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组织特异性mRNA-LNP递送技术的研发策略

宗岩[1], 魏妥[2,3], 程强[1*]

1. 北京大学未来技术学院, 生物医学工程系, 北京 100871;
2. 中国科学院动物研究所, 干细胞与生殖生物学国家重点实验室, 北京 100101;
3. 北京干细胞与再生医学研究院, 北京 100101
* 联系人, E-mail: qiangcheng@pku.edu.cn

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摘要　新型冠状病毒疫苗的成功研发将信使RNA(messenger RNA, mRNA)技术在全球范围推到了全新高度, 更是直接推动了我国mRNA医药产业的快速崛起和发展. mRNA技术成功的背后离不开安全、高效的递送载体, 尤其是脂质纳米颗粒(lipid nanoparticle, LNP), 已获批(包括正式获批和紧急使用授权)的mRNA新冠疫苗大多受益于此递送体系, 临床在研的mRNA药物项目超90%也同样依赖于LNP. 作为mRNA递送领域炙手可热的明星载体, LNP自身的研发因此备受关注. 当前mRNA-LNP技术在肝脏递送、肌肉注射的相关生物医学应用中较为成熟, 但针对肝外组织的靶向递送相对处在研究早期. 为了最大化扩宽mRNA药物的应用场景, 研究者已经采取多种技术手段研发LNP靶向递送技术. 基于此, 本文重点讨论了当前组织靶向mRNA-LNP技术的研究策略, 包括LNP处方优化、筛选新型脂质分子、靶向抗体修饰、给药方式优化等, 并简要展望本领域未来的可能发展方向.

关键词　脂质纳米颗粒, mRNA治疗, 组织靶向递送, 纳米医学

自2019年末起, 新型冠状病毒病(coronavirus disease 2019, COVID-19)肆虐全球, 造成了全球性的公共卫生难题. 新冠病毒基因组序列公布不到1年的时间, mRNA疫苗脱颖而出, 以极快的速度研发成功并在欧洲和美国获批上市, 使得mRNA疫苗和mRNA药物研发引发全球范围内高度关注. 2021年, *MIT Technology Review*评选mRNA疫苗为年度"十大突破性技术"; 2023年, *Nature*持续将mRNA疫苗研发列为值得关注的研究方向之一; 资本市场也纷纷投入巨资进入该领域, 仅仅2021年内, 全球mRNA技术领域内的投融资事件就多达30多起, 涉及金额20多亿美元. 这些事件无不表明, mRNA技术正在迎来前所未有的发展速度, 正在展现出强有力的应用前景.

mRNA药物的应用主要存在两个关键问题: 一方面是mRNA分子本身, 其免疫原性能激活一些固有免疫不良反应. 当前这一问题可通过引入非天然核苷酸(如假尿嘧啶、N1-甲基-假尿嘧啶等)进行修饰得到很大改善[1], mRNA修饰技术先驱Katalin Karikó及Drew Weissman教授也因此共享2021年拉斯克临床医学奖. 另一方面是安全和高效递送, 由于mRNA分子量大、携带负电荷且容易被酶降解, 因此需要递送载体的保护以及依靠递送载体协助mRNA进入细胞中进行翻译表达[2]. 脂质纳米颗粒(lipid nanoparticle, LNP)递送载体的不断发展为mRNA疫苗的快速获批发挥了极为关键的作用[3]. 事实上, 在整个RNA药物研发历程中, LNP都举足轻重. 2018年, 由Alnylam公司研发的Onpattro获得美国

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Zong Y, Wei T, Cheng Q. Recent advances in strategies for developing tissue-selective mRNA-LNP technology (in Chinese). Chin Sci Bul, 2023, doi: 10.1360/TB-2023-0613

食品药品监督管理局(Food and Drug Administration, FDA)批准用于治疗由遗传性转甲状腺素蛋白淀粉样变性引起的多发性神经病变, 这是历史上第一个小干扰核糖核酸(small interfering RNA, siRNA)疗法, 具有里程碑意义, 其中所用递送载体正是MC3 LNP(D-Lin-MC3-DMA, 简称MC3)[4]; 2020年底, 两款COVID-19 mRNA疫苗获得紧急使用授权(此后均已获得正式批准, 商品名称分别为Comirnaty和Spikevax)用于抵抗全球新型冠状病毒感染大流行, 实为历史上mRNA药物的首次问世, 同样依赖LNP递送体系. 这些RNA药物0到1的里程碑事件足以说明, LNP递送载体是推动RNA药物发展的最好选择之一.

经典的脂质纳米颗粒主要由4种类型的脂质分子组成, 包括可离子化阳离子脂质(关键脂质)、辅助性磷脂、胆固醇和聚乙二醇偶联脂质(图1)[5]. mRNA-LNP的制备过程大致如下: 首先, 将mRNA分子溶解在酸性缓冲液中(如pH 4.0柠檬酸钠溶液); 其次, 将各种脂质分子按照计算好的比例溶解于乙醇中; 最后, 利用微流控芯片将水相和乙醇相(3:1, 体积比)快速混合即可完成mRNA-LNP的制备. 由于水相为酸性环境, 可离子化阳离子脂质被质子化而带正电荷, 可以通过静电相互作用与携带负电荷的mRNA分子结合, 进而实现对mRNA的包裹. 随后, 用在pH 7.4的磷酸盐缓冲液透析处理, 可将体系改变为中性, 促使可离子化阳离子脂质转变回不带电状态, 此时LNP的表面一般呈现微弱负电状态. 一旦该mRNA-LNP进入细胞内涵体/溶酶体(酸性环境)中, LNP则再次带正电荷, 并随后与生物膜发生融合促使mRNA释放到胞质中进行高效翻译[6]. 上述这些特性使得LNP具有安全、高效递送RNA的能力, 这也是LNP能最终成功的关键因素所在.

