are needed. A breakthrough came when it was demonstrated that DSB in mammalian genome, generated by transient expression of engineered homing nuclease, can enhance homology directed repair (HDR) efficiency by more than two orders of magnitude.[83] This demonstration motivated the field to uncover and develop such molecular scissors with adequate specificity, efficiency, and more importantly, programmability that can be utilized in human cells. The development of zinc finger nucleases (ZFN) by fusing DNA-binding zinc finger domains to the cleavage domain of FokI restriction endonuclease represents one of the first successes of such programmable endonucleases.[84–86] Redesigning ZFNs to target different sequences remains technically challenging even today and requires protein engineering expertise that most individual laboratories do not possess; however, in studies where ZFNs were used, the system has demonstrated remarkable capability to enhance gene targeting efficiency including in patient-specific human iPSCs.[87,88] The search for more easily reprogrammable endonucleases continued. Soon after a family of transcription activator-like effector (TALE) proteins was described in plant pathogens,[89,90] it was realized that their modular DNA recognition code can be utilized to construct designer TALE nucleases (TALENs) by replacing the zinc finger domains in ZFNs.[91,92] It was rapidly shown that this class of genome editing enhancing tool can be widely applied to many biological systems including human pluripotent stem cells.[93–95] TALENs are much easier to design and construct than ZFNs, and would have been much more widely used in biological and medical research if not for the recent explosion of genome editing tools based on CRISPR (an abbreviation for clustered regularly interspaced palindromic repeats) systems. CRISPR and the CRISPR-associated (Cas) proteins are important parts of a type of prokaryotic adaptive immune system found in many bacteria and archaea.[96,97]

As sequence-restricted endonucleases, CRISPR-Cas use RNA molecules (termed guide RNAs or gRNAs) to determine binding and cleavage specificity of the Cas protein.[98] The CRISPR-Cas9 system was the first to be adapted to make sequence-specific DNA cleavages in test tubes,[7] and soon after, in mammalian systems including human cells.[99,100] In the following years, other CRISPR-Cas

systems such as Cas12 and Cas13 have been discovered and adapted to serve as DNA or RNA editing tools.[101–103]

There are several notable advantages of CRISPR-Cas over other genome editing tools. Because the target specificity of CRISPR systems is determined by complementarity between guide RNA and its DNA or RNA targets, repurposing of CRISPR-Cas only requires a change in the target-specific spacer sequence (~20 nt in the case of CRISPR-Cas9) in guide RNA, which is significantly easier and cheaper than re-designing and/or assembling a protein component as required in ZFN and TALEN systems. Numerous studies have also demonstrated the robustness of CRISPR-Cas in targeting various plant and animal genomes. Moreover, it has been shown in patient-specific iPSCs that CRISPR/Cas9 can specifically target either the mutant or the wild-type allele with little disruption at the other allele differing by a single nucleotide, giving it an advantage in allele-specific genome editing of point mutations.[104] In addition, the relatively small size of specificity-determining guide RNAs, compared to proteins as in the cases of ZFNs and TALENs, makes it easier to deliver multiple components targeting different genomic sequences simultaneously. This enhanced multiplexed targeting capacity is another important advantage over all other designer nucleases.[105] Because of its unprecedented simplicity, efficiency and robustness, CRISPR/Cas has rapidly become the most popular genome editing system in biological, medical, and agricultural research.

One of the most exciting recent progresses in CRISPR genome editing is the development of tools that do not rely on creating DNA double strand breaks (DSBs) for precise genome editing. As discussed in the previous section, the basic underlying mechanisms of modern-day genome editing tools are to create chromosome breaks at predefined location/sequence and stimulate cellular DNA repair machinery including HDR, which can incorporate new sequences near the break site in the presence of a homology donor template. In the absence of a repair template, the DSBs can be rejoined through a nonhomologous end joining (NHEJ) mechanism, which often introduces short DNA insertions or deletions (indels) that can effectively disrupt gene functions. The reliance on cellular DNA repair pathways, which are often cell type- and cell cycle-dependent, is a rate-limiting factor in improving genome editing efficiency. The development of base editing and prime editing are two examples of remarkable protein engineering successes that were aimed at expanding the CRISPR genome editing toolbox and achieving precise genome editing without creating DSBs and with less dependency on cellular DNA repair machinery. Base editors were designed to make single nucleotide changes and were developed by fusing engineered, catalytically impaired Cas proteins to DNA/RNA modifying enzymes such as nucleoside deaminases.[106,107] Depending on the enzymatic components used, base editors can be categorized into two main classes, cytosine base editors mediate C:G to T:A transitions, while adenine base editors can convert A:T to G:C.[106,107] Efficiency of base editing has been demonstrated in various biological systems.[108]

Prime editing is a more recent development that utilizes a different approach to achieve defined genetic modifications beyond single nucleotide changes.[109] In a prime editor, a nickase form of Cas9 is fused to an engineered reverse transcriptase (RT) enzyme, while the guide RNA is extended to form a prime editing gRNA (pegRNA). After the DNA nicking events occur at a genomic locus defined by the gRNA, a portion of the pegRNA anneals to the complementary DNA strand near the nick site. This displaces the non-targeted strand, allows the nicked DNA to serve as a primer to initiate the reverse transcription process catalyzed by the RT activity from the prime editor, using another portion of the pegRNA as the RT template. The result of this reverse transcription is the transfer of sequence information from the pegRNA into the target DNA strand.[109] The advantage of this approach is that it can mediate targeted insertions and deletions beyond base-to-base conversions, while still without the need for generating DSBs or exogenous donor DNA templates. Both base editing and prime editing do not rely on generating DNA DSBs to mediate editing effect, therefore potentially improving their safety profile by reducing the incidence of unwanted insertions, deletions, and chromosomal rearrangement.

Applications of genome editing in human organoid studies

As the genome editing technologies were being advanced, they have been widely used in all areas of life sciences including human stem cell research and the stem cell-derived organoid research.[110] It is anticipated to continue playing important roles in advancing studies on human organoids, including those derived from iPSCs and from primary cell types (Table 1). One of the reported genome editing applications in human organoids is to create knock-in reporters by targeted and in-frame insertion of a fluorescence reporter to the gene of interest.[55,111] Expression of the fluorescence reporter, whether directly fused to the endogenous gene, or separated by a "self-cleaving" 2 A peptide, will be under the control of the endogenous promoter and enhancer. This is a useful approach to analyze kinetics of gene expression in real-time. By targeting an endogenous gene that has a tissue-specific expression pattern, this approach can be used to monitor lineage commitment in the 3D cultures without the need of, or as a complementary approach to, immunohistochemistry. It is particularly beneficial for studying intracellular proteins such as transcription factors that often require cell/tissue permeabilization for immunostaining. This approach has been successfully used in tagging the cytokeratin 7 locus with mCherry to study 3D bile duct differentiation from human iPSCs,[55] and in tagging the AFP locus with mNeon to trace hepatic development in hepatocyte organoids.[111] Using an improved CRISPR-based HDR-independent knock-in method, TUBB::mNEON; CDH1::tdTomato double-knock-in hepatocyte organoids were achieved, which made it feasible for the first time to conduct human hepatocyte division dynamics study by reporter-based mitotic spindle analyses.[111]

Table 1. Application of genome editing in human organoids – Representative studies.

References	Tissue origin	Cell source	Purpose of study	GE tools	Types of modification	GE component delivery	GE efficiency and specificity
Woo *et al.*[112]	Intestinal	Patient-derived iPSCs containing DKC1 A386T mutation	Modeling dyskeratosis congenita	CRSIPR-Cas9n (D10A) nickase	HDR-mediated point mutation correction or generation using ssDNA donor templates	Lipofectamine-mediated transfection to undifferentiated iPSCs	5%–15% editing efficiency among puro-resistant clones
Drost *et al.*[121]	Intestinal	Human intestinal stem cells isolated from duodenal biopsy	Modeling sequential adeno-carcinoma cancer gene activation	CRSIPR-Cas9	HDR-mediated mutagenesis and NHEJ-mediated gene disruption	Lipofectamine-mediated transfection of Cas9 and gRNA plasmids to dissociated ISC single cells	Mutant organoids selected by removing individual growth factors in culture medium. Off-target analysis by *in silico* prediction and amplicon deep sequencing
Fujii *et al.*[119]	Intestinal	Primary intestinal stem cells	Establishing electroporation methods to genetically modify ISCs	CRSIPR-Cas9	HDR-mediated generation of hotspot mutations in oncogenes (KRAS and PIK3CA)	Electroporation of genome editing plasmids to single cells dissociated from organoids.	N.D. Edited clones selected by puromycin
Matano *et al.*[120]	Intestinal	Normal primary intestinal stem cells	Modeling adeno-carcinoma mutant gene activation in colorectal cancers	CRSIPR-Cas9	NHEJ-mediated mutagenesis in tumor suppressor genes APC, SMAD4, and TP53. HDR-mediated gain of function mutations in oncogenes KRAS and PIK3CA	Electroporation of genome editing reagents into single cells dissociated from organoids	Efficiency N.D. Edited clones were selected using altered culture conditions. Exome sequencing and *in silico* analyses were used to identify off-target sites
Verissimo *et al.*[122]	Intestinal	Primary cells from colorectal cancer patient	Evaluating K-Ras inhibitors and drug combinations in a preclinical setting	CRSIPR-Cas9	HDR-medicated $KRAS^{G12D}$ mutation introduction, and NHEJ-mediated inactivation of the 2nd allele of KRAS	Transfection of genome editing plasmids into dissociated cells.	Edited cells selected by withdrawing EGF and adding the EGFR inhibitor gefitinib
Drost *et al.*[123]	Intestinal	Normal primary colon epithelium	To explore the origin of cancer-associated mutational signatures	CRISPR-Cas9	HDR-mediated insertion of puromycin-resistance cassette into the second exon of MLH1	lipofectamine transfection of editing plasmids into dissociated single cell suspension	N.D. Puromycin selection.
Yan *et al.*[124]	Intestinal	Primary cell cultures	To model early onset colorectal cancer	CRISPR-Cas9	NHEJ-mediated disruption of APC, RNF43, and TP53	Electroporation of single cells from organoids	Edited cells selected by altered culture conditions
Kawasaki *et al.*[129]	Colonic	Primary normal colon tissue/cells	Modeling traditional serrated adenoma	CRISPR-Cas9	Chromosome rearrangement; NHEJ-mediated TP53 disruption; HDR-mediated modification of BRAF	Electroporation of genome editing reagents into single cells dissociated from organoids. PiggyBAC	N.D. Edited clones enriched by puro selection

(continued)

Table 1. Continued.

