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向阳飞，上海科技大学生命科学与技术学院研究员，博导，干细胞与神经生物学实验室负责人，国家重点研发计划首席科学家。主要研究方向是人脑类器官模型构建及应用，在过去的十多年研究中，陆续建立了不同的脑区特异类器官及整合不同脑区、不同细胞类型的复合脑类器官，国际上较早实现了跨脑区连接、神经–血管共发育、亚脑区特异类器官、多组织复合类器官等体外模型的构建。

复合脑类器官技术及其在发育与疾病模拟中的应用

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摘要 脑类器官是由多能干细胞经体外诱导分化形成的能再现脑结构特征和生理功能的三维神经组织。脑区和亚脑区特异性类器官能再现特定脑区或核团的结构特征、细胞类型和生理功能。复合脑类器官技术包含多脑区和多谱系类器官技术，前者可用于研究神经元迁移、神经环路连接、脑区发育等科学问题，后者则包含血管化、免疫细胞互作等类器官技术。目前脑类器官技术已用于神经疾病模拟、机制解析和治疗策略开发。该文重点综述了现阶段建立的脑区特异性类器官模型，以及复合脑类器官技术的发展与应用。

关键词 脑类器官; 脑区特异性类器官; 复合脑类器官; 神经发育; 血管化; 疾病模拟

Advances in Integrated Brain Organoids and Their Applications in Modeling Development and Disease

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Abstract Brain organoids are three-dimensional neural cultures derived from pluripotent stem cells, which can recapitulate certain structural and functional features of the brain. Region- or subregion-specific brain organoids closely resemble the cell types, structures, and physiological functions of specific brain regions or nuclei. Integrated

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brain organoids contain multiple brain regions or cells from distinct lineages. The former can be used to study neuronal migration, neural circuit connections, and brain region development, among others, while the latter includes vascularization, brain-immune interactions, and others. Brain organoids have been utilized to model neurological disorders, dissect disease mechanisms, and discover therapeutic strategies. This review focuses on recent progress in brain organoid technologies, including the development and application of integrated brain organoids.

Keywords brain organoids; region-specific brain organoids; integrated brain organoids; neurodevelopment; vascularization; disease model

脑类器官是指干细胞在体外分化、自组织形成的三维(three-dimensional, 3D)培养物, 可以重现人脑的细胞类型、组织结构以及生理功能。与二维培养相比, 脑类器官能展现更为复杂的组织结构以及更多样的细胞类型。目前脑类器官技术主要分为非定向分化构建的脑类器官, 以及定向诱导分化构建的脑区特异性类器官。LANCASTER等[1]于2013年报道了非定向分化的脑类器官, 其具有类似大脑结构特征。除了非定向分化的脑类器官外, 脑区特异性类器官如模拟皮层[2-3]、丘脑[4]、下丘脑[5-6]、纹状体[7]、中脑[8-9]、小脑[10]等区域的方案也逐渐得到建立。脑区特异性类器官虽然更精确地代表特定脑区结构, 但忽略了在发育过程和生理病理过程中, 脑区之间以及脑区和非神经谱系细胞的互作关系。而复合脑类器官技术则弥补了这个缺陷, 复合脑类器官包含多脑区类器官和多谱系类器官, 前者是将多种脑区特异性类器官进行组装实现不同脑区间的连接, 后者则是将脑类器官与血管组织等其他谱系细胞进行整合, 以用来更好地模拟脑区发育以及脑区间、组织间互作。

1 脑类器官技术概述

脑类器官技术的建立主要基于神经发育理论的积累以及干细胞技术的发展。脑作为人体器官中最为复杂的器官之一, 难以直接被研究, 且由于物种差异, 以传统模式动物作为模型来研究人脑发育和疾病机制的解释度有限, 而体外诱导脑类器官则为脑科学研究提供了有效的研究模型。随着干细胞技术的出现和发展, ZHANG等[11]在2001年发现神经干细胞可形成模拟神经管形态的玫瑰花环结构。但二维培养体系中细胞类型较为简单, 难以再现复杂的体内组织结构。研究者开始探索构建体外三维培养体系。在2013年, LANCASTER等[1]成功建立了首个基于人多能干细胞(human pluripotent stem cells, hPSCs)非定向分化的三维脑类器官模型, 该模型可再现人脑皮层发育的特征。同期, TAISUKE等[12]通过人胚胎干细胞(human embryonic stem cells, hESCs)的定向分化, 模拟了大脑新皮层发育过程中的复杂动态。2017年报道的三项研究, 首次在人类多能干细胞空间组装的基础上构建复合类器官, 将不同类器官进行融合以实现功能整合, 再现神经元的迁移过程[3,13-14]。XIANG等[4]在2019年建立的首个三维脑类器官投射模型再现了丘脑–皮层之间的双向投射。随后, 复合类器官模型逐渐向多元拓展, 越来越多涵盖不同脑区、跨组织的复合脑类器官模型被构建。

目前, 脑类器官体系主要可分为两类: 非定向性脑类器官与区域特异性脑类器官。在无血清、无其他外源因子条件下, 多能干细胞可自发分化为神经外胚层, 经神经上皮扩增、长期成熟后即可得到非定向分化脑类器官。自2013年首个基于人多能干细胞非定向分化的脑类器官模型被构建后, 多种非定向分化方法也陆续被建立[15-19]。非定向分化脑类器官更适用于宽泛地研究人脑。但人脑中不同脑区、核团中细胞组成不同, 分别执行着不同的生理功能。因此, 开发可用于研究特定脑结构的脑区特异性类器官模型具有重要意义。脑区特异性类器官的诱导是通过在体外模拟体内脑区发育所需的形态发生信号和模式形成信号通路实现的。例如, 目前利用SMAD抑制骨形态发生蛋白(bone morphogenetic protein, BMP)通路和转化生长因子-β(transforming growth factor-beta, TGF-β)通路来诱导神经外胚层身份, 通过激活音猬因子(sonic hedgehog, SHH)信号促进腹侧化, 激活WNT信号激活尾侧化等。近期, 有研究将拟胚体暴露在形态发生素的正交梯度环境中, 从而构建了不同脑区的类器官[20]。通过模式发生调控因子来影响三维分化中细胞命运决定是目前广泛认可的脑区特异性类器官的诱导方法。

2 脑区特异性类器官技术

脑区特异性类器官通过模拟不同脑区、核团的体内发育调控来进行体外诱导, 以实现对特定脑区结构的再现, 以期更为细致地针对不同脑区、核团进行研究。从胚胎发育角度来看, 中枢神经系统(central nervous system, CNS)由神经管发育而来, 神经管根据发育轴可分为前脑(包含端脑、间脑、视泡)、中脑和后脑(包含小脑、脑桥、延髓)。目前, 已经建立了一系列脑区特异性类器官体系, 它们可在一定程度上再现人脑特定区域的体内特征和功能(图1和表1)。

2.1 前脑类器官

由于大脑皮层是人们最为关注的脑区之一, 同时也是最早成功建立的脑区特异性类器官, 因此皮层类器官方法的建立受到了众多研究者的广泛关注。自2013年起, 一系列皮层类器官构建方法先后被建立[2-3,12,21-29]。研究者通过抑制SMAD通路, 诱导干细胞向神经外胚层分化, 通过添加WNT通路抑制剂使分化命运限制在端脑谱系[12], 该阶段的拟胚体可自发模拟背侧端脑模式发生[30], 即自发分化为皮层类器官。PAŞCA等[22]通过抑制SMAD通路, 添加成纤维细胞生长因子2(fibroblast growth factor 2, FGF2)等小分子, 成功构建出含有星型胶质细胞以及功能性皮层深层和表层神经元的皮层类器官。XIANG等[3]通过抑制SMAD通路以及WNT通路, 构建了皮层类器官。QIAN等[27]通过建立微型生物反应器、优化神经诱导因子等, 建立了可模拟六个皮层神经元发生的脑类器官, 可再现人大脑皮层发育的关键特征, 并通过切片培养减少了脑类器官培养过程中内部缺氧导致的细胞坏死[2]。

