间充质干细胞来源的外泌体治疗缺血性脑卒中的研究进展

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摘要 外泌体是直径为30~150 nm的细胞外囊泡, 内含细胞来源的核酸和蛋白质等生物活性分子, 在细胞间通讯过程中发挥重要作用。间充质干细胞来源的外泌体可有效转运mRNA、microRNA及蛋白质等生物活性物质, 具有促进血管生成、减轻炎症反应、调节自噬水平、抑制细胞凋亡和焦亡等重要生物学功能, 其在改善神经系统疾病预后方面有着良好的临床应用前景。该文就间充质干细胞来源的外泌体对缺血性脑卒中的神经保护作用及机制进行了综述, 并讨论了靶向修饰的外泌体在治疗缺血性脑卒中的应用, 以期为后续研究提供参考。

关键词 外泌体; 缺血性脑卒中; 神经保护; 靶向修饰

Research Progress of Exosomes Derived from Mesenchymal Stem Cells in the Treatment of Ischemic Stroke

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Abstract Exosomes are extracellular vesicles with a diameter of 30~150 nm, containing bioactive molecules such as nucleic acids and proteins derived from cells, which play pivotal roles in intercellular communication processes. Exosomes derived from mesenchymal stem cells can effectively transport bioactive substances, such as mRNA, microRNA, protein and so on. Exosomes have many important biological functions such as promoting angiogenesis, reducing inflammatory response, regulating autophagy levels, inhibiting apoptosis and pyrolysis. It has a good prospect of clinical application in improving the prognosis of neurological diseases. In this paper, the neuroprotective effects and mechanisms of exosomes derived from mesenchymal stem cells on ischemic stroke were summarized, and the applications of targeted and modified exosomes in the treatment of ischemic stroke were discussed, in order to provide a reference for subsequent studies.

Keywords exosomes; ischemic stroke; neuroprotection; targeted modification

脑卒中是具有高发病率、高致残率和高死亡率的急性脑血管病, 严重威胁人类生命健康。缺血性脑卒中(ischemic stroke, IS)更为多见, 常因颅内动脉狭窄或脑动脉栓塞引起的脑供血和供氧不足而发生, 可导致继发性神经功能障碍[1]。目前, 缺血性脑卒中的治疗选择有限, 重组组织纤溶酶原激活剂

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(recombinant tissue plasminogen activator, rt-PA)是唯一获得FDA批准的静脉溶栓剂。考虑到4.5 h内有限的溶栓时间窗和与rt-PA相关的脑出血和再灌注损伤事件的风险, 许多患者仍无法达到最佳治疗效果[2]。此外, 血脑屏障(blood-brain barrier, BBB)是一种高度选择性的屏障, 可为大脑提供保护作用。但是, 血脑屏障也限制了许多治疗药物以有效浓度到达中枢神经系统(central nervous system, CNS)[3]。因此, 目前科研人员正在研究克服这些问题的方法。

间充质干细胞(mesenchymal stem cells, MSCs)具有自我复制和分化能力, 可从脐带、骨髓和脂肪等组织中获得。MSCs还可以产生旁分泌因子, 如细胞因子、趋化因子和生物活性因子等[4]。越来越多的证据表明, MSCs主要通过旁分泌因子发挥治疗作用, 而外泌体(exosomes)是细胞旁分泌作用的关键介质[5-6]。研究发现, 骨髓间充质干细胞(bone marrow mesenchymal stem cells, BMSCs)来源的外泌体(BMSC-Exos)和脂肪间充质干细胞(adipose-derived mesenchymal stem cells, ADSCs)来源的外泌体(ADSC-Exos)均可参与修复IS受损的组织[7-8]。IS是中枢神经系统最常见的疾病之一, 其氧化应激反应、炎症反应和神经细胞凋亡是缺血性脑损伤的重要病理机制。此外, 小胶质细胞作为中枢神经系统的第一道防线, 在IS病理过程中发挥着不可或缺的作用。小胶质细胞可以响应不同的微环境干扰而被激活为M1或M2表型。前者产生大量促炎细胞因子并加剧缺血性脑损伤。相反, 后者通过分泌各种抗炎细胞因子和神经营养因子对大脑表现出神经保护作用[9]。间充质干细胞来源的外泌体(MSC-Exos)可通过调节小胶质细胞的极化以减轻缺血性脑卒中的炎症反应。在这篇综述中, 我们讨论了MSC-Exos作为细胞旁分泌因子对缺血性脑卒中的治疗潜力。

