Organoids well recapitulate organ-specific functions from their tissue of origin and remain fundamental aspects of organogenesis. Organoids are widely applied in biomedical research, drug discovery, and regenerative medicine. There are various cultivated organoid systems induced by adult stem cells and pluripotent stem cells, or directly derived from primary tissues. Researchers have drawn inspiration by combination of organoid technology and tissue engineering to produce organoids with more physiological relevance and suitable for translational medicine. This review describes the value of applying organoids for tumorigenesis modeling and tumor vaccination. We summarize the application of organoids in tumor precision medicine. Extant challenges that need to be conquered to make this technology be more feasible and precise are discussed.

Keywords: organoids, tumor-initiating cell, tumorigenesis, precision medicine, tumor vaccine

INTRODUCTION

Organoids are three-dimensional cell complexes with a particular spatial structure cultured in vitro (1, 2). It is amplified and maintains certain structural and functional features of their source tissue (3). Organoids develop from stem-like cells or initiating cells including embryonic stem cells (ESCs) (4, 5), adult stem cells (ASCs) (6), induced pluripotent stem cells (iPSCs), and progenitor cells (7–9). ESCs are cells selected from the intraembryonic cell mass or obtained by inhibiting primordial germ cells in vitro, which has the ability of multidirectional differentiation (10, 11). ASCs are undifferentiated cells existing in various differentiated tissues that are responsible for repair and regeneration after tissue injury (5). Progenitor cells can repair and regenerate following tissue damage (12). In this review, we demonstrate organoid platforms derived from primary tissues, ASCs or iPSCs, summarize the organoid bioengineering advancement, and describe the possibility of...
applying organoids for carcinogenesis modeling (Figure 1). Current challenges about the broad application of organoids are also discussed.

**ORGANOID CULTURE FROM PRIMARY TISSUES**

**Organoid Culture From Stem Cells**

Organoid culture became a major technological advance in 2009. Hans Clevers reconstructed a suitable niche better for maintaining intestinal stem cells in vitro (13). This system contains a key conditioned medium supplemented with multiple growth factors including Wnt agonists R-spondin-1 or -3, Wnt3a, epidermal growth factor (EGF), and noggin. Mouse primary intestine cells were embedded into hydrogel [i.e., extracellular matrix (ECM)] followed by gut organoid formation with villus structure owing to the self-renewal capacity of intestinal stem cells, which has pioneered the field of organoid cancer biology (13). The system guaranteed continuous proliferation of organoids, not only ensured stability and purification of mouse genome but also had an advantage of amplification (14). Research fellows have cultivated diverse organoid systems derived from tissues with epithelial origination, such as bladder, colon, rectum, endometrium, fallopian tube, kidney, liver, lung, esophagus, oral mucosa, pancreas, prostate, salivary gland, skin epidermis, stomach, and taste buds (15–25). Researchers have also generated organoids from normal cells in the urinary tract and bronchial lavage, rather than parenchymatous organs (26, 27). Organoids derived from ASCs maintain phenotypic and genetic stability, better reflecting their primary tissue genome (28, 29). Genomic mutation in organoids can be investigated by immunohistochemistry staining, whole-exome sequencing (WES), and RNA sequencing (30). Organoid is driven by the inherent ability of stem cells themselves (13). Their self-assembly ability allows organoids to produce functional mature “organs” by precise spatial and temporal order (31, 32). Similarly, organoids run self-assembly processes in vitro by changing cytokine constituents of culture media, simulating organoid differentiation and maturation (14, 33, 34). Gabriel et al. (35) observed that brain organoids assembled optic vesicles, including primitive corneal epithelial and lens-like cells. This study confirmed that the self-assembly process was carried out via a multistep process in the early stage of organoid genesis.

Organoid culture varies from different tissues of origin; several organs require more efforts to establish a stable condition (i.e., heart and immune organs) (36–38). It has been studied to generate complex and highly structured cardiac organs by embedding human iPSCs into matrix glue, regulating small molecule directional cardiac differentiation through the biphasic Wnt pathway (39). The lacrimal secretion of neurotransmitters by lacrimal gland organoids was verified by orthotopic transplantation in mice (40).