除了皮层类器官外, 其他前脑脑区特异性类器官方法也在过去几年逐渐被建立, 包括腹侧端脑类器官[3,13], 视杯类器官[31-32]、脉络丛类器官[33-35]、海马体类器官[33,35-38]、纹状体类器官[7,39]、丘脑类器官[4,29,40-42]、下丘脑类器官[5-6,27]等。前脑包含视泡, 视泡的远端内陷形成视杯, 视杯进一步发育为视网膜, 视泡的近端部分发育为视神经。视杯和视网膜类器官的研究引起了广泛关注[31-32,43-49]。2011年EIRAKU等[32]建立了含有多种视网膜细胞类型的小鼠视杯类器官。2012年NAKANO等[31]通过先抑制WNT通路, 再激活SHH通路和WNT通路, 成功构建了人视杯类器官。GABRIEL等[43]生成了具有两侧对称视泡的脑类器官, 其中包含原始角膜上皮细胞、晶状体类似细胞、视网膜色素上皮、视网膜祖细胞等发育视泡的细胞类型。ZHONG等[49]成功诱导产生具有成熟光感受器细胞的视网膜类器官。ELDRED

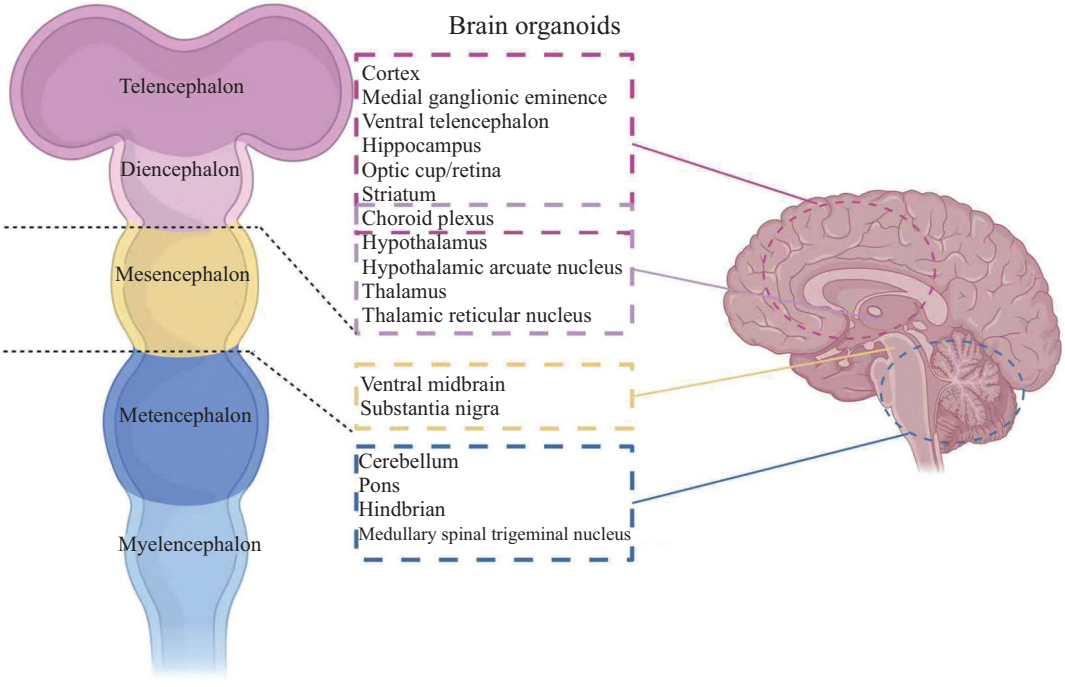


图1 多能干细胞衍生的脑区特异性类器官模型

Fig.1 Brain region-specific organoid models derived from pluripotent stem cells

表1 脑区特异性类器官的培养条件

Table 1 Culture conditions for region-specific brain organoids

脑类器官类型 Organoid type	脑区 Target region	起始状态 Starting status	诱导分子 Inductive molecule	模式化分子 Patterning molecule	基质或其他成分 Matrix or other components	长期培养成分 Long term culture	参考文献 References
Forebrain organoid	Cortex	Detached colonies	Dorsomorphin A83-01 SB431542 WNT-3A CHIR99021	NA	Matrigel	BDNF GDNF Ascorbic acid SpinΩ cAMP TFGβ	QIAN et al., 2016 [27]
Cortical organoid	Cortex	Dissociated cells	IWR1e SB431542	NA	Matrigel	Static with 40% O_2	KADOSHIMA et al., 2013 [12]
Cortical organoid	Cortex	Dissociated cells	SB431542 XAV939 LDN193189	NA	No matrix	cAMP Orbital shaker BDNF Ascorbic acid	XIANG et al., 2017 [3]
Cortical spheroid	Cortex	Detached colonies	Dorsomorphin SB431542	NA	FGF2 EGF	Static BDNF NT3	PASCA et al., 2015 [22]
Cortical organoid	Cortex	Dissociated cells	Dorsomorphin SB431542	FGF2	FGF2 EGF	Rotation BDNF GDNF NT-3 Ascorbic acid cAMP	TRUJILLO et al., 2019 [23]
Forebrain organoid	Cortex	Dissociated cells	IWR1 SB431542	NA	Matrigel	Orbital agitation FBS Heparin	VELASCO et al., 2019 [24]
Cortical organoid	Cortex	Dissociated cells	Dorsomorphin SB431542 XAV-939	NA	EGF FGF2	BDNF NT3	YOON et al., 2019 [25]
Cortical organoid	Cortex	Dissociated cells	Noggin	NA	No matrix	Static Ascorbic acid	MARIANI et al., 2015 [26]

续表1

脑类器官类型 Organoid type	脑区 Target region	起始状态 Starting status	诱导分子 Inductive molecule	模式化分子 Patterning molecule	基质或其他成分 Matrix or other components	长期培养成分 Long term culture	参考文献 References
Cortical organoid	Cortex	Dissociated cells	Dorsomorphin SB431542	NA	FGF EGF	Orbital shaker BDNF GDNF NT-3 Ascorbic acid cAMP	FITZGERALD et al., 2024 [28]
Cortical organoid	Cortex	Dissociated cells	SB431542 Dorsomorphin IWR1e Thiazovivin	IWR1e SB431542	bFGF EGF Matrigel	Orbital shaker BDNF GDNF cAMP Thiazovivin DAPT Ascorbic acid FBS	NITYANANDAM et al., 2025 [29]
MGE organoid	MGE	Dissociated cells	SB431542 XAV939 LDN193189	Purmorphamine SHH	NA	BDNF cAMP Ascorbic acid Orbital shaker	XIANG et al., 2017 [3]
Subpallium spheroid	Ventral telencephalon	Detached colonies	Dorsomorphin SB431542	IWP-2 SAG	FGF2 EGF	BDNF NT3 Static	BIREY et al., 2017 [13]
Thalamus organoid	Thalamus	Dissociated cells	BMP7 MEKi SAG FGF8b	NA	bFGF EGF Matrigel	Orbital shaker BDNF GDNF cAMP Thiazovivin DAPT Ascorbic acid FBS	NITYANANDAM et al., 2025 [29]
Thalamus organoid	Thalamus	Dissociated cells	SB431542 LDN193189 Insulin	BMP7 PD0325901	NA	Orbital shaker BDNF Ascorbic acid	XIANG et al., 2019 [4]
Thalamus organoid	Thalamus	Dissociated cells	SB431542 LDN193189 Insulin	BMP7 PD0325901	NA	Orbital shaker BDNF Ascorbic acid	SHIN et al., 2024 [40]

