Genes & Diseases (2022) 9, 585–597

Available online at www.sciencedirect.com

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REVIEW ARTICLE

Kidney organoids as a promising tool in nephrology

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Received 7 November 2020; received in revised form 15 January 2021; accepted 18 January 2021
Available online 30 January 2021

KEYWORDS
Biomedical research;
Disease modelling;
Kidney organoids;
Precision medicine;
Stem cells

Abstract Kidney disease has become a global public health problem affecting over 750 million people worldwide and imposing a heavy economic burden on patients. The complex architecture of the human kidney makes it very difficult to study the pathophysiology of renal diseases *in vitro* and to develop effective therapeutic options for patients. Even though cell lines and animal models have enriched our understanding, they fail to recapitulate key aspects of human kidney development and renal disease at cellular and functional levels. Organoids can be derived from either pluripotent stem cells or adult stem cells by strictly regulating key signalling pathways. Today, these self-differentiated organoids represent a promising technology to further understand the human kidney, one of the most complex organs, in an unprecedented way. The newly established protocols improved by organ-on-chip and coculture with immune cells will push kidney organoids towards the next generation. Herein, we focus on recent achievements in the application of kidney organoids in disease modelling, nephrotoxic testing, precision medicine, biobanking, and regenerative therapy, followed by discussions of novel strategies to improve their utility for biomedical research. The applications we discuss may help to provide new ideas in clinical fields.

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Peer review under responsibility of Chongqing Medical University.
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https://doi.org/10.1016/j.gendis.2021.01.003

Introduction

The human kidney is a complex organ consisting of approximately 1 million nephrons. The nephron is the functional unit of the kidney; it contains a renal corpuscle to filter and a renal tubule to reabsorb. Any change in the structure of the nephron can greatly affect the kidney's function. Multiple cell types and remarkable architectural complexity slow down the study of kidney organogenesis. Additionally, the human kidney plays an important role in whole-body homeostasis, regulating acid-base balance, electrolyte concentrations, extracellular fluid volume, and blood pressure.[1] Kidney disease can be attributed to genetic disorders but can also arise after chronic injuries, such as inflammatory disease, high blood pressure, and diabetes.[2] As a major target organ of toxic side effects the kidney is also highly vulnerable to drug-induced renal impairment.[3] Currently, biological studies in nephrology principally depend on two-dimensional (2D) cell lines. However, cell lines lack multiple cell types and cell—scaffold interactions. Cells in a monolayer have no support for spreading in the vertical dimension, which may result in abnormal polarity for specific cells. The absence of oxygen, nutrients and factors in conventional 2D culture medium also restricts its accuracy in reflecting the *in vivo* environment. To better understand kidney disease, effective models are in urgently needed.

Organoids are *in vitro*-derived three-dimensional (3D) cell aggregates, characterized by self-renewal and self-organization, and they exhibit similar organ functionality as the tissue of origin. They can be generated from both induced pluripotent stem cells (iPSCs) and adult stem cells (ASCs). In addition to the multicellular structures known as organoids, the culture system of organoids also includes various growth factors selected on the basis of their role(s) in kidney development. Extracellular matrix (ECM) is also needed for cell differentiation and orientation, which not only provides mechanical support for organoids but also participates in cell growth, migration, differentiation, and cell survival.[4]

Over the past few years, numerous organoids have been generated to model organs derived from different germlines. These organoids have been employed to study various diseases, especially monogenic hereditary diseases. In addition, tumour organoids are emerging as a powerful tool to clarify the mechanism of cancer initiation and progression. Recently, some researchers have also attempted to exploit organoids to study the complex tumour microenvironment.

Kidney organoids provide a novel approach to study nephrology. In this review, we will mainly focus on the application of kidney organoids in the fields of kidney development and disease, drug testing, and precision medicine. We will also describe the achievements and limitations of new technologies developed in the past 5 years, such as single-cell RNA sequencing and microfluidic devices, to highlight new horizons in basic research and clinical therapy.

Bibliometric analysis of researches on kidney organoids

A brief introduction and purpose of bibliometric analysis

Again, kidney organoids played a pivotal role in modelling disease, groping after novel and effective methods to dissect disease pathology and promote the development of precision medicine. In recent years, with the continuous progress of 3D organoids, especially in kidney medicine, surrounding this subject, the researchers in or outside the country have published many theses by deepening the exploration into the etiology, pathogenesis, application et al. To make this review more systematically and comprehensively, we combed and summarized the published studies by Bibliometric analysis.

Methods

Data source and collection

Search strategies were performed in the Web of Science up to 31st August 2020 by using keywords "kidney organoids", "stem cells", "disease modelling" and "precision medicine".

Inclusion and exclusion criteria and literature selection

We included studies on kidney organoids and excluded reduplicative publications, Conference Address and coverages. Two reviewers evaluated the keywords, authors, countries and institutions respectively; solely, the discrepant results were evaluated by the third reviewer.

Data analysis by visualization VOSviewer

We used VOSviewer 1.6.11 (Leiden University, Leiden, Netherlands) to extract the authors, countries, institutions and keywords of included literatures; then, network maps and cluster analysis were generated. Additionally, we generated a density map of keywords. Of note, in network maps the nodes were considered as being behalf of representing the number or frequency of analyzed objects and the connects among nodes indeed represented relations including co-occurrence and etc; besides, different clusters were differentiated by varied colors. Equally, in the density map, the brightness of the color was diametrically related to the frequency of the keywords.

Results

Search results

There were 863 publications retrieved, of which 469 from core journals. We excluded 15 publications in accordance with the exclusion criteria, and in the end, 454 publications were included.

Keywords

2029 keywords were extracted from the included studies. And we summarized the keywords with top 20 frequency (see Table 1).

A density map was generated for the keywords with frequency >5 (see Fig. 1). One hundred and forty-eight nodes were presented in the density map and the brightest two were organoids and pluripotent stem-cells where located in the centre of map and surrounded by stem-cells, generation, *in-vitro*, intermediate mesoderm, nephron progenitors et al. As shown, the margin of the map was reversely dark including blinding, Igr5, colon, pathway et al.

