综述

外泌体载体治疗在中枢神经系统疾病中的研究进展

陈德义[1] 苏刚[2] 刘骥飞[1] 吴琼慧[1] 陈玮[1] 张振昶[1]*

([1]兰州大学第二医院神经内科, 兰州 730030; [2]兰州大学基础医学院遗传研究所, 兰州 730000)

摘要 中枢神经系统疾病包括脑血管疾病、神经退行性疾病和脑肿瘤等。血脑屏障(blood-brain barrier, BBB)阻碍了大多数通过血液循环系统输送到大脑来治疗和预防中枢神经系统疾病的药物。外泌体在细胞间物质运输和信号交流中发挥重要作用, 由于其具有较小的体积、高递送效率、低免疫原性和良好的生物相容性等特点, 可以通过正常的内吞作用和转胞吞作用进入脑内皮细胞, 进而穿过血脑屏障转运内容物。为提高外泌体靶向性, 对其膜进行工程改造, 从而产生具有靶向能力的囊泡是今后外泌体载体研究的重要方向。该文就外泌体的生物学特征、工程化修饰及其作为治疗载体在中枢神经系统疾病中的研究进行综述。

关键词 外泌体; 中枢神经系统疾病; 工程化修饰; 载体

Research Progress of Exosomes as Vehicles in the Therapy of Central Nervous System Diseases

CHEN Deyi[1], SU Gang[2], LIU Jifei[1], WU Qionghui[1], CHEN Wei[1], ZHANG Zhenchang[1]*

([1]*Department of Neurology, Lanzhou University Second Hospital, Lanzhou 730030, China*;
[2]*Institute of Genetics, School of Basic Medical Sciences, Lanzhou University, Lanzhou 730000, China*)

Abstract Central nervous system diseases include cerebrovascular diseases, neurodegenerative diseases and brain tumors. The BBB (blood-brain barrier) hinders the delivery of most drugs treated and prevented central nervous system diseases to the brain through the blood circulatory system. Exosome plays important roles in intercellular substance transport and signal communication. Due to its small volume, high delivery efficiency, low immunogenicity, and good biocompatibility, it can enter brain endothelial cells through normal endocytosis and transcytosis, and then transport contents across the BBB. In order to improve the targeting of exosomes, engineering their membranes and producing vesicles with targeting ability are essential research directions for exosomes as vesicles in the future. This article reviews the biological characteristics, engi-

收稿日期: 2021-03-23 接受日期: 2021-06-30

国家自然科学基金面上基金(批准号: 31870335)、甘肃省基因功能重点实验室科技重大专项合作项目(批准号: BA2016036)、甘肃省卫计委卫生行业计划基金(批准号: GSWSKY2016-17)、甘肃省科学技术厅自然科学基金(批准号: 20JR5RA344)、兰州市科技发展指导性计划项目(批准号: 2019-ZD-51)和兰州大学第二医院“萃英研究生导师应聘者”培训项目(批准号: 201802)资助的课题

*通讯作者。Tel: 13893647595, E-mail: 13893647595@163.com

Received: March 23, 2021 Accepted: June 30, 2021

This work was supported by the National Natural Science Foundation of China (Grant No.31870335), the Science and Technology Major Special Collaboration Project of Gansu Provincial Key Laboratory of Gene Function (Grant No.BA2016036), the Health Industry Planning Project of Gansu Provincial Health and Family Planning Commission (Grant No.GSWSKY2016-17), the Natural Science Foundation of Gansu Provincial Department of Science and Technology (Grant No.20JR5RA344), the Lanzhou Science and Technology Development Guiding Plan Project (Grant No.2019-ZD-51) and the “Cuiying Graduate Supervisor” Applicant Training Program of Lanzhou University Second Hospital (Grant No.201802)

*Corresponding author. Tel: +86-13893647595, E-mail: 13893647595@163.com

neered modifications of exosomes and their research as vehicles in the therapy of central nervous system diseases.

Keywords exosomes; central nervous system diseases; engineering modification; vehicles

外泌体可以通过多种途径传递生物活性物质, 并安全高效地转移生物活性物质参与细胞代谢, 如组织修复[1]、免疫调控[2]以及肿瘤治疗[3]。外泌体可以携带遗传物质, 具有稳定的脂膜结构, 在体液中分布广泛等递送载体的基本特征, 作为治疗载体其逐渐成为疾病研究的重要方向[4]。中枢神经系统存在复杂的血脑屏障(blood-brain barrier, BBB), 使其疾病治疗具有一定的局限性[5]。血脑屏障是一种高度选择性的半通透性屏障, 可为大脑提供保护作用, 然而某些治疗药物也难以穿过血脑屏障[6]。而外泌体借助自身的递送载体优势, 可以跨血脑屏障转运内容物。因此, 基于外泌体的药物递送研究至关重要[7]。研究外泌体的细胞摄取机制可以提高外泌体对核酸、蛋白质以及小分子药物的递送效率。

