治疗性外泌体的研究进展

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摘要: 外泌体是一种活细胞分泌的囊性小泡, 携带大量具有组织或细胞特异性的蛋白质、脂质及遗传物质, 可调控不同的生理活动, 因此作为一类新兴的治疗药物被广泛研究。间充质干细胞 (mesenchymal stem cells, MSCs) 和树突状细胞 (dendritic cells, DCs) 衍生的外泌体是研究较为广泛的两类外泌体, 已有许多临床前及临床研究表明其在肺部疾病、肝脏疾病、神经系统疾病及肿瘤等疾病中展现出良好的治疗效果。另外, 巨噬细胞、肿瘤细胞和植物细胞等众多其他细胞衍生的外泌体也因其治疗潜力受到越来越多的关注。除了天然来源的外泌体, 工程化外泌体的研究也取得许多进展。已报道的外泌体工程化手段种类繁多, 如外泌体靶向修饰、外泌体包载活性成分等。本文总结了不同来源的治疗性外泌体的研究进展, 并讨论了外泌体的应用前景与未来可能遇到的挑战。

关键词: 外泌体; 生物工程; 药物治疗; 生物治疗; 临床研究

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Research progress of therapeutic exosomes

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Abstract: Exosomes are a kind of endosomal vesicles that are secreted by most if not all living cells. Due to their capability of delivering a variety of cargos, such as tissue- or cell-specific proteins, lipids, and genetic materials, and their broad biological activities, exosomes have gained substantial attention as emerging therapeutics. Exosomes derived from mesenchymal stem cells (MSCs) and dendritic cells (DCs) are two types of exosomes that are widely studied. Many preclinical and clinical studies have shown that they have a satisfactory treatment effect in lung diseases, liver diseases, nervous system diseases, tumors, and other diseases. In addition, exosomes from macrophages, tumor cells, plant cells, and many other cells are getting more attention due to their therapeutic potential. Besides natural exosomes, research on engineered exosomes has also made plenty of progress. There have been several engineering methods of exosomes, such as targeting modification and loading of active ingredients. In this review, we summarize the research progress of therapeutic exosomes from different sources, and further discusses the application prospects of exosomes and possible challenges in the future.

Key words: exosome; bioengineering; drug therapy; biological therapy; clinical study

由细胞所分泌的细胞外囊泡可根据其大小、内容物和形成机制差异分为凋亡小体、微泡、外泌体[1]。凋亡小体和微泡从质膜中直接出芽产生, 而外泌体是起源于细胞胞内体的纳米级脂质双层囊泡, 大小约为30～150 nm, 形似杯状。外泌体最先于体外培养的绵羊网织红细胞的上清液中被发现[2], 但最初仅被视为细胞清除不必要蛋白质的外排方式。后来研究证实, 外泌体是生物信息交流的重要媒介, 几乎所有细胞在不同的生理和病理状态下均会分泌这一物质。外泌体表面富含胆固醇、神经鞘磷脂和神经酰胺等脂类物质, 内载蛋白质、核酸等生物信息, 另外还含有代谢产物和氨基

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酸等多种物质。外泌体根据是否经过人工修饰分为天然外泌体和工程化外泌体，前者可分为动物源性外泌体和植物源性外泌体，而动物源性外泌体又可细分为正常外泌体和肿瘤外泌体[3]。因此，目前外泌体的来源是其主要的分类依据，但未来可能会考虑从生物学分布、免疫原性等方面进行分类[4]。

外泌体的形成机制至今仍未明确，目前主流观点认为细胞质膜内陷首先形成内体 (endosome)，继而内体向腔内出芽形成腔内囊泡转化为多泡体 (multivesicular bodies, MVB)，MVB 与质膜融合释放出的内部囊泡即为外泌体[5]。作为天然携带和传递细胞间信息的载体，外泌体保留了母体细胞的部分生物学功能，可调节复杂的生物活动，在多种疾病中显示出巨大的治疗价值。同时，因其具有免疫原性低、尺寸小、具有高渗透长滞留效应 (enhanced permeability and retention, EPR) 等优势，被广泛应用于药物递送，如小分子化学药物、蛋白质和核酸等[6]。本文围绕治疗性外泌体，就外泌体的细胞来源、外泌体工程化、临床应用及其前景和挑战展开重点介绍 (图 1)。

