胡慧丽, 山东大学基础医学院教授, 山东省泰山学者青年专家, 基础医学院遗传学系副主任, 山东大学干细胞及再生医学中心常务副主任。于山东大学生命科学院获得学士学位, 山东大学基础医学院获得博士学位。博士后工作师从荷兰皇家科学院CLEVER教授。长期从事成体干细胞与组织再生机制研究, 运用类器官对细胞可塑性、疾病发病机制进行深入探究, 并探讨其在疾病治疗中的应用。建立了人/小鼠肝细胞来源的3D类器官培养模型, 为阐明肝细胞再生机制、揭示肝脏疾病发生机制等基础研究问题提供了关键技术, 也为实现个体化医疗提供了可能。目前的课题方向包括: (1) 运用类器官、小鼠模型、CRISPR筛选和单细胞测序进行成体干/祖细胞命运调控机制研究; (2) 肝脏疾病及衰老干预机制探究; (3) 肝脏微器官的构建。
http://www.genetics.sdu.edu.cn/info/1005/1039.htm

类器官在发育与再生中的研究进展

郭贝贝 胡慧丽*

(山东大学基础医学院遗传学系, 实验畸形学教育部重点实验室,
山东大学干细胞与再生医学研究中心, 济南 250012)

摘要 类器官(organoid)作为体外模拟器官结构和功能的三维培养体系, 已经广泛应用于发育研究、疾病建模和药物筛选。类器官在再生医学中具有重要的应用前景。胚胎干细胞、诱导多能性干细胞和多组织成体干/祖细胞来源的类器官再现了发育分化、稳态自我更新和组织损伤再生过程, 为揭示发育和再生调控机制、明确生理病理进程提供了可能。近年来, 多细胞类型的新型培养模式和单细胞测序等技术的应用促进了类器官的发展。该文总结了类器官在发育与再生中的最新研究成果, 并就前沿技术在类器官研究中的应用进行了综述与展望。

关键词 类器官; 干细胞分化; 损伤再生; 细胞可塑性

Research Advances of Organoids in Development and Regeneration

GUO Beibei, HU Huili*

(*Key Laboratory of Experimental Teratology, Ministry of Education, Department of Molecular Medicine and Genetics, School of Basic Medical Sciences, Stem Cell and Regenerative Medicine Research Center, Cheeloo College of Medicine, Shandong University, Jinan 250012, China*)

收稿日期: 2021-04-11 接受日期: 2021-04-22
国家重点研发计划(批准号: 2019YFA0111400)、国家自然科学基金(批准号: 31501179、31970779、9206810312)和山东省软科学研究计划项目(批准号: 2019RKE27005)资助的课题
*通讯作者。Tel: 0531-88382043, E-mail: huhuili@sdu.edu.cn
Received: April 11, 2021 Accepted: April 22, 2021
This work was supported by the National Key Research and Development Program of China (Grant No.2019YFA0111400), the National Natural Science Foundation of China (Grant No.31501179, 31970779, 9206810312) and the Soft Science Research Program of Shandong Province (Grant No.2019RKE27005)
*Corresponding author. Tel: +86-531-88382043, E-mail: huhuili@sdu.edu.cn

Abstract Organoid is the *in vitro* three-dimensional culture system remodeling the structure and function of its original organ. Organoids have been widely used in developmental research, disease modeling and drug screening and have potential key application in regenerative medicine. Organoids derived from embryonic stem cells, induced pluripotent stem cells, and adult stem/progenitor cells from tissue recapitulate differentiation during development, self-renewal in homeostasis, and regeneration after tissue injury, respectively. Organoids opened up new avenues to reveal the molecular mechanism of development and regeneration. It provides possibilities to clarify the physiological and pathological progression. Recently, scientists focus on establishing and characterizing new organoid models with multiple cell types by novel co-culture methods and new technology such as single-cell RNA sequencing, which facilitates the development of organoids. This review summarizes the latest research in development and regeneration using organoids. This review also gives an outlook for the application of cutting-edge technologies in organoid research.

Keywords organoid; stem cell differentiation; injury and regeneration; cell plasticity

围绕全球人口老龄化、恶性肿瘤高发和重大慢性疾病治疗等亟待解决的科学问题, 国家“十四五”规划和《中华人民共和国国民经济和社会发展第十四个五年规划和2035年远景目标纲要》对发病机制基础研究、健康干预和再生医学等前沿技术进行了布局[1]。类器官是近年来迅速发展的三维(three dimensions, 3D)培养技术, 高度模拟了来源组织或器官的结构和功能。一方面, 类器官可用于研究干细胞分化、组织自我更新与再生修复, 是阐述细胞命运、细胞互作与微环境, 特别是人类细胞生理与病理调控分子机制的良好模型[2]。另一方面, 类器官可以为组织器官再造提供“种子细胞”, 在衰老、疾病干预、个体化和精准医疗中具有潜在的转化应用前景[3]。

近年来, 类器官的基础研究集中于在分子水平上阐述干细胞或祖细胞的精确细胞行为, 包括其如何高效分离扩增、细胞异质性分析和定向诱导分化, 并逐渐向构建多细胞类型互作的复杂仿生微器官新型培养模式过渡。

1 类器官模拟器官发育过程

胚胎干细胞(embryonic stem cells, ESCs)和诱导多能性干细胞(induced pluripotent stem cells, iPSCs)来源的类器官在体外可模拟发育过程。将多能性干细胞(pluripotent stem cells, PSCs)置于小分子抑制剂或生长因子的培养条件下, 诱导细胞向特定的细胞命运定向分化, 在形态和空间构成上模拟器官发生, 从而揭示发育相关的分子机制[4-6]。目前, 已成功构建多种ESCs或iPSCs来源的3D类器官模型, 包括外胚层发育形成器官视杯/视网膜、脑, 内胚层发育形成器官肠、胃、肝脏、胰腺、气管、肺, 以及中胚层发育形成器官血管、心脏、肾脏、膀胱等。