续表1

脑类器官类型 Organoid type	脑区 Target region	起始状态 Starting status	诱导分子 Inductive molecule	模式化分子 Patterning molecule	基质或其他成分 Matrix or other components	长期培养成分 Long term culture	参考文献 References
Ventralized human thalamic organoid	Thalamic reticular nucleus	Dissociated cells	SB431542 LDN193189 Insulin	PD325901 BMP7	SHH	Orbital shaker BDNF Ascorbic acid	KIRAL et al. 2023 [41]
Thalamus organoid	Thalamus	Dissociated cells	SB431542 Insulin Dorsomorphin SAG FGF8b	PD325901 BMP7	Matrigel bFGF EGF	Orbital shaker BDNF GDNF cAMP DAPT Ascorbic acid	PATTON et al., 2024 [42]
Hypothalamus organoid	Hypothalamus	Detached colonies	LDN193189 SB431542	Purmorphamine SHH WNT3A	Matrigel	SpinΩ CTNF FGF2	QIAN et al., 2016 [27]
Hypothalamus organoid	Hypothalamus	Dissociated cells	KSR BMP4 SAG	KSR SAG 40% O_2	NA	KSR SAG 40% O_2	KASAI et al., 2020 [5]
Arcuate organoid	Hypothalamic arcuate nucleus	Detached colonies	LDN A83-01 IWR1-endo SAG Purmorphamine SHH	IWR1-endo SAG Purmorphamine SHH	Mouse hypothalamus astrocytes	Orbital shaker BDNF GDNF Dibutyryl-cAMP Ascorbic acid	HUANG et al., 2021 [6]
Striatum organoid	Striatum	Dissociated cells	SB431542 DMH1	SHH	FBS	NA	WU et al., 2024 [7]
Striatum organoid	Striatum	Dissociated cells	DMH1 SB431542	SHH	FBS	NA	LIU et al., 2022 [39]
Striatum organoid	Striatum	Dissociated cells	SAG IWP2	NA	Matrigel	Orbital shaker Ascorbic acid Insulin	REUMANN et al., 2023 [8]
Choroid plexus organoid	Choroid plexus	Dissociated cells	KSR SB431542 IWR1e	CHIR99021 BMP4	FBS	Static with 40% O_2	SAKAGUCHI et al., 2015 [33]
Choroid plexus organoid	Choroid plexus	Dissociated cells	STEMdiffTM cerebral organoid kit (NI media)	CHIR99021 BMP4	Matrigel	Orbital shaker Ascorbic acid	PELLEGRINI et al., 2020 [34]

续表1

脑类器官类型 Organoid type	脑区 Target region	起始状态 Starting status	诱导分子 Inductive molecule	模式化分子 Patterning molecule	基质或其他成分 Matrix or other components	长期培养成分 Long term culture	参考文献 References
Choroid plexus organoid	Choroid plexus	Detached colonies	KSR LDN193189 SB431542 IWP-2	CHIR99021 BMP7	NA	Orbital shaker BDNF GDNF	JACOB et al., 2020 [35]
Hippocampal organoid	Hippocampus	Detached colonies	LDN193189 Cyclopamine SB431542 XAV939	CHIR99021 BDNF	NA	CHIR99021 BDNF	POMESHCHIK et al., 2020 [36]
Hippocampal organoid	Hippocampus	Dissociated cells	KSR SB421542 XAV939 LDN193189 Cyclopamine FBS BMP4 CHIR99021	WNT3a Purmorphamine	NA	BDNF GDNF	WU et al., 2024 [37]
Hippocampal organoid	Hippocampus	Detached colonies	DKK1 Cyclopamine Noggin SB431542	FGF2 WNT3a BDNF	Hippocampal astrocytes Laminin	FGF2 BDNF Ascorbic acid cAMP FBS	YU et al., 2014 [38]
Optic cup	Optic cup	Dissociated cells	IWR1e	CHIR99021 SAG FBS	Matrigel	40% O_2 Retinoic acid FBS	NAKANO et al., 2012 [31]
Optic vesicle organoid	Optic vesicle	Dissociated cells	Insulin Retinol acetate SB431542	Retinol acetate Dorsomorphin SB431542	Matrigel	FGF2	GABRIEL et al., 2021 [43]
Retinal organoid	Retina	Dissociated cells	BMP4 LDN193189 FBS	FBS	Taurine	Retinoic acid BDNF DHA	HARKIN et al., 2024 [45]
Retinal organoid	Retina	Dissociated cells	BMP4	FBS	Taurine PolyHEMA	Retinoic acid	CAPOWSKI e al., 2019 [46]
Retinal organoid	Retina	Dissociated cells	NA	FBS	Taurine Matrigel	Retinoic acid	COWAN et al., 2020 [47]

续表1

脑类器官类型 Organoid type	脑区 Target region	起始状态 Starting status	诱导分子 Inductive molecule	模式化分子 Patterning molecule	基质或其他成分 Matrix or other components	长期培养成分 Long term culture	参考文献 References
Retinal organoid	Retina	Dissociated cells	IWR1e	SAG	Matrigel	Retinoic acid DAPT	ELDRED et al., 2018 [48]
Retinal organoid	Retina	Detached colonies	NA	NA	Taurine	FBS Retinoic acid	ZHONG et al., 2020 [49]
Substantia nigra organoid	Substantia nigra	Dissociated cells	SB431542 DMH1 SHH CHIR	SAG SHH CHIR FGF8b	NA	NA	WU et al., 2024 [7]
Midbrain organoid	Ventral midbrain	Dissociated cells	Noggin SB431542 CHIR99021 SAG FGF8	SAG FGF8	Matrigel	Orbital shaker Ascorbic acid Insulin	REUMANN et al., 2023 [8]
Midbrain organoid	Ventral midbrain	Dissociated cells	SB431542 Noggin SHH-C24II CHIR99021	FGF8b	Laminin-111	BDNF Ascorbic acid GDNF cAMP DAPT	NOLBRANT et al., 2017 [9]
Midbrain organoid	Ventral midbrain	Dissociated cells	SB431542 Noggin SHH-C24II CHIR99021	FGF8b	Matrigel	BDNF Ascorbic acid GDNF cAMP DAPT	FIORENZANO et al., 2021 [50]
Midbrain organoid	Ventral midbrain	Dissociated cells	SB431542 Noggin SHH CHIR99021	FGF8b	Matrigel	BDNF Ascorbic acid GDNF cAMP DAPT	SOZZI et al., 2022 [51]
Midbrain organoid	Ventral midbrain	Detached colonies	SB431542 DMH1 SHH CHIR99021	SAG FGF8b	Matrigel	SHH FGF8b	ZHU et al., 2022 [52]

