mRNA肺部递送系统研究进展

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摘要: 肺部疾病是全球性的重大公共卫生问题, 持续威胁着人类的生命健康并造成巨大的经济负担。基于信使RNA (messenger RNA, mRNA) 的吸入制剂高效靶向肺部细胞, 可克服传统疗法不良反应大、肺部生物利用度低、目标蛋白难以体外合成等问题, 为治疗肺部疾病提供了新思路。然而, 肺部生理结构复杂且mRNA难以进入细胞, 促使人们尝试利用合适的纳米递送系统以提高递送效率。本文介绍了肺部递送需克服的屏障, 讨论了mRNA肺部递送系统最新研究进展, 包括脂质纳米粒、聚合物纳米粒、多肽和外泌体, 总结了mRNA肺部递送系统的局限性并对mRNA吸入制剂应用前景进行展望。

关键词: mRNA; 吸入制剂; 肺部递送; 肺部屏障; 纳米递送系统; 疫苗

中图分类号: R943 **文献标识码**: A **文章编号**: 0513-4870(2024)11-3074-11

Research progress on mRNA pulmonary delivery systems

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Abstract: As a major global public health problem, pulmonary diseases threaten human life and health while causing a huge economic burden. The messenger RNA (mRNA)-based inhalation preparation, which effectively targets pulmonary cells can overcome the problems of traditional therapy, such as high side effects, low pulmonary bioavailability, and difficulty in synthesizing target proteins *in vitro*, thus providing new ideas for the treatment of pulmonary diseases. However, as the lung structure is complex and mRNA has trouble entering cells, researchers have been attempting to develop a suitable nano delivery system for higher delivery efficiency. This review introduces the barriers to pulmonary delivery, discusses the recent research development of the mRNA pulmonary delivery systems, including lipid nanoparticles, polymeric nanoparticles, polypeptides and exosomes, summarizes their limitations and looks forward to the application of mRNA inhalation preparations.

Key words: mRNA; inhalation preparation; pulmonary delivery; pulmonary barrier; nano delivery system; vaccine

收稿日期: 2024-02-18; 修回日期: 2024-09-04.

基金项目: 国家自然科学基金资助项目 (32371440, 32101157, 82104105); 中国博士后基金 (2021M693966); 国家重点研发计划 (2023YFC2605000).

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DOI: 10.16438/j.0513-4870.2024-0139

肺部感染、肺部肿瘤、肺部遗传性疾病等肺部疾病, 是全球性的重大公共卫生问题, 威胁着人类的生命健康并造成巨大的经济负担[1,2]。临床中常采用口服、注射和吸入的给药方式治疗肺部疾病, 其中吸入给药能够将药物直接输送到肺部, 是治疗肺部疾病最直接有效的给药途径[3]。随着获得FDA批准的两款信使RNA (messenger RNA, mRNA)药物BNT16b2与mRNA-1273在控制新型冠状病毒疫情中大放光彩[4], mRNA疗法成为基因治疗领域中的热点。目前, 已有超过17种mRNA候选药物正在进行临床试验[5]。以mRNA为基础的吸入制剂, 旨在高效靶向肺部特定细胞、在细胞内表达功能性蛋白以达到治疗肺部疾病的目的, 已成为多种肺部疾病的潜在疗法。然而, 肺黏膜组织作为人类抵御外界环境的天然屏障, mRNA药物的递送受到极大阻碍。此外, mRNA为一种带负电荷的亲水性大分子, 在体内半衰期短、自身难以进入细胞, 选择合适的递送系统尤为重要[6]。本文介绍了肺部递送mRNA治疗的优点与肺部递送需克服的屏障, 讨论了mRNA肺部纳米递送系统的最新研究进展, 包括脂质纳米颗粒、聚合物、多肽和外泌体, 总结了mRNA纳米递送系统的局限性并对mRNA药物应用前景进行展望。

