分子生物医学

骨髓间充质干细胞治疗脊髓损伤应用方法的多样性

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摘要：脊髓损伤（SCI）会导致损伤节段以下肢体的运动、感觉和内脏功能永久性障碍，目前尚无有效治疗方法。针对 SCI 的治疗和康复已成为神经科学领域的重要课题和难题。骨髓间充质干细胞（BMSCs）具有神经营养功能，还可调节免疫平衡、跨胚层分化为神经细胞等，移植 BMSCs 对 SCI 后的功能恢复具有显著的促进作用。且 BMSCs 治疗 SCI 的应用方法具有多样性，即 BMSCs 可以通过多种不同的应用方法，以多种不同的机制发挥治疗 SCI 的作用。相信随着对 BMSCs 治疗 SCI 研究的深入，不同应用方法的特点和优势也将更加清晰，从而为临床 SCI 患者的不同需求提供最佳的治疗方案。

关键词：脊髓损伤；骨髓间充质干细胞；应用方法

Diversity of Application Methods of Bone Marrow Mesenchymal Stem Cells in Treatment of Spinal Cord Injury

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Abstract：Spinal cord injury（SCI） can cause permanent impairment of limb movement，sensation and visceral functions below the injured spinal cord segment，so far there is no effective treatment method. SCI therapy and rehabilitation has become an important topic and problem in the field of neuroscience. Bone marrow mesenchymal stem cells（BMSCs） have neurotrophic function，can regulate immune balance，and trans-differentiate into neural cells，etc. BMSC transplantation has a significant role in promoting functional recovery after SCI. Moreover，BMSCs have diversified application methods in the treatment of SCI，that is，BMSCs can perform the therapeutic function on SCI through various application methods with different mechanisms. It is believed that with the in-depth study of BMSCs for SCI treatment，the characteristics and advantages of different application methods will become clearer，so as to provide the optimal treatment scheme according to the different needs of the SCI patients in clinical practice.

Key words：Spinal cord injury；Bone marrow mesenchymal stem cells；Application methods

脊髓损伤（spinal cord injury，SCI）是一类因创伤、肿瘤、缺氧、炎症等因素造成的脊髓完整性和连续性被破坏的疾病。近年来，SCI 患者呈逐年增多趋势，且以青壮年居多。中枢神经系统神经元损伤后难以再生，造成 SCI 患者肢体和内脏功能恢复困难，给患者造成极大痛苦，同时也给患者的家庭和社会带来沉重的经济负担。自骨髓间充质干细胞（bone marrow mesenchymal stem cells，BMSCs）被发现以来，其在中枢神经系统疾病中的作用一直是学者们研究的热点。移植 BMSCs 对于 SCI 患者具有显著的治疗作用，可改善 SCI 患者的预后，其内在机制可能由于 BMSCs 具有显著的神经营养、调节免疫平衡、抗氧化应激和抗凋亡等作用；在体外培养条件下 BMSCs 还具有跨胚层分化的能力，可提高突触传递功能，促进神经网络构建，改善后肢运动功能；

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此外,BMSCs 可自身获得,因此可以有效避免免疫排斥反应[1-2]。这些特性使 BMSCs 成为治疗 SCI 的理想候选细胞。BMSCs 治疗 SCI 有多种不同的应用方法,通过不同的机制发挥作用,产生不同的治疗效果。现就 BMSCs 治疗 SCI 的应用方法的多样性予以综述。

1 BMSCs 概述

间充质干细胞(mesenchymal stem cells, MSCs)是一类源于中胚层且具有自我更新和多项分化潜能的多能干细胞,其中 BMSCs 最早被发现,而骨髓也被认为是 MSCs 临床应用的主要来源之一[3]。1970 年,Friedenstein 等[4]首次发现来自骨髓的某种细胞可以在塑胶培养基上形成集落;1991 年,Caplan[5]将这种细胞命名为 BMSCs,同时发现 BMSCs 具有分化形成骨、软骨和脂肪的潜能。目前,国际细胞治疗协会建议通过以下标准识别 BMSCs:可以黏附在塑胶上;在标准的体外分化条件下可分化为软骨细胞、成骨细胞和脂肪细胞;可表达细胞表面标志物 CD105、CD73 和 CD90,且不表达 CD45、CD34、CD14、CD11b、CD79a、CD19 以及人类白细胞抗原 DR 等[6]。

