干细胞治疗年龄相关性黄斑变性的临床研究进展

祝 贺[1,2] 王 磊[2] 吴 骏[2] 郝 捷[2]*

([1]中南民族大学, 湖北省武陵山区资源植物种质改良与利用重点实验室, 武汉 430074;
[2]中国科学院动物研究所, 干细胞与生殖生物学国家重点实验室, 北京 100101)

摘要 年龄相关性黄斑变性(age-related macular degeneration, AMD)是造成60岁以上人群失明的主要原因之一, 目前尚无有效的治疗方法。干细胞具有自我更新和分化成多种组织细胞的特性, 为治疗该类不治之症带来了潜在的希望。如今, 国内外已经相继开展了很多利用干细胞相关产品治疗AMD的临床试验研究, 并取得了令人鼓舞的结果。但是, 真正利用干细胞治疗AMD还存在着诸多问题。该文将对干细胞治疗AMD的移植方法、细胞类型和存在问题进行综述。

关键词 干细胞; 视网膜色素上皮细胞; 年龄相关性黄斑变性; 干细胞治疗

Clinical Research Progress in Stem Cell-based Therapies for Age-related Macular Degeneration

Zhu He[1,2], Wang Lei[2], Wu Jun[2], Hao Jie[2]*

([1]*Hubei Provincial Key Laboratory for Protection and Application of Special Plants in Wuling Area of China, South-Central University for Nationalities, Wuhan 430074, China*; [2]*State Key Laboratory of Stem Cell and Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China*)

Abstract Age-related macular degeneration (AMD) is one of the main causes of blindness in people over 60 years old, but to date there's no effective treatment. Stem cells hold characteristics of self-renewal and differentiate into a variety of tissue cells. This poses a potential hope for the treatment of these incurable diseases. Today, there have been a lot of clinical trials using stem cell related products for the treatment of AMD at home and abroad, and encouraging results have been showed. However, there are still many problems should be solved for the real application of stem cells to treat AMD. Herein, stem cells transplantation method, cells types and existing problems will be reviewed.

Keywords stem cells; retinal pigment epithelium; age-related macular degeneration; stem cell therapy

年龄相关性黄斑变性(age-related macular degeneration, AMD)是造成60岁以上人群失明的主要原因之一, 在过去几十年里发病率呈不断上升的趋势[1-2]。我国是世界第一人口大国, 每年有大量的患者因AMD丧失视力。根据脉络膜新生血管存在与否, 可将AMD分为干性和湿性两大类, 其中干性AMD占80%左右, 湿性AMD占20%左右。干性AMD是由于视网膜色素上皮萎缩导致光感受器损伤, 疾病进展期间无新生血管侵入。相反, 湿性AMD则是因为脉络膜新生血管侵入视网膜, 引起视网膜内渗血、出血、瘢痕形成等一系列病理改变, 尤其病变区累及眼底黄斑时, 将严重影响视力, 进而导致失明。

近年来, 随着再生医学的蓬勃发展, 细胞移植治疗为根治AMD带来了潜在的希望。视网膜色素上皮细胞(retinal pigment epithelium, RPE)是生长在位于神经视网膜和脉络膜血管层之间Bruch's膜上的一类单层细胞[3], 在维持视网膜和光感受器功能方面起着非常重要的作用, 包括血–视网膜屏障的形成、神经视网膜营养的供给、光感外节的吞噬、

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*通讯作者。Tel: 010-62558737, E-mail: haojie@ioz.ac.cn
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*Corresponding author. Tel: +86-10-62558737, E-mail: haojie@ioz.ac.cn
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细胞代谢物的清除、视觉色素的重建、杂散光的过滤与吸收以及视觉感知转换的驳接等[3]。同时, RPE分泌拮抗血管新生的因子(vascular endothelial growth factor, VEGF)和胞外基质, 抑制脉络膜新生血管向视网膜内的侵入生长[4-5]。因此, 理论上RPE移植是治疗AMD的有效方法。2009年, 美国FDA批准ACT(Advanced Cell Technology)公司(现已更名为Ocata Therapeutics公司)开展人胚胎干细胞(human embryonic stem cells, hESCs)来源的RPE移植治疗干性AMD和Stargardt黄斑营养不良的临床试验, 结果显示, RPE具有临床安全性与一定的治疗效果[6-7]。2014年, 日本理化研究所(RIKEN)开展自体人诱导多能干细胞(human induced pluripotent stem cells, hiPSCs)来源的RPE移植治疗AMD的临床试验[8]。迄今为止, 国内外已开展了16项细胞移植治疗AMD的临床试验(表1和表2)。在此, 本文将对

