神经干细胞外泌体——神经疾病治疗的新途径

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摘要 外泌体(exosomes)几乎由所有类型的细胞释放, 不同细胞来源的外泌体携带不同的蛋白质、核酸和脂质, 参与细胞间的信息交流。最近的研究表明, 神经干细胞(neural stem cells, NSCs)分泌的外泌体可参与神经性疾病生理和病理的变化过程, 并发挥其潜在的神经调节和修复功能。因此, NSCs分泌的外泌体可以起到治疗神经系统疾病的作用。该文阐述了外泌体的生物合成, NSCs分泌的外泌体的特性、功能及其治疗神经系统疾病的研究进展; 讨论了外泌体在神经系统疾病治疗方面的应用潜力和面临的挑战。

关键词 神经干细胞; 外泌体; 神经系统疾病; 神经再生

Neural Stem Cell-Derived Exosomes — a New Approach for the Treatment of Neurological Diseases

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Abstract Exosomes are released by nearly all cell types and carry a cargo of proteins, nucleic acids and lipids that vary by the origin of cell. Exosomes participate in the communication of information between cells. Recent studies have shown that NSC (neural stem cell)-derived exosomes can participate in the physiological and pathological changes of neurological diseases and play their potential neuroregulatory and repair functions. Thus, NSC-derived exosomes can also afford therapeutic benefits. First, this review describes the biosynthesis of exosomes. Then, the properties and functions of NSC-derived exosomes and advances in the treatment of neurological diseases are summarized. Finally, the potential and challenges of exosomes in the treatment of neurological diseases are discussed.

Keywords neural stem cells; exosomes; nervous system disease; neuroregeneration

细胞外囊泡(extracellular vesicles, EVs)是由细胞分泌的具有双层磷脂膜结构的囊泡小体, 可以分为2个不同的类别: 来源于多泡内体(multivesicular endosomes, MVEs)的外泌体(exosomes, 40~150 nm)和来源于细胞质膜的微泡(microvesicles, 150~1 000 nm)[1-2]。其中, 外泌体是过去十年来最受关注的一种胞外囊泡[3-4], 几乎所有类型的细胞在体外培养过程中均可分泌, 外泌体还出现在血液、唾液、尿液和母乳等体液中[5-9]。早在1981年, TRAMS等[10]首次用胞外囊泡描述细胞在体外培养过程中通过脱落产生且具有胞外酶活性的小

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泡。两年后, HARDING等[11]发现大鼠网织红细胞能够释放小囊泡。不久之后, PAN等[12]利用电子显微镜观察到了绵羊红细胞通过胞吐作用产生约50 nm的小泡, 证明了其大小和在核内体中的初始起源。随后, JOHNSTONE等[13]率先分离了这些纳米囊泡, 并命名为“exosomes”。最初的研究认为, 外泌体是细胞释放的垃圾产物[14], 但后来研究发现, 外泌体参与细胞间的信息交流[1,3-4]。其中, 外泌体中携带蛋白质、脂质和核酸等多种生物活性成分, 可介导体内细胞间远程通讯[1], 传递非编码RNA和编码RNA[15], 传递免疫信号[16], 甚至可作为完全独立的代谢单元发挥作用[17]。因此, 研究者对外泌体在体内的起源和功能, 以及对外泌体在疾病诊断和治疗中发挥的作用的研究兴趣日益增长, 尤其在神经系统疾病的诊断和治疗领域。

中风(stroke)、脊髓损伤(spinal cord injury, SCI)、帕金森病(Parkinson’s disease, PD)、阿尔茨海默病(Alzheimer’s disease, AD)等神经系统退行性和损伤性疾病, 日趋普遍和严重, 但目前仍然缺乏有效的治疗方法。外泌体不仅参与凝血、抗炎、干细胞扩增和神经元通讯等多种生理和病理过程[18], 而且在抗原呈递、细胞死亡和血管生成方面也发挥重要作用[19], 其携带与神经退行性疾病相关的特定成分[20]。神经干细胞(neural stem cells , NSCs)分泌的外泌体可增强神经可塑性, 促进神经再生和损伤后功能的恢复, 通过调节神经元和胶质细胞在局部微环境和远处靶细胞的功能来提供治疗效果[21]。因此, NSCs分泌的外泌体具有巨大的临床应用潜力, 是一种潜在的神经系统疾病治疗的新途径。