References	Tissue origin	Cell source	Purpose of study	GE tools	Types of modification	GE component delivery	GE efficiency and specificity
						vectors expressing puro^{R} gene co-transfected for selection purpose	
Artegiani *et al*.[111]	Intestinal, hepatic	Primary cells from intestinal and hepatic tissues	Establishing method for organoid genome editing	CRISPR-Cas9	CRISPR–Cas9-mediated homology-independent knock-in	Organoids were directly co-electroporated with plasmids expressing Cas9 and gRNA, followed by single cell dissociation and clonal growth of KI organoid	Absolute efficiency N.D. Higher efficiency as compared to conventional HDR.
Tian *et al*.[55]	Bile duct	Healthy donor-derived iPSCs	To improve bile duct tissue generation from human iPSCs	CRISPR-Cas9	HDR-dependent reporter knock-in at cytokeratin 7 locus	Electroporation of dissociated undifferentiated iPSCs	N.D. Puromycin selection to enrich targeted iPSCs
Liu *et al*.[128]	Esophageal	Patient-derived Barrett epithelium (BE)-specific iPSCs	To model Wnt signaling activation in BE neoplastic transformation	CRISPR-Cas9	NHEJ-mediated gene disruption of adenomatous polyposis coli (APC)	Lipofectamine-mediated transfection of Cas9 and gRNA plasmids to dissociated BE organoids	N.D. Mutant organoids selected by altering culture conditions.
Ogawa *et al*.[125]	Brain	Human ESC line H9	Modeling glioma formation	CRSIPR-Cas9	HDR to target an HRas^{G12V}-IRES-tdTomato construct into the TP53 locus	Editing plasmids were electroporated directly into cerebral organoids	N.D. Edited cells showed growth advantage.
Blair *et al*.[117]	Brain	hESC line WIBR3; TSC patient-derived fibroblasts reprogrammed into iPSCs	To determine the developmental origin of tuberous sclerosis cells	CRSIPR-Cas9	TSC1 and TSC2 loss-of-function by HDR-mediated deletion of exon 17 of TSC1 or exon 5 of TSC2 (frameshift and premature stop codon); Introduction of second-hit allele by Cre-loxP insertion	Dissociation individual cells electroporated with plasmid vectors	N.D. Manually picked colonies subjected to PCR genotyping and screening for positive clones (monoallelic or biallelic); LoxPed exon 5 of TSC2 allele (second conditional allele) subjected to puromycin selection;
Khan *et al*.[113]	Neuronal	iPSCs from healthy donor used for genome editing (iPSCs from patients with 22q11DS used for other part of the study)	Role of DGCR8 in 22q11DS neuropsychiatric disease	CRSIPR HiFi Cas9	NHEJ-mediated indel in DGCR8;	Electroporation, of HiFiCas9 and sgRNA RNP into undifferentiated iPSCs	Efficiency N.D. Specificity analyzed by *in silico* prediction and Sanger sequencing
Bian *et al*.[126]	Cerebral	Human ESC line H9	Modeling brain tumor formation (glioblastoma)	CRISPR–Cas9	NHEJ-mediated mutagenesis in tumor-suppressor genes	CRISPR plasmids nucleofected directly into developing EB/organoids	N.D.

(continued)

Table 1. Continued.

References	Tissue origin	Cell source	Purpose of study	GE tools	Types of modification	GE component delivery	GE efficiency and specificity
Deng *et al.*[114]	Retina	iPSCs derived from Retinitis pigmentosa patient with RPGR mutations	Evaluating roles of RPGR mutation in diseased photoreceptor, and effect of gene repair	CRSIPR-Cas9	HDR-mediated mutation correction	Plasmid components of genome editing nucleofected into dissociated iPSCs	N.D. Targeted cells enriched by G418 selection
Freedman *et al.*[115]	Kidney	Human ESC line H9	Modelling kidney disease phenotypes	CRSIPR-Cas9 (GFP-tagged Cas9)	HDR-mediated gene targeting, knockout of podocalyxin, KOs of polycystic kidney disease genes PKD1 or PKD2	Plasmid component of genome editing component transfected into dissociated H9 hESCs	N.D. flow cytometric sorting of GFP+ cells to enrich targeted cells
Forbes *et al.*[118]	Kidney	iPSCs derived from nephronophthisis patient with compound-heterozygous IFT140 variants	Modeling NPHP-related ciliopathies with IFT140 mutation/ variants	CRISPR-Cas9 (mRNA)	One-step reprogramming and gene-editing protocol	Genome editing components and reprogramming factors electroporated into patient dermal fibroblasts	N.D.
Seino *et al.*[127]	Pancreatic	iPSCs from normal donor	Modeling pancreatic tumor phenotypes	CRSIPR-Cas9	NHEJ-mediated mutagenesis	Electroporation of Cas9 and sgRNA plasmids or lentiviral-mediated delivery of Cas9 and gRNA into organoids	N.D. Editing efficiency improved by cold shock treatment. Edited cells enriched by puro selection and growth factor-withdrawn
Schwank *et al.*[116]	Intestinal	Intestinal stem cells from CF patients	Feasibility of genome editing human primary stem cells with inherited mutation	CRISPR-Cas9	NHEJ-mediated mutagenesis; HDR-mediated gene correction of CFTR	Plasmids of genome editing components transfected into dissociated organoid by lipofectamine	Efficiency N.D. A puro-resistance gene is included in donor template to facilitate enrichment of targeted cells. Off-targets assessed by sequencing of *in silico*-predicted sites
Geurts *et al.*[132]	Pulmonary, intestinal	CF airway cells of nasal-brush-derived epithelial organoid; CF patient-derived intestinal organoid biobank	Experimental therapeutics on CFTR mutations	CRSIPR-Cas9; spCas9-ABE; xCas9-ABE	ABE-mediated base editing; HDR mediated by Cas9 and ssDNA donor	Genome editing components electroporated into single cells dissociated from organoids	Editing efficiency of SpCas9-ABE was 5-fold higher compared to conventional CRISPR/Cas9-mediated HDR. Editing specificity evaluated by genome-wide analysis.

GE: genome editing; N.D.: not determined; NHEJ: non-homologous end joining; HDR: homologue directed repair; CF: cystic fibrosis; CFTR: cystic fibrosis transmembrane conductance regulator; DGCR8: DiGeorge syndrome chromosomal [or critical] region 8; ABE: adenine base editor; CBE: cytosine base editor; NPHP: nephronophthisis; TSC: tuberous sclerosis complex.

In the majority of the recent human organoid studies that incorporated genome editing, the objective was to understand genetic basis of diseases. Specifically, CRISPR system has been used to achieve gene knock-out or genetic mutation repair to facilitate disease modeling (Table 1). Organoids generated from genome-edited patient-specific iPSCs were used to evaluate genetic contributions to diseases such as DKC1 mutation in dyskeratosis congenita,[112] 22q11 deletion in neuropsychiatric disease,[113] RPGR mutations in retinitis pigmentosa,[114] roles of podocalyxin, PKD1 and PKD2 in kidney development and polycystic kidney disease,[115] CFTR in cystic fibrosis,[116] TSC2 in tuberous sclerosis complex,[117] as well as IFT140 variants in nephronophthisis-related ciliopathies.[118] 3D organoid culture has also seen increasing applications in cancer research. Organoids created from cancer patient cells, sometimes termed "tumoroids", have been reported to model the formation, progression, and phenotypes of tumors.[72] As cancer development is often associated with genetic aberrations, genome editing becomes an essential tool and has been applied in human iPSC-, ESC-, and primary cell/tissue-derived organoids to model a variety of tumorigenesis and the contributions of genetic variations such as those in TP53, SMAD4, APC, KRAS, PIK3CA, MLH1, RNF43, and CDKN2A.[119–128]

In addition to the conventional HDR-based gene correction and NHEJ-mediated small indel formation, some organoid studies have also employed the latest editing tools or unconventional editing approaches to achieve research goals. In a study on traditional serrated adenoma (TSA) modeling, CRISPR-Cas9 with a pair of guide RNAs was used to facilitate chromosome rearrangements by generating DSBs at the two intended chromosomal fusion breakpoints.[129] Using this approach, the investigators successfully created human organoid models of TSA that contain several R-spondin gene fusions. Unsurprisingly, the investigator observed that successful propagation of organoids with detectable intra-chromosomal deletions can only be achieved when the organoids are defective in TP53, or when the medium contains IGF1 and FGF2. This observation is consistent with previous reports that p53-mediated growth arrest, in response to CRISPR-Cas mediated DSBs, negatively affects editing outcomes.[130,131]

A recent study examined the feasibility of adenine base editors (ABEs) in correcting genetic defects associated with disease progression in patient-derived organoids.[132] By analyzing cystic fibrosis transmembrane regulator (CFTR) mutations in a CF intestinal organoid biobank, Geurts *et al*. determined that only 1.2% of the organoid samples carry mutations that are suitable for spCas9-ABE-mediated editing. These targetable sequences have the mutated nucleotides that can be functionally corrected by A:T-to-G:C transitions, and are located within the base editor editing window. In addition, they need to be able to tolerate bystander editing of nearby A:T pairs that are also located in the editing window. By targeting a R785X mutation, the study shows that a SpCas9-ABE achieved an editing efficiency of 8.88%, which is 5-fold higher than the conventional Cas9-mediated HDR using single-stranded donor oligonucleotides. Functional rescue of CFTR activity was demonstrated in a forskolin-induced swelling assay.[132] xCas9-ABE,[133] a further engineered version that has a more relaxed protospacer adjacent motif (PAM) sequence requirement than spCas9-ABE, achieved editing efficiencies of 1.43% at two additional mutations W1282X and R553X,[132] which were not suitable targets of spCas9. Although an improvement in absolute efficiency was not observed in xCas9-ABE, the much broader targeting range (19% in the organoid biobank now have targetable mutations) demonstrated its advantage and the value of research on expanding Cas variants with altered and/or relaxed PAM constraints.[134–138] This study also evaluated the editing specificity, an issue that majority of human organoid genome editing studies have not yet assessed, using genome-wide analysis and demonstrated negligible off-target effect of ABEs.[132]

iPSC systems for evaluating genome editing

While the advances in genome editing have facilitated cutting edge studies on human development and disease mechanisms using stem cell-derived organoids, it is reasonable to anticipate that iPSC-derived organoids can contribute to safety and efficiency evaluation of genome editing technology as new and more sophisticated tools emerge.