1.1 外胚层来源器官(脑、视网膜)

探究大脑和神经的发育机制, 揭示精神疾病致病机理是人类亟待解决的重大科学问题之一, 但脑科学研究缺乏良好的模型。从2013年LANCASTER等[7]建立了包含多个脑区的首个“脑类器官(cerebral organoids, COs)”开始, 目前已经有多种方案将PSCs来源的神经外胚层细胞自组装成与胚胎组织类似且包含不同脑区的脑类器官。采用单细胞转录组测序(single-cell RNA sequencing, scRNA-seq)对培养不同时间点的脑类器官进行基因表达和细胞聚类分析发现, 其模拟了发育过程中的细胞类型变化, 再现了胚胎新皮质发育过程[8-9]。通过对4个月培养期间单细胞转录组和染色质状态的拟时分析, 以及对脑类器官的形成过程进行动态监测, 他们描述了多能干细胞逐步分化成神经干细胞并形成前脑(背侧、腹侧脑和间脑)、中脑、后脑(菱脑)和视网膜等不同脑区的过程[10]。在长期培养过程中, 类器官细胞逐渐发育成熟, 并产生类似于人脑的脑电波信号, 为研究神经网络活动在皮层中的作用提供了可能[11]。脑类器官中存在兴奋性和抑制性的异质性神经元, 再现了神经元迁移、皮层分层及神经环路建立等生理功能[9-10,12]。除了神经元, “迷你皮层”也包含神经元与中枢神经系统的星形胶质细胞和少突胶质细胞, 可用于各细胞类型通讯研究、髓鞘罕见病如Pelizaeus-Merzbacher病机制研究和治疗的药物开发[13]。通过诱导hESCs分化成丘脑类器官, 可建立与皮层类器官融合的轴突连接, 模拟丘脑皮层回路等复杂的脑

活动[14]。与人脐静脉内皮细胞(human umbilical vein endothelial cells, HUVECs)共培养、圆盘片状化类器官或在COs中异位表达ETV2转录因子, 均可以克服脑类器官内部氧气和营养物质供应不足的局限, 加速细胞分化成熟, 为模拟血脑屏障(blood-brain barrier, BBB), 实现脑类器官在小头畸形、精神发育迟滞等脑疾病机制中的探究提供可能[15-17]。

来源于神经外胚层的视网膜与视觉感知密切相关, 其发育异常会导致视力受损或疾病发生。ESCs在包含Y27632、CHIR99021等小分子化合物的分化培养基中聚集形成视网膜类器官(retinal organoids, ROs)。scRNA-seq结果显示, 视网膜类器官细胞类型复杂, 关键细胞如视杯细胞呈现有序性分化, 按时间顺序分别形成神经节细胞、感光细胞前体、水平细胞、无长突细胞、双极细胞和müller细胞[18-20]。早期视网膜发育经历视野细胞–视泡、视杯细胞–视网膜色素上皮细胞和感光细胞三个阶段。类器官与其在体内的发育过程一致, 光感受器与视网膜层细胞类型的形成决定了其具备视网膜的生理功能[21-24]。视网膜类器官分泌的甲状腺激素对类器官分化形成感知红色和绿色的视锥细胞至关重要[25]。近年来, 为了进一步优化ROs培养体系, OSAKADA等[26]利用小分子化合物成功诱导分化出感光细胞与视网膜细胞, 摆脱了ROs对动物或大肠杆菌细胞中产生的重组蛋白的依赖。ZHONG等[27]建立了iPSCs来源的高度自主分化的类器官, 该类器官可形成更为成熟的感光细胞和分层的视网膜类组织。通过高通量单细胞测序对不同时间点的ROs进行分析, 他们精细地描绘了视网膜发育轨迹和类器官分子网络变化, 并进一步结合染色质开放程度深入分析了类器官与胚胎视网膜在基因表达特征上的异同。这些结果表明, ROs在细胞类型、细胞形态和分子水平方面高度模拟了视网膜的发育过程, 为视网膜疾病机制研究和药物筛选提供了新途径[28]。

1.2 内胚层来源器官(肠道、肺、肝)

PSCs来源的胚状体在外源性活化素A(Activin A)的诱导下产生终末内胚层[29-30]。Wnt和FGF信号协同激活与BMP信号抑制有利于内胚层定向向后肠谱系分化, 形成原始肠管。2011年, SPENCE等[30]构建了人ESC来源的肠道类器官(human intestinal organoids, HIOs)。通过添加EGF、Noggin等组分, 在3D培养条件下促进高度折叠结构的后肠球体阶段性定向分化出隐窝样–绒毛的3D结构[30]。与成体Lgr5$^+$干细胞来源的类器官不同, HIOs不仅包含黏膜上皮细胞, 也包含黏膜下层间质细胞如成纤维细胞、平滑肌细胞等, 并在转录水平和细胞功能特征上持续处于胚胎状态[31-32]。近几年, 包含外胚层来源的肠道神经丝的无动物源性Mini-gut的建立, 弥补了在HIOs和成体干细胞类器官肠道中的罕见细胞类型缺乏的不足, 为类器官应用于临床移植再造器官提供了可能, 但存在维持期较短以及无法机械传代的缺陷[33]。

在PSCs中抑制TGFβ、BMP信号、Wnt信号, 可高效产生腹前前肠内胚层[34-35]。再次激活Wnt和FGF信号可促进内胚层分化形成前肠球体[30,36-37], 进一步激活SHH信号促进球体向肺谱系分化[38]。其中, 成纤维细胞生长因子10(fibroblast growth factor 10, FGF10)在诱导前肠球体祖细胞标记物表达减少、肺泡标记物表达增加过程中至关重要[39]。2015年, 科学家诱导干细胞形成第一个肺类器官。肺类器官可分化形成基底细胞、杯状细胞、Clara、纤毛、I型肺泡(PDPN$^+$APQ5$^+$)和II型肺泡上皮细胞(SP-B$^+$SP-D$^+$ABCA3$^+$)等细胞类型, 同时包含未分化的肺泡祖细胞[40-42]。随后科学家建立了包含分支气道和肺泡结构的类器官模型, 且可用于体外模拟妊娠中期胚胎的不同发育程度的肺。研究发现, 在肺类器官中引入*HPS1*基因突变, 可引起细胞早发肺纤维化改变, 重现纤维化疾病发生[43]。同时, 将远端肺脏类器官和肺类器官——SARS-CoV-2伪病毒共培养模型应用于药物筛选中, 可为新冠病毒临床试验提供数据支持[44-45]。