1 外泌体概述

几乎所有细胞都可释放细胞外囊泡(extracellular vesicles, EVs)到胞外空间, 根据EVs的生物发生过程、释放途径、大小和功能, 可将EVs分为微囊泡、凋亡小体和外泌体[10]。其中, 微囊泡和凋亡小体主要是以细胞膜出芽的方式形成。外泌体起源于细胞内, 具有脂质双层结构, 细胞膜内陷形成的多泡体(multivesicular body, MVB)与质膜融合, 并向胞外空间释放其包含的腔内囊泡(intraluminal vesicles, ILVs), 这些被释放到细胞外的ILVs, 即为外泌体[11]。

外泌体包裹多种生物活性分子, 包括蛋白质、脂质、mRNA和miRNA (microRNA), 这些生物活性分子介导外泌体细胞间的信号交流。外泌体主要是通过内吞作用、配体–受体相互作用或直接膜融合的内化作用模式特异性作用于靶细胞, 将其活性分子释放到受体细胞中, 从而调节其生物学功能[12]。外泌体蛋白包括膜转运相关蛋白和融合蛋白, 如Rab蛋白、膜联蛋白(annexin)、四跨膜蛋白、转运所需的内体分选复合物(endosomal sorting complex required for transport, ESCRT)和热休克蛋白(heat shock protein, HSP)等[13]。其中, 四跨膜蛋白家族成员(CD9、CD63、CD81)已被用作外泌体鉴定的表面标志物; ESCRT在分选加载蛋白质-miRNA复合物到外泌体的过程中起着重要作用。外泌体由脂质双层结构组成, 脂质膜可促进受体细胞对外泌体的摄取, 并阻止其内容物在运输过程中被破坏[14]。在外泌体生物发生过程中, miRNA会被选择性地加载到外泌体中。miRNA通过作用于mRNA 3′UTR来调控基因转录和蛋白质翻译过程。因此, 外泌体可通过携带不同的miRNA来影响受体细胞的转录过程, 并在细胞间信号交流和功能调节上发挥作用[15-16]。

2 外泌体治疗缺血性脑卒中的潜在机制

2.1 促进脑组织重塑

IS患者的大脑修复涉及一系列高度交互的过程, 如血管生成、神经发生、突触生成和轴突生长, 它们共同促进脑组织重塑以协调神经功能恢复[17]。大脑中动脉闭塞(middle cerebral artery occlusion, MCAO)模型常用于IS的体内实验研究。研究发现, MSC-Exos可促进大脑皮质和纹状体缺血边界区域的血管生成、神经发生和神经元存活, 这些变化有利于脑组织重塑[7]。上述有益改变也可以由MSC-Exos携带的miRNA-17-92通过靶向下调*PTEN*, 进而激活磷脂酰肌醇-3-激酶/蛋白激酶B/雷帕霉素/糖原合酶激酶3β(PI3K/Akt/mTOR/GSK-3β)信号通路来实现[18-19]。MSC-Exos被静脉注射后, 可以迁移到小鼠MCAO模型缺血病变区域并与神经元融合, 进一步的研究发现, 外泌体通过促进血管生成和神经发生来发挥神经保护作用[20]。最近的一项研究表明, MSC-Exos可有效促进老年大鼠MCAO模型皮层缺血区域的血管生成和神经发生, 进而促进神经功能

