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mRNA疫苗的突破与药物研发革新

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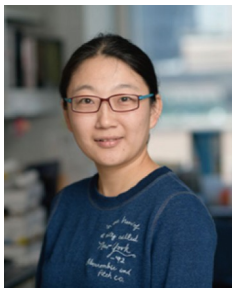
2023年度诺贝尔生理学或医学奖授予美国宾夕法尼亚大学科学家卡塔林·卡里科(Katalin Karikó)和德鲁·魏斯曼(Drew Weissman), 以表彰他们所发现的核苷碱基修饰方式, 使得开发有效的针对新型冠状病毒的mRNA疫苗成为可能. 他们的开创性工作, 促成了针对新型冠状病毒的高效mRNA疫苗从基础研发到临床应用的快速转化, 挽救了上千万人的生命, 并为开发靶向其他类型传染病以及重大疾病的预防性或治疗性RNA药物树立了典范.

1 mRNA疫苗简介

区别于传统意义上的灭活疫苗、减毒疫苗以及重组疫苗、腺病毒疫苗等, mRNA疫苗将编码抗原的mRNA分子以脂质体包裹的方式递送到细胞中, 该mRNA分子可作为模板来指导细胞内的核糖体合成病原体的特定蛋白质(如新型冠状病毒的刺突蛋白). 这些蛋白质抗原可刺激机体的适应性免疫反应产生中和抗体, 识别并中和侵入宿主细胞的病原体. 2013年, 全球第一款mRNA疫苗——抗狂犬病毒糖蛋白疫苗CV7201在健康人群中开展了临床试验[1]. 接下来的几年时间里, 研究人员陆续开展了针对其他传染病病原体(如流感病毒、寨卡病毒、巨细胞病毒和基孔肯雅热病毒等传染病)的mRNA疫苗临床试验.

2019年12月, 新型冠状病毒感染暴发, 并在世界范围内快速传播. 2020年1月9日, 随着致病病原体SARS-CoV-2基因组序列的确定, BioNTech和Moderna公司迅速完成了针对新型冠状病毒的mRNA疫苗设计、生产和临床试验[2~4], 并几乎同时获得了该mRNA疫苗的紧急使用许可. 2020年12月2日, 英国药品和健康产品管理局(Medicines and Healthcare Products Regulatory Agency, MHRA)成为历史上第一个批准mRNA疫苗的全球药品监管机构, 对辉瑞和BioNTech联合研发的新型冠状病毒mRNA疫苗BNT162b2给予了紧急使用授权. 2020年12月11日, 美国食品药品监督管理局(Food and Drug Administration, FDA)也对BNT162b2给予了紧急使用授权, 并在1周后, 对Moderna公司研发的新型冠状病毒mRNA疫苗mRNA-1273给予了使用许可.



魏绿 中国科学院生物物理研究所副研究员. 研究方向为RNA结合蛋白和长非编码RNA调控肿瘤发生、发展、转移的分子机制.

薛愿超 中国科学院生物物理研究所研究员, 博士生导师, 课题组组长. 研究方向为非编码RNA的功能机制及相关技术研发, 主要关注RNA-蛋白质复合物在转录和表观遗传层面如何调控基因表达以及如何在细胞分化和转分化等命运决定过程中发挥作用.

2 真核细胞mRNA的结构与修饰

1953年, 詹姆斯·沃森(James Watson)与弗朗西斯·克里克(Francis Crick)提出了DNA双螺旋结构模型[5]. 1957年, 克里克提出了著名的中心法则(central dogma), 即遗传信息从DNA传递至RNA, 再由RNA传给蛋白质的单向不可逆过程[6]. 中心法则的提出, 标志着现代分子生物学的开端, 其作为分子生物学的核心理论在此后的半个多世纪里指导了一系列的重大科学发现.