1 外泌体的起源与生物发生

细胞外囊泡(extracellular vesicles, EVs)包括凋亡小体(apoptotic body)、微囊泡(microvesicles)和外泌体(exosomes)三个亚型, 其中外泌体受到人们的广泛关注[8]。外泌体是直径为30~150 nm的脂质双层膜囊泡[9]。TRAMS等[10]统称质膜来源的囊泡为外泌体, 并首次提出“外泌体”的概念, 认为其是具有5′-核苷酸酶活性的膜囊泡, 可能具有生理功能并起源于各种细胞系培养物的渗出液。JOHNSTONE等[11]在网织红细胞成熟过程中追踪了转铁蛋白受体, 发现外泌体的形成与成熟红细胞中转铁蛋白受体的丢失有关。为了将它们与其他类型的EVs区别开来, 故命名这种膜囊泡为“外泌体”[12]。

外泌体的生物合成始于细胞膜内陷, 质膜(plasma membrane, PM)内陷形成早期内体, 逐渐成熟为晚期内体。晚期内体膜向内出芽, 产生具有腔内囊泡(intraluminal vesicles, ILVs)的多泡体(multivesicular body, MVB)。在此过程中, 核酸和蛋白质被包裹到外泌体中[13-14]。当MVB与PM融合时将ILVs释放到细胞外, 即外泌体[15-16](图1)。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(tetraspanin)家族成员(CD9、CD63、CD81、CD82)、转运所需的内体分选复合物(endosomal sorting complexes required for transport, ESCRT)成员(TSG101、Alix)、热休克蛋白(heat shock protein, Hsp)成员(Hsp60、Hsp70、Hsp90)和主要组织相容性复合体(major histocompatibility complex, MHC)蛋白等外泌体生物合成相关的蛋白[17]。外泌体还通过携带不同类型的核酸来影响受体细胞的转录过程, 并在细胞间信号交流、器官发育和生理功能上发挥调节作用[18-19]。正是由于外泌体携带许多蛋白质和核酸, 为其工程化改造奠定了基础。

2 外泌体的载体特性及工程化改造机制

工程外泌体包括装载有特定内容物或经过表面修饰的外泌体[20]。天然外泌体存在靶向性差的问题, 经过工程改造的外泌体其靶向能力大大提高[21]。外泌体还具有高递送效率、低免疫原性、良好的生物相容性和易于穿过血脑屏障等特点。在外泌体天然脂质双层结构的基础上, 使用特定的表面分子对其进行工程改造, 这种工程化策略不仅保留了外泌体的固有特征, 更重要的是, 经过工程改造的外泌体还可以有效包裹治疗药物(图2A), 包括装载miRNA[22]、姜黄素(curcumin, Cur)[23]和多巴胺[24]等, 进而靶向大脑递送。

对树突状细胞(dendritic cell, DC)进行基因工程改造, 以表达由溶酶体相关膜糖蛋白2b(lysosome associated membrane glycoprotein 2b, Lamp2b)和靶向神经元的狂犬病病毒糖蛋白(rabies virus glycoprotein, RVG)肽组成的融合蛋白, 从而使经过改造的细胞产生带有RVG肽的外泌体[25](图2B)。该RVG肽可能通过与烟碱乙酰胆碱受体(nicotinic acetylcholine receptor, nAChR)结合, 从而选择性靶向神经元细胞和脑内皮细胞[26]。值得注意的是, RVG肽修饰的外泌体被注射到小鼠体内后, 表现出穿过血脑屏障的靶向能力[25]。这些发现将为外泌体用作基于核酸和蛋白疗法的跨血脑屏障递送载体的研究奠定基础。

