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综 述

间充质干细胞来源的外泌体治疗炎症性肠病研究进展*

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摘要 间充质干细胞主要通过免疫调控和旁分泌在炎症性疾病的治疗中发挥功能。MSC 的旁分泌效应是通过分泌可溶性因子并释放外泌体而发挥作用。外泌体将 DNA、蛋白质/肽、mRNA、microRNA、脂质和细胞器等成分转移到受体细胞中直接发挥功能。MSC-Exo 替代 MSC 为炎症性肠病的治疗提供了一种新策略。总结不同组织(骨髓、脐带和脂肪)来源的 MSC- Exo 用于治疗炎症性肠病的研究进展。

关键词 间充质干细胞 外泌体 炎症性肠病

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间充质干细胞(mesenchymal stem cells,MSC)是通过旁分泌机制释放外泌体(exosome,Exo)等载体介导细胞间通讯交流、激活靶细胞信号通路,以影响微环境中细胞的生物学行为,从而诱导缺损组织的再生。采用不同来源的间充质干细胞的外泌体治疗炎症性肠病已取得初步成果并具备潜在的临床应用价值,但仍有亟待解决的问题,包括不同来源间充质干细胞外泌体异质性、间充质干细胞外泌体的产量、外泌体的标准以及外泌体在临床前/临床应用中的使用剂量和频率等。比较不同间充质干细胞外泌体在治疗炎症性肠病的免疫调节作用,明确间充质干细胞外泌体的作用机制,筛选出适合炎症性肠病治疗的间充质干细胞及其外泌体将为利用外泌体治疗炎症性肠病奠定重要的转化基础。

1 炎症性肠病

炎症性肠病(inflammatory bowel disease,IBD)包括克罗恩病(crohn's disease,CD)和溃疡性结肠炎(ucerative colitis,UC),是多因素导致的复杂慢性复发性疾病。IBD 在临床上表现为体重减轻、血性黏膜粪便、腹痛、直肠萎缩和易复发等症状[1-4]。近年来全球 IBD 发病率呈现上升趋势,患病率高于 0.3%[5],中国 IBD 发病率近年来增加显著,预计 2025 年将达到 150 万人,且发病人群偏年轻化[6]。IBD 的病因和发病机制被认为与遗传易感性、肠道菌群紊乱、氧化应激、免疫失调和环境等因素有关[7-13]。目前 IBD 的治疗药物主要有氨基水杨酸类药物、糖皮质激素、抗生素、免疫调节剂和抗体类药物。患者服用传统的氨基水杨酸类药物和糖皮质激素后出现较多不良反应,如粒细胞减少、自身免疫性溶血和再生障碍性贫血等,而长期服用免疫抑制和抗体类药物会导致抗微生物和抗肿瘤免疫力下降,并增加感染、肌肉萎缩和恶性肿瘤等风险[14]。因此,临床上迫切需要针对 IBD 有效和安全的新型的治疗方法[15-16]。

2 间充质干细胞来源的外泌体

间充质干细胞来源于中胚层,主要分布于人体骨髓、脐带、脂肪、胎盘等多种组织和器官间质中[17]。MSC 具有较强的多向分化潜能及自我更新和增殖的能力[18]。在不同诱导条件下,能够分化为脂肪、骨和软骨等多种组织细胞。此外,MSC 还具有低免疫原性和免疫调节的特性,能在炎症环境中抑制免疫反应,发挥抗炎作用[18]。因此 MSC 移植被认为是治疗自身免疫性疾病[如移植物抗宿主病(graft-versus-host disease,

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GvHD)]和炎性疾病(如肛周瘘管)的潜在新疗法[18-22]。但有几个因素可能是导致 MSC 治疗效果欠佳的原因:(1)MSC 进入体内会发生经血液介导的炎症反应(instant blood-mediated inflammatory reaction,IBMIR),损害了植入细胞的寿命和治疗效力[23-25];(2)细胞在体内的驻留能力不足以及引起微血管阻塞,限制了 MSC 的治疗能力[26-30];(3)细胞进入体内受到周围微环境的影响使其恶性分化及引起机体排斥反应。虽然目前 MSC 的体内移植相对安全,但仍面临成瘤风险的安全问题[31]。

研究表明,MSC 主要通过细胞的旁分泌作用发挥免疫调节功能[32-36]。MSC 的旁分泌物由可溶性成分和细胞外囊泡(extracellular vesicles,EV)组成,其中,外泌体是目前公认研究最多且功能较明确的一类 EV,其直径约 30~200 nm。因此,在 MSC 的细胞移植过程中,MSC-Exo 从质膜萌芽后释放到细胞外环境中,以旁分泌的方式发挥生物学作用[37-39]。