恢复和脑组织重塑[21]。这些研究数据表明，MSC-Exos是促进脑组织重塑的有效策略。

2.2 抑制细胞凋亡和细胞焦亡

脑缺血后，神经细胞的稳态会发生一系列变化，例如活性氧的产生、Ca^{2+}超载和兴奋性毒性，最终可能引发细胞凋亡或坏死。缺血半影区的神经细胞凋亡可能在缺血后数小时内发生[22]。因此，积极抑制细胞凋亡进程以挽救神经元的存活，对治疗IS有着重要意义。氧和葡萄糖剥夺(oxygen and glucose deprivation, OGD)是一种广泛用于研究IS的体外实验模型[23]。MSC-Exos可减轻OGD诱导的原代大鼠脑内皮细胞凋亡[24]。MSC-Exos还可以通过激活AMPK磷酸化和下调JAK2/STAT3/NF-κB信号通路，进而改善MCAO模型的神经元凋亡进程[25]。此外，ZHANG等[26]发现，ADSC-Exos可抑制OGD诱导的原代皮层神经元凋亡，进一步的研究发现，野生型*KDM6B*基因的3′UTR可与miR-22-3p互补结合，*KDM6B*是miR-22-3p的靶基因。外泌体携带的miR-22-3p通过靶向抑制*KDM6B*介导的BMP2/BMF轴来减轻神经元凋亡。

细胞焦亡是一种不同于凋亡的程序性细胞死亡，在维持细胞稳态方面有着重要作用[27]。已有研究证实，NLRP3炎症小体可以介导细胞焦亡的发生[28]。BMSC-Exos通过抑制NLRP3炎症小体介导的细胞焦亡，进而减轻OGD诱导的原代皮层神经元和神经母细胞瘤N2a细胞损伤[29]。此外，线粒体自噬在细胞焦亡过程中也发挥着重要作用。BMSC-Exos通过增加AMPK依赖性自噬通量来抑制NLRP3炎症小体介导的细胞焦亡，从而减轻OGD诱导的PC12细胞损伤[30]。HU等[31]发现，MSC-Exos通过上调FOXO3a表达水平以增强线粒体自噬，从而抑制OGD诱导的小胶质细胞焦亡并减轻随后的神经元损伤。因此，通过调节线粒体自噬来抑制细胞焦亡可能是治疗IS的重要策略。

2.3 维持血脑屏障完整性

位于中枢神经系统和外周循环系统之间的BBB有助于建立和维持CNS微环境的稳态。BBB作为神经血管单元(neurovascular unit, NVU)的一部分，主要是由内皮细胞、基底膜、周细胞和星形胶质细胞通过紧密连接(tight junction, TJ)组成的特殊结构[32]。NVU还可以调节BBB通透性和脑血流。

在缺血性脑卒中的早期阶段，BBB表现出通透性和破坏性的增高，进而导致免疫细胞和炎症因子的渗透增多，比如中性粒细胞、巨噬细胞和T细胞通过细胞旁和跨细胞途径迁移穿过BBB并浸润脑实质来加剧炎症反应[33]。此外，星形胶质细胞和小胶质细胞等局部免疫细胞被激活并产生基质金属蛋白酶9(matrix metalloproteinase-9, MMP-9)等物质，而MMP-9的过度表达可导致TJ蛋白claudin-5的显著降解[34-35]。在新生小鼠缺氧缺血性脑损伤模型中，间充质干细胞衍生的细胞外囊泡会促进脑内皮细胞增殖，并抑制星形胶质细胞和小胶质细胞激活[36]。这可能有助于减少MMP-9等物质的产生以减轻BBB渗漏。MSC-Exos还可以通过上调TJ蛋白ZO-1和claudin-5表达水平以维持BBB完整性[37]。因此，MSC-Exos可通过维持BBB的完整性来发挥神经保护作用。