1 肺部递送mRNA治疗的优势

口服或注射给药治疗肺部疾病时, 药物需跨过肺血管屏障到病灶中发挥作用, 肺部有效药物浓度低且易引发不良反应, 而吸入给药用于肺部疾病治疗时: ① 递送路程短并绕过肝脏首过效应, 起效快的同时局部药物浓度高, 能够增强药物递送效率并最大限度降低不良反应[7]; ② 肺泡巨大的表面积可显著改善药物的吸收, 减小给药剂量, 降低用药成本[8]; ③ 作为非侵入性的局部给药方式, 操作简便且允许患者自我使用[9]; ④ 用作疫苗时可引发黏膜免疫反应, 产生分泌型免疫球蛋白A (secretory immunoglobulin A, sIgA), 加强疫苗免疫效果[10,11]。由于吸入给药能实现高效肺部递送, 已被广泛应用于肺部疾病防治, 包括哮喘[12]、肺纤维化[13]、囊性纤维化[14]、肺部感染、慢性阻塞性肺病[15]、肺癌[16]和疫苗[17,18]等。

mRNA是生命遗传信息的载体, 能将遗传信息从DNA转移到核糖体, 并被翻译成蛋白质从而执行功能[19]。当mRNA进入细胞质后, 可利用宿主细胞的蛋白质合成机制表达目标蛋白, 从而实现异常蛋白的功能补偿或引发特异性免疫反应[20,21]。与其他药物相比, mRNA药物具有以下几方面优势: ① 高效灵活, 仅需修改mRNA序列就能实现在人体内表达不同类型的治疗性蛋白[22]; ② mRNA在细胞质发挥作用, 不会整合到宿主基因中, 安全性良好[23]; ③ 研发周期短, 尤其是面对新发突发传染病时能快速大规模制备疫苗[24]; ④ 此外, 由于肺部与空气接触, 其表面环境中的核酸酶活性低于血清中的核酸酶活性[25], 利于维持mRNA的稳定性并最大限度提高药物效果[26]。

2 肺部屏障

2.1 空气动力学要求

吸入颗粒的空气动力学直径决定颗粒沉积的位置, 是影响肺部给药的主要制剂因素[27]。药物在肺中的沉积机制主要有3种: 惯性碰撞、重力沉降和布朗扩散[28,29]。空气动力学直径大于5 μm的颗粒由于惯性碰撞在口咽部和上呼吸道沉积[30]; 1～5 μm的颗粒多遵循重力沉降机制在下呼吸道沉积[31]; 小于1 μm的颗粒被直接呼出[32]。因此, 吸入制剂药物的空气动力学直径应尽量处于1～5 μm内以实现肺深部沉积[33,34] (图1A)。

2.2 黏液纤毛清除

肺黏液层由杯状细胞和黏膜下腺体分泌产生, 包括黏蛋白、水和表面活性物质[35]。黏液中带负电荷的黏蛋白, 在气道表面形成具有50～180 nm可变空隙率的黏蛋白纤维网状结构, 以捕获吸入的刺激物或药物颗粒, 在纤毛细胞的摆动下将困在黏液中的颗粒向喉咙处推送, 随后通过咳嗽排出或被吞咽[36]。部分通过黏蛋白纤维网状结构的颗粒随即进入孔隙为20～40 nm的纤毛周层, 进入该层后的颗粒不易被纤毛运动冲走(图1B)。一般来说, 表面亲水且呈电中性的纳米颗粒会具有较好的黏液层渗透能力。然而mRNA递送通常依赖于亲脂性和带正电的载体, 需进行修饰以提高其黏膜递送能力。纳米颗粒的表面聚乙二醇 (polyethylene glycol, PEG)化是提高黏液层渗透能力最成功的方案[37], PEG高度亲水性和电中性的表面可使PEG化纳米颗粒“隐身”于黏液中。改变PEG链的长短和表面密度可调节颗粒肺部分布、黏膜渗透和肺停留时间[38,39]。例如, 在一项研究中, Lokugamage等[40]考察了不同脂质成分对脂质纳米粒 (lipid nanoparticle, LNP)雾化给药效果的影响, 作者指出, 高摩尔比的PEG搭配阳离子脂质显著改善了LNP的肺部递送效果。此外, 氟碳化合物也可以通过减少与黏蛋白之间的相互作用, 改善黏液渗透。例如, Ge等[41]开发胍基化和氟化双功能螺旋肽, 结果显示, 氟化的多肽将黏液渗透能力增强了约240倍。

2.3 肺表面活性物质

肺中的支气管经多次分枝形成无数细支气管, 细支气管末端膨大成囊, 囊的四周有很多突出的小囊泡, 即为肺泡, 成人约有3～4亿个肺泡, 总面积近100平方米[42,43]。肺泡上皮由肺泡上皮细胞 (alveolar epithelial

