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hiPSC-Based Tissue Organoid Regeneration

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Abstract

Induced pluripotent stem cells (iPSCs) are generated from terminally differentiated cells and have the potential to differentiate to any organs originated from the embryonic germ layers. Extensive effort has been made to establish protocols for direct *in vitro* conversion of human iPSCs (hiPSCs) to different cell types/organs. Importantly, hiPSCs can be generated from patients with known genetic mutations that predispose to high-risks of specific disease development. Thus, the hiPSCs technology provides unlimited resources for creating patient-specific disease models. hiPSC-derived three-dimensional “organoid” models have recently emerged as a powerful tool to recapitulate the physiologically-relevant process of disease progression *in vitro*. In this chapter, we will discuss the current advancement of organoid regeneration from hiPSCs and the applications of hiPSCs-derived organoids. The limitations and challenges of this approach will also be discussed here.

Keywords: disease modeling, induced pluripotent stem cells, organoid, organ-on-chip, tissue regeneration

1. Introduction

Induced pluripotent stem cells (iPSCs), generated directly from terminally differentiated cells [1], can differentiate toward all three embryonic germ layers - ectoderm, mesoderm, and endoderm. iPSCs can give rise to diverse cell types such as neurons, cardiomyocytes, and hepatocytes under defined conditions [2–4], and thus may provide a useful tool for studying human organ development. Human iPSCs (hiPSCs) also open new avenues for patient-specific or personalized disease modeling and therapies [5]. In the following sections, we will summarize the current advances in hiPSC-derived organoid differentiation and discuss the applications of these hiPSC-derived organoids in pre-clinical and clinical areas.

2. Generation of hiPSC

2.1. Protocols for generating hiPSCs

Dr. Yamanaka first reported the generation of hiPSCs from fibroblasts using four transcriptional factors (POU5F1, SOX2, KLF4, and MYC) [6]. There are many protocols to further improve the original method. The first improvement was to minimizing the integration risks such as using non-integrating adenoviral vectors, transfection of mRNA, and using cell-penetrating peptide-tagged reprogramming factors [7]. Transgene-free hiPSC generation protocols have been published by multiple groups [8]. Using small molecules such as valproic acid, sodium butyrate, PD0325901, and others to create iPSCs has been reported [9–11]. Haase et al. reported a new non-transgenic protocol to generate hiPSCs from patient cord blood CD34+ cells using CytoTune™ Sendai reprogramming vectors under the exclusive usage of animal-derived component-free (ADCF) materials and components [12]. Recently, non-integrative and non-viral mRNA reprogramming technology has been reported for hiPSC generation [13]. Rapid, efficient, and safe strategies which are compliant with standard Good Manufacturing Practice (GMP) regulations pave the way for hiPSC clinical applications.

2.2. Genome editing of hiPSCs

Genome editing in hiPSCs provides a valuable tool for disease modeling, mechanism study, and gene therapy. A line of technology utilizing engineered nucleases consisting of sequence-specific DNA-binding domains attached to a non-specific DNA nuclease have been developed. These cutting-edge technologies allow researchers to manipulate entire genomes, including specific genes, intergenic regions, promoters, enhancers, silencers, and insulators. After zinc finger nucleases (ZFNs, first-generation) and transcription activator-like effector nucleases (TALENs, second-generation), the clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) technology is the third-generation editing tool. Despite the difference in the nucleases, the common mechanisms involve inducing DNA double-strand breaks (DSBs) in targeted DNA. Compared to TALEN and ZFN, CRISPR/Cas9 has become the system of choice because of its features such as high feasibility, high affordability, and precise targeting.

3. hiPSC-based tissue regeneration

hiPSC-derived organoids are valuable resources and tools for disease modeling, organ development research, and therapy screening. The current established hiPSC-derived organoids are listed in **Table 1** (adapted from Shi et al. [1]).