受限于细胞中大量存在的核糖体RNA, 直到1961年, mRNA分子才被发现. 早期mRNA被定义为不稳定的核糖核酸中间体分子, 可将遗传信息从基因传递给核糖体合成蛋白[7,8]. 随着mRNA、tRNA的发现以及密码子与反密码子规律的揭示[9,10], mRNA的结构与功能研究逐渐被人们所关注. 真核细胞中mRNA分子由RNA聚合酶II转录生成, 编码功能

性蛋白质分子. 大多数真核细胞的mRNA分子发生共转录/转录后修饰, 通常在其5′末端加上帽子结构(m7pppG cap), 3′末端加上多聚腺苷化尾(poly(A) tail)[11,12]. 这两种修饰方式极大提高了mRNA的稳定性和翻译效率. 除此之外, mRNA中的5′和3′非翻译区(untranslated region, UTR)协同调控mRNA的半衰期, 直接影响其翻译效率.

1961~1971年, 科学家发现, 从细胞中分离纯化的mRNA分子可在体外无细胞环境或者在体内翻译产生蛋白质[13]. 1984年, mRNA加帽酶和RNA合成酶的发现及应用[14,15], 使mRNA的体外合成成为可能. 这些进步促使科学家思考如何利用体外合成的mRNA分子在体内产生蛋白质, 从而达到疾病的预防和治疗目的. 但是早期的尝试都极不成功: 无论是单独向特定组织注射mRNA分子, 或者使用脂质纳米颗粒包裹mRNA递送到特定的细胞类型中, 虽然mRNA分子编码的抗原能够得到表达, 但是mRNA整体表现出极度的不稳定, 并会引起机体严重的炎症反应[16,17]. 这极大地限制了mRNA疫苗和药物的研发进程.

3 卡里科和魏斯曼的开创性工作: mRNA上核苷修饰会避免激活天然免疫反应

1985年, 卡塔林·卡里科博士毕业后来到了美国天普大学(Temple University)进行博士后研究, 并随后转去宾夕法尼亚大学(University of Pennsylvania)与德鲁·魏斯曼合作, 继续其mRNA修饰与成药研究工作. 1998~2005年, 她发表了一系列工作, 旨在增强mRNA在体内的稳定性, 并解决其较高的免疫原性问题. 她们发现: (1) 细胞内的Toll样受体3/7/8(Toll-like receptor, TLR)能够识别体外制备的mRNA分子, 是导致炎症反应的主要感受器(图1); (2) 天然RNA分子(如从哺乳动物细胞中提取的mRNA或tRNA)极少地诱发机体产生炎症反应, 但体外转录的RNA分子则具有较强的免疫原性, 猜测这可能与天然RNA分子中广泛存在的核苷修饰有关; (3) 向体外转录的RNA分子中引入特定核苷修饰(如假尿嘧啶修饰)后, 能够极大降低TLR介导的免疫激活响应, 并抑制树突状细胞(dendritic cells, DCs)中炎症性细胞因子的产生[18]; (4) 含有特定修饰核苷的mRNA更加稳定, 并具有更强的翻译效率[19,20]. 修饰核苷的发现及其应用为mRNA疫苗研究提供了坚实的理论基础, 并为mRNA成药进程扫清了障碍.

4 mRNA药物: 高效性、稳定性、靶向性

卡里科和魏斯曼的工作在很大程度上解决了一直困扰mRNA成药的核心问题, 即外源导入的mRNA分子的高免疫原性. 但是, mRNA疫苗或药物还需要解决其他几个关键问题, 包括: (1) 抗原的稳定与高效表达; (2) 能否实现靶向特定类型的细胞或者组织. 1978年, Dimitriadis[21]首次报道通过脂质体包裹编码兔球蛋白mRNA, 在小鼠淋巴细胞中成功地实现了蛋白翻译. 随后的几十年中, 脂质体(liposome)、脂质纳