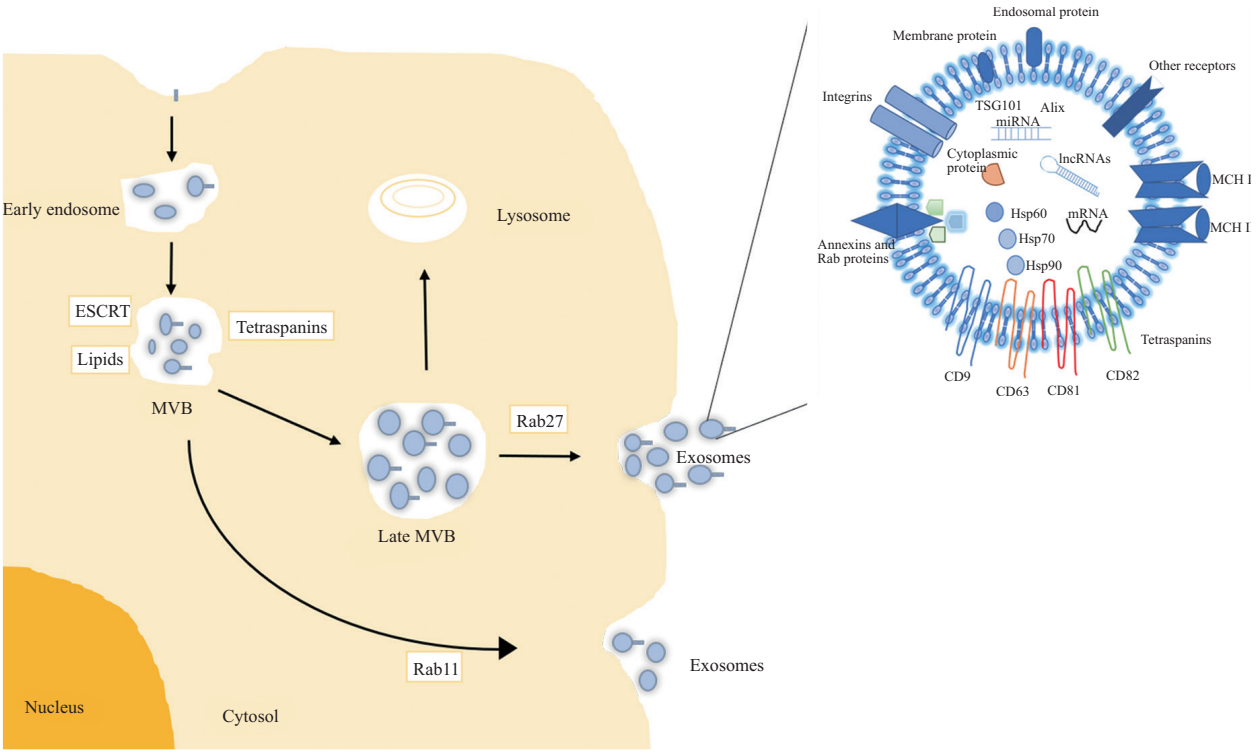
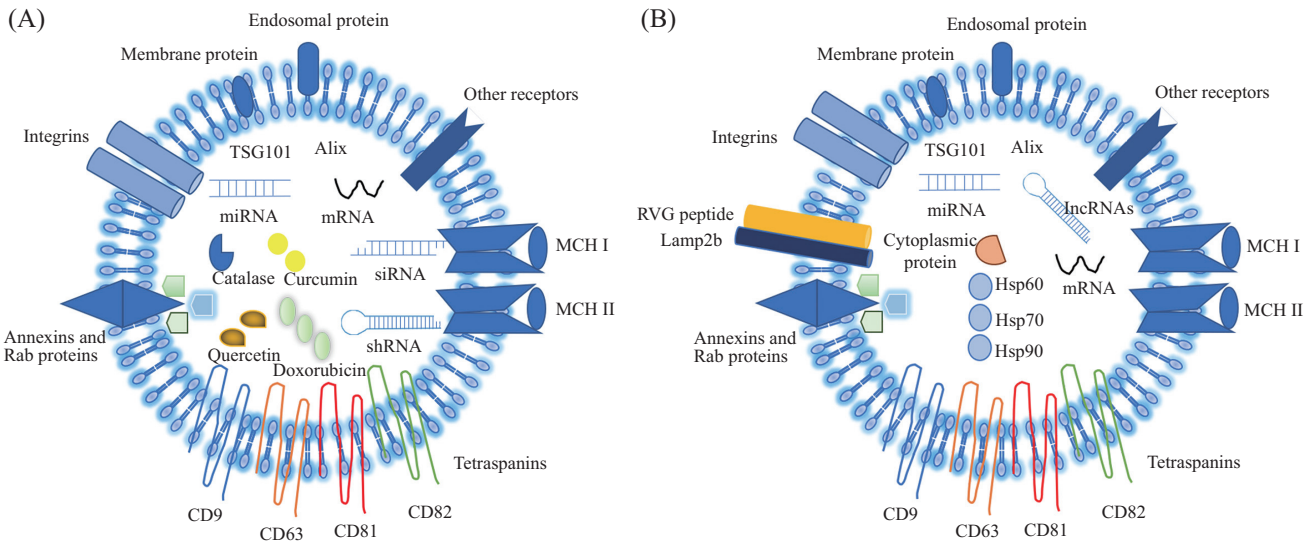


图1左侧为外泌体的生物发生过程。外泌体是内体和MVB中形成的ILVs, ESCRT蛋白和四跨膜蛋白也参与了ILVs的形成。当MVB与质膜融合时释放ILVs, 即“外泌体”, 而不同的Rab蛋白介导了外泌体的分泌过程。右侧为外泌体的结构示意图。

Fig.1 shows the biogenesis process of exosomes on the left. Exosomes are ILVs formed in endosomes and MVB, and ESCRT proteins and tetraspanins are also involved in ILVs formation. ILVs, the “exosomes”, are released when MVB fuses with the plasma membrane, whereas different Rab proteins mediate the secretory process of the exosomes. The structure diagram of exosomes is shown on the right.

图1　外泌体的生物发生、分泌和结构(根据参考文献[15-16]修改)

Fig.1　Biogenesis, secretion and structure of exosomes (modified from references [15-16])



A: 外泌体携带药物、蛋白质和核酸; B: 外泌体携带靶向神经元的RVG肽。

A: exosomes carry drugs, proteins and nucleic acids; B: exosomes carry RVG peptides that target neurons.