3.1. Ectoderm-derived tissues

Ectoderm is one of the three germ layers and the most exterior layer in the human embryo. It covers the outside of the embryo. The ectoderm gives rise to the central nervous system

Organoids	Applications	Refs.
iPSC derived organoid model		
Brain organoids	Modeling autism disorder	[14]
	Modeling ALS disease	[15]
	Modeling Parkinson's disease	[16]
	Modeling Zika virus infection	[17]
	Modeling Seckel syndrome	[18]
Brain-region specific organoids	Modeling Zika virus infection and human brain development disease	[19]
Breast organoid	Breast cancer research	[20]
Cystic organoids	Modeling Alagille syndrome, polycystic liver disease and cystic fibrosis	[21]
Fallopian tube organoids	Ovarian cancer research	[22]
Liver bud	Organ-bud transplantation for regenerative medicine	[23]
Lung organoids	Lung development and lung disease modeling	[22, 25]
Pancreas	Pancreatic disease model	[26]
Retinal organoids	Modeling glaucoma	[27]

Table 1. Summary of hiPSC- and ESC-derived organoids, adapted from Shi et al. [1].

(the brain and spinal cord), the peripheral nervous system, the sensory epithelia of the eye, ear, and nose, the epidermis and its appendages (the nails and hair), the mammary glands, the hypophysis, the subcutaneous glands, and the enamel of the teeth (**Figure 1**).

Figure 1. Summary of the organs originated from ectoderm.

3.1.1. Neuronal tissue regeneration

Several protocols have been developed for the *de novo* differentiation of hiPSC into cell types comprising the central nervous system (**Figure 2**). In general, protocols utilize either a monolayer culture condition, in which the neuroectoderm is further pushed toward spinal or cortical fates via neural rosette formation, or a three-dimensional culture system leading to the formation of neural organoids that again possess features of either cortical or spinal cell types. Here, we discuss the critical components of cortical and spinal organoid differentiation protocols.

Successful formation of a cortical organoid depends upon the appropriate temporal- and regional-specific expression of several proteins and transcripts. The first method of this differentiation was presented by Lancaster et al. [28] in which the group relied upon intrinsic

Figure 2. Specification of iPSC-derived neural tissue and exogenous factors used for derivation of organoids from iPS cells. Arrows indicate the temporal flow of tissue lineage as described by human development. Colored text denotes the small molecules that have been used to derive organoid models of each tissue (in gray) from iPSC spheroid or EB culture. Brain regions: CTX=Cortex and PNS: Peripheral nervous system. Growth factor and small molecules: BDNF, Brain-Derived Neurotrophic Factor; CNTF, Ciliary Neurotrophic Factor; CycA, Cyclophilin A; DSM, Dorsomorphin; EGF, Epidermal Growth Factor; FGF, Fibroblast Growth Factor; GDNF, Glial Cell-Derived Neurotrophic Factor; IWP2, WNT inhibitor; LDN, LDN-193189; NT3, Neurotrophin-3; SAG, Smoothened Agonist; SB, SB431542; SDF, Stromal cell-derived factor; SHH, Sonic Hedgehog; PMP, Purmorphamine (Adapted from Lullo and Krigstein [29]).

self-organization, a droplet of gelatinous matrix, and spinning bioreactors to drive neural-specific embryoid bodies toward developing cerebral structures.

Based on this initial protocol, several groups have published tangential methods that have improved the cortical organoid model. Pasca et al. [30] developed a differentiation method resulting in the generation of exclusively excitatory neurons reminiscent of the dorsal telencephalon, as well as the derivation of non-reactive astrocyte-like cells. Dual SMAD inhibition has been used to induce neural-ectoderm differentiation in suspension, which results in high-efficiency temporal and spatial organization of forebrain organoids [19, 30, 31]. Groups have also implemented transcriptional profiling to investigate the cell type composition of the mature organoid and to compare the overall maturity to that of the developing human brain [19, 30].

One of the most critical features for defining or characterizing a neuron from iPSCs is the neuron's function and physiology. Many groups have assayed the physiology of the developed organoids, implementing calcium imaging or patch-clamp physiology to describe spontaneous activity and expression of specific neurotransmitter receptors [19, 28, 30]. With the addition of an exogenous matrix built of hydrogel, another model of cerebral organoids could reach a larger size over a shorter duration in culture and express the oligodendroglial-like marker Olig2 [32].

In addition to modeling the brain and its specific regions, neural organoids have also been developed for modeling the immature spinal cord and motor nerve units [33, 34]. Experimentation with different extracellular matrix components and rigidity led to the dorsal-ventral patterning of neural cysts within nine days of culture [33], resulting in the immature modeling of the human spinal cord. Another group described a method for generating motor nerve organoids that developed a polarized axon fascicle [34]. Although the above protocols have their limitations, it is evident that organoid technology is rapidly moving toward the goal of forming nervous systems.