Authors

A total of 2,894 authors have been busy with relevant studies on kidney organoids, Among them, 740 (25.6%) authors have only one study published; surprisingly, 24 (0.8%) authors have engaged in more than five publications. We generated the network map of authors with frequency >5, of which 26 authors relating to four clusters meet the thresholds (see Fig. 2A). On the right side of network map contains Miyoshi Tomoya, Bonventre Joseph V and Morizane Ryuji (#3, three authors); cluster on the left of the map was the biggest cluster including eight authors (#1), and Melissa H was an vital node which connected cluster #1, cluster #2 and cluster #4. The greem cluster (#2) containing six authors showed close links to cluster #3 and Freedman Benjamin.S was the important node who connected cluster #2 to cluster #3.

Countries and institution

Forty-two countries all over the world were included in researches on kidney organoids and a network map was generated for basic visualization (see Fig. 2B). As shown, the network included 42 nodes and 150 links. The cooperation among countries were correspondingly tightening, and the USA (150/216 69.4%) was the largest node which closely related to Canada, Australia, Scotland, Egypt, *etc*.

Table 1 Keywords with top 20 frequency.

Rank	Keywords	Frequency
1	organoids	128 (87.0%)
2	pluripotent stem-cells	90 (61.2%)
3	expression	78 (53.0%)
4	generation	78 (53.0%)
5	differentiation	77 (52.4%)
6	*in-vitro*	63 (42.9%)
7	model	52 (35.4%)
8	kidney organoids	51 (34.7%)
9	stem-cells	50 (34.0%)
10	mouse	49 (33.3%)
11	disease	48 (32.7%)
12	kidney	42 (28.6%)
13	culture	36 (24.5%)
14	intermediate mesoderm	30 (20.4%)
15	nephron progenitors	30 (20.4%)
16	cells	28 (19.0%)
17	cancer	27 (18.4%)
18	transplantation	27 (18.4%)
19	directed differentiation	26 (17.7%)
20	identification	25 (17.0%)

Additionally, 717 institutions contributed to the publications on kidney organoids, and 338 of whom engaged in only one study. We summarized the organizations which published more than eight publications in Table 2. As shown, the organizations are comprised of sixteen universities, two companies and four research institutes, We also generated network map of organizations with frequency>5 (see Fig. 2C). The above-mentioned network map contained 49 nodes and 6 clusters. The three biggest nodes were Harvard Medical School (35/717 4.88%), Univ Melbourne (30/717 4.18%) and Brigham & Womens Hosp (22/717 2.93%). The largest cluster #1 was comprised of Leiden Univ, Murdoch Childrens Res Inst, Royal Childrens Hosp, Univ Cambridge, Univ Edinburgh, Univ Manchester, Univ Melbourne, Univ Michigan, Univ Queensland, Univ Southern Calif and Washington Univ. And the second largest cluster #2 was principally consisted of the affiliations of Harvard, such as Harvard University, Harvard Stem Cell Inst and Harvard Med Sch. The thord cluster #3 was consisted of nine universities including Baylor Coll Med, Chinese Acad Sci, Cincinnati Childrens Hosp Med Ctr, Icahn Sch Med Mt Sinai, Johns Hopkins Univ, Mem Sloan Kettering Canc Ctr, Univ Chicago, Univ Penn and Weill Cornell Med. And the above-mentioned universities all connected tightly with other universities. As demonstrated, Univ Queensland was an important node connrcting cluster #1, cluster #2, cluster #3, cluster #4 and cluster #6.

Discussion

The above-mentioned study analyzed the keywords, authors, institutions and countries of researchers on kidney organoids on the basis of Bibliometric analysis which presented a current situation of studies on kidney organoids in and outside the world. For the countries involved, more than 61.9% of countries published over three studies, distinctly, close cooperation among varied countries has been noticed, which is beneficial to speeding up the quantitative and qualitative change of scientific and technical payoffs. In general, we can focus on interdisciplinary cooperation to improve catalysts to speed them up.

Application of kidney organoids

Unveiling kidney organogenesis

One of the greatest potential applications of the organoid model is in the study of kidney development. Until now, analysing kidney organogenesis has been mostly based on animal models. Although the mouse model has indeed bettered our understanding of human development, there is no denying that the fundamental differences between humans and animals have hindered further study.[5] As compared with animal models, organoids are easier to genetic engineering and allow the study of developmental diseases more accurately (see Fig. 3). Although, the human embryo is considered the best material, such work on the embryo as molecular and cellular model is usually inhibited in most countries due to ethical issues. Organoids, as an emerging technology, are thus becoming a revolutionary way forward for developmental studies.[6]

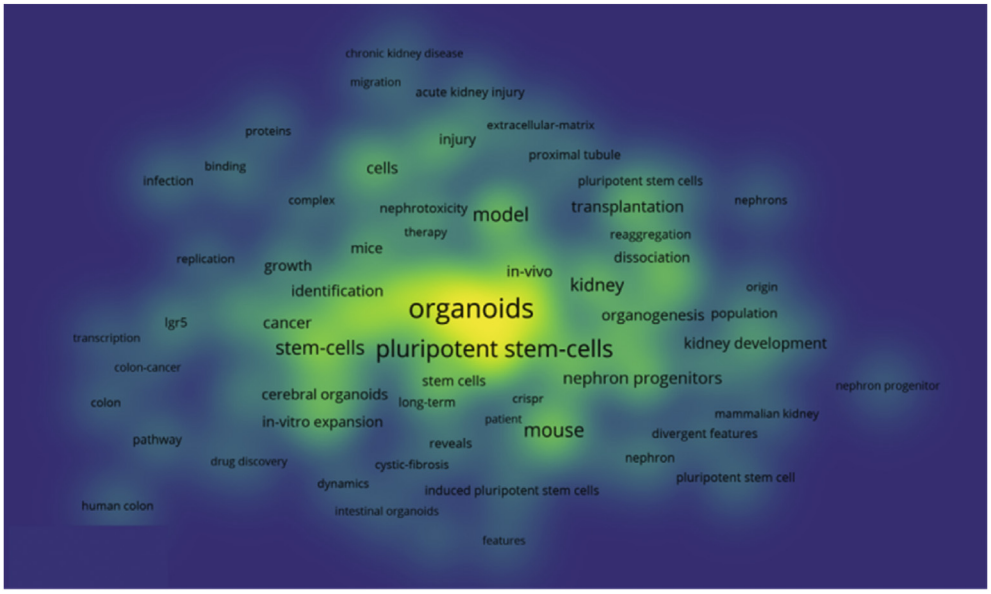


Figure 1 Density map of main keywords. Note: The brightness of the color is positively correlated with the frequency of keywords.