图2　外泌体工程改造

Fig.2　Engineering renovation of exosomes

KHONGKOW等[27]用pcDNA GNSTM-3-RVG-10-Lamp2b-HA载体转染产生外泌体的细胞, 使得工程改造的细胞产生表面上带有RVG和GNSTM肽的外泌体, 收集这些工程外泌体, 然后使用连续挤压法制备包裹金纳米颗粒((silver nanoparticles, AuNPs)的外泌体。研究结果表明, 经过表面修饰的外泌体包裹AuNPs比未经修饰的外泌体具有更强的血脑屏障穿透能力。这项研究可能会加快AuNPs表面修饰以穿过血脑屏障通用设计原理的开发, 为脑部疾病提供新的治疗策略。

3 外泌体载体治疗在中枢神经系统疾病中的应用

3.1 外泌体作为药物载体

脑卒中是中枢神经系统最常见的疾病之一, 其炎症反应、氧化应激和神经细胞凋亡是缺血性脑损伤的重要病理机制。姜黄素具有抗炎、抗氧化应激、抗凋亡等多种生物活性, 由于水溶性差和体内代谢不稳定等问题, 其临床应用受到限制。而外泌体可以包裹姜黄素进行大脑递送, 继而改善缺血性脑损伤。HE等[28]以巨噬细胞为供体细胞, 将其与3 μg/mL姜黄素在37 °C条件下共孵育24 h, 通过超速离心获得了载有姜黄素的巨噬细胞外泌体(Exo-Cur)。Exo-Cur可显著减少活性氧(reactive oxygen species, ROS)的积聚, 并通过激活occludin、claudin-5等紧密连接蛋白的表达以维持血脑屏障的完整性。TIAN等[29]通过表面修饰环辛炔的外泌体与叠氮化多肽之间的生物正交反应, 将功能性配体RGDyk环肽缀合到外泌体表面, 用其装载姜黄素后可靶向到达卒中小鼠模型的脑缺血部位, 有效抑制病灶区域的炎症反应和细胞凋亡。KALANI等[30]以小鼠胚胎干细胞(mouse embryonic stem cell, mESC)为供体细胞, 将其与姜黄素在室温下孵育15 min, 并快速冻融循环2至3次, 通过超速离心获得装载有姜黄素的mESC外泌体。与对照组相比, 负载姜黄素的外泌体可显著提高卒中模型组的神经功能评分, 减少脑梗死面积, 改善炎症反应, 并减少星形胶质细胞增生和*N*-甲基-*D*-天冬氨酸受体1(*N*-methyl-*D*-aspartate receptor 1, NMDAR1)表达。此外, 负载姜黄素的外泌体可增加药物溶解度、稳定性和生物利用度, 同时抑制脂多糖(lipopolysaccharide, LPS)诱导小胶质细胞活化产生的脑部炎症[31]; 经鼻内途径给药后, 装载有姜黄素的外泌体也可以被小胶质细胞吸收, 从而减少LPS诱导的脑部炎症[32]。载药外泌体这类纳米制剂和鼻内给药途径的实验结果为未来卒中潜在的非侵入性的治疗提供了希望。

阿尔茨海默病(Alzheimer's disease, AD)是最常见的神经退行性疾病, 磷酸化Tau蛋白的逐渐积累是AD的标志性特征。WANG等[33]发现, 巨噬细胞外泌体负载姜黄素后, 可通过Akt/GSK-3β信号通路抑制冈田酸(okadaic acid, OA)诱导的Tau蛋白磷酸化, 进而改善AD小鼠的认知功能。该研究表明, 巨噬细胞来源的外泌体还可能从其亲代细胞中遗传了淋巴细胞功能相关抗原-1(lymphocyte function-associated antigen 1, LFA-1), 该蛋白与内皮细胞间黏附分子-1(intercellular adhesion molecule-1, ICAM-1)相互作用, 从而介导了外泌体跨血脑屏障的迁移, 增强了姜黄素的血脑屏障穿透能力。QI等[34]设计了负载槲皮素(quercetin, Que)的血浆外泌体(Exo-Que), 以改善药物的生物利用度和增强Que的大脑靶向性。他们还发现, Exo-Que通过抑制细胞周期蛋白依赖性激酶5(cyclin-dependent kinase 5, CDK5)介导的Tau蛋白磷酸化来减少不溶性神经原纤维缠结(neurofibrillary tangle, NFT)的形成, 从而改善AD模型小鼠的认知功能。

外泌体还可以包裹紫杉醇和阿霉素等以治疗脑肿瘤[35-36]。将负载阿霉素的胶质母细胞瘤(glioblastoma, GBM)来源的外泌体与GBM在37 °C条件下共孵育4 h, 与单独的阿霉素处理相比, 负载阿霉素的外泌体处理更有效地抑制了GBM的增殖[37]。这同时表明, GBM外泌体可能具有归巢作用, 自体外泌体可作为治疗药物向本体细胞递送的理想载体[38]。

3.2 外泌体作为蛋白载体

外泌体还可以加载外源性蛋白进行大脑递送, 进而改善脑卒中发生后的神经细胞凋亡情况。色素上皮衍生因子(pigment epithelium-derived factor, PEDF)是一种多功能蛋白, 具有抗炎、抗氧化和神经保护特性[39]。HUANG等[40]用PEDF过表达的载体转染脂肪间充质干细胞(adipose-derived mesenchymal stem cells, ADSCs), 然后用外泌体提取试剂盒获得载有PEDF的外泌体, 经脑室内注射后, 发现外泌体携带的PEDF可以调节自噬相关蛋白表达水平, 显著改善脑缺血神经细胞的凋亡情况。YUAN等[41]将巨噬细胞外泌体和脑源性神经营养因子(brain-

derived neurotrophic factor, BDNF)在10 mmol/L磷酸盐缓冲液中混合, 把BDNF作为模型蛋白加载到外泌体中, 经静脉给药后, 其可以穿过血脑屏障, 并将BDNF递送至大脑以减轻神经炎症。该团队发现, 在存在脑部炎症的情况下, 这种递送作用增强。该发现对使用巨噬细胞外泌体作为纳米载体进行治疗性蛋白的大脑递送, 进而治疗中枢神经系统疾病具有重大意义。

