Perspectives of Metabolic Syndrome-Related Organoids

Subjects: Cell Biology

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Organoids are spontaneously formed multicellular structures that provide a reliable model for studying early development and certain diseases. MetS is a systemic disease that affects multiple organs and tissues throughout the human body. A single organoid is not a good model for studying metabolic syndrome, as it lacks the organ-to-organ and system-to-system interactions necessary to study the disease. Secondly, the current immaturity of organoids and the inability to produce them on a large scale and in a standardized manner have created significant limitations for the study of various diseases, especially systemic diseases such as Mets. However, the combination of organoids with other technologies is expected to break the metabolic syndrome research bottleneck.

Keywords: metabolic syndrome ; pancreatic organoids ; β-cells ; diabetes

1. CRISPR-Based Gene Editing

Compared to animal models and primary cell models, PSC-derived organoids can be altered by CRISPR-Based, an efficient gene editing technology, to alter the expression of a gene in organoids. This method can help to improve the efficiency or maturity of organoid construction and can also be used to explore the impact of the gene on the whole process of disease development, providing strong support for the molecular pathogenesis of related diseases and the subsequent development of gene therapy.

For example, l'Hortet et al. used this technique to confirm the important role of the SIRT1 gene in human fatty liver formation, found that increased fatty acid biosynthesis exacerbated fat accumulation by differentiating edited iPSCs into hepatocytes and knocking out SIRT1, and established a human fatty liver model with human SIRT1 knockout iPSC-derived hepatocytes that obtained a pro-inflammatory phenotype and shared a similar lipid and metabolic profile to the human fatty liver [1]. Just recently, Hendriks' group used this technique to knock out the APOB and MTTP genes in human fetal hepatocyte-derived organoids, deletions of which are responsible for two monogenic lipid disorders predisposing to NAFLD: familial hypolipoproteinemia and abetalipoproteinemia. $APOB^{-/-}$ and $MTTP^{-/-}$ mutant organoids constitute a natural steatosis organ model and can be maintained in long-term culture at levels that maintain a stable level of steatosis. The group used the lipotropic organoids to build a CRISPR-Based screening platform, through which FADS2 was found to be a key regulator of lipotrophy. While FADS2 deficiency exacerbated the steatosis phenotype, overexpression of FADS2 resulted in reduced steatosis [2].

2. 3D Synthetic Scaffolds

Currently, effective expansion of organoids requires matrix or basement membrane extraction (BME). However, most organoid cultures use Matrigel as BME. Matrigel is derived from mouse sarcomas, and its composition is heterogeneous and varies significantly from batch to batch, making it impossible to standardize organoid models for large-scale culture and the reproduction of results more difficult [3]. Compared to animal-derived matrices, protein- or polysaccharide-based biopolymers can be recombinantly produced with reduced variability. In addition, synthetic matrices offer the opportunity to experimentally isolate the stiffness, bioactivity, and variability of the environment in which the organoid grows, allowing screening methods to be developed to investigate the impact of each parameter on stem cell fate [4][5][6].

For example, enrichment of certain ECM components, such as laminin, promotes the conversion of bipotential pancreatic progenitor cells to endocrine cell specification, whereas exposure to other ECM components induces ductal cell differentiation, implying that stage-specific scaffolds may promote endocrine differentiation in vitro and improve induction efficiency [7].

3. 3D Bioprinting

Bioprinting is a promising and innovative biomanufacturing strategy for precisely locating biological agents, including living cells and extracellular matrix (ECM) components, in defined 3D layered tissues to create artificial multicellular tissues/organs [8]. An engineering approach using bioprinting to control initial cell density, size, and shape of cell aggregates, cell–ECM interactions, and biochemical gradients will provide more precise guidance for the generation of PSC-derived organoids [9].