Undifferentiated human iPSCs have long been used in evaluating genome editing tools because they are more physiologically-relevant than other immortalized cell line, are more readily available than many types of primary human cells, and are more feasible for clonal expansion than adult stem cells. One of the biggest safety concerns over the conventional CRISPR-Cas systems has been the potential of causing unintended genetic changes or off-target cleavage, which was first reported in studies of immortalized human cell lines, soon after the initial demonstrations of CRISPR-Cas9-mediated mammalian genome editing.[139–141] To further address the off-target issue in a physiologically relevant system, several studies analyzed clonally selected genome-edited human iPSCs using whole genome sequencing approach. Data from these studies suggest that although genetic variants are observed, correctly edited iPSC clones with no or few unintended modifications caused by CRISPR-Cas9 can be obtained.[142–144]

Human iPSC studies also contributed to the understanding of base editor safety profiles. Based on the evidence on endogenous cytidine deaminases causing mutations in human cancers, unbiased genome-wide analysis was conducted in base-edited iPSC clones to determine whether such mutagenesis activity remains in the engineered base editors that contain deaminase domain. Together with two other reports on cytosine base editors using plant and mouse systems,[145,146] the study reveals that global unintended mutations enriched for C:G->T:A transitions can occur at genomic locations with local sequence context consistent with the APOBEC mutagenesis signature.[147] Human iPSCs will remain valuable tools for assessing safety profiles of further evolved base editors[148,149] and other emerging genome editing tools.

As summarized in Table 1, genome editing technologies have been applied in iPSC-based organoids to further their power for understanding human organogenesis, normal physiology, and disease pathology. One can also envision that the organoid culture systems can be used as an evaluation tool to facilitate development of genome editing tools. Traditional pre-clinical evaluation of candidate therapeutics, particularly those for gene and cell therapies, often includes *ex vivo* functional studies in cell lines and, when feasible, *in vivo* studies using animal models. *In vitro* studies and animal studies are meant to complement each other, as both have advantages and disadvantages. Animal studies are generally necessary because they provide the physiological complexity that does not exist in cell lines. It had been increasingly realized, however, that physiological differences between experimental animals (such as rodents and large animals) and humans are beyond simply size; genetic differences, which result in distinct organ organization, tissue composition, cellular metabolism and signaling, can complicate safety and efficacy predictions of candidate therapeutics based on animal studies.[150,151] It is a particularly important issue in evaluating genome editing tools, as difference in genomic sequences can have more consequential impact on procedure outcomes. For example, a 90% DNA sequence homology between a human transcriptional factor and its mouse ortholog may not result in functional difference in any significant way, but certainly could result in a need for different targeting strategy (e.g. different gRNAs) when being edited. Human cell lines and primary human cells, on the other hand, provide human-relevant systems. Cell lines are also easier and more economical to maintain and manipulate, and not as ethically controversial as animal models. However, cell lines have one significant disadvantage in that they do not have the complex physiological behavior representing what is occurring *in vivo*, which makes the use of animal models necessary. With their human origin and the complex three-dimensional tissue-like structures, human organoid culture systems may address some of the limitations of the current evaluation tools and, with further improvement, may lead to the reduction in the use of animal models for proof of concept studies.

Potential advantages of iPSC organoid systems for assessing genome editing

With the rapid expansion of the genome editing toolbox, investigators now have more options to edit a genome more efficiently. In comparison, development of methods for functional evaluation of genome editing outcomes is lagging far behind. Determining the biological consequences of genetic changes, including intended genetic modifications and off-target editing, remains one of the biggest challenges. The commonly used *in vitro* assays, such as the IL-2 independent T cell proliferation assay, simply could not provide adequate biochemical microenvironments for most genetic alterations to manifest.[152] Optimization of human iPSC organoid technologies could provide a significant and much needed boost to genome editing evaluation, for the following reasons: (1) In the 3D multicellular organoids, more complicated cellular functions and physiological features can be assessed than those in 2D culture conditions. (2) Genome-edited iPSCs can theoretically generate an array of organoids recapitulating all major tissue/organ types, making it possible to capture tissue-specific manifestation of a genetic alteration. (3) Organoids can be generated from patient-specific iPSCs or from iPSC banks with defined genetic diversity, to examine genotype-specific functional outcomes and facilitate personalized therapy or precision medicine. (4) Functional organoids with multiple integrated cell types can also be used to assess certain *in vivo* genome editing procedures, particularly in cases that the genome editing components are either delivered by vehicles with cell/tissue specificity (e.g. nanoparticles or viral vectors with cellular tropisms) or expressed under tissue-specific promoters.

Limitations and future possibilities of organoid-based genome editing evaluation

Technologies for creating miniature tissues or organs in cultures have greatly improved over the last decades; however, for the system to be effective in evaluating genome editing technologies, technical improvements are still needed.

Most of the reported organoid structures so far recapitulate some structural and physiological features of certain tissue types, but rarely have the complexity or carry out compete functions of their respective organs. This feature has advantages and disadvantages. On one hand, a simplified structure with limited major cell types makes it easier to isolate and identify genome editing events, which would make it particularly advantageous for single-cell RNA-seq or other characterizations relying on a relatively pure cell population. On the other hand, a more complete assessment of the biological consequences of a given genetic modification can only be made when cells carrying the edits have the necessary environmental cues to carry out the full spectrum of their natural functionality. For the organoid systems to be able to replace or reduce animal uses, further cell engineering is needed to push these *in vitro* cultured structures closer to behaving like actual organs.

In iPSC-derived organoids, maturity of the cells is an issue. In organoids derived from primary tissue stem/progenitor cells, these miniatures are structurally and functionally close to their tissues of origin. However, in organoids through directed differentiation from iPSCs, maturation and functionality remain a prominent problem in many tissue types at present.[153–156] This is owing to the molecular mechanisms governing developmental trajectory of organs remain largely unknown. It is anticipated that, with more molecular mechanisms being deciphered in iPSCs, more mature iPSC-derived organoids can be achieved.

Sizes of current organoids are mostly in the range of millimeters, far from the actual sizes of most organs, so it may not be ideal yet to evaluate efficiency and accuracy of tissue-specific delivery of genome editing tools.[157] Of note, many organ systems are largely replicas of structural units at a similar size scale of organoids, such as the lobules of liver and nephrons of kidney. How to functionally organize

each individual organoids through tissue engineering is a critical next step toward building an organ-like surrogate for research and replacement therapies.

A lack of vasculature and blood circulation is a common feature of current organoid systems. Without functional vasculature, organoids of larger sizes may undergo hypoxia and suffer from nutrient depletion in the inner cores. It also leads to another important issue that is the lack of immune cell penetration in the mesenchymal and epithelial tissues. Given the ubiquitous involvement of immune system in cell behavior, a thorough evaluation of genome editing consequences would be difficult to achieve with the current technology.[158] It is conceivable that organoids derived from somatic stem cells (such as those from intestinal crypts) are not best suited to recapitulate individual villus with a centralized blood vessel, owing to the tissue-restricted properties of these cells. iPSCs and some of their progeny with considerable differentiation potential may offer some advantages in inducing vasculogenesis during organoid formation. The alternative, and not mutually exclusive, approach is through advanced tissue engineering. Progress made in addressing this issue includes incorporating mesenchymal stromal cells, endothelial cells in organoid culture, ectopically expressing pro-angiogenic gene, transplantation of *in vitro*-generated organ buds, and utilizing 3D fabrication or microfluidic technologies to create vascular-like structures.[59,159–163]

One significant disadvantage of most organoid systems, compared to animal models, is the absence of interactions between organs or even between different types of tissues. Fortunately, active ongoing research in approaches such as organs-on-a-chip and body-on-a-chip systems are providing opportunities to potentially address this issue and improve communications between organoids as well as between organoids and their environments.[164–166]

For certain mutations, their phenotypical manifestations at cellular, tissue, or organ levels may not become instantaneously detectable and will require long-term culture of the mutant cells. The development of organoid culture technology is an improvement over the traditional 2D culture in maintaining cells with stem/progenitor cell phenotypes. However, few studies have addressed the culture conditions for long-term steady state maintenance of human organoids, although some organoids have been reported to be maintained in culture for over 100 days.[167] Like many other issues with organoids, it will benefit from a better understanding of molecular mechanisms underlying tissue homeostasis and repair, as well as advanced engineering approaches.

In order for organoids to serve as an evaluation platform, certain technical standards should be established, and the system needs to be able to generate functional units with predefined size range and consistent functionality. So far, most of the methods used to create 3D tissues rely to certain degrees on spontaneous aggregation, which results in variations in size, transcriptional landscape, and functionality among individual spheres and between batches.[62] Biomedical engineering approaches, such as bioprinting and microfabrication,[168–170] may be highly valuable for improving the consistency of organoid formation pave the way pushing the organoids into more practical use.

Summary

As we witnessed over the past decade or so, technical advancements in generating normal and patient-derived iPSCs have significantly enhanced our understanding of human developmental dynamics and plasticity. Applications of these knowledge also substantially expanded our ability to advance regenerative medicine, with implementing *in vitro* organoid miniatures being a critical milestone in developing advanced tools for tissue replacement therapies and drug development. Meanwhile, the emergence of tailored genome editing technologies tremendously potentiates the precision-demanding genetic manipulations of human genomes. While this breakthrough is having an unprecedented impact on nearly all biological research areas, ongoing and future improvements of genome editing technology could also greatly benefit from the physiological relevance of human stem cells and organoids when they are implemented as evaluation tools. It is anticipated that synergies among these technologies will continue to accelerate the transformation of translational research in biomedicine.