HANEY等[42]用编码过氧化氢酶(catalase)的质粒DNA转染巨噬细胞, 获得了载有过氧化氢酶合成所需物质(包括DNA、mRNA、转录因子或过氧化氢酶蛋白)的外泌体, 这些内容物可以被外泌体有效地转移到神经元并在帕金森病(Parkinson's disease, PD)模型小鼠中发挥神经保护作用。该研究表明, 外泌体作为一种高效的递送系统, 可将蛋白质和遗传物质递送到靶细胞。此后, HANEY等[43]又以小鼠巨噬细胞为供体细胞, 使用超速离心法分离纯化外泌体, 并评估了室温孵育、冻融循环、超声处理和连续挤压四种外泌体加载过氧化氢酶的方法。然后使用凝胶过滤色谱法分离了负载过氧化氢酶的外泌体。这些载有过氧化氢酶的外泌体经鼻内途径给药后, 显著降低了小鼠脑内小胶质细胞和星形胶质细胞增生, 促进了PD模型小鼠神经元的存活。该研究表明, 外泌体是治疗性蛋白过氧化氢酶的有效载体。过氧化氢酶被包裹到外泌体中, 可长时间保留其生物活性、延长其血液循环时间和降低其免疫原性, 从而提高治疗效果。

3.3 外泌体作为核酸载体

在中枢神经系统疾病中, 有效利用外源性外泌体, 如干细胞来源外泌体, 携带遗传物质, 特别是microRNA的有效作用靶细胞, 可以改善脑卒中发生后神经细胞的凋亡进程。间充质干细胞来源外泌体(exosomes derived from mesenchymal stem cells, MSC-EXO)携带的miR-17-92, 通过激活PI3K/蛋白激酶B/雷帕霉素/糖原合酶激酶3β信号通路来改善神经发生和神经细胞凋亡进程[44]。MSC-EXO携带的miR-29b-3p还可以通过激活PTEN介导的Akt信号通路, 改善脑卒中模型大鼠的血管生成和神经元凋亡进程[45]。YANG等[46]发现, RVG肽与Lamp2b融合修饰的外泌体可以有效地将miR-124递送至脑缺血部位, 并促进缺血脑组织的神经发生。该研究表明, 工程外泌体可能是转运基因药物治疗卒中的最佳候选载体。ZHANG等[47]发现, 工程修饰的外泌体作为递送载体可以防止miRNA降解来改善卒中症状, 该团队将RGDyk环肽修饰的外泌体装载miR-210经尾静脉注射后, 其可以靶向到达小鼠缺血脑组织, 进而增强病变区域的血管内皮生长因子(vascular endothelial growth factor, VEGF)表达和血管生成。此外, miR-133b修饰的外泌体还可以显著抑制脑出血(intracerebral hemorrhage, ICH)卒中后RhoA表达并激活ERK1/2-CREB信号通路, 从而发挥神经保护作用[48]。

在抑制神经炎症的治疗中, 小胶质细胞来源的外泌体可以将miR-146a-5p转移至神经元, 进而下调miR-146a-5p的突触靶标以减轻神经炎症[49]。LAI等[50]通过尾静脉注射RVG肽修饰的外泌体, 将其携带的miR-193b-3p递送至蛛网膜下腔出血(subarachnoid hemorrhage, SAH)小鼠的大脑, 从而抑制SAH后组蛋白脱乙酰基酶3(histone deacetylase 3, HDAC3)的表达和促进NF-κB p65乙酰化来减轻神经炎症。该研究为外泌体递送miRNA治疗SAH提供了新策略。

在神经退行性疾病治疗中, JAHANGARD等[51]将携带miR-29b的外泌体注射到AD大鼠模型的海马中, 可以通过降低靶基因*NAV3*和*BIM*的表达水平来改善AD动物的空间学习和记忆能力。通过表面电穿孔使外泌体负载*BACE1* siRNA, 静脉给药后抑制了AD模型小鼠中BACE1 mRNA和蛋白的表达, 从而减少了AD小鼠中β-淀粉样蛋白(amyloid-beta, Aβ)肽产生[25]。RVG肽修饰的外泌体, 还可以将siRNA递送至脑组织中, 继而降低PD模型小鼠中α-突触核蛋白(alpha-synuclein, α-Syn) mRNA和蛋白质水平来发挥保护作用[52]。此外, 负载短发夹RNA微环(short hairpin RNA microcircle, shRNA-MC)的外泌体, 经尾静脉注射后可将内容物递送至PD模型小鼠脑组织中, 进而降低α-Syn聚集的水平和减少多巴胺能神经元的损失[53]。