目前, RNA-LNP技术已经在肝脏递送(FDA批准的Onpattro)、肌肉注射(新型冠状病毒疫苗)相关应用中取得巨大成功, 这主要得益于肝脏靶向和局部给药LNP技术的成熟. 为了充分发挥mRNA技术优势, 如低成本、高效率、周期短等, 开发组织靶向LNP至关重要, 有望加速扩宽mRNA药物的应用场景. 下文将重点介绍领域内的几种研究策略和当前进展, 并在最后进行了适当讨论和展望, 期望该文能够为领域的发展提供些许有用信息.

1 LNP处方优化

如前文所述, LNP通常由4种脂质成分组成, 这一特性使得LNP制剂相对复杂且具有多样性, 也为研究多样化LNP提供了简单易行的途径. 静脉注射后, LNP会在肝脏具有非常明显的富集, 这也使得肝靶向递送相对容易. 前文提到FDA批准的MC3 LNP即为肝脏靶向递送中最具代表性载体(结构式见图2), 对它的研究非常充分且机制明确[4]. 该种LNP进入血液中循环后, 吸附各种不同的血浆蛋白形成“蛋白冠”. 其中, 发挥关键作用的是载脂蛋白E(apolipoprotein E, ApoE), ApoE与其对应的肝细胞表面受体(低密度脂蛋白受体, low density lipoprotein receptor, LDLR)结合, 从而实现肝靶向递送[7,8]. 此外, 各种肝脏靶向的LNP被陆续开发, 例如C12-200 LNP[9]、5A2-SC8 LNP[10]等, 递送机制也都是源于ApoE蛋白介导的“内源靶向”而实现. 最早获批的两款mRNA新冠疫苗所使用的SM-102 LNP(Moderna)和ALC-0315 LNP(Pfizer-BioNTech), 在静脉注射后也主要会在肝脏部位进行mRNA翻译表达(SM-102的结构式见图2).

相比于肝脏递送, 肝外组织靶向递送的研究相对处于早期. 先前已有研究表明, 优化LNP的配方可很大程度地提高mRNA的体内外递送功效[11,12], 因此科学家期望通过对肝靶向LNP的配方优化找到一种简单易行的肝外靶向递送策略. 2020年, Siegwart教授团队[13]报道了一种名为“选择性器官靶向”(selective organ targeting, SORT)LNP递送技术, 实现了精准、简易、通用的肝外靶向mRNA递送策略(图3(a)). 他们在传统的四组分肝靶向LNP基础上, 创新性地加入了第五组分(命名为SORT分子), 制备出五组分LNP体系(代表性SORT分子的化学结构见图2). 当SORT分子为带正电荷的脂质时(如DOTAP), LNP可将mRNA特异递送到肺部表达; 当SORT分子为带负电荷脂质时(如18PA), LNP则将mRNA特异递送进入脾脏表达; 而SORT分子为可离子化阳离子脂质分子时(如DODAP), 则LNP会增强mRNA在肝脏的表达. 值得一提的是, 他们的研究表明, SORT分子的选取不依赖脂质分子的结构、给药剂量、检测时间等因素, 只需保证带电性即可; 同时, 这种策略在5A2-SC8 LNP、MC3 LNP、C12-200 LNP中均实现了快速、高效的肺、脾、肝靶向递送, 具有非常高的普适性. 这一发现为肝外mRNA组织特异性递送提供了一定的指导原则和解决办法, 领域内多个课题组运用该技术成功获得并研发出多种多样的组织靶向LNP载体, 进一步证明其普适性[14~16]. 通过靶向肺部方式递送编码抗原、抗体或基因编辑工具的mRNA,

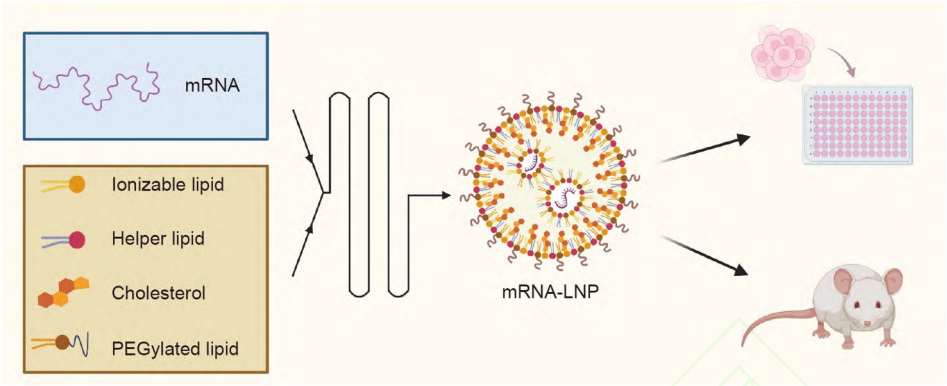

图 1 (网络版彩色)mRNA-LNP的制备以及体外、体内评价示意图. Created with BioRender.com
Figure 1 (Color online) Schematic diagram of preparation and evaluation of mRNA-LNP formulation. Created with BioRender.com