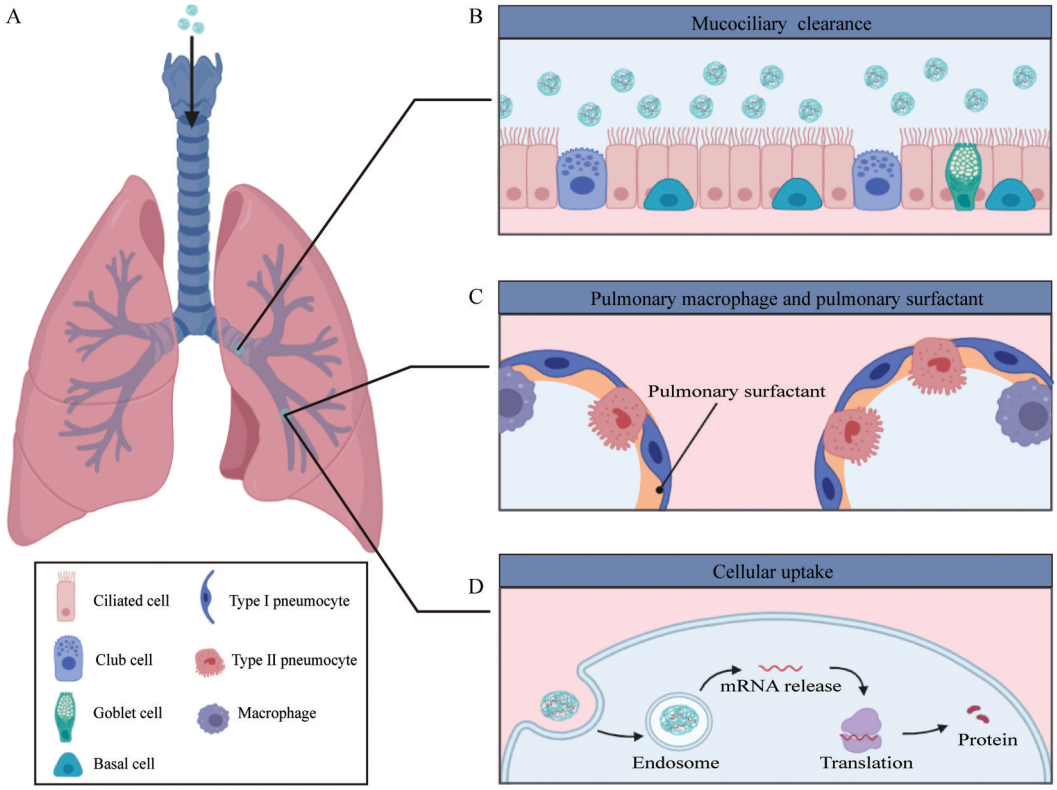

Figure 1 Schematic illustration of the pulmonary barriers that affect mRNA delivery. A: The optimal aero dynamic diameter for efficient lung deposition typically is in a range between 1−5 μm; B: Mucociliary clearance; C: Alveolar macrophages and pulmonary surfactant; D: Cellular and intracellular barriers

cells, AEC) I型和II型上皮细胞组成, 其中AEC I参与氧气和二氧化碳的交换[44], AEC II分泌的肺表面活性物质 (pulmonary surfactant, PS) 维持肺表面张力并阻止颗粒或病原体进入肺上皮细胞。PS的主要成分是二棕榈酰卵磷脂DPPC (90%) 和表面活性物质结合蛋白 (pulmonary surfatcant-associated protein, SP) (10%)。SP共有4种类型: SP-A、SP-B、SP-C和SP-D。SP-A和SP-D是较大的亲水性蛋白质, 是肺先天免疫系统的关键组成部分, 可将病原体和颗粒输送到免疫细胞。SP-B和SP-C是疏水蛋白, 可降低肺泡表面张力, 防止呼吸末肺泡塌陷, 与磷脂结合时维持正常的呼吸功能[45,46]。吸入进肺泡的mRNA递送系统需要特殊设计以使其在PS的存在下保持稳定性和功能性 (图1C)。例如, 有研究将仿生肺表面活性剂 (bio-PS) 添加至递送载体中, 成功将mRNA递送进AECs并诱导黏膜免疫应答[47]。

2.4 肺巨噬细胞

肺泡巨噬细胞 (alveolar macrophages, AMs) 位于肺泡, 占驻留肺泡免疫细胞总量的95%以上, 到达肺泡的颗粒可通过调理素依赖的机制或清道夫受体机制被AMs清除[48] (图1C)。颗粒的粒径与表面性质影响AMs的吞噬效率。AMs易吞噬的颗粒粒径范围在1.5 μm以内, 并且其摄取效率是粒径依赖性的, 利用纳米递送载体可以显著降低AMs的吞噬效率[49]。对颗粒表面进行修饰可增加空间屏障, 减少蛋白质吸附与巨噬细胞的识别。例如, 利用聚乙二醇进行表面修饰, 提供刷状的空间屏障, 从而降低巨噬细胞清除率[50]。