3.1.2. Non-neural tissue regeneration

3.1.2.1. Skin

Skin is the largest organ of the body and provides a barrier to protect the interior from the external environment. Human skin is the first barrier system that is vital for homeostasis. Protocols using hiPSCs to generate human skin or skin components have been developed. Regeneration of human skin typically composed of 2D and 3D methods. Keratinocytes are major epithelial components in the skin. Researchers have developed protocols using defined medium and chemical/cytokines generate keratinocyte them. Itoh et al. used a combination of retinoic acid and bone morphogenetic protein (BMP) 4 to induce ectoderm epithelial cell differentiation from the patient-derived hiPSCs [35]. The same group later generated hiPSC-derived dermal fibroblasts, together with keratinocytes, to build 3D skin equivalents using an air-liquid interface culture [36]. The skin contains not only keratinocytes and fibroblasts, but also other skin appendages (eg. sweat glands, sebaceous glands, and hair follicle) and cells from different germ layer origins (fat cells, neurons, immune cells, muscles, blood vessels, and melanocytes. So far, the *in vitro* differentiation protocols have only been successful in developing dermal fibroblasts, keratinocytes, and melanocytes [37, 38]. Creating a full layer of skin tissue is still unfulfilled (**Figure 3**).

Figure 3. The hiPSC-based regeneration strategy for a full-layered human skin.

3.1.2.2. Mammary gland

Mammary epithelial cells originate from non-neural/surface ectoderm cells, which co-exist with neural ectoderm cells at the same embryonic stage. Although mouse mammary gland development has been well studied, the human breast development is still poorly understood due to numerous differences between the mammary glands of the two species. In addition, questions regarding human mammary stem cell identity, mammary epithelial differentiation hierarchy, and the effects of ovarian hormones on mammary development are major obstacles for *in vitro* mammary gland regeneration.

Taking a cue from the understanding of human embryonic mammary gland development [39, 40], Qu et al. conceptualized that the first step for *in vitro* induction of mammary differentiation from hiPSCs was to pattern hiPSCs into non-neural ectoderm, thus enriching mammary progenitors. The group developed a reliable two-step protocol to generate human mammary-like organoids from hiPSCs [20]. These organoids express luminal, basal, and breast-specific markers. Despite these novel findings, this *in vitro* system needs to be improved to fully recapitulate the formation of mammary ductal and alveolar structures.

3.2. Mesoderm-derived tissues

The mesoderm is formed through a process called gastrulation around the third week of embryonic development. Initially, mesoderm is segmented into three crucial compartments; the paraxial mesoderm (PM), the intermediate mesoderm (IM), and the lateral plate mesoderm (LPM) (**Figure 4**). The PM, also known as presomitic or somitic mesoderm, gives rise to embryonic structures of the sclerotome, myotome, and dermomyotome, which later develop into many

Figure 4. Mesoderm subdivision and mesoderm-originated tissue development.

adult tissues, including most of the axial skeleton, skeletal muscles, and connective tissues of the skin. The IM, which lies between the PM and LPM, differentiates into the urogenital duct system and gives rise to the kidneys, gonads, Wolffian (male) or Müllerian (female) ducts, fallopian tube, uterus, and the adrenal glands. The LPM is located on the side of the IM and is split horizontally into two layers: splanchnic mesoderm and somatic mesoderm. These layers contribute to the formation of the heart, blood vessels, and blood cells as well as to the connective tissue of the limbs and the space between these layers develop into the body cavity. This section summarizes the current hiPSC-derived 3D organoid differentiation research for tissues of mesodermal origin.

3.2.1. Kidney

Adult humans have a limited number of nephrons which do not increase during life but rather decay with age through attrition or disease. Currently, there is no known treatment accessible for nephron renewal in patients with chronic or end-stage kidney disease. Recent hiPSC-based tissue regeneration studies have provided the novel sources for nephron progenitor cell (NPC) production and potentially kidney regeneration. This section summarizes the current protocols to generate and maintain NPCs and 3D human kidney organoids.

