Cancer Letters 457 (2019) 20–27

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

# Organoid technology in cancer precision medicine

Xinyi Xia[a,b,c], Fei Li[a,b,c], Juan He[a,b,c], Rebiguli Aji[a,b,c], Dong Gao[a,b,c,*]

[a] State Key Laboratory of Cell Biology, University of Chinese Academy of Sciences, 320 Yueyang Road, Shanghai, 200031, China
[b] CAS Key Laboratory of Systems Biology, University of Chinese Academy of Sciences, 320 Yueyang Road, Shanghai, 200031, China
[c] CAS Center for Excellence in Molecular Cell Science, Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, University of Chinese Academy of Sciences, 320 Yueyang Road, Shanghai, 200031, China

ARTICLE INFO

*Keywords:*
Patient-derived organoid
Precision medicine
Preclinical model
3D culture

ABSTRACT

Organoid technology has been remarkably improved over the last decade. Various organoids have been derived from different types of tissues and recapitulate their organ-specific gene expression signatures, particular tissue spatial structures and functions of their original tissue. The patient-derived organoids (PDOs) have been used to elucidate crucial scientific questions, including the relationships between genetic/epigenetic alterations and drug responses, cell plasticity during disease progressions, and mechanisms of drug resistances. With the great expectations, PDOs will be widely used to facilitate the personalized medical decisions, which have the potential to profoundly improve patient outcomes. In this review, we will discuss the developmental details, current achievements, applications and challenges of organoid technology in precision cancer medicine.

## 1. Introduction

Genotype-based cancer precision medicine is deeply and widely studied to facilitate cancer treatments in order to improve patient outcomes. However, the genotype-based predictions of cancer therapies are not unequivocal. First, both intra-tumor and inter-tumor heterogeneity may increase diagnosis difficulties and thus contribute to therapeutic resistances. Secondly, tumor development is a highly dynamic process. Mutations are usually accumulated through the rapid DNA replications of tumor cells, including chromosome instability, microsatellite instability and the changes in epigenetic modifications. In addition, the tumor evolution is made by participations of the abnormal microenvironments, which are established by cancer-associated fibroblasts, immunocytes and the multiple cells composed vascular networks. Furthermore, the diverse ranges of human tumor cells in both molecular heterogeneity and plasticity present new challenges for personalized precision medicine, which may be addressable through making the relative guidance for drug testing on the patients derived preclinical models.

Maintenance of heterogeneity in original tumors and restoration of tumor microenvironments are the two major challenges for establishing preclinical models, which may ultimately influence the conformance of drug responses and clinical outcomes. The patients-derived cancer cell lines (PDCs) can be rapidly established for drug discoveries and high throughput screenings. However, lacks of the information on both tumor architecture and microenvironment limit their application potentials as the preclinical models. On the other hand, the patient-derived tumor xenografts (PDXs) can maintain the particular tumor heterogeneities as well as the interactions between the tumor cells and their surrounding stromal cells. Indeed, they have been utilized for preclinical drug evaluation and biomarker identification. Nonetheless, they also carry some shortcomings to hamper their practical applications for precision medicine, such as, the successful initiation rate varies among tumors [1], and the required over-long time period for preparations, etc. Compared to the above two type of models, the patient-derived tumor organoids (PDOs) have the special property. They can be rapidly generated with limited tumor samples and largely recapitulate the nature of their tumor origins. Currently, organoid technology has shown tremendous potentials for applications in personalized medicine (Table 1). In general, organoids are organ-like structures that can be efficiently generated either from the harvested samples of patients or from the induction of pluripotent stem cells. Their advantages for genetic and structural similarities with their original tissues highlight the special potentials of organoid technology for precision medicine. Usually, the heterogeneous diseases, especially the tumors, often consist with various cell subpopulations with distinct gene alterations, which are difficult for the clinical treatments and also hard for reserving in cancer models. As the preclinical models, self-organized three-dimensional organoid can largely recapitulate the heterogeneous composition of its origin tumor [2,3], and keep to maintain the

* Corresponding author. Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, China.
*E-mail address:* dong.gao@sibcb.ac.cn (D. Gao).

https://doi.org/10.1016/j.canlet.2019.04.039
Received 24 January 2019; Received in revised form 22 April 2019; Accepted 25 April 2019

**Table 1**
Comparison of preclinical cancer models.

| Cancer models | Advantages | Challenges |
|---|---|---|
| PDCs, | *Easy to maintain<br>* Propagated rapidly<br>* Amenable for genetic manipulation<br>* Suitable for high-throughput drug screening<br>* Cost-effective | * Lack of heterogeneity<br>* Lack of microenvironment, such as tumor-stromal interaction and immune system<br>* Long term culture may increase genome instability<br>* Change of cell morphology in 2D culture may influence cell identity<br>* Lack germline mutation control from the origin patient, making it difficult to distinguish between oncogenic gene alterations and alterations gained from long-term culture |
| PDXs, | * Recapitulate the tumor-stromal interaction<br>* Maintain tumor architecture and heterogeneity | * Long propagation time<br>* Successful rate varies among tumors<br>* Unsuitable for genetic manipulation<br>* Unsuitable for high-throughput drug screening<br>* Lack of immune cells because tumor are embedded in immune-deficient mice<br>* PDX derived from single biopsy, which may not represent the whole tumor |
| PDOs, | * High successful initiation rate<br>* Suitable for high-throughput drug screening<br>* Amenable for genetic manipulation<br>* Maintain tumor architecture and heterogeneity<br>* Suitable for biobank establishment | * Lack of tumor-stromal interaction and immune system<br>* Organoid culture depend on matrigel, which is not well-defined |

mutation patterns during long-term culture without genetic alterations [4]. Another key advantage of PDO is reflected from organoid cultures. The organoid in culture have high accessibility and visibility, which makes it more suitable for studying dynamic process, such as tumor development. Furthermore, organoid can be built with very few starting cells and propagated rapidly under proper culture condition. This property allows the rapid and efficient establishments of particular preclinical model even with very limited amount of harvested tissue. In conclusion, the organoid technology supplies a new generation of platform for precision medicine, especially for the personalized medical decisions, drug screening and signaling pathway study, etc.

## 2. History of three-dimensional (3D) cell culture

During the past century, 3D cell culture system has been gradually established based on collective experiences from two-dimensional (2D) cell culture at the earlier time. Although the establishment of 2D cell culture reflected a significant revolutionary progress, the more developed and novel 3D cell culture system enables strict recapitulation of the *in vivo* cell signatures, which is urgently needed for the studies on the basic cell biology and human diseases in nowadays. As early as in 1907, Wilson et al. described the self-organization of sponge cells *in vitro* [5]. Results from their study demonstrated the possibility of generating whole organism *in vitro*. At same time, “organoid” refers to the small epithelial fragments taken from animals and removed with most stromal components. For achieving the *in vitro* culture of organoid, the hanging drop culture method was developed by Harrison in 1907 [6]. Remarkably, the embryo nerve cord was cultured in a liquid drop of lymph to hold the tissue in a properly fixed position. The hanging drop culture technology was widely used since then [7,8]. After the successes in application of collagenase for tissue digestion in 1950s, researchers were able to culture cells with 3D structures using dissociated cells [9,10]. After the successful isolation of Matrigel in 1980s, various 3D culture technologies were rapidly established and broadly used in the study of organ morphogenesis [11–14].

Although 3D culture was described over a hundred years ago [5], the first single stem cell-derived organoid was only established in 2009. The single stem cell-derived organoid has special characters of self-proliferation capability and differentiation potential to form organ like structures. Sato et al. reported that the $Lgr5^+$ intestine stem cells self-organized to intestine crypt–villus structures without mesenchymal niche in Matrigel [15]. Later on, the numerous groups started to focus on the establishments of organoid biobanks for many kinds of tissues based on their interested studies, both in the fields of researches of healthy human physiology and the pathology with diseases [16].

## 3. Different stem cell origins for deriving organoids

Up to now, several kinds of organoids have been successfully derived from different stem cells, including pluripotent stem cells (PSCs) of both embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), and various adult stem cells (ASCs). Under the proper niches built by growth factor cocktails, they can display the capability of self-organization and differentiation. With the gene-editing technology, some models can be successfully used to study tumor-initiation and basic mechanisms of cancer biology.

### 3.1. Organoids derived from PSCs

With high ability of self-renewal and differentiation, PSCs (both ESCs and iPSCs) are broadly used in reconstructions of organ structures *in vitro* in the form of organoids.

#### 3.1.1. Brain organoids from PSCs

PSCs have been broadly used in reconstruction of tissue structures for different brain regions and for the whole brain. Lancaster et al. first reported the protocol to generate the cerebral organoids [17]. These cerebral organoids consisted a broad diversity of cells representing various brain regions [18] and recapitulate *in vivo*-like topography [19–21]. Optic cup organoids and retina organoids were successfully generated from ESCs and iPSCs respectively [22–27]. These organoids differentiated into wide ranges of retinal cell types after transplantation into mice [28] or primate [29] retinal degeneration models, which implicated their potential in clinical applications. In another report, functional adenohypophysis organoids were generated form mouse ESCs [30]. The establishments of several brain region-specific organoids were further reported, including forebrain [31,32], cerebellum [33], cortex [34,35], hippocampus [36], midbrain [37,38] and hypothalamus [31].