**Organoid Bioengineering**

The inherent self-organizing of stem cells does not signify that organoid might form fine tissue under any condition (41). This process emphasizes that fate guides organoid to develop into mimic-tissues in a highly environment-dependent manner (42). Those established organoid-forming approaches have considerable defects: when cultured for too long, stem cells would uncontrollably develop into a circular cystic closed structure, with a short life span and non-physiological shape, resulting in inconsistency between organoid and organs in anatomy and physiology (43, 44). To solve this issue, bioengineering cultivated organoids into a variety of biomaterials that can promote their better proliferation, precise differentiation, and exact function (43, 45, 46). Tissue bioengineering uses bioactive substances to regenerate or repair tissues through in vitro construction (Figure 2) (47). It is accomplished by controlling the process of organoids and establishing the next generation with high physiological correlation (48). Researchers at the EPFL Institute have constructed an intestinal geometric scaffold with hydrogel, providing an appropriate place to guide organoid to form a true intestinal organ (49). In this method, stem cells were cultured in scaffolds simulating the surface of natural tissue and then combined into microfluidic chips. Due to their inherent self-organization, organoids grew on tubular scaffolds and self-organized to form intestines (47). Organoids would gradually form continuous cell layers with recess structure and villous-like domain and form “mini-intestines” in vitro, which maintain the same functional features as primary organs in vivo.
Improved methods have been used for organoid generation (i.e., stomach, liver, kidney, etc.) (52–55). Microfluidic approach and organoid chip integrate mechanical and physiological parameters, expanding the usage of organoids for recapitulating the physiological function of their source organs (Figure 2) (37). Using organ setting on a chip, a single gastric organoid has been successfully established (56). Not singly but in pairs, the use of lumen flow and pressure circulation to induce peristaltic movement showed the feasibility of integrating engineering in organoid culture (57). Coincidentally, kidney organoids on chips were exposed to shear stress by applying fluid flow, thereby mimicking the kidney environment in vivo (58). The existence of fluid flow not only promoted maturation of renal organoids but also was conducive to formation of vascular network with perfusion lumen. Creative combination of organoids and tissue engineering allows organoid growth, which may overcome space constraints and promote shape-guided morphogenesis and physiology. Furthermore, researchers used microfabricated cell rejection microporous (an apparatus for culturing organoids using tissue engineering) to culture and monitor homogeneous liver organoids from dissociated human iPSCs (54). Organoid bioengineering improves experimental replication, optimizes model quality, and realizes clinical transformation. Decembrini et al. (59) have proven that production of retinal organoids was accelerated and standardized in the best physicochemical microenvironment. A biomimetic hydrogel composed of circular bottom microwell arrays and optimized media formulation not only facilitated mouse ESC formation but also aggregated retinal organoids in a stereotypical manner, leading to an unlimited source of retinal neurons (59). Combining organoid culture with tissue engineering, organ devices accurately control organoid growth, while organoids simulate real physiology more in line with the situation in vivo (60–62).

ORGANOID AS A MODEL FOR TUMORIGENESIS

Compared with traditional two-dimensional culture of tumor cells, tumor organoids maintain better cell heterogeneity, retain tumor characteristics, show less loss of tumor niche components, and provide a more authentic environment for clinical treatment (63, 64). In contrast to patient-derived tumor xenografts (PDXs), the success rate of tumor organoid construction is way higher (50%–90% vs. 10%–30%), organoids are maintained for a longer time with lower cost, so it is easy to gene editing and large-scale drug screening (65–68). There are two kinds of organoid construction techniques, one is derived from differentiation of iPSCs (16) and the other is directly derived from tumor tissues (69). Constructing tumor organoids from iPSCs largely depends on tumor types, and the culture operation is more complex (70). Tumor organoids obtained by iPSC differentiation lose the complexity of the tumor microenvironment (TME) (71). The more common organoid culture method is to directly use primary tissues, supplemented by cytokines, tumor matrix, and other components (70, 72). Tumor organoids such as colorectal, breast, pancreatic, prostate, liver, and gastric cancers have been successfully constructed (73–77). Researchers have obtained a diverse collection of tumor organoids with different characteristics through in vitro culture to constitute a living organoid biobank (78). Through histochemical observation of the morphology of tumor organoids, their internal structure was...
like that of primary tissue (1, 4, 6). Simultaneously, genome and single-cell sequencing was carried out to explore the variation between organoids and primary tumors in the gene mutation spectrum (79).

