干细胞与视网膜变性微环境互作对干细胞命运决定的影响

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摘要 干细胞治疗视网膜变性疾病的安全性和有效性得到大量实验证据的支持, 已进入临床研究阶段。然而, 干细胞移植变性视网膜后, 治疗效果往往在后期出现消退甚至消失。除干细胞自身的特性, 植入干细胞所处的微环境是决定干细胞命运的关键因素。变性视网膜中的胶质疤痕、慢性炎症以及异常代谢微环境, 往往导致植入视网膜的干细胞不能长期存活、正确分化和整合, 这可能是干细胞治疗视网膜变性疾病效果不能长期维持的重要原因。另一方面, 植入视网膜的干细胞也能通过旁分泌作用、物质交换等方式主动调控视网膜变性微环境使其有利于植入干细胞的命运。未来在临床应用中可以通过不同种类干细胞的联合移植或结合调控微环境的方式延长干细胞的治疗效果。本文阐述了干细胞与视网膜变性微环境的相互作用及其对干细胞命运决定的影响机制。

关键词 干细胞, 视网膜变性, 微环境, 炎症, 胶质化, 代谢

视网膜变性疾病是以视网膜色素上皮细胞(retinal pigment epithelium, RPE)和视网膜神经元丢失为特征的一大类致盲性眼病, 主要包括年龄相关性黄斑变性(age related macular degeneration, AMD)、视网膜色素变性(retinitis pigmentosa, RP)、青光眼和糖尿病视网膜病变(diabetic retinopathy, DR)等。视网膜变性疾病是致盲的首要原因, 影响了全世界超过3亿的人口, 极大地影响了人们的生活质量, 造成严重的社会经济负担^[1~3]。目前针对此类致盲性眼病所导致的视力丧失尚

无有效的治疗方法。虽然视网膜变性疾病的发病机制不尽相同, 但它们具有相似的终末病理改变, 即视网膜感光细胞或神经节细胞等神经元的变性死亡, 最后导致不可逆的视力丧失^[1~4]。干细胞治疗因其有望修复和再生受损的视网膜神经元, 恢复视网膜细胞结构和功能, 被认为是最具前景的治疗方法。截至目前, 国内外已开展多项干细胞治疗视网膜变性疾病的临床试验。已公开的临床研究结果显示, 干细胞对AMD病人和RP病人均显示出良好的安全性和短期疗效(网络版

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附表1)。远期观察显示, 这些干细胞临床试验的疗效难以长期维持, 其中的重要原因可能是植入的干细胞或干细胞来源的治疗细胞受变性视网膜微环境的影响, 不能长期存活、分化和替代受损神经元。视网膜变性疾病微环境往往出现共同的病理生理改变, 包括反应性胶质增生、免疫异常和代谢紊乱等(图1), 影响移植干细胞的命运^[5~12]。另一方面, 某些特定类型的干细胞, 如骨髓间充质干细胞(bone marrow mesenchymal cells, BMSCs)、神经干细胞等, 也可以通过主动改善视网膜变性微环境对视网膜变性疾病产生治疗作用^[13,14]。深入探讨干细胞与视网膜变性微环境的互动机制将有助于促进干细胞移植治疗视网膜变性疾病的策略改进, 加速临床转化。本文将结合最新临床研究和实验进展回顾干细胞与视网膜变性微环境间的相互作用及其对干细胞命运的影响和机制。

1 干细胞治疗视网膜变性疾病临床研究现状

干细胞移植是目前治疗视网膜变性疾病最具前景的方法。植入的干细胞除了早期的营养作用及神经保护作用外, 也可以与视网膜细胞发生融合/物质交换或者分化为变性视网膜中丢失的RPE细胞、感光细胞和神经节细胞并整合入原有神经环路而发挥再生修复作用。同时, 视网膜也是进行干细胞移植治疗的理想部位^[15]。因为相对于大脑、脊髓等移植部位而言, 视网膜干细胞移植手术简单, 仅依赖玻璃体腔注药术或常规的玻璃体切割手术即可完成。其次, 植入的干细胞或其衍生细胞能通过眼底镜、OCT等高分辨率成像设备进行无创追踪和观察。同时, 视网膜血-视网膜屏障构成独特的封闭环境, 能限制植入细胞的全身扩布。视网膜的相对免疫赦免特性也使得视网膜移植具有更轻的排斥反应。以上优势使得视网膜移植成为人胚胎干细胞(human embryonic stem cells, hESCs)来源治疗细胞移植最早成功应用于临床的范例。

1.1 治疗干细胞的来源

干细胞具有自我更新能力, 也具有分化为多种细胞类型的能力。根据干细胞的来源不同, 可以将用于治疗视网膜变性疾病的干细胞来源分为两大类: (i) 胚胎干细胞(embryonic stem cells, ESCs)和诱导性多

能干细胞(induced pluripotent stem cells, iPSCs); (ii) 组织特异性干细胞, 包括人胚胎视网膜组织来源的视网膜前体细胞、神经干细胞、骨髓间充质干细胞、造血干细胞等。其中, 骨髓间充质干细胞取材于供者自身, 伦理学风险较小, 植入后可以避免排斥反应, 但BMSCs作为中胚层来源的细胞分化视网膜细胞效率较低。采用视网膜组织特异性干细胞进行移植显示出更好的视网膜分化潜能, 同时成瘤风险低。多项临床试验采用人胚胎视网膜来源的视网膜前体细胞(human fetal retinal progenitor cells, hfRPCs)进行干细胞移植^[16], 均取得较好的治疗效果。但人胚胎组织标本获取困难, 且具有伦理学限制, 阻碍了hfRPCs的临床转化应用。通过不同的培养方法能将多能干细胞(iPSCs和ESCs)分化成供干细胞移植治疗的多种细胞类型, 包括RPE细胞、视网膜前体细胞、感光细胞和神经节细胞等, 提供了丰富的治疗细胞来源^[17,18]。iPSCs细胞虽然可以从病人自身的体细胞诱导而成, 可以避免伦理学问题, 但部分视网膜变性的患者往往存在致病突变, 用其自身细胞制备的iPSCs及其分化的治疗细胞同样也存在基因缺陷。最新报道显示, 自体iPSCs植入供体自身后仍然能产生免疫排斥反应, 并且自体iPSCs制备细胞需要较高的时间经济成本, 因此, 目前提倡采用HLA配型后的同种异体iPSCs作为治疗细胞来源^[19]。ESCs取材于胚胎内细胞团, 具有一定的伦理学问题, 但一旦建立成稳定的细胞系, 可在体外大量扩增, 易于基因操作, 且基因操作后仍具有向各种细胞分化的潜能, 是合适的治疗细胞来源^[20]。