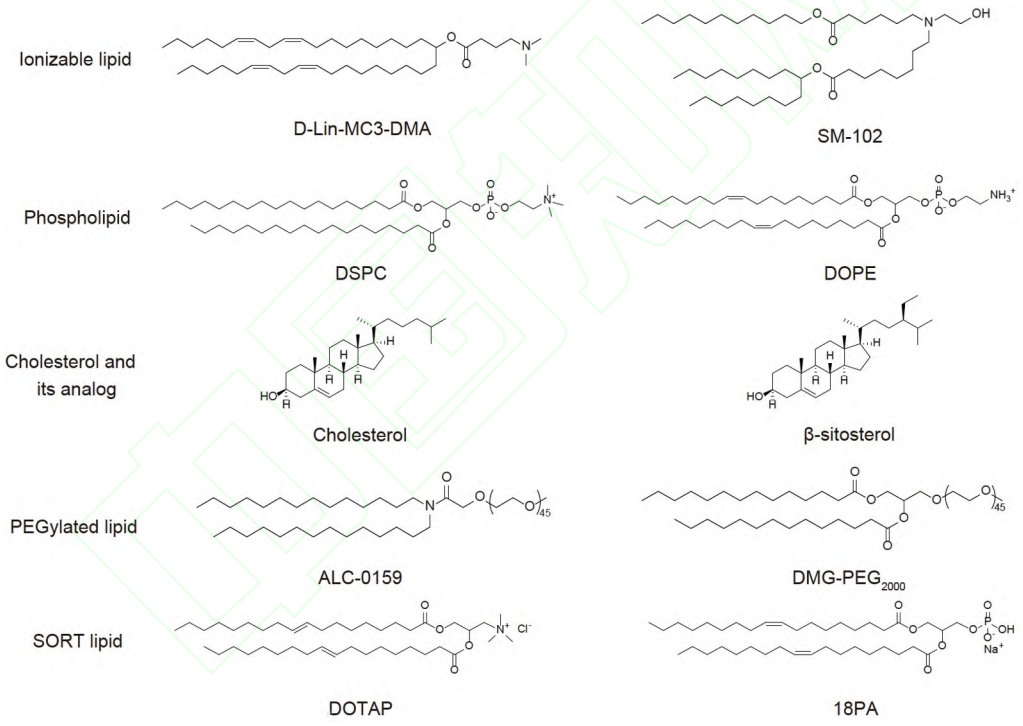

图 2 代表性可离子化阳离子脂质、磷脂、胆固醇、聚乙二醇(polyethylene glycol, PEG)偶联脂质和SORT脂质分子的化学结构
Figure 2 The chemical structures of representative ionizable cationic lipids, phospholipids, cholesterol, PEGylated lipids and SORT lipids

用于抵抗包括新冠在内的由呼吸道病毒引发的相关病也极具应用前景. Dong、Liu和Wang等团队[14]合作, 利用肺靶向SORT LNP技术特异递送了能降解肺组织蛋白酶L的Cas 13d编辑系统, 实现了对新冠病毒感染的有效预防和治疗. 随后, Siegwart教授团队[17]对SORT技术靶向机制进行了探索(图3(b)). 他们猜测, 跟肝脏靶向机制类似, 不同类型SORT分子可能导致LNP吸附不同种类血浆蛋白, 进而实现组织特异性mRNA递送. 通过对不同SORT LNP蛋白冠的质谱分析, 发现肝脏、肺脏和脾脏LNP吸附的主要蛋白分别为ApoE、vitronectin和β2-glycoprotein I, 这一结果为揭示肝外组织靶向机制提供了可能. 然而, 文中并没有直接的数据验证该结果, 还有待进一步研究和讨论.

除上述设计外, 利用通量筛选也是研发组织特异性LNP的常规办法. 为了增加筛选通量和简化筛选流程, Dahlman教授团队[18]利用DNA条形码(DNA bar-