续表1

脑类器官类型 Organoid type	脑区 Target region	起始状态 Starting status	诱导分子 Inductive molecule	模式化分子 Patterning molecule	基质或其他成分 Matrix or other components	长期培养成分 Long term culture	参考文献 References
Midbrain organoid	Midbrain	Dissociated cells	Noggin SB431542 CHIR99021	SHH FGF8	Matrigel	BDNF GDNF Ascorbic acid cAMP Orbital shaker	JO et al., 2016 [53]
Midbrain organoid	Midbrain	Detached colonies	LDN193189 SB431542 SHH Purmorphamine FGF8	LDN193189 CHIR99021 SHH Purmorphamine FGF8	Matrigel	BDNF GDNF Ascorbic acid SpinΩ cAMP TGFβ	QIAN et al., 2016 [27]
Cerebellum organoid	Cerebellum	Dissociated cells	SB431542 FGF2 Insulin	FGF19	SDF1	NA	MUGURUMA et al., 2015 [54]
Cerebellum organoid	Cerebellum	Dissociated cells	SB431542 Noggin CHIR99021 FGF8b	NA	Matrigel	Orbital agitation T3 BDNF Matrigel	ATAMIAN et al., 2024 [10]
Hindbrain organoid	Hindbrain	Dissociated cells	RA Purmorphamine	NA	Matrigel	Orbital agitation BDNF	VALIULAHI et al., 2021 [55]
Hindbrain organoid	Hindbrain	Dissociated cells	SB431542 CHIR99021 DMH1	CHIR99021 SHH FGF4	Laminin	Orbital shaker GDNF BDNF TGFβ3 IGF-I Ascorbic acid	ZIVKO et al., 2024 [56]
Brainstem organoid	Brainstem	Dissociated cells	Dorsomorphin SB431542 Insulin Progesterone	bFGF Insulin Progesterone	EGF	Orbital shaker Ascorbic acid cAMP BDNF GDNF NT-3	EURA et al., 2020 [57]
Medullary spinal trigeminal nucleus organoid	Medullary spinal trigeminal nuclei	Dissociated cells	LDN193189 SB431542 CHIR99021 Retinoic acid	CHIR99021	Matrigel EGF NGF	BDNF Ascorbic acid Orbital shaker	PANG et al., 2024 [58]

NA: 类器官培养中, 除常规培养基成分外, 未额外添加此类成分。

NA: no extra such molecules were used in organoid culture except the normal components of the culture medium.

等[48]发现甲状腺激素可以调控视网膜类器官中视锥细胞亚型的分化命运，这增加了对人类视网膜发育机制的了解，且有望在治疗和视力修复方面发挥作用。近年来，视网膜类器官在不同细胞系的可重复性也得到了关注[45-46]。

脉络丛对于维持CNS的稳定和正常生理功能至关重要。SAKAGUCHI等[33]在原构建新皮层类器官的分化方案基础上[12]，通过联用CHIR99021和骨形态发生蛋白4(bone morphogenetic protein 4, BMP4)成功构建了脉络丛类器官。PELLEGRINI等[34]开发的脉络丛类器官具有高度复杂的折叠组织形态，可形成含有类似脑脊液的腔室。JACOB等[35]构建的脉络丛类器官具有与体内相似的基因表达情况，他们发现其对SARS-CoV-2具有高易感率，该模型可用于研究SARS-CoV-2感染模式。因为与海马体发育相关的内侧皮层位于脑室脉络丛附近，研究者通过调整脉络丛类器官分化方案中背侧化处理的时机、CHIR99021或BMP4的浓度以及处理时间构建了海马体类器官，该类器官含有功能性海马体颗粒状和锥体状神经元[33]。

此外，XIANG等[4]通过抑制SMAD通路、添加胰岛素实现神经外胚层组织尾侧化，进一步添加骨形态发生蛋白7(bone morphogenetic protein 7, BMP7)等诱导产生了丘脑类器官。SHIN等[40]在此分化方法基础上，发现22q11.2缺失会导致丘脑神经元和胶质细胞转录失调，并会导致丘脑轴突的过度生长。QIAN等[27]通过抑制SMAD通路，激活WNT3A、SHH信号通路构建了下丘脑类器官。XIANG等[3]先抑制SMAD通路以及WNT通路，再通过添加视黄酸(retinoic acid, RA)、SHH、Purmorphamine构建了腹侧端脑内侧神经节隆起(medial ganglionic eminence, MGE)类器官。

2.2 中脑类器官

中脑多巴胺能神经元与多种神经退行疾病相关，如帕金森病(Parkinson's disease, PD)、亨廷顿病(Huntington's disease, HD)等，因此中脑类器官受到广泛关注，目前已经有多种中脑类器官分化方案[7-9,50-53]。JO等[53]于2016年首次构建含有功能性中脑多巴胺能神经元的中脑类器官模型，在此模型中可以检测到多巴胺的产生，且能观察到类似神经黑色素的颗粒，可能与体内黑质区域有关。NOLBRANT等[9]通过抑制SMAD通路，利用SHH和CHIR99021分别诱导腹侧化和尾侧化，并添加成纤维细胞生长因子8b(fibroblast growth factor 8b, FGF8b)确定中脑后脑边界，成功建立了含有多巴胺能神经元的中脑类器官。而REUMANN等[8]则将SHH替换为Smoothened激活剂(smoothened agonist, SAG)来诱导腹侧化。ZHU等[52]联用SHH和SAG来诱导含有多巴胺能神经元的中脑类器官。WU等[7]在此基础上调整分化条件，成功在体外诱导出了中脑黑质类器官，与纹状体类器官组装再现了亨廷顿病的神经元投射缺陷，这为研究神经退行性疾病提供了新的平台。

2.3 后脑类器官

后脑可分为脑桥、小脑和延髓。小脑细胞类型复杂、结构精细，目前报道的小脑类器官方法较少。MUGURUMA等[54]通过添加成纤维细胞生长因子2(fibroblast growth factor 2, FGF2)、胰岛素(insulin)促进类器官尾部化，建立了中后脑边界结构，并通过进一步添加成纤维细胞生长因子19(fibroblast growth factor 19, FGF19)、基质细胞衍生因子-1(stromal cell-derived factor-1, SDF-1)促进了小脑上皮样组织的产生。ATAMIAN等[10]于2024年建立了含有功能性浦肯野细胞的小脑类器官模型，研究者使用FGF8b确定中后脑边界特征，并利用CHIR99021激活WNT通路，使其尾侧化，促进神经外胚层向小脑命运分化。该小脑类器官能暂时地表现出类似小脑的层状结构，且具有相对完整的神经功能网络。VALIULAHI等[55]提出了一种从hPSCs诱导生成含有5-羟色胺(5-hydroxytryptamine, 5-HT)神经元的后脑类器官模型方法，研究者通过添加RA促进尾侧化，并通过添加Purmorphamine激活SHH信号通路来促进腹侧化，获得了含有5-羟色胺神经元的后脑类器官。ZIVKO等[56]通过添加SHH和FGF4，建立了含有5-HT能神经元的后脑类器官模型，并将其应用于评估阿尔茨海默病(Alzheimer's disease, AD)患者的神经精神症状的发展和药物治疗的个体差异，为其他神经精神症状相关疾病的体外评估提供了参考。脑干是大脑的后部区域，连接大脑皮层与脊髓，由中脑、脑桥和延髓三部分组成。EURA等[57]成功建立了含中脑/后脑祖细胞、去甲肾上腺素能及胆碱能神经元、多巴胺能神经元以及神经嵴谱系细胞的脑干类器官。