AUTHORS' CONTRIBUTIONS

ZY and YYJ designed the review paper structure and layout. LW, ZY, and YYJ contributed to the preparation of the manuscript.

DECLARATION OF CONFLICTING INTERESTS

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REFERENCES

1. Wu J, Izpisua Belmonte JC. Stem cells: a renaissance in human biology research. *Cell* 2016;**165**:1572–85
2. Thomson J, Itskovitz-Eldor J, Shapiro S, Waknitz M, Swiergiel J, Marshall V, Jones J. Embryonic stem cell lines derived from human blastocysts. *Science* 1998;**282**:1145–7
3. Drukker M, Benvenisty N. The immunogenicity of human embryonic stem-derived cells. *Trends Biotechnol* 2004;**22**:136–41
4. Bradley J, Bolton E, Pedersen R. Stem cell medicine encounters the immune system. *Nat Rev Immunol* 2002;**2**:859–71
5. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006;**126**:663–76

6. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007;**131**:861–72
7. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* 2012;**337**:816–21
8. Thomas KR, Capecchi MR. Introduction of homologous DNA sequences into mammalian cells induces mutations in the cognate gene. *Nature* 1986;**324**:34–8
9. Evans M, Kaufman M. Establishment in culture of pluripotential cells from mouse embryos. *Nature* 1981;**292**:154–6
10. Doetschman T, Gregg RG, Maeda N, Hooper ML, Melton DW, Thompson S, Smithies O. Targetted correction of a mutant HPRT gene in mouse embryonic stem cells. *Nature* 1987;**330**:576–8
11. Revazova E, Turovets N, Kochetkova O, Kindarova L, Kuzmichev L, Janus J, Pryzhkova M. Patient-specific stem cell lines derived from human parthenogenetic blastocysts. *Cloning Stem Cells* 2007;**9**:432–49
12. Mai Q, Yu Y, Li T, Wang L, Chen MJ, Huang SZ, Zhou C, Zhou Q. Derivation of human embryonic stem cell lines from parthenogenetic blastocysts. *Cell Res* 2007;**17**:1008–19
13. Lin G, OuYang Q, Zhou X, Gu Y, Yuan D, Li W, Liu G, Liu T, Lu G. A highly homozygous and parthenogenetic human embryonic stem cell line derived from a one-pronuclear oocyte following in vitro fertilization procedure. *Cell Res* 2007;**17**:999–1007
14. Tachibana M, Amato P, Sparman M, Gutierrez NM, Tippner-Hedges R, Ma H, Kang E, Fulati A, Lee HS, Sritanaudomchai H, Masterson K, Larson J, Eaton D, Sadler-Fredd K, Battaglia D, Lee D, Wu D, Jensen J, Patton P, Gokhale S, Stouffer RL, Wolf D, Mitalipov S. Human embryonic stem cells derived by somatic cell nuclear transfer. *Cell* 2013;**153**:1228–38
15. de Wert G, Mummery C. Human embryonic stem cells: research, ethics and policy. *Hum Reprod* 2003;**18**:672–82
16. Park I, Zhao R, West J, Yabuuchi A, Huo H, Ince T, Lerou P, Lensch M, Daley G. Reprogramming of human somatic cells to pluripotency with defined factors. *Nature* 2008;**451**:141–6
17. Kim J, Zaehres H, Wu G, Gentile L, Ko K, Sebastiano V, Araúzo-Bravo M, Ruau D, Han D, Zenke M, Schöler H. Pluripotent stem cells induced from adult neural stem cells by reprogramming with two factors. *Nature* 2008;**454**:646–50
18. Aasen T, Raya A, Barrero M, Garreta E, Consiglio A, Gonzalez F, Vassena R, Bilić J, Pekarik V, Tiscornia G, Edel M, Boué S, Belmonte J. Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. *Nat Biotechnol* 2008;**26**:1276–84
19. Haase A, Olmer R, Schwanke K, Wunderlich S, Merkert S, Hess C, Zweigerdt R, Gruh I, Meyer J, Wagner S, Maier L, Han D, Glage S, Miller K, Fischer P, Schöler H, Martin U. Generation of induced pluripotent stem cells from human cord blood. *Cell Stem Cell* 2009;**5**:434–41
20. Giorgetti A, Montserrat N, Aasen T, Gonzalez F, Rodríguez-Pizà I, Vassena R, Raya A, Boué S, Barrero M, Corbella B, Torrabadella M, Veiga A, Izpisua Belmonte J. Generation of induced pluripotent stem cells from human cord blood using OCT4 and SOX2. *Cell Stem Cell* 2009;**5**:353–7
21. Loh Y, Agarwal S, Park I, Urbach A, Huo H, Heffner G, Kim K, Miller J, Ng K, Daley G. Generation of induced pluripotent stem cells from human blood. *Blood* 2009;**113**:5476–9
22. Ye Z, Zhan H, Mali P, Dowey S, Williams DM, Jang YY, Dang CV, Spivak JL, Moliterno AR, Cheng L. Human-induced pluripotent stem cells from blood cells of healthy donors and patients with acquired blood disorders. *Blood* 2009;**114**:5473–80
23. Brown ME, Rondon E, Rajesh D, Mack A, Lewis R, Feng X, Zitur LJ, Learish RD, Nuwaysir EF. Derivation of induced pluripotent stem cells from human peripheral blood T lymphocytes. *PLoS One* 2010;**5**: e11373
24. Staerk J, Dawlaty MM, Gao Q, Maetzel D, Hanna J, Sommer CA, Mostoslavsky G. Jaenisch R. Reprogramming of human peripheral blood cells to induced pluripotent stem cells. *Cell Stem Cell* 2010;**7**:20–4
25. Choi SM, Liu H, Chaudhari P, Kim Y, Cheng L, Feng J, Sharkis S, Ye Z, Jang YY. Reprogramming of EBV-immortalized B-lymphocyte cell lines into induced pluripotent stem cells. *Blood* 2011;**118**:1801–5
26. Liu H, Ye Z, Kim Y, Sharkis S, Jang YY. Generation of endoderm-derived human induced pluripotent stem cells from primary hepatocytes. *Hepatology* 2010;**51**:1810–9
27. Yu J, Vodyanik M, Smuga-Otto K, Antosiewicz-Bourget J, Frane J, Tian S, Nie J, Jonsdottir G, Ruotti V, Stewart R, Slukvin I, Thomson J. Induced pluripotent stem cell lines derived from human somatic cells. *Science* 2007;**318**:1917–20
28. Rajesh D, Dickerson SJ, Yu J, Brown ME, Thomson JA, Seay NJ. Human lymphoblastoid B-cell lines reprogrammed to EBV-free induced pluripotent stem cells. *Blood* 2011;**118**:1797–800
29. Kotini AG, Chang CJ, Boussaad I, Delrow JJ, Dolezal EK, Nagulapally AB, Perna F, Fishbein GA, Klimek VM, Hawkins RD, Huangfu D, Murry CE, Graubert T, Nimer SD, Papapetrou EP. Functional analysis of a chromosomal deletion associated with myelodysplastic syndromes using isogenic human induced pluripotent stem cells. *Nat Biotechnol* 2015;**33**:646–55
30. Chao MP, Gentles AJ, Chatterjee S, Lan F, Reinisch A, Corces MR, Xavy S, Shen J, Haag D, Chanda S, Sinha R, Morganti RM, Nishimura T, Ameen M, Wu H, Wernig M, Wu JC, Majeti R. Human AML-iPSCs reacquire leukemic properties after differentiation and model clonal variation of disease. *Cell Stem Cell* 2017;**20**:329–44.e7
31. Liang G, Zhang Y. Genetic and epigenetic variations in iPSCs: potential causes and implications for application. *Cell Stem Cell* 2013;**13**:149–59
32. Nishizawa M, Chonabayashi K, Nomura M, Tanaka A, Nakamura M, Inagaki A, Nishikawa M, Takei I, Oishi A, Tanabe K, Ohnuki M, Yokota H, Koyanagi-Aoi M, Okita K, Watanabe A, Takaori-Kondo A, Yamanaka S, Yoshida Y. Epigenetic variation between human induced pluripotent stem cell lines is an indicator of differentiation capacity. *Cell Stem Cell* 2016;**19**:341–54
33. Keller GM. In vitro differentiation of embryonic stem cells. *Curr Opin Cell Biol* 1995;**7**:862–9
34. Eiraku M, Watanabe K, Matsuo-Takasaki M, Kawada M, Yonemura S, Matsumura M, Wataya T, Nishiyama A, Muguruma K, Sasai Y. Self-organized formation of polarized cortical tissues from ESCs and its active manipulation by extrinsic signals. *Cell Stem Cell* 2008;**3**:519–32
35. Estes JD, Wong SW, Brenchley JM. Nonhuman primate models of human viral infections. *Nat Rev Immunol* 2018;**18**:390–404
36. Anisimov VN, Ukraintseva SV, Yashin AI. Cancer in rodents: does it tell us about cancer in humans? *Nat Rev Cancer* 2005;**5**:807–19
37. Jakel RJ, Schneider BL, Svendsen CN. Using human neural stem cells to model neurological disease. *Nat Rev Genet* 2004;**5**:136–44
38. Iefremova V, Manikakis G, Krefft O, Jabali A, Weynans K, Wilkens R, Marsoner F, Brandl B, Muller FJ, Koch P, Ladewig J. An organoid-based model of cortical development identifies non-cell-autonomous defects in wnt signaling contributing to Miller-Dieker syndrome. *Cell Rep* 2017;**19**:50–9
39. Bershteyn M, Nowakowski TJ, Pollen AA, Di Lullo E, Nene A, Wynshaw-Boris A, Kriegstein AR. Human iPSC-derived cerebral organoids model cellular features of lissencephaly and reveal prolonged mitosis of outer radial glia. *Cell Stem Cell* 2017;**20**:435–49 e4
40. Linkous A, Balamatsias D, Snuderl M, Edwards L, Miyaguchi K, Milner T, Reich B, Cohen-Gould L, Storaska A, Nakayama Y, Schenkein E, Singhania R, Cirigliano S, Magdeldin T, Lin Y, Nanjangud G, Chadalavada K, Pisapia D, Liston C, Fine HA. Modeling patient-derived glioblastoma with cerebral organoids. *Cell Rep* 2019;**26**:3203–11.e5
41. Xiang Y, Tanaka Y, Cakir B, Patterson B, Kim KY, Sun P, Kang YJ, Zhong M, Liu X, Patra P, Lee SH, Weissman SM, Park IH. hESC-derived thalamic organoids form reciprocal projections when fused with cortical organoids. *Cell Stem Cell* 2019;**24**:487–97 e7
42. Parfitt DA, Lane A, Ramsden CM, Carr AJ, Munro PM, Jovanovic K, Schwarz N, Kanuga N, Muthiah MN, Hull S, Gallo JM, da Cruz L, Moore AT, Hardcastle AJ, Coffey PJ, Cheetham ME. Identification and correction of mechanisms underlying inherited blindness in human iPSC-derived optic cups. *Cell Stem Cell* 2016;**18**:769–81