在脑肿瘤的治疗中, 携带miR-133b的MSC-EXO可通过抑制*Zeste 2*基因增强子(enhancer of *Zeste 2*, *EZH2*)破坏Wnt/β-catenin信号转导途径, 从而抑制GBM的增殖、侵袭和迁移[54]。外泌体携带的miR-199a也可通过下调ArfGAP的GTP酶结构域、锚蛋白重复序列和PH结构域2(ArfGAP with GTPase domain, ankyrin repeat and PH domain 2, AGAP2)抑制神经胶质瘤的进展[55]。此外, 负载有miR-146b-5p

的外泌体可与神经胶质细胞中的表皮生长因子受体(epidermal growth factor receptor, *EGFR*) mRNA结合并抑制其表达, 进而降低神经胶质瘤的侵袭性和恶性程度[56]。也有研究发现, 在GBM生长和侵袭过程中, 外泌体中的miR-1表达降低, 促进了GBM的生长和侵袭[57]。因此, 基于外泌体携带的microRNA进行深入研究, 有助于发现治疗脑肿瘤的新策略。

4 前景与展望

血脑屏障的存在阻碍了药物对中枢神经系统疾病的有效治疗。几乎所有大分子药物和98%以上的小分子药物都不能通过血脑屏障。安全有效地递送药物是治疗中枢神经系统疾病的重要策略, 而外泌体是能够穿过血脑屏障的细胞外囊泡, 是细胞间信息交流的重要载体, 因此可以作为治疗药物进行大脑递送的载体。大量研究表明, 巨噬细胞来源的外泌体可能从其亲代细胞中遗传了LFA-1, 该蛋白与ICAM-1相互作用, 从而介导载药外泌体跨血脑屏障的迁移和穿透, 这种递送作用在存在脑部炎症的情况下增强。因此, 基于巨噬细胞外泌体的研究, 将可能为中枢神经系统疾病提供突破性的治疗策略。

尽管外泌体在药物载体领域具有巨大的应用潜力, 但天然外泌体的产量较低、易被自身免疫系统清除等问题限制了其进一步的临床应用。此外, 通过表面修饰实现外泌体的高效靶向并非易事, 需要严格控制反应条件, 避免外泌体的聚集和破损。需要进一步的研究来确定外泌体作为载体治疗中枢神经系统疾病过程的精确机制。针对不同疾病如何选择合适的细胞作为外泌体的来源, 如何高效地装载治疗药物等问题仍需要深入研究。期望随着外泌体大规模生产方法的出现以及外泌体工程修饰的发展, 在不久的将来可能建立与外泌体相关的治疗和诊断策略。

参考文献 (References)

[1] NEWTON W C, KIM J W, LUO J Z Q, et al. Stem cell-derived exosomes: a novel vector for tissue repair and diabetic therapy [J]. J Mol Endocrinol, 2017, 59(4): R155-65.

[2] ANEL A, GALLEGO-LLEYDA A, DE MIGUEL D, et al. Role of exosomes in the regulation of T-cell mediated immune responses and in autoimmune disease [J]. Cells, 2019, 8(2): 154.

[3] MASAOUTIS C, MIHAILIDOU C, TSOUROUFLIS G, et al. Exosomes in lung cancer diagnosis and treatment. From the translating research into future clinical practice [J]. Biochimie, 2018, 151: 27-36.

[4] KALLURI R, LEBLEU V S. The biology, function, and biomedical applications of exosomes [J]. Science, 2020, 367(6478): eaau6977.

[5] DONG X. Current strategies for brain drug delivery [J]. Theranostics, 2018, 8(6): 1481-93.

[6] THOMSEN M S, HUMLE N, HEDE E, et al. The blood-brain barrier studied *in vitro* across species [J]. PLoS One, 2021, 16(3): e0236770.

[7] KUMAR A, ZHOU L, ZHI K, et al. Challenges in biomaterial-based drug delivery approach for the treatment of neurodegenerative diseases: opportunities for extracellular vesicles [J]. Int J Mol Sci, 2020, 22(1): 138.

[8] KOOIJMANS S A, VADER P, VAN DOMMELEN S M, et al. Exosome mimetics: a novel class of drug delivery systems [J]. Int J Nanomedicine, 2012, 7: 1525-41.

[9] JOO H S, SUH J H, LEE H J, et al. Current knowledge and future perspectives on mesenchymal stem cell-derived exosomes as a new therapeutic agent [J]. Int J Mol Sci, 2020, 21(3): 727.

[10] TRAMS E G, LAUTER C J, SALEM N, Jr, et al. Exfoliation of membrane ecto-enzymes in the form of micro-vesicles [J]. Biochim Biophys Acta, 1981, 645(1): 63-70.

[11] PAN B T, JOHNSTONE R M. Fate of the transferrin receptor during maturation of sheep reticulocytes *in vitro*: selective externalization of the receptor [J]. Cell, 1983, 33(3): 967-78.

[12] JOHNSTONE R M, ADAM M, HAMMOND J R, et al. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes) [J]. J Biol Chem, 1987, 262(19): 9412-20.

[13] DEMORY BECKLER M, HIGGINBOTHAM J N, FRANKLIN J L, et al. Proteomic analysis of exosomes from mutant KRAS colon cancer cells identifies intercellular transfer of mutant KRAS [J]. Mol Cell Proteomics, 2013, 12(2): 343-55.