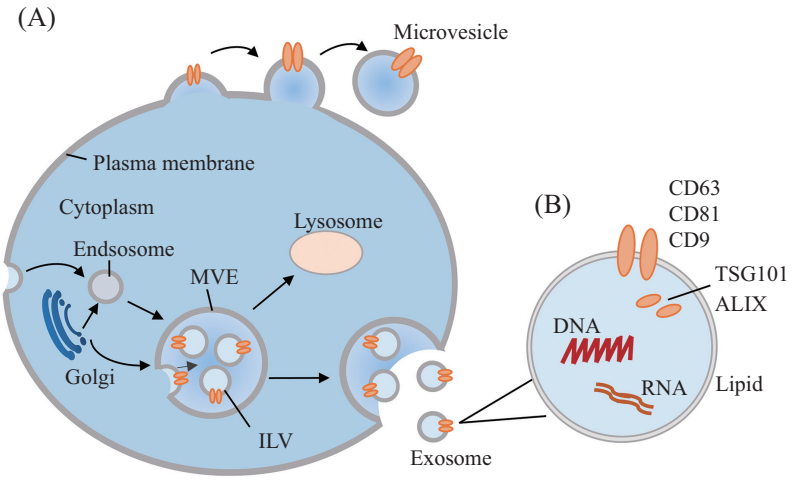
1 外泌体的生物合成

外泌体和微泡的生物发生都涉及膜运输过程, 但二者有不同的生物发生模式(图1A): RNA、DNA和脂质选择性地整合到腔内囊泡(intraluminal vesicles, ILVs), 这些小泡位于MVEs中, 是外泌体的前体; 内体膜向内出芽会导致胞质蛋白和其它成分被吞噬到ILVs的腔内; MVEs与细胞质膜融合后, 将ILVs作为外泌体释放到细胞外空间。而微泡则直接通过质膜的向外出芽脱落产生[22]。已有研究表明, 每种细胞类型根据其生理状态调节外泌体的生物发生, 并释放含有特定脂质(胆固醇、磷脂酰丝氨酸、磷脂酰肌醇、鞘磷脂和磷脂酰胆碱等[23])、蛋白质(TSG101、CD63、CD81、CD9和ALIX等[24])和核酸(mRNA、microRNA和非编码RNA[15])的外泌体(图1B)[25]。

2 NSCs及其分泌的外泌体

2.1 NSCs的特性和应用局限

NSCs是神经系统不同发育阶段的初级祖细胞, 能够自我更新, 可分化为神经元、星形胶质细胞和少突胶质细胞[26]。因此, NSCs对中枢神经系统的形成起着至关重要的作用。从脑组织中分离, 或者从多能干细胞诱导扩增的NSCs可用于治疗大脑神经损伤[27-30], 这主要是通过介导神经再生、可塑性, 以



A: 外泌体和微泡的生物合成过程; B: 外泌体的结构和组成成分。

A: biosynthesis of exosomes and microvesicles; B: structure and composition of exosomes.

图1 细胞外囊泡的合成和释放(根据参考文献[2]修改)

Fig. 1 Schematic diagram of extracellular vesicle formation and release (modified from the reference [2])

及减弱神经炎症等途径来发挥作用[31-33]。虽然NSCs移植治疗有极大的应用前景, 但细胞去分化、免疫排斥、干细胞致瘤、干细胞存活率较低等问题限制了NSCs的临床应用。这使NSCs分泌的外泌体的替代疗法受到了极大的关注可用于治疗神经损伤和神经退行性疾病。

2.2 NSCs分泌的外泌体

NSCs及其它细胞分泌的外泌体主要通过5种途径分离获得: 超速离心法, 包括差速离心、结合蔗糖或碘克沙醇的密度梯度离心; 超滤和尺寸排阻色谱法; 多聚物沉淀法, 采用亲水聚合物, 如聚乙二醇; 免疫亲和捕获层析法; 微控流芯片分离法[34-35]。目前, 研究者普遍采用的是超速离心法。

许多神经退行性疾病的病理分析显示, 外泌体可充当疾病发展的介质[20]。据报道, 神经细胞分泌的外泌体, 通过将特定的致病颗粒, 如阿尔茨海默病中的β-淀粉样蛋白肽和tau蛋白[36-37], 帕金森病中的α-突触核蛋白[38], 从产生它们的原始细胞传递到其它细胞, 从而介导了疾病的发展。但另一方面, 外泌体亦可发挥神经保护作用, 促进周围神经再生和神经损伤的修复[21]。此外, NSCs外泌体携带并传递具有生物活性的RNA、蛋白质和脂质, 可参与突触传递和神经元去极化[39]、神经元和神经胶质细胞之间的信息交流[40], 还能调节突触功能, 维持神经血管完整性和髓鞘形成[41]。在此背景下, NSCs释放的外泌体受到了广泛的关注。