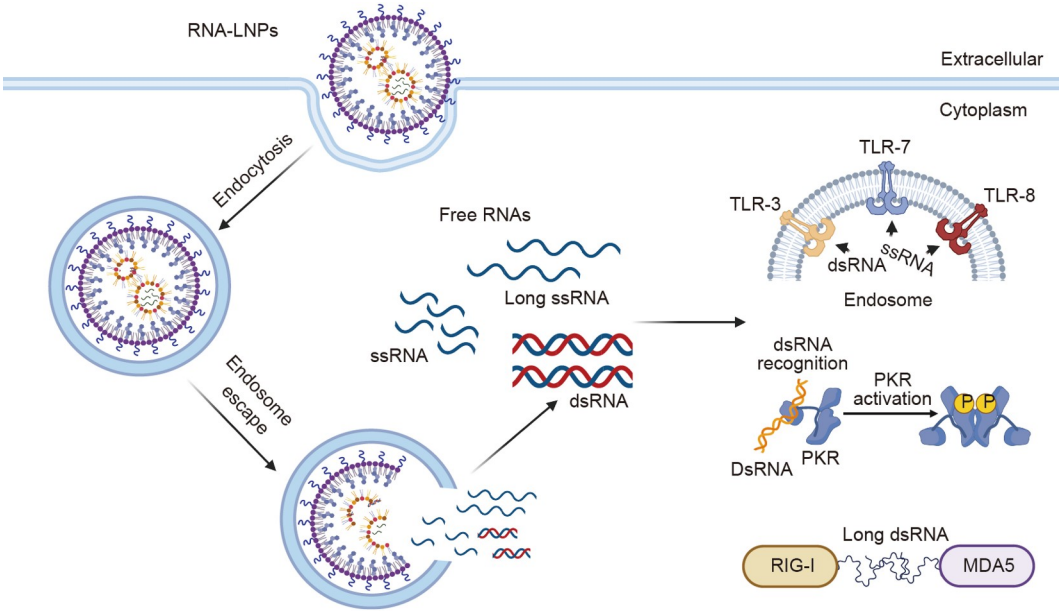


图 1 (网络版彩色)细胞质中的外源RNA感受器. 由脂质纳米颗粒包裹的不同类型的RNA分子, 通过胞吞的方式进入细胞质, 随后裸露的RNA分子发生内体逃逸和释放. TLR-3/7/8是膜定位的主要RNA分子感受器. PKR特异性地识别并结合双链RNA分子. RIG-I/MDA5结合长双链RNA分子. TLR: Toll样受体; PKR: 干扰素诱导的双链RNA依赖的蛋白激酶; RIG-I: 视黄酸(维甲酸)诱导基因蛋白I; MDA5: 抗黑色素瘤分化相关基因5

Figure 1 (Color online) Exogenous RNA sensors in cytoplasm. Different types of exogenous RNA molecules are packaged by lipid nanoparticles and delivered into the cytoplasm by endocytosis, and then naked RNAs escape from endosomes. TLR-3/7/8 are primary RNA sensors anchored on cell membranes. PKR specifically recognizes double-stranded RNAs. RIG-I/MDA5 binds long double-stranded RNAs. PKR: Interferon-induced dsRNA-dependent protein kinase; TLR: Toll-like receptor; RIG-I: Retinoic acid inducible gene-I; MDA5: Melanoma differentiation associated gene 5

米颗粒(lipid nanoparticle, LNP)作为mRNA分子的高效载体, 被广泛用于细胞水平和活体水平的mRNA分子递送. 脂质体、脂质纳米颗粒包装的mRNA稳定性更高[22,23]、半衰期长[24,25], 可通过静脉注射或肌肉注射稳定地进入血液、肌肉组织, 从而发挥其生物学活性[26,27]. 与此同时, 脂质纳米颗粒表面可以通过特定的化学修饰手段, 添加功能性基团和靶向特定受体的配体分子, 如叶酸分子与叶酸受体[28]. 这种具有靶向性的脂质纳米颗粒在肿瘤的靶向治疗中具有重要意义.

5 mRNA药物: 从基础到临床

在解决了外源mRNA导入机体的不稳定性和高免疫原性后, mRNA疫苗的开发和大规模临床使用成为可能. 1997年, 全球首个mRNA药物公司Merix Bioscience在美国成立(现为Argos Therapeutics). 随后, 2008和2010年, BioNTech公司和Moderna公司先后在德国和美国成立, 专攻mRNA疫苗的开发, 并积极促成全球范围内临床试验的开展. 2010~2020年的10年时间里, 大大小小的RNA制药公司成立, 致力于靶向传染性疾病、肿瘤等的mRNA疫苗和药物开发.

