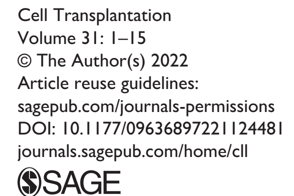
Review (invited)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Modeling Human Organ Development and Diseases With Fetal Tissue–Derived Organoids

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Abstract

Recent advances in human organoid technology have greatly facilitated the study of organ development and pathology. In most cases, these organoids are derived from either pluripotent stem cells or adult stem cells for the modeling of developmental events and tissue homeostasis. However, due to the lack of human fetal tissue references and research model, it is still challenging to capture early developmental changes and underlying mechanisms in human embryonic development. The establishment of fetal tissue–derived organoids in rigorous time points is necessary. Here we provide an overview of the strategies and applications of fetal tissue–derived organoids, mainly focusing on fetal organ development research, developmental defect disease modeling, and organ–organ interaction study. Discussion of the importance of fetal tissue research also highlights the prospects and challenges in this field.

Keywords

organoids, human fetal tissues, organ development, developmental defect diseases

Introduction

Over the past decade, innovations in organoid technology have upgraded *in vitro* biological research from two-dimensional cell lines to three-dimensional (3D) multilineage cell cultures, providing a revolutionary platform for studying human organ development and diseases. In general, organoids are grown from adult tissue stem cells (ASCs) or directionally differentiated pluripotent stem cells (PSCs) through self-organization; thus, their cell type composition, structure, and function are to some extent similar to those of native organs. The two categories of organoids have been summarized by Yoshiki Sasai[1] and Hans Clevers[2], who are the creators of the PSC-derived cortical organoids[3] and the ASC-derived intestinal organoids[4] respectively. Currently, human organoids have been established for a wide range of organs from all three germ layers, according to the reported chronological order including the intestine and colon[5,6], optic cups/retina[7–10], brain[11,12], kidney[13,14], prostate[15,16], gastric[17,18], lung[19–21], liver/pancreas[22], uterus[23], inner ear[24,25], vascular network[26], and heart[27–29], among others. Many subsequent studies have also done a multifaceted optimization on this basis. For example, the long-term expansion of functional liver hepatocyte organoids was achieved by optimizing the culture condition of liver ductal organoids[30,31]; co-culture system of mesenchymal cells and endothelial progenitors was introduced to create vascularized epithelium organoids[32–34]; more specific differentiation guidance was used to generate multiple brain organoids with region-specific identities, including midbrain organoids[35], hypothalamic organoids[36], hippocampal organoids[37], and others.

Of note, not all types of organoids could be derived from ASCs. ASCs from tissues with high cellular turnover, such as intestine[5], colon[6], and lung[20,21], are most likely to be constructed into organoids successfully. They are commonly used to mimic *in vivo* homeostasis of their original tissues. In contrast, for adult tissues with quiescent state or slow physiological turnover, it is quite difficult to establish organoids and recapitulate homeostatic self-renewal and differentiation *in vitro* through ASCs, which limits the application of ASC organoid strategies. Alternatively, organoids could be established by directing PSC differentiation through sequential signaling manipulation. The PSC differentiation strategy

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allows *de novo* acquisition of specifically differentiated cells and avoids the repeated consumption of primary human tissues in the generation of organoids, which facilitates the establishment of organoids that mimic proliferative-restricted tissues, such as brain[11,35,38,39] and heart[27,29]. Therefore, the cooperation of ASC and PSC strategies has resulted in the establishment of organoid models for the vast majority of human tissues and organs.

Several limitations need to be vetted in PSC-derived organoid models. PSC-derived organoids are often defined by the molecular features of their cells and partial tissue-specific functions. However, even if we can determine the cell identities in PSC-derived organoids with lineage markers, more information is needed to support the consistency of various fates and maturation states between PSC-derived organoids and their *in vivo* counterparts, especially the recapitulation of those different physiological states in exact time points. In addition, heterogeneity of pluripotency among PSCs makes it difficult to ensure state equivalence when producing differentiated cells[40]. The presence of off-target cell types after differentiation is another challenge. Therefore, studies have compared organoids derived from PSCs with the age-matched human fetal tissues in the transcriptome for more supporting information. For instance, RNA-seq analysis showed that PSC-derived human gastric organoids shared a very similar transcriptional profile with human fetal stomach tissue, but distinct from human fetal intestine or adult stomach[17]; comparison of *in vitro* and *in vivo* cortical single-cell transcriptomes elucidated the similarity between human cerebral organoids and the fetal neocortex[41]; and transcription profiles showed that PSC-derived kidney organoids have the highest congruence with the first trimester human kidney[42]. There is no doubt that PSC organoids are beneficial to study organogenesis and developmental events that lead to tissue formation. However, the lack of human references and faithful models across actual human early development is still an obstruction for usage of PSC-derived organoids in modeling human fetal organ development. Moreover, studies showed that PSC-derived organoids resemble early fetal-stage tissues, challenging the achievement of an adult tissue stage *in vitro*. This leaves a huge gap that remains to be filled between the organoids derived from ASCs and PSCs.

For a long time, human embryonic and fetal tissues have been used for development and disease research via a variety of techniques, including short-term culture, immunostaining, gene expression profile, and biochemistry, under the permission of medical ethics. Clinically, fetal tissues were initially used for experimental surgery because of their growth ability and low graft rejection, and were once transplanted into the brain of an adult who suffered from Parkinson's disease[43] or Huntington's disease[44]. Although this approach has been questioned for its merits, it laid the foundation for the development of stem cell–based therapies. For fetal tissues, start from any specified organ origins at any definite gestational weeks (GWs); they could be used to study more accurate human developmental events of organogenesis. Importantly, fetal tissues are the gold standard for the validation of newly emerging PSC-based organoids[29,45]. For that, there are no alternatives for fetal tissue, not up to this point.