43. Li Y, Muffat J, Omer A, Bosch I, Lancaster MA, Sur M, Gehrke L, Knoblich JA, Jaenisch R. Induction of expansion and folding in human cerebral organoids. *Cell Stem Cell* 2017;**20**:385–96.e3
44. Nakano T, Ando S, Takata N, Kawada M, Muguruma K, Sekiguchi K, Saito K, Yonemura S, Eiraku M, Sasai Y. Self-formation of optic cups and storable stratified neural retina from human ESCs. *Cell Stem Cell* 2012;**10**:771–85
45. Takebe T, Sekine K, Kimura M, Yoshizawa E, Ayano S, Koido M, Funayama S, Nakanishi N, Hisai T, Kobayashi T, Kasai T, Kitada R, Mori A, Ayabe H, Ejiri Y, Amimoto N, Yamazaki Y, Ogawa S, Ishikawa M, Kiyota Y, Sato Y, Nozawa K, Okamoto S, Ueno Y, Taniguchi H. Massive and reproducible production of liver buds entirely from human pluripotent stem cells. *Cell Rep* 2017;**21**:2661–70
46. Miura S, Suzuki A. Generation of mouse and human organoid-forming intestinal progenitor cells by direct lineage reprogramming. *Cell Stem Cell* 2017;**21**:456–71 e5
47. Mithal A, Capilla A, Heinze D, Berical A, Villacorta-Martin C, Vedaie M, Jacob A, Abo K, Szymaniak A, Peasley M, Stuffer A, Mahoney J, Kotton DN, Hawkins F, Mostoslavsky G. Generation of mesenchyme free intestinal organoids from human induced pluripotent stem cells. *Nat Commun* 2020;**11**:215
48. Munera JO, Sundaram N, Rankin SA, Hill D, Watson C, Mahe M, Vallance JE, Shroyer NF, Sinagoga KL, Zarzoso-Lacoste A, Hudson JR, Howell JC, Chatuvedi P, Spence JR, Shannon JM, Zorn AM, Helmrath MA, Wells JM. Differentiation of human pluripotent stem cells into colonic organoids via transient activation of BMP signaling. *Cell Stem Cell* 2017;**21**:51–64.e6
49. Dye BR, Youngblood RL, Oakes RS, Kasputis T, Clough DW, Spence JR, Shea LD. Human lung organoids develop into adult airway-like structures directed by physico-chemical biomaterial properties. *Biomaterials* 2020;**234**:119757
50. Chen YW, Huang SX, de Carvalho A, Ho SH, Islam MN, Volpi S, Notarangelo LD, Ciancanelli M, Casanova JL, Bhattacharya J, Liang AF, Palermo LM, Porotto M, Moscona A, Snoeck HW. A three-dimensional model of human lung development and disease from pluripotent stem cells. *Nat Cell Biol* 2017;**19**:542–9
51. Miller AJ, Hill DR, Nagy MS, Aoki Y, Dye BR, Chin AM, Huang S, Zhu F, White ES, Lama V, Spence JR. In vitro induction and in vivo engraftment of lung bud tip progenitor cells derived from human pluripotent stem cells. *Stem Cell Rep* 2018;**10**:101–19
52. McCauley KB, Hawkins F, Serra M, Thomas DC, Jacob A, Kotton DN. Efficient derivation of functional human airway epithelium from pluripotent stem cells via temporal regulation of wnt signaling. *Cell Stem Cell* 2017;**20**:844–57 e6
53. Varzideh F, Pahlavan S, Ansari H, Halvaei M, Kostin S, Feiz MS, Latifi H, Aghdami N, Braun T, Baharvand H. Human cardiomyocytes undergo enhanced maturation in embryonic stem cell-derived organoid transplants. *Biomaterials* 2019;**192**:537–50
54. Mills RJ, Parker BL, Quaife-Ryan GA, Voges HK, Needham EJ, Bornot A, Ding M, Andersson H, Polla M, Elliott DA, Drowley L, Clausen M, Plowright AT, Barrett IP, Wang QD, James DE, Porrello ER, Hudson JE. Drug screening in human PSC-cardiac organoids identifies pro-proliferative compounds acting via the mevalonate pathway. *Cell Stem Cell* 2019;**24**:895–907.e6
55. Tian L, Deshmukh A, Ye Z, Jang YY. Efficient and controlled generation of 2D and 3D bile duct tissue from human pluripotent stem cell-derived spheroids. *Stem Cell Rev Rep* 2016;**12**:500–8
56. Mun SJ, Ryu JS, Lee MO, Son YS, Oh SJ, Cho HS, Son MY, Kim DS, Kim SJ, Yoo HJ, Lee HJ, Kim J, Jung CR, Chung KS, Son MJ. Generation of expandable human pluripotent stem cell-derived hepatocyte-like liver organoids. *J Hepatol* 2019;**71**:970–85
57. Takeishi K, Collin de l'Hortet A, Wang Y, Handa K, Guzman-Lepe J, Matsubara K, Morita K, Jang S, Haep N, Florentino RM, Yuan F, Fukumitsu K, Tobita K, Sun W, Franks J, Delgado ER, Shapiro EM, Fraunhoffer NA, Duncan AW, Yagi H, Mashimo T, Fox IJ, Soto-Gutierrez A. Assembly and function of a bioengineered human liver for transplantation generated solely from induced pluripotent stem cells. *Cell Rep* 2020;**31**:107711
58. Sun L, Wang Y, Cen J, Ma X, Cui L, Qiu Z, Zhang Z, Li H, Yang RZ, Wang C, Chen X, Wang L, Ye Y, Zhang H, Pan G, Kang JS, Ji Y, Zheng YW, Zheng S, Hui L. Modelling liver cancer initiation with organoids derived from directly reprogrammed human hepatocytes. *Nat Cell Biol* 2019;**21**:1015–26
59. Takebe T, Sekine K, Enomura M, Koike H, Kimura M, Ogaeri T, Zhang RR, Ueno Y, Zheng YW, Koike N, Aoyama S, Adachi Y, Taniguchi H. Vascularized and functional human liver from an iPSC-derived organ bud transplant. *Nature* 2013;**499**:481–4
60. Yoshihara E, O'Connor C, Gasser E, Wei Z, Oh TG, Tseng TW, Wang D, Cayabyab F, Dai Y, Yu RT, Liddle C, Atkins AR, Downes M, Evans RM. Immune-evasive human islet-like organoids ameliorate diabetes. *Nature* 2020;**586**:606–11
61. Hohwieler M, Illing A, Hermann PC, Mayer T, Stockmann M, Perkhofer L, Eiseler T, Antony JS, Muller M, Renz S, Kuo CC, Lin Q, Sendler M, Breunig M, Kleiderman SM, Lechel A, Zenker M, Leichsenring M, Rosendahl J, Zenke M, Sainz B, Jr., Mayerle J, Costa IG, Seufferlein T, Kormann M, Wagner M, Liebau S, Kleger A. Human pluripotent stem cell-derived acinar/ductal organoids generate human pancreas upon orthotopic transplantation and allow disease modelling. *Gut* 2017;**66**:473–86
62. Phipson B, Er PX, Combes AN, Forbes TA, Howden SE, Zappia L, Yen HJ, Lawlor KT, Hale LJ, Sun J, Wolvetang E, Takasato M, Oshlack A, Little MH. Evaluation of variability in human kidney organoids. *Nat Methods* 2019;**16**:79–87
63. Takasato M, Er PX, Chiu HS, Maier B, Baillie GJ, Ferguson C, Parton RG, Wolvetang EJ, Roost MS, Chuva de Sousa Lopes SM, Little MH. Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis. *Nature* 2015;**526**:564–8
64. Low JH, Li P, Chew EGY, Zhou B, Suzuki K, Zhang T, Lian MM, Liu M, Aizawa E, Rodriguez Esteban C, Yong KSM, Chen Q, Campistol JM, Fang M, Khor CC, Foo JN, Izpisua Belmonte JC, Xia Y. Generation of human PSC-Derived kidney organoids with patterned nephron segments and a de novo vascular network. *Cell Stem Cell* 2019;**25**:373–87 e9
65. Artegiani B, Clevers H. Use and application of 3D-organoid technology. *Hum Mol Genet* 2018;**27**:R99–R107
66. Drost J, Clevers H. Organoids in cancer research. *Nat Rev Cancer* 2018;**18**:407–18
67. Rossi G, Manfrin A, Lutolf MP. Progress and potential in organoid research. *Nat Rev Genet* 2018;**19**:671–87
68. Little MH, Combes AN. Kidney organoids: accurate models or fortunate accidents. *Genes Dev* 2019;**33**:1319–45
69. Bhaduri A, Andrews MG, Kriegstein AR, Nowakowski TJ. Are organoids ready for prime time? *Cell Stem Cell* 2020;**27**:361–5
70. Del Dosso A, Urenda JP, Nguyen T, Quadrato G. Upgrading the physiological relevance of human brain organoids. *Neuron* 2020;**107**:1014–28
71. Kim J, Koo BK, Knoblich JA. Human organoids: model systems for human biology and medicine. *Nat Rev Mol Cell Biol* 2020;**21**:571–84
72. Tuveson D, Clevers H. Cancer modeling meets human organoid technology. *Science* 2019;**364**:952–5
73. Monteil V, Kwon H, Prado P, Hagelkruys A, Wimmer RA, Stahl M, Leopoldi A, Garreta E, Hurtado Del Pozo C, Prosper F, Romero JP, Wirnsberger G, Zhang H, Slutsky AS, Conder R, Montserrat N, Mirazimi A, Penninger JM. Inhibition of SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE2. *Cell* 2020;**181**:905–13 e7
74. Lamers MM, Beumer J, van der Vaart J, Knoops K, Puschhof J, Breugem TI, Ravelli RBG, Paul van Schayck J, Mykytyn AZ, Duimel HQ, van Donselaar E, Riesebosch S, Kuijpers HJH, Schipper D, van de Wetering WJ, de Graaf M, Koopmans M, Cuppen E, Peters PJ, Haagmans BL, Clevers H. SARS-CoV-2 productively infects human gut enterocytes. *Science* 2020;**369**:50–4
75. Zhou J, Li C, Liu X, Chiu MC, Zhao X, Wang D, Wei Y, Lee A, Zhang AJ, Chu H, Cai JP, Yip CC, Chan IH, Wong KK, Tsang OT, Chan KH, Chan JF, To KK, Chen H, Yuen KY. Infection of bat and human intestinal organoids by SARS-CoV-2. *Nat Med* 2020;**26**:1077–83