2 BMSCs 治疗 SCI 的多种应用方法及其作用机制

BMSCs 可以跨胚层分化为属于外胚层来源的神经干细胞、神经元和胶质细胞[7],并具有免疫调节、抗氧化应激和抗凋亡等作用,因此在减轻 SCI 后的继发性损伤方面具有广阔的应用前景,是一种被广泛研究的成体干细胞[8]。目前 BMSCs 治疗 SCI 具有多种应用方法,主要包括:直接移植体外培养扩增的 BMSCs、仅应用 BMSCs 分泌的活性物质治疗 SCI、体外诱导分化 BMSCs 为神经干细胞或前体细胞后再移植、用特定基因干预 BMSCs 后再移植以及 BMSCs 移植联合其他方法治疗 SCI 等。

2.1 直接移植体外培养扩增的 BMSCs 目前可以通过多种途径直接移植 BMSCs 治疗 SCI,主要包括将 BMSCs 直接注射到损伤脊髓局部、鞘内注射、静脉注射或腹腔注射[9-10]。BMSCs 具有“归巢”的特性,移植后会向损伤位点迁移[1]。移植体外培养扩增的 BMSCs 后,脊髓局部的表现和功能会显著改善,炎症反应缓解,细胞凋亡减轻[11];出血反应与瘢痕纤维化减轻[12];血脊髓屏障的完整性得到巩固[13];轴突再生和再髓鞘形成能力提高,神经通路的重新构建增强[14]。在这种情况下,BMSCs 发挥治疗作用主要通过神经营养、调控免疫平衡以及抑制氧化应激反应和神经元凋亡等机制。BMSCs 可以通过分泌生长因子和神经营养因子(neurotrophin, NT)起到神经营养的作用,促进神经元再生[15-18]。SCI 后,过度的炎症反应是引起继发性 SCI 的重要原因之一,调控免疫平衡、抑制过度的促炎症反应以及增强有利于愈合和再生的因子的合成和释放,是治疗 SCI 的重要策略之一,而移植 BMSCs 能够发挥这种作用[19-20]。氧化应激反应是引起 SCI 后继发性损伤的另一重要因素,如何抑制氧化应激反应也是治疗 SCI 的重要策略之一,而 BMSCs 可以通过抑制氧化应激,减轻继发性损伤,达到治疗的目的[21]。SCI 后,一系列引起继发性损伤的因素均会导致残存的神经元凋亡,进一步加重损伤,而移植的 BMSCs 可以通过多种途径抑制神经元凋亡[22-24],促进 SCI 大鼠的功能恢复。学者们陆续开展了多项临床试验,初步结果显示移植 BMSCs 后患者的运动、感觉以及膀胱功能改善,表明移植 BMSCs 安全可行,且能提高 SCI 患者的生活质量[1-2,9,25]。

2.2 应用 BMSCs 分泌的活性物质治疗 SCI SCI 后,损伤区的微环境不利于移植细胞的存活,且移植细胞在体内的存活和分化方向难以控制,阻碍了移植细胞作用的发挥。直接利用 BMSCs 分泌的活性物质治疗 SCI,可避免这些不利因素。BMSCs 可以分泌众多有利于组织修复和再生的物质,包括多种营养因子、免疫调节因子、含有生物活性分子的外泌体等[1]。研究发现,给大鼠注射 BMSCs 的条件性培养液,可以显著减轻 SCI,促进 SCI 后的功能恢复[26-29],说明 BMSCs 条件性培养液中含有 BMSCs 分泌的促进脊髓再生和修复的物质。实验证实,条件性培养液可以减轻凋亡和氧化应激反应[30]。进一步检测发现,条件性培养液中至少含有 120 种蛋白质分子,包括表皮细胞生长因子、成纤维细胞生长因子(fibroblast growth factor, FGF)、血管内皮生长因子、血管紧张素、脑源性神经营养因子(brain-derived neurotrophic factor, BDNF)、胶质细胞源性神经营养因子(glial cell line-derived neurotrophic factor, GDNF)、睫状生长因子、白细胞介素-10 以及白细胞介素受体拮抗剂等众多促进神经再生和组织修复的分子[31]。蛋白质组学证实,条件性培养液中含有丰富的神经营养、血管生成和免疫调节相关的蛋白分子[32]。近年研究还发现,

静脉注射 BMSCs 分泌的外泌体也可以促进 SCI 后的功能修复[33]。BMSCs 来源的外泌体可以通过抑制 A1 型星形胶质细胞活化[34]或作用于 M2 型巨噬细胞发挥作用[35],促进损伤脊髓环境中星形胶质细胞和巨噬细胞向有利于再生和修复的表型转化。

2.3 体外诱导 BMSCs 分化为神经前体细胞后再移植 虽然神经干/前体细胞是治疗神经系统疾病的一种良好的候选细胞,但伦理、法律和来源的制约限制了神经干/前体细胞的应用。而 BMSCs 可以由自身获得,避免了免疫排斥反应和伦理、法规的制约,因此很多研究通过 BMSCs 跨胚层分化来获取神经干/前体细胞,然后再移植治疗不同的中枢神经系统疾病。