In the past few years, scientists have developed multiple protocols with human induced pluripotent stem cell-derived (hiPSC-derived) kidney organoids to recapitulate kidney development *in vivo*. A combination of small molecules is added to culture systems to induce *in vitro* renal lineage differentiation.[7,8] Takasato and colleagues reported a multi-step protocol to culture 3D kidney organoids from iPSCs. They varied the duration of initial Wnt signalling before addition of FGF9, a kind of growth factor that can induce the formation of the intermediate mesoderm, and they found that the time of exposure to Wnt signalling regulated the direction of cell differentiation, confirming the role of Wnt signalling in kidney organogenesis. These results greatly increase our understanding of kidney embryogenesis. hiPSC-derived kidney organoids are also used to study podocyte maturation.[9] The migration of tight junction components such as ZO-1 from the apical to the basement membrane was captured. To characterize podocyte development, scRNA-Seq was performed on podocytes from human foetal kidney and organoid-generated podocytes to compare the transcriptional profiles. Despite the absence of vasculature, organoid-generated podocytes still exhibited highly similar, progressive transcriptional profiles.[10]

Another potential application is in the study of self-organization. Cellular self-organization entails a change in cell behaviour during the interaction with other cells. The information flow in such highly interconnected networks often contains feedback loops rather than linear pathways.[11] This inherent non-linearity is, therefore, regarded as the basis for the emergence of tissue-level phenomena. Kidney organogenesis includes the complex temporal and special connection between the ureteric bud and metanephric mesenchyme where self-organization plays a special role. Due to technological restrictions, deciphering the underlying mechanisms remains a challenge.

However, the advancements in organoids have opened up innovative approaches to this field. Taguchi et al[12] established a protocol for reconstituting higher-order organ structures that can be used to recapitulate embryonic branching morphogenesis. They integrated the branching epithelium into an organoid as an organizer of tissue geometry and confirmed that, as the progenitor niche, the branching epithelium is essential for the expansion of the organ size. Their reassembly method together with kidney organoids will be a powerful approach to recapitulate organotypic architecture.

All in all, because they can bypass ethical issues to some extent, organoids from iPSCs will serve as a remarkable method to yield insights into kidney development. The constant optimization of organoids may promote further improvements in developmental study. Below we discuss the potential applications of kidney organoids in disease modelling, nephrotoxic testing, precision medicine, biobanking and regenerative therapy (see Fig. 4).

Disease modelling

Recently, studies have described several successful attempts at using kidney organoids as a novel disease-modelling platform. Compared with conventional cell lines that are lineage-restricted, organoids induced from iPSCs or adult kidney tubular epithelium are genetically diverse and resemble the complex *in vivo* environment. The presence of multiple cell types makes it possible to study the microenvironment of disease *in vitro*. Moreover, the 3D cell cultures used with organoids are an important step in a trend towards ever more physiologically relevant tissue models.[13] In the case of glomeruli for example, 3D organoid-derived glomeruli displayed enhanced gene expression associated with slit diaphragm components, renal filtration cell differentiation, and glomerular development in comparison to that in 2D cultures.[14] Base on the present technology, many