Organoids maintain tumor heterogeneity within and among tumors (80). Patient-derived organoids (PDOs) maintains the diversity and complexity of tumor origin in terms of cell heterogeneous, histology, gene mutation, transcription spectrum, and even metabolism (39, 81, 82). Organoids not only reflect characteristics of primary tumors, but also exert advantages for explorations of tumorigenesis, cell communication, epigenetics, and invasion (34, 77).

Due to various sources of tumor samples and slightly discrepant culture system among laboratories, it is not easy to duplicate the experimental findings, and this hinders further verification and reference of obtained data. A study conducted by Dr. Manel Esteller, director of the Josep Carreras leukemia Institute (IJC), used epigenetic Infinium MethylationEPIC BeadChip (EPIC), a microarray chip from Illumina that interrogates more than 850,000 CpG sites to analyze DNA methylation status of 25 human cancer organoids (83). This data indicated that tumor organoids highly sustain biological properties and heterogeneity of tumor tissue in situ.

**Tumorigenesis in Organoids**

Tumor originates from accumulation of gene mutation in normal somatic cells; while not all mutations have access to induce tumorigenesis, tolerance of different tissues to the same mutation is widely divergent. Numerous cell and animal experiments have clarified key factors and decisive mechanisms initiated from gene mutation to tumorigenesis, fully understanding that such process is artificial due to the failure of monitoring and intervening the earliest process of tumor development. Innovative organoid system makes it possible to understand the transformation process from normal tissue to tumor (84). The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 system has revolutionized genetic engineering, allowing gene editing of normal organoids by inducing oncogenes to obtain tumor organoids and track the carcinogenic process from initial to advanced stage (85–87). Mutated tumor-related genes KRAS, CDKN2A, TP53, and SMAD4 in normal pancreatic organoids eventually developed to a state recapitulating pancreatic ductal adenocarcinoma (80, 88, 89). Lannagan et al. (90) found that mutated intestinal organoids could grow and reproduce without relying on any exogenous cytokines. When transplanting organoids with four mutations into mice, these organoids induced by colorectal cancer were invasive (90). This approach can amplify the effects of driving mutations in the same genetic background. Organoids from normal tissues mimic tumor pathogenesis by continuously introducing cancer-driven mutations (91, 92). Combination of organoid system and CRISPR/Cas9 strategy is powerful to study the origin of tumor (93, 94).

It is generally believed that cancer is a progressive disease caused by accumulation of abnormal gene mutation. These genetic anomalies include tumor suppressor/oncogene mutations and chromosome abnormalities (95). It is increasingly clear that tumors can be induced by epigenetic changes. Epigenetics refers to heritable change of gene function without a change of genetic material, eventually leading to different phenotypes (96). It mainly includes DNA methylation, histone modification, non-coding RNA regulation, and chromatin structure reconstruction. Potential tumor suppressor genes are inhibited or silenced at the transcriptional level by DNA methylation, promoting malignant transformation from normal cells (97, 98). It has begun to dig influence and underlying mechanisms that modulate methylation and chromatin states of tumor suppressor gene or tumor oncogene during tumor formation (96, 99). Epigenetic changes bring abundant precancerous cell expansion. They first occur in precancerous cells, determining subsequent genetic changes that promote malignant transformation and tumor cell clonal expansion (98, 99). Aloia et al. (100) have demonstrated that bile duct cells underwent epigenetic modification of genome-wide DNA methylation during tissue damage and subsequent organoid construction. This work that was inspired by those epigenetic changes of organoids from normal to malignant cell transition was able to determine subsequent uncontrollable modification of genetic machinery, eventually leading to malignant occurrence and development (95, 98, 100).