In recent years, fetal progenitor–derived organoids have become essential models for the study of human organ development and developmental disease because of their unique properties both in multipotent progenitor composition and in architecture of fetal organs. Unlike ASCs and PSCs, cells derived from fetal tissues have undergone specification during development and maintain a proliferative progenitor state, which allows them to expand substantially and further differentiate and mature *in vitro*. Fetal tissue–derived organoids characterize the specificity of tissues and organs at different developmental stages and determine the mechanisms of cell fate transition, which greatly improves the understanding of human organ development. And the knowledge in high confidence level of human organ development also serves as a gold-standard reference for PSC-derived cells and organoids, thus enabling fidelity assessment for *in vitro* models while improving clinical relevance and utility for PSC-derived lineages.

In this Review, we will focus on the investigations conducted through fetal tissue–derived organoid models. To show the superiority of fetal tissues in studying human organ development, we first introduce the applications of fetal tissue–derived organoids in developmental research works of fetal organs. After that, we discuss developmental defect disease models established by fetal tissue–derived organoids, with the ultimate aim for clinical guidance. We then discuss a co-culture system of fetal tissue–derived organoids to mimic interaction between immature organs in the fetal stage, which can provide detailed information for understanding the fetal microenvironment. Finally, we address the importance, challenges, and future developments in this field.

Fetal Tissue–Derived Organoids in Fetal Organ Development Research

During human embryonic development, the fertilized egg undergoes a series of division and differentiation events to form three germ layers (endoderm, mesoderm, and ectoderm), which then form specific cell lineages in organogenesis. These specific cell lineages preserve stem/progenitor properties in fetal life until organ maturation. In postnatal life and adulthood, although tissues have limited self-renewal, small numbers of tissue-specific stem cells are found in differentiated organs. The homeostasis maintenance and damage repair capacity of adult tissues are driven by the proliferation and differentiation of resident stem cells. Because of the accessibility of adult tissues, stem cell studies of human adult organs have been extensively reported[46]. Nevertheless, the evolutionary relationship from fetal progenitors to adult stem cells remains unclear. A recent series of studies have focused

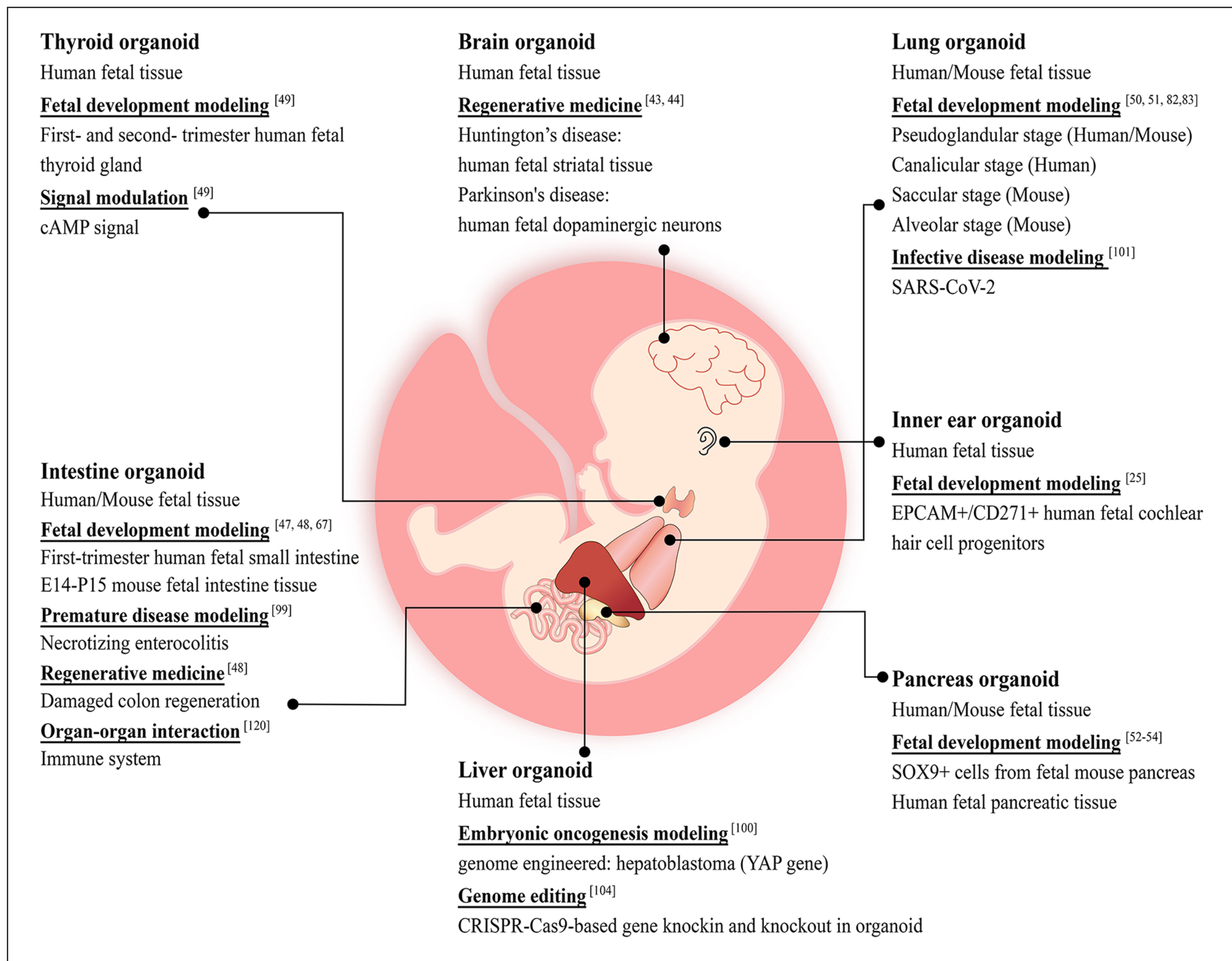

Figure 1. Schematic of various organoids that are grown from fetal tissues. Organoids can be derived from multiple fetal tissues (eg, thyroid, intestine, brain, liver, lung, pancreas, and inner ear). These fetal-derived organoids are widely used in studies of fetal organ development, developmental defect disease modeling, and organ–organ interaction studies. Various applications of tissue-specific organoids are indicated above.