此外, 类器官技术是近年发展起来的前沿科学技术, 可将ESCs或iPSCs在体外自发形成视网膜类器官, 从这些类器官中分离出结构和功能更接近体内发育状态的治疗细胞, 这成为干细胞治疗视网膜变性疾病新的治疗细胞来源^[21,22]。研究结果提示, 视网膜类器官来源的RPE细胞和感光前体细胞比二维分化的细胞有优势, 移植后能形成更加成熟的细胞结构^[23,24]。目前采用多能干细胞来源RPE细胞治疗视网膜变性疾病的临床研究开展较多, 但尚无多能干细胞来源RPCs和感光前体细胞的临床研究报道。相较RPE细胞而言, 多能干细胞来源RPCs种子细胞异质性较大, 混杂致瘤性细胞的可能性较高。本课题组^[17]前期提出, 采用表面抗原分选策略能够从hESCs来源视网膜类器官中获得同时具备安全性和有效性的治疗种子细胞, 有望为干细胞

图 1 视网膜变性微环境对干细胞命运的影响。A: 正常视网膜; B: 视网膜变性微环境对植入干细胞命运的影响。I: Müller细胞反应性增生。激活的Müller细胞增生肥大形成胶质瘢痕、分泌抑制性细胞外基质蛋白抑制植入干细胞迁移、整合; Müller细胞分泌因子支持植入干细胞存活, 形成结构支持引导干细胞迁移整合; II: 星形胶质细胞反应性增生阻隔植入细胞迁移; III: 小胶质细胞激活。IIIa: 激活的M1型小胶质细胞吞噬植入干细胞; IIIb: 分泌炎症因子形成炎症微环境影响植入细胞的正确分化; M2型小胶质细胞分泌保护性细胞因子促进干细胞存活; IV: 异常代谢微环境(氧化应激、糖代谢、脂代谢和氨基酸代谢紊乱)对植入干细胞命运(存活、自我更新、分化和整合)的影响

Figure 1 Influence of degenerative retinal microenvironment on the fate of stem cells. A: Normal retina; B: influence of the degenerative retinal microenvironment on the fate of grafted stem cells. I: Reactive gliosis of Müller cell. Activated Müller cells form glial scar and secrete inhibitory extracellular matrix proteins to inhibit the migration and integration of grafted stem cells. Müller cell secretory factors support the survival of grafted stem cells and form structural support to guide the migration and integration of stem cells. II: Reactive gliosis of astrocyte blocks the migration of grafted cells. III: Activation of microglial cells. IIIa: Activated M1-type microglial cells phagocytose the grafted stem cells. IIIb: Activated microglial cells produce inflammatory factors to form an inflammatory microenvironment that affects the correct differentiation of the grafted cells. M2-type microglia secrete protective cytokines to promote stem cell survival. IV: Effects of abnormal metabolic microenvironments (oxidative stress, glucose metabolic disorders, lipid metabolic disorders, and amino acid metabolic disorders) on the fate of grafted stem cells (survival, self-renewal, differentiation, and integration)

治疗视网膜变性疾病提供新的治疗细胞来源。

1.2 干细胞治疗视网膜变性疾病临床研究结果

(1) hiPSCs-RPE/hESC-RPE. hiPSCs-RPE/hESC-RPE细胞是第一个多能干细胞来源应用于视网膜变性疾病临床试验的种子细胞。早在十年前, 美国(Advanced Cell Technology, ACT)公司联合美国加州大学洛杉矶分校(University of California, Los Angeles, UCLA)进行了用hESC-RPE治疗Stargardt病和干性AMD的I/II期临床试验^[25]。该研究发现, hESC-RPE体外可分化为纯度99%以上的RPE细胞, 采用细胞悬液经视网膜下腔移植到9例Stargardt病和9例干性AMD病人中, 共有13例病人在移植后12个月时视功能提高或者保持稳定, 初步证明了hESC-RPE移植的短期安全性。

本实验室^[26]采用临床级hESC-RPE来源的RPE细胞悬液移植3例湿性AMD病人, 在12个月的观察期内, 3例病人的视功能均得到不同程度的改善。另一项来自英国伦敦大学的hESC-RPE治疗Stargardt病实验结果提示干细胞移植安全性好, 患者视力短暂提升, 但在12个月时的微视野检查提示视力无改善^[27]。单层RPE细胞移植片被认为是更合适的治疗形式, 因为RPE细胞只有形成单层、极性的细胞层才能发挥其生理功能。目前已经有三项临床试验采用RPE细胞移植片的方式进行^[28-30]。日本科学家Takahashi团队^[28]采用一例湿性AMD病人iPSCs来源的单层RPE细胞薄片移植回病人视网膜下腔, 一年后该病人视力保持稳定, 但出现黄斑水肿等并发症。美国辉瑞公司(Pfizer)与英国伦敦大学学院的Coffey实验室^[29]将hESC-RPE诱导分化为单层RPE

细胞的薄片移植入两例晚期湿性AMD病人, 在移植后12个月, 两例病人的视力均比较术前提高. 美国南加州大学Humayun团队^[30]将hESCs-RPE细胞在材料膜片上培养成单层结构, 再将RPE细胞膜片移植治疗入4例进展期干性AMD病人, 治疗后4~12个月的观察期内, 有1眼最佳校正视力得到改善, 有2眼固视能力得到明显改善. 采用HLA配型后的同种异体hiPSCs-RPE细胞移植治疗湿性AMD病人, 结果显示, 在所有病人术后1年期内均能观察到RPE移植物存活, 且不伴有异常增殖, 但术后观察到的并发症较多^[19].

(2) hfRPCs. hfRPCs是一种神经祖细胞, 表现出许多类似干细胞的特征. 动物实验提示, hfRPCs移植后能分化成感光细胞, 挽救视网膜变性大鼠视力^[16]. 因此, hfRPCs有望在移植后替代受损感光细胞从而恢复视力. 陆军军医大学第一附属医院眼科率先在国内开展hfRPCs治疗RP临床试验^[16]. 首期试验采用hfRPCs治疗8例RP病人, 其在观察期1年内未发生瘤和免疫排斥等不良反应, 展示出较好的安全性, 其中5例病人在移植后2~6个月视力得到改善. 2020年7月, 美国jCyte公司也公布了采用hfRPCs治疗RP的IIb期临床试验结果^[31]. 结果显示, 在74例满足受试条件的受试患者中, 所有受试眼未出现严重的不良事件, 48例接受hfRPCs细胞移植的眼视功能较基线保持稳定或者提高. 以上结果证实了hfRPCs作为治疗细胞的潜力.