2.4 调节小胶质细胞极化

缺血后炎症反应是加剧IS病理进展的重要环节，而小胶质细胞是脑中促炎细胞因子水平升高的主要细胞来源。MSC-Exos可通过调节小胶质细胞极化以抑制神经炎症反应。ZHAO等[38-39]发现，MSC-Exos通过逆转CysLT2R-ERK1/2介导的小胶质细胞M1极化，促进小胶质细胞向抗炎M2表型转化，进一步的研究发现，外泌体miR-223-3p显著降低了CysLT2R的mRNA和蛋白表达水平。该研究表明，外泌体携带的miR-223-3p可通过抑制*CysLT2R*表达来介导小胶质细胞极化过程。LI等[40]研究发现，MSC-Exos携带的miR-26b-5p通过靶向*CH25H*抑制TLR信号通路来减弱缺血诱导的小胶质细胞M1极化和炎症反应，进而减轻脑缺血后的神经损伤。BMSC-Exos还可通过将小胶质细胞从促炎的M1表型转化为抗炎的M2表型来抑制NLRP3炎症小体介导的细胞焦亡和炎症反应[41]。因此，通过外泌体途径调节小胶质细胞极化是治疗IS的重要策略。专注于抑制小胶质细胞M1极化或促进小胶质细胞从M1表型转变为M2表型，将可能是缓解IS炎症反应有希望的治疗策略。

2.5 递送miRNA

miRNA是一类小的非编码RNA，可调节靶细胞中的基因表达以介导蛋白质翻译和细胞整体功能[42]。miRNA通过作用于mRNA 3′UTR来调节基因转录及蛋白质翻译过程[43]。不同的miRNA对其靶细胞的表型和生理状态具有不同的影响，比如促进血管生成、抑制炎症反应和细胞凋亡进程。此外，

表1 外泌体miRNA在缺血性脑卒中治疗中的作用

Table 1 The role of exosomal miRNA in the treatment of ischemic stroke

外泌体来源(给药途径) Source of exosomes (route of administration)	miRNA	靶基因 Target gene	神经保护作用 Neuroprotective effects	参考文献 References
BMSCs (tail vein injection)	miR-17-92	*PTEN*	Promote angiogenesis and neurogenesis	[19]
ADSCs (intracerebroventricular injection)	miR-22-3p	*KDM6B*	Inhibit neuronal apoptosis	[26]
BMSCs (tail vein injection)	miR-223-3p	*CysLT2R*	Promote polarization of M2 microglia	[39]
MSCs (tail vein injection)	miR-26b-5p	*CH25H*	Inhibit polarization of M1 microglia	[40]
ADSCs (–)	miR-181b-5p	*TRPM7*	Promote angiogenesis	[45]
BMSCs (–)	miR-138-5p	*LCN2*	Inhibit apoptosis and inflammation	[47]
BMSCs (intracerebroventricular injection)	miR-221-3p	*ATF3*	Inhibit apoptosis and inflammation	[48]
MSCs (–)	miR-542-3p	*TLR4*	Inhibit inflammation of glial cells	[49]

–是指研究未明确指出具体给药途径。

– refers to studies that do not specify a specific route of administration.

MSC-Exos作为miRNA的递送载体, 可长时间保留其生物活性, 延长其血液循环时间并使其能够被远端细胞内化, 从而调节受体细胞的功能[44]。

在OGD模拟的IS体外实验模型中, YANG等[45]发现, ADSC-Exos促进了脑微血管内皮细胞的血管生成, 进一步的研究发现, *TRPM7*是miR-181b-5p的直接靶基因, 外泌体携带的miR-181b-5p通过靶向下调TRPM7的mRNA和蛋白表达水平, 从而上调血管内皮生长因子(vascular endothelial growth factor, VEGF)的蛋白表达水平。ZHANG等[46]发现, MSC-Exos与BV2小胶质细胞共培养, 显著降低了IL-6、TNF-α和IL-1β的mRNA和蛋白质表达水平, 进一步的实验结果表明, MSC-Exos携带的miR-146a-5p通过抑制IRAK1/TRAF6信号通路减轻小胶质细胞介导的神经炎症反应。此外, BMSC-Exos通过递送miR-138-5p下调*LCN2*表达水平, 从而促进星形胶质细胞增殖并抑制细胞凋亡和炎症反应; 双荧光素酶报告实验结果显示, miR-138-5p可以特异性结合*LCN2*的3′UTR并下调*LCN2*的基因表达量, 表明*LCN2*是miR-138-5p的靶基因[47]。