AMs也是治疗肺部疾病的有效靶点[51]。AMs既可以通过内吞作用和吞噬作用表现出抗炎状态, 还可通过模式识别受体识别病原体相关分子模式表现出促炎状态[52]。通过靶向AMs的药物递送系统, 可调节AMs的免疫状态, 从而缓解肺部炎症反应[53]。

2.5 细胞屏障

mRNA递送至细胞内的效率是其发挥药效的关键, 在此过程中mRNA面临着两道障碍: 细胞摄取和释放到细胞质[54,55] (图1D)。通常药物通过内吞作用被细胞摄取。内吞作用有4种机制, 包括网格蛋白介导的内吞作用、细胞膜穴样凹陷 (小窝蛋白) 介导的内吞、巨胞饮作用、与质膜直接融合[56]。由于mRNA带负电荷、亲水性强, 无法被带负电荷的细胞膜直接摄取, 而且其自身易被降解、稳定性差[57], 因此选择合适的递送系统尤为重要。亲脂性载体可提高细胞摄取效

率[58], 例如, 胆固醇作为细胞膜的组成成分, 常被用作脂质锚, 从而提高载体的稳定性和细胞摄取效率并降低载体的细胞毒性[59]。利用表面功能化修饰递送载体以提高细胞摄取效率也是一种常见的策略。例如, 小鼠的支气管肺泡灌洗液中含有丰富的白蛋白, 能与肺上皮细胞上高表达的FcRn结合。Hartwell等[60]将白蛋白亲和的脂质偶联到抗原上, 通过“白蛋白搭便车”的原理使其被FcRn跨黏膜转运到肺组织中, 增强了抗原的肺部渗透并提高了疫苗的保护效果。

细胞摄取后, 药物被包裹在细胞膜形成的囊泡(内涵体) 中, 必须在内涵体成熟之前逃离出内涵体/溶酶体, 并将mRNA完整地释放到细胞质中才能发挥治疗效果[61]。具体内涵体逃逸的策略, 将在下文递送载体中进行阐述。

3 递送系统

由于mRNA极其不稳定、电负性强而无法被细胞摄取, 且肺部的天然生物屏障作用进一步增加了mRNA递送难度, 因此肺部递送mRNA需谨慎选择合适的递送系统。理想的递送系统应满足以下几点: ① 安全性好; ② 保护mRNA不受核酸酶降解; ③ 能顺利穿过肺部屏障; ④ 提高细胞摄取并促进内体逃逸; ⑤ 将mRNA释放到细胞质中并成功表达出相应蛋白。本文综述了近期用于肺部递送mRNA的递送系统, 包括LNP、聚合物纳米颗粒、外泌体和多肽 (图2), 对各个递送系统的作用机制、优点与缺点以及最新研究成果进行总结与讨论。

3.1 LNP

LNP是迄今为止应用于临床的最先进的核酸药物递送平台, 目前已有三款基于LNP-RNA的疗法获得FDA批准, 分别是通过静脉注射向肝细胞递送小干扰RNA的Patisiran[62], 和两款新冠mRNA疫苗[63,64]。LNP常由4个组分构成: 可电离脂质、辅助磷脂、胆固醇和聚乙二醇修饰的脂质 (PEG化脂质), 各个组分的修饰与不同比例, 决定了脂质纳米粒mRNA递送效率和器官分布[65,66]。