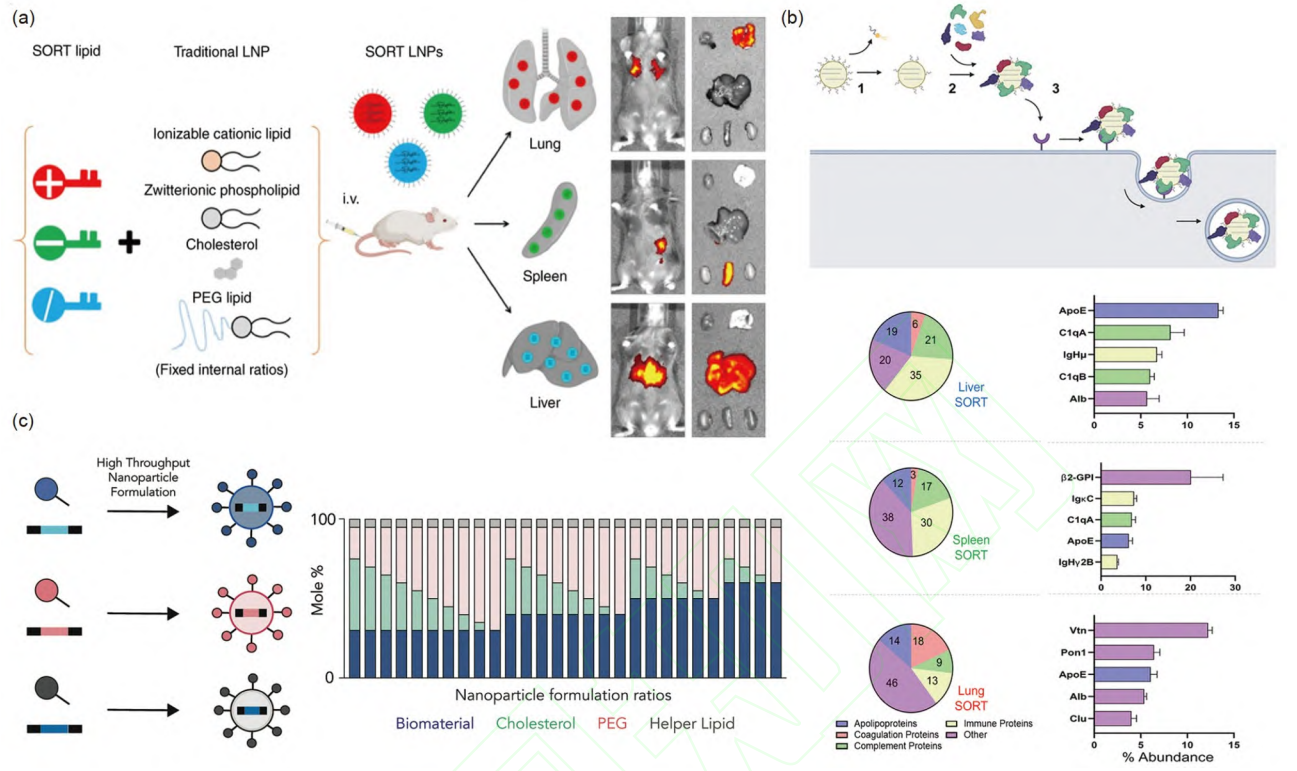

图 3 (网络版彩色)通过LNP处方优化实现mRNA的组织特异性递送. (a) 选择性器官靶向(SORT)技术可实现肝脏、肺脏和脾脏的特异性mRNA递送. Reprinted with permission[13]. Copyright © 2020, Springer Nature. (b) SORT LNP介导的器官靶向机制可能是由于蛋白冠中特异性成分决定的. Reprinted with permission[14]. Copyright © 2021, National Academy of Sciences. (c) 通过DNA条形码技术对LNP处方进行通量筛选, 能实现组织特异性mRNA-LNP递送. Reproduced under terms of the CC-BY license[15]. Copyright © 2018, Melissa P. Lokugamage et al, published by Elsevier

Figure 3 (Color online) Tissue-specific mRNA delivery can be achieved through optimizing LNP formulation. (a) Selective organ targeting (SORT) technology achieves liver-, lung- and spleen-specific mRNA delivery. Reprinted with permission[13]. Copyright © 2020, Springer Nature. (b) The mechanism of SORT LNP organ targeting may be determined by specific components of the protein corona. Reprinted with permission[14]. Copyright © 2021, National Academy of Sciences. (c) High-throughput LNPs screening by DNA barcoding technology reaches tissue-specific mRNA delivery. Reproduced under terms of the CC-BY license[15]. Copyright © 2018, Melissa P. Lokugamage et al, published by Elsevier

code)和深度测序技术开发了一种名为JORDAN(joint rapid DNA analysis of nanoparticles)的研究策略, 该策略可通过包载独特DNA序列对LNP处方进行高通量筛选(图3(c)). 每一种LNP处方对应一种独特的DNA条形码序列, 通过流式细胞分选技术、二代测序技术可同步分析数百种LNP处方, 并找出LNP与组织、细胞特异性递送之间的关系[19]. 在近期的研究报道中, 该课题组进一步利用DNA条形码高通量筛选技术得到了具有一定肿瘤特异性的LNP递送体系[20]. 此外, 该课题组在转基因td-Tomato小鼠中, 通过LNP共包裹DNA条形码以及Cre mRNA(编码Cre重组酶, 可以激活td-Tomato荧光蛋白的表达), 使该技术的应用不仅可以分析LNP在组织和细胞中的分布, 更可以同步检测mRNA的表达. 他们将这种共递送的策略命名为FIND(fast identification of nanoparticle delivery). 利用这一技术, 他们筛选了不同胆固醇类似物, 分析LNP处方对mRNA-LNP递送效率和组织特异性的影响[21]并扩展到其他应用方面[22]. 这种DNA条形码通量筛选技术大大缩短了LNP的筛选周期, 提高了筛选效率.

