定量与工程生物学在mRNA基因疗法中的应用

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摘要 信使RNA(messenger RNA, mRNA)作为基因信息的瞬时载体, 是一种多功能、灵活及安全的基因治疗手段, 但由于mRNA分子的不稳定性和免疫原性, 这种基因疗法最初并没有得到普及. 在过去几十年里, 定量与工程生物学学科交叉融合使得以mRNA为基础的基因治疗方法逐渐从概念验证阶段走向临床治疗阶段. 本文从mRNA的功能结构、体外合成mRNA的设计和技术创新等方面阐述了定量与工程生物学在mRNA基因治疗中的应用. 借助一些实验室和临床应用案例展示了定量与工程生物学方法指导mRNA药物设计, 解决当代医疗问题. 有效的工程设计方法将有助于针对各种适应症和遗传背景建立精准的mRNA基因治疗平台. 目前, 尽管基于mRNA的基因治疗方法在大多数适应症中仍处于预临床阶段, 但各种工程与定量生物设计方法的积累可能会为新一代mRNA基因疗法拓宽道路.

关键词 定量生物学, 合成生物学, 化学修饰mRNA, 基因治疗

信使RNA(messenger RNA, mRNA)是遗传信息的中间体和生物表达蛋白质的模板. 因此, 可以将外源mRNA引入靶细胞表达感兴趣的蛋白. 在20世纪70年代初, Gurdon等人^[1]首次利用微注射方法在爪蟾卵母细胞中验证了上述概念. 以mRNA为基础的基因治疗方法的优点在于: 首先, mRNA疗法具有更广泛的应用, mRNA不需要进入细胞核就可以在细胞质内表达蛋白质实现其功能, 因此, mRNA可以在缓慢分裂或不分裂的细胞中发挥作用; 其次, mRNA疗法具有更高的安全性, 由于其表达的瞬时性和基因组整合极低的可能性, 降低了与基因治疗相关的两个主要风险(宿主基因组的整合和关键区域的突变)^[2]; 最后, 体外合成

mRNA的半衰期和表达丰度可以通过结构和组分设计来确定, 使得mRNA类药物的药代动力学(pharmacokinetics, PK)被更好地控制^[3]. 这些优势为mRNA类合成药物的药品生产质量管理规范(good manufacturing practice, GMP)创造了有利条件. 此外, 利用细胞自然机制合成的治疗性蛋白质具有天然的翻译后修饰和适宜的蛋白质折叠效果, 这比体外合成的重组蛋白质更有优势.

近年来, mRNA作为一种治疗手段, 在基因治疗领域日益受到重视. 体外转录(*in vitro*-transcribed, IVT) mRNA已应用于临床前及临床试验, 包括癌症治疗^[4]、疫苗研发^[5,6]和蛋白替代药物研发^[7,8]等. 与癌症和疫苗

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治疗相比,大多数使用mRNA的蛋白替代疗法仍处于临床前开发阶段(<https://clinicaltrials.gov/>). 进展较快的mRNA疗法的临床代表案例整理在表S1.

合成mRNA驱动目标蛋白在体内表达俨然成为一种替代传统基因疗法的新兴基因疗法. 尽管mRNA已成为一类潜在的治疗药物,但一些影响mRNA治疗效果的因素必须得到改善,包括可翻译性、稳定性、免疫原性和细胞内递送等,以进一步实现临床的广泛应用^[9]. 近年来,合成mRNA技术及相关研究发展迅猛,这得益于过去的几十年里,代表定量化的定量生物学与代表工程化的合成生物学两者高度互补结合. mRNA基因治疗要达到特定的目的,需要优化设计以可预测和定量的方式发挥作用,而定量与工程生物学提高了我们有效设计和优化生物系统以执行设计任务的能力. 本文阐述了mRNA基因疗法的应用优势和前景,借助一些案例展示了定量与工程生物学方法应用于mRNA基因治疗以及指导mRNA药物设计,解决当代医疗问题.

1 mRNA的主要结构及其生物学功能

具有翻译功能的真核生物mRNA的结构通常包含5'帽子结构(5' cap)、5'-非翻译区(5'-untranslated regions, 5'-UTR)、开放阅读框(open reading frame, ORF)、3'-非翻译区(3'-untranslated regions, 3'-UTR)和多聚腺苷酸尾(poly(A) tail). 如果翻译的目标蛋白是分泌型蛋白,在设计mRNA结构时通常考虑引入前导肽(leader peptide)或信号肽(signal peptide, SP)的核酸序列(图1(b)).