It has been demonstrated that the effectiveness of differentiating hPSCs into SC-β cells is closely related to cell density, cell line, and induction protocol [10][11]. In 2018, Memon et al. were able to improve the induction efficiency of $PDX1^{+}/NKX6.1^{+}$ pancreatic progenitor cell populations by manipulating the replating density [12]. In 2019, Bernal et al. demonstrated that volumetric bioprinting via optical tomography could shape gelatin hydrogels containing organoids into complex centimeter-scale 3D structures in less than 20 s [13]. Last year, Daly et al. demonstrated a bioprinting method that transfers high-resolution spheroids into homogeneous supporting hydrogels, allowing them to be patterned and fused into high-cell-density microtissues with defined spatial organization [14].

4. Organoids in a Microfluidic Device

The problem of inaccessibility during organoid culture has been a major problem for researchers, and the usual solution is to periodically disassemble and reseed the organoids onto the culture medium, which makes them unsuitable for long-term research observations. Microfluidic devices are a promising tool for integrating channels for nutrient supply and waste removal within organoids and enabling autonomous control of experimental conditions.

In 2020, Liu et al. developed a droplet microfluidic system for the regulated fabrication of hybrid hydrogel capsules, which permits large-scale 3D culture and the formation of functional and uniform islet-like organoids derived from hiPSC. The produced hybrid capsules exhibit high homogeneity and are stable, biocompatible, and infiltrative [15].

5. Organoids on a Chip

In 2018, Koike's team developed a scheme for the sequential construction of liver, biliary, and pancreatic (HBP) structures from three-dimensionally cultured human pluripotent stem cells (PSCs). Unfortunately, such a scheme cannot yet be clearly discernible spatially and requires further development of maturation [16], which, in combination with organoid microarray technology, may be used to model connectivity between organoids from other stem cell sources [17].

In 2022, Tingting Tao's team designed a liver and islet-like organ co-culture system on a chip capable of studying organ–organ interactions under perfusion co-culture conditions for up to 30 days. The system provides a powerful method to study the feedback loop within the human liver–pancreatic islet axis that maintains glucose levels in the normoglycaemic range in vitro, a result that cannot be achieved in single organ culture; both liver and islet organoids exhibit mitochondrial dysfunction and reduced glucose transport capacity under hyperglycaemic conditions, which can be alleviated by metformin treatment, suggesting that the liver-islet organoids system is able to mimic the key pathological features of T2DM. A distinct advantage of this system is its ability to mimic the human-relevant functional coupling of liver and islet organs in response to external hyperglycemic stimuli and drugs, which is not easily studied in monolayer cell cultures or animal models [18].

6. Biobanks and MetS

MetS and genetic susceptibility are closely related, and genetic polymorphisms play an important role in MetS [19][20]. Extracted ASC-derived organ tissues from MetS patients can preserve the inherited characteristics of the disease, and the resulting organ tissues can be used for the construction of biobanks. This will facilitate global MetS research and personalized treatment, such as the development of appropriate diet, exercise, and medication plans for a particular nucleotide polymorphism. As early as 2008, genome-wide association scans identified PNPLA3 (rs738409[G], encoding I148M) to be closely associated with increased levels of liver fat and liver inflammation [21]. In a newly published paper by Hendriks' group, demonstrating that genetic susceptibility to NAFLD affects the efficacy of relevant drugs, they found that carrying the PNPLA3 I148M variant attenuates organ response to fatty liver drugs, which is particularly evident in the FXR–FGF19 drug axis, and this research provides evidence for possible future personalized medicine for NAFLD [2].

The establishment of such biobanks has been the subject of global efforts. Zeng et al. created isogenic human ESCs (hESCs) with mutations in type 2 diabetes susceptibility genes identified by genome-wide association studies (GWAS). In

pancreatic β-like cells derived from these cell lines, CDKAL1, KCNQ1, and KCNJ11 mutations were found to cause impaired glucose secretion in vitro and in vivo, consistent with defective glucose homeostasis [16]. Recently, Kimura et al. designed combinations of human organoids for steatohepatitis from a multi-donor human progenitor cell bank to investigate the effect of metabolic status on genotype–phenotype associations. Precision hepatology is supported by a comprehensive arsenal of mechanistic, discriminative, and therapeutic reasoning [22].

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