(3) 其他组织特异性干细胞. 其他组织来源的干细胞包括骨髓间充质干细胞、骨髓来源造血干细胞及脐带间充质干细胞(umbilical cord mesenchymal stem cells, UCMSCs)等, 已经广泛用于干细胞治疗视网膜变性的临床试验(网络版附表1). 虽然动物实验结果提示, 非视网膜组织来源的干细胞分化为视网膜感光细胞的概率较小, 但其仍可以通过旁分泌作用延缓视网膜变性. 几项采用骨髓来源的干细胞治疗视网膜变性疾病的临床试验显示出较好的安全性, 部分结果提示短期内能提高患者的视力, 改善生活质量^[32~41]. 本课题组^[40]前期研究结果表明, 静脉输注UCMSCs对晚期RP患者是安全的, 大多数患者在术后12月的视力能够改善或维持, 术后3个月生活质量明显改善, 提示静脉输注UCMSCs可能是治疗晚期RP患者的一种有效方法. 同样地, 采用静脉输注自体BMSCs可显著降低糖尿病视网膜病变患者的空腹血糖和C反应蛋白水平^[38]. 结果显示, 增殖期糖尿病视网膜病变病人接受自体

BMSCs输注后最佳矫正视力得到显著改善, 黄斑水肿减轻, 且在治疗期间所有病例无急性反应, 随访期间无严重不良事件发生. 以上结果证实静脉输注是干细胞治疗视网膜变性疾病的可选方案.

1.3 干细胞治疗视网膜变性疾病临床试验的安全性和有效性

大多数在正规机构开展的干细胞治疗视网膜变性疾病的临床试验安全性得到初步证实, 但也有部分试验结果提示, 干细胞移植后术眼可能出现黄斑水肿、视网膜前膜、玻璃体增殖性病变和视网膜脱离等并发症^[28,42,43]. 目前, 一些未经审批和监管的干细胞治疗试验也在悄悄进行. 这些干细胞疗法用于治疗视网膜变性疾病缺乏安全性试验证据, 会引起严重的不良事件, 导致患者的视力丧失^[44]. 优化干细胞移植治疗策略, 严格审批和监管干细胞治疗临床试验, 提高治疗的安全性是干细胞移植治疗视网膜变性的首要目标.

目前多数干细胞治疗视网膜变性疾病在短期内能观察到一定的治疗效果, 但干细胞治疗的长期效果难以维持^[16,25,30]. 本团队^[16]前期采用hfRPCs治疗RP, 临床试验结果显示, 5例病人在移植后2~6个月视力得到改善, 但疗效在移植12个月后均出现显著下降直至消失. 并且不同类型的疾病及不同的疾病阶段, 干细胞治疗的效果反馈也不同. 当然, 干细胞治疗的有效性(干细胞存活、迁移、分化和整合等细胞命运)一方面与干细胞本身的特性有关, 另一方面也取决于其所在的视网膜微环境.

2 视网膜微环境和干细胞命运决定

2.1 微环境决定干细胞命运

干细胞微环境由细胞、相邻的细胞外基质和可溶性分子组成, 这些因素共同决定了干细胞的命运, 即干细胞的存活、增殖、迁移、分化和神经环路的形成^[45]. 在视网膜发育过程中, 视网膜祖细胞分化命运受视网膜微环境的复杂调控, 最终分化形成视网膜组织. 体外培养时, 采用不同的诱导因子构建不同的干细胞分化微环境, 能让干细胞定向分化为不同的视网膜细胞^[18]. 相较二维培养而言, 视网膜类器官之所以能分化出更加成熟的视网膜细胞结构, 如感光细胞外节, 可能是因为三维培养的组织存在干细胞分化所需

的复杂微环境, 这种微环境促进细胞的发育成熟^[21,22].

在干细胞修复再生损伤视网膜的过程中, 干细胞命运的决定也与所处的视网膜微环境密切相关. 处于发育阶段的视网膜微环境可能更有利于移植干细胞的存活和分化^[46], 而年老和疾病的微环境则不利于干细胞的存活和正确分化^[47]. 某些视网膜损伤微环境也能促进移植干细胞的整合^[48,49]. 非视网膜源性的干细胞在视网膜微环境的作用下也能向视网膜细胞方向分化^[50], 提示微环境对干细胞分化命运的重要性. 移植到视网膜的干细胞在迁移和分化过程中出现的有趣现象是, 当干细胞迁移至视网膜不同的区域定植时, 即表达这一区域细胞的标记, 提示干细胞的命运受定植区域视网膜微环境影响^[17]. 最新研究表明, 干细胞移植治疗损伤的视网膜, 植入干细胞和受者视网膜发生融合/物质交换的概率也受视网膜变性微环境的影响^[51]. 不同的视网膜变性微环境可能对干细胞命运具有不同的作用, 因此需要深入研究视网膜正常微环境和变性微环境的特性及其对植入干细胞的命运影响.

2.2 视网膜正常微环境的组成

视网膜微环境是由视网膜细胞(包括视网膜神经元、RPE和胶质细胞)、细胞外基质和可溶性分子组成的层状复杂网络系统. 视网膜微环境中的各种组分协同作用, 维持整个视网膜正常稳态.

(1) 细胞. (i) 视网膜神经元. 五种类型的视网膜神经元组成了层状的视网膜神经组织结构, 其中包括两种类型的感光细胞(视锥细胞和视杆细胞)、水平细胞、双极细胞、无长突细胞和神经节细胞. 视网膜神经元协同作用负责视觉信号的形成、加工和向上级中枢的传递. 视网膜神经元通过其分泌的神经营养因子和神经递质构成神经分化的微环境. (ii) RPE. RPE是一层立方上皮细胞, 其一侧与感光细胞紧密相连, 另一侧与Bruch's膜相连. 视网膜色素上皮细胞主要参与光吸收、视色素循环、营养物质和离子转运及感光细胞外节吞噬等视网膜生理过程, 是视网膜代谢微环境稳态的关键细胞, 同时也参与视网膜免疫豁免微环境的构建^[52]. (iii) 视网膜胶质细胞. 视网膜包含三种类型的胶质细胞: 大胶质细胞(Müller胶质细胞、星形胶质细胞)和小胶质细胞. 三种胶质细胞不仅提供结构上的支持, 而且通过调节新陈代谢、吞噬神经元碎片、参与神经递质循环和营养因子的释放, 参与维持视网

膜复杂的微环境稳态^[5,11].

Müller细胞起源于神经视网膜, 呈放射状横跨整个神经视网膜, 参与形成神经视网膜的内外界膜, 支撑视网膜结构, 同时也联系了视网膜神经元、血管和玻璃体之间的解剖结构^[5]. 除了结构支持, Müller细胞还参与视网膜营养代谢、维持离子平衡和神经递质的循环. Müller胶质细胞具有吞噬功能, 能够吞噬清除视网膜死亡神经元和碎片, 从而维持视网膜微环境稳态^[53]. 此外, Müller细胞在视网膜免疫微环境稳态和神经再生微环境形成中也发挥重要作用^[5,54].