1.1 5'帽子结构

5'帽子结构能够与真核起始因子4E(eukaryotic initiation factor 4E, eIF4E)结合,对mRNA的高效翻译至关重要^[10]. 没有帽子结构的mRNA不能被翻译,且具有免疫原性. 5'帽子结构能保护mRNA免受核酸外切酶的切割,是招募mRNA前体剪接、聚腺苷酸化和核输出的蛋白因子唯一识别锚点^[11]. 合成IVT mRNA通常使用帽类似物. 常用的帽类似物主要有5类: m7GpppG帽类似物(cap0)、m7GpppGm帽类似物(cap1)、m7GpppGm2帽类似物(cap2)、经修饰的抗逆转帽类似物(antireverse cap analogs, ARCA)和 β -S-ARCA(图1(a)).

在体外转录过程中, m7GpppG结合的方向不定会导致帽子结构的识别效率偏低,进而造成mRNA的翻译效率降低^[12]. 通过工程学方法,在m7鸟苷(m7G)的

C2'-或C3'-位上进行修饰,开发了ARCA. ARCA不能以相反的方向整合,因此在一定程度上提高了IVT mRNA的翻译效率^[13]. 为了进一步提高mRNA对去帽酶的抵抗力,同时增加其对eIF4E的亲合力,将ARCA进行化学修饰,如在 β -磷酸中使用硫代磷酸酯修饰的帽类似物(β -S-ARCA). 研究显示,与ARCA或m7GpppG相比, β -S-ARCA在树突细胞(dendritic cells, DCs)中既能提高mRNA的翻译效率又能延长mRNA的半衰期^[14]. β -S-ARCA能抵抗清除剂脱帽焦磷酸酶(scavenger decapping pyrophosphatase, DcpS)去帽酶的作用,从而延长mRNA的半衰期^[15]. 不过,这种含硫代磷酸键的 β -S-ARCA帽子类似物合成的mRNA,其产生的抗原特异性反应要大于标准ARCA合成的mRNA^[14]. 此外, β -S-ARCA还具有细胞选择性,如在小鼠乳腺上皮细胞(HC11)和不成熟的DCs细胞中, β -S-ARCA加帽的mRNA可以表达蛋白,而在成熟的DCs细胞中则不能^[14].

先天免疫系统胞质RNA传感器,如RIG-I(retinoic acid-inducible gene I)和IFIT1(interferon-induced proteins with tetratricopeptide repeat 1)等能识别外源mRNA并产生一定的免疫响应,这种传感器介导的免疫刺激反过来可能抑制mRNA的翻译^[16]. RIG-I能被cap0加帽mRNA激活,但不被cap1加帽的mRNA激活,而IFIT1与cap0的亲合力更强^[17,18].

1.2 mRNA非翻译区

mRNA非翻译区(untranslation region, UTR)包括5'-UTR和3'-UTR,其中包含多种调控元件,这些元件对mRNA的稳定性和蛋白质的翻译至关重要. 真核生物mRNA的UTRs在转录后调控中起着关键作用,包括调节mRNA稳定性^[20]、调控mRNA的亚细胞定位^[21]、控制mRNA的出核转运以及翻译效率^[22,23]等. 图1(c)展示了非翻译区中主要结构元件的功能特征. 5'-UTR短而无结构,因为稳定的次级基序阻碍了小核糖体亚基对起始密码子的识别^[24]. UTR组合文库的研究显示,5'-UTR序列对于蛋白质表达最关键,3'-UTR是mRNA半衰期的关键驱动力^[25].