2019年全球新型冠状病毒肆虐, 促成了全球首个针对新型冠状病毒刺突蛋白的mRNA疫苗的临床使用. 截至2023年初, 全球已经开展临床试验的mRNA药物多达60个, 治疗的疾病类型涵盖: (1) 新型冠状病毒及其变异株; (2) 肿瘤免疫疗法以及个体化疗法, 靶向特定肿瘤表面抗原以及肿瘤个体化新抗原; (3) 传染性疾病疫苗, 如寨卡病毒、EB病毒、流感病毒、带状疱疹病毒、乙型肝炎病毒等; (4) 作为蛋白缺陷性疾病的替代性疗法, 如mRNA编码鸟氨酸氨甲酰基转移酶, 来治疗鸟氨酸氨甲酰基转移酶缺乏症; (5) 罕见病、肿瘤特异性基因突变的遗传改造, 基因编辑相关[29].

6 RNA疗法: 机遇与挑战

mRNA疫苗从理论到临床应用的快速突破提示我们, 基于mRNA疗法的新型疾病治疗策略和药物研发思路会极大地改变我们现有的疾病诊断和治疗方式(图2). 的确, mRNA药物相较于传统小分子药物、抗体药物, 具有明显的优势: (1) 相对更加容易的靶点选择和药物设计, 极大地推进了药物研发周期[30]; (2) mRNA药物主要在细胞质中发挥功能, 极少整合进基因组[31]; (3) mRNA药物的半衰期相对较短, 代谢产物纯天然, 没有持续和累积毒性[32].

值得一提的是, 编码蛋白质的mRNA序列仅占人类基因组的2%, 其余98%都为非编码核酸序列, 它们经过广泛转录产生了海量的非编码RNA分子[33]. mRNA的非编码区域(如5′UTR和3′UTR), 广泛地参与调控mRNA的稳定性、转录效率、转录后修饰和蛋白翻译效率. 在许多重要生命过程中(如发育、衰老、肿瘤等), 非编码RNA也可调控mRNA的稳定性、翻译和降解. 已知90%以上的疾病相关变异均位于基因组非编码区域[34]. 对这些非编码区域的深入研究将为开发高效、稳定的mRNA药物奠定基础. 此外, 探索非编码RNA的结构、功能以及其在重要生命过程中扮演的角色, 对深刻