2 新型可离子化阳离子脂质筛选

LNP中可离子化阳离子脂质分子最为重要, 与mRNA的包裹效率、递送效率息息相关, 也是专利申请中最重要的环节之一, 因而对它的结构研究也最为丰富. 近年来, 一些具有肝外组织靶向性的可离子化阳离子脂质也在不断地被发现和鉴定出来(图4). Xu教授团队[23]在该方向做了诸多贡献. 2022年, 该团队利用迈克尔加成反应设计合成具有酰胺键结构的可离子化阳离子脂质分子(如306-N16B、113-N16B), 将它们与磷脂、胆固醇和PEG脂质组合制备成N-系列LNP(图4

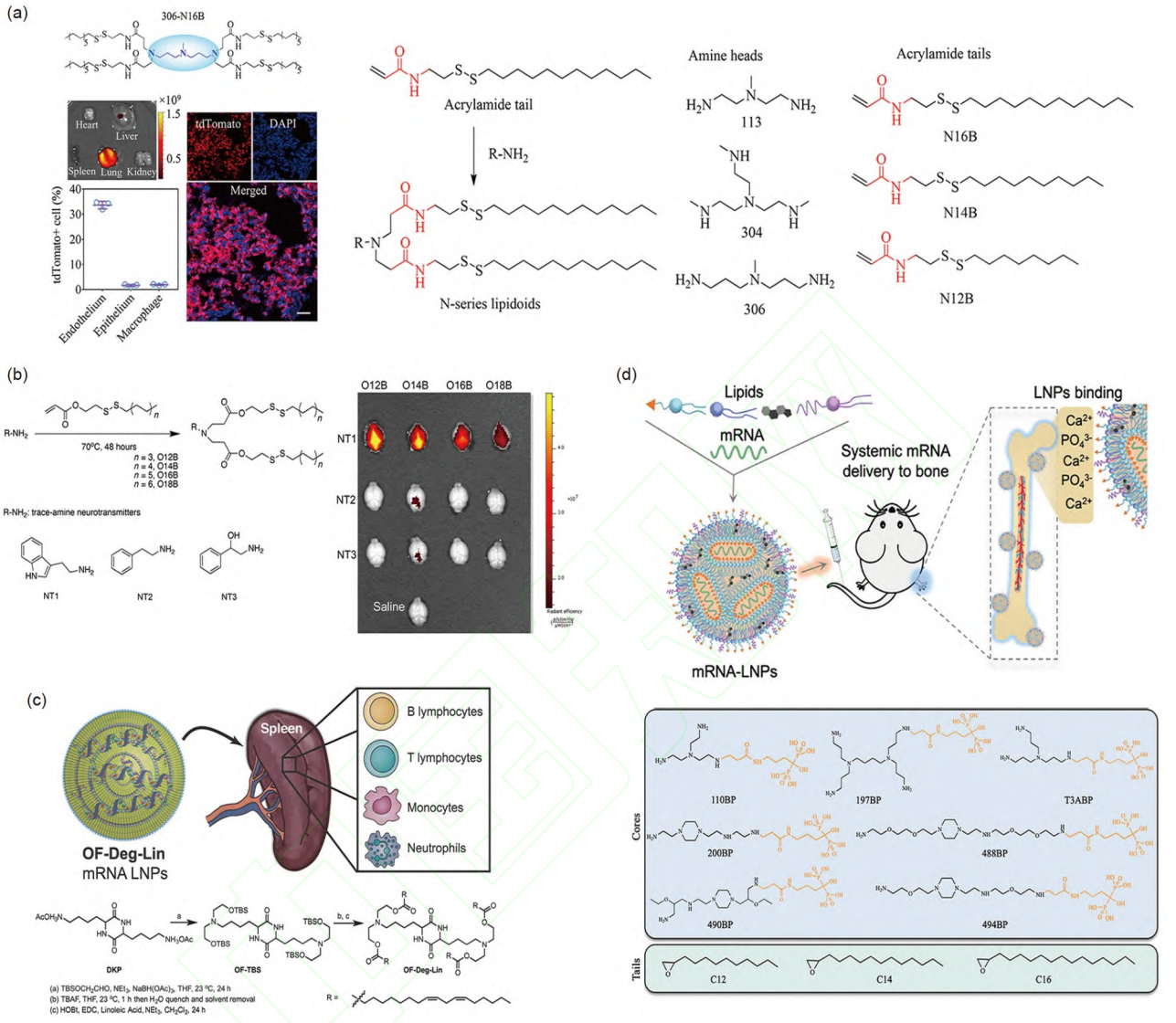

图 4 (网络版彩色)通过筛选、开发新的可离子化阳离子脂质实现mRNA组织特异性递送. (a) 肺靶向mRNA递送的N-系列可离子化阳离子脂质. Reproduced under terms of the CC-BY-NC-ND license[23]. Copyright © 2022, Min Qiu et al, published by National Academy of Sciences. (b) 修饰神经递质的可离子化阳离子脂质分子实现脑靶向核酸药物递送. Reproduced under terms of the CC-BY-NC license[26]. Copyright © 2020, Feihe Ma et al, published by The American Association for the Advancement of Science. (c) 脾脏靶向mRNA递送的可离子化阳离子脂质(OF-Deg-Lin). Reprinted with permission[25]. Copyright © 2017, John Wiley and Sons. (d) 通过在可离子化阳离子脂质氨基头部偶联双磷酸基团将mRNA递送至骨微环境. Reprinted with permission[27]. Copyright © 2019, American Chemical Society