#### 3.1.2. Organoids of foregut organs from PSCs

Theoretically, the endoderm shapes into a primitive gut tube, which will give rise to foregut, midgut and hindgut after gastrulation [39]. The foregut further differentiates into thyroid, lung, esophagus, liver, biliary tree, pancreas and stomach [40]. As reported, foregut organoids could be generated from PSCs through a step-wise differentiation process that resembles development stages *in vivo*. Agreeing with the principles in developmental biology, almost all kinds of foregut

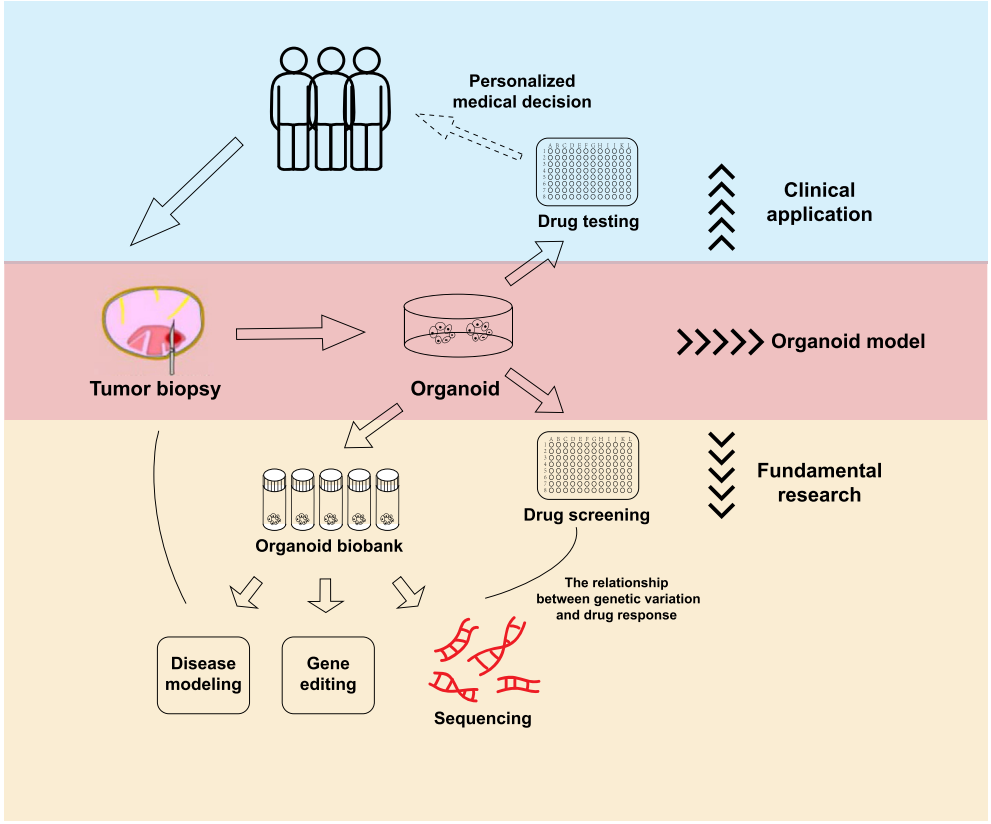


**Fig. 1.** The applications of patient-derived organoids in precision medicine. Organoids can be generated from patient-derived cancer cells with a high successfully rate. The high throughput drug screening in patient-derived organoids can assist personalized medical decision in clinical. In parallel, patient-derived organoids can also be preserved as biobanks. Organoid biobanks can facilitate fundamental research through disease modeling, gene editing and linking the relationship between genetic variations and drug responses.

organoids could be established from PSCs, including liver [41], stomach [40,42], pancreas [43,44], thyroid [45] and lung [46–50].

#### 3.1.3. Organoids of small intestine and colon from PSCs

Robust and efficient generation of intestinal organoid from PSCs can be achieves through the temporal manipulation of growth factors that mimic the intestinal development *in vitro* [51]. These organoids have the capacity to reconstructe the intestine tissues, which was confirmed by transplantation assay with mouse model of damaged colonic epithelium [52–54].

### 3.2. Organoids derived from ASCs

Adult stem cells are regarded to exist in almost every type of tissues, responsible for tissue maintenance and injury repair. Theoretically, they have the advantage in reconstructing the mature and functional architect than both ESCs and iPSCs [55]. They have been broadly used to establish many types of organoid lines, especially for surface ectoderm glandular tissues [56]. For example, Lgr5 (leucine rich repeat containing G-protein-coupled receptor 5), a Wnt targeted gene, has been proved to be a stem cell marker in various kinds of epithelium [57].

#### 3.2.1. Organoids of small intestine and colon from ASCs

The actively proliferating $Lgr5^+$ stem cells were found to located at the intestine crypt structure with a delicate niche composed of site-specific paneth cells, transit-amplifying cells, goblet cells etc [58]. It was reported that intestine organoids were successfully cultured by adding growth factor cocktail to mimic the delicate niche *in vivo* [15]. Both Wnt3a and R-spondin, the Wnt signal enhancer, were employed to activate the Wnt signal. In theory, Wnt signal is a key factor for the stabilization of β-catenin, needed for both stem cell fate maintenance and paneth cells differentiation [59]. In addition, Notch signal contributes to the maintenance of the undifferentiated state of stem cells [60]. Other key factors in small intestine organoid culture include the mitosis accelerator EGF, the crypt structure promotor Noggin etc. Therefore, the discovery of these essential factors enabled the establishment of small intestine organoids under defined conditions [61–64].

#### 3.2.2. Organoids of liver and pancreas from ASCs

Liver and pancreas are both originated from foregut and cooperate to regulate metabolism [65]. They have been found to harbor common adult stem cells based on the results that transplantation of mouse pancreatic cells rescues liver failure [66], and that hepatocyte-like cells could be induced into pancreatic cells [67]. In addition, they both self-renewal in a slow rate under homeostasis [55]. In accordance with the slow proliferation rate, $Lgr5^+$ cells were known to be very rare under physiological conditions, but increased after injury [68]. Based on the results of small intestine organoid in culture, it was reported that single $Lgr5^+$ stem cell in liver and pancreas had the capacity to form functional liver and pancreas organoids respectively *in vitro* [69,70].

#### 3.2.3. Other ASCs derived organoids

Based on the reported landmark work on the culture of small intestine organoids, several organoids were successfully derived from other organs [15]. Karthaus [71] and Chua [72] cultured genetically stable prostate organoids that were derived from luminal cells and basal cells. Their work identified a new approach to study prostate cells *in vitro*. Afterwards, various tissue-specific organoids were successfully generated from their own ASCs, including mammary gland organoids [73], fallopian tube epithelium organoids [74], taste bud organoids [75], gastric organoids [57,76] and airway organoids [77–80].

## 4. Current organoid models for cancers and other diseases

Nowadays, precision medicine advocates that every patient should be treated differently based on the personal differences, including individual genome and transcriptome landscape, environment and life

style. However, due to the lack of understanding in the relationships between genetic mutations and drug responses, particular treatment of certain mutation with its targeted drugs cannot always receive the expected outcomes. To overcome the challenges in cancer therapy, personalized disease models have been addressed. Organoids have recently emerged as a powerful disease model in precision medicine. It sheds light on a new approach to improve drug responses and minimize side effects on the highly heterogeneous diseases, especially cancers. The patients-derived cancer organoids, which maintain both the heterogeneity and genetic features of their original cancer tissues might be broadly used in the future of personalized cancer medicine (Fig. 1).

Organoid biobanks have been established to model various kinds of tumors, including colorectal cancer [59,81], pancreatic cancer [82], prostate cancer [71], liver cancer [83], bladder cancer [84,85], etc. Based on their previous study on mouse small intestine organoid culture, Sato et al. cultured human intestinal adenoma organoids in 2011 [59]. After that, we established a protocol to culture advanced prostate cancer organoids [71]. Different with some other common malignant tumors, prostate cancer lacks *in vitro* models because of difficulty on the propagation of prostate cancer cells. Using organoid culture system, we were able to recapitulate frequent mutations in prostate cancer and enabled genetic and pharmacologic studies of prostate cancer in a new approach. In another study, Boj et al. successfully modeled ductal pancreatic cancer using organoid culture [82]. Since the mortality of ductal pancreatic cancer is extremely high due to the difficulty in the early diagnosis, an optimal model of pancreatic cancer is greatly expected to study its tumorigenesis. Right now, pancreatic organoids can be rapidly generated from benign or malignant tissues based on their findings. Wetering et al. developed an organoid biobank for colorectal cancer and the adjacent normal tissues in 2015 [86]. Furthermore, based on the study of colorectal cancer organoids derived from different clinical stages, Shimokawa et al. demonstrated the correlation between niche-independent growth and disease progression [87]. Recently, organoid biobanks for liver tumor [83], mammary tumor [88,89], bladder cancer [84] and brain tumor [90] were subsequently generated. In summary, orgnaoid biobanks have become a powerful platform to understand the deadly malignancy and facilitate new drug development.

As expected, organoid technology has gradually supplied the promising models for various types of tumors, which can recapitulate *in vivo* natures of diseases or injuries under *in vitro* conditions [91]. Since organoid contains most types of cells from its organ source, and it can recapitulate the pathological changes of human organs in the more faithful way [92,93]. For examples, H. pylori is a bacterium which can colonize in the gastric mucosa and cause acute gastritis *in vivo*, however, H. pylori stopped proliferating and died gradually in 2D gastric cancer cell culture system [40,94]. While the injection of H. pylori into the lumen of gastric organoids can maintain the viability of the bacteria and cause acute inflammatory response in epithelial cells [95]. Huch et al. successfully modeled a1-antitrypsin deficiency and Alagille syndrome by culturing patient-derived organoids [70]. Morizane et al. mimicked kidney development by culturing organoids from human PSCs and successfully modeled kidney injury *in vitro* [96]. Qian et al. employed the region specific brain organoid platform to model Zika virus infection, which include cell death and neuronal cell-layer volume decrease [31]. Neurodevelopmental disorders are often difficult to mimic with mouse models. Lancaster et al. cultured reprogrammed patient-derived fibroblasts to form cerebral organoids, which showed features of microcephaly [17]. Therefore, organoid technology has gradually become a powerful tool for understanding human pathology.