大多数影响mRNA稳定性的顺式作用元件通常位于3'-UTR,因此具有较短3'-UTR的mRNA会丧失这些调控元件,并表现出较大的稳定性^[20]. AU-富集元件(AU-rich elements, AREs)和GU-富集元件(GU-rich elements, GREs)是3'-UTR里最常见的不稳定序列^[26,27]. 在合成mRNA时,需要避免这些不稳定基序,同时引入稳

图1 (网络版彩色)mRNA的主要结构及其工程设计。(a) 常用的帽类似物。(b) mRNA的主要结构。5' cap: 5'帽子结构; 5'-UTR: 5'-非翻译区; SP: 信号肽; ORF: 开放阅读框; 3'-UTR: 3'-非翻译区; poly(A) tail: 多聚腺苷酸尾。(c) 非翻译区内结构元件及其功能。♠, 转录控制; ♥, 亚细胞定位; ♦, mRNA的稳定性; ♣, 聚腺苷酸化。图(c)改编自文献[19]

Figure 1 (Color online) The main structure of mRNA and its engineering design. (a) Commonly used cap analogues. (b) The main structure of mRNA. 5' cap: 5' cap structure; 5'-UTR: 5'-untranslated region; SP: signal peptide; ORF: open reading frame; 3'-UTR: 3'-untranslated area; poly(A) tail: poly A tail. (c) Structural elements and their functions of untranslated region. ♠, Transcription control; ♥, subcellular localization; ♦, mRNA stability; ♣, polyadenylation. Figure (c) adapted from Mignone et al. [19]

定的元件以改善mRNA的半衰期。IVT mRNA多使用珠蛋白(globin)的UTR, 含 α -和 β -globin的3'-UTR mRNA的半衰期超过1 d, 这是由于其3'-UTR含有不连续的嘧啶富集稳定元件^[28,29]以及核仁蛋白结合元件(nucleolin-binding element)^[30]。终止密码子后面紧跟G或A, 能有效地区终止翻译^[31]。

1.3 ORF

ORF是编码目标蛋白的区域。同样, 在编码区需要避免AREs或UA和UU二核苷酸富集的不稳定RNA元

件。编码区GC含量也是一个重要的参数。此外, ORF序列具有一定的免疫刺激作用并影响翻译效率, 富U序列和一些RNA高级结构具有免疫刺激性, 能与各种RNA传感器, 如Toll样受体(Toll-like receptors, TLR)、RIG-I和蛋白激酶R(protein kinase R, PKR)等相互作用, 影响mRNA的稳定性^[32~34]。体外转录的mRNA免疫原性阻碍了mRNA疗法的发展。

由于必须维持蛋白编码的信息, 因此ORF不能像其他结构元件一样自由调整。ORF可以利用密码子的简并性进行优化。密码子优化是基于宿主tRNA的偏好, 将丰

度高、频繁使用的密码子替换为稀有密码子。然而,密码子优化并不都有利,密码子优化可能影响蛋白质构象和功能、增加免疫原性并降低功效,也可能破坏了原有RNA二级结构和潜在的剪接基序。虽然也有报道显示,可以通过密码子优化显著提高蛋白质产量,但并不是所有情况都适用。某些蛋白质需要缓慢的翻译才能进行适当的折叠和修饰,这也是稀有密码子存在的意义。

1.4 poly(A)尾

poly(A)尾与5'帽子结构和其他多种决定因素协同调控mRNA的翻译效率和稳定性^[35]。合成mRNA的poly(A)尾可以在体外转录过程中产生,也可以在转录后通过附加的酶反应产生。然而,酶加尾难以控制poly(A)尾的长度,从而难以统一单批次的加尾反应。相反,当线性DNA模板中含有poly(A)尾转录序列时,不同批次体外转录反应均可产生稳定长度的poly(A)尾。由于在预临床和临床应用中的目标是以高可重复性的方式精确生产药物,因此直接产生poly(A)尾的方法更受欢迎。

大多数研究表明,当将poly(A)尾的长度从大约60~70 nt增加到100~150 nt时,mRNA的翻译都可以增强。对DCs细胞中poly(A)尾长度的系统研究发现,蛋白质的表达随poly(A)尾长度的增加而增加,而含有120个腺苷poly(A)尾mRNA的蛋白质产量最高^[36]。

2 体外合成mRNA稳定性和表达效率的设计

2.1 化学修饰核苷酸改善mRNA的稳定性、免疫原性和翻译效率

mRNA是遗传信息的瞬时载体,但由于RNA分子的普遍不稳定性和免疫原性等原因,以mRNA为基础的基因疗法方法最初并没有得到普及,而核苷酸修饰是一种改善的策略。

RNA的转录后修饰在自然界中频繁发生,但在各个物种中相对保守。这些自然发生的修饰分为内源mRNA分子和外来入侵微生物的RNA分子。到目前为止,已经发现了172种修饰核苷酸,然而大多数核苷酸的修饰类型是比较罕见的^[37]。密码子工程与化学修饰核苷酸的结合,以及模拟自然发生的细胞内修饰等已被证明可以降低免疫原性和增加翻译效率^[38]。有研究显示,核苷酸修饰可以通过增加RNA分子的稳定性和降低其免疫原性来大大增强mRNA的功能属性(Kariko paradigm)^[39,40],这对mRNA基因治疗方法的发展具有