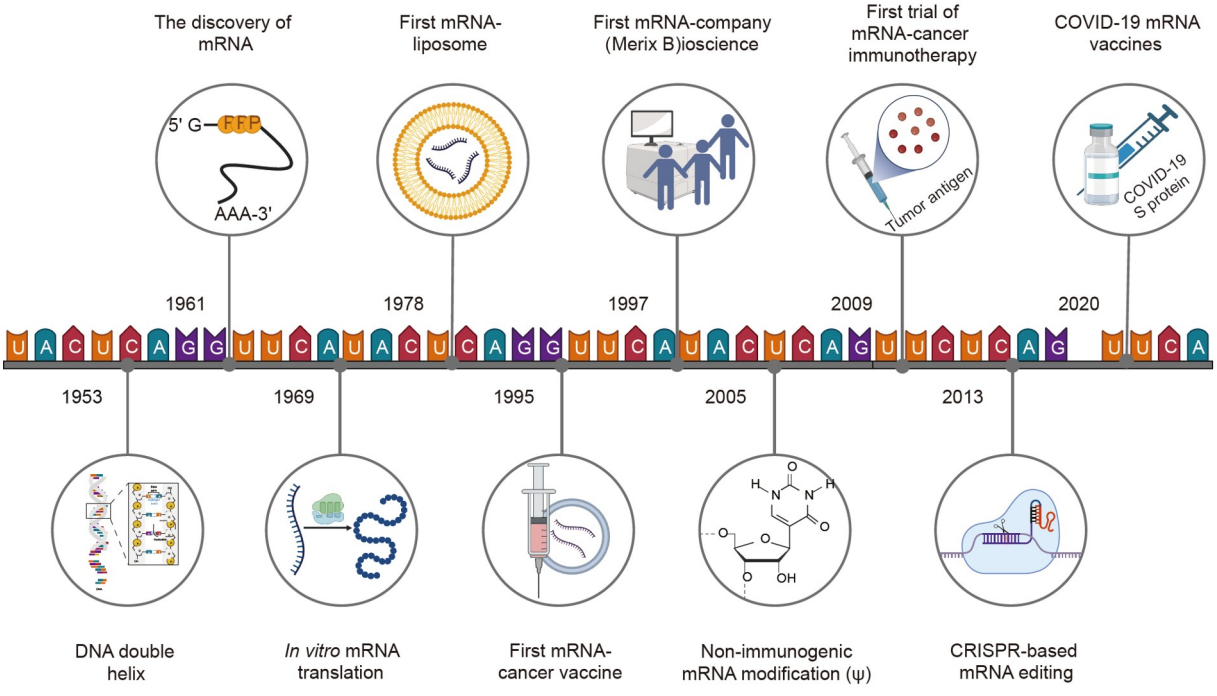


图 2 (网络版彩色)mRNA疫苗研发历程大事年表
Figure 2 (Color online) Landmark events during the research and development of mRNA vaccines

理解特定生命过程的分子机制、疾病发生发展的规律至关重要.

近年来, 非编码RNA的功能机制研究如火如荼. 在细胞内, 非编码RNA通过折叠成特定的高级结构, 招募特定的蛋白因子(如转录因子、染色质重塑因子、组蛋白修饰因子等), 或者靶向其他类型的RNA分子(如特定的mRNA分子、microRNA分子等), 实现对于特定基因表达的调控[35]. 深入探索与特定非编码RNA相互作用的蛋白、核酸分子, 将有助于我们理解非编码RNA通过调控mRNA分子而调节生命健康的规律. 靶向这些特定的功能性非编码RNA, 也可促进新型RNA药物如siRNA、反义寡核苷酸药物(antisense oligonucleotides, ASO)等的研发. 此外, 针对重大疾病中高表达的mRNA分子, 也可设计siRNA和反义寡核苷酸药物进行干预, 从而达到治疗的目的.

RNA在细胞内都不是裸露的, 而是在RNA结合蛋白的帮助下折叠成复杂的高级结构. 系统解析mRNA和各种非编码RNA的原位构象, 捕获细胞内的RNA-RNA、RNA-蛋白相互作用位点, 研究结构与mRNA翻译效率和稳定性的关系, 对以RNA为靶点的药物研发至关重要. 2016年, Howard Chang实验室[36]开发了PARIS(psoralen analysis of RNA interactions and structures)技术, 可在活细胞中鉴定RNA二级结构以及远距离RNA-RNA相互作用. 2020年, 中国科学院生物物理研究所薛愿超实验室开发了RIC-seq(RNA *in situ* conformation sequencing)技术, 能够全景式捕获细胞中mRNA和非编码RNA的原位构象[37,38]. 在此基础上开发的vRIC-seq技术, 可解析SARS-COV-2基因组RNA在病毒颗粒内的二级结构和三级构象. 通过对病毒基因组结构的深入解析, 选取单链区域进行靶向ASO以及siRNA的设计, 能够在细胞内实现病毒的高效清除[39]. RNA结构解析技术的应用, 将为我们揭示RNA结构调控基因表达的新规律[40], 为靶向RNA分子的智能药物设计奠定基础.

7 结语

mRNA疫苗在新型冠状病毒疫情预防中的卓越表现, 给予了药物研发企业极大的信心和动力, 持续开发以RNA为中心的新型疗法, 拓展mRNA药物应用场景, 可为不同疾病的预防和治疗提供新策略. 2023年7月26日, Moderna公司和默沙东联合宣布, 首个mRNA个性化肿瘤疫苗mRNA-4157和抗PD-1单抗(Keytruda)组合疗法推进到了III期临床试验. 这是全球首个进入III期临床试验的mRNA癌症疫苗. 此前披露的中期临床数据表明, 对于高风险黑色素瘤患者来说, 相较于单独使用抗PD-1单抗, 该联合疗法能够将患者复发或死亡风险降低44%[41].