多种因素可以促进 BMSCs 向神经干/前体细胞的跨胚层分化。BDNF、神经生长因子、GDNF、FGF-2 等多种生长因子可以促进 BMSCs 向神经元或神经前体细胞分化[36-37]。声波、电刺激、抗氧化剂等多种方法也可以诱导 BMSCs 的跨胚层分化[38-40]。不同类型的 RNA[如长链非编码 RNA[41]和微 RNA(microRNA,miRNA)[42]]也被证实在 BMSCs 向神经元分化过程中发挥重要作用。还有研究发现,SCI 患者和多发性硬化患者血液中的自身反应性 T 细胞均可在体外诱导 BMSCs 跨胚层分化[43]。

BMSCs 可以跨胚层分化为多种神经细胞,如多巴胺能神经元[44]、运动神经元样细胞[45]、少突胶质细胞样细胞[46]、5-羟色胺能神经元[47]、神经前体细胞[37]、神经元样细胞与胶质样细胞[48]等,体现出通过细胞替代方法治疗帕金森病、运动神经元疾病、脱髓鞘疾病、5-羟色胺失调的精神失常性疾病、SCI 等疾病的应用前景。Ye 等[49]用脑脊液诱导 BMSCs 分化为星形胶质细胞和神经元样细胞,并将其移植到大鼠损伤的脊髓,结果发现,BDNF、神经生长因子及神经营养因子-3(neurotrophin-3,NT-3)的含量均增加,功能恢复也得到显著改善。de la Garza-Castro 等[50]研究发现,与直接移植 BMSCs 相比,将 BMSCs 转化为施万细胞前体细胞后再移植治疗的 SCI 大鼠活动能力和被移植细胞的迁移率均进一步提高。由此可见,与移植脑和脊髓来源的神经干/前体细胞相比,BMSCs 跨胚层分化获取的神经干/前体细胞避免了伦理、法规与来源的约束,而与直接移植 BMSCs 相比,移植 BMSCs 跨胚层分化获取的神经干/前体细胞对 SCI 患者的治疗针对性更强,具有一定的临床意义。

2.4 基因干预 BMSCs 后再移植 将某些有利于神经再生的基因转染至 BMSCs 后再移植到 SCI 患者体内,对于 SCI 后的功能恢复具有更好的疗效。如将分别转染了音猬因子[51]、碱性 FGF[52]、血管内皮生长因子[53]、GDNF[54]、miR-124[55]、Bcl-2[56]或 miR-200a[57]基因的 BMSCs 移植到 SCI 大鼠,均有效促进了 BMSCs 的存活,增加神经纤维的再生和轴突生长,促进了运动和感觉功能的恢复;此外,miR-544[58]、miR-29b[59]或 miR-126[60]基因修饰的 BMSCs 释放的外泌体亦更加有效地促进了 SCI 大鼠的轴突再生和运动功能的恢复。这些研究均提示,BMSCs 经过基因干预后再移植,在治疗 SCI 方面具有良好的应用前景。

2.5 BMSCs 移植联合其他方法治疗 SCI

2.5.1 BMSCs 移植联合应用生长因子 生长因子是通过与特异性细胞膜受体结合,调节细胞生长与其他功能效应的多肽类物质。研究证实,将生长因子与 BMSCs 移植联合治疗 SCI 是一种有效的治疗方法[2]。Shin 等[61]将 FGF-2 与 BMSCs 移植联合应用,发现 FGF-2 对移植的 BMSCs 具有增殖、保护和神经诱导作用,可提高 BMSCs 移植后的存活率,并显著改善了 SCI 症状。黄兴锐等[62]将碱性 FGF 与 BMSCs 联合应用后发现,胶质纤维酸性蛋白的表达减少,神经丝蛋白 200 的表达上调,残余轴突受到保护,促进了修复与再生,大鼠的运动功能也得到有效改善。表明 FGF-2 与 BMSCs 移植联合应用,一方面可以促进移植的 BMSCs 存活,另一方面通过叠加或协同发挥治疗作用,是一种有效的治疗策略。

2.5.2 BMSCs 移植联合电针刺激 电针疗法是指在针刺入人体穴位后,在针上通以接近人体生物电的微量低频脉冲电流进行刺激来防治疾病的一种疗法,与 BMSCs 移植联合应用,可以促进 SCI 后的功能恢复。王文峰和叶红晖[63]研究发现,电针联合 BMSCs 移植可促进 BMSCs 存活以及向神经元样细胞分化。Ding 等[64]通过移植经酪氨酸激酶 C 基因修饰的 BMSCs 并联合电针疗法治疗 SCI,结果发现其可通过提高脊髓 NT-3 水平,上调神经源性因子层粘连蛋白和生长相关蛋白-43 的表达,下调胶质纤维酸性蛋白和硫酸软骨素蛋白聚糖的表达,增加皮质运动诱发电位的传导,提高后肢运动功能。Liu 等[65]还发现,