1 外泌体的分离技术

由于外泌体的大小、来源、内容物和功能均具有异质性，目前尚无标准的、普适的外泌体分离技术，这些原因极大制约了外泌体功能性方面研究内容的深入开展。大多数的分离技术无法将外泌体与具有相似物理特性的脂蛋白和非内体途径的细胞外囊泡完全分离[3]。目前常用的分离方法有超速离心法、聚合物沉淀法、超滤法、尺寸排阻色谱法、免疫亲和色谱法等[7]。超速离心法是目前使用最广泛的方法，其原理是根据溶液中颗粒的物理性质、溶剂的密度和黏度依次将组分分离。其优点是无需试剂即可用于提取大体积的样本，但是提取过程耗时较长且得率低。聚合物沉淀法的原理是通过添加聚合物从而改变外泌体的溶解度和分散性，最终借助较低转速的离心方法得到外泌体。该方法操作简便、无需超速离心机、产率高，然而易引入沉淀试剂和脂蛋白等杂质，可能会影响下游分析。超滤法和尺寸排阻色谱法是基于外泌体和其他囊泡之间存在大小尺寸差异而实现分离的方法。超滤法快速、纯度中等，但由于膜的吸附作用可能造成外泌体损失。尺寸排阻色谱分离的外泌体纯度高，保留了完整性和生物活性，缺点是分离过程耗时长、不适合大规模生产、价格偏高。免疫亲和色谱法可分离高纯度的外泌体及外泌体亚群，但需要选择合适的标志物，用于分离总外泌体时可能由于不用亚群之间标志物的表达差异造成偏倚。总而言之，外泌体的分离需要综合考虑样本体积、纯度、成本、仪器、时间和研究目的等因素选择最合适的方法。

2 间充质干细胞来源的外泌体治疗

间充质干细胞 (mesenchymal stem cells, MSCs) 是再生医学领域中使用最广泛的干细胞，离体增殖能力强，能够从多种组织中被分离得到，如骨髓、脂肪、脐带和胎盘等。免疫调节特性和高生产能力是 MSCs 所具有的两个显著特征。初步数据表明，间充质干细胞衍生的外泌体 (MSC-derived exosomes, MSC-Exos) 也具有 MSCs 的一些免疫调节特性。同时，MSCs 被认为是产生外泌体能力最强的细胞[8]，已越来越多地被应用于临床治疗。自首次报道以来，MSC-Exos 可以作为 MSCs“无细胞”治疗剂，能够有效增强药物诱导的肝损

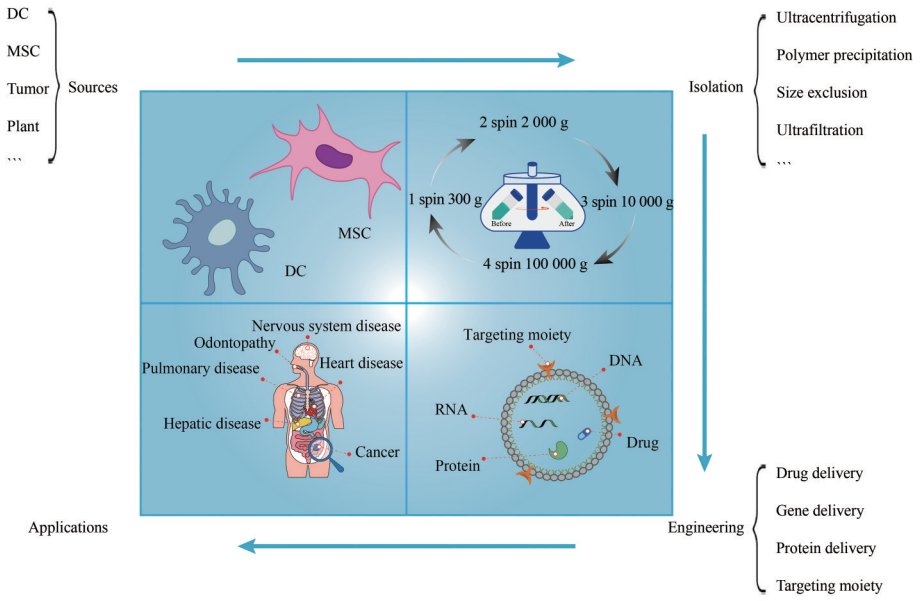

Figure 1 The sources, isolation, engineering and applications of exosomes. MSC: Mesenchymal stem cell; DC: Dendritic cell

伤中的肝再生[9]、促进皮肤伤口愈合[10]、骨骼肌再生[11]、骨折愈合和软骨再生等[12]。

2.1 间充质干细胞外泌体治疗肺部疾病

肺部慢性和急性疾病, 如慢性阻塞性肺疾病、肺纤维化和肺动脉高压, 被认为是世界范围内的主要健康问题。MSCs的细胞疗法与无细胞疗法为慢性和急性肺部疾病提供了一种新的治疗方法, 该方法具有抗炎、免疫调节、再生、促血管生成和抗纤维化的特性。线粒体功能障碍是吸烟引起肺部炎症和慢性阻塞性肺疾病的机制之一[13], 已有研究表明MSCs和MSC-Exos的联合治疗具有抗炎作用, 并且能够减少线粒体损伤反应的发生, 在由香烟烟雾诱导的肺损伤小鼠模型中显示出治疗效果[14]。支气管肺发育不良 (bronchopulmonary dysplasia, BPD) 是一种多因素慢性肺部疾病。基于MSC-Exos疗法的BPD临床前研究表明, MSC-Exos不仅可以预防BPD, 还可逆转肺损伤[15]。MSC-Exos的作用机制与调节肺巨噬细胞的表型有关, 通过抑制巨噬细胞促炎M1态和增强抗炎M2态, 从而抑制肺部炎症和免疫反应[16]。在低氧诱导的肺部炎症反应的实验模型中, MSC-Exos通过抑制信号传导与活化转录因子3 (signal transducers and activators of transcription, STAT3) 介导的信号传导发挥肺部保护作用并抑制肺动脉高压[17]。