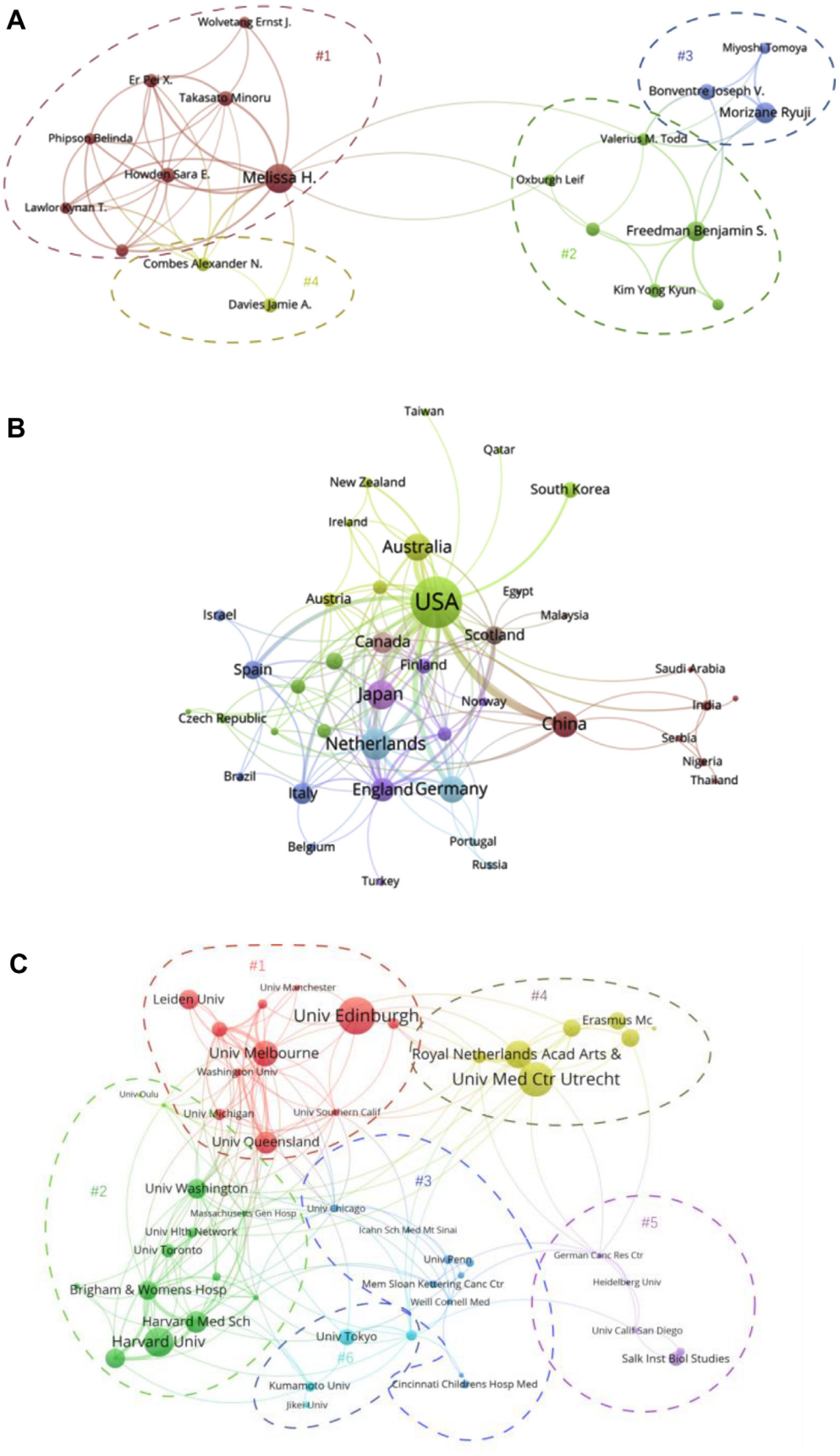


Figure 2 Network map of studies on kidney organoids around the world. (**A**) Network map of 26 authors with frequency greater than five. (**B**) Countries involved studies on kidney organoids. (**C**) Network map of 49 institutions with frequency greater than five. Note: The nodes represent the number of frequency, the links between nodes represent collaboration, and different colors of nodes represent different clusters.

Table 2 Institutions Published More Than eight Studies on kidney organoids Rank.

Rank	label	cluster	Frequency
1	Harvard Medical School	1	35 (4.88%)
2	Univ Melbourne	4	30 (4.18%)
3	Brigham & Womens Hosp	1	22 (2.93%)
4	Harvard Stem Cell Inst	1	21 (2.93%)
5	Murdoch Childrens Res Inst	4	21 (2.93%)
6	Univ Med Ctr Utrecht	2	19 (2.93%)
7	Univ Washington	3	19 (2.93%)
8	Harvard Univ	1	17 (2.37%)
9	Kumamoto Univ	5	15 (2.09%)
10	Royal Netherlands Acad Arts & Sci	2	15 (2.09%)
11	Univ Edinburgh	3	14 (1.95%)
12	Univ Queensland	4	13 (1.81%)
13	Univ Toronto	1	13 (1.81%)
14	Leiden Univ	4	10 (1.39%)
15	Princess Maxima Ctr Pediat Oncol	2	10 (1.39%)
16	Stanford Univ	5	10 (1.39%)
17	Washington Univ	3	10 (1.39%)
18	Royal Childrens Hosp	4	9 (1.26%)
19	German Cancer Research Center	2	8 (1.12%)
20	Massachusetts Institute of Technology (MIT)	1	8 (1.12%)
21	Univ Michigan	3	8 (1.12%)
22	Univ Southern Calif	3	8 (1.12%)

researchers have applied kidney organoids to renal disease modelling (see Table 3).

PSC-derived organoids

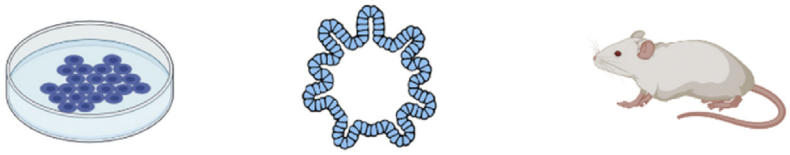
PSC-derived organoids are generated using a combination of directed differentiation, morphogenetic processes, and the intrinsically driven self-assembly of cells.[20] They have the potential to form structures through processes that only occur during embryonic development, thus, they have an advantage in hereditary disease modelling.

The kidney is an extremely complex organ with a wide range of abnormalities, many of which have a significant genetic component. Genetic disorders of renal structure and function might result in end-stage renal disease (ESRD), for which treatments are of limited availability and efficacy.[21]

Within the monogenic renal diseases, autosomal dominant polycystic kidney disease (ADPKD) draws much attention due to its high incidence rate affecting over 12.5 million people worldwide.[22] It is characterized by the abnormal development and growth of multiple cysts within the kidney. Mutations in PKD, which encodes the protein polycystin that is normally largely expressed in the primary cilium, are responsible for more than 90% of ADPKD cases.[23] However, the role of these mutations in the early stages remains to be explored. Freeman et al[15] was the first to establish renal-like structures *in vitro* and recapitulated a PKD-relevant phenotype through a combination of iPSC-derived kidney cells and CRISPR/Cas9 gene editing. Renal organoids were induced from PKD-iPSCs with biallelic, truncating mutations in PKD1 or PKD2 using CRISPR/Cas9 gene editing. Several weeks later, large, translucent, cyst-like structures were observed alongside kidney organoids in PKD hPSCs, which were absent in unmodified controls. At approximately 35 days, PKD cysts first became noticeable and continued to expand for the duration of the culture. Such findings validated that PKD mutations independently lead to abnormal cystogenesis.[15] High-throughput screening of this gene-edited model further indicated that polycystins may normally function to positively regulate actomyosin activation within the tubular epithelium, strengthening and tightening the tubule to prevent the formation of a cyst.[24] The results partly clarified the molecular functions of the polycystin proteins in ADPKD.

Unlike ADPKD for which genetic mutations can be easily observed in living patients, the incidence of PODXL mutations in podocytopathies is relatively low due to its embryonic or perinatal lethality, making it more difficult to study the role of PODXL mutations in disease.[25] In addition, podocytes are also challenging to study in culture systems because they are prone to dedifferentiate.[26] Kim et al[9] made full use of abundant iPSC-podocytes derived from PODXL$^{-/-}$ iPSCs in kidney organoids, and compared them to developing podocytes in tissue sections, revealing that iPSC-podocytes phenocopy podocytes at the capillary loop stage (CLS). HPSC-podocytes exhibited a reduction in microvilli and lateral spaces, implying that podocalyxin is essential for podocyte microvillus formation. More importantly, these phenotypes were also consistent with results in podocalyxin-deficient mice at the CLS.[9] Hence, iPSC-podocytes are an attractive candidate to mimic the podocytopathies of a specific stage of podocytes *in vivo*.