76. Yang L, Han Y, Nilsson-Payant BE, Gupta V, Wang P, Duan X, Tang X, Zhu J, Zhao Z, Jaffre F, Zhang T, Kim TW, Harschnitz O, Redmond D, Houghton S, Liu C, Naji A, Ciceri G, Guttikonda S, Bram Y, Nguyen DT, Cioffi M, Chandar V, Hoagland DA, Huang Y, Xiang J, Wang H, Lyden D, Borczuk A, Chen HJ, Studer L, Pan FC, Ho DD, tenOever BR, Evans T, Schwartz RE, Chen S. A human pluripotent stem cell-based platform to study SARS-CoV-2 tropism and model virus infection in human cells and organoids. *Cell Stem Cell* 2020;**27**:125–36.e7
77. Jacob F, Pather SR, Huang WK, Zhang F, Wong SZH, Zhou H, Cubitt B, Fan W, Chen CZ, Xu M, Pradhan M, Zhang DY, Zheng W, Bang AG, Song H, Carlos de la Torre J, Ming GL. Human pluripotent stem Cell-Derived neural cells and brain organoids reveal SARS-CoV-2 neurotropism predominates in choroid plexus epithelium. *Cell Stem Cell* 2020;**27**:937–50
78. Ramani A, Muller L, Ostermann PN, Gabriel E, Abida-Islam P, Muller-Schiffmann A, Mariappan A, Goureau O, Gruell H, Walker A, Andree M, Hauka S, Houwaart T, Dilthey A, Wohlgemuth K, Omran H, Klein F, Wieczorek D, Adams O, Timm J, Korth C, Schaal H, Gopalakrishnan J. SARS-CoV-2 targets neurons of 3D human brain organoids. *EMBO J* 2020;**39**:e106230
79. Zhang BZ, Chu H, Han S, Shuai H, Deng J, Hu YF, Gong HR, Lee AC, Zou Z, Yau T, Wu W, Hung IF, Chan JF, Yuen KY, Huang JD. SARS-CoV-2 infects human neural progenitor cells and brain organoids. *Cell Res* 2020;**30**:928–31
80. Ramani S, Crawford SE, Blutt SE, Estes MK. Human organoid cultures: transformative new tools for human virus studies. *Curr Opin Virol* 2018;**29**:79–86
81. Zwaka T, Thomson J. Homologous recombination in human embryonic stem cells. *Nat Biotechnol* 2003;**21**:319–21
82. Urbach A, Schuldiner M, Benvenisty N. Modeling for Lesch-Nyhan disease by gene targeting in human embryonic stem cells. *Stem Cells* 2004;**22**:635–41
83. Rouet P, Smih F, Jasin M. Introduction of double-strand breaks into the genome of mouse cells by expression of a rare-cutting endonuclease. *Mol Cell Biol* 1994;**14**:8096–106
84. Kim YG, Cha J, Chandrasegaran S. Hybrid restriction enzymes: zinc finger fusions to fok I cleavage domain. *Proc Natl Acad Sci U S A* 1996;**93**:1156–60
85. Smith J, Bibikova M, Whitby FG, Reddy AR, Chandrasegaran S, Carroll D. Requirements for double-strand cleavage by chimeric restriction enzymes with zinc finger DNA-recognition domains. *Nucleic Acids Res* 2000;**28**:3361–9
86. Bibikova M, Carroll D, Segal DJ, Trautman JK, Smith J, Kim YG, Chandrasegaran S. Stimulation of homologous recombination through targeted cleavage by chimeric nucleases. *Mol Cell Biol* 2001;**21**:289–97
87. Zou J, Maeder M, Mali P, Pruett-Miller S, Thibodeau-Beganny S, Chou B, Chen G, Ye Z, Park I, Daley G, Porteus M, Joung J, Cheng L. Gene targeting of a disease-related gene in human induced pluripotent stem and embryonic stem cells. *Cell Stem Cell* 2009;**5**:97–110
88. Hockemeyer D, Soldner F, Beard C, Gao Q, Mitalipova M, DeKelver R, Katibah G, Amora R, Boydston E, Zeitler B, Meng X, Miller J, Zhang L, Rebar E, Gregory P, Urnov F, Jaenisch R. Efficient targeting of expressed and silent genes in human ESCs and iPSCs using zinc-finger nucleases. *Nat Biotechnol* 2009;**27**:851–7
89. Boch J, Scholze H, Schornack S, Landgraf A, Hahn S, Kay S, Lahaye T, Nickstadt A, Bonas U. Breaking the code of DNA binding specificity of TAL-type III effectors. *Science* 2009;**326**:1509–12
90. Moscou MJ, Bogdanove AJ. A simple cipher governs DNA recognition by TAL effectors. *Science* 2009;**326**:1501
91. Christian M, Cermak T, Doyle EL, Schmidt C, Zhang F, Hummel A, Bogdanove AJ, Voytas DF. Targeting DNA double-strand breaks with TAL effector nucleases. *Genetics* 2010;**186**:757–61
92. Cermak T, Doyle EL, Christian M, Wang L, Zhang Y, Schmidt C, Baller JA, Somia NV, Bogdanove AJ, Voytas DF. Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. *Nucleic Acids Res* 2011;**39**:e82
93. Joung JK, Sander JD. TALENs: a widely applicable technology for targeted genome editing. *Nat Rev Mol Cell Biol* 2013;**14**:49–55
94. Hockemeyer D, Wang H, Kiani S, Lai CS, Gao Q, Cassady JP, Cost GJ, Zhang L, Santiago Y, Miller JC, Zeitler B, Cherone JM, Meng X, Hinkley SJ, Rebar EJ, Gregory PD, Urnov FD, Jaenisch R. Genetic engineering of human pluripotent cells using TALE nucleases. *Nat Biotechnol* 2011;**29**:731–4
95. Choi SM, Kim Y, Shim JS, Park JT, Wang RH, Leach SD, Liu JO, Deng C, Ye Z, Jang YY. Efficient drug screening and gene correction for treating liver disease using patient-specific stem cells. *Hepatology* 2013;**57**:2458–68
96. Mojica FJ, Ferrer C, Juez G, Rodriguez-Valera F. Long stretches of short tandem repeats are present in the largest replicons of the archaea Haloferax mediterranei and Haloferax volcanii and could be involved in replicon partitioning. *Mol Microbiol* 1995;**17**:85–93
97. Jansen R, Embden JD, Gaastra W, Schouls LM. Identification of genes that are associated with DNA repeats in prokaryotes. *Mol Microbiol* 2002;**43**:1565–75
98. Deltcheva E, Chylinski K, Sharma CM, Gonzales K, Chao Y, Pirzada ZA, Eckert MR, Vogel J, Charpentier E. CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III. *Nature* 2011;**471**:602–7
99. Mali P, Yang L, Esvelt KM, Aach J, Guell M, DiCarlo JE, Norville JE, Church GM. RNA-guided human genome engineering via Cas9. *Science* 2013;**339**:823–6
100. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, Zhang F. Multiplex genome engineering using CRISPR/cas systems. *Science* 2013;**339**:819–23
101. Zetsche B, Gootenberg JS, Abudayyeh OO, Slaymaker IM, Makarova KS, Essletzbichler P, Volz SE, Joung J, van der Oost J, Regev A, Koonin EV, Zhang F. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. *Cell* 2015;**163**:759–71
102. Abudayyeh OO, Gootenberg JS, Essletzbichler P, Han S, Joung J, Belanto JJ, Verdine V, Cox DBT, Kellner MJ, Regev A, Lander ES, Voytas DF, Ting AY, Zhang F. RNA targeting with CRISPR-Cas13. *Nature* 2017;**550**:280–4
103. Liu JJ, Orlova N, Oakes BL, Ma E, Spinner HB, Baney KLM, Chuck J, Tan D, Knott GJ, Harrington LB, Al-Shayeb B, Wagner A, Brotzmann J, Staahl BT, Taylor KL, Desmarais J, Nogales E, Doudna JA. CasX enzymes comprise a distinct family of RNA-guided genome editors. *Nature* 2019;**566**:218–23
104. Smith C, Abalde-Atristain L, He C, Brodsky BR, Braunstein EM, Chaudhari P, Jang YY, Cheng L, Ye Z. Efficient and allele-specific genome editing of disease loci in human iPSCs. *Mol Ther* 2015;**23**:570–7
105. Smith CJ, Castanon O, Said K, Volf V, Khoshakhlagh P, Hornick A, Ferreira R, Wu CT, Guell M, Garg S, Ng AHM, Myllykallio H, Church GM. Enabling large-scale genome editing at repetitive elements by reducing DNA nicking. *Nucleic Acids Res* 2020;**48**:5183–95
106. Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature* 2016;**533**:420–4
107. Gaudelli NM, Komor AC, Rees HA, Packer MS, Badran AH, Bryson DI, Liu DR. Programmable base editing of a•T to G•C in genomic DNA without DNA cleavage. *Nature* 2017;**551**:464–71
108. Rees HA, Liu DR. Base editing: precision chemistry on the genome and transcriptome of living cells. *Nat Rev Genet* 2018;**19**:770–88
109. Anzalone AV, Randolph PB, Davis JR, Sousa AA, Koblan LW, Levy JM, Chen PJ, Wilson C, Newby GA, Raguram A, Liu DR. Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature* 2019;**576**:149–57
110. Driehuis E, Clevers H. CRISPR/cas 9 genome editing and its applications in organoids. *Am J Physiol Gastrointest Liver Physiol* 2017;**312**:G257–G65
111. Artegiani B, Hendriks D, Beumer J, Kok R, Zheng X, Joore I, Chuva de Sousa Lopes S, van Zon J, Tans S, Clevers H. Fast and efficient generation of knock-in human organoids using homology-independent CRISPR-Cas9 precision genome editing. *Nat Cell Biol* 2020;**22**:321–31
112. Woo DH, Chen Q, Yang TL, Glineburg MR, Hoge C, Leu NA, Johnson FB, Lengner CJ. Enhancing a Wnt-Telomere feedback loop restores intestinal stem cell function in a human organotypic model of dyskeratosis congenita. *Cell Stem Cell* 2016;**19**:397–405