The kidney is derived from IM which forms a urogenital ridge on either side of the aorta. Current multistep-directed differentiation methods are intended to recapitulate these crucial stages of renal embryonic development. Studies for the critical transcriptional regulation process and signaling pathways contribute to the better understanding of each stage of renal development (**Figure 5**). Importantly, these studies enable us to recognize the factors that direct cell fate decisions and have been the basis for establishing the current differentiation protocols and culture conditions [41, 42].

hiPSC-derived 3D human kidney organoids that led to the generation of more complex and partially self-organizing organoids [43–46] have been established (**Figure 6**). Takasato et al. [43, 44]

Figure 5. Schematic representation of the differentiation stages into NPCs and kidney organoids and markers that identify each stage. GATA3, GATA Binding Protein 3; LAM, laminin; LHX1, LIM homeobox 1; LPM, lateral plate mesoderm; MIXL, Mix Paired-Like Homeobox; OCT4, POU class 5 homeobox1; OSR1, odd-skipped related transcription factor 1; PAX2, paired box 2; PAX8, paired box 8; SALL1, spalt-like transcription factor 1; SIX2, SIX homeobox 2; SOX2, SRY-box 2; WT1, Wilms tumor 1.

Figure 6. Protocols for the directed differentiation of hiPSC into kidney organoid. The timescale is shown at the top (shortened after 20 days) and Day 0 marks the hiPSC stage. Oval images represent the obtained cell types-like in each stage, namely: Ep, epiblast; LPS, late primitive streak; NM, nascent mesoderm; PNM, posterior nascent mesoderm; PIM, posterior intermediate mesoderm; UE, ureteric epithelium; PA, pre-tubular aggregate; RV, renal vesicle. Growth factors and small molecules; BMP4, Bone Morphogenetic Protein bone 4; CHIR, CHIR99021; FGF, Fibroblast Growth Factor; RA, Retinoic Acid; VitD3, vitamin D3; WNT3A, Wingless Type Family member 3A; mTeSR1, defined iPSC medium and B27-Serum free cell culture supplements. Figure adapted from (Jacqueline Kai et al. [47]).

reported the generation of self-organizing kidney organoids using CHIR99021 and fibroblast growth factor (FGF) 9/heparin in a monolayer culture followed by cell pelleting. The transcriptional profiling of resulting organoids exhibits significant similarity to the first-trimester embryonic human kidney.

On the other hand, Morizane et al. [45] have patterned mesoderm into PM, IM, and LPMs and generated NPCs by mediating graded signals of GSK-3 β inhibitor CHIR99021, Noggin, and Activin A. In this study, renal vesicle was formed by transiently treating the NPCs with the CHIR99021 and FGF9, following self-organizing differentiation into podocytes, proximal tubules, loop of Henle, and distal tubules in both 2D and 3D culture. Alternatively, a shorter

and more straightforward protocol has been developed by the group of Bonventre and collaborators. Bonventre group *et al.* demonstrated the formation of nephron-like structures as well as endothelial-like cells that were arranged into cords and expressed the endothelial markers CD31 and von Willebrand factor [46].

3.2.2. Cardiomyocytes

Cardiovascular disease remains the leading cause of death worldwide. It encompasses an extensive range of clinical conditions due to genetics, physiologic and metabolic circumstances as well as drug toxicity. Most heart diseases are associated with severe damage to, or loss of, cardiomyocytes (CMs), and mammalian CMs have a limited regenerative capacity [48]. The recent advancements in the field of hiPSC-derived CMs (hiPSC-CM) offer unique opportunities for not only disease modeling and personalized drug efficacy/toxicity screening but also for stem cell-based cardiac regenerative therapy [49–51].

CMs arise from mesoderm, which is further specified into cardiac mesoderm and cardiac progenitor cells by three families of extracellular signaling molecules: WNT, FGF and TGF β superfamily ligands (WNT3a, bone BMP4, Nodal and Activin A). The expression of these ligands in a spatiotemporal manner defines the mesodermal cell fate and prime CM differentiation [52, 53]. Several groups successfully mimic these signaling processes *in vitro* to generate hiPSC-CM, which is summarized in **Table 2** (adapted from Smith et al. [54] and Burrridge et al. [55]).