As more and more novel genetic mutations from patients are identified, it is difficult to discriminate whether these mutations are single nucleotide polymorphism (SNPs) or disease-causing mutations using cell lines or model organisms. Animal models have limitations, such as genetic differences between species and the inability to account for the possibility of the observed variation resulting from modifier genes that are present in the affected individual but not in the animal model. Three D patient-derived renal cellular models have somewhat overcome these limitations, and they may offer opportunities to validate novel disease-related mutations and to study their cellular mechanism. For example, kidney organoids were generated from proband iPSCs lines, which contain compound-heterozygous variants in IFT140[17]. This proband was identified as nephronophthisis (NPHP), a monogenic disease with inevitable decline to ESRD. Although previous studies have found that IFD140 is associated with NPHP-related ciliopathies, the mechanism remains unknown.[27] Patient-derived organoids exhibited significantly shorter cilia, consistent with defective retrograde IFT. Gene enrichment analysis of these patient-derived organoids found abnormal expression of multiple downstream genes that play a part in cellular cytoskeletal interaction pathways and adhesion, consistent with the proband phenotype. Of note, kidney organoid tubules from gene-corrected PSCs restored normal ciliary morphology, verifying the IFD140 variant as disease-causing.[17]



	2D cell lines	kidney organoids	aninal models
Cost of culture	++	+	+++
Physiologic structure	+	++	+++
Cell-cell contact	+	++	+++
Vascularization and nerve	–	–	+++
wild cell types	–	–	–
Long-term genetic stability	+	++	++
High-thought screening	+++	++	–
Genetic engineering	+++	++	+
Biobanking	++	+++	+
Modelling kidney development and disease	+	+++	++

+++:best; ++:suitable; +:possible

Figure 3 Characteristics of the three mainstream preclinical kidney diseases models.

Tubuloids

Fully differentiated epithelia are responsible for the replacement of damaged cells in kidney proximal tubule through dedifferentiation. Tubuloids are derived from adult kidney tubular epithelia, which were induced to a stem cell/progenitor state. They have limited complexity and only reflect the epithelial parts of organs with absence of blood vessels and stromal cells. These organoids invariably give rise to cystic and highly polarized epithelial structures containing the entire assortment of differentiated, functional epithelial cells with architectural aspects of the original epithelium. Tubuloids can be rapidly produced and the genetic stability makes them more suitable to expand over longer passages.[19]

Schutgens F et al. [19]applied tubuloids to study BK virus infection. Tubuloids infected with BK virus exhibited enlarged nuclei, a typical pathological presentation of BK virus nephropathy. Furthermore, BK virus expanded stably in kidney tubuloids. This study demonstrated that kidney tubuloids can be potentially employed to model infectious diseases.[19] Meanwhile the team also applied the tubuloid culture protocols to study cancer modelling. Traditional cancer cell lines have long been used for cancer modelling. The types of cell lines are limited as only a subset of tumour cells are amenable to expansion on plastic tissue culture substrates. Likewise, the genes in cancer cell lines are unstable, resulting in cumulative mutations over a lifetime.[28] Although animal cancer models have provided an important research tool to explore basic mechanisms of cancer, their generation can be time-consuming, and it is argued that these models often fail to faithfully recapitulate disease progression in patients. While patient-derived tumour xenografting (PDTX) can recapitulate each patient's cancer pathology, its time-consuming and expensive nature also present challenges. In addition, PDTX models are less amenable to genetic manipulation.[29] Conversely, tumour organoids from patient biopsies can be generated efficiently from tumour tissue and capture tumour heterogeneity and with less cost and time. In 2019, Schutgens F et al. [19]cultured tumour-derived organoids from two patients with Wilms' tumour, which is the most common type of renal cancer in the paediatric age group and is a type of nephroblastoma.[30] The tumour organoids included stroma, blastema, and epithelium similar to the original tumour tissue. A different morphology was observed between the tumour-derived structure and organoids derived from healthy tissues and in particular, a stromal (non-epithelial) compartment only appeared in the tumour-derived system. Researchers also found identical copy number variations (CNVs) in both the tumour organoids and the primary tumour tissue, indicating

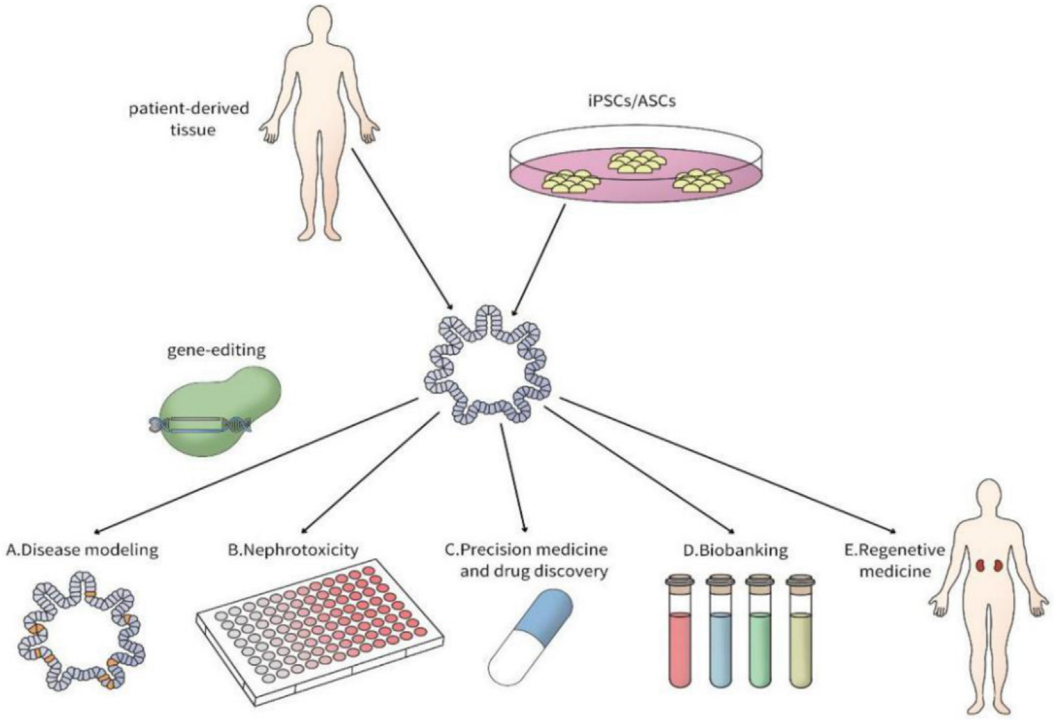


Figure 4 Potential applications in disease modelling, nephrotoxic testing, precision medicine, biobanking and regenerative therapy. (**A**) Organoids from patient-derived tissues or iPSCs/ASCs can be used to model diseases through genetic manipulations. (**B**) Nephrotoxicity testing can be constructed on a large scale. (**C**) Organoids facilitate the development of personalized medicine and drug discovery. (**D**) Organoids serve as a renewable resource in biobanking. (**E**) The ultimate goal of organoids is to generate functional human organs for regenerative medicine.

that tumour organoids retain the genetic features of the primary tumour.