TAKEBE等[46]建立了首个PSCs来源的肝类器官, 将iPSCs与基质细胞、人脐静脉内皮细胞和间充质干细胞进行2D共培养, 细胞间的相互作用激活FGF和BMP信号, 促使3D结构的自组装并形成与胚肝表达谱一致的3D肝芽结构。PSCs分化形成终末内胚层和前肠祖细胞后, 通过加入肝细胞生长因子(hepatic growth factor, HGF)、抑瘤素M(oncostatin M, OSM)诱导类器官向肝细胞命运分化, 或通过加入FGF10、维甲酸(retinoic acid, RA)和Activin A等可诱导其向胆管细胞命运分化。此外, 包含FGF2和维生素C的MTeSR培养基可诱导iPSCs源性类器官实现肝细胞和胆管细胞样细胞的共分化[47]。ESCs衍生的人胎肝类器官(human embryonic hepatic organoids,

hEHOs)模型的建立, 为实现肝细胞大规模扩增生产和细胞治疗提供了可能[48]。

1.3 中胚层来源器官(肾脏、心血管)

通过调节BMP4、Activin A以及FGF信号诱导iPSCs形成“原始条纹”, 即中胚层和内胚层的祖细胞群体。持续激活Wnt信号促进其向前中胚层输尿管上皮细胞分化, 短时间的Wnt信号激活更有利于祖细胞向后中胚层的后肾方向分化。Wnt、FGF信号的适当激活, 同时BMP4信号的抑制诱导PSCs形成可分化为收集管和功能肾单位潜能的两种祖细胞群, 在3D培养条件下可形成输尿管上皮和后肾间质, 以及包含集合管、早期远端小管、早期近端小管和肾小球四部分的肾脏类器官[49-50]。肾类器官中各细胞群空间排列接近肾脏组织, 且具备成熟的生理功能[51]。对PSCs来源的3D肾脏类器官分化过程进行scRNA-seq显示, 肾单位祖细胞是肾脏血管的一个非常规来源, 可以生成血管化的肾脏类器官, 为生物人工肾的开发奠定基础[52-53]。

近两年, 类器官在心脏与血管研究领域也取得了突破性进展[54-58]。中胚层经过VEGF-A、FGF2等生长因子诱导后在3D条件下形成首个血管类器官。移植血管类器官到小鼠体内后, 类器官发育形成完善的血管系统, 包括动脉和毛细血管[54]。小鼠和人多能性干细胞来源的心脏类器官发育过程再现了体内胚胎心脏发育过程中的形态变化和功能结构[56-58]。CHIR17和IWP2诱导分化的3D心脏形成类器官(heart-forming organoid, HFO)直径约为2 mm。研究人员通过NKX2.5-eGFP绿色荧光报告基因验证了HFO可精确模拟早期心脏发育过程[55]。

2 类器官模拟自我更新与损伤再生过程

成体干细胞(adult stem cells, ASCs)或组织块来源的类器官培养模拟了来源组织的自我更新或损伤再生过程。小肠、结肠、胃等自我更新速度较快的组织器官, 含有明确的成体干细胞群。例如, Lgr5$^+$肠道干细胞在体外3D培养条件下形成隐窝–绒毛样的类器官, 可分化形成快速增殖(transit-amplifying, TA)细胞、潘氏细胞、肠道吸收细胞和分泌细胞等[59-62]。肠道类器官还表现出一定的损伤再生能力。构建基于图像的表型筛选平台, 绘制调节类器官分子的功能性遗传互作图谱, 为深入理解肠道再生机制提供了理论依据[63]。在多种小分子化合物(8因子)培养条件下建立的新型小肠类器官模拟了“增生态”的隐窝形态, 主要表达损伤相关基因。其中, VPA和EPZ6438通过激活YAP通路下游基因, 对类器官获得再生特征发挥关键作用。与ENR(egf-noggin-rspodin)传统培养条件形成的类器官传代扩增能力相比, 新型类器官的传代扩增能力大大增强, 为运用类器官研究损伤再生和疾病损伤药物筛选提供了新途径[64]。

在胰岛、肝脏等自我更新速度较慢的器官中, 成体干细胞的存在和位置一直存在争议。哺乳动物成体胰岛可在3D培养体系中扩增[65]。最新研究表明, 成年小鼠的胰岛中存在Procr$^+$细胞类群, 在正常生理状态下能够分化形成胰岛全部细胞类型, 并在血管共培养的3D培养体系中形成功能胰岛类器官[66]。尽管多项研究表明, 肝脏不同分区包括肝门静脉周围的Axin2$^+$、TERT^{High}、Sox9$^+$和损伤激活的Lgr5$^+$等细胞被报道负责肝脏自我更新和损伤再生[67-70], 但活体监测细胞增殖新技术-ProTracer(proliferation tracer)和多Cre谱系示踪技术证实, 肝小叶中间区域的增殖细胞是肝脏稳态维持的细胞来源[71-72]。原代肝细胞重塑后形成的祖细胞可在3D培养条件下形成肝类器官[73]。肝细胞来源的类器官与肝细胞结构、功能和基因表达谱高度一致, 模拟了肝切除后的再生过程[74-75]。具有双向分化潜能的Lgr5$^+$祖细胞, 在成体未损伤肝脏中几乎不存在, 而当损伤发生后出现在胆管细胞附近。体外培养Lgr5$^+$形成胆管类器官可诱导分化成肝细胞[76]。TET1介导的DNA羟甲基化在胆管类器官形成的细胞命运重塑中发挥重要作用, 模拟了小鼠体内肝脏损伤中胆管细胞的响应[76-77]。胰岛类器官、肝脏类器官和回肠类器官[78]移植可改善糖尿病小鼠胰岛功能、重建小鼠损伤肝脏和重塑大鼠小肠结构和功能, 是类器官在再生医学和健康干预中的应用的重要方向。