NSCs分泌的外泌体具有以下功能(图2): (1)介导生物学效应, 主要通过其特异miRNA,调控细胞的生长和凋亡, 具有神经保护性。STEVANATO等[42]通过测序发现, 人NSCs分泌的外泌体有113种miRNAs, 结果显示, hsa-miR-1246、hsa-miR-4488、hsa-miR-4508、hsa-miR-4492和hsa-miR-4516等5种miRNAs表达量较高, 其中hsa-miR-1246是一种靶向p53的miRNA[43], 在调节细胞生长和凋亡中发挥重要作用[44]。此外, 其他研究显示, miR-21a、miR-125b在NSCs分泌的外泌体中高表达, 能够促进神经细胞的分化和再生[45-46]。(2)介导NSCs与微环境的通讯。COSSETTI等[47]通过研究发现, 外泌体介导小鼠NSCs与微环境之间的通讯, 促炎因子激活NSCs中的干扰素-γ(interferon gamma, IFN-γ)信号转导通路, 导致IFN-γ途径的特定成分通过外泌体作用于靶细胞[47]。(3)通过非受体的方式介导病毒进入细胞。例如, 将NSCs分泌的外泌体加入柯萨奇–腺病毒受体(coxsackie virus and adenovirus receptor, CAR)缺陷细胞和5型腺病毒(adenovirus type 5, Ad5)中进行共培养, 能够促进Ad5进入CAR-缺陷细胞[48]。(4)可以作为独立的代谢单元发挥作用, 改变微环境中关键营养素的浓度, 从而影响周围细胞的生理功能[17]。(5)影响衰老过程。研究发现, 出生后小鼠下丘脑NSCs通过释放携带特定miRNA的外泌体进入脑脊液进而影响衰老过程[49]。(6)影响小胶质细胞的形态形成。最近的研究表明, 小鼠脑室下区NSCs分泌的外泌体中富含miRNA let-7、miR-9、miR-26和miR-181, 这些miRNAs特异性靶向小胶质细胞, 影响其出生后在神经系统中的形态、功能和增殖[50-51]。因此, 利用NSCs分泌的外泌体来治疗神经系统疾病具有巨大的应用前景。