Method	Culture condition	Differentiation format	Mesoderm induction	Cardiac specification factors	Cardiac Differentiation factors	Ref.
Suspension EB in StemPro34	Knock-out serum Replacement (KSR)/FGF2	StemPro 34	Activin A, BMP4	VEGFA, DKK1	VEGFA, FGF2	[56]
			FGF2	VEGFA, DKK1, SB431542		[57]
				Dorsomorphin		
Monolayer Differentiation	Monolayer on Matrigel with MEF	RPM1 plus B27	Activin A, BMP4	IWR1	Tri-iodothyroline	[58]
			Activin A, BMP4, FGF2	RPM1 plus B27	RPM1 plus B27	[59]
			Activin A, BMP4, FGF2	Noggin, RAi, DKK1	DKK1	[60]
	(KSR)/FGF2 on MEF	RPM1 plus B27(–insulin)	Activin A, BMP4, FGF2	VEGFA, DKK1	VEGFA, FGF2	[61]
		LI-APEL	Activin A, BMP4, FGF2, VEGFA, SCF	LI-BEL	LI-BEL	[62]
		mTeSR	Activin A, BMP4	IWR1 or IWP4	RPM1 plus B27	[63]
	Chemically defined E8	RPM1 plus B27(–insulin)	CHIR	IWR1 or IWP4	RPM1 plus B27	
		CDM3	CHIR/WNTC59	CDM3	CDM3	[65]

Table 2. Methods for hiPSC-CM differentiations (adapted from Smith et al. [54] and Burrridge et al. [55]).

The first generation of CM differentiation was established using ESCs and successfully applied to hiPSCs. While the traditional embryoid body protocol engaged serum-derived spontaneous differentiation into CM, its efficiency was very low, (~1–5%). The second generation of CM differentiation aimed to recapitulate the embryonic developmental sequences *in vitro* by modulating stage-specific activation/inhibition of signaling pathways with recombinant protein, details described in **Table 2**. At the molecular level, each stage of iPSC-CM differentiation is characterized by sequential expression of specific sets of genes [53]. These protocols were much more efficient; however, they were expensive and exhibited high batch-to-batch variation.

The third-generation hiPSC-CM protocol is composed of sequential modulation of the Wnt signaling pathway: activation at an early stage with small molecules such as CHIR-99021 and then inhibition at a late stage with small molecules such as IWP2 [64–66]. These monolayer-based directed differentiation protocols generate CMs with high efficiency. On the other hand, maturation of these CMs became a major challenge for the use of *de novo* CMs in heart research, especially for disease modeling and drug testing [67, 68]. Thus, several studies used prolonged cell culture, electrical stimulation, mechanical stretch or hormonal stimulations to induce CM maturity [69–72].

hiPSC-CM technology has transformed the field of cardiovascular research, especially the study of inherited and acquired cardiovascular diseases. Several heart diseases including long QT syndromes, catecholaminergic polymorphic ventricular tachycardia, and familial hypertrophic cardiomyopathy have been modeled using hiPSC-CM [73–75]. Patient-specific CM regeneration may hold the promise for stem cell-based cardiac therapy.

3.2.3. Fallopian tube

Ovarian cancer is the leading cause of gynecologic cancer-related deaths in the United States. Fallopian tube epithelia (FTE) has been identified as the origin of ovarian cancer [76]. The discovery of serous tubal intraepithelial carcinoma (STIC) lesions, a preneoplastic finding in the fallopian tube fimbriae of patients with BRCA mutations, supports the model of FTE origin of serous “ovarian” carcinoma [77].

Yucer et al. [22] developed a hiPSC-derived 3D human FTE model, mimicking the FTE development process via various intermediate stages toward mature FTE in 3D organoid culture. Female reproductive tract structures including fallopian tube epithelium arise from the Müllerian duct in parallel to the urinary system from IM of the urogenital ridge in the posterior primitive streak. Therefore, Yucer et al. [22] recapitulated Müllerian development starting with IM generation and further developed into fallopian tube epithelial precursors using pro-Müllerian growth factors. Each step of this differentiation is monitored through the expression of established markers (**Figure 7**). Further differentiation of the fallopian tube epithelial lineage was attained on a 3D growth platform, which enables the FTE organoid to self-organize into a convoluted luminal structure with secretory and ciliated cellular components [22].