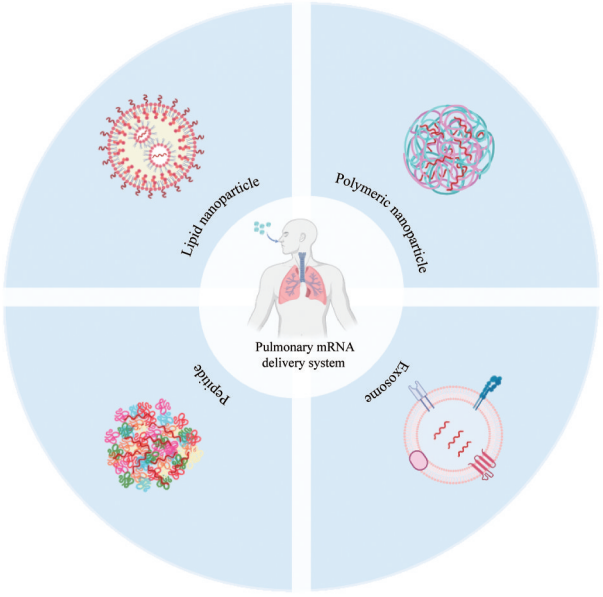

Figure 2 mRNA pulmonary delivery system

每种成分在递送中起着不同的作用。可电离脂质是最关键的组分, 通常含有至少一个叔胺头部结构和疏水的烷基链结构, 用于装载mRNA并介导内体逃逸[67]。最初的LNP技术使用阳离子脂质装载核酸, 阳离子脂质具有出色的转染效率, 但存在正电荷引起的溶血问题[68]和激活非特异性免疫导致的毒性问题[69,70]。这推动人们寻找电荷随pH值改变而改变的可电离脂质 (图3): 此类脂质在低pH值下叔胺键质子化, 携带正电荷, 但在生理pH值下呈现电中性, 因此可以在酸性缓冲液中与mRNA通过静电作用组成复合物[71], 在生理条件下转变为电中性, 从而降低细胞毒性[72]。可电离脂质的pH值敏感性对mRNA的内涵体逃逸至关重要, 当LNP被细胞摄取后, 在晚期内涵体的酸性环境下, 可电离脂质中的叔胺质子化, 磷脂形成一个较小的两性离子头部和一个疏水链尾部, 变成一个锥形结构, 从而扰乱内涵体膜, 促进内涵体逃逸, 将核酸释放到细胞质中[73,74]。通过引入不饱和键, 可以提高生物降解性或递送效率。例如, 适合用于肺部递送mRNA的可电离脂质RCB-4-8, 其含有的炔烃和碳酸酯基团分别促进核酸的内涵体逃逸和加速脂质在体内的降解[75]。

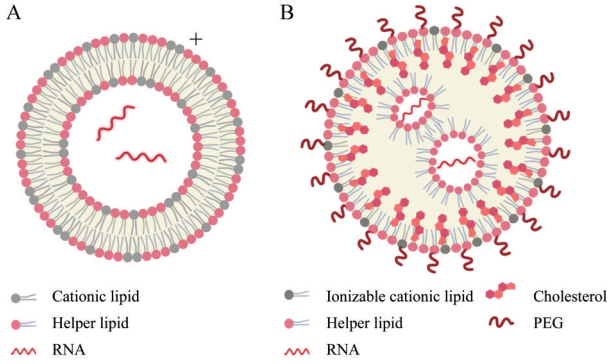

Figure 3 Schematic representation of the most commonly used lipid nanoparticles (LNPs) for RNA delivery. A: Liposomes prepared by cationic lipid; B: LNPs prepared by ionizable lipid

除了可电离脂质, 其他3种脂质成分也促进了纳米粒子的形成和功能。胆固醇具有较强的膜融合能力, 可促进细胞摄取[76]。研究发现, 胆固醇的尾部烷基链的结构和构象对转染效率具有显著影响。例如, 与

胆固醇相比, C24烷基取代的胆固醇类似物可以将LNP转染性能提高11～211倍。研究人员通过低温透射电镜观察到, 经过类似物替换后的LNP出现多面状态, 由此推测, 可能是胆固醇C24烷基造成的引起脂质体膜结构缺陷, 导致在内涵体中LNP膜的不稳定, 促使LNP的结构解离, 有助于核酸的释放[77]。

辅助磷脂可支持层状脂质双层结构的形成并稳定其结构排列[78]。磷脂与生物膜结构同源, 优化辅助磷脂的结构具有提高mRNA内体逃逸的潜力。例如, Liu等[79]合成了含有pH可调两性离子头和疏水尾的多尾可电离磷脂 (ionizable phospholipids, iPhos), 相较于普通磷脂, iPhos具有更好的mRNA递送能力和蛋白质表达能力。亲水性的PEG化脂质锚定于脂质纳米粒表面以提高稳定性, 限制脂质融合、调节纳米颗粒大小并促进渗透黏液层[80], 更重要的是, PEG脂质可通过减少巨噬细胞的摄取而增加纳米颗粒半衰期[81]。最近, Ongun等[82]还发现PEG脂质的摩尔比会影响雾化LNPs的递送效率。