3 外泌体治疗神经疾病的潜力

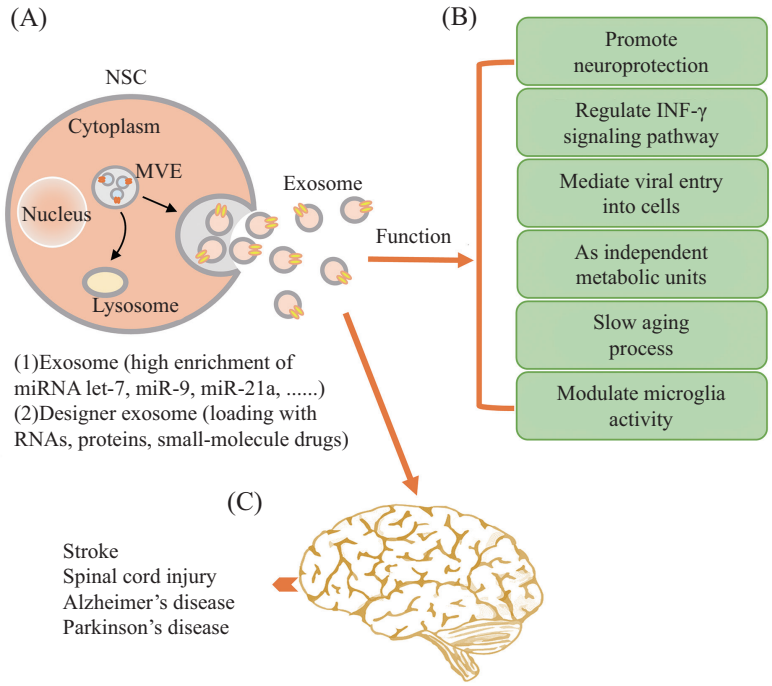
3.1 使用NSCs外泌体的优点

由于干细胞分泌的外泌体似乎比干细胞本身更有利于组织再生和修复[52], 因此, NSCs分泌的外泌体已被用于多种神经疾病治疗的研究。与NSCs移植治疗相比, 利用其分泌的外泌体进行治疗有如下优势: (1)外泌体可以通过滴鼻的方式传递到大脑的不同区域[53]。(2)外泌体因在体内无法复制, 释放药物后会迅速解体, 所以使用外泌体进行治疗后几乎不会发生肿瘤或恶性转化[54]。(3)外泌体是小囊泡, 将其经静脉注射后导致小血管阻塞的可能性极低, 而且能够通过血脑屏障[55-56]。(4)免疫原性较低[56]。由此可见, 通过大型细胞工厂生产外泌体用于治疗疾病是可行的[57]。NSCs分泌的外泌体提供了一种治疗神经系统疾病的无细胞疗法, 且不会产生明显的副作用。

3.2 外泌体在中风治疗中的应用

中风是造成死亡和残疾的主要原因之一, 但目前市场上仍没有能促进神经功能恢复的药物。对大多数中风幸存者来说, 需要漫长的康复过程和长期的药物治疗。因此, 需要一种能够修复中风患者脑损伤的替代疗法。

WEBB等[58]的研究表明, 在小鼠血栓栓塞性卒中后静脉注射NSCs分泌的外泌体可促进小鼠受损细胞、组织和功能的恢复。NSCs外泌体促进了小胶质细胞极化成M2表型, 进而促进碎片清除和减少



A: NSCs分泌的外泌体; B: NSCs外泌体的功能; C: NSCs外泌体在神经疾病治疗中的应用。外泌体主要通过两种方式用于神经疾病的治疗: NSCs分泌的外泌体富含miRNA let-7、miR-9、miR-21a等miRNA, 可直接用于治疗疾病; 将特异RNA、蛋白质和小分子药物加载到外泌体中, 用经过修饰的外泌体靶向治疗神经系统疾病。

A: exosomes derived from NSCs; B: functions of NSC-derived exosomes; C: application of NSC-derived exosomes in treatment of neurological diseases. Exosomes are mainly used for the treatment of neurological diseases in two ways. NSC-derived exosomes are enriched with miRNA let-7, miR-9, miR-21a, etc., which can be directly used for treatment. In addition, specific RNAs, proteins and small molecule drugs are loaded into exosomes, which target the treatment of nervous system diseases with modified exosomes.

图2 NSCs分泌的外泌体的功能和在神经疾病治疗中的应用(根据参考文献[42-51]修改)

Fig.2 Biological function of NSC-derived exosomes and their therapeutic potential in neurological disease (modified from the references [42-51])

慢性炎症[59-60], 能够提供神经保护, 减轻运动和记忆障碍[58]。随后, WEBB等[61]继续研究了NSCs分泌的外泌体在猪中风模型中的疗效。通过静脉注射外泌体进行治疗发现, 猪中风模型在受损组织和功能水平上得到了显著改善, 维持了脑白质的完整性。综上所述, 用NSCs分泌的外泌体治疗中风有以下5个特点: 具有神经保护性, 能够改善行为和活动力, 可消除缺血性病变的颅内出血转化, 减少脑损伤体积和脑肿胀, 促进损伤后功能的恢复。虽然关于NSCs衍生的外泌体治疗脑中风的研究较少, 但也显示出了其巨大的治疗潜力。

3.3 外泌体在脊髓损伤治疗中的应用

脊髓损伤常常导致受损脊柱下方肢体的严重功能障碍甚至致命[62], 破坏神经元或切断轴突。此外, 由于炎症引起的延迟性和继发性损伤, 可能导致水肿、神经细胞凋亡、空洞化和反应性神经胶质增生等[63-64]。脊髓损伤的治疗正在迅速发展, 目前一些实验性的治疗方法还不确定是否能安全地改善预后[65]。而外泌体携带参与信号转导的物质[66], 可调节微环境和大脑功能的各个方面[16-17,48-49]。因此, NSCs分泌的外泌体为脊髓损伤的治疗提供了一种新途径。