表1 在临床试验注册网站(ClinicalTrials.gov)注册的干细胞治疗AMD相关试验汇总

Table 1 List of stem cell treatments of AMD from ClinicalTrials.gov

注册号 ID	国家 Country	研究阶段 Phase	细胞类型 Cell type	疾病 Disease	开展日期 Date
NCT00401713	Austria	Not provided	autologous RPE	AMD and CNV	2004.02－2008.09
NCT01226628	USA	Phase 1	Human umbilical tissue-derived cells (CNTO 2476)	AMD-GA	2010.09－2015.03
NCT01632527	USA	Phase 1/2	HuCNS-SC	AMD-GA	2012.06－2015.06
NCT02137915	USA	Phase 2	HuCNS-SC	AMD-GA	2014.04－2019.06
NCT02467634	USA	Phase 2	HuCNS-SC	AMD-GA	2015.06－2017.12
NCT01344993	USA	Phase 1/2	hESC-PRE (MA09-hRPE)	Advanced dry AMD	2011.04－2014.11
NCT02463344	USA	Phase 1/2	hESC-RPE (MA09-hRPE)	AMD	2012.07－2029.12
NCT02445612	USA	Phase 1/2	hESC-RPE (MA09-hRPE)	Stargardt macular dystrophy	2012.07－2029.12
NCT01674829	South Korea	Phase 1/2	hESC-PRE	Advanced dry AMD	2012.09－2016.02
NCT02286089	Israel	Phase 1/2	hESC-PRE	Advanced dry AMD	2015.02－2016.08
NCT01691261	UK	Phase 1	hESC-RPE	Acute wet AMD and recent rapid vision decline	2015.02－2017.06
NCT02464956	UK	Observational	iPSC-RPE	Late wet AMD or dry AMD	2015.05－2016.04
NCT02590692	USA	Phase 1/2	hESC-RPE (CPCB-RPE1)	Dry AMD and geographic atrophy	2015.10－2018.09

HuCNS-SC: 人中枢神经系统干细胞。

HuCNS-SC: human central nervous system stem cell.

表2 中国临床试验注册中心中干细胞治疗AMD相关试验汇总

Table 2 List of stem cell treatments of AMD from ChiCTR

注册号 Registration number	单位 Institution	注册题目 Public title	研究类型 Study type	注册日期 Date of registration
ChiCTR-OCB-15006423	Southwest hospital	Clinical study of subretinal transplantation of human embryo stem cell derived retinal pigment epitheliums in treatment of macular degeneration diseases	Observational study	2015.05.12
ChiCTR-OPC-15006757	The first affiliated hospital of nanjing medical university	Treatment of Dry Age-related Macular Degeneration using fetal retinal pigment epithelium	Observational study	2015.07.16
ChiCTR-OCB-15007054	Institute of zoology, chinese academy of sciences	Clinical study of subretinal transplantation of clinical human embryonic stem cells derived retinal pigment epitheliums in treatment of dry age-related macular degeneration diseases	Observational study	2015.09.13

ChiCTR: 中国临床试验注册中心(www.chictr.org.cn)。

ChiCTR: Chinese Clinical Trial Registry (www.chictr.org.cn).

利用RPE移植治疗AMD的相关研究进展进行综述与讨论。

1　RPE移植

1.1　移植技术

Open-sky是首次报道的RPE移植技术。该技术通过将视网膜前段打开, 然后制造一个视网膜皮瓣, 暴露出RPE层, 将组织原有的RPE细胞通过胰蛋白酶消化和轻轻地摩擦移除, 再将^{3}H-胸腺嘧啶标记的人RPE移植到猴Bruch's膜上[9-10]。Open-sky技术可以使供体RPE与Bruch's膜连接, 但无法将Bruch's膜与视网膜重新整合。

Close-eye技术解决了Open-sky技术无法重新整合视网膜的缺陷。根据移植途径, Close-eye技术分为内路法和外路法两种类型。内路法又称经玻璃体视网膜密闭式移植法, 是经睫状体平坦部将玻璃微细吸管刺入视网膜, 注入100~200 μL平衡盐溶液, 利用形成的气泡空洞将神经视网膜分离, 充分暴露Bruch's膜层, 最后将RPE悬液注入气泡空洞完成移植[11]。该方法在兔[11]、大鼠[12]、猪[13]、猫[14]、狗[15]以及猴[16]的实验中得到验证和应用。外路法通过切断上直肌充分暴露巩膜, 在移植区作一近全层巩膜瓣, 由视网膜相切方向将显微注射器刺入脉络膜, 到达视网膜下腔, 注入RPE细胞悬液[17-19]。综上所述, 外路法对眼球正常组织结构破坏性大, 且手术难度高, 故内路法更适合人体移植手术[20]。