另外, MSC-Exos在急性肺损伤和急性呼吸窘迫综合征中同样发挥重要作用[18]。2020年, 新型冠状病毒肺炎 (coronavirus disease 2019, COVID-19) 大流行对全球公共卫生和国际经济产生了重大影响, 迫切需要一种有效的疗法来治疗COVID-19患者。目前已开展针对COVID-19的MSC及其外泌体治疗的安全性和有效性的多项临床试验评估, 结果显示MSC-Exos治疗能够改善某些COVID-19患者的病情。其治疗作用在于减少细胞因子风暴, 增强肺泡液清除能力, 并促进上皮和内皮的恢复[19]。因此, MSCs及其外泌体有望成为新冠肺炎治疗候选药物。此外, 外泌体还可以作为生物标志物、疫苗和药物输送系统, 用于治疗COVID-19患者[20-22]。

2.2 间充质干细胞外泌体治疗肝脏疾病

MSC-Exos在许多损伤修复中起到重要作用, 最近的多项研究显示出其在肝病动物模型中的治疗作用。Tan等[9]研究结果表明, MSC-Exos主要通过激活肝细胞增殖、上调Bcl-xL (B-cell lymphoma-extra large) 蛋白表达, 从而抑制肝细胞凋亡, 发挥在肝损伤的保护作用。Qu等[23]发现miR-181-5p修饰的脂肪间充质干细胞 (miR-181-5p-modified adipose-derived mesenchymal stem cells, miR-181-5p-ADSCs) 衍生的外泌体具有抗肝纤维化的作用。研究者将ADSCs经过工程改造以表达miRNA-181-5p, 并通过外泌体将miRNA-181-5p递送至小鼠肝星状细胞 (mouse hepatic stellate cells, HST-T6) 或CCl_4诱导的纤维化肝脏。结果表明, 来自miR-181-5p-ADSCs的外泌体通过下调STAT3和Bcl-2表达并激活HST-T6细胞的自噬, 防止肝纤维化的发生。Liu等[24]研究脂肪组织来源的间充质干细胞衍生的外泌体 (exosomes from adipose tissue-derived MSCs, AMSC-Exos) 对急性肝衰竭 (acute liver failure, ALF) 治疗的作用及其机制。结果表明, miR-17修饰的AMSC-Exos可通过减少巨噬细胞中的NLRP3炎性小体活化来改善脂多糖和*D*-半乳糖胺 (lipopolysaccharide and *D*-galactosamine, LPS/GalN) 诱导的ALF, 而miR-17敲除的AMSC-Exos则显示明显减弱的ALF的治疗作用。这项研究为MSC-Exos疗法及其潜在机制提供了新的见解, AMSC-Exos可能是预防暴发性肝炎及其他TXNIP/NLRP3炎性小体增加的相关炎性肝病的新型策略。

2.3 间充质干细胞外泌体治疗神经系统疾病

外泌体在神经系统疾病的治疗和损伤修复中也有巨大的潜能。虽然MSCs不能通过分化为神经细胞和替代神经细胞来实现其再生作用, 但是MSCs衍生的外泌体能够携带修复脑损伤的物质。Chen等[25]研究表明, EP_4 (E-type prostanoid receptor 4) 拮抗剂可诱导MSCs外泌体中2',3'-环核苷酸3'-磷酸二酯酶 (CNP) 上调, 进而促进受损大脑中神经元和神经突触形成, 以及记忆、认知和学习功能的恢复。近年来, 外泌体作为脑卒中的治疗手段也经历了迅速的发展。以MSCs为基础的治疗手段能够在脑卒中的亚急性期促进大脑功能恢复, MSC-Exos是介导其组织修复作用的关键[26]。MSC-Exos具有治疗脑卒中的功效, 同时, 外泌体可能是反映疾病病理进展的生物标志物[27]。在体外, miR-17-92能够促进少突胶质细胞生成、神经发生和轴突生长。Xin等[28]研究表明, 用富含miR-17-92的MSC-Exos治疗脑卒中可以增强脑卒中后神经可塑性并促进神经功能恢复。另外, 富含miR-133b的MSC-Exos也能够促进大鼠脑卒中后的神经重构和功能恢复[29]。在实验性脑出血与脑缺血动物模型中, 接受外泌体治疗的动物在病变大小、白质修复和功能恢复等方面显示出更好的结果[30,31]。