3 前沿技术在类器官研究中的应用

3.1 单细胞测序技术

scRNA-seq的迅猛发展, 与ATAC、蛋白组学、代谢组学等多组学结合, 为阐明类器官复杂细胞组分、基因表达特征, 探讨细胞相互作用提供了重要技术保障。在类器官研究领域中的应用主要包括以下几个方面。(1) 探究类器官是否高度模拟来源组织, 包括生理和病理特征[79-81]。(2) 运用类器官探究时序

性发育关键事件, 优化培养条件、鉴定分化顺序及确定细胞功能[53,82]。(3) 发现新的细胞亚群, 如对克罗恩病患者来源的小肠类器官进行单细胞测序, 结果显示类器官的形成主要基于OLFM4^{+}与SLC12A2^{+}干细胞群体[83]。同时, scRNA-seq联合空间转录组学、单分子荧光原位杂交(single-molecule RNA fluorescence *in situ* hybridization, smRNAFISH)、顺序杂交测序(sequential fluorescence *in situ* hybridization, seqFISH)以及空间条形码测序等技术可识别空间上定义的细胞类型, 揭示同一组织的不同区域发生不同调控的分子机制[84-87]。

3.2 谱系示踪技术

遗传谱系示踪技术(genetic lineage tracing)是研究体内特定细胞类型起源及命运最常用且有效的方式。类器官结合谱系示踪技术, 主要应用于鉴定类器官干细胞群及示踪异质性细胞群体细胞命运。利用Aldh1b1^{CreERT2};Rosa26^{LSLtdTomato}双杂合子小鼠与Aldh1b1$^{fl/fl}$;Rosa26$^{CreERT2/CreERT2}$小鼠示踪发现, Aldh1b1^{+}细胞是形成胰腺类器官的主要细胞来源[88]。Prorc^{+}细胞具有上皮细胞向间充质细胞转化的特征, 稳态条件下可分化为β、α、δ和PP四种分泌细胞类型[66]。利用Bmi1^{CreERT2};Rosa26-YFP小鼠, 发现位于胃峡部的Bmi1^{+}细胞是具有管腔和基底双向分化潜能的对5-FU敏感的新的干细胞群。Bmi1^{+}细胞受损后可快速增殖, 是体外胃类器官形成的重要来源[89]。位于胃峡部腺体底部的Troy^{+}细胞也可以在体外培养形成类器官[90]。

3.3 基因编辑技术

类器官培养实现了多来源组织的功能再现, 结合基因编辑技术优势, 可以实现“从头”研究基因具体作用。(1) 模拟肿瘤发生进程。在人类肠道类器官中运用CRISPR/Cas9技术引入结直肠癌常见基因(*APC*、*P53*、*KRAS*和*SMAD4*)突变, 并在不同培养条件下进行筛选, 可对驱动基因突变在肿瘤不同进程中对细胞行为的影响进行深入探讨[91]。在人hiHep肝类器官中引入肝细胞癌致癌因子的基因如*c-MYC*和*RAS*, 探究致癌基因功能并证实胆管细胞癌肿瘤起源[92]。(2) 提供基因筛选平台。在人肠道类器官中通过全基因组CRISPR文库筛查TGFβ抗性的潜在肿瘤抑制因子, 并在正常和*APC*突变的类器官中进行验证[93]。结合独特的分子识别物(unique molecular identifier, UMI), 可以实现在类器官标记和移植后体内克隆的特征研究[94]。(3) 开发新型类器官可视化技术。新型基因工具“CRISPR-HOT”的开发, 实现了对人源类器官中的特定基因的荧光可视化标记, 突破了类器官中实时动态观测的瓶颈[95]。

3.4 器官芯片

随着类器官技术的迅猛发展, 类器官培养中的缺陷和不足也逐渐显现出来。(1) 多数类器官只包含上皮细胞组分, 不含血管和免疫细胞, 无法进行供氧, 因此生长受限。(2) 类器官供体差别大, 难以实现标准化, 与真实器官在大小、空间构成上存在巨大差异。(3) 无法模拟器官的生物物理环境与器官协同作用。

因此, 以微流控等为代表的器官芯片技术, 在推动类器官完善上或起到不可或缺的重要作用。通过小分子或重组蛋白浓度差异, 模拟生理信号梯度区域变化建立血管化的类器官, 解决营养与氧气供应的不足[96-97]。引入生物力学模拟器官真实硬度[98]。将多器官共培养模拟肠–肝轴等, 实现不同器官细胞之间的信号串扰, 再现复杂的器官间生理活动和细胞动态相互作用[99]。此外, 基于多孔滤膜支持物或基质胶分区的气液界面(air liquid interface, ALI)培养方式, 将上皮细胞暴露在周围空气中, 使其通过顶端分泌物产生并分化出顶端微环境, 能更好地模拟与空气直接接触的皮肤表皮细胞、呼吸细胞和肠道上皮细胞[100-102]。这些技术的应用对建立标准化和仿生微器官意义重大。

4 结语与展望

来源于ESCs/iPSCs/ASCs和通过病理组织建立的多种类器官, 再现了发育分化、稳态自我更新和组织损伤再生过程, 为揭示发育和再生调控机制、明确生理病理进程提供了可能, 在遗传性疾病[103]、感染性疾病宿主–病原体互作[104]、肿瘤[6]等疾病的机制研究中应用广泛。新技术与新生物工程手段的应用有利于克服类器官供体差异、成熟度与组织结构性模拟不足、细胞类型缺乏等弊端。单细胞测序技术的发展和普及, 建立了类器官与原器官转录谱的密切联系, 有助于深入认识类器官中不同的细胞类型, 构建了更加完善的培养体系[105]; 空间转录组学的兴起将提高我们对原位组织区域的结构认知[86], 结合器官芯片和3D打印技术构建结构上更加仿生的类器官模型[106]; 小分子标记探针影像的发展对动态掌握并获取类器官内

部不同细胞类型的位置信息有重要促进作用[107]; 生物反应器的改进为类器官规模化产业化培养提供了可能[108]。

参考文献 (References)

[1] 中华人民共和国国民经济和社会发展第十四个五年规划和2035年远景目标纲要[N]. 人民出版社, 2021-03-13(1).