## 5. Applications of organoid technology

### 5.1. Organoids for drug screenings

The establishment of organoid culture system provides an opportunity to rapidly and efficiently model the human cancers and other diseases, to enable the high throughput drug screenings specific for individual patient. As a successful example, Dekkers et al. employed organoid-based drug screening in cystic fibrosis [97]. Cystic fibrosis is usually caused by the mutation of CFTR, which is a epithelial anion channel protein encoding gene. There are nearly 2000 identified CFTR mutations, but only a few of them have been successfully targeted. The drug testing in patient-derived organoids enabled the prediction of particular drug response in individual patient with the rare and uncharacterized CFTR mutations.

With the establishment of tumor organoid biobanks, organoid technology gradually shows the powerful potentials to facilitate personalized cancer medicine. Two organoid biobanks were derived from colon cancer and their adjacent healthy tissue, representing common gene alterations in colon cancer, which revealed by DNA and RNA sequencing. 83 clinically used drugs were screenned on those organoids. The screening results are consistent with known gene-drug associations, which showed the potential of organoid as a high-throughput drug screening platform [86,87]. Vlachogiannis et al. first compared the relationship of drug responses between patients and their metastatic gastrointestinal tumor organoids. This endeavor represented the valuable application of tumor organoids for setting the guidance during drug prescription in precision cancer medicine [98]. Afterward, Tiriac et al. tested chemotherapy in patient-derived pancreatic cancer organoids. The therapeutic profiles of pancreatic cancer organoids paralleled patients outcomes and the longitudinal assessment of PDOs reflected the clinical course for an individual patient [99]. Through the co-culture of patient-derived glioma stem cells and human ESCs-derived cerebral organoids, glioblastoma organoids can closely phenocopy glioblastoma in patients, providing the platform for high-throughput drug screening [100]. Henceforth, the subsequent drug screenings will be greatly helped by the tumor organoids biobanks, during which the relationships between genetic landscapes and therapeutic outcomes for patients will be better understood [101–104].

Besides, organoid models may also be used in drug toxicity testing in the future. For example, hepatocyte derived liver organoids showed the potential in studying drug metabolism and toxic reactions to complement animal testing [70]. When cisplatin, a nephrotoxicant, was used to treat kidney organoids in culture, the mature proximal tubules-specific apoptosis could be caused, indicating that the application of kidney organoids has been successfully performed in drug response tests [105].

### 5.2. Organoids for tissue engineering

Genetic manipulation can be employed to further broaden the scope of utilizing organoids in drug screenings, regenerative medicine and signaling pathway studies [106]. For instance, the accurate gene editing technology such as CRISPR-Cas9 can be used to correct the gene mutations in patient-derived organoids. Transplantation of those fully functionally modified organoids back into patients may efficiently accelerate tissue repair in patients. Schwank et al. have tried to put this strategy into the clinical practice through correcting gene mutation in cystic fibrosis [107], a hereditary disease caused by single-gene mutation. As reported, the CRISPR-Cas9 mediated DNA repair restored the normal function in patient-derived organoids. Right now, this is just at initial stage of an early attempt to treat the currently incurable hereditary diseases by the transplantation of genetic engineered organoids.

## 6. Current challenges of organoid technology in precision cancer medicine

### 6.1. Challenges in reconstruction of tumor microenvironment

Although organoid is regarded as a promising model *in vitro*, it still has limitations on many aspects. It cannot fully recapitulate the

complicated structures of tumor microenvironment. It lacks the blood vessels, immune cells and neurons. Both disease progression and drug resistance for most tumors not only depend on gene alterations of tumor themselves, but are also influenced by the tumor microenvironments, such as the surrounding fibroblasts and immunocytes.

The healthy stromal cells functions as a barrier against tumor, while the cancer associated fibroblasts facilitate tumor progression. Cancer associated fibroblasts (CAFs) often harbor unique cytokine secretion profiles, have an increased capability of cell proliferation and produce an abnormal extracellular matrix [108,109]. Those properties contribute to tumor remodeling and pathogenic angiogenesis, which promoted tumor progressions and eventually associated with poor outcomes [110]. Through the addition of stromal components during establishment process of organoid in culture, stromal microenvironment in organoids could be partially restored. Direct input of human endothelial cells into the co-culture of breast cancer organoids in mice generated functional network of capillary vessel, which could connect to mice circulatory system [111]. PSCs-derived human blood vessel organoids can be self-assembled *in vitro* into a capillary network with endothelial cells and pericytes. This study showed the possibility of rebuilding the vascular system in organoid *in vitro* [112,113]. Workman et al. incorporated PSCs-derived neural crest cells into intestine organoids [114]. Those PSCs-derived nerve cells incorporated into organoids to successfully form neuroglial structures that resembled enteric nervous system in intestine and had functional neural activities.

Immune system can be tumor-promoting and tumor-preventing under different context. Although healthy immune system mount antitumor responses, unbalanced immune cells could become a microenvironment as a powerful contributor to tumor development [115–117]. For this regard, some efforts have been made to restore the immune microenvironment in organoid system. Lymphocytes were co-cultured with cells for the establishment of intestinal epithelial organoids. These lymphocytes showed significantly sustained activities of proliferation and active movement [118]. In addition, macrophage-enteroid was established by co-culture of intestinal stem cells with monocyte-derived macrophages. It was found that epithelia and macrophages could cooperate to regulate cytokine secretion and respond after virus infection [119,120]. Under modified situations, tumor organoids could also entrain peripheral blood derived T cells to a tumor-specific reactive state [121]. With the 3D organoid culture system, the PSCs can be induced as a T cell with anticancer function, which provides an possible way to produce therapeutic T cells [122]. Neal et al. employed an air-liquid interface method to generate patient-derived organoids that preserve tumor stromal cells and T cells [123].

Nowadays, it is still an insurmountable challenge to reconstitute the tumor microenvironment with patients-derived CAFs, immunocyte and capillary system in 3D organoid cultures, mimicking the disease progression and evolution.

### 6.2. Challenges for requirements of more defined matrices

The matrix resembling basement membrane used for 3D culture was successfully isolated from murine chondrosarcoma as early as 1977 [40]. The bioactive molecules containing extracellular matrix scaffolds provide the physical and biochemical supports necessary for 3D culture. Those matrix supports the tissue reconstruction by creating a favorable microenvironment, which enabled 3D assemble of cells in cultures.

In present, most of current protocols of organoid culture depend on animal-derived Matrigel or collagens as supports of 3D matrix. Certainly, both Matrigel and collagens are powerful matrix to mimic extracellular matrix *in vivo* and maintain the stability of undifferentiated state for some stem cells. However, they are composed of complex components including laminin, collagen IV, and entactin etc. These components contain multiple undefined growth factors that may affect cellular activities unexpectedly. And current 3D scaffolds are still not suitable for the controlled modifications due to the undefined property. For consideration of clinical applications, the risks in immunogen or pathogen transformation are still existed concerns. Therefore, new generation of matrices with known components is needed to broaden the applications of organoids [37,124–126]. For example, poly(ethylene glycol) (PEG) macromers was found in study to mimic collagenase substrates in ECM [127]. In another work, the conjugation of hydrophilic polymers with fibrinogen, collagen, or albumin could be used to generate new semi-synthetic hydrogels [128].

## 7. Conclusion

Organoid technology has already emerged as a new platform that facilitates the studies of complicated diseases, especially many cancers. Particularly, patient-derived organoids serve as preclinical models in the practice of precision medicine, which are proved to have great potentials of applications in cancer treatments and improvements of patients' outcomes. To reach this long-term goal, drug screenings in preclinical models might broadly use to predict treatment responses in future. Patient-derived organoids can be used in the prediction of drug response of particular individual patient. Furthermore, organoids are amendable for applying genetic manipulations on the cells before entering into organoid establishment, which enables the organoid-based fundamental researches. In the future, organoid technology will become a more powerful platform for understanding of human diseases and the discovering of new drugs.

## Conflicts of interest

There are no conflicts of interest to disclose.

## Acknowledgements

This study was supported by grants from the Strategic Priority Research Program of the Chinese Academy of Sciences (XDA16020905 and XDB19000000), the National Key Research and Development Program of China (No. 2017YFA0505500), and the National Natural Science Foundation of China (81830054 and 81772723 to DG).

## References

[1] A.T. Byrne, D.G. Alferez, F. Amant, D. Annibali, J. Arribas, A.V. Biankin, A. Bruna, E. Budinska, C. Caldas, D.K. Chang, R.B. Clarke, H. Clevers, G. Coukos, V. Dangles-Marie, S.G. Eckhardt, E. Gonzalez-Suarez, E. Hermans, M. Hidalgo, M.A. Jarzabek, S. de Jong, J. Jonkers, K. Kemper, L. Lanfrancone, G.M. Maelandsmo, E. Marangoni, J.C. Marine, E. Medico, J.H. Norum, H.G. Palmer, D.S. Peeper, P.G. Pelicci, A. Piris-Gimenez, S. Roman-Roman, O.M. Rueda, J. Seoane, V. Serra, L. Soucek, D. Vanhecke, A. Villanueva, E. Vinolo, A. Bertotti, L. Trusolino, Interrogating open issues in cancer precision medicine with patient-derived xenografts, Nat. Rev. Canc. 17 (2017) 254–268.