2.4 间充质干细胞外泌体的其他治疗作用

MSC-Exos还有许多其他的治疗作用, 如促进皮肤伤口愈合、软组织修复和缺血心肌修复, 抑制疤痕形成、干预肿瘤的发生发展过程等。

皮肤修复和再生是动态而复杂的过程, 外泌体在皮肤伤口愈合中发挥重要作用[32,33]。Hu等[34]发现表

明, AMSC-Exos可以通过优化成纤维细胞的特性促进皮肤伤口愈合, 在伤口愈合的早期, 外泌体能够增加胶原蛋白I和III的产生, 而在后期, 外泌体可能通过抑制胶原蛋白的表达以减少疤痕的形成。此外, 调节巨噬细胞M2极化与转运microRNA可能是加快皮肤损伤愈合的另外途径。

在体外和体内进行MSC-Exos治疗时也观察到其具有心脏保护作用, 心肌细胞中EZH2的抑制或miR-25-3p的过度表达是其可能的机制[35]。在与心肌梗塞(MI) 治疗剂的共同作用下, MSC-Exos能够发挥促进血管生成和免疫调节作用, 从而保护心脏并诱导随后的心脏再生过程[36]。

在骨相关疾病损伤中, MSC-Exos通过增强细胞增殖和浸润, 减少细胞凋亡和调节免疫反应来介导软骨修复[37]。一系列体内研究结果表明, 给予MSC-Exos有效地减少了软骨细胞中炎性细胞因子的产生, 增加了软骨细胞外基质成分的表达, 并最终增强了软骨组织的再生[38]。MSC-Exos的治疗作用是通过多种细胞的协调动员和功能激活来实现的。

另外, 已有研究表明MSC-Exos在肿瘤发生发展过程中发挥“双刃剑”的作用, 即MSC-Exos对肿瘤细胞有着促进或抑制作用。人脐带间充质干细胞来源的外泌体通过转移MMP-2酶、改变细胞功能和重塑肿瘤微环境来促进卵巢癌和乳腺癌细胞的增殖[39]。源自胃癌组织的MSC-Exos, 在体内外均能促进胃癌肿瘤细胞的生长和迁移[40]。来自胎盘的MSC-Exos能够促进胎盘微血管内皮细胞迁移和血管生成[41]。另外的研究表明经紫杉醇修饰的MSC-Exos在胰腺腺癌中能够发挥强大的抗肿瘤效果[42], 经miR-143修饰的MSC-Exos能够减少骨肉瘤细胞的迁移[43], 经miR-122修饰的AMSC-Exos能够增加肝肿瘤细胞对化疗药物的敏感性[44]。

3 树突状细胞来源的外泌体治疗

作为一类经典的免疫系统抗原呈递细胞, 树突状细胞 (dendritic cells, DCs) 广泛分布于黏膜部位和皮肤中, 在启动抗原特异性免疫和耐受中发挥重要作用。DCs分泌的外泌体 (dendritic cell-derived exosomes, Dex) 的分子组成与DCs相似, 包括主要组织相容性复合物 (major histocompatibility complex, MHC)、共刺激分子和与免疫细胞相互作用的其他功能成分[45]。Dex通过旁分泌、自分泌和内分泌等途径作用于效应靶标, 参与机体的免疫应答、细胞增殖分化、细胞凋亡和肿瘤发生发展等病理、生理过程。

在基于DCs的免疫疗法的研究探索中, Dex凭借其特性展现出潜在的临床应用价值。首先, Dex保留了DCs基本的免疫刺激能力; 其次, 外泌体的膜稳定性使其拥有生物制剂通常不具备的天然保存优势; 再者, 此类无细胞疗法规避了常规的病毒和细胞疗法可能存在的安全隐患 (如体内复制的风险)[46]。

3.1 树突状细胞源性外泌体治疗肿瘤

当前基于树突状细胞的免疫疗法研究火热, 主要类型包括DCs的免疫调节剂、DCs疫苗和Dex等。其中, Dex克服了DCs疫苗免疫原性差、容易诱导肿瘤耐受的缺点, 兼具摄取处理呈递肿瘤抗原、刺激T细胞特异性免疫的能力, 从而实现长期抗肿瘤效应。

Lu等[47]发现高表达甲胎蛋白的Dex能够触发抗肿瘤免疫反应、重塑肝细胞癌 (hepatocellular carcinoma, HCC) 小鼠的肿瘤微环境, 尤其在无胸腺和缺乏$CD8^+$ T淋巴细胞的小鼠中效果明显, 这为HCC免疫治疗提供了无细胞疫苗这一新思路。尽管在癌症免疫疗法中DCs疫苗显示出强大的治疗效果, 但仍然存在许多不良反应, 而Dex疫苗恰可克服这一缺点。居里研究所推进Dex疫苗治疗非小细胞肺癌的临床研究, 其I期临床试验显示在Dex疫苗接种后, 机体中的自然杀伤 (natural killer, NK) 细胞数目显著增加, 这与Dex通过自然杀伤细胞2族成员D配体 (natural killer group 2 member D ligands, NKG2DLs) 介导NK细胞的激活有关[48]。II期临床试验表明第二代Dex疫苗干扰素-γ (IFN-γ) -Dex通过IFN-γ上调Dex上的BAG6表达, 增加与NKp30的结合量继而活化更多NK细胞, 因此具有更强的免疫刺激能力, 实现了对非小细胞肺癌诱导化疗后的维持免疫治疗[49]。