2.4 核团特异性类器官

虽然目前已经建立了多种脑区特异性类器官，但能再现特异性核团的类器官模型仍处于早期阶段[6,41,58]。KIRAL等[41]在此前建立的丘脑类器官方法基础上[4]，通过添加SHH激活腹侧化信号通路，成功建立了腹侧丘脑类器官，并再现了丘脑网状核特征。HUANG等[6]通过多种小分子联合激活SHH信号通路，并抑制WNT通路，生成了具有弓状核样特征的下丘脑类器官。PANG等[58]通过补充CHIR99021和RA来激活尾侧化信号，开发了一种具有三叉神经脊束核(spinal trigeminal nucleus, SpV)特征的延髓类器官，可用于再现背侧延髓的三叉神经脊束核的特征，填补了延髓体外模型的空缺。

3 复合类器官技术

人体的许多生理和病理过程与跨器官间的交流密切相关，包括经典内分泌系统中的激素分泌和反馈调节[59]，以及CNS中通过大脑介导的神经递质和神经肽传递。虽然传统的单一类器官模型可以模拟特定组织的发育特征，但难以重现器官间复杂的系统互作，尤其是神经系统疾病中多脑区协同功能障碍。随着复合类器官技术的出现，将单独培养的脑区特异性类器官融合，可生成“多脑区组装体”(表2)。这种组装体可以结合多种类器官类型，解析区域间相互作用，甚至可直接将缺失的细胞类型合并到同一类器官中进行研究[60-61]。如XIANG团队[58]利用定向分化策略构建了SpV类器官模型，将其与丘脑、皮层类器官进行整合，成功在SpV神经元与丘脑神经元之间建立了连接。复合类器官技术的出现为研究特定神经元细胞间的相互作用、重现脑区间信号转导及神经环路的发育提供了新途径[62]。

3.1 神经元迁移模拟

神经元迁移是大脑发育的核心环节。神经元一旦形成，则会迁移到大脑的特定区域以促进神经元间短距离和长距离连接的建立。人类神经元迁移特有的长时程、高复杂性使得其体内迁移微环境比其他模式动物(如小鼠)更加难以模拟[63]。而基于多脑区组装、功能单元整合及动态调控的复合脑类器官技术，为深入研究神经元迁移过程提供了更有效的模型。

基于人类多能干细胞的空间组装技术，复合类器官构建的发展推动了跨脑区神经元迁移的研究，特别是γ-氨基丁酸能中间神经元(GABAergic interneurons)的迁移网络。研究人员通过将诱导生成的腹侧端脑MGE类器官与背侧皮层类器官进行三维共培养[13]，成功构建了具有功能性神经连接的跨脑区复合类器官模型。这类三维培养系统能模拟神经元发育微环境，并动态解析中间神经元迁移中的细胞互作、分子调控及神经环路整合机制。BAGLEY等[14]利用背腹轴复合类器官，揭示了CXCR4信号通路在迁移中的作用；XIANG团队[3]以MGE类器官为研究对象，再现了非肌肉型肌球蛋白II对迁移运动的调控。

此外，组装体还可以用来研究神经嵴细胞迁移等复杂过程。神经嵴细胞的迁移受分泌分子、细胞外基质成分及细胞间相互作用的调控[64-65]。对神经嵴细胞与神经类器官组装进行研究，可以揭示调控神经嵴细胞命运决定的信号通路与特定分子在迁移中的作用[66]。值得注意的是，融合类器官中的神经元表现出更高的放电频率，这提示跨脑区的整合可诱导产生单一类器官中不存在的功能性神经元特征。

3.2 环路连接模拟

神经发育中，神经元迁移至目标区域定位后[67]，胚胎会分泌特定的趋化因子促进轴突在神经元微环境中的延伸[68]。这一关键发育过程依赖于轴突导向机制和细胞黏附分子之间的协同调控[69-70]。CNS中神经元组织成具有一定空间特异性的网络或环路，其功能活动由不同脑区和局部神经环路的精准协调和整合机制共同决定[71]。因此类器官组装体模型的出现可以有效模拟神经细胞间的动态相互作用，尤其是轴突间的引导。2019年，XIANG等[4]在体外建立了首个三维脑类器官投射模型，通过hESCs构建了丘脑类器官，并与皮层类器官进行了融合，首次再现了丘脑–皮层之间的双向投射，为神经环路的研究提供了新视角。近年来，体外构建神经元环路的研究持续深入且进展显著。研究人员融合人类大脑皮层类器官(human cortical spheroids, hCSs)与纹状体类器官(human striatal spheroids, hStrSs)，成功构建皮层–纹状体组装体模型，再现hCSs神经元向hStrSs的定向轴突投射和突触连接过程[72]。在此基础上，研究人员开发由腹侧中脑、纹状体和皮层组织组成的复合类器官，以建立腹侧中脑多巴胺能神经元向目标区域投射的模型[8]。基于中脑–纹状

表2 组装体类型与应用

Table 2 Applications of assembloid models

组装体类型 Assembloids	发育模式 Patterns	应用场景 Applications	参考文献 References
SpV-thalamus-cortex assembloid	Fusion of SpV-specific organoids with thalamic and cortical organoids	Modeling axonal projections from SpV to thalamus and subsequent synaptogenesis	[58]
Ventral telencephalon-dorsal cortex assembloid I	Fusion of MGE with cortical organoids	Modeling migratory defects observed in Timothy syndrome	[13]
Ventral telencephalon-dorsal cortex assembloid II	Fusion of hCS (human cortical spheroids) with hSS (human subpallial spheroids)	Reproducing CXCR4-mediated migratory signaling	[14]
Ventral telencephalon-dorsal cortex assembloid III	Fusion of MGE with cortical organoids	Reproducing the role of nonmuscle myosin II in migration of human MGE progenitors	[3]
Thalamus-cortex assembloid	Fusion of thalamic and cortical organoids	Modeling reciprocal axon connectivity between human thalamus and cortex	[4]
Cortex-striatum assembloid	Fusion of hCSs with hStrSs	Modeling axonal projections from cortical neurons to striatal organoids and subsequent synaptogenesis	[72]
Midbrain-striatum-cortex assembloid	Fusion of ventral midbrain, striatal and cortical tissues	Modeling the dopaminergic projections into striatal and cortical tissues	[8]
Cortico-motor assembloid	Fusion of cortical, spinal cord, and skeletal muscle organoids	Forming an intact, three-component cortico-motor circuit	[74]
Ascending neural sensory pathway assembloid	Fusion of peripheral sensory neurons, dorsal spinal cord (and hindbrain) projection neurons, thalamic neurons and glutamatergic cortical neurons	Modeling a four-component ascending sensory circuit going from peripheral sensory neurons to cortical neurons	[75]
Neuromusculoskeletal tri-tissue organoids	Co-differentiation of hNMSOs (human pluripotent stem cells into NMS tri-tissue organoids)	Modeling motor neuron regulation of skeletal muscle contraction via NMJs (neuromuscular junctions)	[76]
Vascularized cortical organoid I	Ectopic expression of ETV2 in cortical organoids to induce endothelial cell generation	Forming functional vascular networks, enhancing cellular viability	[81]
Vascularized cortical organoid II	Co-culture of hESCs with HUVECs (human umbilical vein endothelial cells)	Modeling neuro-vascular interactions in cortical development	[82]
Neural-perivascular assembloid	Fusion of PLCs with cortical organoids	Modeling of SARS-CoV-2 neuropathology	[84]
Vascularized brain organoid I	Fusion of vessel and brain organoids	Modeling interactions of neurovascular	[86]
Vascularized brain organoid II	Fusion of brain and blood vessel organoids	Modeling blood-brain barrier formation and cerebral cavernous malformations in human PSC-derived organoids	[87]
Microglia-brain organoid assembloid	Co-culture of brain organoids with primitive-like macrophage	Modeling interaction between neuronal cells and microglia	[88]
Adhesion brain organoids system	Long-term culture system enriched with astrocytes	Modeling interaction between microglia and neurons and mechanisms of Alzheimer's disease	[89]