[2] C. Pauli, B.D. Hopkins, D. Prandi, R. Shaw, T. Fedrizzi, A. Sboner, V. Sailer, M. Augello, L. Puca, R. Rosati, T.J. McNary, Y. Churakova, C. Cheung, J. Triscott, D. Pisapia, R. Rao, J.M. Mosquera, B. Robinson, B.M. Faltas, B.E. Emerling, V.K. Gadi, B. Bernard, O. Elemento, H. Beltran, F. Demichelis, C.J. Kemp, C. Grandori, L.C. Cantley, M.A. Rubin, Personalized in vitro and in vivo cancer models to guide precision medicine, Cancer Discov. 7 (2017) 462–477.

[3] F. Weeber, M. van de Wetering, M. Hoogstraat, K.K. Dijkstra, O. Krijgsman, T. Kuilman, C.G. Gadellaa-van Hooijdonk, D.L. van der Velden, D.S. Peeper, E.P. Cuppen, R.G. Vries, H. Clevers, E.E. Voest, Preserved genetic diversity in organoids cultured from biopsies of human colorectal cancer metastases, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) 13308–13311.

[4] M. Schutte, T. Risch, N. Abdavi-Azar, K. Boehnke, D. Schumacher, M. Keil, R. Yildiriman, C. Jandrasits, T. Borodina, V. Amstislavskiy, C.L. Worth, C. Schweiger, S. Liebs, M. Lange, H.J. Warnatz, L.M. Butcher, J.E. Barrett, M. Sultan, C. Wierling, N. Golob-Schwarzl, S. Lax, S. Uranitsch, M. Becker, Y. Welte, J.L. Regan, M. Silvestrov, I. Kehler, A. Fusi, T. Kessler, R. Herwig, U. Landegren, D. Wienke, M. Nilsson, J.A. Velasco, P. Garin-Chesa, C. Reinhard, S. Beck, R. Schafer, C.R. Regenbrecht, D. Henderson, B. Lange, J. Haybaeck, U. Keilholz, J. Hoffmann, H. Lehrach, M.L. Yaspo, Molecular dissection of colorectal cancer in pre-clinical models identifies biomarkers predicting sensitivity to EGFR inhibitors, Nat. Commun. 8 (2017) 14262.

[5] H.V. Wilson, A new method by which sponges may be artificially reared, Science 25 (1907) 912–915.

[6] R.G. Harrison, Observations on the living developing nerve fiber, Exp. Biol. Med. 1