3.2 树突状细胞衍生外泌体治疗

DCs常作为自身免疫疾病治疗的主要靶点, 而Dex拥有与DCs相似的生物学功能, 因而应用于探究自身免疫性疾病治疗。不同于成熟Dex, 未成熟Dex和免疫抑制性Dex均具有抗炎特性[50]。类风湿性关节炎 (rheumatoid arthritis, RA) 是一种慢性自身免疫疾病, 目前治疗手段只能缓解症状, 无法根治该疾病。Yang等[51]在研究RA疗法时发现, 经IL-10或IL-4处理过的Dex或基因工程修饰表达死亡配体 (如FasL和TRAIL) 的免疫抑制性Dex, 比常规RA抑制剂更能显示出在RA模型中的抗炎效力, 能达到根治RA的目的。尽管目前尚无应用于治疗自身免疫性疾病外泌体疫苗的临床试验研究, 但是现有的动物实验和疾病模型表明, 未成熟Dex能够减少依赖于T细胞的免疫激活效应, 减轻自身免疫性疾病的临床表现, 延长移植物的存活时间[50]。另外, 经基因修饰 (如在供体DC过表达FasL、IL-4、IL-10) 或经IL-10处理过的Dex, 有望为治疗系统性红斑狼疮和其他自身免疫性疾病提供更为安全的治疗策略[52]。

3.3 树突状细胞衍生的外泌体与其他疾病治疗

Dex在皮肤移植、肝损伤、心梗和神经疾病等治疗领域展现出强大效力。Li等[53]在研究移植排斥时发现, 与microRNA-Exosome组相比, DC-Exosome组的$CD11c^+$ DCs数量减少, 而HLA^-G^+ DCs的比例显著增加, 且靶向DCs的Dex可在皮肤移植后数天内可诱导免疫耐受, 继而发现Dex在治疗皮肤移植后的免疫排斥中有应用价值。Zheng等[54]发现骨髓来源的Dex可以通过调节Tregs和Th17细胞二者的平衡来减轻缺血再灌注后的肝损伤。现有研究表明$CD4^+$ T细胞活化可以促进心梗后的伤口愈合, Liu等[55]在小鼠尾静脉注射Dex后发现脾$CD4^+$ T细胞的趋化因子和炎症因子表达增加, 而且心脏射血分数、左室短轴缩短率和左室后壁厚度三项心功能指标得到明显改善。在人脑疾病领域, Dex仍然具有巨大的应用价值。实验表明, 经IFN-γ刺激后的Dex在应用于海马切片培养时, 可以显著改善髓鞘的形成过程, 同时减少大脑的氧化应激程度, 改善偏头痛的情况, 因此被视为潜在的对抗抑郁症的新型疗法[56]。

3.4 其他细胞分泌的外泌体与疾病治疗

除了MSCs和DCs分泌的外泌体, 还有众多其他细胞来源的外泌体已被报道作为治疗疾病的候选药物。McDaniel等[57]研究表明, 肝脏干细胞来源的外泌体可以通过传递miRNA let-7改善小鼠导管反应和胆汁淤积性纤维化。相比于肿瘤裂解物, Wang等[58]揭示源自肿瘤细胞的外泌体可以更有效促进DCs成熟并增强MHC交叉呈递, 以增强DCs疫苗的抗肿瘤效应。Zhu等[59]揭示了源自脂肪干细胞的外泌体可以增加脂肪类移植物的存活率和褐变情况, 促使极化的巨噬细胞 (M2) 分泌儿茶酚胺以促进米色脂肪的再生, 为整形治疗提供了新思路。

除了动物可以分泌外泌体外, 植物中提取得到外泌体的相关报道开启了这一研究领域的新篇章。植物来源的天然可食用外泌体是近年新兴的药物疗法, 已有研究发现生姜外泌体内含靶向乳杆菌中各种基因的microRNA, 可以通过依赖于IL-22的机制修复小鼠的肠道屏障继而治疗结肠炎[60]。此外, 植物外泌体样纳米囊泡还可依据治疗目的工程化为具有免疫调节、再生修复、抗炎、抗肿瘤等作用的生物治疗物质或药物递送载体, 因而在未来临床实践上具有广阔的应用价值[61]。