Nephrotoxicity testing

Nephrotoxicity refers to the toxic effects on kidney tissue usually induced by chemotherapeutic drugs, and it usually results in acute kidney injury (AKI), a global health problem that lacks targeted therapies.[31] Toxicity analysis is necessary for any drug before it enters the market. As a novel and economical tool, kidney organoids may serve as an excellent platform on which to monitor and assess the biological effects of drugs. Recently, He C et al[32] provided evidence that kidney organoids are more sensitive to drugs than 2D cell culture. They assessed the nephrotoxicity of Quantum dots using kidney organoids and HK2 human renal tubular epithelial cells in a 2D culture system. Lower IC50 values in organoid models validated their higher sensitivity. Cisplatin is a chemotherapy medication used to treat numerous human cancers; however, the antitumour efficacy of cisplatin is severely limited by cisplatin-induced kidney injury.[33] To test the toxic effects of cisplatin more accurately, large numbers of iPSC-derived kidney organoids were generated and exposed to cisplatin with doses ranging from 5 to 100 μM. After cisplatin treatment, the expression of AKI markers increased and kidney organoids responded with dose-dependent DNA damage and cell death. Strikingly, cisplatin predominately targets the stromal compartment instead of the proximal tubule, which might be attributed to the lower expression of cisplatin transporters in proximal tubule cells of kidney organoids.[34] However, despite successful attempts at nephrotoxicity testing using organoids, iPSC-derived organoids are still immature and represent a foetal stage of differentiation. Future studies are needed to make kidney organoids a more practicable model for screening nephrotoxicity.

The application of regenerative medicine

Currently, kidney transplantation is the most effective treatment for end-stage renal failure. However, the global shortage of organs for transplantation prevents it from becoming a routine therapy.[35] Furthermore, patients with organ transplants often depend on long-term immunosuppressive drugs, which also brings about an increased risk of infection and malignancy.[36] Human organoids avoid the basic problems of tissue rejection and ethical issues, and thus show promise in terms of kidney regeneration in humans.

Generating a completely functional organ is the ultimate goal of organoids. Although Takasato successfully generated iPSC-derived organoids containing multiple cell types, including glomeruli, renal tissue, stromal and endothelial cells, RNA sequencing analysis indicated that these organoids resembled the first trimester kidney and single-cell transcriptomics of kidney organoids cells also demonstrated that the kidney cell types were immature when benchmarked against foetal and adult human single-cell

Table 3 Applications of organoids in renal disease modeling.

Source	Diseases modelled	Gene mutations	Physiological studied	Limitations	Ref
Human iPSCs	Polycystic kidney disease	PKD-1 and PKD-2	Organoids can recapulate the process of cyst formation	Organoids culture system lacks vascularized glomerulus	[15]
Human iPSCs	Polycystic kidney disease	PKD-1 and PKD-2	Adherent forces limit tubular deformation and subsequent cyst formation in organoids with PKD mutation	Collecting ducts cannot be examined due to immaturity	[16]
Human iPSCs	Disease in Podocyte Development	PODXL	Podocalyxin is required for efficient microvillus formation in podocytes	Endothelial cells were relatively low in abundance	[9]
Patient-derived iPSCs	Nephronophthisis-related ciliopathy	IFT-140	IFT-140 affects ciliary length, unstable mRNA transcript can be reversed by gene correction	Renal stroma was not sufficiently characterized	[17]
Patient-derived iPSCs	Alport Syndrome	COL4A5	Podocytes differentiated from iPSCs indicate dysfunctional potassium channel activity	Podocyte-like cells did not show functional properties of podocytes *in vitro*	[18]
Patient-derived ASCs	Cystic fibrosis	CFTR	Cystic fibrosis organoids respond to forskolin swelling slightly	Organoids culture system lacks interstitial cells and vasculature	[19]

datasets.[8,37] To promote the development and maturation of organoids, iPSC-derived nephron progenitor spheres were induced *in vitro* to initiate tubulogenesis, followed by transplantation to beneath the kidney capsule of immunodeficient mice.[38] For the first time, this improved technology led to the successful generation of vascularized iPSC-derived podocytes. ScRNA-seq analyses also identified that podocytes nucleated vascular (host-derived) and mesangial (graft-derived) developmental programs.[10] An alternative approach for vasculature development is to implant kidney organoids into chick chorioallantoic membranes (CAMs), which are a naturally immunodeficient environment. CAM implanted organoids also exhibited higher levels of vascularization and maturation.[39]

Another option is to transplant kidney organoids into lymph nodes (LDs). There are abundant LNs in the human body, making them more easily accessible. More importantly, LNs have ready access to the bloodstream, which supplies nutrients and oxygen to the cells. Hormones and signalling agents can also be transported to the LNs, regulating cell growth. Active neoangiogenesis at this site guarantees sustainable cell survival and engraftment as well.[40] Based on this, mouse renal embryonic tissue was transplanted into jejunal lymph nodes. Not only were fully mature nephrons vascularized by host arterioles observed, but glomerular cysts were also present in some mice.[41]

A similar approach was applied to iPSC-derived kidney organoids. Glomerular-like structures containing mature podocytes were observed. This work demonstrated that LNs might act as a bioreactor and provide a more suitable site for ectopic kidney organogenesis.[42]

The human kidney is an exquisitely complex structure with the presence of more than 26 different specialized cells. Such diversity of cell types has greatly increased the difficulty in producing transplantable kidneys *in vitro*. Taken together, transplanting kidney organoids into a highly vascularized site not only induces vascularization and maturation but also diminishes off-target cells.[43] Whether kidney organoids will be able to integrate into clinical settings as a strategy for regenerative medicine relies on their safety, reproducibility, and scalability, as well as their cost-effectiveness. With the advancement of multiple technologies applicable to kidney organoids, future efforts will no doubt bring them closer to the ultimate goal of using them to replace or repair functional tissue units.