on the organ developmental simulation via organoids derived from fetal progenitor cells of several endoderm tissues (intestine[47,48], thyroid[49], lung[50,51], and pancreas[52–54]) and ectoderm tissues (inner ear[25]), intending to reveal stem cell states exist in fetal stage and to discover the mechanisms of tissue differentiation and maturation (Fig. 1).

Intestine

The intestinal epithelium is the highly cellular turnover tissue in mammals with a tight hierarchical organization to maintain tissue homeostasis. The crypt-villus units constitute the intestinal epithelial structure. Inside the crypt, continuously dividing stem cells give rise to progenitor cells while maintaining their own population simultaneously, by rapidly proliferating and differentiating into absorptive (enterocytes) and secretory (Paneth, goblet, enteroendocrine, and tuft cell) cell types[55].

Signature gene identification greatly improved the understanding of adult intestinal stem cell biology during homeostasis and regeneration. Lgr5 is found to be specifically expressed in crypt stem cells of mouse small intestine by the Clevers group[56]. Lineage tracing in mice showed that $Lgr5^+$ cells are multipotent stem cells that generate all cell types of the epithelium, representing Lgr5 as a genuine marker of intestine[56]. Although lineage tracing can evaluate stem cell population in animal models, the real-time monitoring of stem cells and their descendants is difficult to be applied to human tissues. To break through that bottleneck, an intestinal organoid culture system for monitoring lineage changes of individual cells *in vitro* was first generated using mouse intestinal tissues[4]. Subsequently, organoids are wildly employed in basic biology research of intestine[57–60] and even in exploring regenerative therapeutics. Successful methods have been established for isolating, culturing, and transplanting $Lgr5^+$ colon stem cells from mice[61]. At the fourth week

after transplantation, the transplanted organoids adhered to and covered superficially damaged colon tissue of colitis mice. The transplanted recipient mice had higher body weights than untransplanted controls, implying a functional engraftment of cultured colon organoids.

Although the intestine of mouse and human has similar architecture, cell type composition, and self-renewal dynamics[62], the long-term culture system of adult human intestinal organoid is much more complex[5]. An alternative approach is to generate human intestinal organoid culture from human-induced pluripotent stem cells (hiPSCs). By manipulating critical signalings with defined combination of grow factors, hiPSCs differentiated into intestinal organoids with villus-like structures and crypt-like stem cell zones, containing enterocytes, goblet cells, Paneth cells, and enteroendocrine cells[63]. This culture system is highlighted as a remarkable model to study human intestinal development. By comparing with fetal mouse gut, PSC-derived intestine organoids are concluded to mimic fetal gut development morphologically. Moreover, morphological change from spherical organoids to budding organoids indicates that PSC-derived intestine organoids could undergo enhanced maturation *in vitro* by changing the chemical or physical environment[63,64].

To illustrate the real gut development, time-course changes of original developing intestine need to be investigated. The straightforward approach is to use fetal tissues. Morphologically, the embryonic development of the murine intestine has been well investigated. Briefly, the primitive gut tube forms polarized epithelium at around embryonic day (E) 9.5. Then the epithelium undergoes rapid proliferation and initiates the conversation to villus cells at approximately E14.5 in the mouse[65]. Until E16.5, the crypt structure can be clearly distinguished[66]. In line with the morphological structure development, the relative proportion of spheroids (represent an undifferentiated state) and organoids (represent a differentiated state) generated from cultured small intestine decreases from fetal (E14) to postnatal (P15) period[47]. Specifically, intestine epithelial cells from E14 to E15 generated almost exclusively spheroids *in vitro*, whereas those from P5 generated almost exclusively organoids, indicating the critical time window of the cell type transformation. Intriguingly, human fetal intestine epithelial cells at GW 10 form spheroids *in vitro* as well, resembling the mouse intestine at around E15–E16, when villus formation started[48]. This observation suggests that the relative abundance of spheroid-generating cells is clearly associated with the developmental stage. Compared with organoids, undifferentiated spheroids showed much lower expression level of several adult intestinal stem cell markers, including Lgr5, Olfm4, Smoc2, and Cdx1, as well as the Wnt target gene Axin2[47,48]. Moreover, the receptor Lgr4, but not Lgr5, is essential for spheroid growth[47], which indicates that Wnt inactivation permits the growth of fetal intestine organoids[48]. Importantly, among the most upregulated genes in spheroids versus organoids, Trop2 and Cnx43 were found to be related to the developing intestine. Further lineage tracing confirmed that Trop2/Cnx43$^+$ cells act as stem cells responsible for prenatal villus formation, whereas Lgr5$^+$ cells maintain postnatal intestinal homeostasis[4,56]. Upon stimulation of exogenous Wnt[48,67] or gamma secretase inhibitor DAPT[47], fetal spheroids differentiated into adult tissue-like organoids with similar "crypt-villus" structure and expression profile, revealing the maturation of fetal spheroids in a dish. In addition to developmental stages, the different regions along the developing intestine represent distinct morphological features. The fetal intestine epithelial cells in distal region generate more organoids, whereas those in proximal region generate more spheroids, suggesting a distal to proximal wave of intestine maturation[48]. These phenomena can only be proven by fetal tissue at present. Altogether, investigation of fetal intestine organoids provides new insights into the cellular behaviors and mechanisms of mammalian intestine development.