起源于中枢神经系统的星形胶质细胞沿视神经迁移至视网膜, 主要位于视网膜神经节细胞层和神经纤维层, 它们的突起覆盖视网膜血管表面形成视网膜血管内屏障^[55,56]. 星形胶质细胞在微环境的离子稳态维持、神经元信号传导和视网膜内皮屏障的形成中发挥关键作用^[55]. 和Müller细胞类似, 星形胶质细胞也具备吞噬能力, 通过吞噬轴突和细胞器来维持细胞外环境稳态^[57].

小胶质细胞是视网膜中的唯一原位免疫细胞, 被认为起源于胚胎卵黄囊的原始巨噬细胞, 而后迁移定植于视网膜. 在视网膜发育期, 小胶质细胞在控制发育相关的细胞凋亡、血管生成和突触修饰等方面, 发挥至关重要的作用^[58]. 在成年期, 静息态小胶质细胞主要定位于视网膜内、外丛状层, 细胞体小呈圆形, 周围有许多分支. 小胶质细胞对外界环境具有较高的敏感度和可塑性, 能够积极地与视网膜神经元和大胶质细胞进行通讯, 监视周围环境并做出反应, 在维持视网膜微环境稳态方面发挥着至关重要的作用^[11].

(2) 细胞外基质. 细胞外基质是由细胞分泌到细胞外的蛋白形成的三维结构, 在结构和功能上支持细胞和组织^[59]. 细胞外基质由各种结构蛋白组成, 包括层黏连蛋白、胶原蛋白、纤维连接蛋白和蛋白多糖. 除了提供结构支持, 这些蛋白还可以结合整合素受体复合物, 调节细胞的分化、增殖、极性、存活和迁移等细胞命运决定过程. 视网膜Müller细胞终足及其分泌的细胞外基质成分组成了视网膜的内界膜^[5]. 内界膜向内阻隔玻璃体形成视网膜独立的微环境. 紧邻RPE层的Bruch's膜是视网膜外屏障的重要组成部分. 内界膜、视网膜外屏障及视网膜内皮屏障的阻隔构成了视网膜独立的微环境. 此外, 感光细胞外基质(interphotoreceptor matrix, IPM)是位于感光细胞和RPE细胞

之间的高度有序的组织结构, 其相互连接的区域围绕着视锥细胞和视杆细胞, 并延伸到整个视网膜下腔。IPM被认为能充当生长因子的受体、调节视磺酸的转运, 参与周围细胞的细胞骨架组织以及氧气和营养物质的转运, 从而维持视网膜正常的微环境^[60]。

(3) 代谢物和信号分子. 视网膜微环境中存在大量细胞代谢物、电解质、神经递质和信号分子, 以上物质协同作用, 参与维持视网膜营养代谢、神经信号传递和各种信号通路的调控, 以维持视网膜的正常微环境。

3 视网膜变性微环境及其对干细胞命运的影响

3.1 反应性胶质增生及其对干细胞命运的影响

(1) Müller细胞的反应性增生对干细胞命运的影响. 在视网膜变性或损伤时, Müller细胞会激活发生反应性胶质增生, 表现为细胞的增大增粗、突起的伸长和细胞中胶质纤维酸性蛋白(glial fibrillary acidic protein, GFAP)及波形蛋白(vimentin)的表达上调^[5]。在损伤早期, Müller细胞激活后能分泌营养因子和抗氧化物质以修复损伤的视网膜^[61]。研究提示, Müller细胞在视网膜变性早期也能通过吞噬作用清除死亡的感光细胞^[62]。随着损伤进展, Müller细胞的持续激活会释放有害因子, 进一步加剧神经元的损伤和阻碍受损视网膜组织的再生^[63]。同时, 增生的Müller细胞在视网膜下腔形成胶质瘢痕, 阻止向视网膜神经元的营养物质输送^[64,65]。此外, Müller细胞反应性增生还会导致玻璃体视网膜增殖, 进一步导致视网膜脱离^[66]。反应性增生的Müller细胞也能产生趋化因子或者作为迁移支架促进单核巨噬细胞/小胶质细胞的浸润, 进一步加剧视网膜变性^[63,67]。

(i) 物理屏障. 视网膜Müller细胞过度激活形成的胶质瘢痕可作为物理屏障阻碍移植细胞的迁移和整合。研究表明, 无论是采用视网膜片还是细胞悬液方式进行细胞移植, Müller细胞的过度激活都会影响移植供体中的细胞向视网膜内迁移, 导致移植细胞的整合率降低^[47,64]。另外, 移植时受者动物的年龄及视网膜变性的病变阶段也可影响植入细胞的整合效率, 可能是因为老年或变性晚期视网膜胶质细胞增生形成胶质疤痕, 阻碍了植入细胞的整合^[68]。如果调整视网膜变

性鼠中Müller细胞反应蛋白GFAP的表达量, 感光前体细胞移植后的整合率与GFAP表达水平呈显著的负相关^[68,69]。不容忽视的是, Müller细胞支撑整个视网膜并且其足突与感光细胞内节形成紧密连接, 组成了视网膜外界膜。当采用GFAP^{-/-}Vim^{-/-}敲除鼠清除小鼠Müller细胞, 导致视网膜细胞原本由Müller占据的间隙产生和外界膜的破坏后, 可提高移植入视网膜细胞的整合^[69,70]。研究证实, 当视网膜外界膜的完整性被破坏时, 整合的感光细胞数量显著增加^[51]。

(ii) 化学屏障. 此外, 当Müller细胞反应性增生时, 可产生抑制神经再生的细胞外基质蛋白^[71,72], 如硫酸软骨素蛋白聚糖(chondroitin sulfate proteoglycans, CSPGs), CSPG形成的化学屏障可阻碍移植细胞的存活、迁移和整合^[73]。多种视网膜变性微环境中均呈现出明显的CSPGs异常升高^[47,72-74]。通过软骨素酶chondroitinase ABC或者基质金属蛋白酶可抑制或者降解视网膜变性微环境中的CSPGs, 从而增加干细胞存活和并促进植入细胞和受者细胞的突触形成^[75-78]。反应性胶质增生也会影响移植干细胞的分化命运。损伤视网膜Müller细胞的反应性增生, 会导致移植的神经干细胞更倾向于向胶质细胞分化, 而不是分化成感光细胞^[48]。

(iii) Müller细胞的反应性增生对干细胞命运的有利影响. Müller细胞在视网膜损伤/变性早期和晚期的作用截然不同, Müller细胞胶质增生对干细胞命运的影响亦具有两面性。在视网膜损伤早期, Müller细胞能分泌bFGF, CNTF等促进干细胞存活和分化的细胞因子^[48]。在视网膜变性后期, Müller细胞发生重塑, 其突起的重排会帮助视锥细胞形成花环样结构, 这有助于残存的视锥细胞存活^[79]。Müller细胞贯穿视网膜全层, 在发育过程中可能作为支架帮助前体细胞迁移分化^[80]。但损伤/变性视网膜微环境中, Müller细胞对移植干细胞的结构支持及引导作用, 尚待进一步阐明。此外, 受者视网膜中Müller细胞是否通过吞噬作用影响移植干细胞命运, 尚无研究报道。