体类器官工程平台, GFP标记的中脑神经元可以有效穿透纹状体类器官并形成高效突触连接, 证实类器官间通路(inter-organoid pathway, IOP)的存在[73]。

目前, 该技术平台能模拟多种神经功能, 包括再现控制肌肉运动[74], 处理疼痛、瘙痒等信息的感觉通路[75]。皮层类器官、后脑/脊髓类器官与骨骼肌类器官的整合构建了"皮层–运动三维组装体"[74], 可用于模拟大脑皮层对肌肉收缩的调控; 在感觉神经回路中, 背根神经节与脊髓类器官的连接, 可建立模拟外周感觉信息传导至中枢神经系统的神经环路[75]。

复合类器官技术在不同脑区可再现神经元轴突延伸和突触连接的形成。通过多组织共发育策略, 还可以模拟体外发育与神经环路构建间的相关性。在最近的研究中, XIANG团队[76]利用人类多能干细

胞实现了神经、肌肉、骨骼三种组织的协同分化，创建了运动神经元通过功能性神经肌肉接头精确调节骨骼肌收缩的模拟系统。该模型不仅再现了神经环路的发育过程，还为研究跨脑区间神经互作提供了重要的体外平台。

3.3 血管化类器官构建

神经元与周围细胞的相互作用对其发育和成熟至关重要[77]，通常涉及多胚层细胞(如中胚层来源的血管组织和免疫细胞)的相互作用。神经系统的血管化在大脑发育过程中发挥关键作用，在确保氧气、营养物质与生长因子运输的同时[78]，还参与微环境调节与代谢废物的清除。传统脑类器官因缺乏功能性血管，易出现中心区域细胞缺氧、营养物质匮乏和类器官体积受限等问题，阻碍对大脑发育过程的模拟[79]。科研人员通过共培养体系构建、类器官融合技术等方法，可将不同胚层来源的细胞整合进行三维培养，模拟细胞间相互作用[80]，实现复合脑类器官的血管化。

目前，血管重构技术已存在多种方案。CAKIR等[81]在hESCs与野生型细胞中表达ETV2，定向诱导皮层类器官中血管内皮细胞的生成。将干细胞分化而来的血管内皮细胞或人静脉内皮细胞与脑类器官共培养可模拟血管化形成[82-83]。类似的共培养方案也被用于引入与脑血管相关的其他细胞如周细胞[84]。WANG等[84]将周细胞样细胞(pericyte-like cells, PLCs)整合到皮层类器官中，构建了神经–血管复合模型，从而模拟了SARS-CoV-2感染所引发的神经病理变化，为研究神经退行性疾病的机制提供了新的技术路径。在最新的研究中，QIN团队[85]将微流控技术整合到多细胞培养系统中，并结合人脑微血管内皮细胞、星形胶质细胞、小胶质细胞和神经，成功复现了血脑屏障结构和神经血管单元的动态互作。

分别构建血管类器官与脑类器官并融合培养，也可以实现神经组装体的血管化并呈现出血脑屏障特征[86]。通过该方法构建的血管化类器官可以形成完整的血管结构，与神经组织紧密连接。研究人员还将直径为3~4毫米的脑类器官与直径约为1毫米的血管类器官融合，以模拟血脑屏障形成[87]。在体外培养条件下整合脑类器官与血管系统有助于缓解长期培养中核心坏死问题，为脑血管疾病及其他病理研究提供更接近生理状态的体外模型。

3.4 胶质细胞发育和组织中心调节

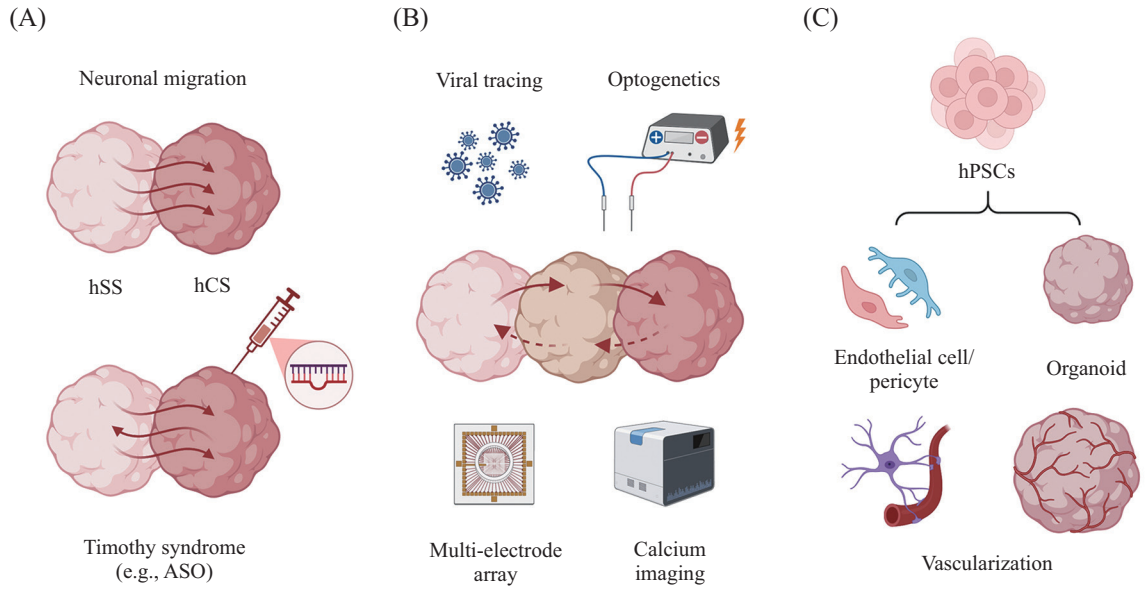
复合类器官技术已被应用于包括神经元迁移、环路模拟、血管生成等研究，同时也在胶质细胞发育和组织中心调节等方面取得了进展。将人类诱导多能干细胞培养的小胶质细胞与脑类器官共培养，揭示了小胶质细胞与神经元祖细胞间由脂质介导的相互作用途径[88]。CHEN等[89]开发的黏附脑类器官平台可将脑类器官培养时间延长至一年以上，且富含星形胶质细胞，能维持小胶质细胞长期存活并促进其分支发育，为研究神经元与胶质细胞、胶质细胞间的相互作用以及阿尔茨海默病等疾病的发病机制提供了全新模型。神经管在大脑发育过程中背腹轴模式化的建立依赖于BMP(背侧信号)和SHH(腹侧信号)的逆浓度梯度进行调控[90]。由LUO等[91]开发的“ORDER”技术，通过在神经外胚层细胞团两端分别植入过表达BMP4和SHH的细胞簇作为背腹侧信号源，形成了自发性反平行的扩散梯度，并产生了与人类胚胎脊髓高度相似的神经管类器官。该技术提供了一个精准的模型来研究神经管发育，但作为人工信号源的细胞簇在培养中可能存在位置不稳定性，还需未来进一步优化。