Precision medicine and drug discovery

The last two decades have seen remarkable development in the field known as "precision medicine", fuelled by researchers in academia and industries creating innovative therapeutic strategies. Precision medicine aims at personalized treatment and improved outcomes through a greater understanding of individual data.[44] Kidney tubuloids derived from the urine of patients with cystic fibrosis can be used to measure cystic fibrosis transmembrane conductance regulator function (CFTR) by forskolin swelling assays, which allows for the identification of individuals who will benefit from CFTR-potentiator drug treatment.[19] As previously discussed, bulk tumours might include a diverse collection of cells harbouring distinct molecular signatures with differential levels of sensitivity to treatment. Such tumour heterogeneity provides the fuel for drug resistance leading to low treatment efficacy.[45] To date, our understanding of cancer drug response and resistance mainly depends on experiments in limited human cancer cell lines. These cell lines lack tumour

heterogeneity, thereby hampering drug development. Such a small number of effective anticancer drugs calls for more effective preclinical models. Grassi et al[46] collected freshly explanted samples of clear cell renal cancer (ccRCC) tissue and matched normal counterparts and generated organoids from both tissues following previously described protocols. Organoids capture tumour heterogeneity, thus, they reflect the original patient tissue better than cell lines. Grassi et al then used tumour and matched normal organoids isolated from two patients to test the effect of anticancer therapies based on multi-kinase inhibition, i.e. SU11274, Foretinib, Cabozantinib, and Levantinib in combination with Everolimus, which were in on-going trials. In the experiment, ccRCC organoids were affected by Foretinib and SU11274, while the expression of target genes pAKT S437 and Perk T202/Y204 was reduced. Additionally, only Foretinib consistently increased the expression of cleaved caspase-3, indicating a high apoptotic rate. For the Covid-19 pandemic, ACE2 is regarded as a key receptor, which is expressed in multiple organs or tissues including alveolar epithelial type II cells, blood vessels, kidneys, heart, and intestine and may lead to the multi-organ failure observed in most severe cases.[47–51] To test whether SARS-CoV-2 can damage human tubular kidney cells directly, Monteil V et al generated kidney organoids to mimic the process of infection. Single-cell RNA sequencing of kidney organoids showed tubular kidney cells and podocytes in kidney organoids expressed ACE2, in a similar manner *in vivo*. Of note, the supernatant of infected kidney organoids could infect Vero E6 cells successfully showing the presence of infectious progeny virus in the engineered kidney organoids. Monteil V and their colleagues then tested the potential therapeutic effect of human recombinant soluble ACE2 (hrsACE2) on the infected organoids. They found that hrsACE2 significantly reduced SARS-CoV-2 levels in a dose-dependent manner and had no toxic effects to kidney organoids in the monitored 3 days. These results demonstrated that hrsACE2 could inhibited the infection by blockade of ACE2 in early stages. As ACE2 also exerts some beneficial or protective influence on other kidney diseases, especially diabetic nephropathy, the established human kidney organoids represent a potential step forward for to evaluate new therapies for various diseases.[52–54] Collectively, A sophisticated system of these results are evidence of the attractiveness of organoid application in drug discovery.

Biobanking

Living biobanks of healthy and diseased human organoids allow organoid models to serve as a renewable resource. Large sample sizes are necessary in translational research to ensure that drug screening results are reliable, and organoid biobanks may bridge the gap between basic research and translational medicine.[44] Additionally, organoid biobanks will have particular benefit for the study of less frequently occurring cancers.[46,55] A newly established organoid biobank for childhood kidney cancers collected tumours and matched normal kidney organoids from a large group of patients with different subtypes of kidney cancer, including Wilms tumours and other less common types of childhood kidney tumours such as malignant rhabdoid tumours, congenital mesoblastic nephroma, and renal cell carcinomas. High-throughput screening of a panel of 150 compounds identified the top 25 most effective compounds for four Wilms tumour organoids, with most of the compounds belonging to the MEK and HDAC inhibitor families. To validate that the most potent MEK and HDAC inhibitors were tumour-specific, Romidepsin, Panobinostat, and PD0325901 were further tested using both normal and tumour organoids. Interestingly, researchers identified a less toxic therapeutic strategy for Wilms tumours, as it was shown that Wilms tumour organoids were exquisitely sensitive to Panobinostat (pan-HDAC inhibitor). Moreover, organoids with severely hampered P53 function demonstrated high sensitivity to Idasanulin, a therapeutic P53 stabilizing agent, indicating that P53-activating agents are particularly applicable to this kind of tumour.[56]

In summary, unlike clinical tumour samples, which contain massive amounts of necrotic tissue as wells as non-tumour cells, tumour organoids are pure tumour cultures. In addition, they provide limitless availability of material due to the ability to propagate them. Organoid biobanks include a large sample size of different kinds of tumours, which thus allows drug screening on a large scale. Genetic manipulation makes it possible to design personalized therapy and the data from biobanks may assist in making a therapeutic plan. The established paediatric cancer organoid biobank may pave the way for improved therapeutic strategies.

Towards a new generation of kidney organoids

Organoids-on-a-chip

As mentioned above, kidney organoids generated using conventional protocols are far from immature. For example, the glomerular structures are largely avascular and only a small fraction of the transcription factors that have been identified in adult cell types are expressed in both proximal tubule cells and podocytes derived from organoids.[8,37,38,57] Moreover, longer organoid incubation did not facilitate differentiation but increased the proportion of off-target cells and reduced the expression of terminal markers.[58] Although researchers have generated kidney organoids by transplantation with a perfusable vasculature that induced nephron epithelial maturation, generating a vascular network and terminally differentiated cells *in vitro* remains challenging.