Thyroid

The thyroid gland develops from ventral foregut endoderm. Using Nkx2-1 knock-in reporter mice and embryonic stem cell line, Longmire et al.[68] developed differentiation protocols of pure Nkx2-1 endodermal progenitors demonstrating that thyroid epithelium shares Nkx2-1$^+$ progenitors with the lung, and the presence of Pax8 expression indicates thyroid lineage specification. Subsequent studies have made efforts to create self-formation of thyroid follicular structures by overexpression of the defined transcription factors NKX2-1 and PAX8 in embryonic stem cells (ESCs)[69], which simulates the organogenesis process of the thyroid gland. These ESC-derived follicular organoids expressed thyrocyte marker genes and showed the ability of iodization. Although previous studies have reported successful differentiation of ESCs into thyrocytes in modified culture conditions[70–72], this is the first study to establish thyroid follicular organoids with self-organizing ability *in vitro*[69]. In 2015, Kotton's group[73] reported a growth factor combination, which mimicked the sequential signals inducing thyroid lineage specification during embryonic development, for the differentiation of ESCs/iPSCs into thyrocytes. During this process, they confirmed the critical roles of bone morphogenetic protein (BMP) and fibroblast growth factor (FGF) signals in guiding the thyroid lineage specification from endoderm and determined the mechanisms of early thyroid organogenesis, which are conserved across species from *Xenopus* to mouse and human. Further research demonstrated that transient overexpression of NKX2-1 coupled with precise modulation of BMP and FGF signals is sufficient for the conversion of anterior foregut endoderm to thyroid epithelium[74]. More recently, researchers utilized single-cell RNA-sequencing (scRNA-seq) technology to deconstruct the molecular combinations of different steps in the transforming process of PSCs into thyroid organoids *in vitro* and identified transforming growth

factor beta (TGF-β) as a negative regulator of thyroid maturation[75]. Therefore, although the extent to which the PSC-induced thyroid cells correspond to the bona fide thyroid cells *in vivo* has been questioned, PSC-derived thyroid organoids still appear to have great potential for thyroid developmental studies.

The turnover rate of thyroid follicular cells in adult homeostasis is quite low. Although specific stem cell population has yet to be discovered, hyperplasia followed by subtotal thyroidectomy suggests that the adult thyroid does have regenerative ability[76]. The culture system of adult tissue–derived thyroid organoids has been established *in vitro*. Thyrocytes isolated from mouse and human were successfully developed into organoids resembling the structure of thyroid gland[77–79]. These organoids maintain a proliferative capacity and partial thyroid functions including thyroglobulin synthesis, iodide uptake, and the production of small amounts of thyroid hormone. However, over time in culture, the thyrocytes in organoids exhibited decreased organoid-forming ability and dedifferentiation features[78]. The modified protocol proposed by Kurmann et al.[73] might solve the dedifferentiation trouble of thyroid organoids by promoting maturation *in vitro*[78]. However, whether this maturation mechanism is consistent with that of the fetus development needs to be answered.

To give insight into human fetal thyroid development, Liang et al.[49] established the long-term organoid culture of human fetal thyroid, in which fetal tissue fragments at 12 GWs grew into follicle structure *in vivo*. These human fetal thyroid organoids (hFTOs) expressed the key transcription factors and specific markers of thyroid follicular cells, including NKX2-1, PAX8, TG, TPO, and TSHR. By using scRNA-seq technology to compare thyroid gland from 12 GWs with 16 GWs, they elaborated the cell atlas of fetal thyroid gland and captured a subpopulation with increased cAMP level related to thyroid hormone synthesis. Interestingly, cAMP activation in hFTOs by forskolin indeed boosts follicle maturation and thus promotes thyroid hormone secretion *in vitro*. The maturation process of hFTOs recapitulates the development of fetal thyroid *in vivo* in many aspects, including the increase in mature thyroid markers, activation of maturation-related transcriptional factors, emergence of hormone-related cell populations, and initiation of hormone secretion. By employing this *ex vivo* system, the authors preliminarily dissected how enhanced chromatin accessibility of thyroid maturation gene loci, key transcriptional factors, and regulatory network determine human thyroid development.

Overall, the use of human fetal thyroid tissues and organoids provides a new understanding of the characteristics and mechanisms during the fetal developmental process, making another step forward in human thyroid development research. It is also feasible to investigate the roles of critical signaling pathways and specific genes in organoids. A more comprehensive developmental timeline of human fetal thyroid development would be expected in further studies.