4 复合类器官技术及疾病模拟

脑类器官能够比模式动物或二维细胞培养更好地再现人脑发育过程，且具有更强的增殖能力和更多样的细胞亚型[1,92]。利用患者来源的诱导多能干细胞(induced pluripotent stem cells, iPSCs)培养的脑类器官，可以克服由遗传背景差异、物种间脑发育差异导致的疾病表型区别[1,93-94]。在此基础上，复合脑类器官技术通过在三维环境中培养多种细胞或组织，可构建出更加复杂且高度模拟人体生理状态的模型(图2)。这些特性使复合脑类器官技术在疾病发育模拟方面更具优势，有助于深入探究疾病机制，并加速新型治疗策略的开发。目前国内外学者已利用该技术成功模拟出多种人脑发育疾病。

4.1 神经退行性疾病

神经退行性疾病是指由中枢神经系统(脑和脊髓)中神经元及胶质细胞持续退化引发的病变。由于这类细胞在终末分化后再生能力有限，一旦发生凋亡、细胞行为或代谢异常，就会导致不可逆的组织损伤，促发神经退行性疾病的产生[95]。常见的神经退行性疾病包括：AD、PD、HD、肌萎缩侧索硬



A: 神经元迁移模拟及疾病应用。人皮层球状体(hCS)与皮层下球状体(hSS)融合, 可在体外重建GABA能中间神经元向皮层迁移的过程。通过注射反义寡核苷酸(ASO), 可修复Timothy综合征等神经发育疾病的迁移缺陷及神经环路异常。B: 神经环路功能解析。图中为三脑区融合模型, 可模拟多区域往返投射(实线、虚线箭头)构建环路模型。常用的环路检测手段包括病毒示踪、光遗传学、微电极阵列(MEA)和钙成像等。C: 血管化模型构建。人多能干细胞可诱导分化为血管内皮细胞/周细胞, 并与脑类器官共培养形成血管化网络, 提高类器官的成熟度。

A: modeling neuronal migration and disease applications. The fusion of hCS (human cortical spheroids) and hSS (human subpallial spheroids) enables the reconstruction of GABAergic interneuron migration towards the cortex *in vitro*. The ASOs (administration of antisense oligonucleotides) can rescue migration deficits and associated neural circuit abnormalities observed in neurodevelopmental disorders such as Timothy syndrome. B: functional neural circuit analysis. The schematic illustrates a tri-regional fusion model capable of simulating reciprocal projections (indicated by solid and dashed arrows) to establish complex circuit models. Common techniques for circuit interrogation include viral tracing, optogenetics, MEA (multi-electrode array), and calcium imaging. C: construction of vascularized models. hPSCs can be differentiated into endothelial cells and pericytes. Subsequent co-culture with brain organoids facilitates the formation of functional vascular networks, enhancing the maturity and complexity of the organoids.

图2　复合脑类器官模型的构建与应用

Fig.2　Integrated construction and application of brain organoids

化症(amyotrophic lateral sclerosis, ALS)、多发性硬化症(multiple sclerosis, MS)等。

近年来, 复合脑类器官技术的发展显著提升了疾病病理模型的仿生度。PARK等[96]整合神经元、星形胶质细胞与小胶质细胞, 观测到小胶质细胞向病变区域定向迁移及促炎因子(如IL-6、TNF-α)释放与神经元丢失的时空关联性, 从而克服了传统阿尔茨海默病模型中缺乏神经免疫互作的局限性。WU等[7]利用HD患者来源的iPSCs构建了纹状体类器官和黑质类器官的组装体, 在体外再现了纹状体的中棘神经元投射障碍, 为体外研究HD提供了有效的研究模型。此外, 研究团队使用人中脑类器官模型评估骨髓间充质干细胞(BM-MSC)分泌物及不同给药途径在帕金森病中的治疗潜力, 发现BM-MSC分泌物可通过多种途径促进神经元的生长和存活[97]。在最近的研究中, 科研人员开发了人源中脑类器官–外周血T细胞的共培养模型, 模拟了T细胞与中脑神经组织的相互作用, 为探究帕金森病的神经免疫调控机制提供了更接近生理条件的平台[98]。血管网络的整合不仅改善了类器官的代谢微环境, 还以更真实的方式模拟了体内疾病作用机制。

4.2　神经发育障碍

神经发育障碍是胎儿期或婴儿时期, 因遗传或环境因素导致的中枢神经系统异常发育的一类疾病[99-100]。大量的医学遗传学证据表明这类疾病是高度遗传的。目前, 只有一类神经发育障碍疾病的遗传因素相对清楚, 即综合征性神经发育障碍。常见的这类疾病主要包括: 唐氏综合征、Rett综合征、脆性X综合征、天使综合征、Timothy综合征等。

神经发育障碍的研究因有限的模型和诊断条件而处于瓶颈, 但复合脑类器官技术的发展为研究未知遗传因素提供了新方法。以Timothy综合征为例, 在前脑组装体模型中观察到患者皮层GABA能中间神经元存在迁移异常, 表现为神经元迁移跳跃

长度缩短而跳跃频率增加[101]。在此基础上, 研究团队开发了基于反义核苷酸的治疗策略, 通过外显子替换技术修复了模型内中间神经元的迁移缺陷及神经环路异常[102]。神经元迁移缺陷也在Rett综合症患者的组装体模型中被观察到。SAMARAS-INGHE等[103]利用复合类器官技术生成大脑皮层–神经节隆起(Cx+GE)组装体, 在功能上对兴奋性和抑制性神经元实现了整合, 证实了神经网络功能障碍可通过非传统神经调节药物TP53抑制剂治疗得到部分挽救。

4.3 精神疾病

精神疾病是一类在生物学、心理学及社会环境因素影响下, 大脑功能失调导致认知功能失调、精神发育迟滞、人格心理障碍等临床表现的疾病。常见的精神疾病主要包括: 自闭症谱系障碍、抑郁症、精神分裂症、双相情感障碍、强迫症、妄想症等。

研究表明, 人类纹状体在神经发育过程中极易出现功能障碍, 而TAC3通路常与精神分裂症相关联[104-105]。尽管目前这些神经元迁移的起源尚不明确, 但构建含TAC3中间神经元的类器官模型并与纹状体类器官进行组装, 有望揭示其在发育、进化及精神疾病中的作用机制[62]。此外, 研究团队在体外重建了人类大脑皮层–纹状体–丘脑–皮层回路的细胞模型[106], 可用于研究与自闭症谱系障碍和抽动秽语综合征相关的*ASH1L*基因突变的病理机制, 且他们观察到了异常同步的神经元活动。这为研究人类早期发育和疾病中的皮层–纹状体–丘脑–皮层回路提供了新的平台。

4.4 脑肿瘤

除上述围绕神经退行与发育等的疾病模拟外, 复合脑类器官技术还被应用于脑肿瘤等方面的研究。科研人员通过模拟肿瘤细胞与神经微环境的复杂互作, 可以构建基于脑肿瘤样本或基因编辑策略的多种恶行肿瘤类器官模型, 为理解肿瘤的侵袭机制和开发靶向疗法提供新思路。

其中, 胶质母细胞瘤(glioblastoma, GBM)作为临床最常见、最具侵袭性的原发性恶性肿瘤, 其发生与大脑微环境密切相关。而复合脑类器官技术通过整合患者来源的肿瘤细胞与人脑区特异性类器官, 可以再现原发肿瘤的病理学特征、细胞异质性及与复杂微环境的互作[107-108]。例如, AMANDA等[109]通过荧光标记的患者来源胶质瘤干细胞与正常hESCs来源的脑类器官进行共培养, 在体外模拟了GBM在正常脑组织中的侵袭。在最近的研究中, MEGHAN等[110]通过将GBM类器官与患者自体CAR-T细胞共培养, 可以评估CAR-T细胞治疗对GBM微环境的影响变化, 从而推动个体化免疫治疗的发展。