(2010) 116–128.
[7] A. Carrel, M.T. Burrows, Cultivation of adult tissues and organs outside the body, J. Am. Med. Assoc. 55 (2010) 1379–1381.
[8] D. Thomson, Controlled Growth en masse (Somatic Growth) of Embryonic Chick Tissue in vitro, Proc. Roy. Soc. Med. 7 (1914) 71–75.
[9] E.Y. Lasfargues, Cultivation and behavior in vitro of the normal mammary epithelium of the adult mouse: II. Observations on the secretory activity, Exp. Cell Res. 13 (1957) 553–562.
[10] M.N. Berry, D.S. Friend, High-yield preparation of isolated rat liver parenchymal cells: a biochemical and fine structural study, JCB (J. Cell Biol.) 43 (1969) 506–520.
[11] M.H. Barcelloshoff, J. Aggeler, T.G. Ram, M.J. Bissel, Functional differentiation and alveolar morphogenesis of primary mammary cultures on reconstituted basement membrane, Development 105 (1989) 223–235.
[12] M. Simian, Y. Hirai, M. Navre, Z. Werb, A. Lochter, M.J. Bissell, The interplay of matrix metalloproteinases, morphogens and growth factors is necessary for branching of mammary epithelial cells, Development 128 (2001) 3117–3131.
[13] J.E. Fata, H. Mori, A.J. Ewald, H. Zhang, E. Yao, Z. Werb, M.J. Bissell, The MAPK (ERK-1,2) pathway integrates distinct and antagonistic signals from TGFalpha and FGF7 in morphogenesis of mouse mammary epithelium, Dev. Biol. 306 (2007) 193–207.
[14] C.M. Nelson, M.M. Vanduijn, J.L. Inman, D.A. Fletcher, M.J. Bissell, Tissue geometry determines sites of mammary branching morphogenesis in organotypic cultures, Science 314 (2006) 298–300.
[15] T. Sato, R.G. Vries, H.J. Snippert, M. van de Wetering, N. Barker, D.E. Stange, J.H. van Es, A. Abo, P. Kujala, P.J. Peters, H. Clevers, Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche, Nature 459 (2009) 262–265.
[16] M. Simian, M.J. Bissell, Organoids: a historical perspective of thinking in three dimensions, J. Cell Biol. 216 (2017) 31–40.
[17] M.A. Lancaster, M. Renner, C.A. Martin, D. Wenzel, L.S. Bicknell, M.E. Hurles, T. Homfray, J.M. Penninger, A.P. Jackson, J.A. Knoblich, Cerebral organoids model human brain development and microcephaly, Nature 501 (2013) 373–379.
[18] G. Quadrato, T. Nguyen, E.Z. Macosko, J.L. Sherwood, S. Min Yang, D.R. Berger, N. Maria, J. Scholvin, M. Goldman, J.P. Kinney, E.S. Boyden, J.W. Lichtman, Z.M. Williams, S.A. McCarroll, P. Arlotta, Cell diversity and network dynamics in photosensitive human brain organoids, Nature 545 (2017) 48–53.
[19] G.Y. Cederquist, J.J. Asciolla, J. Tchieu, R.M. Walsh, D. Cornacchia, M.D. Resh, L. Studer, Specification of positional identity in forebrain organoids, Nat. Biotechnol. 37 (2019) 436–444.
[20] Y. Miura, S.P. Pasca, Polarizing brain organoids, Nat. Biotechnol. 37 (2019) 377–378.
[21] A.A. Pollen, A. Bhaduri, M.G. Andrews, T.J. Nowakowski, O.S. Meyerson, M.A. Mostajo-Radji, E. Di Lullo, B. Alvarado, M. Bedolli, M.L. Dougherty, I.T. Fiddes, Z.N. Kronenberg, J. Shuga, A.A. Leyrat, J.A. West, M. Bershteyn, C.B. Lowe, B.J. Pavlovic, S.R. Salama, D. Haussler, E.E. Eichler, A.R. Kriegstein, Establishing cerebral organoids as models of human-specific brain evolution, Cell 176 (2019) 743–756 e717.
[22] T. Nakano, S. Ando, N. Takata, M. Kawada, K. Muguruma, K. Sekiguchi, K. Saito, S. Yonemura, M. Eiraku, Y. Sasai, Self-formation of optic cups and storable stratified neural retina from human ESCs, Cell Stem Cell 10 (2012) 771–785.
[23] M. Eiraku, N. Takata, H. Ishibashi, M. Kawada, E. Sakakura, S. Okuda, K. Sekiguchi, T. Adachi, Y. Sasai, Self-organizing optic-cup morphogenesis in three-dimensional culture, Nature 472 (2011) 51–56.
[24] X. Zhong, C. Gutierrez, T. Xue, C. Hampton, M.N. Vergara, L.H. Cao, A. Peters, T.S. Park, E.T. Zambidis, J.S. Meyer, D.M. Gamm, K.W. Yau, M.V. Canto-Soler, Generation of three-dimensional retinal tissue with functional photoreceptors from human iPSCs, Nat. Commun. 5 (2014) 4047.
[25] A. Kuwahara, C. Ozone, T. Nakano, K. Saito, M. Eiraku, Y. Sasai, Generation of a ciliary margin-like stem cell niche from self-organizing human retinal tissue, Nat. Commun. 6 (2015) 6286.
[26] K.J. Wahlin, J.A. Maruotti, S.R. Sripathi, J. Ball, J.M. Angueyra, C. Kim, R. Grebe, W. Li, B.W. Jones, D.J. Zack, Photoreceptor outer segment-like structures in long-term 3D retinas from human pluripotent stem cells, Sci. Rep. 7 (2017) 766.
[27] P.J. Susaimanickam, S. Maddileti, V.K. Pulimamidi, S.R. Boyinpally, R.R. Naik, M.N. Naik, G.B. Reddy, V.S. Sangwan, I. Mariappan, Generating minicorneal organoids from human induced pluripotent stem cells, Development 144 (2017) 2338–2351.
[28] J. Assawachananont, M. Mandai, S. Okamoto, C. Yamada, M. Eiraku, S. Yonemura, Y. Sasai, M. Takahashi, Transplantation of embryonic and induced pluripotent stem cell-derived 3D retinal sheets into retinal degenerative mice, Stem Cell Reports 2 (2014) 662–674.
[29] H. Shirai, M. Mandai, K. Matsushita, A. Kuwahara, S. Yonemura, T. Nakano, J. Assawachananont, T. Kimura, K. Saito, H. Terasaki, M. Eiraku, Y. Sasai, M. Takahashi, Transplantation of human embryonic stem cell-derived retinal tissue in two primate models of retinal degeneration, Proc. Natl. Acad. Sci. U. S. A. 113 (2016) E81–E90.
[30] H. Suga, T. Kadoshima, M. Minaguchi, M. Ohgushi, M. Soen, T. Nakano, N. Takata, T. Wataya, K. Muguruma, H. Miyoshi, S. Yonemura, Y. Oiso, Y. Sasai, Self-formation of functional adenohypophysis in three-dimensional culture, Nature 480 (2011) 57–62.
[31] X. Qian, H.N. Nguyen, M.M. Song, C. Hadiono, S.C. Ogden, C. Hammack, B. Yao, G.R. Hamersky, F. Jacob, C. Zhong, K.J. Yoon, W. Jeang, L. Lin, Y. Li, J. Thakor, D.A. Berg, C. Zhang, E. Kang, M. Chickering, D. Nauen, C.Y. Ho, Z. Wen, K.M. Christian, P.Y. Shi, B.J. Maher, H. Wu, P. Jin, H. Tang, H. Song, G.L. Ming, Brain-region-specific organoids using mini-bioreactors for modeling ZIKV exposure, Cell 165 (2016) 1238–1254.
[32] T. Kadoshima, H. Sakaguchi, T. Nakano, M. Soen, S. Ando, M. Eiraku, Y. Sasai, Self-organization of axial polarity, inside-out layer pattern, and species-specific progenitor dynamics in human ES cell-derived neocortex, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 20284–20289.
[33] K. Muguruma, A. Nishiyama, H. Kawakami, K. Hashimoto, Y. Sasai, Self-organization of polarized cerebellar tissue in 3D culture of human pluripotent stem cells, Cell Rep. 10 (2015) 537–550.
[34] A.M. Pasca, S.A. Sloan, L.E. Clarke, Y. Tian, C.D. Makinson, N. Huber, C.H. Kim, J.Y. Park, N.A. O'Rourke, K.D. Nguyen, S.J. Smith, J.R. Huguenard, D.H. Geschwind, B.A. Barres, S.P. Pasca, Functional cortical neurons and astrocytes from human pluripotent stem cells in 3D culture, Nat. Methods 12 (2015) 671–678.
[35] J.A. Bagley, D. Reumann, S. Bian, J. Levi-Strauss, J.A. Knoblich, Fused cerebral organoids model interactions between brain regions, Nat. Methods 14 (2017) 743–751.
[36] H. Sakaguchi, T. Kadoshima, M. Soen, N. Narii, Y. Ishida, M. Ohgushi, J. Takahashi, M. Eiraku, Y. Sasai, Generation of functional hippocampal neurons from self-organizing human embryonic stem cell-derived dorsomedial telencephalic tissue, Nat. Commun. 6 (2015) 8896.
[37] N. Gjorevski, N. Sachs, A. Manfrin, S. Giger, M.E. Bragina, P. Ordonez-Moran, H. Clevers, M.P. Lutolf, Designer matrices for intestinal stem cell and organoid culture, Nature 539 (2016) 560–564.
[38] A.S. Monzel, L.M. Smits, K. Hemmer, S. Hachi, E.L. Moreno, T. van Wuellen, J. Jarazo, J. Walter, I. Bruggemann, I. Boussaad, E. Berger, R.M.T. Fleming, S. Bolognin, J.C. Schwamborn, Derivation of human midbrain-specific organoids from neuroepithelial stem cells, Stem Cell Reports 8 (2017) 1144–1154.
[39] A.M. Zorn, J.M. Wells, Vertebrate endoderm development and organ formation, Annu. Rev. Cell Dev. Biol. 25 (2009) 221–251.
[40] K.W. McCracken, E.M. Cata, C.M. Crawford, K.L. Sinagoga, M. Schumacher, B.E. Rockich, Y.H. Tsai, C.N. Mayhew, J.R. Spence, Y. Zavros, J.M. Wells, Modelling human development and disease in pluripotent stem-cell-derived gastric organoids, Nature 516 (2014) 400–404.
[41] T. Takebe, K. Sekine, M. Enomura, H. Koike, M. Kimura, T. Ogaeri, R.R. Zhang, Y. Ueno, Y.W. Zheng, N. Koike, S. Aoyama, Y. Adachi, H. Taniguchi, Vascularized and functional human liver from an iPSC-derived organ bud transplant, Nature 499 (2013) 481–484.
[42] T.K. Noguchi, N. Ninomiya, M. Sekine, S. Komazaki, P.C. Wang, M. Asashima, A. Kurisaki, Generation of stomach tissue from mouse embryonic stem cells, Nat. Cell Biol. 17 (2015) 984–993.
[43] C. Greggio, F. De Franceschi, M. Figueiredo-Larsen, S. Gobaa, A. Ranga, H. Semb, M. Lutolf, A. Grapin-Botton, Artificial three-dimensional niches deconstruct pancreas development in vitro, Development 140 (2013) 4452–4462.
[44] K.W. McCracken, E. Aihara, B. Martin, C.M. Crawford, T. Broda, J. Treguier, X. Zhang, J.M. Shannon, M.H. Montrose, J.M. Wells, Wnt/beta-catenin promotes gastric fundus specification in mice and humans, Nature 541 (2017) 182–187.
[45] A.A. Kurmann, M. Serra, F. Hawkins, S.A. Rankin, M. Mori, I. Astapova, S. Ullas, S. Lin, M. Bilodeau, J. Rossant, J.C. Jean, L. Ikonomou, R.R. Deterding, J.M. Shannon, A.M. Zorn, A.N. Hollenberg, D.N. Kotton, Regeneration of thyroid function by transplantation of differentiated pluripotent stem cells, Cell Stem Cell 17 (2015) 527–542.
[46] B.R. Dye, D.R. Hill, M.A. Ferguson, Y.H. Tsai, M.S. Nagy, R. Dyal, J.M. Wells, C.N. Mayhew, R. Nattiv, O.D. Klein, E.S. White, G.H. Deutsch, J.R. Spence, In vitro generation of human pluripotent stem cell derived lung organoids, Elife 4 (2015).
[47] B.R. Dye, P.H. Dedhia, A.J. Miller, M.S. Nagy, E.S. White, L.D. Shea, J.R. Spence, A bioengineered niche promotes in vivo engraftment and maturation of pluripotent stem cell derived human lung organoids, Elife 5 (2016).
[48] A.P. Wong, C.E. Bear, S. Chin, P. Pasceri, T.O. Thompson, L.J. Huan, F. Ratjen, J. Ellis, J. Rossant, Directed differentiation of human pluripotent stem cells into mature airway epithelia expressing functional CFTR protein, Nat. Biotechnol. 30 (2012) 876–882.
[49] S.X. Huang, M.N. Islam, J. O'Neill, Z. Hu, Y.G. Yang, Y.W. Chen, M. Mumau, M.D. Green, G. Vunjak-Novakovic, J. Bhattacharya, H.W. Snoeck, Efficient generation of lung and airway epithelial cells from human pluripotent stem cells, Nat. Biotechnol. 32 (2014) 84–91.
[50] A.J. Miller, B.R. Dye, D. Ferrer-Torres, D.R. Hill, A.W. Overeem, L.D. Shea, J.R. Spence, Generation of lung organoids from human pluripotent stem cells in vitro, Nat. Protoc. 14 (2019) 518–540.
[51] J.R. Spence, C.N. Mayhew, S.A. Rankin, M.F. Kuhar, J.E. Vallance, K. Tolle, E.E. Hoskins, V.V. Kalinichenko, S.I. Wells, A.M. Zorn, N.F. Shroyer, J.M. Wells, Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro, Nature 470 (2011) 105–109.
[52] R.P. Fordham, S. Yui, N.R. Hannan, C. Soendergaard, A. Madgwick, P.J. Schweiger, O.H. Nielsen, L. Vallier, R.A. Pedersen, T. Nakamura, M. Watanabe, K.B. Jensen, Transplantation of expanded fetal intestinal progenitors contributes to colon regeneration after injury, Cell Stem Cell 13 (2013) 734–744.
[53] C.L. Watson, M.M. Mahe, J. Múnera, J.C. Howell, N. Sundaram, H.M. Poling, J.I. Schweitzer, J.E. Vallance, C.N. Mayhew, Y. Sun, G. Grabowski, S.R. Finkbeiner, J.R. Spence, N.F. Shroyer, J.M. Wells, M.A. Helmrath, An in vivo model of human small intestine using pluripotent stem cells, Nat. Med. 20 (2014) 1310–1314.
[54] L. Hartl, G. Huelsz-Prince, J. van Zon, S.J. Tans, Apical constriction is necessary for crypt formation in small intestinal organoids, Dev. Biol. (2019) PMID: 30914321.
[55] G. Rossi, A. Manfrin, M.P. Lutolf, Progress and potential in organoid research, Nat.