Existing methods rely on 3D culture of stem cells with the addition of growth factors. However, these culture systems fail to produce a dynamic microenvironment including tissue-tissue interactions, cyclic mechanical forces and shear stress, which are crucial to organogenesis. To improve the present culture system, researchers have attempted to integrate organoids with organ-on-a-chip technology, a microfabricated cell culture device used to model the functional units of human organs.[59] Musah et al[60] designed a kidney-glomerulus-on-a-chip that can recapitulate the tissue-tissue interface of the human glomerular capillary wall, which has not been achieved before. Cyclic strains together with fluid flow contributed to an increase in the number of podocyte cell processes, indicating a high level of maturation. Glomerulus-on-a-chip also reconstituted the normal filtration barrier

in vitro with restricted permeability of larger molecules. Additionally, iPSC-derived podocytes secreted a higher level of VEGF-A, an important factor in glomeruli development and vascular patterning *in vivo*. Furthermore, tubuloids cultured on an organ-an-chip allowed extended expansion and transporter activity analysis, enabling personalized molecule studies in tubuloids.[19] Recently, Homan and colleagues cocultured kidney organoids with human endothelial cells using a millifluidic culture system. Their research focused more on the effect of fluidic shear stress (FSS) on the maturation of both glomerular and tubular structures. High FSS induced vascularization of glomerular compartments and increased formation and maturation of tubular epithelia, opening up new avenues for investigating organogenesis, tubular and glomerular disease, and kidney regeneration.[61] Multiorgan models using a microfluidic array to coculture intestine, liver, brain, and kidney organoids have also been generated to model multi-organ interactions. Notably, the four organ models were derived from a single donor and shared the same genetic background, permitting more accurate and precise drug testing.[62] In the future, the integration of biosensing and mechanical automation to control organoid culture will further promote the reproducibility of organoids-on-a-chip.

Organoids for immunotherapy

The tumour microenvironment (TME) comprises various cell types and extracellular components that surround tumour cells. The TME is being increasingly recognized as a key factor participating in tumour initiation, progression, and metastasis. A growing body of evidence suggests that the TME also has a profound effect on cancer treatment responses.[63] Recapitulating the tumour microenvironment *in vitro* has been a major problem. Tumour organoids are composed exclusively of tumour cells and lack cancer-associated stromal and immune components that have important roles in disease pathogenesis, however, organoid systems make it possible to study interactions between immune cells and cancer cells. A recent report described patient-derived tumour organoids cultured in an air-liquid interface system. The organoids preserved not only the genetic alterations of their original tumour tissues but also the complex TME architecture including diverse immune populations. Immune checkpoint inhibitor treatment was performed and resulted in the expansion and activation of antigen-specific TILs. PD-1/PDL-1 checkpoint blockade promoted tumour epithelial cell killing, indicating that human PDO tumour-infiltrating lymphocytes (TILs) functionally recapitulated the PD-1-dependent immune checkpoint.[64] As immunotherapies have shown therapeutic efficacy in some specific human and are revolutionizing cancer therapy, the difference of clinical efficacy from tumor types significantly limited its utility. To clarify the interaction between tumor cells and endogenous T cells, a strategy to generate a large amount of tumor reactive cells is needed especially for epithelial tumor. Tumour organoids obtained from patients who display resistance to PD-1 blockade were cocultured with peripheral blood lymphocytes to get T-cell populations. Their protocol makes it easier to access the efficacy of T cell-based immunotherapy *in vitro*[65] Patient-derived tumour organoids provide a more effective platform to test the effects of such novel therapies and will further advance personal cancer immunotherapy.

Conclusion and outlook

Kidney organoids have a promising future in various fields due to their accessibility and manoeuvrability. To date, much has been done to recapture kidney development through the process of directing the differentiation of pluripotent stem cells into early kidney cells. Meanwhile, the application of organoids in cell organization studies also presents unprecedented potential to help elucidate the underlying mechanisms and further predict the behaviour of nephrogenesis in response to different conditions. The potential of kidney organoids as tools to model diverse diseases is an emerging but promising field. With patient-derived organoids, the possibility of finding biomarkers for the risk and progression of some genetic diseases may be greatly increased.[66] For decades, research into kidney cancer has relied on transgenic mouse models; however, the emergence of kidney organoids not only overcomes the bottleneck presented by the heterogeneous genomic landscape of kidney cancers but they may also be an important model for studying the importance and influence of the crosstalk between tumour cells and their surrounding stroma. Creating organoid biobanks covering a broad spectrum of genetic variation will eventually facilitate the design of powerful screening platforms. Moreover, organoids also represent a powerful tool for developing personalized medicine, which will be beneficial in exploring more effective drugs with minimal side effects.

Despite the strengths of organoids in nephrology, we must acknowledge the limitations of current kidney organoid protocols. Currently, most kidney organoids lack a vascular network, which poses a key challenge for regenerative medicine. Furthermore, the absence of endothelial-epithelial crosstalk hinders their maturity and restricts the functionality of kidney organoids. To overcome these limitations, studies are underway to combine organoids with new technologies such as microfluidic devices and mechanical automation. The optimal culture system for coculturing multiple cell types will also require further investigation.

Taken together, the tremendous advances in organoid technologies have forged a path towards a deeper understanding of human kidney development, disorders, and drug studies. Increasing exploration will ensure that these models are continuously improved. In combination with the integration of multiple technologies, such as biosensing and mechanical automation, we can foresee that a highly accurate and reproducible culture model could emerge and overcome existing obstacles, thereby accelerating the transition from bench to bedside.

Author contributions

MW and PZ drafted the manuscript, TH generated the figure, JS and FS performed the background research. MI

Nasser and QH reviewed and edited the figures. MZ reviewed and edited the manuscript. All authors have read and approved the content of the manuscript.

Conflict of interests

The authors declare no conflict of interest.

Funding

This study was supported by grants from Undergraduate Innovation and Entrepreneurship Project (No. 202321007), Hunan Innovative Provincial Construction Project (No. 2019SK2211), Changsha Science, Technology Plan Project (No. kq2001044), the National Natural Science Foundation of China (No. 81970248), National Key Research and Development Program of China (No. 2018YFA0108700, 2017YFA0105602), NSFC Projects of International Cooperation and Exchanges (No. 81720108004), National Natural Science Foundation of China (No. 81974019), The Research Team Project of Natural Science Foundation of Guangdong Province of China (No. 2017A030312007), The Key Program of Guangzhou Science Research Plan (No. 201904020047), and The Special Project of Dengfeng Program of Guangdong Provincial People's Hospital (No. DFJH201812, KJ012019119, KJ012019423).

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