4 工程化外泌体应用于疾病治疗

除了天然来源的外泌体, 近年来, 越来越多的研究表明工程化外泌体在疾病治疗中具有巨大的潜力。目前, 已报道的外泌体工程化手段包括外泌体靶向修饰和外泌体包载活性成分等。

4.1 外泌体靶向修饰

天然外泌体的靶向能力不足, 限制了其临床应用, 而在外泌体表面修饰特异性受体能够赋予或增强外泌体靶向性。Fu等[62]使用慢病毒载体向T细胞转入编码嵌合抗原受体 (chimeric antigen receptor, CAR) 的基因, 并纯化得到携带CAR的外泌体CAR-Exo, 研究发现CAR受体可介导外泌体对肿瘤的靶向杀伤效应, 且相比于CAR-T疗法, CAR-Exo具有安全性高、不受免疫检查点PD-L1抑制等优势。Shi等[63]向HEK293细胞转入CD3和人表皮生长因子受体2 (human epidermal growth factor receptor 2, HER2) 的特异性受体。该工程化HEK293细胞的外泌体可双重靶向T细胞CD3分子和乳腺癌相关抗原HER2, 进而引导细胞毒性T细胞杀伤HER2阳性乳腺癌细胞。

除基因修饰, 外泌体还可通过化学手段连接靶向基团。Tian等[64]利用一种简单、快速和生物正交的化学方法, 在外泌体表面连接靶向肽c (RGDyK) 作为靶向脑缺血的药物载体。体内研究证实该外泌体可穿越血脑屏障, 在脑缺血的损伤区域富集, 抑制炎症反应和细胞凋亡。

4.2 外泌体包载活性成分

4.2.1 加载小分子药物 将药物加载于外泌体有利于提高药物的稳定性、溶解度、生物利用度和靶向性, 进而增强药物疗效, 减少不良反应。加载小分子药物的方法有共孵育、挤压、超声、反复冻融、微流控和供体细胞共培养等。疏水性小分子药物如紫杉醇 (paclitaxel, PTX)、多柔比星 (doxorubicin, DOX) 和姜黄素等, 比亲水性药物更适合采用共孵育的方法加载。Sun等[65]率先使用共孵育的方法将姜黄素载入小鼠淋巴瘤细胞系EL-4衍生外泌体。经测定, 该方法可在1 g外泌体中载入2.9 g姜黄素。体内外实验结果表明, 相比于游离药物, 外泌体姜黄素向活化单核细胞的递送能力显著增强, 可提高姜黄素对脂多糖诱导的脓毒症休克的保护效力。在另一项研究中, Yang等[66]同样采用共孵育的方法, 加载小分子抗肿瘤药物PTX或DOX。经高效液相色谱法测定, 1 μg外泌体可载入 (7.3 ± 1.1) ng PTX或 (132.2 ± 2.9) ng DOX。值得注意的是, 外泌体是一种非常特殊的纳米载体, 本身已携带大量的蛋白质和核酸, 或许会导致装载能力的降低[67]。为了提高包封率, Thakur等[68]研究了微流控辅助药物装载。使用该方法, DOX和PTX的包封率分别达到19.7%和17.7%, 显著高于共孵育的方法。

除直接向外泌体加载药物, 外泌体载药的另一策略是将药物与活的供体细胞共混, 使母代细胞分泌出载有“货物”的外泌体, 即内源性载药方式。Pascucci等[42]

发现间充质干细胞可在体外摄入PTX并释放含PTX的外泌体。该研究表明活细胞或许可作为生物工厂生产外泌体药物。

4.2.2 加载蛋白质 在外泌体腔内加载蛋白质最常用的方法是工程改造供体细胞。为了提高sgRNA:Cas9核糖核酸蛋白复合物加载效率, Ye等[69]开发了一种基于蛋白−蛋白相互作用的方法, 将绿色荧光蛋白 (green fluorescent protein, GFP) 与外泌体标志分子CD63融合, 将GFP纳米抗体与目标蛋白Cas9融合, 利用GFP和GFP纳米抗体之间的亲和力引导CRISPR-Cas9系统进入外泌体。Yim等[70]报道了一种名为EXPLORs的蛋白加载系统, 通过蓝光控制蛋白−蛋白相互作用, 从而将目标蛋白与内源性外泌体生物发生过程整合, 主动地将蛋白引导至新生成的外泌体。除上述两种方法外, 已有多种蛋白质加载系统被相继开发。然而, 许多方法在免疫原性、包装效率和稳定性等方面存在局限性, 如何高效地将功能蛋白分子装载到外泌体仍有待进一步探索[71]。

