Review Article

Research progress of organoids derived from normal tissues

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Abstract: Organoids are self-organizing three-dimensional tissue structures formed from stem cells extracted from healthy or diseased individuals *in vitro*. Organoid technology plays an important role in disease modeling, tissue development research, drug testing and regenerative medicine. This article reviews the development history, culture methods, classification, application, discussion, conclusions and prospects of organoids derived from normal tissues.

Keywords: Organoids; stem cells; regenerative medicine; three-dimensional culture

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Introduction

Organoids are self-organizing three-dimensional tissue structures formed from stem cells extracted from healthy or diseased individuals *in vitro* (1), and they simulate the key structural and functional characteristics of the corresponding organs in the body (2,3). Organoid technology plays an important role in disease modeling, tissue development research, drug testing and regenerative medicine. Organoids can be divided into organoids derived from normal tissues and organoids derived from cancer or precancerous lesions. This paper mainly introduces the organoids derived from normal tissues.

A brief history of development and culture methods of normal tissue-derived organoids

A brief history of development

The first popularity of the word "organoids" was in the years 1965–1985, represented by the increase of the PubMed search word "organoids" (3). Organoids were mainly used in classical developmental biology experiments that describe organogenesis through cell dissociation and regrouping experiments (2). Li *et al.* (4) found that mammalian cells have the potential to differentiate into their own tissues through the study of mouse mammary epithelial cells. Sato *et al.* (5) successfully established the first three-dimensional organoid model derived from mouse intestinal stem cells in 2009. The establishment of the first organoid had a cross-era significance and opened a new chapter in the history of organoids. In the past ten years, the innovative three-dimensional model of organoid has sprung up rapidly, and it is becoming more and more common among researchers (6).

Culture methods

There are currently three general methods for generating organoids *in vitro*, including: (I) organoids are produced on extracellular matrix (such as Matrigel) scaffolds (5); (II) using a rotating bioreactor to agitate the embryoid body (EB) to produce organoids (7); (III) organoids are formed by air-liquid interface culture (8).

Classification of normal tissue-derived organoids

Normal tissue-derived organoids take advantage of the unlimited expansion of normal stem cells in culture (3). They originate from two main types of stem cells, namely

adult stem cells (ASCs) and embryonic stem cells (ESCs) and their induced pluripotent stem cells (iPSCs) (3). Pluripotent stem cells (PSCs) include ESCs and iPSCs (9-11). Normal tissue-derived organoids include ASCs-derived organoids and PSCs-derived organoids.

ASCs-derived organoids

ASCs are pluripotent undifferentiated cells found throughout adult mammals (12). They have the ability to self-renew and can produce all cell types of specific tissues, which ensures the replacement of apoptotic cells and regeneration of damaged tissues (12).

The first establishment of a mouse intestinal organoid model in 2009 was a major technological advance in the field of organoids derived from ASCs (5). In this study, a single $Lgr5^+$ stem cell was isolated from mouse intestinal epithelium and cultured in a simple three-dimensional culture system with Matrigel as the extracellular matrix (5). In order to maintain the self-renewal, proliferation and differentiation of stem cell populations, some key growth factors (such as WNT, Noggin, R-respondin, and EGF) were also added (5). These stem cells were later found to form a continuously expanding crypt-villi structure that exhibited a near-physiological epithelial structure similar to the normal intestine of mice (5). Dekkers *et al.* (13) then used a similar culture system to generate human small intestine organoids. On this basis, $Lgr5^+$ stem cells from other organs were later found to be able to form different organoids, including gastric organoids (14), colonic organoids (15), pancreatic organoids (16), and liver organoids (17). In addition, ASCs-derived organoid technology is also applied to multiple organs, such as the esophagus (18), tongue (19), lung (20), taste buds (21), salivary glands (22), prostate (23), breast (24), and gallbladder (25).