Lung

The lung forms out of the primitive foregut endoderm. During embryonic period, organogenesis of lung occurs at around GW 4–7 in humans (E9.5–12 in mouse). Soon afterward into the fetal period, lung development involves four stages: pseudoglandular (human: GW 5–17; mouse: E12–16.5), canalicular (human: GW 16–26; mouse: E16.5–17.5), saccular (human: GW 24–38; mouse: E17.5–P4), and alveolar stages (human: GW 36–3 years; mouse: P4–21)[80]. Organoids derived from adult lung stem cells and PSCs have been reported to model respiratory cell type transition, cell fate specification, and pulmonary diseases[81]. As a thorough understanding of the cellular and molecular processes in the developing lung is lacking, these models are unable to recapitulate all the four stages (pseudoglandular, canalicular, saccular, and alveolar stages) in fetal lung development. Original embryonic and fetal lung tissues from specific stages may serve as the best source of immature cells with the potential to mimic cell fate determination and underlying molecular mechanisms. Employing fetal pulmonary organoids derived from E17.5 fetal mouse lung, Mondrinos et al.[50] demonstrated exogenous FGF signals play roles in driving *de novo* fetal pulmonary alveolar morphogenesis. Rodent lung organoids derived from earlier pseudoglandular[82] or later alveolar[83] lung tissues have also been developed for normal lung development studies. To determine whether human lung develops in a similar way as mouse lung does, Nikolic et al.[51] developed a long-term renewable human tip organoid derived from human GW 5–9 lungs and captured differentiative behavior of tips toward bronchiolar or alveolar lineages *in vitro*. This process is consistent with mouse lung development in which the distal tip cells function as potent progenitors producing bronchiolar and alveolar epithelial cells[84]. This indicates that the mouse model can replace human model to some extent in the study of lung development. The research findings of fetal lung via organoids significantly improve understanding of the underlying human *in vivo* differentiation mechanisms and provide references for directional differentiation of PSCs. However, through scrupulous comparison, Nikolic et al.[51] pointed out the differences between mouse and human in signaling requirements for long-term self-renewal. Other studies also demonstrated that the cellular and molecular processes during fetal lung development are distinct between mice and humans[85–87]. Therefore, more accurate human lung model needs to be established for human lung development research.

Pancreas

Expression of Pdx1 in foregut endoderm, prior to the outgrowth of dorsal and ventral pancreatic buds, indicates the specification of the pancreas. Genetic experiments in mice have demonstrated that multipotent progenitor cells (MPCs), coexpressing Pdx1 and Sox9, constitute the common origin

of all pancreatic lineages, both endocrine (the islets of Langerhans) and exocrine (acinar and ductal)[88,89].

β cells in the islets of Langerhans are responsible for insulin secretion upon glucose stimulation and cooperate with other hormone-producing cells to regulate whole-body glucose homeostasis[90]. It is well known that absolute or relative deficiency of insulin can lead to type 1 diabetes (T1D) and type 2 diabetes (T2D), respectively. Transplantation of PSC-derived precursor cells, as a leading strategy, has enabled generation of mature insulin-producing cells *in vivo* and effectively ameliorated hyperglycemia in diabetic mice[91–94]. Very recently, Du et al.[95] optimized the differentiation strategy based on their previous protocol and generated islets derived from human PSCs at high efficiency with an impressive effect in a nonhuman primate model of diabetes, which further demonstrated the safety and efficacy of these new β cells derived from PSCs for therapeutic application in clinical treatment of diabetes.

To elucidate the pathogenesis of diabetes, a complete understanding of the mechanism concerning the maintenance of MPCs and their potential to differentiate into all pancreatic lineages is required. Utilization of fetal-derived pancreatic tissues offers the possibility to identify the regulators for those events in early pancreatic development. Greggio et al.[52] established a 3D culture system, in which embryo-derived pancreatic progenitors could expand *in vitro*, differentiate into endocrine lineages, and recapitulate pancreas morphogenesis. Similarly, by reconstituting organogenesis with purified single fetal mouse pancreas progenitors, Sugiyama et al. established a unique system for dissecting regulatory genes in pancreas development[96]. Here, it is important to emphasize that the systems mentioned above could not guarantee the long-term expansion of pancreatic progenitors. By using a previously developed culture system[97], Bonfanti et al.[53] represented an efficient model for *in vitro* long-term expansion of fetal human pancreas organoids and identified epidermal growth factor as a gatekeeper to modulate *in vitro* expansion and differentiation of fetal pancreas progenitors. Moreover, Goncalves et al.[54] recently developed a robust expansion and differentiation culture system using human fetal pancreas or human PSCs, focusing on trapping progenitors *in vitro* closely resembling those *in vivo*. Briefly, this method highlights the maintenance and long-term expansion of pancreatic progenitors *in vitro*, which allows for the existence of more closely resembled fetal-like pancreatic progenitors and, more importantly, the possibility for high-throughput screening for small molecules with high reproducibility. Noteworthy, Li and coworkers recently identified that both dorsal and ventral pancreatic progenitors originate from midgut, contrary to the traditional concept that pancreatic progenitors have both ventral and dorsal parts of foregut origin[98], thus highlighting the necessity to dissect the mechanism underlying early development of pancreas with high-resolution techniques, which may bring permanent changes in our current understanding of pancreas development.

Taken together, although the differentiation methods from PSC to produce β cells hold great potential in cell-based therapy for diabetes, to obtain β cells of full maturity requires the detailed investigations into the mechanisms underlying pancreas development and characterization of multiple pancreatic lineages. The use of fetal-derived organoids, together with applications of other techniques, such as scRNA-seq, will help to identify crucial contributors in pancreas development for a better PSC differentiation protocol and pave the way to the treatment of diabetes.

Fetal Tissue–Derived Organoids in Modeling Developmental Defect Diseases

A unique advantage of fetal tissue–derived organoids in disease modeling, compared with those derived from ASCs or PSCs, is their ability to mimic pathologies restricted to defined developing stages. Several disease models based on fetal tissue–derived organoids, that recapitulate host–pathogen interactions[99], genetic diseases[100], and virus infection[101], have already been developed. These studies in principle demonstrate that organoids can exhibit certain disease features, reproducing developmental pathological process, and serve as a targeting drug discovery platform. In addition, fetal tissue–derived organoids are emerging as a promising source of transplantable tissues for regenerative medicine[48].