113. Khan TA, Revah O, Gordon A, Yoon SJ, Krawisz AK, Goold C, Sun Y, Kim CH, Tian Y, Li MY, Schaepe JM, Ikeda K, Amin ND, Sakai N, Yazawa M, Kushan L, Nishino S, Porteus MH, Rapoport JL, Bernstein JA, O'Hara R, Bearden CE, Hallmayer JF, Huguenard JR, Geschwind DH, Dolmetsch RE, Pasca SP. Neuronal defects in a human cellular model of 22q11.2 deletion syndrome. *Nat Med* 2020;**26**:1888–98
114. Deng WL, Gao ML, Lei XL, Lv JN, Zhao H, He KW, Xia XX, Li LY, Chen YC, Li YP, Pan D, Xue T, Jin ZB. Gene correction reverses ciliopathy and photoreceptor loss in iPSC-Derived retinal organoids from retinitis pigmentosa patients. *Stem Cell Reports* 2018;**10**:1267–81
115. Freedman BS, Brooks CR, Lam AQ, Fu H, Morizane R, Agrawal V, Saad AF, Li MK, Hughes MR, Werff RV, Peters DT, Lu J, Baccei A, Siedlecki AM, Valerius MT, Musunuru K, McNagny KM, Steinman TI, Zhou J, Lerou PH, Bonventre JV. Modelling kidney disease with CRISPR-mutant kidney organoids derived from human pluripotent epiblast spheroids. *Nat Commun* 2015;**6**:8715
116. Schwank G, Koo BK, Sasselli V, Dekkers JF, Heo I, Demircan T, Sasaki N, Boymans S, Cuppen E, van der Ent CK, Nieuwenhuis EE, Beekman JM, Clevers H. Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. *Cell Stem Cell* 2013;**13**:653–8
117. Blair JD, Hockemeyer D, Bateup HS. Genetically engineered human cortical spheroid models of tuberous sclerosis. *Nat Med* 2018;**24**:1568–78
118. Forbes TA, Howden SE, Lawlor K, Phipson B, Maksimovic J, Hale L, Wilson S, Quinlan C, Ho G, Holman K, Bennetts B, Crawford J, Trnka P, Oshlack A, Patel C, Mallett A, Simons C, Little MH. Patient-iPSC-derived kidney organoids show functional validation of a ciliopathic renal phenotype and reveal underlying pathogenetic mechanisms. *Am J Hum Genet* 2018;**102**:816–31
119. Fujii M, Matano M, Nanki K, Sato T. Efficient genetic engineering of human intestinal organoids using electroporation. *Nat Protoc* 2015;**10**:1474–85
120. Matano M, Date S, Shimokawa M, Takano A, Fujii M, Ohta Y, Watanabe T, Kanai T, Sato T. Modeling colorectal cancer using CRISPR-Cas9-mediated engineering of human intestinal organoids. *Nat Med* 2015;**21**:256–62
121. Drost J, van Jaarsveld RH, Ponsioen B, Zimberlin C, van Boxtel R, Buijs A, Sachs N, Overmeer RM, Offerhaus GJ, Begthel H, Korving J, van de Wetering M, Schwank G, Logtenberg M, Cuppen E, Snippert HJ, Medema JP, Kops GJ, Clevers H. Sequential cancer mutations in cultured human intestinal stem cells. *Nature* 2015;**521**:43–7
122. Verissimo CS, Overmeer RM, Ponsioen B, Drost J, Mertens S, Verlaan-Klink I, Gerwen BV, van der Ven M, Wetering MV, Egan DA, Bernards R, Clevers H, Bos JL, Snippert HJ. Targeting mutant RAS in patient-derived colorectal cancer organoids by combinatorial drug screening. *Elife* 2016;**5**:e18489
123. Drost J, van Boxtel R, Blokzijl F, Mizutani T, Sasaki N, Sasselli V, de Ligt J, Behjati S, Grolleman JE, van Wezel T, Nik-Zainal S, Kuiper RP, Cuppen E, Clevers H. Use of CRISPR-modified human stem cell organoids to study the origin of mutational signatures in cancer. *Science* 2017;**358**:234–8
124. Yan HHN, Siu HC, Ho SL, Yue SSK, Gao Y, Tsui WY, Chan D, Chan AS, Wong JWH, Man AHY, Lee BCH, Chan ASY, Chan AKW, Hui HS, Cheung AKL, Law WL, Lo OSH, Yuen ST, Clevers H, Leung SY. Organoid cultures of early-onset colorectal cancers reveal distinct and rare genetic profiles. *Gut* 2020;**69**:2165–79
125. Ogawa J, Pao GM, Shokhirev MN, Verma IM. Glioblastoma model using human cerebral organoids. *Cell Rep* 2018;**23**:1220–9
126. Bian S, Repic M, Guo Z, Kavirayani A, Burkard T, Bagley JA, Krauditsch C, Knoblich JA. Genetically engineered cerebral organoids model brain tumor formation. *Nat Methods* 2018;**15**:631–9
127. Seino T, Kawasaki S, Shimokawa M, Tamagawa H, Toshimitsu K, Fujii M, Ohta Y, Matano M, Nanki K, Kawasaki K, Takahashi S, Sugimoto S, Iwasaki E, Takagi J, Itoi T, Kitago M, Kitagawa Y, Kanai T, Sato T. Human pancreatic tumor organoids reveal loss of stem cell niche factor dependence during disease progression. *Cell Stem Cell* 2018;**22**:454–67.e6
128. Liu X, Cheng Y, Abraham JM, Wang Z, Wang Z, Ke X, Yan R, Shin EJ, Ngamruengphong S, Khashab MA, Zhang G, McNamara G, Ewald AJ, Lin D, Liu Z, Meltzer SJ. Modeling Wnt signaling by CRISPR-Cas9 genome editing recapitulates neoplasia in human Barrett epithelial organoids. *Cancer Lett* 2018;**436**:109–18
129. Kawasaki K, Fujii M, Sugimoto S, Ishikawa K, Matano M, Ohta Y, Toshimitsu K, Takahashi S, Hosoe N, Sekine S, Kanai T, Sato T. Chromosome engineering of human colon-derived organoids to develop a model of traditional serrated adenoma. *Gastroenterology* 2020;**158**:638–51 e8
130. Ihry RJ, Worringer KA, Salick MR, Frias E, Ho D, Theriault K, Kommineni S, Chen J, Sondey M, Ye C, Randhawa R, Kulkarni T, Yang Z, McAllister G, Russ C, Reece-Hoyes J, Forrester W, Hoffman GR, Dolmetsch R, Kaykas A. p53 inhibits CRISPR-Cas9 engineering in human pluripotent stem cells. *Nat Med* 2018;**24**:939–46
131. Haapaniemi E, Botla S, Persson J, Schmierer B, Taipale J. CRISPR-Cas9 genome editing induces a p53-mediated DNA damage response. *Nat Med* 2018;**24**:927–30
132. Geurts MH, de Poel E, Amatngalim GD, Oka R, Meijers FM, Kruisselbrink E, van Mourik P, Berkers G, de Winter-de Groot KM, Michel S, Muilwijk D, Aalbers BL, Mullenders J, Boj SF, Suen SWF, Brunsveld JE, Janssens HM, Mall MA, Graeber SY, van Boxtel R, van der Ent CK, Beekman JM, Clevers H. CRISPR-based adenine editors correct nonsense mutations in a cystic fibrosis organoid biobank. *Cell Stem Cell* 2020;**26**:503–10.e7
133. Hu JH, Miller SM, Geurts MH, Tang W, Chen L, Sun N, Zeina CM, Gao X, Rees HA, Lin Z, Liu DR. Evolved Cas9 variants with broad PAM compatibility and high DNA specificity. *Nature* 2018;**556**:57–63
134. Kleinstiver BP, Sousa AA, Walton RT, Tak YE, Hsu JY, Clement K, Welch MM, Horng JE, Malagon-Lopez J, Scarfò I, Maus MV, Pinello L, Aryee MJ, Joung JK. Engineered CRISPR-Cas12a variants with increased activities and improved targeting ranges for gene, epigenetic and base editing. *Nat Biotechnol* 2019;**37**:276–82
135. Walton RT, Christie KA, Whittaker MN, Kleinstiver BP. Unconstrained genome targeting with near-PAMless engineered CRISPR-Cas9 variants. *Science* 2020;**368**:290–6
136. Chatterjee P, Jakimo N, Lee J, Amrani N, Rodriguez T, Koseki SRT, Tysinger E, Qing R, Hao S, Sontheimer EJ, Jacobson J. An engineered ScCas9 with broad PAM range and high specificity and activity. *Nat Biotechnol* 2020;**38**:1154–8
137. Kleinstiver BP, Prew MS, Tsai SQ, Topkar VV, Nguyen NT, Zheng Z, Gonzales AP, Li Z, Peterson RT, Yeh JR, Aryee MJ, Joung JK. Engineered CRISPR-Cas9 nucleases with altered PAM specificities. *Nature* 2015;**523**:481–5
138. Nishimasu H, Shi X, Ishiguro S, Gao L, Hirano S, Okazaki S, Noda T, Abudayyeh OO, Gootenberg JS, Mori H, Oura S, Holmes B, Tanaka M, Seki M, Hirano H, Aburatani H, Ishitani R, Ikawa M, Yachie N, Zhang F, Nureki O. Engineered CRISPR-Cas9 nuclease with expanded targeting space. *Science* 2018;**361**:1259–62
139. Cradick TJ, Fine EJ, Antico CJ, Bao G. CRISPR/Cas9 systems targeting *β*-globin and CCR5 genes have substantial off-target activity. *Nucleic Acids Res* 2013;**41**:9584–92
140. Fu Y, Foden JA, Khayter C, Maeder ML, Reyon D, Joung JK, Sander JD. High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. *Nat Biotechnol* 2013;**31**:822–6
141. Hsu PD, Scott DA, Weinstein JA, Ran FA, Konermann S, Agarwala V, Li Y, Fine EJ, Wu X, Shalem O, Cradick TJ, Marraffini LA, Bao G, Zhang F. DNA targeting specificity of RNA-guided Cas9 nucleases. *Nat Biotechnol* 2013;**31**:827–32
142. Smith C, Gore A, Yan W, Abalde-Atristain L, Li Z, He C, Wang Y, Brodsky RA, Zhang K, Cheng L, Ye Z. Whole-Genome sequencing analysis reveals high specificity of CRISPR/Cas9 and TALEN-based genome editing in human iPSCs. *Cell Stem Cell* 2014;12–5 3
143. Suzuki K, Yu C, Qu J, Li M, Yao X, Yuan T, Goebl A, Tang S, Ren R, Aizawa E, Zhang F, Xu X, Soligalla RD, Chen F, Kim J, Kim NY, Liao HK, Benner C, Esteban CR, Jin Y, Liu GH, Li Y, Izpisua Belmonte JC. Targeted gene correction minimally impacts whole-genome mutational load in human-disease-specific induced pluripotent stem cell clones. *Cell Stem Cell* 2014;**15**:31–6