PSCs-derived organoids

PSCs are capable of infinite proliferation, self-renewal, and differentiation into almost all cell types in the organism (26). PSCs include ESCs and iPSCs. ESCs are derived from totipotent cells of early mammalian embryos,and they have the ability to differentiate into multiple germ layers, and they can proliferate infinitely *in vitro* (10). As a major breakthrough in biomedical research, iPSCs were discovered by Takahashi and Yamanaka in 2006 (11). This study successfully reprogramed terminally differentiated adult cells into iPSCs by introducing a mixture of nuclear transcription factors (Oct4, Sox2, Klf4, c-myc) (11). The iPSCs were very similar to human embryonic stem cells (hESCs) (11). Compared with ASCs-derived organoids, PSCs-derived organoids are not restricted in the availability of major human tissues (27). The first organoid derived from mouse and human PSCs was cortical tissue, which demonstrated a patterned structure with very high similarity to early cortical development (28). Organoids derived from PSCs have been reported in the stomach (29), intestine (30), liver (31), lung (32), inner ear (33), brain (7), pituitary (34), and kidney (35).

Application of normal tissue-derived organoids

Disease modeling

Organoids are highly similar in shape and function to corresponding individual organs. The organoid disease models have been widely used to study the occurrence and development of diseases. Organoid technology helps study the interactions between gastrointestinal and enteroviruses (36). Pompaiah *et al.* (37) explored the infection mechanism of *Helicobacter pylori* by establishing a gastric organoid disease model derived from ASCs or PSCs. Lancaster *et al.* (7) modeled cerebral organoids to simulate brain development and microcephaly, and proved that neurons in patients' organoids were prematurely differentiated. This defect could help explain the disease phenotype (7).

Organoids can also serve as a platform for the research of certain genetic diseases biology. Retinitis pigmentosa is a hereditary and irreversible disease that is mainly caused by mutations in the *PRGR* gene (38). Deng *et al.* (38) derived a pigmented retinitis organoid with PRGR mutation from iPSCS, and subsequently found defects in photoreceptor cell morphology, localization, transcription profile, and electrophysiological activity.The CRISPR-Cas9-mediated correction of RPGR mutation rescued the structure and electrophysiological characteristics of photoreceptors (38). This study used patient-specific organoids to reproduce the pathogenesis of RPGR and provided theoretical evidence for targeted gene therapy of RPGR mutations (38).

Tissue development research

Organoids can self-organize to reproduce the development of embryos and tissues *in vitro* (39). This model is superior to the traditional two-dimensional cell culture method

in reflecting the functional, structural and geometric characteristics of living tissue (39). McCracken *et al.* (40) derived gastric organoids from human pluripotent stem cells (hPSCs). The study found that the development of gastric organoids was highly similar to that of gastric organs in the body, and they also went through a series of key stages such as endoderm induction and foregut formation (40). Chen *et al.* (41) derived lung bud organoids containing mesoderm and pulmonary endoderm from hPSCs and differentiated into branched airways and early alveolar structures after xenotransplantation and three-dimensional culture of Matrigel. Their expression analysis and structural characteristics showed that these branched structures had reached the level of the second trimester of human pregnancy (41). Miller *et al.* (42) implanted hPSCs-derived three-dimensional organoids with human lung bud tip-like domains into the airways of immunocompromised adult mice. HPSCs-derived epithelial bud tip-like structures survived in the mouse airways for 6 weeks and showed the potential to differentiate into multiple proximal lineages, including differentiation into mucus-producing cells, ciliated cells, and neuroendocrine cells. Zhang *et al.* (43)used hPSCs-derived basal progenitor cells for three-dimensional culture *in vitro* to reproduce the normal development of esophageal stratified squamous epithelium and revealed the key role of Notch signal. Camp *et al.* (44) found that the expression levels of most genes in cerebral organoids were similar to those in human fetal cerebral cortex. Wang *et al.* (45) developed a brain organoid-on-a-chip system derived from hiPSCs to simulate fetal brain development in early pregnancy after exposure to nicotine. The brain organoid showed clear key characteristics in the early stages of brain development, such as neurodifferentiation, regionalization and cortical tissue (45). When it was exposed to nicotine, it showed premature neuronal differentiation and its axonal growth showed abnormal neuronal differentiation and migration (45). These results suggested that nicotine exposure can lead to neurogenesis damage in fetal brain development in the early stage of pregnancy (45).

Drug testing

In many cases, factors such as hepatotoxicity and cardiotoxicity of drugs may adversely affect human organs (46). Reliable cell technology is needed to simulate the function of human physiological organs *in vitro* to improve the safety, repeatability and efficacy of the test platform (47), thus promoting the development of organoid technology. Organoids derived from normal tissues play an important role in testing the toxicity and efficacy of drugs.