[2] MCKINLEY K L, CASTILLO-AZOFEIFA D, KLEIN O D. Tools and concepts for interrogating and defining cellular identity [J]. Cell Stem Cell, 2020, 26(5): 632-56.

[3] TIRIAC H, PLENKER D, BAKER L A, et al. Organoid models for translational pancreatic cancer research [J]. Curr Opin Genet Dev, 2019, 54: 7-11.

[4] LANCASTER M A, KNOBLICH J A. Organogenesis in a dish: modeling development and disease using organoid technologies [J]. Science, 2014, 345(6194): 1247125.

[5] SHI Y, INOUE H, WU J C, et al. Induced pluripotent stem cell technology: a decade of progress [J]. Nat Rev Drug Discov, 2017, 16(2): 115-30.

[6] TUVESON D, CLEVERS H. Cancer modeling meets human organoid technology [J]. Science, 2019, 364(6444): 952-5.

[7] LANCASTER M A, RENNER M, MARTIN C A, et al. Cerebral organoids model human brain development and microcephaly [J]. Nature, 2013, 501(7467): 373-9.

[8] CAMP J G, BADSHA F, FLORIO M, et al. Human cerebral organoids recapitulate gene expression programs of fetal neocortex development [J]. Proc Natl Acad Sci USA, 2015, 112(51): 15672-7.

[9] GIANDOMENICO S L, MIERAU S B, GIBBONS G M, et al. Cerebral organoids at the air-liquid interface generate diverse nerve tracts with functional output [J]. Nat Neurosci, 2019, 22(4): 669-79.

[10] KANTON S, BOYLE M J, HE Z, et al. Organoid single-cell genomic atlas uncovers human-specific features of brain development [J]. Nature, 2019, 574(7778): 418-22.

[11] TRUJILLO C A, GAO R, NEGRAES P D, et al. Complex oscillatory waves emerging from cortical organoids model early human brain network development [J]. Cell Stem Cell, 2019, 25(4): 558-69,e7.

[12] XIANG Y, TANAKA Y, PATTERSON B, et al. Fusion of regionally specified hpsc-derived organoids models human brain development and interneuron migration [J]. Cell Stem Cell, 2017, 21(3): 383-98,e7.

[13] MADHAVAN M, NEVIN Z S, SHICK H E, et al. Induction of myelinating oligodendrocytes in human cortical spheroids [J]. Nat Methods, 2018, 15(9): 700-6.

[14] XIANG Y, TANAKA Y, CAKIR B, et al. hESC-derived thalamic organoids form reciprocal projections when fused with cortical organoids [J]. Cell Stem Cell, 2019, 24(3): 487-97,e7.

[15] CAKIR B, XIANG Y, TANAKA Y, et al. Engineering of human brain organoids with a functional vascular-like system [J]. Nat Methods, 2019, 16(11): 1169-75.

[16] QIAN X, SU Y, ADAM C D, et al. Sliced human cortical organoids for modeling distinct cortical layer formation [J]. Cell Stem Cell, 2020, 26(5): 766-81,e9.

[17] SHI Y, SUN L, WANG M, et al. Vascularized human cortical organoids (vOrganoids) model cortical development *in vivo* [J]. PLoS Biol, 2020, 18(5): e3000705.

[18] COLLIN J, QUEEN R, ZERTI D, et al. Deconstructing retinal organoids: single cell RNA-Seq reveals the cellular components of human pluripotent stem cell-derived retina [J]. Stem Cells, 2019, 37(5): 593-8.

[19] COWAN C S, RENNER M, DE GENNARO M, et al. Cell types of the human retina and its organoids at single-cell resolution [J]. Cell, 2020, 182(6): 1623-40,e34.

[20] KIM S, LOWE A, DHARMAT R, et al. Generation, transcriptome profiling, and functional validation of cone-rich human retinal organoids [J]. Proc Natl Acad Sci USA, 2019, 116(22): 10824-33.

[21] IKEDA H, OSAKADA F, WATANABE K, et al. Generation of $Rx^+/Pax6^+$ neural retinal precursors from embryonic stem cells [J]. Proc Natl Acad Sci USA, 2005, 102(32): 11331-6.

[22] MEYER J S, SHEARER R L, CAPOWSKI E E, et al. Modeling early retinal development with human embryonic and induced pluripotent stem cells [J]. Proc Natl Acad Sci USA, 2009, 106(39): 16698-703.

[23] NAKANO T, ANDO S, TAKATA N, et al. Self-formation of optic cups and storable stratified neural retina from human ESCs [J]. Cell Stem Cell, 2012, 10(6): 771-85.

[24] OSAKADA F, IKEDA H, MANDAI M, et al. Toward the generation of rod and cone photoreceptors from mouse, monkey and human embryonic stem cells [J]. Nat Biotechnol, 2008, 26(2): 215-24.

[25] ELDRED K C, HADYNIAK S E, HUSSEY K A, et al. Thyroid hormone signaling specifies cone subtypes in human retinal organoids [J]. Science, 2018, 362(6411): eaau6348.

[26] OSAKADA F, JIN Z B, HIRAMI Y, et al. *In vitro* differentiation of retinal cells from human pluripotent stem cells by small-molecule induction [J]. J Cell Sci, 2009, 122(Pt 17): 3169-79.