**Tumor Microenvironment in Organoid System**

TME is an internal condition for the production and survival of tumor cells, including immune and inflammatory cells, fibroblasts, vascular endothelial cells (101). Bidirectional communication between tumor cells and TME possesses an indispensable position in tumor promotion (36). Tumor-infiltrating lymphocytes (TILs) are heterogeneously composed of different lymphocytes; their phenotypic and functional characteristics largely correlate with interaction with tumor cells (102). Researchers cultured tumor organoids through air-liquid interface to reproduce TME *in vitro*. Air-PDOs successfully retained inherent fibrous matrix and a variety of immune cell components of primary tumor tissue (103). Through integrated culture, the *in situ* tumor essence and matrix were reserved, including functional TILs. By continuously coculturing cancer organoids and peripheral blood monocytes (PBMCs) in the presence of T cell-stimulating growth factors, antigen-specific cytotoxic T cells were selected and amplified in about half of total samples (Figure 2). T cells expanded from adjacent healthy epithelial tissues resulted in undisturbed organoid expansion without a significant level of organoid cytotoxicity (104).

Cancer-associated fibroblasts (CAFs) secrete miscellaneous cytokines, chemokines, and growth factors to create a conducive microenvironment for tumor progression (105). The prominent role of CAFs is considered to shape stem cell niche to cultivate cancer stem cells (CSCs), while two-dimensional culture of cell lines is far from satisfactory in summarizing general characteristics of CSCs (106). The first coculture model of
CAFs and organoids was established in pancreatic cancer, followed by in liver, colorectal, prostate, esophageal, and breast cancers (107–110). Recently, it is reported that organoids and CAFs from diethylnitrosamine (DEN)-induced mouse liver tumors can be cultured together; CAFs promoted organoid growth through paracrine signals. Cotransplantation of CAFs with liver tumor organoids promoted tumor growth in xenotransplantation models; CAFs may not regulate the efficiency of organoid initiation but accelerated its growth (111).

The deficiency of blood vessels in the organoid system brings challenges (112). One study emphasized that endothelial cells can be replaced by adaptable angiogenic cells to form a perfusion plastic vascular plexus for restrictive synthetic semipermeable membrane by organ chip system, and this provided a physiological platform for tumor organoid vascularization (113). This minimized hypoxia in tumor organoids provided a ponder over influence and mechanism of vascular endothelial cells on tumor occurrence and development (Figure 2) (112, 114).

APPLICATION OF ORGANOIDS IN PRECISION TUMOR ONCOTHERAPY

Tumors are generally heterogeneous, and there is no fixed treatment for all types of tumors, which makes precision medicine a new direction for tumor therapy, that is, patients with different stages of tumors are entitled to discrepant treatment schemes (115, 116). Tumor organoids replicate complex signaling pathways and cell-to-cell relationship and more accurately reflect tumor genetic features (79). Organoids become a personalized treatment channel with its unique experimental advantages—high level of physiological relevance and convenience of in vitro operation (4, 65, 117).

Application of Organoids in Tumor Medication

Organoid technology for precision medicine refers to drug screening in vitro in PDOs and formulation of individual medication (32, 85). Tumor organoids largely maintain heterogeneity between source tumors and different patients, individual morphology and scale of organoids remain such uniform (3, 38, 118). These organoids not only remove confusing variables that may be introduced from animal models but also provide greater complexity than homogenized cell cultures (1, 44, 66). The valuable tumor model established by patients’ iPSCs is able to understand tumor pathogenesis and disease progression (3, 9). The established liiving biobank of tumor organoids has infinite predictive value for determining the distinct drug response of patients. In turn, it gives access to large-scale drug screening (63, 119). Tumor organoids swiftly select the optimal therapeutic schedule for patients, accompanied by reducing side effects and tumor recurrence (24, 70, 120). They significantly shorten the preclinical test period, working as a key slot in drug discovery, providing a large amount of biological data and a high-quality platform (6, 33). With the progress and standardization of organoid culture, the accessibility of tumor organs will be more widely realized (25, 30, 121).