由于LNP的肝靶向性, 目前仅有少数用于治疗肺部疾病的LNPs进入临床试验 (表1)。如何增加肺部递送效率, 减少肝非特异性靶向是目前研究的主要方向。采用肺部给药是降低LNP肝脏分布、提高肺部疾病治疗药效的方法之一。另一方面, 通过调整LNP的组成能够改变LNP的靶向性, 多项研究表明, 增加阳离子脂质组分有利于mRNA在肺中表达。例如, Kranz等[83]利用阳离子脂质制备LNP用于递送Luc-RNA, Luc荧光信号主要出现在肺部。Liu等[79]将iPhos与阳离子脂质结合, 提高递送效率的同时实现了mRNA的肺部表达。Cheng等[84]开发了一种新型器官选择靶向纳米颗粒平台——SORT-LNP。SORT-LNP通过在传统的LNP基础上添加阳离子脂质作为第五组分, 可实现对小鼠肺特异性靶向递送。Cheng等[84]指出, 纳米颗粒表面形成的蛋白冠可能影响LNP的器官靶向性: 当PEG化脂质从SORT-LNP表面解离, 暴露出的SORT脂质会被不同的血清蛋白识别并吸附, 吸附的蛋与靶器官中的受体相互作用, 从而促进LNP具有不同的器官靶向性[85]。Qiu等[86]研究发现, 改变脂质分子结构中的官能团可以改变脂质纳米颗粒的器官靶向性: 当疏水性尾与亲水性头部通过酯键链接时, LNP倾向于靶向肝 (O系列), 当疏水性尾与亲水性头部通过酰胺键连接时, LNP倾向于靶向肺 (N系列)。改变N系列LNPs脂质头部结构还可以靶向不同的肺细胞亚群。此外, 研究者通过蛋白质组学分析靶向肺部LNPs冠层中的蛋白质, 同样发现器官的靶向性可能与LNP蛋白冠有关, 但是具体潜在的机制仍需要进一步研究。

影响雾化LNPs递送因素众多, 数以千计的不同结构的脂质组分需在体外逐个考察, 同时体外数据并不能很好地与体内数据相匹配也限制着其临床转化, 如何高效筛选具有更高体内转染效率的LNPs-RNA系统是一道难题。Lokugamage等[40]开发了一种基于簇的工作流技术, 这种技术先对LNPs进行多维筛选后在体内进行量化评估, 提高了雾化LNPs-mRNA药物的筛选效率, 有望应用于更多的LNPs-mRNA递送领域。

3.2 聚合物纳米颗粒

用于递送mRNA的聚合物纳米颗粒大多由阳离子聚合物构成。聚合物纳米颗粒通过静电作用与mRNA形成复合物, 被细胞摄取后利用“质子海绵效应”[87,88]促进核酸的内涵体逃逸[89]。相较于LNP, 聚合物纳米颗粒结构简单, 修饰容易, 载药量相对较高[90,91]。然而, 具有高效核酸转染效率的同时, 聚合物纳米颗粒也存在阳离子导致的高细胞毒性、低胶束稳定性[92]等缺点。如何保持阳离子聚合物高转染效率的同时, 降低细胞毒性并增加肺部递送稳定性是重要的研究方向。

利用PEG进行修饰是一种降低毒性并提高稳定性的有效策略。例如, Ke等[93]利用PEG修饰优化聚合物纳米颗粒, 在胶束表面形成PEG电晕, 以屏蔽表面电荷提高胶束稳定性, 并在肺部展现出特异性递送与高转染效率。

聚合物具有多种拓扑结构, 包括线形、接枝和超支化结构, 改变聚合物拓扑结构以调节聚合物特性是优化mRNA雾化递送效率的重要策略[94]。相对于线性聚合物, 超支化聚合物拥有大量末端功能团, 可显著增加胺基基团的密度[95]。此外, 超支化聚合物拥有更宽的分子质量分布, 更高的支化度, 更好的分子链柔性, 在雾化过程中展现出更好的稳定性。例如, Patel等[96]将超支化PBAE与线性PBAE相比, 发现超支化PBAE胶束在雾化前后具有更稳定的粒径和低分散性, 可通过吸入的方式将mRNA有效递送至肺上皮细胞。

Table 1 Clinical trials of pulmonary mRNA delivery drugs

Drug name	Target protein	Disease	Stage	NCT number
MRT-5005	Cystic fibrosis transmembrane conductance regulator	Cystic fibrosis	Phase 2 (completed)	NCT03375047
VX-522	Cystic fibrosis transmembrane conductance regulator	Cystic fibrosis	Phase 1/2 (active, not recruiting)	NCT05668741
ARCT-032	Cystic fibrosis transmembrane conductance regulator	Cystic fibrosis	Phase 1 (recruiting)	NCT05712538
RCT-1100	DNAI1	Ciliary motility disorders	Phase 1 (recruiting)	NCT05737485