[27] ZHONG X, GUTIERREZ C, XUE T, et al. Generation of three-dimensional retinal tissue with functional photoreceptors from human iPSCs [J]. Nat Commun, 2014, 5: 4047.

[28] XIE H, ZHANG W, ZHANG M, et al. Chromatin accessibility analysis reveals regulatory dynamics of developing human retina and hiPSC-derived retinal organoids [J]. Sci Adv, 2020, 6(6): eaay5247.

[29] D'AMOUR K A, AGULNICK A D, ELIAZER S, et al. Efficient differentiation of human embryonic stem cells to definitive endoderm [J]. Nat Biotechnol, 2005, 23(12): 1534-41.

[30] SPENCE J R, MAYHEW C N, RANKIN S A, et al. Directed differentiation of human pluripotent stem cells into intestinal tissue *in vitro* [J]. Nature, 2011, 470(7332): 105-9.

[31] AURORA M, SPENCE J R. hPSC-derived lung and intestinal organoids as models of human fetal tissue [J]. Dev Biol, 2016, 420(2): 230-8.

[32] FINKBEINER S R, HILL D R, ALTHEIM C H, et al. Transcriptome-wide analysis reveals hallmarks of human intestine development and maturation *in vitro* and *in vivo* [J]. Stem Cell Rep, 2015, 4(6): 1140-55.

[33] UCHIDA H, MACHIDA M, MIURA T, et al. A xenogeneic-free system generating functional human gut organoids from pluripotent stem cells [J]. JCI Insight, 2017, 2(1): e86492.

[34] GREEN M D, CHEN A, NOSTRO M C, et al. Generation of

anterior foregut endoderm from human embryonic and induced pluripotent stem cells [J]. Nat Biotechnol, 2011, 29(3): 267-72.

[35] LOH K M, ANG L T, ZHANG J, et al. Efficient endoderm induction from human pluripotent stem cells by logically directing signals controlling lineage bifurcations [J]. Cell Stem Cell, 2014, 14(2): 237-52.

[36] CHEN Y J, FINKBEINER S R, WEINBLATT D, et al. *De novo* formation of insulin-producing "neo-beta cell islets" from intestinal crypts [J]. Cell Rep, 2014, 6(6): 1046-58.

[37] XUE X, RAMAKRISHNAN S, ANDERSON E, et al. Endothelial PAS domain protein 1 activates the inflammatory response in the intestinal epithelium to promote colitis in mice [J]. Gastroenterology, 2013, 145(4): 831-41.

[38] SERLS A E, DOHERTY S, PARVATIYAR P, et al. Different thresholds of fibroblast growth factors pattern the ventral foregut into liver and lung [J]. Development, 2005, 132(1): 35-47.

[39] VOLCKAERT T, CAMPBELL A, DILL E, et al. Localized Fgf10 expression is not required for lung branching morphogenesis but prevents differentiation of epithelial progenitors [J]. Development, 2013, 140(18): 3731-42.

[40] DYE B R, DEDHIA P H, MILLER A J, et al. A bioengineered niche promotes *in vivo* engraftment and maturation of pluripotent stem cell derived human lung organoids [J]. eLife, 2016, 5: e19732.

[41] DYE B R, HILL D R, FERGUSON M A, et al. *In vitro* generation of human pluripotent stem cell derived lung organoids [J]. eLife, 2015, 4: e05098.

[42] HUANG S X, ISLAM M N, O'NEILL J, et al. Efficient generation of lung and airway epithelial cells from human pluripotent stem cells [J]. Nat Biotechnol, 2014, 32(1): 84-91.

[43] CHEN Y W, HUANG S X, DE CARVALHO A, et al. A three-dimensional model of human lung development and disease from pluripotent stem cells [J]. Nat Cell Biol, 2017, 19(5): 542-9.

[44] HAN Y, YANG L, DUAN X, et al. Identification of candidate COVID-19 therapeutics using hPSC-derived lung organoids [J]. bioRxiv, 2020, doi: 10.1101/2020.05.05.079095.

[45] SALAHUDEEN A A, CHOI S S, RUSTAGI A, et al. Progenitor identification and SARS-CoV-2 infection in human distal lung organoids [J]. Nature, 2020, 588(7839): 670-5.

[46] TAKEBE T, SEKINE K, SEKINE K, et al. Vascularized and functional human liver from an iPSC-derived organ bud transplant [J]. Nature, 2013, 499(7459): 481-4.

[47] WU F, WU D, REN Y, et al. Generation of hepatobiliary organoids from human induced pluripotent stem cells [J]. J Hepatol, 2019, 70(6): 1145-58.

[48] WANG S, WANG X, TAN Z, et al. Human ESC-derived ex-pandable hepatic organoids enable therapeutic liver repopulation and pathophysiological modeling of alcoholic liver injury [J]. Cell Res, 2019, 29(12): 1009-26.

[49] TAGUCHI A, KAKU Y, OHMORI T, et al. Redefining the *in vivo* origin of metanephric nephron progenitors enables generation of complex kidney structures from pluripotent stem cells [J]. Cell Stem Cell, 2014, 14(1): 53-67.

[50] TAKASATO M, ER P X, CHIU H S, et al. Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis [J]. Nature, 2016, 536(7615): 238.

[51] TAGUCHI A, NISHINAKAMURA R. Higher-order kidney organogenesis from pluripotent stem cells [J]. Cell Stem Cell, 2017, 21(6): 730-46,e6.

[52] LOW J H, LI P, CHEW E G Y, et al. Generation of human PSC-derived kidney organoids with patterned nephron segments and a *de novo* vascular network [J]. Cell Stem Cell, 2019, 25(3): 373-87,e9.

[53] WU H, UCHIMURA K, DONNELLY E L, et al. Comparative analysis and refinement of human PSC-derived kidney organoid differentiation with single-cell transcriptomics [J]. Cell Stem Cell, 2018, 23(6): 869-81,e8.