Figure 7. Schematic of iPSC derive FTE organoid model. The stepwise differentiation of FTE via various intermediate stages which are characterized by specific molecular signatures. BMP4, Bone Morphogenetic Protein bone 4; CHIR, CHIR99021; E2, Estrogen; P4, Progesterone; WNT, Wingless Type Family member.

hiPSC-derived 3D-FTE organoids model offers a faithful *in vitro* platform to investigate the fallopian tube origin of ovarian cancer and to explore early cancer pathogenesis and progression. This platform can also be used to study high-risk germline mutations including BRCA1/2, to identify the molecular signature and genetic alteration involved in carcinogenesis and ultimately uncover novel drug targets.

3.3. Endoderm-derived tissues

The endoderm gives rise to the epithelial lining of the gastrointestinal and respiratory tracts; the parenchyma of the tonsils, the liver, the thymus, the thyroid, the parathyroids, and the pancreas; the epithelial lining of the urinary bladder and urethra; and the epithelial lining of the tympanic cavity, tympanic antrum, and auditory tube (Figure 8).

Figure 8. Summary of organs originated from endoderm.

3.3.1. Small intestine, stomach, and colon

The small intestinal tissue is composed of a single-layer of epithelial cells which form a lumen that is surrounded by connective tissue. Functionally, the small intestine plays a central role in digestion and absorption of nutrients. There are different cell lineages (enterocytes, goblet cells, Paneth cells, tuft cells and enteroendocrine cells) in the small intestine with various functions such as exocrine, absorption, and protection. While diseases of the small intestine, such as tumor, inflammatory bowel disease (IBD), lactose intolerance are common, the study of these diseases have encountered difficulties due to the limitation of *in vitro* modeling systems.

The protocols used for intestinal organoid differentiation have been published by different groups [78, 79]. In general, hiPSCs are directed to differentiate into definitive endoderm followed by intestinal fate specification and development using Wnt3A and FGF4. Intestinal organoids, usually cultured in Matrigel, show a polarized, columnar epithelium that is patterned into villus-like structures and crypt-like proliferative zones that expresses intestinal stem cell markers. The epithelium contain functional enterocytes, as well as goblet, Paneth and enteroendocrine cells with a layer of mesenchymal cells. Yu et al. [80] reported a refined, non-Matrigel scaffold and 3D intestinal organoid culture protocol. The matrix-free system may improve the yield, decrease the time, and facilitate high-throughput approaches. The protocols used to generate intestines from hiPSCs are summarized in **Figure 9**.

Gastric ulcer and gastric cancer affect 10% of the world’s population and there is no experimental model of the normal human gastric mucosa. The lack of proper models has hindered mechanistic studies, preventive approach testing, and disease modeling. Kyle et al. developed the first protocol directing hiPSCs to 3D gastric organoids by manipulating FGF, WNT, BMP, retinoic acid and EGF signaling pathways [81]. These organoids formed primitive gastric gland- and pit-like domains, proliferative zones containing LGR5-expressing cells, surface and antral mucous cells, and a diversity of gastric endocrine cells.

Figure 9. The protocols used to generate intestinal organoids using hiPSCs.

Recent studies have successfully generated colonic organoids from hiPSCs. Following the similar differentiation path to intestinal organoids, hiPSCs were sequentially differentiated into definitive endoderm, hindgut endoderm, and colonic organoids. The colonic organoid differentiation was conducted in a Matrigel 3D culture. Jorge et al. [82] modified the protocol used for small intestinal organoid differentiation by adding BMP2 in the Matrigel culture stage. BMP signaling can promote posterior fate in human gut tube cultures. Another group [83] reported a different approach by supplementing the inhibitors CHIR99021 and LDN193189, and EGF in the Matrigel culture stage. These hiPSCs-derived colonic organoids exhibit crypt-like structure formed by a polarized epithelium consisting of colon stem cells, goblet, and endocrine cells and a layer of supportive mesodermal tissue.

3.3.2. Lung

The regeneration of lung epithelial cells/organoids has applications in regenerative medicine, modeling of lung disease, drug screening and studies of human lung development. The lung is composed of endoderm-derived epithelial cells surrounded by mesenchymal-derived stromal cells. Lung epithelial cell differentiation follows the path of definitive endoderm to anterior foregut endoderm. Then the Nkx2.1+ endoderm will bud from the ventral side of the anterior foregut to form the primitive lung bud, which will form the respiratory tree. Signals from the mesenchyme to the epithelium are critical in cell specification, determination, and differentiation, and are essential for proper development and maturation of the lung [84].