AI等[48]发现, BMSC-Exos携带miR-221-3p通过抑制*ATF3*来减轻炎症反应和神经元凋亡, 进一步的研究发现, 野生型*ATF3*基因的3′UTR存在miR-221-3p互补结合位点, *ATF3*是miR-221-3p的靶基因。此外, MSC-Exos包裹的miR-542-3p还可通过抑制TLR4来减轻缺血诱导的神经胶质细胞炎症反应[49]。PAN等[50]发现, 携带miR-132-3p的MSC-Exos进入大脑后, 可通过下调靶蛋白RASA1, 上调Ras表达及PI3K磷酸化, 进而降低血管氧化应激和细胞凋亡水平。

综上, MSC-Exos通过递送不同的miRNA与相应的靶基因结合来调控炎症反应和细胞凋亡进程(表1)。因此, 外泌体作为间充质干细胞的旁分泌因子, 其包裹的miRNA可能是其发挥神经保护作用的潜在机制(图1)。这些研究结果揭示了MSC-Exos作为一种无细胞治疗策略保护缺血性脑卒中的巨大潜力。

3 靶向和修饰外泌体用于治疗缺血性脑卒中

外泌体具有高递送效率、低免疫原性和良好的生物相容性, 可作为药物载体通过血脑屏障到达脑内病变部位。外泌体被修饰后也可作为递送药物或miRNA的靶向载体, 为治疗IS提供新的策略(表2)。

外泌体可通过相关靶标蛋白质或肽的融合来实现靶向作用。例如, 狂犬病病毒糖蛋白(rabies virus glycoprotein, RVG)与外泌体蛋白溶酶体相关膜糖蛋白2b(lysosome associated membrane glycoprotein 2b, Lamp2b)的融合可以有效地将miR-124递送到脑缺血部位, 进而改善皮层神经发生来减轻缺血性损伤[51]。RVG可能是通过与烟碱型乙酰胆碱受体(nicotinic acetylcholine receptor, nAChR)结合, 从而选择性靶向神经元细胞和脑内皮细胞[52]。外泌体也可以作为载体, 在表面修饰后运输生物活性物质。脑缺血后, 高迁移率族蛋白B1(high mobility group box-1, HMGB1)被释放到细胞外空间, 可导致随后的炎症反应。KIM等[53]通过电穿孔将HMGB1-siRNA加载到RVG修饰的外泌体(RVG-Exos)中, RVG-Exos被静脉注射后可有效减少HMGB1的表达量, 并降低神经细胞凋亡水平。因此, 装载有HMGB1-siRNA

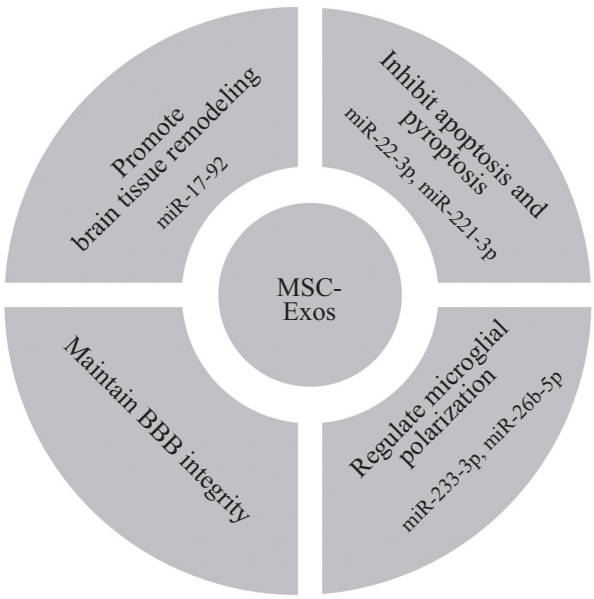

图1 外泌体治疗缺血性脑卒中的潜在机制

Fig.1 Potential mechanisms of exosomes in the treatment of ischemic stroke

表2 靶向和修饰外泌体治疗缺血性脑卒中

Table 2 Targeted and modified exosomes for the treatment of ischemic stroke

外泌体的表面修饰 Surface modification of exosomes	外泌体的内容物修饰 Content modification of exosomes	神经保护作用 Neuroprotective effects	参考文献 References
RVG	miR124	Promote neurogenesis	[51]
RVG	HMGB-siRNA	Inhibit apoptosis	[53]
RGDyk	miRNA-210	Promote angiogenesis	[54]
–	PEDF	Promote autophagy and inhibit neuronal apoptosis	[56]
–	IONP	Promote angiogenesis and inhibit inflammation	[57]