MSC-Exo 膜富含胆固醇、鞘磷脂、神经酰胺和脂质筏蛋白[40],能够与靶细胞进行膜融合[37]。MSC-Exo 到达靶细胞后,通过类似受体-配体相互作用的方式来触发信号或以内吞作用传递其内含物(mRNA、miRNA、酶、细胞因子、趋化因子、免疫调节因子和生长因子),从而调节免疫细胞的表型和功能[41]。外泌体的治疗效果取决于细胞来源和能够归巢到特定的部位[42]。研究 Exo 在体内的转归,需要对 Exo 进行标记,主要有以下几种方式:(1)装载 Cre-LoxP 系统的 Exo 表达绿色荧光蛋白 GFP[43];(2)亲脂性染料(如 DiD、DiL、DiR、ICG 和罗丹明 123)标记[44-48],发光剂(如 DPA-SCP)标记[49-50];(3)酶与底物反应的生物发光剂(如 Gluc、Fluc 和 Rluc)标记[51-57];(4)放射性核素(^{125}I、^{111}In 和^{99m}Tc)标记[58-63];(5)金纳米颗粒(gold nanoparticles,GNPs)标记[64-65]。在炎症性肠病的研究中,对 MSC-Exo 进行 ICG 标记后发现 MSC-Exo 归巢到了结肠部位[47]。

与移植 MSC 相比,MSC-Exo 治疗疾病没有细胞输入,避免了 MSC 体内成瘤的风险,同时克服了由于同种异体免疫反应而导致的细胞不受控制的分化、恶性改变或排斥反应[39,66];可以避免单核细胞和巨噬细胞的吞噬作用,并在循环过程中保持稳定[67];具有特有的靶向性[68];可以透过血脑屏障[69]。因此,MSC-Exo 有望成为治疗自身免疫性疾病和炎性疾病的新疗法[39]。

3　间充质干细胞外泌体与炎症性肠病

骨髓间充质干细胞(bone marrow derived mesenchymal stemcells,BMSC)、脐带间充质干细胞(umbilical cord derived mesenchymal stem cells,UCMSC)和脂肪间充质干细胞(adipose derived mesenchymal stem cell,ADMSC)是最常见的 MSC。不同来源 MSC 形态和生物学功能非常相似[70-72],研究也发现他们之间存在着差异。Valencia 等[73]对来自同一供体的 BMSC 和 ADMSC 与 NK 细胞共培养,发现二者对 NK 细胞的增殖、细胞因子分泌、活化受体及细胞毒性的免疫调节作用等无明显差异,但 ADMSC 抑制树突状细胞分化的能力更强。另一项研究表明,BMSC 和 UCMSC 可促进巨噬细胞向抗炎表型(M2 型)极化,而 ADMSC 对促进巨噬细胞极化无明显调节作用[74]。由此说明不同组织来源的 MSC 对免疫细胞调节能力存在差异。

通过旁分泌发挥免疫调节功能可能是 MSC 治疗 IBD 的主要途径。目前已有不少利用动物模型开展 MSC-Exo 治疗 IBD 的研究。对不同组织来源的 MSC-Exo 治疗 IBD 的研究进行比较,并对受到调控的细胞因子和信号通路进行总结(表 1)。基于炎症性肠病动物模型使用的 MSC-Exo 多为超速离心方法所提取,但在治疗过程中注射的剂量与频率各不相同,目前尚没有统一或最适合的使用标准(表 2)。

3.1　骨髓间充质干细胞来源的外泌体

Yang 等[75]构建了 2,4,6-三硝基苯磺酸(TNBS)诱导的 SD 大鼠的 IBD 模型,尾静脉注射 BMSC 来源的外泌体(BMSC-Exo)后,体内的促炎性细胞因子水平降低,抑制细胞凋亡和 NF-κBp65 信号转导途径,调节抗氧化剂与氧化剂的平衡。Wu 等[76]用人 BMSC-Exo 在葡聚糖硫酸钠(DSS)和 TNBS 诱导的小鼠 IBD 模型中开展的治疗研究表明:尾静脉注射 BMSC-Exo 可使 $M_2\beta$ 巨噬细胞极化,抑制炎症反应并维持结肠的屏障完整性。BMSC-Exo 主要作用于结肠中的巨噬细胞,巨噬细胞产生 IL-10 与 BMSC-Exo 的作用有关。Cao 等[77]用 BMSC-Exo 在体外可以促进 LPS 诱导的巨噬细胞增殖并抑制炎症反应,包括促炎因子下调和抗炎因子上调。在 DSS 诱导的小鼠 IBD 模型中,腹腔注射 BMSC-Exo 可以减轻 IBD 的症状(包括促进 M_2 巨噬细胞的极化、增加结肠长度以及修复结肠黏膜损伤)。此外,对 IBD 的修复作用或与 JAK1/STAT1/STAT6 信号通路有关。Yang 等[78]在利用外泌体尾静脉注射治疗 DSS 诱导的小鼠 IBD 模型中,BMSC 经 IFN-γ 预处理后产生的 Exo 通过促进 miR-125a 和 miR-125b 的表达,再与 Stat3 的 3′-UTR 结合以抑制 Th17 细胞分化,从而减轻 IBD 的症状。