Blanchard等[97]利用超支化PBAE雾化递送CRISPR-Cas13a的mRNA, 在小鼠流感病毒模型和仓鼠新冠病毒模型中显示出良好治疗效果。此外, Rotolo等[98]在超支化PBAE基础上加入二硫醇合成聚β氨基硫脂, 获得更优异的安全性和耐受性, 并成功将mRNA雾化递送到小鼠、仓鼠、雪貂、奶牛和恒河猴的肺部, 在1/20的低剂量下达到与全身中和抗体治疗相似的疗效。总而言之, 超支化结构更有助于聚合物纳米颗粒雾化递送时的稳定性并提高肺部递送的效率, 越来越受到研究者们的青睐。

3.3 外泌体

外泌体是活细胞分泌的天然纳米颗粒 (直径在20～150 nm)。外泌体的形成起源于细胞膜经过内陷形成的内体, 内体再次内陷形成多囊泡体 (multivesicular body, MVB), 最后通过胞吐作用将囊泡释放到胞外[99,100](图4)。因此, 外泌体内包含了从亲本细胞中获得的各类分子信息 (核酸、蛋白质、脂质和代谢物等), 在细胞间通讯中起着至关重要的作用[101]。相较于人工合成的载体, 外泌体具有免疫原性低、易穿透黏液层、可避开内涵体–溶酶体途径和具有天然靶向性等优势, 被认为是理想的新一代药物递送载体[102-105]。目前, 在不破坏外泌体膜完整性的情况下提高外泌体载药量与细胞转染效率仍是一个巨大挑战[106,107]。

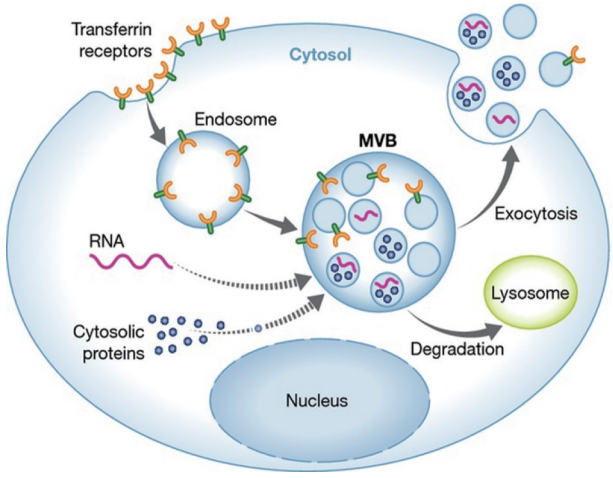

Figure 4 Biogenic mechanism of exosomes. Adapted from Ref. 100 with permission. Copyright © 2022 Frontiers

现有的载药方法可分为分泌前载药法和分泌后载药法。分泌前载药法通常通过向亲代细胞转染质粒或与亲代细胞共培养的方式, 得到含有mRNA的外泌体, 然后通过合适的提取方法即可获得载药的外泌体, 对提取的外泌体几乎不进行处理, 能较好的保留外泌体的完整性, 但载药效率相对较低[108]。分泌后载药方法通常需要先提取出外泌体, 再通过物理或化学的方式将核酸载入其中, 载药效率相对较高, 但可能会损害外泌体的完整性[109]。Yang等[110]开发了一种名为细胞纳米孔 (cellular nanoporation, CNP) 的技术, 利用CNP的纳米孔和瞬时电刺激, 将质粒DNA转染细胞中并刺激细胞释放包含内源性转录mRNA的外泌体, 大大简化治疗性外泌体产生的整个过程, 有望推进外泌体的临床应用。

在肺部递送方面, 利用肺细胞源外泌体似乎是更好的选择。Popowski等[111]证明, 相对于LNP和人胚胎肾细胞外泌体, 肺球细胞来源外泌体递送的mRNA具有更好的肺部分布与蛋白表达水平。Dinh等[112]证明采用的肺球状细胞来源外泌体 (LSC-Exo) 在降低肺纤维化和恢复肺功能方面优于间充质干细胞来源外泌体 (MSC-Exo), 并且LSC-Exo本身没有引起任何局部或全身免疫反应。

通过对外泌体进行工程化改造也是提高其转染效率的重要手段。在外泌体表面修饰CD47受体是一种逃避巨噬细胞的潜在方法, 该受体与信号调节蛋白α相互作用, 产生“不吃我”信号, 从而阻止巨噬细胞摄取[113]。除此之外, 创新性地将脂质体与外泌体进行膜融合, 可显著提高装载mRNA的能力并降低外泌体用量[114]。