A number of studies show the differentiation of hiPSCs into lung epithelial cells in 2D [84–88]. However, 3D lung organoid differentiation has become the trend. Briana et al. [24] reported a breakthrough in the stepwise differentiation of human lung organoids from hiPSCs that consist of both epithelial and mesenchymal components. These lung organoids possess upper airway-like epithelium with basal cells and immature ciliated cells surrounded by smooth muscle and myofibroblasts as well as an alveolar-like domain with appropriate cell types. Later, Chen et al. reported the generation of lung bud organoids (LBOs) that contain mesoderm and pulmonary endoderm and develop into branching airway and early alveolar structures after xenotransplantation and Matrigel 3D culture [89].

The application of acellular lung matrices has been reported in 3D lung tissue reconstruction. Decellularized lung matrix supports the culture and lineage commitment of hiPSC-derived lung progenitor cells [90]. The rotating bioreactor was also used to provide an air-liquid interface, which is a potent inducer of type I epithelial differentiation for both hiPSC-Alveolar epithelial type (AT) II and ATI cells [91]. The bioreactor system provides a method for large-scale production of alveolar epithelium for tissue engineering and drug discovery. Another improvement for lung regeneration from hiPSCs is the use of biomaterials [25].

4. Application of iPSC-based tissue regeneration

hiPSC-derived cells or organoids are becoming promising resources for disease modeling and therapeutical applications. In general, somatic cells from patients can be reprogrammed to hiPSCs. In turn, patient-specific hiPSCs can be converted into target organs using established

protocols. These *in vitro* derived organs can be used for multiple purposes, including patient-specific disease modeling, drug testing, therapy screening, and transplantation.

4.1. Personalized disease modeling

The biggest advantage of the hiPSC technology lies in its patient-specific feature. hiPSC-derived 3D organoid models have recently emerged as a powerful tool to recapitulate and investigate the physiologically-relevant process of disease onset and progression *in vitro*. This model system leverages the self-renewal and multi-lineage differentiation capability of multipotent stem cells and their intrinsic self-organization regenerative ability to form 3D tissue architecture. Importantly, hiPSCs can be derived from patients with known hereditary genetic mutations that are associated with a higher risk of a particular disease. This provides a valuable approach to determine whether additional genetic alterations are needed to interact with the known mutations, thereby contributing to disease susceptibility, initiation, and progression [92].

Several hiPSC-derived, inherited human disease models have been used to reproduce cancers associated with those high-risk patients [93, 94]. A hiPSC-derived osteosarcoma model for Li-Fraumeni syndrome has yielded promising results in displaying disease pathogenesis and carcinogenesis events commonly found in relevant human cells [95]. Cystic Fibrosis (CF) is an inherit disease of secretory glands. Among all the organs, pancreas is the earliest and most severely affected organs impacted by CF. hiPSC-derived pancreatic epithelial cells can be used to study personalized CF development [96]. Kyle et al. [81] used hiPSC-derived gastric organoids to model the pathophysiological response of the human stomach to *H. pylori* infection. In addition, Miguel et al. reported using hiPSC-derived colonic organoids to model family APC mutation-associated colon cancer initiation [83]. More and more hiPSCs-based disease models will be established.

4.2. Therapeutic applications

4.2.1. Drug screening

Organoids differentiated from patient-derived hiPSCs can be used to build a screening platform to develop and validate therapeutic approaches. hiPSC-derived organoids have a line of features that make them suitable models. Using a defined protocol, hiPSC-derived organoids become an unlimited resource for a specific patient. The *in vitro* direction of organ differentiation allows the rapid and robust generation of organoids with identical features. Most importantly, the organoids are 3D based mini-tissues that consist of multiple cell types, and that recapitulate the tissue structures *in vivo*. Thus, the drug screening results are more applicable *in vivo*. As an example, hiPSC-based drug screening for Huntington's disease has been established [97] developed. The applications of hiPSCs that have been reprogramed from patients of heritable, genetic diseases has been summarized by Wonhee Suh in a review paper [98].

Biomimetic tissues on a chip have been developed for drug discovery [99]. Organ-on-a-chip is based on microfluidic technology and has been proposed as a novel cell-based assay tool in pre-clinical studies. Furthermore, the concept of body-on-a-chip, which stands for multiple organs connected through microfluid devices, can mimic multiple interactions between organs [100]. Applying hiPSC research to the concept of organ-on-a-chip has provided a promising future for the development of the patient-specific body-on-a-chip [101]. Drug

screening is no longer a process that is limited by the responses of targeted organ, it can also provide an evaluation of systemic responses.