Figure 4 (Color online) Tissue-specific mRNA delivery can be reached by screening ionizable cationic lipids. (a) Lung-targeted mRNA delivery was achieved by N-series ionizable cationic lipids formed LNP. Reproduced under terms of the CC-BY-NC-ND license[23]. Copyright © 2022, Min Qiu et al, published by National Academy of Sciences. (b) Neurotransmitter-modified ionizable cationic lipids enabled brain-targeted nucleic acid delivery. Reproduced under terms of the CC-BY-NC license[26]. Copyright © 2020, Feihe Ma et al, published by The American Association for the Advancement of Science. (c) The OF-Deg-Lin ionizable cationic lipid formed LNP mediated spleen-targeted mRNA delivery. Reprinted with permission[25]. Copyright © 2017, John Wiley and Sons. (d) mRNA-LNP can target the bone microenvironment based on diphosphate group-coupled ionizable cationic lipids. Reprinted with permission[27]. Copyright © 2019, American Chemical Society

(a)). 该LNP可以实现高效的mRNA肺部靶向递送[23], 其表面蛋白冠成分也与肝靶向LNP有着显著差别, 这一结论与SORT LNP类似, 同样暗示了血清蛋白冠在组织靶向递送中的关键作用. 此外, 该课题组还报道, 具有咪唑结构的可离子化阳离子脂质可帮助LNP实现脾脏及T细胞mRNA递送[24]. 与之类似, Anderson教授团队[25]曾报道OF-Deg-Lin脂质结构, 基于该脂质的LNP可将mRNA靶向递送至脾脏及脾脏中B淋巴细胞(图4

(c). 受到神经递质分子可以穿透血脑屏障的启发, Xu教授团队[26]设计合成了偶联神经递质的可离子化阳离子脂质分子, 实现了核酸、蛋白分子的脑部递送(图4(b). 同理, Mitchell教授团队[27]在可离子化阳离子脂质上修饰双磷酸基团, 通过双磷酸基团与骨微环境中钙离子的络合实现LNP针对骨微环境的mRNA递送(图4(d). 以上这些研究充分证明, 通过对新型可离子化阳离子脂质结构的设计和筛选, 可有效获得肝外组织mRNA-LNP靶向递送体系(图4).

3 靶向性抗体修饰

抗体和抗原的特异性识别为研发新型靶向LNP提供了可行性. 近些年, 运用抗体修饰LNP靶向递送mRNA已经有很多报道(图5). Epstein教授团队[28]在LNP表面修饰了CD5抗体(CD5主要表达在T细胞表面), 将编码嵌合抗原受体(chimeric antigen receptor, CAR)的mRNA递送至体内循环T细胞并在体内生成原位CAR-T细胞, 可有效治疗心肌纤维化, 恢复心脏正常功能. 除了CD5抗体, 其他研究团队还利用了CD3抗体[29]或CD4抗体[30]来修饰LNP, 将mRNA特异性地递送至体内的T淋巴细胞, 实现在体T细胞活化或者重编程改造. 这些重要研究引发国内外研究热潮, 通过mRNA-LNP技术实施原位CAR-T疗法的研究已在产业界取得广泛关注和快速推进. 除T细胞靶向外, Muzykantov团队[31]在LNP的表面修饰CD31抗体(CD31为内皮细胞表面标志物)实现了肺部靶向性mRNA递送, 并且他们发现这种递送方式是非ApoE依赖的. 最近, Anderson团队[32]利用CD117抗体修饰LNP, 在体内实现了高效的造血干细胞和祖细胞mRNA递送以及基因编辑, 为造血干细胞基因疗法提供了新的策略. 以上抗体修饰LNP的方法有效实现了肝外器官/细胞mRNA递送, 不过大多数LNP仍旧会在肝脏有较强mRNA表达(脱靶表达), 这也是该技术手段亟待解决的问题之一.

4 给药方式选择

改变给药方式也是实现组织特异性递送的重要手段之一. 如图6所示, 除静脉注射, 还可以使用肌肉注射、皮下注射、吸入制剂等不同方式完成mRNA-LNP的给药. 目前获批的新型mRNA疫苗采用肌肉注射方式, 该方式下抗原递呈细胞能够快速摄取mRNA-LNP, 从而激发特异免疫反应; 同样, 有报道称皮下给药方式则可能更好实现针对淋巴结系统的靶向递送, 有助于mRNA-LNP疫苗的研制[33]. 不同的给药方式对于机体抗LNP抗体的产生也有着较大的影响[34]. 此外, 针对肺靶向递送, 尤其是期望进入气道上皮细胞, 雾化吸入、气管内给药则可能是更好的给药途径. Dahlman教授团队[35]此前针对雾化吸入对LNP处方进行了优化, 并且在流感病毒模型中证明雾化吸入mRNA-LNP(表达中和抗体)对小鼠具有很好的保护性. 不久前, Anderson教授团队[36]筛选了用于气管内给药的可离子化阳离子脂