3.4 多肽类

基于肽的递送系统具有生物相容性好、设计通用性强、易于合成、免疫原性低等优势[115]。该递送系统通常含有赖氨酸和精氨酸残基, 在生理pH值下具有正电荷, 通过静电相互作用与mRNA结合[116]。

3.4.1 短链肽 Qiu等[117]制备了模拟肺表面活性蛋白B的阳离子多肽——KL4肽, 用于小干扰RNA (siRNA)的肺部递送。为提高KL4肽溶解性并使其能递送mRNA, 他们将KL4与PEG共价结合以提高亲水性, 开发出吸入型PEG_{12}KL4用于mRNA肺部递送[118]。Xu等[119]进一步探讨了PEG链长度对PEG化阳离子肽递送mRNA至肺的影响, 研究表明, 一定长度PEG链可显著提高mRNA肺转染效率, 但过长的PEG链会降低mRNA肺转染效率, 并且降低其生物相容性。

3.4.2 核糖体蛋白 核糖体蛋白是一组与核糖体RNA (ribosomal RNA, rRNA) 结合形成的内源性蛋白, 具有等电点种类多, 异源性交叉反应风险低等优势[120]。Zhang等[121]验证了核糖体蛋白用于肺部递送mRNA的可行性和效率, 构建了核糖体蛋白–基质金属蛋白酶mMMP13复合物在内, 双功能肽和PEG化角化细胞生长因子在外的纳米颗粒。其中, 核糖体蛋白的电荷变化具有pH敏感性, 可促进mRNA的内涵体逃逸。研究显示, 吸入给药后能逆转博莱霉素诱导的小鼠肺纤维化。

3.4.3 合成肽 Guan等[122]开发了一系列由3个功能片段组成的合成肽: 含有疏水基团的锚定域, 用于与泊洛沙胺相互作用; 由碱性氨基酸组成的阳离子域, 用于包装mRNA并介导内涵体逃逸; 含有特定靶向片段的靶向域。这类肽与mRNA、泊洛沙胺自组装形成三元复合物纳米颗粒。泊洛沙胺的两亲性与接近电中性的性质, 使合成肽具有黏液惰性, 有效克服肺黏液的捕获, 可显著增强囊性纤维化小鼠肺部mRNA药物的递送。

4 总结与展望

近年来人们在mRNA修饰与mRNA递送载体上取得了里程碑式的进步, 使mRNA技术愈发成熟, 包括更低的免疫排斥、更高的mRNA稳定性和细胞摄取效率[123-125]。本文介绍的各类递送系统显示出优越的mRNA肺部递送能力, 在治疗特发性肺纤维化、先天肺部疾病及疫苗等领域展现出应用潜力, 但目前仍有一些问题限制着临床转化: ① 缺乏针对不同组织的可选择的靶向手段[126], 深入研究纳米颗粒与体内蛋白质相互作用的分子机制有利于预测递送载体在体内的靶向行为; ② 存在正电荷相关的血浆蛋白聚集、非特异性免疫[127]; ③ PEG诱导产生PEG特异性抗体加快PEG化药物的血液清除或引发超敏反应[128]; ④ 稳定性低, 对运输要求极高[129]; ⑤ 生物来源材料产量低、生产成本高, 且缺乏批次间差异的控制和质量标准[130]; ⑥ 吸入装置及赋性剂对制剂稳定性、空气动力学粒径分布、载药量等存在影响[131]; ⑦ 体内研究所采用的实验动物的肺部结构和生理特性与人类不完全相同, 有必要在临床试验之前选择非人灵长类动物进行更进一步的研究。

许多研究者在此方向上进行着不懈的努力, 并取得了瞩目的成果, 通过蛋白冠假说解释了LNP在体内分布的潜在机制[132]; 通过破坏细胞 (如机械挤压、超声、冻融等处理), 可获得纳米尺寸的, 外观及结构类似于外泌体的细胞衍生的纳米囊泡 (cell-derived nanovesicles, CNV), 提高了产量, 降低了生产成本[133,134]; 此外, 冻干技术的发展极大的提高了mRNA药物的稳定性和生产放大的可行性[135,136]。相信随着更多研究的深入和载体材料的进一步扩展, mRNA吸入制剂一定会在防治肺部疾病领域中展现出强大的应用前景。

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利益冲突: 本文所有作者声明不存在利益冲突关系。

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