Wang *et al.* (48) proposed a new strategy to construct hiPSCs-derived liver organoids in a three-dimensional perfusion chip system by combining stem cell biology and microengineering technology, and then established a liver organoid-on-a-chip system. Under perfusion culture conditions, liver organoids showed improved cell viability and higher expression of endoderm genes (SOX17 and FOXA2) and mature liver genes (ALB and CYP3A4) (48). When liver organoids were exposed to acetaminophen, they showed dose-dependent and time-dependent hepatotoxicity (48). Eder *et al.* (49) cultured cardiomyocytes derived from hiPSCs into three-dimensional cardiac tissue and applied them to preclinical drug screening. Takasato *et al.* (50) derived kidney organoids through hiPSCs. When the kidney organoids were treated with cisplatin, their proximal convoluted tubules showed cytophagic dextran and differential apoptosis (50). Kitsuka *et al.* (51) derived cardiac organoids from hiPSCs, and used cardiac organoids to test a newly synthesized adenosine analog, COA-Cl. It was found that COA-Cl can inhibit phosphodiesterase and increase the contraction of cardiac organoids (51). It was concluded that COA-Cl might be used as a cardiotonic agent in the future (51).

Regenerative medicine

The purpose of modern regenerative medicine is to replace diseased tissue with corresponding healthy tissue through allotransplantation. The insufficient supply of healthy donor tissue and the inherent immunological rejection pose a challenge to the long-term survival and function of transplanted tissues in recipients. Organoid technology enables researchers to obtain isogenic or human lymphocyte antigen-matched organoids from few easily accessible or biopsied tissues of patients (6). Relevant research on normal tissue-derived organoids in regenerative medicine has been carried out and many meaningful results have been obtained.

Injury models have been used in a variety of organ systems to enable primary tissue-derived or hPSCs-derived organoids to be successfully implanted into physiologically related parts of adult mice (39,52). Sugimoto *et al.* (39) successfully transplanted human primary tissue-derived colonic epithelial organoids into the colon of mice with endogenous epithelial damage. Sampaziotis *et al.* (52) implanted organoids derived from primary tissues of extrahepatic bile duct into the injured extrahepatic bile

duct and gallbladder of mice, and found that the damaged gallbladder wall and biliary epithelium were successfully repaired. Voges *et al.* (53) derived cardiac organoids from hESCs. After freeze injury with dry ice probes, cardiac organoids showed an endogenous regenerative response and complete recovery after 2 weeks of acute injury (53). Cardiac organoids were similar to fetal cardiac tissues, and this study showed that immature human cardiac tissue has inherent regenerative capacity (53).

Nie *et al.* (54) obtained high-purity endothelial cells and mesenchymal cells from the umbilical cord of a single donor, and then used endothelial cells to generate hiPSCs. HiPSCs could be further differentiated into endoderm (54). Single donor cell-derived liver organoids (SDC-LOs) were synthesized by endoderm, endothelial cells and mesenchymal cells (54). After SDC-LOs were transplanted into mice with liver failure, the liver function of mice recovered rapidly and their survival rate were significantly improved (54). Lenti *et al.* (55) used lymph node stromal progenitor cells and acellular extracellular matrix scaffolds to generate functional lympho-organoids (LOs). In order to evaluate whether LOs play a role in lymphatic reflux, a surgical anatomical model of axillary/brachial lymphadenectomy in adult mouse was used to simulate lymphadenectomy during cancer operation, which contributed to the development of lymphedema, followed by LOs transplantation (55). Two months later, lymphatic drainage was evaluated by measuring near-infrared (NIR) fluorescence imaging of the axillary area of LOs transplantation, and it was found that lymphatic drainage and perfusion were effectively restored (55).

Discussion

As the most complicated organ of the human body, the study of its related diseases is more complicated than other organ diseases. HPSCs-derived brain organoids offer unprecedented opportunities to simulate human brain development and diseases. Ischemic stroke, as one of the classic diseases originating in the brain, lacks effective treatment options. At present, brain organoids are only used for the research on the pathogenesis of neurological and neuropsychiatric diseases (56,57). No application of brain organoids has been found to document the treatment of ischemic stroke.