mRNA疗法前景广阔, 但是机遇与挑战并行. 在下一阶段, 我们希望看到的是: 基于新型冠状病毒mRNA疫苗的范例, 与全球烈性传染病相关的RNA病毒疫苗的持续开发; HIV病毒引发的获得性免疫缺陷综合征(acquired immune deficiency syndrome, AIDS)的根本性治疗; 以及靶向肿瘤表面抗原的个性化治疗策略的不断开发. 在技术水平上, mRNA药物的体内递送仍旧极大地依赖脂质纳米颗粒方式, 是否还存在其他类型的高生物相容的靶向递送方式? 这是限制RNA药物研发的关键问题. 在核心基础科学问题上, 如何进一步精准地探究RNA分子的二级、高级结构及其与其他核酸、蛋白分子的相互作用模式, 将会在分子层面上揭示RNA参与重要生命过程的全新角色和分子机制, 并为实现上述临床目标提供强有力的理论依据.

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Summary for “mRNA疫苗的突破与药物研发革新”

Breakthroughs in mRNA vaccines and innovations in drug development

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On October 2, 2023, Drs. Katalin Karikó and Drew Weissman from the University of Pennsylvania were awarded the Nobel Prize in Physiology or Medicine “for their discoveries concerning nucleoside base modifications that enabled the development of effective mRNA vaccines against COVID-19”.

mRNA was first discovered in 1961 and characterized as a class of RNA molecules that can transfer genetic information from DNA to ribosomes for protein synthesis. In eukaryotes, mRNAs are transcribed by RNA polymerase II and go through multiple rounds of co- or post-transcriptional modifications, including m7pppG cap at 5′-ends, poly(A) tails at 3′-ends, various reversible chemical modifications in coding regions and 3′ untranslated regions. These modifications can significantly increase mRNA stability, half-life, nuclear export, and translational efficiencies. During 1961–1984, along with the discoveries of tRNAs, codons, anticodons, cell-free mRNA translation systems, and *in vitro* RNA synthesis, researchers began to show broad interest in delivering exogenous mRNAs into the cells or body for disease prevention and treatment.

Since the 1990s, many attempts have been made to achieve such a purpose. However, these attempts are unsuccessful due to the severe inflammatory responses induced by exogenous RNA, which can activate cellular RNA sensors, including Toll-like receptor 3/7/8, PKR, and RIG-I/MDA5. Such limitation severely restricted the clinical applications of RNA-based drug development. Drs. Katalin Karikó and Drew Weissman discovered that introducing RNA modifications, such as pseudouridine, to exogenous RNAs can significantly reduce cellular inflammatory responses and increase RNA stability and translation efficiency. Their groundbreaking works promote the rapid transformation of highly effective mRNA vaccines targeting COVID-19 from basic research to clinical applications.

In December 2020, the FDA approved two mRNA vaccines against COVID-19, BNT162b2 from Pfizer-BioNTech and mRNA-1273 from Moderna, to prevent COVID-19. These vaccines have been proven safe and effective in preventing SARS-CoV-2 infection, saving millions of lives. The success of COVID-19 mRNA vaccines also set an extraordinary standard for other RNA-based drug development. Up to early 2023, more than 60 RNA-based vaccines or drugs have initiated clinical trials. These vaccines or drugs aim to prevent and treat different types of diseases, including COVID-19, cancer immunotherapy, personalized therapy, and infectious diseases such as influenza virus, Zika virus, and hepatitis B virus. Alternatively, several therapies for protein deficiency diseases are under development.

In this paper, we reviewed the journey of mRNA vaccines from the initial discovery of mRNA molecules, the *in vitro* synthesis of mRNA, the various modifications on mRNA, and the specific modification, pseudouridine, which can efficiently prevent the immunogenicity of exogenously introduced RNAs when delivered into the cells. These discoveries together promoted the success of mRNA vaccines against COVID-19. We also discussed the possibilities of setting noncoding RNAs as therapeutic targets and promising drug candidates based on our understanding of their crucial roles in regulating life and health. In contrast to protein- and small-molecule-based drugs, mRNA- and RNA-based therapies are more programmable and druggable. Scientists can easily and quickly design drugs based on RNA primary sequences and higher-order structures, significantly reducing the time and cost of traditional drug research and development. RNA-based therapies will definitely revolutionize drug development and show more substantial power in preventing infectious diseases and cancers.

mRNA vaccines, RNA modifications, COVID-19, RNA drugs

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