(2) 星形胶质细胞的反应性增生对干细胞命运的影响. 与Müller细胞相比, 星形胶质细胞在视网膜变性疾病中的作用受到关注较少。在某些类型的视网膜损伤中, 如视网膜脱离、青光眼和视神经损伤, 星形胶质细胞也会发生反应性增生^[7-9]。在正常视网膜中, 星形胶质细胞表达GFAP^[8], 反应性增生的星形胶质细胞

不仅上调GFAP, 而且在结构上发生重构, 在外观上会变得越来越不规则, 其突起也会深入神经视网膜层. 在视神经头区域的星形胶质细胞激活与青光眼眼内压升高和神经节细胞死亡有关^[9]. 受研究手段的限制, 目前的研究很少单独观察星形胶质细胞激活对干细胞命运的影响. 当抑制变性视网膜中GFAP表达来提高干细胞移植整合率时, 有可能部分是通过抑制星形胶质细胞激活来提高整合率^[69]. 此外, 当采用经玻璃体腔方式移植干细胞时, 干细胞与位于内层的星形胶质细胞也会产生密切接触, 激活的星形胶质细胞可能和Müller细胞、小胶质细胞一起迁移至移植区域, 增殖形成视网膜前膜, 但具体的作用机制尚待阐明^[81]. 此外, 异常的星形胶质细胞可能导致血视网膜屏障(blood-retinal barrier, BRB)的破坏, 从而改变视网膜微环境的稳态, 这也可能会影响干细胞的命运^[82].

3.2 异常免疫微环境及其对干细胞命运的影响

小胶质细胞是视网膜固有免疫防御系统的重要组成部分. 在视网膜损伤和变性过程中, 小胶质细胞扮演了重要的角色^[6]. 以往认为, 小胶质细胞在视网膜损伤早期被激活, 形态由分枝样变成阿米巴样, 募集至受损区域, 吞噬清除凋亡细胞, 行使修复作用^[83]. 研究表明, 在视网膜变性过程中, 小胶质细胞也能吞噬活的感光细胞而加重视网膜变性^[84]. 另外, 小胶质细胞在过度激活时会分泌多种炎症介质, 造成炎性的视网膜微环境, 不利于感光细胞的存活^[85]. 同时激活的小胶质细胞持续合成补体, 沉积在视网膜, 但补体通路激活后究竟是加速视网膜变性还是延缓变性, 目前尚存争议^[86-89]. 此前, 小胶质细胞因其激活后的功能差异被划分为促炎的“M1”型和抗炎的“M2”型^[90]. 近年来随着单细胞测序技术的应用, 对小胶质细胞多样性和可塑性的认识也逐渐加深. 在疾病过程中, 受不同疾病微环境的影响, 小胶质细胞在促炎或抗炎表型之间的转化是一个复杂的动态过程, 呈现疾病相关性的转变^[91-93]. 除了小胶质细胞产生改变, 在损伤/变性的视网膜中, 巨噬细胞和淋巴细胞等外周免疫细胞也通过损伤的血视网膜屏障被募集至视网膜, 延缓或者加重视网膜变性过程^[94-96].

(1) 吞噬作用. 小胶质细胞在疾病环境中呈现的多样性也决定了其对干细胞命运决定的复杂性. 吞噬能力是小胶质细胞的主要功能之一. 小胶质细胞的可

以敏锐识别周围环境的异常, 迁移至损伤区域, 吞噬死亡细胞碎片, 维持微环境稳态. 移植的同种异体或异种干细胞作为外源性细胞进入视网膜时, 小胶质细胞能通过吞噬作用来攻击清除移植细胞, 从而影响移植细胞的存活、迁移和整合^[73,97-99]. 在干细胞移植视网膜后, 在细胞移植区发现大量的小胶质细胞/巨噬细胞浸润, 且其浸润程度与细胞整合数量成负相关^[98]. 小胶质细胞是否影响植入细胞的整合较难界定, 因为移植细胞也可能是发生整合后再受小胶质细胞影响. 此外, 这些被募集的细胞不仅限于视网膜固有小胶质细胞, 同时也可能是循环系统的巨噬细胞^[95]. 目前可以通过细胞谱系追踪和利用特定的细胞标记, 如利用TMEM119初步将视网膜小胶质细胞和循环系统来源的巨噬细胞分开^[100]. 随着对两类免疫细胞亚型研究的深入, 不同亚型的小胶质细胞和巨噬细胞对干细胞命运决定的影响, 将是未来研究的热点.

(2) 炎症微环境. 除了吞噬作用外, 激活的小胶质细胞也可能产生大量不利于细胞生存的炎症因子. 其中一些与移植细胞的迁移有关, 但大部分炎症因子与植入细胞的死亡有关^[101,102]. 视网膜变性过程中激活小胶质细胞所造成的特殊炎症微环境可能影响移植细胞正确分化. 已有研究提示, 激活小胶质细胞来源的IL-6能够阻碍视网膜祖细胞产生神经球^[103], 降低视网膜祖细胞分化成Recoverin⁺类感光细胞的能力^[104].

(3) 小胶质细胞激活对干细胞命运的有利影响. 某些激活态的小胶质细胞也能分泌保护性细胞因子, 这对于干细胞存活和分化是有利的^[90]. 小胶质细胞参与发育视网膜中视网膜祖细胞的分化和突触形成过程, 在成年正常和疾病视网膜中参与视网膜神经环路中的突触修饰过程^[105,106]. 本课题组^[106]前期研究发现, 在视网膜变性过程中, 小胶质细胞能通过吞噬突触后蛋白来抑制视杆细胞的双极细胞的异位突触形成, 这有助于延缓RP早期视力的下降. 干细胞移植应用于视网膜变性疾病治疗时, 移植干细胞分化整合替代的过程, 同样是在疾病环境中实现神经再生和神经环路重建的过程. 小胶质细胞是否也参与移植干细胞整合进入视网膜环路中的突触联系形成, 尚待进一步研究.