Rev. Genet. 19 (2018) 671–687.
[56] H. Clevers, Modeling development and disease with organoids, Cell 165 (2016) 1586–1597.
[57] N. Barker, M. Huch, P. Kujala, M. van de Wetering, H.J. Snippert, J.H. van Es, T. Sato, D.E. Stange, H. Begthel, M. van den Born, E. Danenberg, S. van den Brink, J. Korving, A. Abo, P.J. Peters, N. Wright, R. Poulsom, H. Clevers, Lgr5(+ve) stem cells drive self-renewal in the stomach and build long-lived gastric units in vitro, Cell Stem Cell 6 (2010) 25–36.
[58] N. Barker, J.H. van Es, J. Kuipers, P. Kujala, M. van den Born, M. Cozijnsen, A. Haegebarth, J. Korving, H. Begthel, P.J. Peters, H. Clevers, Identification of stem cells in small intestine and colon by marker gene Lgr5, Nature 449 (2007) 1003–1007.
[59] T. Sato, D.E. Stange, M. Ferrante, R.G. Vries, J.H. Van Es, S. Van den Brink, W.J. Van Houdt, A. Pronk, J. Van Gorp, P.D. Siersema, H. Clevers, Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium, Gastroenterology 141 (2011) 1762–1772.
[60] A. Ootani, X. Li, E. Sangiorgi, Q.T. Ho, H. Ueno, S. Toda, H. Sugihara, K. Fujimoto, I.L. Weissman, M.R. Capecchi, C.J. Kuo, Sustained in vitro intestinal epithelial culture within a Wnt-dependent stem cell niche, Nat. Med. 15 (2009) 701–706.
[61] N. Sachs, Y. Tsukamoto, P. Kujala, P.J. Peters, H. Clevers, Intestinal epithelial organoids fuse to form self-organizing tubes in floating collagen gels, Development 144 (2017) 1107–1112.
[62] R.J. von Furstenberg, A.S. Gulati, A. Baxi, J.M. Doherty, T.S. Stappenbeck, A.D. Gracz, S.T. Magness, S.J. Henning, Sorting mouse jejunal epithelial cells with CD24 yields a population with characteristics of intestinal stem cells, Am. J. Physiol. Gastrointest. Liver Physiol. 300 (2011) G409–G417.
[63] M. Fukuda, T. Mizutani, W. Mochizuki, T. Matsumoto, K. Nozaki, Y. Sakamaki, S. Ichinose, Y. Okada, T. Tanaka, M. Watanabe, T. Nakamura, Small intestinal stem cell identity is maintained with functional Paneth cells in heterotopically grafted epithelium onto the colon, Genes Dev. 28 (2014) 1752–1757.
[64] S. Yui, T. Nakamura, T. Sato, Y. Nemoto, T. Mizutani, X. Zheng, S. Ichinose, T. Nagaishi, R. Okamoto, K. Tsuchiya, H. Clevers, M. Watanabe, Functional engraftment of colon epithelium expanded in vitro from a single adult Lgr5(+) stem cell, Nat. Med. 18 (2012) 618–623.
[65] K.S. Zaret, M. Grompe, Generation and regeneration of cells of the liver and pancreas, Science 322 (2008) 1490–1494.
[66] X. Wang, M. Al-Dhalimy, E. Lagasse, M. Finegold, M. Grompe, Liver repopulation and correction of metabolic liver disease by transplanted adult mouse pancreatic cells, Am. J. Pathol. 158 (2001) 571–579.
[67] M.S. Rao, V. Subbarao, J.K. Reddy, Induction of hepatocytes in the pancreas of copper-depleted rats following copper repletion, Cell Differ. 18 (1986) 109–117.
[68] M. Huch, C. Dorrell, S.F. Boj, J.H. van Es, V.S. Li, M. van de Wetering, T. Sato, K. Hamer, N. Sasaki, M.J. Finegold, A. Haft, R.G. Vries, M. Grompe, H. Clevers, In vitro expansion of single Lgr5+ liver stem cells induced by Wnt-driven regeneration, Nature 494 (2013) 247–250.
[69] M. Huch, P. Bonfanti, S.F. Boj, T. Sato, C.J. Loomans, M. van de Wetering, M. Sojoodi, V.S. Li, J. Schuijers, A. Gracanin, F. Ringnalda, H. Begthel, K. Hamer, J. Mulder, J.H. van Es, E. de Koning, R.G. Vries, H. Heimberg, H. Clevers, Unlimited in vitro expansion of adult bi-potent pancreas progenitors through the Lgr5/R-spondin axis, EMBO J. 32 (2013) 2708–2721.
[70] M. Huch, H. Gehart, R. van Boxtel, K. Hamer, F. Blokzijl, M.M. Verstegen, E. Ellis, M. van Wenum, S.A. Fuchs, J. de Ligt, M. van de Wetering, N. Sasaki, S.J. Boers, H. Kemperman, J. de Jonge, J.N. Ijzermans, E.E. Nieuwenhuis, R. Hoekstra, S. Strom, R.R. Vries, L.J. van der Laan, E. Cuppen, H. Clevers, Long-term culture of genome-stable bipotent stem cells from adult human liver, Cell 160 (2015) 299–312.
[71] D. Gao, I. Vela, A. Sboner, P.J. Iaquinta, W.R. Karthaus, A. Gopalan, C. Dowling, J.N. Wanjala, E.A. Undvall, V.K. Arora, J. Wongvipat, M. Kossai, S. Ramazanoglu, L.P. Barboza, W. Di, Z. Cao, Q.F. Zhang, I. Sirota, L. Ran, T.Y. MacDonald, H. Beltran, J.M. Mosquera, K.A. Touijer, P.T. Scardino, V.P. Laudone, K.R. Curtis, D.E. Rathkopf, M.J. Morris, D.C. Danila, S.F. Slovin, S.B. Solomon, J.A. Eastham, P. Chi, B. Carver, M.A. Rubin, H.I. Scher, H. Clevers, C.L. Sawyers, Y. Chen, Organoid cultures derived from patients with advanced prostate cancer, Cell 159 (2014) 176–187.
[72] C.W. Chua, M. Shibata, M. Lei, R. Toivanen, L.J. Barlow, S.K. Bergren, K.K. Badani, J.M. McKiernan, M.C. Benson, H. Hibshoosh, M.M. Shen, Single luminal epithelial progenitors can generate prostate organoids in culture, Nat. Cell Biol. 16 (2014) 951–961 951-954.
[73] J.R. Linnemann, H. Miura, L.K. Meixner, M. Irmler, U.J. Kloos, B. Hirschi, H.S. Bartsch, S. Sass, J. Beckers, F.J. Theis, C. Gabka, K. Sotlar, C.H. Scheel, Quantification of regenerative potential in primary human mammary epithelial cells, Development 142 (2015) 3239–3251.
[74] M. Kessler, K. Hoffmann, V. Brinkmann, O. Thieck, S. Jackisch, B. Toelle, H. Berger, H.J. Mollenkopf, M. Mangler, J. Sehouli, C. Fotopoulou, T.F. Meyer, The Notch and Wnt pathways regulate stemness and differentiation in human fallopian tube organoids, Nat. Commun. 6 (2015) 8989.
[75] W. Ren, B.C. Lewandowski, J. Watson, E. Aihara, K. Iwatsuki, A.A. Bachmanov, R.F. Margolskee, P. Jiang, Single Lgr5- or Lgr6-expressing taste stem/progenitor cells generate taste bud cells ex vivo, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 16401–16406.
[76] D.E. Stange, B.K. Koo, M. Huch, G. Sibbel, O. Basak, A. Lyubimova, P. Kujala, S. Bartfeld, J. Koster, J.H. Geahlen, P.J. Peters, J.H. van Es, M. van de Wetering, J.C. Mills, H. Clevers, Differentiated Troy+ chief cells act as reserve stem cells to generate all lineages of the stomach epithelium, Cell 155 (2013) 357–368.
[77] J.R. Rock, M.W. Onaitis, E.L. Rawlins, Y. Lu, C.P. Clark, Y. Xue, S.H. Randell, B.L. Hogan, Basal cells as stem cells of the mouse trachea and human airway epithelium, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 12771–12775.
[78] T. Tadokoro, Y. Wang, L.S. Barak, Y. Bai, S.H. Randell, B.L. Hogan, IL-6/STAT3 promotes regeneration of airway ciliated cells from basal stem cells, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) E3641–E3649.
[79] R. Jain, C.E. Barkauskas, N. Takeda, E.J. Bowie, H. Aghajanian, Q. Wang, A. Padmanabhan, L.J. Manderfield, M. Gupta, D. Li, L. Li, C.M. Trivedi, B.L. Hogan, J.A. Epstein, Plasticity of Hopx(+) type I alveolar cells to regenerate type II cells in the lung, Nat. Commun. 6 (2015) 6727.
[80] C.E. Barkauskas, M.J. Cronce, C.R. Rackley, E.J. Bowie, D.R. Keene, B.R. Stripp, S.H. Randell, P.W. Noble, B.L. Hogan, Type 2 alveolar cells are stem cells in adult lung, J. Clin. Investig. 123 (2013) 3025–3036.
[81] J. Mullenders, E. de Jongh, A. Brousali, M. Roosen, J.P.A. Blom, H. Begthel, J. Korving, T. Jonges, O. Kranenburg, R. Meijer, H.C. Clevers, Mouse and human urothelial cancer organoids: a tool for bladder cancer research, Proc. Natl. Acad. Sci. U. S. A. (2019) PMID:30787188.
[82] S.F. Boj, C.I. Hwang, L.A. Baker, I.I. Chio, D.D. Engle, V. Corbo, M. Jager, M. Ponz-Sarvise, H. Tiriac, M.S. Spector, A. Gracanin, T. Oni, K.H. Yu, R. van Boxtel, M. Huch, K.D. Rivera, J.P. Wilson, M.E. Feigin, D. Ohlund, A. Handly-Santana, C.M. Ardito-Abraham, M. Ludwig, E. Elyada, B. Alagesan, G. Biffi, G.N. Yordanov, B. Delcuze, B. Creighton, K. Wright, Y. Park, F.H. Morsink, I.Q. Molenaar, I.H. Borel Rinkes, E. Cuppen, Y. Hao, Y. Jin, I.J. Nijman, C. Iacobuzio-Donahue, S.D. Leach, D.J. Pappin, M. Hammell, D.S. Klimstra, O. Basturk, R.H. Hruban, G.J. Offerhaus, R.G. Vries, H. Clevers, D.A. Tuveson, Organoid models of human and mouse ductal pancreatic cancer, Cell 160 (2015) 324–338.
[83] L. Broutier, G. Mastrogiovanni, M.M. Verstegen, H.E. Francies, L.M. Gavarro, C.R. Bradshaw, G.E. Allen, R. Arnes-Benito, O. Sidorova, M.P. Gaspersz, N. Georgakopoulos, B.K. Koo, S. Dietmann, S.E. Davies, R.K. Praseedom, R. Lieshout, I.J. JNM, S.J. Wigmore, K. Saeb-Parsy, M.J. Garnett, L.J. van der Laan, M. Huch, Human primary liver cancer-derived organoid cultures for disease modeling and drug screening, Nat. Med. 23 (2017) 1424–1435.
[84] S.H. Lee, W. Hu, J.T. Matulay, M.V. Silva, T.B. Owczarek, K. Kim, C.W. Chua, L.J. Barlow, C. Kandoth, A.B. Williams, S.K. Bergren, E.J. Pietzak, C.B. Anderson, M.C. Benson, J.A. Coleman, B.S. Taylor, C. Abate-Shen, J.M. McKiernan, H. Al-Ahmadie, D.B. Solit, M.M. Shen, Tumor evolution and drug response in patient-derived organoid models of bladder cancer, Cell 173 (2018) 515–528 e517.
[85] I. Vasyutin, L. Zerihun, C. Ivan, A. Atala, Bladder organoids and spheroids: potential tools for normal and diseased tissue modelling, Anticancer Res. 39 (2019) 1105–1118.
[86] M. van de Wetering, H.E. Francies, J.M. Francis, G. Bounova, F. Iorio, A. Pronk, W. van Houdt, J. van Gorp, A. Taylor-Weiner, L. Kester, A. McLaren-Douglas, J. Blokker, S. Jaksani, S. Bartfeld, R. Volckman, P. van Sluis, V.S. Li, S. Seepo, C. Sekhar Pedamallu, K. Cibulskis, S.L. Carter, A. McKenna, M.S. Lawrence, L. Lichtenstein, C. Stewart, J. Koster, R. Versteeg, A. van Oudenaarden, J. Saez-Rodriguez, R.G. Vries, G. Getz, L. Wessels, M.R. Stratton, U. McDermott, M. Meyerson, M.J. Garnett, H. Clevers, Prospective derivation of a living organoid biobank of colorectal cancer patients, Cell 161 (2015) 933–945.
[87] M. Fujii, M. Shimokawa, S. Date, A. Takano, M. Matano, K. Nanki, Y. Ohta, K. Toshimitsu, Y. Nakazato, K. Kawasaki, T. Uraoka, T. Watanabe, T. Kanai, T. Sato, A colorectal tumor organoid library demonstrates progressive loss of niche factor requirements during tumorigenesis, Cell Stem Cell 18 (2016) 827–838.
[88] A.A. Duarte, E. Gogola, N. Sachs, M. Barazas, S. Annunziato, J.R.D. Ruiter, A. Velds, S. Blatter, J.M. Houthuijzen, M. Van de Ven, H. Clevers, P. Borst, J. Jonkers, S. Rottenberg, BRCA-deficient mouse mammary tumor organoids to study cancer-drug resistance, Nat Methods 15 (2017) 134–140.
[89] N. Sachs, J. de Ligt, O. Kopper, E. Gogola, G. Bounova, F. Weeber, A.V. Balgobind, K. Wind, A. Gracanin, H. Begthel, J. Korving, R. van Boxtel, A.A. Duarte, D. Lelieveld, A. van Hoeck, R.F. Ernst, F. Blokzijl, I.J. Nijman, M. Hoogstraat, M. van de Ven, D.A. Egan, V. Zinzalla, J. Moll, S.F. Boj, E.E. Voest, L. Wessels, P.J. van Diest, S. Rottenberg, R.G.J. Vries, E. Cuppen, H. Clevers, A living biobank of breast cancer organoids captures disease heterogeneity, Cell 172 (2018) 373–386.
[90] S. Bian, M. Repic, Z. Guo, A. Kavirayani, T. Burkard, J.A. Bagley, C. Krauditsch, J.A. Knoblich, Genetically engineered cerebral organoids model brain tumor formation, Nat. Methods 15 (2018) 631–639.
[91] B.E. Michels, M.H. Mosa, B.M. Grebbin, D. Yepes, T. Darvishi, J. Hausmann, H. Urlaub, S. Zeuzem, H.M. Kvasnicka, T. Oellerich, H.F. Farin, Human colon organoids reveal distinct physiologic and oncogenic Wnt responses, J. Exp. Med. 216 (2019) 704–720.
[92] M. Li, J.C. Izpisua Belmonte, Organoids - preclinical models of human disease, N. Engl. J. Med. 380 (2019) 569–579.
[93] L. Li, H. Knutsdottir, K. Hui, M.J. Weiss, J. He, B. Philosophe, A.M. Cameron, C.L. Wolfgang, T.M. Pawlik, G. Ghiaur, A.J. Ewald, E. Mezey, J.S. Bader, F.M. Selaru, Human primary liver cancer organoids reveal intratumor and interpatient drug response heterogeneity, JCI Insight (2019) 4.
[94] E.D. Segal, S. Falkow, L.S. Tompkins, Helicobacter pylori attachment to gastric cells induces cytoskeletal rearrangements and tyrosine phosphorylation of host cell proteins, Proc Natl Acad Sci U S A 93 (1996) 1259–1264.
[95] S. Bartfeld, T. Bayram, M. van de Wetering, M. Huch, H. Begthel, P. Kujala, R. Vries, P.J. Peters, H. Clevers, In vitro expansion of human gastric epithelial stem cells and their responses to bacterial infection, Gastroenterology 148 (2015) 126–136 e126.
[96] R. Morizane, A.Q. Lam, B.S. Freedman, S. Kishi, M.T. Valerius, J.V. Bonventre, Nephron organoids derived from human pluripotent stem cells model kidney development and injury, Nat. Biotechnol. 33 (2015) 1193–1200.
[97] J.F. Dekkers, G. Berkers, E. Kruisselbrink, A. Vonk, H.R. de Jonge, H.M. Janssens, I. Bronsveld, E.A. van de Graaf, E.E. Nieuwenhuis, R.H. Houwen, F.P. Vleggaar,