1.2　移植方式

RPE移植的方式可以分为细胞悬液注射[6]、细胞片层移植[21]和细胞与生物材料共培养后移植[22]。其中, 目前临床试验中所应用的均为细胞悬液注射的方式。在ACT公司开展的临床试验中[6-7,23], ACT公司最初分别将三种不同剂量的细胞(50 000、100 000、150 000个细胞)悬浮在150 μL的平衡盐溶液(balanced salt solution, BSS)中, 然后移植入患者的视网膜下腔中[6-7]。结果显示, 50 000个细胞的剂量效果较好[7]。细胞片层移植利用激光将细胞切割成细胞薄片, 然后整体移入视网膜下腔中[21]。细胞与生物材料共培养后移植, 是利用生物相容性好、可降解的生物材料, 在体外与RPE细胞共培养一段时间, 然后在整体移入视网膜下腔中[22,24]。但是, 后面两种移植方式还处于动物实验阶段, 其有效性和安全性有待进一步的研究。

2　应用于临床治疗RPE细胞类型

根据RPE来源将其分为两类: 自体来源的RPE和多能干细胞(pluripotent stem cells, PSCs)来源的RPE。

2.1　自体来源的RPE

最早用于移植治疗AMD的RPE为患者自体来源的RPE。收集患者视网膜周边健康的RPE, 然后将它们移植到病变黄斑区域的视网膜下腔[25-27]。研究结果表明, 自体来源的RPE移植治疗AMD具有免疫排斥反应低的优点, 但其疗效并不理想[28-29]。细胞往往因聚集成团或失巢凋亡, 无法抵达Bruch's膜发挥正常生理功能, 甚至引起许多并发症状, 如黄斑水肿、视网膜脱离[28-30]。此外, 患者自体来源的健康RPE数量有限, 很难填充整个黄斑区域[31]。

2.2　PSCs来源的RPE

PSCs具有自我更新和分化成多种类型细胞的特性, 该特性使其成为细胞移植治疗潜在的、无限的种子细胞。人PSCs包括hESCs[32]和hiPSCs[33]两大类型, 分别在1998年和2007年成功获得并稳定建系。目前, 有很多研究小组通过PSCs分化获得RPE, 并证明分化获得的RPE具有与原代RPE相同或类似的细胞形态与生理功能[34-36]。利用PSCs获得成熟RPE的方法可分为自发分化法和小分子诱导分化法。

研究发现, 约1%的hESCs可自发分化为RPE[37], 根据培养方式自发分化可分为贴壁分化法与悬浮分化法两大类型。贴壁分化法是将hESCs进行贴壁培养。hESCs长满皿底、边界互相融合后, 去除hESCs培养液中的bFGF成分后继续培养。随着RPE的自发分化出现棕色斑点, 之后斑点范围不断变大, 颜色不断加深形成黑色区域。待黑色素区域达到一定数量后, 利用机械切割法将黑色素区域细胞传代培养, 经过多次培养传代, 最终获得较纯的RPE细胞。悬浮分化是指将hESCs在低吸附培养板中进行悬浮培养。hESCs形成拟胚体(embryoid body, EB)后去除bFGF继续培养, 1~3周后将EB球消化贴壁培养, 4~8周后出现黑色素区域[38], 此后RPE纯化方法与同贴壁分化法类似[39]。以上两种自发分化方法均能获得大量的、高纯度的RPE。此外, Eiraku等[40]通过悬浮自发分化方法利用小鼠胚胎干细胞成功模拟视网膜发育的过程并获得类似眼杯的结构组织[40]。但是, 上述两种自发分化方法也存在分化效率低, 所获得RPE为发育后期成熟细胞类型, 其自身整合能

力差[31]。

利用小分子定向诱导RPE提高了分化效率, 可大大缩短分化周期。Osakada等[41]添加CKI-1(Wnt通路抑制剂)和SB-431542(TGF-β通路抑制剂)促进hESCs前期向神经外胚层的分化, 最终能够获得(18.1±1.9)%的RPE。Idelson等[35]发现, 烟碱能促进hESCs向神经外胚层分化并提高RPE分化效率, Activin A能提高RPE的产量并在RPE的培养过程中维持其细胞表型。这些发现被Buchholz等[42]加以改进, 仅需14 d便能获得80%的黑色素细胞。此后, 还有许多利用小分子定向诱导PSCs分化成RPE的方法被陆续报道[43-44], 但总体上缺乏统一操作诱导分化流程, 并且小分子种类与添加时间存在很大的差异。而目前应用于临床试验的RPE均为自分化法获得的, 利用多种小分子诱导分化获得RPE的方法, 其安全性、有效性尚待深入研究[34]。