4.2.2. Gene therapy

The nature of the disease and desired genetic modification, efficiency and accuracy of gene repair methodology, as well as cell state will determine the success of gene therapy [102]. In theory, monogenic diseases dictated by a dysfunctional copy of the causative gene would be reversed by introducing a wild-type copy of the gene into cells [103, 104]. Over 80% of rare diseases are considered to have a genetic origin [105], which means the precise gene editing technologies can be practically used to correct these genetic factors. The application of genome editing technologies in therapeutic trials have been reported in many diseases, such as retinal diseases [106], lysosomal storage diseases [107], arthritis [108], and neurological disorders [109, 110]. In contrast, polygenic diseases that require simultaneous multiple alterations of the genome are more challenging to treat with gene therapy [111].

Gang et al. presented a highly efficient and reproducible protocol to edit the genome of hiPSCs through the combined use of the CRISPR/Cas9 RNA-guided nuclease and piggyBac (bacterial artificial chromosome) transposase [112]. Their method can result in efficient, targeted genome editing and concurrent “scarless” transgene excision. Satoru et al. reported using gene editing with engineered site-specific endonuclease technology to treat dominant-negative disorders by targeting only the mutant allele while leaving the normal allele intact [113]. Using precise gene editing technology to correct gene mutations from hiPSCs generated from patients combined with hiPSC differentiation into target cells/organs for transplantation provides an immense promise for the future of gene therapy (**Figure 10**).

Figure 10. The summary of gene therapy applying precise genome editing technology in hiPSCs.

4.2.3. Transplantation

Given that hiPSCs are pluripotent stem cells which can be propagated unlimitedly and protocols for their differentiation into different cells/organoids have been established, hiPSC-derived micro-tissues are a potentially innovative material source for transplantation. In addition, immune rejection will be minimized when essentially returning the hiPSC-derived tissue to the original patient. For mature cells that have no or limited regenerative ability, such as cardiomyocytes, neurons, and pancreatic cells, hiPSC-derived cell/organoids are especially valuable for tissue repair. There are a series of clinical studies evaluating hiPSC-cells/organoids for treatment of neural degeneration, diabetes, heart failure, and retinal cells [114]. Although research on the application of hiPSCs in therapy have shown encouraging progress, there are some concerns involving the safety of hiPSC-based cell transplantation. Tumor risk and acquired gene mutations are major concerns.

5. Future and challenges

The original protocol to generate hiPSCs involves four transcriptional factors, but this method is not suitable because of its effect on genome integrity via the introduction of additional plasmids with exogenous genes. To make hiPSCs and their derivatives applicable for clinical uses, many improvements have been made to optimize the method for iPSC generation. The integration-free and chemical reprogramming protocols have been developed to minimize the risk of jeopardizing genome integrity [115, 116].

In general, the genetic nature of a disease, the molecular editing platform used, the delivery method, and the targeted cells and organs are all factors that influence the efficacy of treatment and determine the likelihood of clinical benefits [117]. The CRISPR/Cas9 molecular scissor system has been used to edit the genomes of a diverse array of mammalian cell types and organisms with high efficiency and precision. Determining and overcoming the actual frequency of off-target activities is challenging, yet critical to the application of the technology in gene therapy. CRISPR/Cas9 technology allows the study of complex genetic diseases, including human cancer, in which multiple mutations and chromosomal translocations are present in the genome [118, 119].

The potential application of hiPSC technology in cancer studies has been proposed, based on the idea of reprogramming cancer cells via hiPSC technology to cancer stem cell (CSC) state. CSCs are well-known as the origin of tumor development, the seeds for distant metastasis, and are critical in therapeutic resistance. Reprogramming the malignant cells back to their original state before the oncogenic transformation occurs [120], may provide tools for exploring the mechanisms of tumor initiation and progression *in vitro*, for studying the heterogeneity and origin of CSCs, and for producing cancer type-specific drug discovery. However, these reprogramming methods remain a challenge because of the cancer-specific epigenetic state and chromosomal aberrations of cancer cells.

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Conflict of interest

All the authors declare no conflict of interest.

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