3.3 异常代谢微环境及其对干细胞命运的影响

视网膜代谢功能障碍被认为是视网膜变性的重要

原因之一。目前研究人员已经确定了一系列与视网膜退行性疾病发病机制相关的关键生物标志物和代谢途径^[107]。

(1) 氧化应激微环境及其对干细胞命运的影响。氧化应激是由抗氧化防御机制和反应性活性氧类(reactive oxygen species, ROS)产生失衡所引起的, 它在多种视网膜疾病的发生发展中发挥关键作用。这是因为视网膜是体内能量消耗最高的器官之一, 特别容易受氧化应激的影响, 视网膜的高氧分压和高多不饱和脂肪酸水平, 增加了视网膜对氧化损伤的敏感性^[108]。ROS的增加会导致视网膜细胞的死亡, 影响视网膜稳态, 导致AMD和青光眼的发生^[109,110]。在DR中, 异常高血糖会导致线粒体电子传递链功能障碍, 最终导致ROS增加和细胞死亡, 这可能与DR的发生或者恶化有关。同样, 病理性高水平的氧化应激会抑制植入神经干细胞分化成神经元, 影响神经的再生效果^[111]。联合抗氧化纳米酶复合支架移植神经干细胞, 则能消除ROS及其对新生神经元的氧化损伤, 从而提高新生神经元的存活率和神经突起的延伸。过量ROS还可导致移植治疗脊髓损伤时BMSCs的凋亡, 使其存活率和移植效率降低, 限制干细胞的治疗效果^[112]。

(2) 糖代谢紊乱及其对干细胞命运的影响。随着衰老的进行, RPE细胞的糖酵解增加并导致感光细胞的死亡, 这是AMD的发病机制之一^[113]。本实验室^[114]前期通过对视网膜变性RCS大鼠视网膜蛋白组学分析发现, 在视网膜变性早期, 存在糖酵解及能量代谢相关蛋白的改变。对渗出性AMD的血浆代谢组学分析也提示疾病过程中线粒体能量代谢的损害和肌肽的缺乏^[115]。糖酵解是干细胞的主要代谢模式, 在眼发育过程中视网膜的增殖性前体细胞进行有氧糖酵解, 糖酵解和糖异生的相关基因在神经干细胞中高表达^[116]。这可能意味着移植的增殖性视网膜前体细胞能响应损伤视网膜的异常有氧糖酵解微环境进行正确的细胞命运决定, 从而发挥治疗作用。在高血糖环境下, 干细胞治疗效果似乎受到损害^[117], 提示糖代谢微环境对干细胞的命运决定发挥重要作用。

(3) 脂代谢紊乱及其对干细胞命运的影响。脂质代谢异常是视网膜变性过程中的重要病理生理改变。AMD中RPE细胞功能受损, 导致脂质的堆积和视网膜损伤。有证据表明, AMD与视网膜胆固醇维持之间存在密切联系, 视循环中RPE细胞内胆固醇氧化

的副产物-氧化固醇的过度积累可导致RPE和感光细胞的死亡^[118]。在晚期AMD中, 过量脂质代谢废物沉积在Bruch's膜上, 可能影响移植细胞存活和分化^[119]。

(4) 兴奋性氨基酸堆积及其对干细胞命运的影响。很多研究发现, 谷氨酸在视网膜变性疾病中会大量积累^[120]。谷氨酸具有神经兴奋毒性作用, 它能刺激NMDA受体, 导致大量Ca⁺进入细胞, 激活磷脂酶、核酸内切酶和蛋白酶, 最终导致视网膜细胞死亡^[121]。同样在DR患者中, 支链氨基酸(BCAA)代谢中亮氨酸、异亮氨酸和缬氨酸的水平也显著增加^[122]。BCAA水平增高被认为与视网膜中谷氨酸的神经毒性有关, 其通过激活mTOR途径, 上调VEGF, 导致Caspase-3表达增加^[123], 从而导致视网膜细胞的凋亡。同时谷氨酸也是诱导视网膜前体细胞分化的关键信号, 能通过上调介导细胞有丝分裂和神经突起生长的蛋白质来诱导成熟大鼠视网膜中的神经祖细胞的形成^[124]。而代谢型谷氨酸受体的激活可以促进大鼠RPCs的增殖分化^[125]。

4 干细胞对视网膜变性微环境的主动调控作用及机制

4.1 干细胞主动调节视网膜变性微环境作用

干细胞的命运不仅被动受视网膜微环境影响, 干细胞也能通过主动改善视网膜变性微环境发挥治疗作用(图2)。目前已经报道的可以调控视网膜变性微环境的细胞类型主要有中枢神经系统来源神经干细胞(neural stem cells, NSCs)、BMSCs、脐带来源干细胞(umbilical cord-derived stem cells, UC-SCs)等干细胞, 另外还有一些特殊类型成体细胞也提示具有微环境改善作用, 如嗅鞘细胞(olfactory ensheathing cells, OECs)^[126~128]。

(1) 干细胞调控反应性胶质增生。BMSCs和NSCs能抑制视网膜变性中的反应性胶质增生^[126,129,130], 表现为下调视网膜组织中的GFAP蛋白表达, 减少Müller细胞的增生肥大。本课题组^[17]前期研究发现, 类器官来源的RPCs也具有主动调控视网膜变性微环境的能力, 能抑制变性过程中的Müller细胞增生, 有利于干细胞存活和发挥功能。干细胞与Müller细胞的作用也受移植方式的影响, 如采用玻璃体腔移植的方式, 则有

图 2 干细胞调节视网膜变性微环境的作用机制。A: 视网膜变性微环境; B: 干细胞进行视网膜下腔移植或玻璃体腔移植, 调节视网膜变性微环境。I: 旁分泌作用。移植的干细胞通过分泌组方式释放细胞外囊泡和分子物质(蛋白质、游离核酸、脂质)等物质作用于视网膜变性微环境。I a: 植入干细胞促进激活的M1型小胶质细胞转变成M2型小胶质细胞, 发挥抑制炎症和保护神经作用; I b: 植入干细胞通过旁分泌效应抑制星形胶质细胞反应性增生; II: 细胞融合。变性视网膜中移植入的干细胞与激活的Müller细胞融合, 逆转反应性胶质增生, 导致Müller细胞重编程为视网膜前体细胞或转分化为神经元; III: 隧道纳米管。移植入的干细胞与视网膜色素上皮细胞形成隧道纳米管, 转运线粒体进入视网膜色素上皮细胞。IV: 细胞替代。干细胞还可以通过分化成具有调控视网膜微环境稳态能力的细胞(视网膜色素上皮细胞、小胶质细胞和Müller前体细胞), 来行使调节微环境的功能

Figure 2 Mechanism of stem cells regulating degenerative retinal microenvironment. A: Retinal degenerative microenvironment. B: The stem cells were transplanted subretinally or intravitreally to regulate the degenerative retinal microenvironment. I: Paracrine. The grafted stem cells release extracellular vesicles and molecular substances (protein, free nucleic acid, and lipid) through secretome to act on the degenerative retinal microenvironment. I a: grafted stem cells promote the transformation of activated M1-type microglia into M2-type microglia, which can inhibit inflammation and protect the neural retina. I b: The grafted stem cells inhibit gliosis of astrocytes through the paracrine effect. II: Cell fusion. The grafted stem cells in the degenerated retina fuse with activated Müller cells to reverse gliosis, leading to reprogramming of Müller cells into retinal progenitors or transdifferentiation into neurons. III: Tunneling nanotubes. The grafted stem cells formed tunneling nanotubes with the retinal pigment epithelial cells and transferred mitochondria into the retinal pigment epithelial cells. IV: Cell replacement. Stem cells can also regulate the microenvironment by differentiating into cells to regulate the homeostasis of the retinal microenvironment (retinal pigment epithelial cells, microglia cells, and Müller precursor cells)