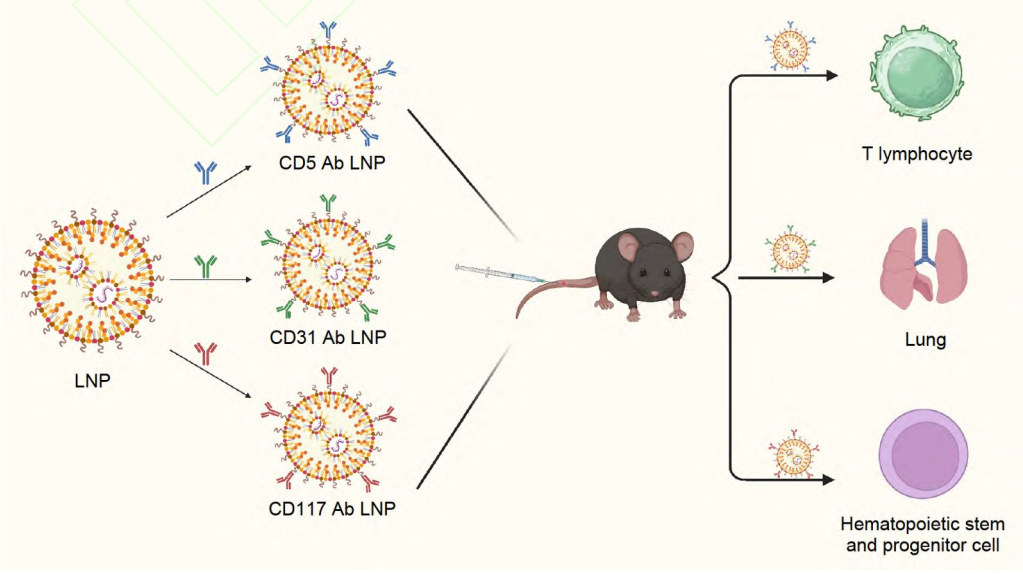

图 5 (网络版彩色)代表性抗体修饰LNP策略以实现器官/细胞的mRNA靶向递送. Created with BioRender.com
Figure 5 (Color online) Representative strategies of antibody-based LNP modification for organ and cell targeting. Created with BioRender.com

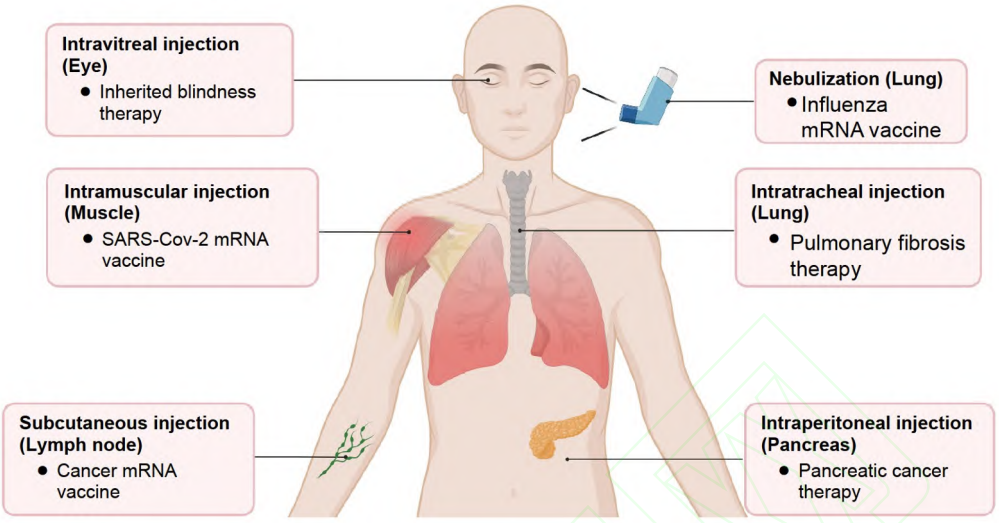

图 6 (网络版彩色)代表性mRNA-LNP的局部给药方式和应用场景. Created with BioRender.com
Figure 6 (Color online) Representative topical routes of administration and applications of mRNA-LNP. Created with BioRender.com

质, 能够实现高效率肺部细胞mRNA递送和基因编辑. 近来, Whitehead教授团队[37]研究发现, 腹腔注射mRNA-LNP可以将mRNA高效递送至胰腺组织, 通过将辅助磷脂替换为带正电的阳离子脂质DOTAP, 腹腔注射后mRNA-LNP胰腺递送的特异性有了显著的提高. Gaurav Sahay教授团队利用玻璃体内注射修饰有感光细胞靶向多肽的LNP实现了针对眼部的mRNA递送[38]. 上述这些给药方式的选择, 为器官靶向递送mRNA-LNP提供了另一途径, 尤其是针对表层组织器官的转染.