Diseases of Premature Infants

It is well documented that the premature infants are at high risk of necrotizing enterocolitis, which links intestinal immaturity to disease. Senger et al.[99] cultured organoids across the fetal age spectrum and determined specific regulated differences in fetal intestinal development related to the onset of necrotizing enterocolitis. They found that lipopolysaccharide treatment stimulated the gene expression of key inflammatory cytokines TNF and CXCL8/IL8 through LPS–TLR4–NF-κB axis in late fetal intestinal organoids, but not in early fetal or adult intestinal organoids, suggesting that late fetal intestinal organoids can be used as a human preclinical model to study the pathogenesis of necrotizing enterocolitis. This model is particularly relevant for studying premature infants' intestinal development and clinical pathology, and could be complementary to other human-derived models.

Embryonic Oncogenesis

The idea that organoids can model fetal pathologies has opened the mind to studies on the oncogenesis caused by abnormal progenitor cells in the developmental pathways. For instance, human hepatoblastoma initiation model was recently established using fetal tissue–derived liver organoids[100]. Hippo-YAP activation in fetal liver organoids led to

hepatoblastoma tumorigenesis with significant upregulation of hepatoblastoma signature genes, including DKK1, COL2A1, THFRSF19, NPNT, MATN3, CST1, PCP4, EDN3, C9orf152, and PEG10. Moreover, according to matched recapitulation of clinical features, the hepatoblastoma organoids displayed spontaneous lung metastasis when transplanted into the mice liver. Detailed mechanism of Hippo-YAP activation–induced metabolic reprogramming revealed the YAP1–G9a axis as a potential target for hepatoblastoma. Importantly, this study using human fetal liver organoids demonstrates that YAP1 activation is sufficient for human hepatoblastoma initiation, which challenges the prior studies in mouse model showing that co-activation of both β-catenin and Yap1 is required[102,103]. Therefore, these results highlight the importance of selecting apposite disease models, and human fetal tissues and organoids are necessary in studying development diseases. Efficient gene knockin and knockout methods based on CRISPR-Cas9 are developed in human fetal hepatocyte organoids[104]. The approach of gene manipulation in human fetal tissue–derived organoid can be extended to studies of other tumorigenesis caused by progenitor abnormalities, such as neuroblastoma[105] and retinoblastoma[106].

Infectious Diseases

SARS-CoV-2 has spread globally for more than 2 years after its initial outbreak in December 2019. SARS-CoV-2 vaccines have indeed greatly reduced the infection rate, but the emergence of multiple SARS-CoV-2 variants brings challenges[107]. An effective infection model of SARS-CoV-2 is necessary for drug screening. Lamers et al.[101] demonstrated that fetal lung bud tip organoids, which potentially differentiate into both airway and alveolar cells, were readily infected by SARS-CoV-2 and have much more increase in infectious virus titers than adult-derived alveolar organoid. This allows for a larger window to observe the anti-SARS-CoV-2 effects of potential drugs. Moreover, organoids mimicking the fetal stage can serve as an excellent virus infection model to determine the mechanisms of prenatal infection. Because fetal tissue is precious and use-restricted, the second-best approach is to employ PSC induction to obtain models that simulate specific developmental stages. Human fetal lung organoids derived from hPSCs, validated to have similar transcriptional profiles to first and second trimester of human fetal lungs, have been used to set up the respiratory syncytial virus (RSV) infection model recapitulating the pathology of prenatal RSV infection in human lung[108]. Another example is the generation of hiPSC-derived cerebral organoids to study the mechanisms of Zika virus infection and the effects on brain development[109–111]. Apparently, it is complicated to determine in what GW-matched developmental state the fetal-like organoids derived from PSCs are. Therefore, we emphasize the irreplaceable application of fetal tissue–derived organoids as developmental disease models.

For Regenerative Medicine

Fetal progenitor cells are also promising resource for regenerative medicine. Transplantation of human fetal tissue has been attempted to treat adult brain disease 20 years ago—for instance, transplantation of human fetal striatal tissue in patients with Huntington's disease and transplantation of human fetal dopaminergic neurons in patients with Parkinson's disease[43,44]. Although no significant difference between the grafted and nongrafted patients was observed over time, those studies support the safety of fetal tissue transplantation and lay the foundations for developing novel progenitor cell therapies.

To assess whether the fetal intestinal progenitors represent a transplantable source, fetal intestine spheroids were applied for repair of colonic injury caused by dextran sulfate sodium (DSS) treatment in adult mice[48]. Following transplantation, fetal intestine spheroids colonized in damaged region and generated heterograft with colonic crypts. Unexpectedly, these crypts expressed colon but not intestine signature genes, suggesting that immature fetal intestine progenitors can respond to the new microenvironment and differentiate into required cell types. It is worth mentioning that, *in vivo* transplantation, cells of fetal intestine spheroids have function on the regeneration of colon injury, but fail to grow under the renal capsule of mice, suggesting that a suitable microenvironment is critical for the growth of grafts and orthotopic transplantation is a more effective method. Several alternative transplantation sites have been tested in animal models to improve transplantation and long-term survival, such as the omentum[112], the epididymal fat pad[113], the lymph node[114,115], and the spleen[116]. All these studies suggest that an ideal site for transplantation is essential for supporting the function of grafted cells as well as the functional maturation of the tissue.

Fetal Tissue–Derived Organoids in Organ–Organ Interaction Study

Organogenesis is a complex and interrelated process, which relies on the immediate niche contributed by self-organization and neighboring tissues. However, it is still unclear how individual, neighboring components coordinate to guide multiorgan initiation, development, and physiological maturation in humans. Multiorganoid integration opens the opportunity for studying the complicated organ–organ interaction *in vitro*. Bagley et al.[12] made attempts to generate dorsal-ventral cerebral organoids modeling complex interactions between different brain regions by the fusion of predifferentiated dorsal and ventral organoids. Koike et al.[117] reported gut spheroids differentiated from human PSCs enable autonomous emergence of hepato-biliary-pancreatic organ, which potentially serves as a model for the study of complicated endoderm organogenesis in humans. Recently, Silva et al.[118] developed a human multilineage iPSC-derived organoid that

recapitulates cooperative cardiac and gut development and maturation. Nevertheless, whether the lineage differentiation and co-development of these PSC-derived multiorganoids recapitulate the real synergistically interrelated benefits of organ development *in vivo* needs further validation. Fetal tissues superior to their specific lineages and defined stages could be valuable resources for studying and verifying organ–organ interactions at the developmental stages.