[14] SKOG J, WÜRDINGER T, VAN RIJN S, et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers [J]. Nat Cell Biol, 2008, 10(12): 1470-6.

[15] KOWAL J, TKACH M, THÉRY C. Biogenesis and secretion of exosomes [J]. Curr Opin Cell Biol, 2014, 29: 116-25.

[16] RINCÓN-RIVEROS A, LOPEZ L, VILLEGAS E V, et al. Regulation of antitumor immune responses by exosomes derived from tumor and immune cells [J]. Cancers, 2021, 13(4): 847.

[17] TAYLOR D D, GERCEL-TAYLOR C. Exosomes/microvesicles: Mediators of cancer-associated immunosuppressive micro-environments [J]. Semin Immunopathol, 2011, 33(5): 441-54.

[18] XIA X, WANG Y, HUANG Y, et al. Exosomal miRNAs in central nervous system diseases: biomarkers, pathological mediators, protective factors and therapeutic agents [J]. Prog Neurobiol, 2019, 183: 101694.

[19] GEIS-ASTEGGIANTE L, BELEW A T, CLEMENTS V K, et al. Differential content of proteins, mRNAs, and miRNAs suggests that MDSC and their exosomes may mediate distinct immune suppressive functions [J]. J Proteome Res, 2018, 17(1): 486-98.

[20] BELLAVIA D, RAIMONDI L, COSTA V, et al. Engineered exosomes: a new promise for the management of musculoskeletal diseases [J]. Biochim Biophys Acta Gen Subj, 2018, 1862(9):

1893-901.

[21] SALUNKHE S, DHEERAJ, BASAK M, et al. Surface functionalization of exosomes for target-specific delivery and *in vivo* imaging & tracking: strategies and significance [J]. J Control Release, 2020, 326: 599-614.

[22] YU X, ODENTHAL M, FRIES J W. Exosomes as miRNA carriers: formation-function-future [J]. Int J Mol Sci, 2016, 17(12): 2028.

[23] OSKOUIE M N, AGHILI MOGHADDAM N S, BUTLER A E, et al. Therapeutic use of curcumin-encapsulated and curcumin-primed exosomes [J]. J Cell Physiol, 2019, 234(6): 8182-91.

[24] QU M, LIN Q, HUANG L, et al. Dopamine-loaded blood exosomes targeted to brain for better treatment of Parkinson's disease [J]. J Control Release, 2018, 287: 156-66.

[25] ALVAREZ-ERVITI L, SEOW Y, YIN H, et al. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes [J]. Nat Biotechnol, 2011, 29(4): 341-5.

[26] HISLOP J N, ISLAM T A, ELEFTHERIADOU I, et al. Rabies virus envelope glycoprotein targets lentiviral vectors to the axonal retrograde pathway in motor neurons [J]. J Biol Chem, 2014, 289(23): 16148-63.

[27] KHONGKOW M, YATA T, BOONRUNGSIMAN S, et al. Surface modification of gold nanoparticles with neuron-targeted exosome for enhanced blood-brain barrier penetration [J]. Sci Rep, 2019, 9(1): 8278.

[28] HE R, JIANG Y, SHI Y, et al. Curcumin-laden exosomes target ischemic brain tissue and alleviate cerebral ischemia-reperfusion injury by inhibiting ROS-mediated mitochondrial apoptosis [J]. Mater Sci Eng C Mater Biol Appl, 2020, 117: 111314.

[29] TIAN T, ZHANG H X, HE C P, et al. Surface functionalized exosomes as targeted drug delivery vehicles for cerebral ischemia therapy [J]. Biomaterials, 2018, 150: 137-49.

[30] KALANI A, CHATURVEDI P, KAMAT P K, et al. Curcumin-loaded embryonic stem cell exosomes restored neurovascular unit following ischemia-reperfusion injury [J]. Int J Biochem Cell Biol, 2016, 79: 360-9.

[31] INGATO D, LEE J U, SIM S J, et al. Good things come in small packages: overcoming challenges to harness extracellular vesicles for therapeutic delivery [J]. J Control Release, 2016, 241: 174-85.

[32] ZHUANG X, XIANG X, GRIZZLE W, et al. Treatment of brain inflammatory diseases by delivering exosome encapsulated anti-inflammatory drugs from the nasal region to the brain [J]. Mol Ther, 2011, 19(10): 1769-79.

[33] WANG H, SUI H, ZHENG Y, et al. Curcumin-primed exosomes potently ameliorate cognitive function in AD mice by inhibiting hyperphosphorylation of the Tau protein through the AKT/GSK-3β pathway [J]. Nanoscale, 2019, 11(15): 7481-96.

[34] QI Y, GUO L, JIANG Y, et al. Brain delivery of quercetin-loaded exosomes improved cognitive function in AD mice by inhibiting phosphorylated tau-mediated neurofibrillary tangles [J]. Drug Deliv, 2020, 27(1): 745-55.

[35] ZHU Q, LING X, YANG Y, et al. Embryonic stem cells-derived exosomes endowed with targeting properties as chemotherapeutics delivery vehicles for glioblastoma therapy [J]. Adv Sci, 2019, 6(6): 1801899.