–是指研究未对外泌体进行表面修饰。

– refers to studies without surface modification of exosomes.

的RVG-Exos具有靶向治疗IS的应用前景。此外，ZHANG等[54]将胆固醇修饰的miR-210加载到缀合RGDyk环肽的BMSC-Exos上，被尾静脉注射后其可靶向到达小鼠的脑组织，进而促进缺血区域的VEGF表达和血管生成。因此，提高外泌体靶向性而对其进行工程改造，产生具有靶向能力的细胞外囊泡是外泌体载体治疗研究的重要方向。

外泌体还可以加载外源性蛋白质进行大脑递送。色素上皮衍生因子(pigment epithelium-derived factor, PEDF)是一种多功能蛋白质，具有抗炎、抗氧化和神经保护功能[55]。HUANG等[56]构建了过表达PEDF的ADSCs并从ADSCs中分离外泌体，发现其携带的PEDF可通过激活细胞自噬和抑制神经元凋亡来治疗IS。最近的一项研究表明，间充质干细胞来源的包裹氧化铁纳米颗粒(iron oxide nanoparticle, IONP)的磁性细胞外囊泡可以显著减轻缺血性脑损伤[57]。

综上，这些研究数据显示了MSC-Exos在细胞通讯中具有重要作用，且使用靶向和修饰的外泌体作为载体来传递特殊的生物材料，包括miRNA，可促进缺血性脑卒中的恢复。这些发现将为外泌体用作基于核酸和蛋白质疗法的跨血脑屏障递送载体的研究奠定基础。

4 小结与展望

MSC-Exos可通过调节细胞中基因和蛋白的表达来诱导神经修复和神经保护，在缺血性脑卒中病理生理过程中发挥重要作用。MSC-Exos携带的物质，特别是miRNA有效地作用于靶细胞，可抑制神经炎症、调节小胶质细胞极化、改善细胞凋亡和焦

亡, 并促进血管生成和神经发生。工程化修饰的外泌体可作为将外源基因、蛋白质和化合物递送至受体细胞的靶向载体。因此, MSC-Exos是具有前景的一种无细胞治疗策略。

尽管外泌体在多项研究中表现出了良好的神经保护特性, 但是, 外泌体在缺血性脑卒中等神经系统疾病治疗中的临床应用转化还存在诸多挑战。首先, 作为一种生物药物, 外泌体的生产标准和质量控制是其安全应用于临床患者的挑战之一, 外泌体分离和储存方法的改进以及可靠的临床试验结果将对外泌体治疗策略的临床转化有很大帮助。其次, 许多研究已通过静脉内给药[58]、鼻内给药[59]、脑室注射给药[60]等方式注射外泌体来治疗缺血性脑卒中。目前, 外泌体治疗的最佳给药方式仍有待进一步商榷。静脉途径给药可能会面临单核吞噬细胞系统(mononuclear phagocyte system, MPS)快速清除的问题, 但是, 外泌体自身的低免疫原性可能会弥补这一缺陷, 并极大地提高药物跨血脑屏障的递送效率; 脑室注射给药在临床应用中可能存在着诸多疾病并发症, 不太易于实现; 而鼻内给药为缺血性脑卒中的非侵入性治疗提供了新的策略。上述外泌体治疗临床转化研究的瓶颈问题仍需进一步的深入探讨。随着外泌体大规模生产方法的出现以及靶向和修饰外泌体技术的发展, 外泌体在缺血性脑卒中治疗中的应用前景将更加广阔。

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