5 讨论与展望

近年来脑类器官研究发展迅速, 但仍然存在许多技术局限。精细核团类器官能够模拟特定核团的神经元特征和组织结构, 能够为核团发育、神经疾病模拟、神经环路构建等研究提供更精细的模型。尽管目前构建脑区特异性类器官的技术已经相对完备, 但构建亚脑区类器官即精细核团类器官的技术仍有较大挑战。此外, 因受限于体外较体内相对简单的发育环境, 脑类器官难以长期培养, 且仍处于胚胎脑阶段。为解决这些问题, 研究人员已开发多种策略。通过改进培养技术、整合血管网络、使用生物材料支架、多细胞类型整合、体内移植等策略来更好地模拟人体生理环境。在中心坏死上, 研究者基于切片气液界面培养[15]、网状管状通道网络构成的血管网络可扩散支架[111]等手段以减少类器官长期培养过程中可能出现的中心坏死, 延长类器官的培养时间; 在提高成熟度上, 研究者将类器官移植到小鼠体内, 通过模拟体内环境来获得更为成熟的类器官[112-115], 也有研究者通过生物材料的引入来构建仿生发育微环境[116-117]。

在多谱系细胞互作上, 现有脑类器官的分化策略难以与其他谱系细胞互作, 如血管系统和免疫系统。在后期培养中, 血管系统的缺失会使类器官中心难以获得足够的氧气和营养, 阻碍类器官长期培养和发育。目前针对类器官血管化问题, 研究者已提出多种方案: 血管细胞与脑类器官的共培养[82,84], 脑类器官中血管内皮细胞的定向分化[81], 血管类器官和脑类器官的融合培养[86-87], 脑类器官体内移植[115,118]等。虽然这些方法已取得一定进展, 但仍难以完全复现体内的功能性血管网络。作为免疫系统中的关键细胞, 如小胶质细胞, 在神经元发育成熟过程中同样发挥着重要作用。以上类似的策略也被应用于免疫系统和脑类器官互作的研究中[88,119-121]。

在发育模拟与疾病治疗上, 脑类器官作为具有特定细胞组成和结构特征的体外人源模型, 在人脑

发育机制研究[92,122-124]、疾病机制研究[7,125-128]、药物评估[102,129-130]、应用治疗[131-134]等方面具备巨大的应用潜力。但是利用脑类器官和组装体来研究人类神经疾病仍处于相对初期阶段。目前，研究人员主要对具有疾病遗传背景来源细胞构建的目标类器官进行组装，以研究神经元间的投射和迁移等生理过程。而如何更好地利用这一模型揭示疾病机制是类器官应用的挑战之一。此外，脑类器官除了可以用于疾病模型构建外，还具有组织再生应用的潜力。近年来，将人类脑类器官移植到小鼠或大鼠体内的研究，揭示了脑类器官可用于治疗脑损伤的应用潜力[113,135]。JGAMADZ等[135]将人前脑皮层类器官移植到成年大鼠视觉皮层的大型损伤腔室中，发现类器官成功地与成年大鼠视觉系统整合。

类器官相较于其他药物筛选平台，具有人类遗传背景，可再现体内组织结构，可批量生产等优势，可以加快药物筛选速度，更高效地和有效地进行药物筛选开发。ANAND等[136]通过建立高质量的人脑类器官模型，筛选到了司美替尼和氟维司群两种药物，并在体外验证了其对于胶质瘤侵袭的抑制性。研究人员利用携带新型SCN2A突变(p.E512K)患者源性的皮层类器官，证明其可作为抗癫痫药物临床前评估的高效平台[137]。PASCA团队[138]借助黄原胶处理的人脑皮质类器官平台，将药物筛选效率提升3倍，首次在人体细胞模型中实现对298种FDA批准药物致畸性的高通量评估。

随着脑类器官的深入研究，伦理问题也逐渐凸显，尤其是与“意识”相关的争议。当脑类器官模型具备一定程度的认知和学习能力时，如何明确其中的意识潜力、道德标准及在研究过程中对伦理边界的界定，也逐渐引发广泛的关注。TRUJILLO等[23]发现体外培养的人脑类器官可产生类似人类早产胎儿的脑电图活动；SAMARASINGHE等[103]发现来自Rett综合征患者的脑类器官具有高度异常和类癫痫样网络活动。这些研究中出现的现象，似乎预示着体外培养的人脑类器官可能具有意识。在众多的理论中，最具竞争力的主要是通过扰动复杂指数以测定人脑类器官的意识[139]。但是现有的脑类器官模型在大小、复杂性和成熟度上都非常有限，且不符合当前界定的意识和意识标准。尽管大脑类器官的复杂性正在随着研究的深入而增加，但是当前构建的大脑类器官与正常人类的大脑仍具有较大的结构和功能差异[140]。因此，随着模型的优化和改进，对意识和意识理解的重新审视和界定也是十分必要的。

随着脑类器官的广泛应用，如何强化脑类器官的质量控制，已成为制约其进一步发展的关键障碍之一。从应用上，一方面需要开发不同类型的脑类器官，另一方面需要加强类器官的质控。不同干细胞系在相同分化方案下发育存在异质性，这可能源于不同细胞系的表观遗传修饰差异和对小分子不同的响应程度等，这严重阻碍了脑类器官的研究与应用。近年来，有研究开始关注不同细胞系的异质性问题[20]：有研究者通过优化培养方案，利用旋转生物反应器实现了大量一致的脑类器官的产生[136]；有研究者通过对原材料标准化对培养基与细胞来源进行统一方案[141]；有研究者利用人工智能和图像分析技术，实现了对类器官结构的高速3D分析，提高了数据稳定性[142]；还有研究者通过开发批次效应校正算法消除非生物变异[143]。但目前尚未明确不同细胞系间异质性的具体机制以及消除不同细胞系间异质性的潜在办法。虽然脑类器官可以在体外再现大脑的部分特征，但由于缺乏体内复杂的发育环境，体外构建的脑类器官在发育上仍处于胚胎脑的早期阶段。仍需继续探索如何构建更为复杂、成熟的脑类器官。

随着多学科交叉发展，3D生物打印、微流控芯片及其他微米/纳米加工技术等新兴技术在脑类器官中的应用，有望加速脑类器官的研究与发展。其中，3D生物打印技术通过将神经干细胞、神经前体细胞或成熟神经元等多种细胞类型打印到三维生物支架中，有望构建包含神经元和胶质细胞的脑类器官模型[144]。而微流控芯片则为类器官提供了微环境动态调控的可能。2023年，PALMA-FLOREZ等[145]在血脑屏障芯片中引入内皮电阻系统，得以实时评估血脑屏障的完整性。此外，人工智能技术的进一步融合也有助于提高脑类器官研究的客观性与准确性，不仅可以在脑类器官中提供准确的数据分析，还可以为药物筛选中的过程监测和结果预测提供有力支持。综上所述，脑类器官作为新兴发展的技术模型，可为解析大脑发育、揭示疾病机制、加快治疗手段开发等方面提供有效的研究模型和工具；同时，进一步开发脑类器官技术，也有望为相关技术提供更为理想的体外模型支持。

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