[36] YANG T, MARTIN P, FOGARTY B, et al. Exosome delivered anticancer drugs across the blood-brain barrier for brain cancer therapy in danio rerio [J]. Pharm Res, 2015, 32(6): 2003-14.

[37] THAKUR A, SIDU R K, ZOU H, et al. Inhibition of glioma cells' proliferation by doxorubicin-loaded exosomes via microfluidics [J]. Int J Nanomedicine, 2020, 15: 8331-43.

[38] QIAO L, HU S, HUANG K, et al. Tumor cell-derived exosomes home to their cells of origin and can be used as trojan horses to deliver cancer drugs [J]. Theranostics, 2020, 10(8): 3474-87.

[39] RIABINSKA A, ZILLE M, TERZI M Y, et al. Pigment epithelium-derived factor improves paracellular blood-brain barrier integrity in the normal and ischemic mouse brain [J]. Cell Mol Neurobiol, 2020, 40(5): 751-64.

[40] HUANG X, DING J, LI Y, et al. Exosomes derived from PEDF modified adipose-derived mesenchymal stem cells ameliorate cerebral ischemia-reperfusion injury by regulation of autophagy and apoptosis [J]. Exp Cell Res, 2018, 371(1): 269-77.

[41] YUAN D, ZHAO Y, BANKS W A, et al. Macrophage exosomes as natural nanocarriers for protein delivery to inflamed brain [J]. Biomaterials, 2017, 142: 1-12.

[42] HANEY M J, ZHAO Y, HARRISON E B, et al. Specific transfection of inflamed brain by macrophages: a new therapeutic strategy for neurodegenerative diseases [J]. PLoS One, 2013, 8(4): e61852.

[43] HANEY M J, KLYACHKO N L, ZHAO Y, et al. Exosomes as drug delivery vehicles for Parkinson's disease therapy [J]. J Control Release, 2015, 207: 18-30.

[44] XIN H, KATAKOWSKI M, WANG F, et al. MicroRNA cluster miR-17-92 cluster in exosomes enhance neuroplasticity and functional recovery after stroke in rats [J]. Stroke, 2017, 48(3): 747-53.

[45] HOU K, LI G, ZHAO J, et al. Correction to: bone mesenchymal stem cell-derived exosomal microRNA-29b-3p prevents hypoxic-ischemic injury in rat brain by activating the pten-mediated AKT signaling pathway [J]. J Neuroinflammation, 2020, 17(1): 203.

[46] YANG J, ZHANG X, CHEN X, et al. Exosome mediated delivery of miR-124 promotes neurogenesis after ischemia [J]. Mol Ther Nucleic Acids, 2017, 7: 278-87.

[47] ZHANG H, WU J, WU J, et al. Exosome-mediated targeted delivery of miR-210 for angiogenic therapy after cerebral ischemia in mice [J]. J Nanobiotechnology, 2019, 17(1): 29.

[48] SHEN H, YAO X, LI H, et al. Role of exosomes derived from miR-133b modified MSCs in an experimental rat model of intracerebral hemorrhage [J]. J Mol Neurosci, 2018, 64(3): 421-30.

[49] PRADA I, GABRIELLI M, TUROLA E, et al. Glia-to-neuron transfer of miRNAs via extracellular vesicles: a new mechanism underlying inflammation-induced synaptic alterations [J]. Acta Neuropathol, 2018, 135(4): 529-50.

[50] LAI N, WU D, LIANG T, et al. Systemic exosomal miR-193b-3p delivery attenuates neuroinflammation in early brain injury after subarachnoid hemorrhage in mice [J]. J Neuroinflammation, 2020, 17(1): 74.

[51] JAHANGARD Y, MONFARED H, MORADI A, et al. Therapeutic effects of transplanted exosomes containing miR-29b to a rat model of Alzheimer's disease [J]. Front Neurosci, 2020, 14: 564.

[52] COOPER J M, WIKLANDER P B, NORDIN J Z, et al. Systemic exosomal siRNA delivery reduced alpha-synuclein aggregates in brains of transgenic mice [J]. Mov Disord, 2014, 29(12): 1476-85.

[53] IZCO M, BLESA J, SCHLEEF M, et al. Systemic exosomal delivery of shRNA minicircles prevents parkinsonian pathology [J]. Mol Ther, 2019, 27(12): 2111-22.

[54] XU H, ZHAO G, ZHANG Y, et al. Mesenchymal stem cell-derived exosomal microRNA-133b suppresses glioma progression via Wnt/β-catenin signaling pathway by targeting EZH2 [J]. Stem Cell Res Ther, 2019, 10(1): 381.

[55] YU L, GUI S, LIU Y, et al. Exosomes derived from microRNA-199a-overexpressing mesenchymal stem cells inhibit glioma progression by down-regulating AGAP2 [J]. Aging, 2019, 11(15): 5300-18.

[56] KATAKOWSKI M, ZHENG X, JIANG F, et al. MiR-146b-5p suppresses EGFR expression and reduces *in vitro* migration and invasion of glioma [J]. Cancer Invest, 2010, 28(10): 1024-30.

[57] BRONISZ A, WANG Y, NOWICKI M O, et al. Extracellular vesicles modulate the glioblastoma microenvironment via a tumor suppression signaling network directed by miR-1 [J]. Cancer Res, 2014, 74(3): 738-50.