Human fetal cells have been used to explore the interaction of the immune system with fetal organs. By investigation of fetal immune cells, McGovern et al.[119] discovered a previously unknown mechanism of immune suppression that they demonstrated human fetuses of 13 weeks' gestational age have functional dendritic cells, which are more likely to activate suppression of immune responses rather than mark the foreign material for annihilation during gestation. This study demonstrates that the immature immune system of the fetus has specific functions distinct from adults.

To explore the effects of immune system on human fetal intestine, Schreurs et al.[120] employed fetal intestinal organoids co-cultured with fetal $CD4^+$ Tem cells and assessed the effects of tumor necrosis factor alpha (TNF-α) produced by fetal $CD4^+$ T cells in intestinal stem cell (ISC) fate determination. They showed that TNF-α has a dose-dependent effect on ISC fetal development. The low number of T cells supported epithelial development, whereas the high number of T cells impaired ISC proliferation. Notably, prenatal $CD4^+$ T cells were specifically observed in human but not in mouse. In addition, the environment exposure is more complex in humans than in model animals; thus, laboratory mouse might not be an optimal model for immune system study, especially during fetal development. In sum, this co-cultured system of fetal intestinal organoids and immune cells provides a new framework for intestinal immune ontogeny and improves the fundamental understanding of the human fetal immune system. In-depth understanding of immune system of the developing fetus could reveal pathogenesis of some abortions and may hold hope for finding ways to suppress the response of the immune system to transplanted organs.

Perspectives

The Importance of Fetal Tissue Research

Although the application of animal models has long led to significant progress in developmental studies, they cannot completely simulate human embryonic organogenesis and further organ development due to species specificity[121]. Another research model, human ASC-derived organoid, representing the postnatal cellular state but not embryonic progenitors specific to their organ of origin, is also unable to mimic human organ development. In contrast, fetal tissue–derived organoids recapitulate the fetal organ architecture and functions, in which progenitor cells are preserved to expand and are able to differentiate into multiple cell types along the developmental trajectory, yielding cell compositions similar to those *in vivo*. Moreover, fetal tissue–derived organoids have robust establishment efficiency (around 70%–100%)[51,53,104,122], independent of their different developmental stages and genetic background, and hold promise for translational research like other organoid systems[123]. Therefore, the use of human fetal tissue–derived organoids has created many advances to investigate human development and diseases, organ–organ interactions, and regenerative medicine and has provided gold references for other research models (Fig. 2).

Although PSC differentiation models the embryonic development and organogenesis[124], it is still important to provide age-matched primary fetal tissues as standard. Hence, the PSC-based organogenesis must have a head-to-head comparison, not only transcriptomic analyses but also tissue-specific performance and functions, with the intended fetal organs. A thorough understanding of fetal tissue–derived organoids will provide references for PSC-derived organoid research and for the directed differentiation of PSCs into functional mature tissues. In the future, fetal tissue research might be replaced by the use of PSCs, but it will take time, and until then, the use of fetal tissues is crucial for human developmental understanding, therapeutic discovery, and stem cell research across many organ systems.

Future and Challenges

Human fetal tissue research holds considerable potential in investigating human development and diseases, and in advanced regenerative medicine. Despite these promising applications, the use of fetal tissue–derived organoids still faces challenges, including the complex ethical issues and lack of standardization in methods.

As the source of fetal tissues is mainly from elective abortions, it faces ethical constraints in research and clinical care. Fortunately, societies have formulated public policies to balance the advancement of biomedical science with the various concerns regarding the use of human embryos and tissues for biomedical research. Much more cautious ethical approaches are needed for the clinical transplantation of organoids.

The defined fetal tissue–derived organoids indeed provide a resource of models that can be used to study the molecular mechanisms and cell fate determinations of the organs during the human developmental process. Considering that the real physiological environment contains multiple cell types, the lack of vascularization and innervation could be a hindrance to developmental research. Alterations of the co-culture system of organoids containing endothelium, neuron, immune, or other lineages will be required for further research. Several types of organoids have successfully established co-culture system with the endothelium[33,34,125], neuron[126], or immune cells[120]. Recently, Eicher et al.[127] have generated complex three-germ-layer human gastric organoids from PSCs. These three-germ-layer organoids contain