[54] WIMMER R A, LEOPOLDI A, AICHINGER M, et al. Human blood vessel organoids as a model of diabetic vasculopathy [J]. Nature, 2019, 565(7740): 505-10.

[55] DRAKHLIS L, BISWANATH S, FARR C M, et al. Human heart-forming organoids recapitulate early heart and foregut development [J]. Nat Biotechnol, 2021, doi: 10.1038/s41587-021-00815-9.

[56] LEE J, SUTANI A, KANEKO R, et al. *In vitro* generation of functional murine heart organoids via FGF4 and extracellular matrix [J]. Nat Commun, 2020, 11(1): 4283.

[57] RICHARDS D J, LI Y, KERR C M, et al. Human cardiac organoids for the modelling of myocardial infarction and drug cardiotoxicity [J]. Nat Biomed Eng, 2020, 4(4): 446-62.

[58] ROSSI G, BROGUIERE N, MIYAMOTO M, et al. Capturing cardiogenesis in gastruloids [J]. Cell Stem Cell, 2021, 28(2): 230-40,e6.

[59] BARKER N, HUCH M, KUJALA P, et al. $Lgr5^+$ stem cells drive self-renewal in the stomach and build long-lived gastric units *in vitro* [J]. Cell Stem Cell, 2010, 6(1): 25-36.

[60] BARTFELD S, BAYRAM T, VAN DE WETERING M, et al. *In vitro* expansion of human gastric epithelial stem cells and their responses to bacterial infection [J]. Gastroenterology, 2015, 148(1): 126-36,e6.

[61] SATO T, STANGE D E, FERRANTE M, et al. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium [J]. Gastroenterology, 2011, 141(5): 1762-72.

[62] SATO T, VRIES R G, SNIPPERT H J, et al. Single Lgr5 stem cells build crypt-villus structures *in vitro* without a mesenchymal niche [J]. Nature, 2009, 459(7244): 262-5.

[63] LUKONIN I, SERRA D, CHALLET MEYLAN L, et al. Phenotypic landscape of intestinal organoid regeneration [J]. Nature, 2020, 586(7828): 275-80.

[64] QU M, XIONG L, LYU Y, et al. Establishment of intestinal organoid cultures modeling injury-associated epithelial regeneration [J]. Cell Res, 2021, 31(3): 259-71.

[65] LIN J Y, CHENG J, DU Y Q, et al. *In vitro* expansion of pancreatic islet clusters facilitated by hormones and chemicals [J]. Cell Discov, 2020, 6(1): 20.

[66] WANG D, WANG J, BAI L, et al. Long-term expansion of pancreatic islet organoids from resident $procr^+$ progenitors [J]. Cell, 2020, 180(6): 1198-211,e19.

[67] HUCH M, DORRELL C, BOJ S F, et al. *In vitro* expansion of single $Lgr5^+$ liver stem cells induced by Wnt-driven regeneration [J]. Nature, 2013, 494(7436): 247-50.

[68] KAWAGUCHI Y. Sox9 and programming of liver and pancreatic progenitors [J]. J Clin Invest, 2013, 123(5): 1881-6.

[69] LIN S, NASCIMENTO E M, GAJERA C R, et al. Distributed

hepatocytes expressing telomerase repopulate the liver in homeostasis and injury [J]. Nature, 2018, 556(7700): 244-8.

[70] WANG B, ZHAO L, FISH M, et al. Self-renewing diploid Axin2$^+$ cells fuel homeostatic renewal of the liver [J]. Nature, 2015, 524(7564): 180-5.

[71] HE L, PU W, LIU X, et al. Proliferation tracing reveals regional hepatocyte generation in liver homeostasis and repair [J]. Science, 2021, 371(6532): eabc4346.

[72] WEI Y, WANG Y G, JIA Y, et al. Liver homeostasis is maintained by midlobular zone 2 hepatocytes [J]. Science, 2021, 371(6532): eabb1625.

[73] ZHANG K, ZHANG L, LIU W, et al. *In vitro* expansion of primary human hepatocytes with efficient liver repopulation capacity [J]. Cell Stem Cell, 2018, 23(6): 806-19,e4.

[74] HU H, GEHART H, ARTEGIANI B, et al. Long-term expansion of functional mouse and human hepatocytes as 3D organoids [J]. Cell, 2018, 175(6): 1591-606,e19.

[75] PENG W C, LOGAN C Y, FISH M, et al. Inflammatory cytokine TNFalpha promotes the long-term expansion of primary hepatocytes in 3D culture [J]. Cell, 2018, 175(6): 1607-19,e15.

[76] HUCH M, GEHART H, VAN BOXTEL R, et al. Long-term culture of genome-stable bipotent stem cells from adult human liver [J]. Cell, 2015, 160(1/2): 299-312.

[77] ALOIA L, MCKIE M A, VERNAZ G, et al. Epigenetic remodelling licences adult cholangiocytes for organoid formation and liver regeneration [J]. Nat Cell Biol, 2019, 21(11): 1321-33.

[78] SUGIMOTO S, KOBAYASHI E, FUJII M, et al. An organoid-based organ-repurposing approach to treat short bowel syndrome [J]. Nature, 2021, 592(7852): 99-104.

[79] CHEN J, LAU B T, ANDOR N, et al. Single-cell transcriptome analysis identifies distinct cell types and niche signaling in a primary gastric organoid model [J]. Sci Rep, 2019, 9(1): 4536.

[80] CHEN K Y, SRINIVASAN T, LIN C, et al. Single-cell transcriptomics reveals heterogeneity and drug response of human colorectal cancer organoids [J]. Annu Int Conf IEEE Eng Med Biol Soc, 2018, 2018: 2378-81.

[81] ROMERO-CALVO I, WEBER C R, RAY M, et al. Human organoids share structural and genetic features with primary pancreatic adenocarcinoma tumors [J]. Mol Cancer Res, 2019, 17(1): 70-83.

[82] SUBRAMANIAN A, SIDHOM E H, EMANI M, et al. Single cell census of human kidney organoids shows reproducibility and diminished off-target cells after transplantation [J]. Nat Commun, 2019, 10(1): 5462.