自噬为脊髓损伤的恢复提供了重要的保护作用[67], RONG等[68]的研究表明, NSCs来源的外泌体通过增加自噬标记蛋白LC3B和beclin-1的表达, 促进自噬体形成, 进而通过激活自噬来减少神经细胞凋亡, 激活小胶质细胞, 抑制神经炎症和促进脊髓损伤模型大鼠的功能恢复。因此, 研究人员认为来自NSCs分泌的外泌体能促进创伤性脊髓损伤后的功能恢复。

3.4 外泌体是治疗神经疾病的“药物”

由于血脑屏障具有高度的选择渗透性, 严格限制了大分子和几乎所有小分子从其他器官运输到大脑[69]。而外泌体因其体积较小和具有生物相容性等

特性, 能够穿过血脑屏障, 在治疗中枢神经系统疾病方面具有很高的灵活性, 可以通过滴鼻给药、静脉、腹腔和颅内注射将蛋白质和RNA传递到大脑[70-71]。HANEY等[70]用exoCAT(catalase-loaded exosomes)经滴鼻给药治疗小鼠帕金森病, 结果表明, exoCAT作为“药物”减弱了帕金森小鼠的神经炎性, 具有显著的神经保护作用。也有研究证明, 通过静脉注射加载了特异miRNA和siRNA的外泌体, 能够靶向大脑中的神经细胞, 并将miRNA和siRNA传递给靶细胞, 用于阿尔兹海默病小鼠的治疗[71-72]。

总而言之, 目前采用2种方法研究外泌体miRNA作为“药物”治疗中枢神经系统疾病。一种方法是直接滴鼻或注射外泌体, 外泌体内已含有对治疗有益的miRNA。另一种方法是将能够调控疾病相关基因表达的miRNA, 或者能够促进神经再生的特异miRNA选择性加载到外泌体中, 然后再进行体内研究。

4 结论与展望

外泌体具有多种生物学功能, 在神经系统疾病的治疗中凸显出强大的潜力。其主要特点为, 外泌体进入目标细胞后不会快速与溶酶体相关膜蛋白结合, 不会被迅速降解, 从而促进了靶细胞中信号通路的激活[47]。由于NSCs分泌的外泌体携带特异性miRNA、蛋白质和脂质等, 且具有抗炎、神经源性和神经营养作用。因此，不同区域NSCs释放的外泌体可能从不同层面影响脑功能, 将其经静脉注射或滴鼻给药有助于治疗多种神经系统疾病。因此, 外泌体靶向治疗神经系统疾病是未来的治疗途径之一。

虽然外泌体在疾病治疗中展现出强大的潜力, 但其应用仍存在许多局限性: (1)外泌体具有明显的异质性, 一方面是由于其体积较小承载能力有限, 导致特异蛋白质的差异分布。另一方面, 昼夜节律、刺激、应激、感染和细胞周期等环境因素诱导的基因表达变化有可能驱动外泌体异质性的发生[73], 影响了外泌体的广泛应用。(2)外泌体存在细胞类型特异性, 受到细胞类型、细胞生理或病理状态、以及某些分子机制的影响[74], 这阻碍了对其特性和功能的操纵。(3)目前研究者们对外泌体的生物功能多样性了解仍然非常有限, 对外泌体与受体细胞相互作用进而对靶细胞进行调控的机制仍无法得出准确的结论。(4)目前仍然缺乏有效的方法区分大小、密度和膜定位与外泌体相似的胞外囊泡。(5)缺乏安全、有效、高产的方法来生产和纯化外泌体, 限制了外泌体用于疾病治疗的临床转化。

基于体外修饰外泌体的方式, 将特定的蛋白质、miRNA、siRNA和药物等加载到外泌体中, 并以其作为药物靶向大脑中特定的神经元类型或区域, 将有助于治疗神经退行性和损伤性疾病, 同时可降低对健康组织产生的副作用[75]。然而, 在外泌体广泛地应用于临床治疗之前，我们仍面临诸多挑战: 首先, 外泌体在特定细胞类型中的特性, 以及不同类型细胞在不同条件下产生的外泌体之间的差异仍有待探索。其次, 寻找合适有效的方法用于外泌体的纯化, 使其符合CGMP(current good manufacture practices)的要求, 且不改变外泌体的生物活性, 能有效地提供一种包含相似的RNA、蛋白质和脂质的外泌体同质群体。最后, 该领域仍然需要构建理想的体内模型, 结合成像方法, 在单囊泡的水平上, 追踪外泌体的释放、运输路线和命运, 进一步阐明外泌体的基本功能, 为未来的临床转化奠定理论基础。

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