联合电针疗法治疗可以促进移植的 BMSCs 分化为少突胶质细胞样细胞,促进轴突再髓鞘化。虽然电针本身的作用机制仍有待进一步研究,但 BMSCs 联合电针治疗 SCI 的效果肯定,电针刺激有可能改善了 BMSCs 发挥作用的微环境,因此也是一种应用前景广泛的治疗策略。

2.5.3 BMSCs 联合其他细胞移植 研究已经证实,单独移植神经干/前体细胞、少突前体细胞或嗅鞘细胞等可以提高 SCI 后的功能恢复[66-68],而与 BMSCs 联合移植,将进一步提高 SCI 的治疗效果。脊髓脱髓鞘是 SCI 中常见的一种病理改变,Kaka 等[69]将 BMSCs 转分化的少突前体细胞与未分化的 BMSCs 联合移植到损伤的大鼠脊髓,结果显示,脊髓空洞化程度降低,BBB(Basso-Beattie-Bresnahan)评分显著提高。Wu 等[70]将嗅鞘细胞与 BMSCs 联合移植到损伤的大鼠脊髓,发现神经修复效果与抗凋亡作用较单纯的嗅鞘细胞或 BMSCs 移植更显著。Stewart 等[71]应用过表达基质细胞衍生因子-1 的 BMSCs 与神经干细胞联合移植治疗 SCI 大鼠,发现通过 BMSCs 过表达产生的基质细胞衍生因子-1 作用于神经干细胞,更有利于轴突再生和运动功能恢复。这些研究提示,BMSCs 与其他细胞联合移植可通过功能互补和协同作用促进 SCI 后的修复。

2.5.4 BMSCs 联合组织工程支架移植 在 SCI 的治疗过程中,脊髓空洞和胶质瘢痕形成的物理屏障限制了移植的 BMSCs 发挥作用,而组织工程支架可以在一定程度上克服物理屏障,为 BMSCs 提供良好的增殖、分化与迁移环境,使 BMSCs 发挥更好的效果。将 BMSCs 作为种子细胞,与生物性可降解支架相结合,在 SCI 的治疗中有巨大的应用前景。Zeng 等[72]将高表达 NT-3 的施万细胞与高表达 NT-3 受体酪氨酸激酶 C 的 BMSCs 在三维明胶海绵支架中共培养 14 d,结果支架中产生了神经网络,全细胞膜片钳记录到自发性突触后电流;将支架与细胞一起移植到大鼠脊髓横断间隙 8 周后,大鼠后肢功能和皮质运动诱发电位均得到改善。Kim 等[73]将 BMSCs 与壳聚糖支架或体外聚乳酸-糖基乙酸支架联合移植,结果显示其细胞移植成功率较单纯 BMSCs 移植高,并具有较好的神经保护作用,运动功能改善显著。Chen 等[74]将具有微通道和 H 型腔的管状支架与 BMSCs 共同移植治疗 SCI,结果发现其促进了神经再生,抑制了神经元凋亡。Wang 等[75]将 BMSCs 植入无细胞脊髓支架治疗 SCI 大鼠,结果证明其可以恢复受损脊髓的功能;且使用具有无细胞脊髓支架的 BMSCs 可减少损伤部位的巨噬细胞,通过早期调控炎症细胞募集,抑制凋亡和继发性炎症,促进功能恢复。以上研究表明,联合应用生物支架有利于移植的 BMSCs 存活、克服物理屏障、调节免疫微环境、抑制神经元凋亡、促进 SCI 部位神经元再生以及功能性突触的形成,同时促进神经网络的构建,对 SCI 损伤后的神经网络重建和功能恢复具有重要作用。

3 小 结

BMSCs 是目前治疗 SCI 理想的候选细胞。BMSCs 治疗 SCI 的应用方法具有多样性,可以直接移植使用或使用其分泌产物,也可以体外跨胚层分化为神经干/前体细胞后再移植,还可以经过基因修饰后再移植,或联合其他方法治疗 SCI。目前还应进一步研究如何在 SCI 后复杂的微环境中提高移植细胞的存活率以及调控移植细胞的分化命运,并对比不同应用方法的优势和特点,针对不同 SCI 患者情况找到更加有效的应用方式,使 BMSCs 发挥最佳的治疗效果。相信未来 BMSCs 可以安全、有效地应用到 SCI 的临床治疗中,为不同 SCI 患者的康复提供有效的个性化治疗策略。

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