144. Veres A, Gosis BS, Ding Q, Collins R, Ragavendran A, Brand H, Erdin S, Talkowski ME, Musunuru K. Low incidence of off-target mutations in individual CRISPR-Cas9 and TALEN targeted human stem cell clones detected by whole-genome sequencing. *Cell Stem Cell* 2014;**15**:27–30
145. Jin S, Zong Y, Gao Q, Zhu Z, Wang Y, Qin P, Liang C, Wang D, Qiu JL, Zhang F, Gao C. Cytosine, but not adenine, base editors induce genome-wide off-target mutations in rice. *Science* 2019;**364**:292–5
146. Zuo E, Sun Y, Wei W, Yuan T, Ying W, Sun H, Yuan L, Steinmetz LM, Li Y, Yang H. Cytosine base editor generates substantial off-target single-nucleotide variants in mouse embryos. *Science* 2019;**364**:289–92
147. McGrath E, Shin H, Zhang L, Phue JN, Wu WW, Shen RF, Jang YY, Revollo J, Ye Z. Targeting specificity of APOBEC-based cytosine base editor in human iPSCs determined by whole genome sequencing. *Nat Commun* 2019;**10**:5353
148. Jin S, Fei H, Zhu Z, Luo Y, Liu J, Gao S, Zhang F, Chen YH, Wang Y, Gao C. Rationally designed APOBEC3B cytosine base editors with improved specificity. *Mol Cell* 2020;**79**:728–40.e6
149. Doman JL, Raguram A, Newby GA, Liu DR. Evaluation and minimization of Cas9-independent off-target DNA editing by cytosine base editors. *Nat Biotechnol* 2020;**38**:620–8
150. Bailey J, Thew M, Balls M. An analysis of the use of animal models in predicting human toxicology and drug safety. *Altern Lab Anim* 2014;**42**:181–99
151. Bailey J, Thew M, Balls M. Predicting human drug toxicity and safety via animal tests: can any one species predict drug toxicity in any other, and do monkeys help? *Altern Lab Anim* 2015;**43**:393–403
152. Corrigan-Curay J, O'Reilly M, Kohn DB, Cannon PM, Bao G, Bushman FD, Carroll D, Cathomen T, Joung JK, Roth D, Sadelain M, Scharenberg AM, von Kalle C, Zhang F, Jambou R, Rosenthal E, Hassani M, Singh A, Porteus MH. Genome editing technologies: defining a path to clinic. *Mol Ther* 2015;**23**:796–806
153. Wu H, Uchimura K, Donnelly EL, Kirita Y, Morris SA, Humphreys BD. Comparative analysis and refinement of human PSC-Derived kidney organoid differentiation with Single-Cell transcriptomics. *Cell Stem Cell* 2018;**23**:869–81.e8
154. Watson CL, Mahe MM, Munera J, Howell JC, Sundaram N, Poling HM, Schweitzer JI, Vallance JE, Mayhew CN, Sun Y, Grabowski G, Finkbeiner SR, Spence JR, Shroyer NF, Wells JM, Helmrath MA. An in vivo model of human small intestine using pluripotent stem cells. *Nat Med* 2014;**20**:1310–4
155. Quinn PM, Buck TM, Mulder AA, Ohonin C, Alves CH, Vos RM, Bialecka M, van Herwaarden T, van Dijk EHC, Talib M, Freund C, Mikkers HMM, Hoeben RC, Goumans MJ, Boon CJF, Koster AJ, Chuva de Sousa Lopes SM, Jost CR, Wijnholds J. Human iPSC-derived retinas recapitulate the fetal CRB1 CRB2 complex formation and demonstrate that photoreceptors and Muller glia are targets of AAV5. *Stem Cell Rep* 2019;**12**:906–19
156. Nam SA, Seo E, Kim JW, Kim HW, Kim HL, Kim K, Kim TM, Ju JH, Gomez IG, Uchimura K, Humphreys BD, Yang CW, Lee JY, Kim J, Cho DW, Freedman BS, Kim YK. Graft immaturity and safety concerns in transplanted human kidney organoids. *Exp Mol Med* 2019;**51**:1–13
157. Cheng Q, Wei T, Farbiak L, Johnson LT, Dilliard SA, Siegwart DJ. Selective organ targeting (SORT) nanoparticles for tissue-specific mRNA delivery and CRISPR-Cas gene editing. *Nat Nanotechnol* 2020;**15**:313–20
158. Charlesworth CT, Deshpande PS, Dever DP, Camarena J, Lemgart VT, Cromer MK, Vakulskas CA, Collingwood MA, Zhang L, Bode NM, Behlke MA, Dejene B, Cieniewicz B, Romano R, Lesch BJ, Gomez-Ospina N, Mantri S, Pavel-Dinu M, Weinberg KI, Porteus MH. Identification of preexisting adaptive immunity to Cas9 proteins in humans. *Nat Med* 2019;**25**:249–54
159. Cakir B, Xiang Y, Tanaka Y, Kural MH, Parent M, Kang YJ, Chapeton K, Patterson B, Yuan Y, He CS, Raredon MSB, Dengelegi J, Kim KY, Sun P, Zhong M, Lee S, Patra P, Hyder F, Niklason LE, Lee SH, Yoon YS, Park IH. Engineering of human brain organoids with a functional vascular-like system. *Nat Methods* 2019;**16**:1169–75
160. Takebe T, Enomura M, Yoshizawa E, Kimura M, Koike H, Ueno Y, Matsuzaki T, Yamazaki T, Toyohara T, Osafune K, Nakauchi H, Yoshikawa HY, Taniguchi H. Vascularized and complex organ buds from diverse tissues via mesenchymal cell-driven condensation. *Cell Stem Cell* 2015;**16**:556–65
161. Sarker MD, Naghieh S, Sharma NK, Chen X. 3D biofabrication of vascular networks for tissue regeneration: a report on recent advances. *J Pharm Anal* 2018;**8**:277–96
162. Jiménez-Torres JA, Beebe DJ, Sung KE. A microfluidic method to mimic luminal structures in the tumor microenvironment. *Methods Mol Biol* 2016;**1458**:59–69
163. Jimenez-Torres JA, Virumbrales-Munoz M, Sung KE, Lee MH, Abel EJ, Beebe DJ. Patient-specific organotypic blood vessels as an in vitro model for anti-angiogenic drug response testing in renal cell carcinoma. *EBioMedicine* 2019;**42**:408–19
164. Takebe T, Zhang B, Radisic M. Synergistic engineering: organoids meet organs-on-a-chip. *Cell Stem Cell* 2017;**21**:297–300
165. Takebe T, Wells JM. Organoids by design. *Science* 2019;**364**:956–9
166. Park SE, Georgescu A, Huh D. Organoids-on-a-chip. *Science* 2019;**364**:960–5
167. Qian X, Nguyen HN, Song MM, Hadiono C, Ogden SC, Hammack C, Yao B, Hamersky GR, Jacob F, Zhong C, Yoon KJ, Jeang W, Lin L, Li Y, Thakor J, Berg DA, Zhang C, Kang E, Chickering M, Nauen D, Ho CY, Wen Z, Christian KM, Shi PY, Maher BJ, Wu H, Jin P, Tang H, Song H, Ming GL. Brain-Region-Specific organoids using mini-bioreactors for modeling ZIKV exposure. *Cell* 2016;**165**:1238–54
168. Reid JA, Palmer XL, Mollica PA, Northam N, Sachs PC, Bruno RD. A 3D bioprinter platform for mechanistic analysis of tumoroids and chimeric mammary organoids. *Sci Rep* 2019;**9**:7466
169. Marti-Figueroa CR, Ashton RS. The case for applying tissue engineering methodologies to instruct human organoid morphogenesis. *Acta Biomater* 2017;**54**:35–44
170. Regier MC, Montanez-Sauri SI, Schwartz MP, Murphy WL, Beebe DJ, Sung KE. The influence of biomaterials on cytokine production in 3D cultures. *Biomacromolecules* 2017;**18**:709–18