[83] SUZUKI K, MURANO T, SHIMIZU H, et al. Single cell analysis of Crohn's disease patient-derived small intestinal organoids reveals disease activity-dependent modification of stem cell properties [J]. J Gastroenterol, 2018, 53(9): 1035-47.

[84] LEIN E, BORM L E, LINNARSSON S. The promise of spatial transcriptomics for neuroscience in the era of molecular cell typing [J]. Science, 2017, 358(6359): 64-9.

[85] MAYR U, SERRA D, LIBERALI P. Exploring single cells in space and time during tissue development, homeostasis and regeneration [J]. Development, 2019, 146(12): dev176727.

[86] MOOR A E, ITZKOVITZ S. Spatial transcriptomics: paving the way for tissue-level systems biology [J]. Curr Opin Biotechnol, 2017, 46: 126-33.

[87] XIA C, FAN J, EMANUEL G, et al. Spatial transcriptome profiling by MERFISH reveals subcellular RNA compartmentalization and cell cycle-dependent gene expression [J]. Proc Natl Acad Sci USA, 2019, 116(39): 19490-9.

[88] MAMEISHVILI E, SERAFIMIDIS I, IWASZKIEWICZ S, et al. Aldh1b1 expression defines progenitor cells in the adult pancreas and is required for Kras-induced pancreatic cancer [J]. Proc Natl Acad Sci USA, 2019, 116(41): 20679-88.

[89] YOSHIOKA T, FUKUDA A, ARAKI O, et al. Bmi1 marks gastric stem cells located in the isthmus in mice [J]. J Pathol, 2019, 248(2): 179-90.

[90] STANGE D E, KOO B K, HUCH M, et al. Differentiated Troy$^+$ chief cells act as reserve stem cells to generate all lineages of the stomach epithelium [J]. Cell, 2013, 155(2): 357-68.

[91] DROST J, VAN BOXTEL R, BLOKZIJL F, et al. Use of CRISPR-modified human stem cell organoids to study the origin of mutational signatures in cancer [J]. Science, 2017, 358(6360): 234-8.

[92] SUN L, WANG Y, CEN J, et al. Modelling liver cancer initiation with organoids derived from directly reprogrammed human hepatocytes [J]. Nat Cell Biol, 2019, 21(8): 1015-26.

[93] RINGEL T, FREY N, RINGNALDA F, et al. Genome-scale CRISPR screening in human intestinal organoids identifies drivers of TGF-beta resistance [J]. Cell Stem Cell, 2020, 26(3): 431-40,e8.

[94] MICHELS B E, MOSA M H, STREIBL B I, et al. Pooled *in vitro* and *in vivo* CRISPR-Cas9 screening identifies tumor suppressors in human colon organoids [J]. Cell Stem Cell, 2020, 26(5): 782-92,e7.

[95] ARTEGIANI B, HENDRIKS D, BEUMER J, et al. Fast and efficient generation of knock-in human organoids using homology-independent CRISPR-Cas9 precision genome editing [J]. Nat Cell Biol, 2020, 22(3): 321-31.

[96] DEMERS C J, SOUNDARARAJAN P, CHENNAMPALLY P, et al. Development-on-chip: *in vitro* neural tube patterning with a microfluidic device [J]. Development, 2016, 143(11): 1884-92.

[97] KIM S, LEE H, CHUNG M, et al. Engineering of functional, perfusable 3D microvascular networks on a chip [J]. Lab Chip, 2013, 13(8): 1489-500.

[98] VINING K H, MOONEY D J. Mechanical forces direct stem cell behaviour in development and regeneration [J]. Nat Rev Mol Cell Biol, 2017, 18(12): 728-42.

[99] DE GREGORIO V, TELESCO M, CORRADO B, et al. Intestine-liver axis on-chip reveals the intestinal protective role on hepatic damage by emulating ethanol first-pass metabolism [J]. Front Bioeng Biotechnol, 2020, 8: 163.

[100] PEZZULO A A, STARNER T D, SCHEETZ T E, et al. The air-liquid interface and use of primary cell cultures are important to recapitulate the transcriptional profile of *in vivo* airway epithelia [J]. Am J Physiol Lung Cell Mol Physiol, 2011, 300(1): L25-31.

[101] PRUNIERAS M, REGNIER M, WOODLEY D. Methods for cultivation of keratinocytes with an air-liquid interface [J]. J Invest Dermatol, 1983, 81(1 Suppl): 28s-33s.

[102] USUI T, SAKURAI M, UMATA K, et al. Preparation of human primary colon tissue-derived organoid using air liquid interface culture [J]. Curr Protoc Toxicol, 2018, 75: 2261-7.

[103] DEKKERS J F, WIEGERINCK C L, DE JONGE H R, et al. A functional CFTR assay using primary cystic fibrosis intestinal

organoids [J]. Nat Med, 2013, 19(7): 939-45.
[104] DANG J, TIWARI S K, LICHINCHI G, et al. Zika virus depletes neural progenitors in human cerebral organoids through activation of the innate immune receptor TLR3 [J]. Cell Stem Cell, 2016, 19(2): 258-65.
[105] WILSON P C, HUMPHREYS B D. Kidney and organoid single-cell transcriptomics: the end of the beginning [J]. Pediatr Nephrol, 2020, 35(2): 191-7.
[106] ZHANG Y S, ARNERI A, BERSINI S, et al. Bioprinting 3D microfibrous scaffolds for engineering endothelialized myocardium and heart-on-a-chip [J]. Biomaterials, 2016, 110: 45-59.
[107] JUNKER J P, NOEL E S, GURYEV V, et al. Genome-wide RNA tomography in the zebrafish embryo [J]. Cell, 2014, 159(3): 662-75.
[108] QIAN X, NGUYEN H N, SONG M M, et al. Brain-region-specific organoids using mini-bioreactors for modeling ZIKV exposure [J]. Cell, 2016, 165(5): 1238-54.