Tumor organoid-based drug screening integrated with next-generation sequencing (NGS) is conducive to oncotherapy, which is combined with clinical treatment to form complementarity (122–124). NGS detects genetic mutations of patients at source and provides drug treatment options, but it alone does not guarantee clinical efficacy (125–128). As a distinct supplement, organoid is advantageous to well investigate this uncertainty (6, 65, 117). Patients with epidermal growth factor receptor amplification were usually treated with cetuximab guided by NGS, yet this consequence was overturned by the organoid system, which was consistent with actual clinical situation (129). Organoid drug screening further picks more effective approaches on the basis of sequencing to give conclusive recommendations and practical biology evidence for patients (20). This technology clarifies particular therapeutics for precision medicine and determines whether a particular group of patients is not suitable for a therapeutic (3, 70).

Application of Organoids in Tumor Vaccination

Organoid culture along with NGS and single-cell sequencing (scRNA-seq) is good to hunt for therapeutic targets and discover mutation-associated neoantigens (MANAs) for fresh targeted remedy or tumor vaccination (Figure 3) (130–134). With continuous innovation and improvement of benchwork for appraising tumor MANAs, such as the recently emerging NeoScreen technology, more previously hidden tumor antigen epitopes have been identified (135–138). Based on bioinformatics data analysis, MANAs reflecting individual disease are unearthed by a machine learning algorithm (138, 139). Using an organoid system, Demmers et al. (140) recently considered that human leukocyte antigen (HLA) class I peptide expression among different clonal cells from the same colorectal cancer patient was variability and its widespread difference in cloning specificity was generally common. By linking organoid proteomics and HLA peptide ligandomics, they discovered that tumor-specific ligands derived from DNA damage and tumor suppressor proteins were remarkably presented in tumor cells, which might be consistent with defunction of their cytoprotective effect. In general, their data demonstrated heterogeneous HLA peptide expression in an individual patient and presumed that a promising multipepptide tumor vaccine may be a feasible option to minimize immune escape risk.

Taking certain mRNAs encoding MANAs as available templates, individualized tumor vaccines against MANAs are artificially synthesized, stimulating tumor-specific T-cell production for the sake of diminishing tumor cells (141–147). These tumor vaccines are theoretically proven to be potential therapeutics for accurate oncotherapy (146, 148, 149). Organoids have been implanted during the development and testing process of vaccines against pathogenic microorganisms including bacteria and viruses (141, 150–152). Researchers developed mini tonsils in vitro from surgery tissues and added coronavirus disease 2019 (COVID-19) candidate vaccines into...
the culture system, a predominant tool to verify vaccine efficacy, to observe whether tonsil organoids incite immune responses, specific immunocytes, and antibodies against viral surface spike proteins (153). It is believed that in the near future, organoids might be exploited in research and development of tumor vaccines (140, 142, 147, 149). PDOs with target gene mutations and autologous or non-autologous immune cells are cocultured. The constructed tumor vaccine candidates are subjected to the coculture system to detect tumor-specific T-cell production and tumor vaccine (145, 148, 154, 155). This emerging platform would be applied to the development of tumor vaccines on a large scale (Figure 3) (3, 36, 37, 65). Tumor organoids provide attractive options for tumor vaccine and are expected to be a unique utensil for tumor precision medicine (146, 153, 156).