可能导致Müller细胞、RPE细胞和小胶质细胞从视网膜迁移到移植干细胞区域, 形成视网膜前膜^[81]。这可能是某些干细胞临床试验出现并发症, 即干细胞移植术后的病人出现严重的玻璃体增殖和黄斑前膜的重要原因^[44,131]。

Müller细胞在发育成熟后, 可能仍保留一定的干细胞特性。在特殊刺激时, Müller细胞能重编程为干细胞或者转分化为视网膜神经元, 这成为利用内源性干细胞修复损伤视网膜的新思路^[54]。当移植干细胞作用

于Müller细胞时, 也可能逆转Müller细胞反应性增生和胶质化的命运, 使其重编程为视网膜前体细胞, 或者使Müller细胞分化成视网膜神经元。Cosma团队^[132]报道, 造血干细胞在移植入视网膜变性小鼠后, 能与视网膜Müller细胞发生融合, 从而使其重编程为感光前体进而分化成感光细胞。Jian等人^[133]的研究也提示, 移植骨髓间充质干细胞进入RCS大鼠视网膜下腔后可以增加GS⁺/CRX⁺细胞数量, 而这些GS⁺/CRX⁺细胞被认为是正在发生重编程的Müller细胞。视网膜干细胞

移植也能增加视网膜变性鼠中CHX10⁺细胞数量, 提示Müller细胞可能发生了重编程^[134]。未来利用单细胞测序及谱系示踪的方法能增加此类研究结果的准确度, 更好地阐释干细胞移植激活内源性Müller细胞重编程的作用机制。

(2) 干细胞调控视网膜免疫微环境。具有免疫调节能力的干细胞能减少变性视网膜中小胶质细胞的激活数量, 使小胶质细胞形态由激活的阿米巴样向分枝静息态转变, 从而改善视网膜免疫微环境^[13,17,135]。本课题组^[13]采用NSCs移植视网膜变性RD1小鼠, 发现NSCs能通过TIMP1抑制视网膜小胶质细胞激活。此外, 类器官来源的RPCs能主动调控视网膜变性微环境, 移植后能抑制视网膜变性鼠小胶质细胞激活, 从而为自身存活提供更佳的微环境, 延长治疗效果^[17]。激活的小胶质细胞过去被分为促炎的M1型和抑炎及具有神经保护作用的M2型。研究表明, 干细胞移植也可以通过促进M1型小胶质细胞向M2型小胶质细胞转换而发挥修复损伤视网膜的作用^[136,137]。此外, 干细胞也能整体下调视网膜疾病微环境中的炎症因子^[14,138], 形成有保护作用的视网膜微环境。干细胞还可以通过募集具有保护作用的外周单核巨噬细胞来改善视网膜损伤微环境^[139]。将内皮祖细胞移植治疗RD1小鼠, 能通过其分泌的CCL2从骨髓将F4/80⁺/Ly6c⁺单核巨噬细胞选择性地募集到视网膜而挽救感光细胞。这些被募集的巨噬细胞高表达神经营养因子, TGF-β1, IGF-1及抗炎介质IL10。

(3) 干细胞调控视网膜异常代谢微环境。移植干细胞能通过旁分泌机制或与受者细胞发生细胞间物质交换主动调节变性视网膜中的异常代谢微环境, 具有减轻氧化应激、减少血脂和降低血糖等功效, 从而恢复有效视功能。

(i) 干细胞调控视网膜氧化应激。氧化应激是多种视网膜变性疾病的发病机制之一。研究发现, 移植的MSCs能通过多种机制降低视网膜细胞中ROS的产生, 促进其存活和再生。干细胞可分泌抗氧化酶和神经营养因子(如CNTF和PEDF)以调节变性视网膜的ROS水平和线粒体发生, 改善线粒体的活性, 增强损伤RPE细胞的抗氧化能力并抑制细胞凋亡^[140~142]。同样MSCs分泌细胞外囊泡(mesenchymal stromal cell (MSC)-derived extracellular vesicles, MSC-EVs), 通过递送mRNA和microRNA, 激活受损视网膜细胞中的自噬

和/或抑制细胞凋亡, 从而促进其存活和再生^[143]。

(ii) 干细胞调控视网膜糖代谢紊乱。BRB是由血管内皮细胞和周细胞共同形成的物理化学屏障, 可维持视网膜微环境稳态^[144]。但在DR中, 血糖升高会诱导P2X7受体激活, 导致周细胞死亡^[145], 周细胞的丢失会导致BRB的破坏和随后威胁视力的血管功能障碍。而脂肪来源的间充质干细胞(adipose mesenchymal stem cells, AMSCs)能通过分化为视网膜中的感光细胞和周细胞并加强视网膜内皮细胞(retinal epithelium cells, RECs)间的细胞连接改善DR中BRB的完整性。此外, 高血糖还会导致线粒体功能障碍而引起视网膜中的氧化应激^[146]。最近的研究表明, MSCs和视网膜细胞可发生细胞间线粒体转移, 接受MSCs线粒体的受体细胞表现出线粒体呼吸能力增强, 线粒体结构和功能相关基因表达增强^[147]。MSCs通过改善线粒体功能降低氧化应激水平, 从而改善糖耐量^[148]。此外, 干细胞及其分泌的因子也可以直接降低血糖, 治疗DR^[38,149]。

(iii) 干细胞调控视网膜脂代谢紊乱。AMD的发病机制包括动脉粥样硬化斑块的形成, 导致血流阻力增加, 进而改变RPE细胞的功能, 导致脂质沉积和视网膜损伤^[150]。MSCs可通过缩减视网膜血管中的动脉粥样硬化斑块, 减少脂质的沉积, 恢复正常血流, 进而改善AMD患者的视力。同时, MSCs通过增加胆固醇外流调节蛋白ABCA1的表达和HIF-1信号的激活, 可更好地处理胆固醇, 进而对小鼠视网膜节细胞功能和存活产生积极影响^[151]。

(iv) 干细胞调控视网膜兴奋性氨基酸堆积。变性视网膜出现大量兴奋性氨基酸的堆积, 会导致视网膜细胞凋亡, 而移植的MSCs能有效对抗谷氨酸兴奋性毒性, 保护视网膜神经节细胞。MSCs通过产生可溶性因子, 抑制NMDA诱导的NMDA受体表达并降低含GluR1的AMPA功能, 降低神经元对谷氨酸的敏感性, 调节谷氨酸诱导的钙通量, 对视网膜节细胞产生神经保护作用^[152]。