J.C. Escher, Y.B. de Rijke, C.J. Majoor, H.G. Heijerman, K.M. de Winter-de Groot, H. Clevers, C.K. van der Ent, J.M. Beekman, Characterizing responses to CFTR-modulating drugs using rectal organoids derived from subjects with cystic fibrosis, Sci Transl Med 8 (2016) 344ra384.

[98] G. Vlachogiannis, S. Hedayat, A. Vatsiou, Y. Jamin, J. Fernándezmateos, K. Khan, A. Lampis, K. Eason, I. Huntingford, R.J.S. Burke, Patient-derived organoids model treatment response of metastatic gastrointestinal cancers, Science 359 (2018) 920–926.

[99] H. Tiriac, P. Belleau, D.D. Engle, D. Plenker, A. Deschenes, T.D.D. Somerville, F.E.M. Froeling, R.A. Burkhart, R.E. Denroche, G.H. Jang, K. Miyabayashi, C.M. Young, H. Patel, M. Ma, J.F. LaComb, R.L.D. Palmaira, A.A. Javed, J.C. Huynh, M. Johnson, K. Arora, N. Robine, M. Shah, R. Sanghvi, A.B. Goetz, C.Y. Lowder, L. Martello, E. Driehuis, N. LeComte, G. Askan, C.A. Iacobuzio-Donahue, H. Clevers, L.D. Wood, R.H. Hruban, E. Thompson, A.J. Aguirre, B.M. Wolpin, A. Sasson, J. Kim, M. Wu, J.C. Bucobo, P. Allen, D.V. Sejpal, W. Nealon, J.D. Sullivan, J.M. Winter, P.A. Gimotty, J.L. Grem, D.J. DiMaio, J.M. Buscaglia, P.M. Grandgenett, J.R. Brody, M.A. Hollingsworth, G.M. O'Kane, F. Notta, E. Kim, J.M. Crawford, C. Devoe, A. Ocean, C.L. Wolfgang, K.H. Yu, E. Li, C.R. Vakoc, B. Hubert, S.E. Fischer, J.M. Wilson, R. Moffitt, J. Knox, A. Krasnitz, S. Gallinger, D.A. Tuveson, Organoid profiling identifies common responders to chemotherapy in pancreatic cancer, Cancer Discov. 8 (2018) 1112–1129.

[100] A. Linkous, D. Balamatsias, M. Snuderl, L. Edwards, K. Miyaguchi, T. Milner, B. Reich, L. Cohen-Gould, A. Storaska, Y. Nakayama, E. Schenkein, R. Singhania, S. Cirigliano, T. Magdeldin, Y. Lin, G. Nanjangud, K. Chadalavada, D. Pisapia, C. Liston, H.A. Fine, Modeling patient-derived glioblastoma with cerebral organoids, Cell Rep. 26 (2019) 3203–3211 e3205.

[101] S.S. Kumar, S.N. Sreedharan, S. Patil, A.T. Raj, The potential role of organoids in pathology and oncology research, Pathol. Oncol. Res. (2019).

[102] N. Phan, J.J. Hong, B. Tofig, M. Mapua, D. Elashoff, N.A. Moatamed, J. Huang, S. Memarzadeh, R. Damoiseaux, A. Soragni, A simple high-throughput approach identifies actionable drug sensitivities in patient-derived tumor organoids, Commun. Biol. 2 (2019) 78.