Application of Organoids in Tumor Chemoprevention and Nanomedicine

Tumor chemoprevention generally refers to inhibition of tumor and vascular cell proliferation, which is expected to maintain its efficacy through the whole stage of chemotherapy for patients (157). It is required to develop advanced approaches for better tumor chemoprevention. The organoid model, a three-dimensional culture platform for primary cells, paves the way of evolution for tumor chemoprevention and provides a practical tool for tumor treatment. A study demonstrated that an A Disintegrin and Metalloprotease 10 inhibitor prevented glioma stem cells from integrating into brain organoids, similar to the mouse xenotransplantation outcome. Temozolomide and Adriamycin treatment reduced the size of tumor organoids by about 30% and 80%, respectively, but had no effect on normal nerve cell organoids. It is worth evaluating the reactivity of glioblastoma multiforme tumor and healthy brain cells when exposed to therapeutics by using the organoid system. They confirmed the biological correlation between organoids and clinical data, providing a basis for high-throughput drug screening and determining the most effective drug timely (158). One additional study indicated that the combination of extracellular signal-regulated kinase 1/2 (ERK1/2) inhibition and autophagy process reduced liver metastases in mice with pancreatic ductal adenocarcinoma PDO transplantation. Over the past decade, vitamin D3 has aroused great interest as a chemoprophylactic agent, especially for treating neoplasms from the digestive system (159). A recent comparative study reported that PDOs revealed the homeostatic effect of vitamin D3 on human intestinal mucosa (160). The organoid technique offers a promising preclinical model for evaluating chemotherapeutic efficacy, a more in-depth mechanistic insight into tumor biology and an organoid biobank system for exploring optimal tumor chemoprevention (161).

Current challenges of organoid application in the clinics include low reproducibility and presence of mixed cell populations, limiting strict experimental examination (38, 42, 77). We have described that the combination of biomaterials and organoids may address the abovementioned deficiencies. Astrocytes and neurons were generated in depart, and they were combined with microplates in the desired proportion and size. This approach accelerated maturation of astrocytes and allowed chemical or genetic manipulation of any cell type before coculture (162, 163). However, how to select certain types of cells as targets for transgenic or epigenetic manipulation, drug delivery, or local extracellular modification is still a bench-side issue (158, 159). Nanoparticles generated from polymer or liposomes are utilized as encapsulation carriers for astrocytes and other cells (164). In another innovative application of nanomedicine, iPSC-derived neural cells and the astrocyte neuron coculture system have been applied as a screening platform to evaluate neurotoxicity. Toxicity is
a major risk for nanomedicine; the safety of any nanoparticles needs to be tested prior to application in vivo (165). An alternative way to reduce toxicity is to use exosomes as a source of nanocarriers, which can increase cell type-specific targeting and enhance their functionality by engineering surface antibodies. Verification of the toxicity of nanomaterials in organoids requires much further attention (158, 159, 164).

CHALLENGES AND PROSPECTS OF ORGANOIDS

Organoid technology has made breakthroughs in regenerative medicine and tumor biology. Organoids have a wide range of potential applications, while they still face technical drawbacks. Most of the existing organoids are derived from epithelial cells; non-epithelial-originated organoids are hard to be established and maintained for a long purpose (i.e., primary glioblastoma) (121). Coculture of tumor organoids with immune cells is a predictable model for cell therapy, but the culture condition requires further optimization. Different tumor types and individual patients maintain heterogeneity; it is difficult to accurately simulate about its dynamic adaptation (81). The classical organoid culture system contains non-human animal products, such as ECM and hydrogel (Matrigel or BME); these may bring undiscovered effects to the organoid biology. Further organoid-bioengineering strategy and conditioned medium developed with artificial substrates may solve the abovementioned limitations (166, 167). The organoid technology potentiates more accurate tumor recapitulation than cell lines and the PDX model (20, 168). The organoid culture system is good to predict drug sensitivity, tumor promotion, and tumor vaccination (119, 169). Having the in vivo modeling accessibility and developing application, organoid technology is expected to have a profound impact on both bench and bedside for precision medicine (84, 120).

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AUTHOR CONTRIBUTIONS

ZW, SZ, and XL wrote the draft. ZL, XX, and AH reviewed the article. GC and JK collected data. ZM and YW provided study materials. DX was responsible for review conception, design, and revision. All authors have read and agreed to the published version of the article.

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