4.2 干细胞调控视网膜变性微环境的机制

(1) 旁分泌作用。干细胞主要通过旁分泌的细胞因子、细胞外囊泡等与视网膜微环境进行互动^[143,153]。采用干细胞的条件培养基和细胞外囊泡也能达到调控视网膜微环境的效果^[143,153,154]。近年来, 组学方法的兴起助力阐明干细胞旁分泌效应, 其中分

泌组(secretome)是指一个细胞分泌到细胞外的所有物质,包括可溶性蛋白、游离核酸、脂质、细胞器和细胞外囊泡等^[155]。蛋白质又包括生长因子、细胞因子、趋化因子、黏附分子、蛋白酶和细胞受体等多种具有功能的物质。细胞外囊泡根据直径大小可分为凋亡小体、细胞微囊泡和外泌体。干细胞通过外囊泡或外泌体中携带的蛋白、细胞因子、microRNA和mRNA在细胞间进行信号传递^[153,156]。这类借助于细胞外囊泡或外泌体分泌的内容物是细胞分泌物的主要组分。进一步揭示不同种类干细胞外囊泡或外泌体中的有效成分,将推进干细胞及其衍生物治疗视网膜变性疾病的临床转化。本课题组前期证实,小鼠和人神经干细胞来源的外泌体具有抑制变性视网膜小胶质细胞过度激活的作用,发现人神经干细胞外泌体microRNA表达谱与小鼠神经干细胞microRNA表达谱高度相似,具有调控包括*TNF- α* 、*IL-1 β* 及*COX-2*在内的炎症相关基因的关键microRNA^[153]。此外,借助蛋白组学和代谢组学进一步研究不同干细胞外囊泡或者外泌体内容物,将有望从多维度解析干细胞调控视网膜变性微环境的机制^[157]。

(2) 物质交换。除了旁分泌效应,植入的干细胞还可能和受者微环境中的细胞通过隧道纳米管(tunneling nanotubes, TNTs)、缝隙连接和细胞融合等方式进行电信号、代谢信息和免疫信号的传递^[147,158,159]。TNT是细胞间的一种特殊的丝状伪足样结构,可以实现一些较大的物质转运,如线粒体、溶酶体等细胞器和大分子蛋白^[160]。研究显示,间充质干细胞能通过TNTs向多种眼细胞(角膜内皮细胞、视网膜色素上皮细胞、感光细胞)转运线粒体,有助于改善受者细胞的能量代谢,挽救细胞的能量代谢损害^[147]。缝隙连接是广泛存在于细胞间的连接形式。在视网膜发育过程中,视网膜祖细胞之间通过缝隙连接形成复杂网络进行细胞间通讯。已有研究表明,干细胞也可以与受者微环境中的终末分化细胞通过缝隙连接进行物质交换。BMSCs能通过缝隙连接向气管内皮细胞转运胞浆物质^[159,161]。神经干细胞可以与脑损伤受者的星形胶质细胞形成缝隙连接,调控星形胶质细胞反应性增生^[158]。因此,干细胞极有可能也是通过缝隙连接与视网膜变性微环境中的细胞进行物质交换以调控其功能。此外,骨髓造血干细胞能与视网膜变性鼠Müller细胞通过细

胞融合的方式促进Müller细胞重编程为感光细胞,从而逆转Müller胶质化的命运^[132]。

(3) 细胞替代。干细胞还可以通过分化成具有调控视网膜微环境稳态能力的细胞,来行使调节微环境的功能。ESC-RPE细胞能通过分泌免疫调节因子及吞噬清除脱落的感光细胞外节来维持视网膜微环境稳态。人多能干细胞诱导成为Müller前体细胞和小胶质前体细胞的技术已经初步建立^[162~164]。多能干细胞来源的Müller前体细胞移植能挽救NMDA损伤后的视功能^[163]。iPSCs来源的小胶质细胞移植到新生小鼠大脑中,其表型和基因表达特征与人大脑静息态小胶质细胞高度相似^[165]。通过外源性移植Müller/小胶质细胞前体的方式来改善视网膜变性微环境有望成为治疗视网膜变性疾病的新策略。

5 展望

干细胞治疗视网膜变性疾病的科研临床转化之路已经走过十余载。在不断的探索中,借助新技术,人们对视网膜变性微环境有了更深入的理解。如何通过改善微环境来提高移植细胞的存活、分化和整合率,将是未来研究的热点问题。鉴于视网膜变性微环境对移植干细胞命运决定至关重要,现在有多种策略旨在通过改善视网膜变性微环境来提高干细胞存活、迁移、分化和整合,包括:(i) 联合细胞移植。某些特殊类型的细胞,如MSCs和OECs具有调节视网膜变性微环境的作用^[127,128,166]。通过联合细胞移植策略能有效促进移植干细胞的存活和分化,提高治疗效果。(ii) 联合免疫调节物。联合具有免疫调节作用的细胞因子、细胞外囊泡、microRNA改善视网膜变性微环境,提高感光细胞整合效率^[153,167]。(iii) 联合生物材料。利用胶原蛋白、层黏连蛋白等制作为生物支架以提供干细胞生长的微环境,有助于干细胞移植后发挥作用^[168,169]。

另外,干细胞也通过多种形式与视网膜变性微环境发生交流。深入了解这种交流模式,并阐释干细胞调节微环境的机制,具有重要意义。总之,深入研究干细胞和视网膜变性微环境的相互作用,有助于更好地探索和优化干细胞移植治疗视网膜变性疾病策略,促进临床转化。

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Effects of interaction between stem cells and degenerative retinal microenvironment on stem cell fate determination

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To date, a substantial amount of experimental data supports the safety and effectiveness of stem cell-based cell therapy in treating retinal degenerative diseases, thus, serving as a foundation for clinical studies. However, its therapeutic effect often declines or even disappears at a later stage. In addition to the characteristics of stem cells, the microenvironment in which stem cells are transplanted is likely a key factor that determines the fate of stem cells. Glial scar, chronic inflammation, and abnormal metabolic microenvironment in the retina often lead to the failure of long-term survival, correct differentiation, and integration of grafted stem cells in the retina, which may be important factors contributing to the failure of long-term therapeutic effect of stem cell treatment for retinal degenerative diseases. Alternatively, grafted stem cells may also actively regulate the degenerating retina through paracrine or material exchange with the host cells, which can help the fate determinant of grafted stem cells in the retina. Thus, the therapeutic effect of stem cell transplantation can be improved by combined-transplantation of different stem cells or by regulating the degenerative retinal microenvironment. This review summarizes the recent progress in interactions between grafted stem cells and degenerative host retina and their impact on stem cell fate determination.

stem cells, retinal degeneration, microenvironment, inflammation, gliosis, metabolism

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