[103] L. Grassi, R. Alfonsi, F. Francescangeli, M. Signore, M.L. De Angelis, A. Addario, M. Costantini, E. Flex, A. Ciolfi, S. Pizzi, A. Bruselles, M. Pallocca, G. Simone, M. Haoui, M. Falchi, M. Milella, S. Sentinelli, P. Di Matteo, E. Stellacci, M. Gallucci, G. Muto, M. Tartaglia, R. De Maria, D. Bonci, Organoids as a new model for improving regenerative medicine and cancer personalized therapy in renal diseases, Cell Death Dis. 10 (2019) 201.

[104] J. Kraiczy, M. Zilbauer, Intestinal epithelial organoids as tools to study epigenetics in gut health and disease, Stem Cell. Int. 2019 (2019) 7242415.

[105] M. Takasato, P.X. Er, H.S. Chiu, B. Maier, G.J. Baillie, C. Ferguson, R.G. Parton, E.J. Wolvetang, M.S. Roost, S.M. Chuva de Sousa Lopes, M.H. Little, Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis, Nature 526 (2015) 564–568.

[106] F. Yu, W. Hunziker, D. Choudhury, Engineering microfluidic organoid-on-a-chip platforms, Micromachines (Basel) 10 (2019).

[107] G. Schwank, B.K. Koo, V. Sasselli, J.F. Dekkers, I. Heo, T. Demircan, N. Sasaki, S. Boymans, E. Cuppen, C.K. van der Ent, E.E. Nieuwenhuis, J.M. Beekman, H. Clevers, Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients, Cell Stem Cell 13 (2013) 653–658.

[108] A. Orimo, P.B. Gupta, D.C. Sgroi, F. Arenzana-Seisdedos, T. Delaunay, R. Naeem, V.J. Carey, A.L. Richardson, R.A. Weinberg, Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion, Cell 121 (2005) 335–348.

[109] J.L. Rinn, C. Bondre, H.B. Gladstone, P.O. Brown, H.Y. Chang, Anatomic demarcation by positional variation in fibroblast gene expression programs, PLoS Genet. 2 (2006) e119.

[110] M. Yamashita, T. Ogawa, X. Zhang, N. Hanamura, Y. Kashikura, M. Takamura, M. Yoneda, T. Shiraishi, Role of stromal myofibroblasts in invasive breast cancer: stromal expression of alpha-smooth muscle actin correlates with worse clinical outcome, Breast Canc. 19 (2012) 170–176.

[111] R. Fernándezperiáñez, I. Molinaprivado, F. Rojo, I. Guijarromuñoz, V. Alonsocamino, S. Zazo, M. Compte, A. Álvarezcienfuegos, Á.M. Cuesta, D. Sánchezmartín, A.M. AlvarezMendez, L. Sanz, L. AlvarezVallina, Basement membrane-rich organoids with functional human blood vessels are permissive niches for human breast cancer metastasis, PLoS One 8 (2013) e72957.

[112] R.A. Wimmer, A. Leopoldi, M. Aichinger, N. Wick, B. Hantusch, M. Novatchkova, J. Taubenschmid, M. Hammerle, C. Esk, J.A. Bagley, D. Lindenhofer, G. Chen, M. Boehm, C.A. Agu, F. Yang, B. Fu, J. Zuber, J.A. Knoblich, D. Kerjaschki, J.M. Penninger, Human blood vessel organoids as a model of diabetic vasculopathy, Nature 565 (2019) 505–510.

[113] C. Mattei, R. Lim, H. Drury, B. Nasr, Z. Li, M.A. Tadros, G.M. D'Abaco, K.S. Stok, B.A. Nayagam, M. Dottori, Generation of vestibular tissue-like organoids from human pluripotent stem cells using the rotary cell culture system, Front Cell Dev. Biol. 7 (2019) 25.

[114] M.J. Workman, M.M. Mahe, S. Trisno, H.M. Poling, C.L. Watson, N. Sundaram, C.F. Chang, J. Schiesser, P. Aubert, E.G. Stanley, A.G. Elefanty, Y. Miyaoka, M.A. Mandegar, B.R. Conklin, M. Neunlist, S.A. Brugmann, M.A. Helmrath, J.M. Wells, Engineered human pluripotent-stem-cell-derived intestinal tissues with a functional enteric nervous system, Nat. Med. 23 (2017) 49–59.

[115] W. Pan, S. Zhu, K. Qu, K. Meeth, J. Cheng, K. He, H. Ma, Y. Liao, X. Wen, C. Roden, Z. Tobiasova, Z. Wei, J. Zhao, J. Liu, J. Zheng, B. Guo, S.A. Khan, M. Bosenberg, R.A. Flavell, J. Lu, The DNA methylcytosine dioxygenase Tet2 sustains immunosuppressive function of tumor-infiltrating myeloid cells to promote melanoma progression, Immunity 47 (2017) 284–297 e285.

[116] G. Mollaoglu, A. Jones, S.J. Wait, A. Mukhopadhyay, S. Jeong, R. Arya, S.A. Camolotto, T.L. Mosbruger, C.J. Stubben, C.J. Conley, A. Bhutkar, J.M. Vahrenkamp, K.C. Berrett, M.H. Cessna, T.E. Lane, B.L. Witt, M.E. Salama, J. Gertz, K.B. Jones, E.L. Snyder, T.G. Oliver, The lineage-defining transcription factors SOX2 and NKX2-1 determine lung cancer cell fate and shape the tumor immune microenvironment, Immunity 49 (2018) 764–779 e769.

[117] E.L. Kuan, S.F. Ziegler, A tumor–myeloid cell axis, mediated via the cytokines IL-1α and TSLP, promotes the progression of breast cancer, Nat. Immunol. 19 (2018) 366–374.

[118] K. Nozaki, W. Mochizuki, Y. Matsumoto, T. Matsumoto, M. Fukuda, T. Mizutani, M. Watanabe, T. Nakamura, Co-culture with intestinal epithelial organoids allows efficient expansion and motility analysis of intraepithelial lymphocytes, J. Gastroenterol. 51 (2016) 206–213.

[119] G. Noel, M. Doucet, J.P. Nataro, J.B. Kaper, N.C. Zachos, M.F. Pasetti, Enterotoxigenic Escherichia coli is phagocytosed by macrophages underlying villus-like intestinal epithelial cells: modeling ex vivo innate immune defenses of the human gut, Gut Microb. (2017) 0.

[120] G. Noel, N.W. Baetz, J.F. Staab, M. Donowitz, O. Kovbasnjuk, M.F. Pasetti, N.C. Zachos, A primary human macrophage-enteroid co-culture model to investigate mucosal gut physiology and host-pathogen interactions, Sci. Rep. 7 (2017) 45270.

[121] K.K. Dijkstra, C.M. Cattaneo, F. Weeber, M. Chalabi, J. van de Haar, L.F. Fanchi, M. Slagter, D.L. van der Velden, S. Kaing, S. Kelderman, N. van Rooij, M.E. van Leerdam, A. Depla, E.F. Smit, K.J. Hartemink, R. de Groot, M.C. Wolkers, N. Sachs, P. Snaebjornsson, K. Monkhorst, J. Haanen, H. Clevers, T.N. Schumacher, E.E. Voest, Generation of tumor-reactive T cells by Co-culture of peripheral blood lymphocytes and tumor organoids, Cell 174 (2018) 1586–1598 e1512.

[122] A. Montel-Hagen, C.S. Seet, S. Li, B. Chick, Y. Zhu, P. Chang, S. Tsai, V. Sun, S. Lopez, H.C. Chen, C. He, C.J. Chin, D. Casero, G.M. Crooks, Organoid-induced differentiation of conventional T cells from human pluripotent stem cells, Cell Stem Cell 24 (2019) 376–389 e378.

[123] J.T. Neal, X. Li, J. Zhu, V. Giangarra, C.L. Grzeskowiak, J. Ju, I.H. Liu, S.H. Chiou, A.A. Salahudeen, A.R. Smith, B.C. Deutsch, L. Liao, A.J. Zemek, F. Zhao, K. Karlsson, L.M. Schultz, T.J. Metzner, L.D. Nadauld, Y.Y. Tseng, S. Alkhairy, C. Oh, P. Keskula, D. Mendoza-Villanueva, F.M. De La Vega, P.L. Kunz, J.C. Liao, J.T. Leppert, J.B. Sunwoo, C. Sabatti, J.S. Boehm, W.C. Hahn, G.X.Y. Zheng, M.M. Davis, C.J. Kuo, Organoid modeling of the tumor immune microenvironment, Cell 175 (2018) 1972–1988 e1916.

[124] A.K. Gupta, J.M. Coburn, J. Davis-Knowlton, E. Kimmerling, D.L. Kaplan, L. Oxburgh, Scaffolding kidney organoids on silk, J. Tissue Eng. Regenerat. Med. (2019) PMID: 30793851.

[125] D. Blondel, M.P. Lutolf, Bioinspired hydrogels for 3D organoid culture, Chimia (Aarau) 73 (2019) 81–85.

[126] T. Rezaei Topraggaleh, M. Rezazadeh Valojerdi, L. Montazeri, H. Baharvand, A testis-derived macroporous 3D scaffold as a platform for the generation of mouse testicular organoids, Biomater. Sci. 7 (2019) 1422–1436.

[127] M.P. Lutolf, J.L. Lauer-Fields, H.G. Schmoekel, A.T. Metters, F.E. Weber, G.B. Fields, J.A. Hubbell, Synthetic matrix metalloproteinase-sensitive hydrogels for the conduction of tissue regeneration: engineering cell-invasion characteristics, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 5413–5418.

[128] L. Almany, D. Seliktar, Biosynthetic hydrogel scaffolds made from fibrinogen and polyethylene glycol for 3D cell cultures, Biomaterials 26 (2005) 2467–2477.