5 总结与展望

新型冠状病毒mRNA-LNP的成功为该技术的快速发展注入了强劲的动力, 与此同时也应注意到当前该技术的不足之处, 国内外同质化竞争非常激烈, 归根到底在于组织靶向mRNA-LNP技术相对不够成熟. 从底层技术着手, 开展组织、细胞靶向mRNA-LNP递送技术, 有望最大化扩宽mRNA技术的应用场景, 并为该领域的发展提供全新动力源泉. 基于此背景, 本文重点总结了4种常见的研发策略, 期望为该研究领域提供些许启发. 总体来说, 各种策略优缺点并存, 我们应该将其有机统一起来进行考虑. 筛选新型可离子化阳离子脂质是化学创新的源头, 然而尚没有非常明确的理论可预测其递送效率以及靶向性, 具有一定的盲目性; LNP处方的通量筛选同样具有工作量大、器官特异性差等缺点, 尽管SORT LNP指出了明确的研发方向, 但阳离子脂质、阴离子脂质的加入是否诱发一定的安全隐患仍需更多数据来证明; 抗体修饰LNP在细胞靶向递送方面有较大优势, 但其组织特异性差、制备工艺复杂等不足之处也亟待解决. 除LNP载体以外, 也应注重mRNA分子本身的设计. 已有研究表明, 通过通量筛选环形RNA分子有望实现组织、细胞选择性表达, 这无疑为研发mRNA-LNP的靶向递送技术增加了全新筹码[39]. 此外, 在大数据、人工智能快速发展的当下, 将其应用到靶向mRNA-LNP开发方面有可能极大程度提升研发效率和成功率.

综上所述, 研发组织靶向mRNA-LNP技术具有重大的科学意义和经济价值. 运用多种LNP研发策略、配合mRNA序列研发、结合先进的人工智能技术, 相信会有更多新型靶向技术涌现而出. 作者也特别期待更多的重要组织器官/细胞被成功递送, 并推动实现mRNA未满足的临床需求.

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Summary for “组织特异性mRNA-LNP递送技术的研发策略”

Recent advances in strategies for developing tissue-selective mRNA-LNP technology

Yan Zong[1], Tuo Wei[2,3] & Qiang Cheng[1*]

[1] *Department of Biomedical Engineering, College of Future Technology, Peking University, Beijing 100871, China;*
[2] *State Key Laboratory of Stem Cell and Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China;*
[3] *Beijing Institute for Stem Cell and Regenerative Medicine, Beijing 100101, China*
* Corresponding author, E-mail: qiangcheng@pku.edu.cn

The approval of mRNA vaccines for coronavirus disease 2019 (COVID-19) has garnered significant attention for mRNA technology worldwide. This milestone has had a profound impact on the industrial transformation of mRNA in China, as evidenced by the increased number of startups and financing events in the past 2-3 years. Regarding mRNA itself, in addition to implementing chemical modifications, such as pseudouridine and N^1–methyl pseudouridine, to avoid innate immune responses, efforts have been made to enhance stability and scale up production to meet clinical needs. Also, the successful application of mRNA technology heavily relies on safe and efficient delivery vehicles, particularly lipid nanoparticles (LNP). The initially approved mRNA COVID-19 vaccines, Comirnaty and Spikevax, have benefited from this delivery system. Furthermore, more than 90% of mRNA drug programs in clinical research, including cancer vaccines, infectious disease vaccines, and therapeutics for inherited genetic disorders, also depend on LNP vectors. Notably, mRNA-4157, an mRNA-LNP cancer vaccine for melanoma developed by Moderna and Merck, demonstrated positive results in clinical trials and was granted Breakthrough Therapy Designation (BTD) by the Food and Drug Administration (FDA). LNP typically consists of four components: ionizable cationic lipid, phospholipid, cholesterol, and PEGylated lipid. Each component plays a crucial role in the stability, function, efficacy, and safety of LNP. The ionizable cationic lipid, in particular, is considered a key component and researchers invest substantial efforts in designing and screening ionizable lipids. These lipids can interact with mRNA molecules (which are negatively charged) through electrostatic interactions, encapsulating them in a low pH buffer. The charge is then neutralized upon buffer exchange to a physiological pH (pH 7.4). Once internalized by cells, the ionizable lipids can regain their positive charge within endosomes/lysosomes to facilitate mRNA release into the cytoplasm for translation. Phospholipid and cholesterol serve as helper lipids, contributing to the formation and stability of lipid nanoparticles, while PEGylated lipid reduces LNP aggregation to prolong in vivo circulation time and decreases phagocytosis by circulating immune cells. Currently, biomedical applications of mRNA-LNP technology are more advanced in liver and muscle targeted delivery. Examples include genome editing for genetic diseases in the liver via intravenous injection and the development of infectious vaccines through intramuscular injection. However, targeted delivery to extrahepatic tissues remains a challenge that is in the early stages of development. In order to maximize the application potential of mRNA drugs, researchers have made significant efforts in this direction, resulting in several reported approaches. This review primarily focuses on discussing the current strategies for developing tissue-targeted mRNA-LNP technologies. These strategies include optimizing LNP formulations, screening novel lipid molecules, modifying specific antibodies on LNP, and exploring suitable administration routes. Additionally, potential future directions in this field are briefly introduced. One of them is delivering therapeutic mRNA to specific cell types. To achieve this goal, further efforts are needed, including understanding the mechanism of tissue/cell targeting for better design of chemical structures, applying antibody modifications on the LNP surface, and exploring novel RNA molecules, such as circular RNA, which is believed to have cell-specific expression profiles. Overall, the information conveyed in this article aims to provide readers with a deeper understanding of mRNA-LNP technology and its potential in the design of targeted mRNA-LNP therapeutics.

lipid nanoparticle, mRNA therapy, tissue-selective delivery, nanomedicine

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