4.2.3 加载核酸 基因治疗是一种利用载体将治疗性基因输送到患者体内的新型疗法, 具有广阔的应用前景。然而, 目前的基因递送载体往往存在高免疫原性、毒性[72]和核酸易降解[73]等问题, 限制了基因治疗的临床应用。外泌体具有天然的向受体细胞递送核酸的能力, 因而作为潜在核酸递送载体受到广泛关注。类似于小分子药物的加载, 核酸的加载同样可分为直接加载和内源性加载两种方式。小的核酸分子 (如miRNA和siRNA) 易直接加载。Alvarez-Erviti等[74]采用了电穿孔的方法, 将siRNA转入DC细胞来源外泌体。同样的方法也用于将miRNA载入骨髓间充质干细胞来源外泌体[75]。然而, 电穿孔可能伴随着siRNA聚集的形成, 导致实际装载到外泌体的siRNA数量被高估[76]。因此, 需要进一步改进该方法或寻找其他替代方案。Brossa等[77]建立了一种新的共孵育的加载方式, 使miRNA附着于外泌体表面的RNA结合蛋白, 有效递送miRNA的同时, 保护miRNA不被RNA酶降解。内源性加载是利用供体细胞向外泌体中富集目标核酸片段, 该方法可提高长片段核酸的加载效率。Hung等[78]报道了一种名为靶向模块化囊泡加载 (TAMEL) 的方法, 在供体细胞中转入溶酶体膜蛋白-2 (lysosome-associated membrane protein 2, Lamp2b) 与MS2噬菌体衣壳蛋白的融合蛋白, MS2噬菌体衣壳蛋白结构域可与目标RNA中同源的MS2茎环结合, 促进货物RNA在外泌体中的富集。在另一研究中, Li等[79]通过构建外泌体膜蛋白CD9与RNA结合蛋白的融合蛋白实现RNA装载。

5 外泌体临床试验研究进展

除了上述临床前研究 (表1[23,24,30,39,41,48,50,55,56,58,60,80-83]), 已有许多外泌体疗法进入临床试验阶段。MSCs和DCs是外泌体的两种主要的细胞来源。MSC-Exos具有分化潜能高、移植后存活率高、无明显不良反应等特点, 已在糖尿病、阿尔茨海默症、干眼症、多器官衰竭、脑血管疾病、急性呼吸窘迫综合征等多种疾病中开展临床研究。许多临床前研究表明, Dex在肿瘤、自身免疫病等多种疾病中具有潜在治疗作用, 相关临床试验

Table 1 Summary of therapeutic exosomes from different sources. BM-MSC: Bone marrow-derived mesenchymal stem cell; ADSC: Adipose-derived mesenchymal stem cell; hucMSC: Human umbilical cord mesenchymal stem cell; pMSC: Placental mesenchymal stem cells

Source of exosomes	Disease/condition	Mechanism	Reference
ADSC	Liver fibrosis	Activate autophagy; down-regulate STAT3 and Bcl-2 in HSC	[23]
ADSC	Acute liver failure	Suppress NLRP3 inflammasome activation in macrophages and reduce the secretion of inflammatory factors	[24]
ADSC	Brain repair, subcortical stroke	Enhance angiogenesis and axon remodeling	[30]
hucMSC	Breast cancer; ovarian cancer	Transfer MMP-2, re-organize the tumor microenvironment	[39]
hucMSC	Diabetes mellitus type 2	Reverse peripheral insulin resistance and relieving β-cell destruction	[83]
pMSC	Placental vascular system development	Mediate microvascular endothelial cell migration and vasculogenesis	[41]
DC	Non-small cell lung cancer	Promote natural killer cell activation and proliferation	[48]
DC	Autoimmune diseases and inflammatory diseases	Reduce immune stimulus that depends on T cells and induce immune tolerance	[50]
DC	Myocardial infarction	Activate $CD4^+$ T cells to facilitate wound healing after myocardial infarction	[55]
DC	Migraine	Improve myelination and reduce oxidative stress	[56]
Tumor cell	Lung tumor	Trigger stronger DC-mediated immune responses and decrease Tregs in the tumor microenvironment	[58]
Plant	Colitis	Vesical microRNAs shape the gut microbiota	[60]
BM-MSC	Multiple myeloma	Transfer of miR-15a	[80]
BM-MSC	Cutaneous wound healing	Promote M2 polarization; transfer of microRNA	[81]
BM-MSC	Breast cancer	Transport tumor regulatory microRNA, proteins, and metabolites	[82]

正逐步开展并初见成效。在晚期不可切除的非小细胞肺癌 (NSCLC) 患者中, Dex能够起维持性免疫治疗作用, 以提高其在4个月时的无进展生存时间 (PFS)。此外, 肿瘤细胞来源、血浆来源的外泌体的临床治疗效果同样受到关注。除人类组织细胞来源的外泌体外, 已有植物来源的外泌体在临床试验中注册。表2总结了不同来源的治疗性外泌体的临床试验研究进展。