划时代的意义。

mRNA的免疫原性在于mRNA可以激活由模式识别受体(pattern recognition receptors, PRR)触发的抗炎反应,诱导促炎性细胞因子和I型干扰素(interferons, IFN)基因的表达^[41],最终导致mRNA的降解和翻译效率的降低。PRR可感应单链RNA(single stranded RNA, ssRNA)、双链RNA(double-stranded RNA, dsRNA)和无帽mRNA。PRR包括TLR^[32]和胞质维甲酸诱导基因I(retinoic-acid-inducible gene I, RIG-I)样受体((RIG-I-like receptors, RLRs)^[42]。化学修饰核苷酸可以降低mRNA的免疫原性。核苷酸修饰的RNA可作为RIG-I反应抑制剂^[43,44]。Durbin等人^[45]发现, RNA修饰可以消除RIG-I信号响应。研究显示, N6甲基腺苷(N6-methyladenosine, m6A)、5甲基尿苷(5-methyluridine, m5U)、2硫尿核苷(2-thiouridine, s2U)、假尿苷(pseudouridine, Ψ)、2'-氟脱氧尿苷(2'-fluoro-deoxyuridine, 2FdU)或2'-氟脱氧胞苷(2'-fluoro-deoxycytidine, 2FdC)可中断不同阶段的RIG-I信号传导途径。同理,不同的RNA修饰也可消除dsRNA和ssRNA引起的PKR激活^[46]。从另一方面看, mRNA的强免疫刺激作用实际上可以用于疫苗接种。据报道,具有mRNA的靶向抗原呈递细胞(antigen presenting cells, APCs)可通过激活T细胞和B细胞来诱导抗肿瘤免疫^[47]。I型IFN对T细胞免疫的影响可能是有益的,也可能是有害的,这取决于它们的诱导动力学、强度和分布。刺激先天免疫可能会提高疫苗的效力,不过仍然需要考虑,不加选择的免疫激活会诱导mRNA的降解并使抗原表达降低。

掺入修饰的核苷酸减少了先天免疫激活反应,但体外合成的化学修饰mRNA仍残留I型IFN和促炎细胞因子的诱导作用。高效液相色谱(high performance liquid chromatography, HPLC)纯化可去除体外转录RNA中的dsRNA、未成功转录的RNA及其他污染物,使体外合成的mRNA产生更高的翻译效率^[48]。因此,将修饰的核苷酸掺入合成mRNA中并随后进行HPLC纯化是目前获得先天免疫沉默mRNA的标准方法。然而,也有研究发现,添加免疫抑制剂(结合I型IFN的牛痘病毒的诱饵受体B18R^[49,50])或免疫逃避蛋白(甲型流感病毒衍生的多功能非结构蛋白NS1^[51])的替代策略也可减轻体外合成mRNA引起的先天免疫反应。

比较常用的化学修饰核苷酸有m6A、Ψ、N1甲基假尿苷(N1-methylpseudouridine, m1Ψ)、s2U、m5U和5甲基胞苷(5-methylcytidine, m5C)等(图2)。这些修饰核

苷酸单独或组合使用都具有一定的效果。Ψ和s2U掺入mRNA可以降低其免疫刺激作用^[52]。m1Ψ是一种天然存在于18S rRNA中的修饰。有研究显示, m1Ψ单独使用或与m5C联合使用大约是经典Ψ合成mRNA编码目的蛋白表达的44和13倍^[53]。此外, 化学修饰核苷酸合成的mRNA也被广泛应用到医学相关研究中。例如, Warren等人^[54]利用m5C/Ψ修饰的IVT mRNA成功对人的体细胞进行重编程和分化; Miki等人^[55]利用m5C/Ψ修饰的mRNA作为筛选多能干细胞衍生特定细胞的纯化工具。除了筛选纯化特定细胞外, Wroblewska等人^[56]利用m5C/Ψ修饰的mRNA构建了一种用于选择性杀死癌细胞的合成mRNA元件; Zangi等人^[57]利用m5C/Ψ修饰的

mRNA通过表达旁分泌因子对心肌梗死的小鼠进行器官修复; Kormann等人^[58]利用m5C/s2U修饰的mRNA治疗遗传性表面活性蛋白B(surfactant protein B, SP-B)缺失引起的先天性致死肺病; Mahiny等人^[59]利用m5C/s2U修饰的mRNA在小鼠中表达锌指核酸酶用于基因组编辑。