3.2 脐带间充质干细胞来源的外泌体

Mao 等[47]发现在 DSS 诱导的 IBD 小鼠模型中，尾静脉注射人 UCMSC 释放的外泌体（UCMSC-Exo）12 h 后，ICG 标记的外泌体归巢到 IBD 小鼠的结肠组织。UCMSC-Exo 除了抑制结肠巨噬细胞中 NF-κB 和 iNOS 的表达外，还抑制 IL-7 信号传导。IL-7 对循环单核细胞显示出强大的趋化特性，使它们大量聚集在发炎的组织中[47,79-80]。UCMSC-Exo 含有 miR-17，通过阻止 Janus 激酶 1 的合成和反式激活来削弱 IL-7 受体的信号传导[79-81]。因此，UCMSC-Exo 显著降低了结肠巨噬细胞中 IL-7 信号通路的活化，从而减轻了炎症[47]。Ma 等[82]用 UCMSC-Exo 与外周血单核细胞（peripheral blood mononuclear cells，PBMC）体外共培养，发现 UCMSC-Exo 促进 PBMC 抗炎因子的分泌并抑制了促炎因子的分泌，但并没有抑制 PBMC 的增殖。继而通过腹腔注射 UCMSC-Exo 治疗 IBD 小鼠后，发现 UCMSC-Exo 通过促进抗炎反应和抑制炎症反应，显著改善小鼠疾病活动指数评分，改善小鼠结肠缩短现象。

3.3 脂肪间充质干细胞来源的外泌体

Chang 等[83]验证了褪黑素（melatonin，MT）和大鼠 ADMSC 来源的外泌体（ADMSC-Exo）尾静脉注射进 DSS 诱导的 SD 大鼠能有效缓解 IBD。与对照组以及单独用 MT 或 ADMSC-Exo 治疗组相比，MT-AMSC-Exo 联合治疗可改善 IBD，体现在炎症因子、氧化应激指标、凋亡蛋白和纤维化标记物的表达降低。

表 1 不同来源的 MSC 外泌体在炎症性肠病中的作用与机制

Table 1 The role and mechanism of MSC exosomes in the treatment of inflammatory bowel disease

组织来源	靶向细胞	调控因子	信号通路	参考文献
BMSC	–	抑制核转录因子：NF-κBp65 下调促炎因子：TNF-α、iNOS、COX-2、IL-1β 上调抗炎因子：IL-10 抑制氧化：MPO、MDA、SOD、GSH 抑制细胞凋亡：caspase-3、caspase-8、caspase-9	NF-κBp65 信号转导途径	[75]
BMSC	M_2β 巨噬细胞	上调抗炎因子：IL-10、金属硫蛋白-2	–	[76]
BMSC	M_2 巨噬细胞	下调促炎因子：TNF-α、IL-6、IL-12、VEGF-A、IFN-γ、CCL-24、CCL-17 上调抗炎因子：IL-10、TGF-β	JAK1/STAT1/STAT6 信号通路	[77]
BMSC	T_{reg} 细胞	下调促炎因子：IL-17、IL-6、TNF-α、IFN-γ 上调抗炎因子：IL-10	–	[78]
UCMSC	结肠巨噬细胞	下调促炎因子：TNF-α、IL-1β、IL-6、iNOS、IL-7 上调抗炎因子：IL-10	IL-7 受体的信号传导	[47]
UCMSC	外周血单核细胞	下调促炎因子：IFN-γ、TNF-α、IL-1β、TGF-β1 上调抗炎因子：IL-10	–	[82]
ADMSC	–	下调炎症因子：MMP-9、TNF-α、NF-κB、IL-1β、IL-6、COX-2、ICAM-1、TLR-4 下调氧化应激指标：NOX-1，NOX-2、NOX-4、氧化蛋白 下调凋亡蛋白：Bax、caspase 3、PARP 下调纤维化标记物：Smad3、TGF-β	–	[83]