6 展望

目前主要根据外泌体的细胞来源对其进行区分, 不同细胞来源的外泌体具有不同的特点和功能, 因此可反映其细胞来源和细胞的生理状态。过去常常认为同一来源的外泌体代表一种均质的囊泡群, 但是, 越来越多的证据表明, 即使在外泌体内部也存在异质性[84], 各亚群具有不同的生物物理特性、*N*-糖基化、蛋白质、脂质、DNA和RNA的组成成分[85,86]。有趣的是, 这些亚群介导了受体细胞中基因差异表达, 从而对受体细胞产生不同的影响。这种外泌体的异质性增加了研究者对其生物发生、分子组成、生物分布和功能理解的难度, 因而需要进一步剖析外泌体亚群和疗效间的关系。

此外, 探索不同细胞来源的外泌体的特点和功能有助于不同疾病的研究。本文关注MSC-Exos与Dex在不同疾病中的治疗潜能, 可以发现, MSC-Exos与Dex在皮肤损伤、肝脏疾病、神经系统疾病和肿瘤等多种疾病中均展现出治疗效果。MSC-Exos因其免疫调节特性、携带包括核酸、蛋白质和脂质的复杂物质、协调动员多种细胞等, 影响疾病的发展进程。而Dex因其具有DC的一些特性, 在自身免疫病和炎性疾病中发挥作用。

另外, 外泌体 (尤其是免疫原性较强的外泌体) 在体内会被快速清除且半衰期短, 这些问题可能限制其临床应用。有研究表明小鼠黑色素瘤细胞系 (B16-BL6) 来源的外泌体, 经静脉给药后半衰期仅为2 min[87]。各种形式的水凝胶, 如壳聚糖/蚕丝水凝胶和亚胺交联水凝胶, 已被用于延长外泌体在局部停留的时间, 增强外泌体的治疗效果[88,89], 然而, 这种方法不适用于延长全身给药的外泌体的半衰期。

目前外泌体的定量方式仍未统一, 采用纳米颗粒跟踪分析技术可对纳米颗粒浓度定量, 但该设备价格昂贵, 不利于大规模使用。更多研究则是通过测定总蛋白的方法对外泌体定量。定量方法上的差异导致给

Table 2 Clinical trials of therapeutic exosomes from different sources. CSTC-Exo: COVID-19 specific T cell derived exosomes; EXO-CD24: Engineered HEK293 cell-derived exosomes CD24-exosomes; Dex: Dendritic cell-derived exosomes; MSC-Exos: MSC-derived exosomes; QD: *Quaque die*

Intervention	Condition	Status	Phase	Dosage	NCT
Senescent melanoma cells derived exosomes	Metastatic melanoma	Unknown	Not applicable	Unknown	NCT02310451
Plasma-derived exosomes	Ulcer	Enrolling by invitation	Phase 1	Unknown	NCT02565264
CSTC-Exo	Coronavirus infection	Active, not recruiting	Phase 1	2.0×10^8 nano vesicles per 3 mL; on day 1 to day 5	NCT04389385
EXO-CD24	SARS-CoV-2	Recruiting	Phase 1	1.0×10^8–1.0×10^{10} exosome particles per 2 mL saline, QD for 5 days	NCT04747574
Adipose derived stem cells exosomes	Periodontitis	Recruiting	Phase 1	Unknown	NCT04270006
Dex	Non-small cell lung cancer	Completed	Phase 2	Unknown	NCT01159288
Plant-derived exosomes	Head and neck cancer oral mucositis	Active, not recruiting	Phase 1	Unknown	NCT01668849
Plant-derived exosomes	Colon cancer	Active, not recruiting	Phase 1	Unknown	NCT01294072
MSC-Exos	Coronavirus	Completed	Phase 1	2.0×10^8 nano vesicles/3 mL	NCT04276987
MSC-Exos	Diabetes mellitus type 1	Completed	Phase 2; Phase 3	1.22×10^6–1.51×10^6 nano vesicles/kg	NCT02138331
MSC-Exos	Alzheimer disease	Recruiting	Phase 1; Phase 2	Low-dose group: 5 μg; mid-dose group: 10 μg; high-dose group: 20 μg	NCT04388982
MSC-Exos	Cerebrovascular disorders	Recruiting	Phase 1; Phase 2	Unknown	NCT03384433
MSC-Exos	Metastatic pancreatic adenocarcinoma; pancreatic ductal adenocarcinoma	Not yet recruiting	Phase 1	Unknown	NCT03608631
MSC-Exos	Multiple organ failure	Not yet recruiting	Not applicable	150 mg once a day for 14 times	NCT04356300

药剂量的计算方式不尽相同, 不同研究间难以进行直接比较。因此, 未来需要对定量方法进一步统一。

外泌体作为一种新型平台, 不仅面临着科学研究方面的挑战, 同时也面临着实践上的挑战。为了更好回答如何准确、有效和选择性地鉴定外泌体, 如何标准化分离、纯化和定量外泌体的问题, 仍然需要进行更多的研究与实践。外泌体领域已经取得了极大的发展, 相信随着外泌体工程和生产工艺的进步, 外泌体治疗的临床应用将成为可能, 外泌体有望成为人类对抗疾病的新手段。

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