Ischemic stroke, also known as cerebral infarction, is one of the three major diseases leading to death and disability in the world (58). At present, there is no effective treatment in clinic. Ischemic stroke can cause damage and necrosis of local nerve cells, decrease of functional neurons and destruction of axonal network around the ischemic area of the brain, followed by secondary brain injury, resulting in irreversible nerve tissue damage, which limits the self-repair of nerve tissue, and eventually leads to permanent loss of local nerve tissue and brain neurological impairment (59-61). In recent years, some scholars have proposed the use of exogenous neural stem cells (NSCs) transplantation to treat ischemic stroke, and conducted some animal experiments. A large number of studies (62,63) have shown that exogenous NSCs can significantly improve the prognosis of cerebral ischemia animals and reduce the infarct volume of animal brain tissues without obvious safety problems. Due to ethical, curative, and safety issues, exogenous NSCs transplantation for the treatment of ischemic stroke is currently almost impossible to apply to the clinic. Kalladka *et al.* (64) completed the world's first clinical trial of exogenous NSCs transplantation. The same type of exogenous NSCs labeled CTX0E03 were injected into 11 patients with ischemic stroke. After follow-up for 2 years, it was found that the neurological functions of the patients with exogenous NSCs transplantation were significantly improved without related adverse reactions (64).

Mansour *et al.* (65) successfully transplanted hPSCs-derived brain organoid into the brain of adult mouse to provide an *in vivo* model of vascularized and functional brain organoid. Organoid grafts showed progressive neuronal differentiation and maturation, glial production, microglia integration, and axons growing to multiple regions of the host brain (65). *In vivo* two-photon imaging showed functional neuron networks and blood vessels in the grafts (65). Finally, extracellular recordings *in vivo* combined with optogenetics showed neuronal activity in the graft and suggested functional synaptic connections between the graft and the host (65). Brain organoids are derived from Embryoid bodies (EBs) that lack the formation of blood vessels, which limits their development and maturation (66). Wang *et al.* (45) found that brain organoids derived from hiPSCs were equivalent to the development of fetuses in early pregnancy.

The above results suggest the feasibility of transplanting human brain organoids into the brains of patients with ischemic stroke. Transplanted human brain organoids may establish neural and vascular connections in patients' damaged brain areas. However, the human brain is different from the mouse brain and is much more complex than brain organoid that is only equivalent to the level of early fetal

development. Therefore, transplantation of brain organoids into human brain injury areas may not have a therapeutic effect. At the same time, considering the problems of ethics, efficacy and safety, it is difficult to treat ischemic stroke by transplanting human brain organoids into the patient's brains. With the advancement of organoid technology and the improvement of safety, the brain organoid technology may be applied in the clinical diagnosis and treatment of ischemic stroke in the future.

Conclusions and prospects

Normal tissue-derived organoids are significantly better than two-dimensional tissue models in simulating the three-dimensional structure, cell composition type, and specific functions of real organs, and they are not restricted by tissue accessibility. Normal tissue-derived organoids have achieved initial results in disease modeling and regenerative medicine, drug testing and so on. Normal tissue-derived organoids show great potential and broad prospects, and their value in the field of biomedicine is inestimable. At the same time, there are many limitations and challenges in normal tissue-derived organoids. In many current studies (45,53), the degree of organoid maturity is low, and its development level is only equivalent to the fetal level. Therefore, it can only simulate the initial development process of the organ and cannot study the complex structure of the organ after maturity. EBs derived from iPSC lacks angiogenesis, which limits the growth and maturation of embryoid body-derived organoids (e.g., brain organoids) (66). At present, the research of organoids on regenerative medicine only stays in the animal experimental stage, and there is still a long way to go before it can be applied to the clinic. The development direction of future organoids includes (8): we should focus on regulating the self-organizing processes of organoids to generate physiologically related shapes and sizes of organoids in a stable and accurate manner; to create mature and functional tissues by prolonging the lifespan of organoids; to carry out pathological multi-factor analysis by adding some important tissues of other organs from the same individual. It is believed that organoid technology will continue to improve and will be applied to the clinic in the near future, bringing the good news to humans.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/dmr.2019.12.04). CZ serves as an unpaid Associate Editor-in-Chief of *Digestive Medicine Research*. The other authors have no conflicts of interest to declare.

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