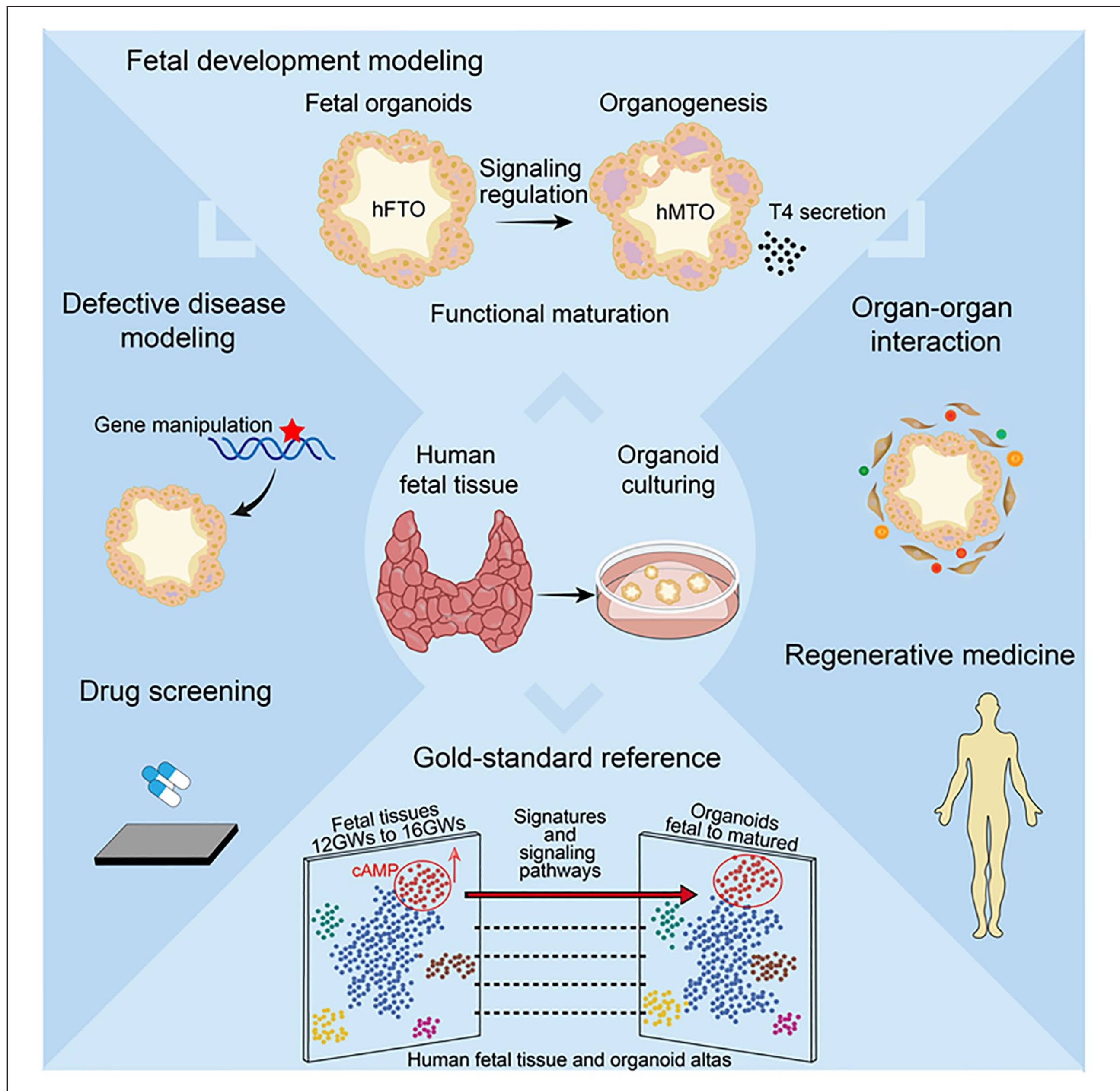

Figure 2. Basic applications of the fetal thyroid tissue–derived organoid. The use of human fetal tissue–derived organoids created many advances to investigate human development and diseases. Take human fetal thyroid gland as an example. Fetal thyroid–derived organoids recapitulate the fetal thyroid architecture and functions, which can be used to understand the principles of organogenesis and the mechanisms of organ–organ interaction, and to develop the gold-standard reference for PSC-derived cells and organoids. In application research, fetal thyroid organoids can serve as useful tools for the study of defective disease through gene manipulation and for drug screening. Based on the characteristics of fetal-derived organoids in which progenitor cells are preserved to expand *in vitro* and with low graft rejection, they could be important resources for regenerative medicine. hFTOs: human fetal thyroid organoids; hMTOs: human maturation thyroid organoids; PSC: pluripotent stem cell.

glandular epitheliums surrounded by oriented layers of smooth muscle innervated by functional enteric nerves. Nevertheless, the composition of microenvironment in different tissues is heterogeneous, which can meet the unique physiological needs of each tissue[128,129]. Therefore, for the co-culture system of fetal organoids, the consistency of developmental state of parenchymal cells and nonparenchymal cells should be considered, as the nervous system and vasculature also continuously develop during the stage of organ development.

Other vascularization techniques devised to embody microenvironments *in vitro* could be adapted for the vascularization

of fetal-derived organoids. Besides conventional microfluidic systems[130], emerging sacrificial networks[131,132], laser ablation technique[133,134], and 3D bioprinting approaches[135,136] have been developed to generate vascularized tissue-like structures. According to the method used, the interactions between vasculature and organoids can occur simultaneously or sequentially, and the compartments and dimensions of spatial relationships can also be designed based on the target tissues. Nowadays, the precision and scalability of 3D microfabrication technology offer foreseeable possibilities to generate free-form vascular structures in organoid models[137]. Although the current studies were performed in simplified cellular systems, future improvements in these methods will help to overcome the lack of vascularization in organoid cultures.

In addition, due to the lack of human reference for the early differentiation events, it is critical to determine whether *in vitro* cultured fetal organoids sustain their developmental age-specific features corresponding to their gestational age. In a word, specific ethical and empirical studies are needed to evaluate the novel artificial culture in recapitulating human organs.

Author Contributions

J. L. drafted the manuscript, X. L. and Y. D. revised the manuscript, and B. Z. edited the manuscript. All the authors read and approved the final manuscript.

Ethical Approval

This study was approved by our institutional review board.

Statement of Human and Animal Rights

This article does not contain any studies with human or animal subjects.

Statement of Informed Consent

There are no human subjects in this article and informed consent is not applicable.

Declaration of Conflicting Interests

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