表 2 不同来源的 MSC 外泌体采取的提取方法、注射剂量及频率

Table 2 Extraction method, injection dosage and frequency of MSC exosomes from different sources

模型种属	组织来源	提取方法	注射剂量（μg）	注射频率	参考文献
Sprague Dawley 大鼠	BMSC	超速离心	50/100 /200	1 次（造模后第 1 天注射，第 4 天取材）	[75]
Sprague Dawley 大鼠	ADMSC	超速离心	50	3 次（造模后第 1 天、第 3 天和第 5 天注射后取材）	[83]
BALB/c 小鼠	BMSC	超速离心	50	7 次（造模后每天注射，注射 7 天后取材）	[77]
KM 小鼠	UCMSC	超速离心	400	3 次（在造模期间第 3 天、第 6 天、第 9 天注射，造模 11 天后取材）	[47]
C57BL/6 小鼠	BMSC	超速离心	200	2 次（造模后第 1 天和第 9 天）	[76]
C57BL/6J 小鼠	BMSC	超速离心	200	3 次（造模后连续注射 3 天，第 10 天取材）	[78]
C57BL/6 小鼠	UCMSC	超速离心	200	1 次（造模后第 1 天注射，第 3 天取材）	[82]

由此可见,MSC-Exo 治疗 IBD 的机制可能是通过抑制促炎因子(TNF-α、IL-1β、IL-6、TGF-β 和 IFN-γ)和促进抗炎因子(IL-10)的分泌,以及调节结肠巨噬细胞来减轻炎症,从而达到治疗 IBD 的效果[47,76-83]。此外,BMSC-Exo 和 ADMSC-Exo 在 IBD 治疗方面还显示了抑制促炎因子 COX-2 分泌、抑制凋亡蛋白 caspase 3 表达的作用,但 UCMSC-Exo 是否具有同样的功能目前还没有研究报道[47,76-83]。基于大、小鼠 IBD 的动物模型研究表明,MSC-Exo 可能是通过调控转录因子表达的机制实现 IBD 的治疗。BMSC-Exo 被证明可能与 NF-κBp65 信号转导途径和 JAK1/STAT1/STAT6 信号通路有关[77];UCMSC-Exo 被证明可能与 IL-7 受体的信号传导有关[47];对于 ADMSC-Exo,目前还没有相关信号通路调控的研究报道[47,76-83]。MSC-Exo 治疗 IBD 的研究中,不同来源的 MSC-Exo,其注射剂量(50 ~ 400 μg)和注射频率(1 ~ 7 次)没有统一标准,小鼠品系的选择与注射剂量和注射频率也是没有统一标准,需要继续进行探讨研究。

4　展　望

目前为止,MSC-Exo 与 IBD 的内在治疗机制还没有完全研究清楚[84],MSC-Exo 是如何在体内抑制 IBD 炎症发生的,仅通过细胞因子调节? 还是有其他途径来调控免疫细胞,继而调控细胞因子? MSC-Exo 治疗炎症性肠病的体内示踪虽然能够看到 Exo 可以到达结肠部位,但相关研究较少,未来在这方面应该会有更多的研究。此外,虽然 MSC-Exo 治疗 IBD 的研究已显示出了初步成效,但为了提高 Exo 靶向性,避免被免疫系统快速清除,还可以对 Exo 进行改造和修饰。常用修饰方法包括添加膜表面肽、靶向蛋白等,这些方法可以使 Exo 具备以下特征:实现 Exo 在组织和器官中的积累;靶向目标组织;高水平的细胞摄取和高效的溶酶体逃逸能力[85]。除了提高 Exo 在炎症性肠病中的治疗效果外,怎样能够稳定地获取大量的 Exo 也是要考虑的。此外,Exo 注射的剂量与频率的标准还需要开展更加深入的研究。

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Progress in the Treatment of Inflammatory Bowel Diseases by Exosomes Derived from Mesenchymal Stem Cells

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Abstract　Mesenchymal stem cells (MSC) play an important role in the treatment of inflammatory diseases through immune regulation and paracrine effect. The paracrine effect of MSC is mediated by secreting soble factors and releasing exosomes (Exo). Exosomes transfer DNA, proteins/peptides, mRNA, microRNA, lipids and organelles into recipient cells and display functions directly. Therefore, MSC-Exo instead of MSC provides a new strategy for the treatment of inflammatory bowel diseases. In this review, we summarize the progress of MSC-Exo derived from various tissues (bone marrow, umbilical cord and adipose) for the treatment of inflammatory bowel diseases.

Keywords　Mesenchymal stem cells　Exosomes　Inflammatory bowel disease