3 小结与展望

通过将RPE移植到视网膜下腔, 无论模型动物还是临床试验患者都获得了不同程度的视觉提升[6-7,21,34]。在移植环境中存活并发挥正常的生理功能是RPE移植获得理想疗效的前提。在视网膜下空间, 正确的细胞功能取决于合适的细胞排列和整合[45]。

目前的移植结果表明, 移植治疗AMD存在如下问题: (1)由于移植区域是一个动态环境, RPE无法固定导致流失; (2)由于RPE为终末分化细胞, 整合能力差导致失巢凋亡; (3)由于AMD患者多为老年人, 其Bruch's膜状态较差, 难以支持新移入的RPE生长[46-47]。

RPE与生物材料共移植可提高细胞的存活率[48], 提示此种RPE移植方法的可行性。最开始人们利用组织分化获得的人羊膜[49]、人晶状体囊[50]和Bruch's膜作为支架。经过几十年的研发, 科学家们获得了第三代可降解医用薄膜支架, 其可用于RPE培养和移植的载体。RPE移植材料需要具有如下特征: (1)生物相容性好, 无免疫原性, 能支持RPE贴附及片层式生长; (2)可降解, 可吸收, 无需替换取出导致二次手术损伤; (3)薄膜状且厚度薄, 由于人体内视网膜下腔空间限制, 支持RPE生长的Bruch's膜一般厚2~4 μm, 相对应材料理想厚度应小于5 μm[24]; (4)通透性佳, 具有多孔超微结构, 保证RPE不掉入材料空隙且呈单片层生长, 同时保证充分的营养物质供应和代谢废物清除; (5)机械强度适宜, 具有一定的刚性, 能耐受外科手术操作, 并具有一定的柔性能避免损伤周围组织。Zi-Bing Jin研究组[24]和Warnke等[51]模仿体内Bruch's膜结构, 利用静电纺丝技术分别将不同的生物可降解材料制作成几微米超薄薄膜材料, 并可促进RPE的黏附与增殖。

此外, 虽然视网膜下腔是一个相对免疫豁免的人体组织区域。但湿性AMD因为脉络膜新生血管侵入视网膜或手术造成的血–视网膜屏障破坏, 导致免疫排斥反应, 进而影响RPE生存质量。在ACT所开展的hESC-RPE治疗黄斑变性的临床试验中, 患者在术前一周开始使用免疫抑制剂他克莫司(tacolimus), 从最低剂量开始, 然后根据患者血液中药物浓度逐渐增加药物剂量, 达到血液终浓度为3~7 ng/mL。在使用他克莫司的同时, 患者还将根据个人情况每日口服不同剂量的吗替麦考酚酯(mycophenolate mofetil, 0.5~2.0 g/d)。患者将在术后持续使用免疫抑制剂6周, 在6周后停用他克莫司, 而继续服用吗替麦考酚酯6周[7]。但结果显示, 在免疫制剂使用过程中出现部分患者由于不良反应而提前终止免疫抑制剂使用的情况[7]。之后, ACT公司首席科学家Robert Lanza等[52]承认, 免疫抑制剂的使用有待进一步优化, 尤其是针对老年患者。此外, 还有研究发现, 免疫反应与RPE移植细胞数量相关联[53]。所以, RPE移植数量以及临床试验中免疫抑制剂给药方案尚待进一步的探索与完善[49]。

成功的视网膜下腔RPE移植会让AMD患者重获光明, 完成这具有划时代意义的治疗需要干细胞生物专家、生物材料专家和资深眼科大夫的协同合作。目前, 多学科交叉融合使干细胞治疗逐步由实验室走向临床, 但同时真正利用干细胞治疗视网膜疾病面临诸多挑战。(1)细胞类型的选择。选择RPE细胞、光感受器细胞、视网膜祖细胞或者其他类型的细胞进行移植, 有待人们对视网膜疾病的病理病因进行更加深入系统的解析。(2)移植物的选择。单细胞悬液、片层细胞或者细胞与生物共培养物, 如何选择适当的材料, 如何验证材料的安全性和细胞材料共同移植物的体内生理功能等, 需要研究者们对细胞和材料进行更加深入的探讨。(3)临床方案的优化。细胞的代次、细胞数量、移植途径、免疫抑制剂给药方案等都需要进一步完善。因此, 在利用干细胞修复视网膜的研究中, 我们还有很长的一段道路需要去探索。

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