2.2 mRNA UTR设计改变蛋白产量和半衰期

在体内递送的外源性mRNA具有相对较短的半衰期是该基因疗法一个主要的缺点。通过工程改造可以解决mRNA半衰期短的问题。UTR的调节是提高mRNA稳定性和翻译效率的一种方法。

图 2 (网络版彩色)mRNA合成常用的天然核苷酸和修饰核苷酸的化学结构。A: adenosine; m1A: N1-methyladenosine; m6A: N6-methyladenosine; U: uridine; Ψ: pseudouridine; m1Ψ: N1-methylpseudouridine; s2U: 2-thiouridine; m5U: 5-methyluridine; mo5U: 5-methoxyuridine; 2FdU: 2'-fluoro-deoxyuridine; C: cytidine; m5C: 5-methylcytidine; mo5C: 5-methylcytidine; hm5C: 5-hydroxymethylcytidine; 2FdC: 2'-fluoro-deoxycytidine; G: guanosine; m2G: 2-methylguanosine

Figure 2 (Color online) Chemical structures of natural nucleotides and modified nucleotides which commonly used for mRNA synthesis. A: adenosine; m1A: N1-methyladenosine; m6A: N6-methyladenosine; U: uridine; Ψ: pseudouridine; m1Ψ: N1-methylpseudouridine; s2U: 2-thiouridine; m5U: 5-methyluridine; mo5U: 5-methoxyuridine; 2FdU: 2'-fluoro-deoxyuridine; C: cytidine; m5C: 5-methylcytidine; mo5C: 5-methylcytidine; hm5C: 5-hydroxymethylcytidine; 2FdC: 2'-fluoro-deoxycytidine; G: guanosine; m2G: 2-methylguanosine

5'-UTR中有蛋白质翻译预启动复合物的起始结合位点,对蛋白质的翻译效率起着关键作用^[60]. mRNA的5'-UTR和3'-UTR可以替换或改变以增加mRNA的翻译效率和稳定性. Globin mRNA能够产生大量的蛋白,且具有较长的半衰期^[61,62]. 研究显示,人 α -和 β -globin的3'-UTR可增强mRNA的稳定性并促进翻译,将人 β -globin的3'-UTR从头到尾方向排列可以额外增加mRNA的稳定性^[63]. 研究发现,不仅在mRNA 3'-UTR处插入一个拷贝的 β -珠蛋白3'-UTR核酸序列能增加目标蛋白的翻译,而且背对背引入两个拷贝的 β -珠蛋白3'-UTR核酸序列也能进一步加强这种效果^[62]. 然而,这种加强效果只出现在未成熟的DCs细胞中,而非成熟的DCs细胞. Warren等人^[54]在成纤维细胞重新编程为诱导的多能干细胞过程中,使用了包含强Kozak翻译信号的人工5'-UTR核酸序列和 α -globin的3'-UTR核酸序列来提高mRNA的蛋白产量.

不同的治疗目的需要对应的治疗方案,未来UTR的研究将使用特定的疾病模型来详细研究药代动力学/药效动力学(pharmacodynamics, PD),这将有助于了解mRNA分子的应用频率和治疗窗口. 根据治疗的需求,针对半衰期较长的蛋白质,如果其需求量较小,应用的频率较低,可以利用工程学方法降低mRNA的翻译效率,如将AREs元件整合到3'-UTR使对应的mRNA序列快速降解以缩短半衰期^[64].

Sample等人^[65]结合深度学习和多核糖体表达谱技术建立了一种5'-UTR预测模型,该模型可以准确地预测特定序列的核糖体负载水平,从而精确调控蛋白质的表达,并且该模型可以应用到基于化学修饰mRNA的基因治疗中. 目前的研究为UTRs的优化提供了重要的信息,UTRdb^[66]收录了大量真核mRNA的UTR序列,同时,借助人工智能和机器学习筛选方法,获得了诸多特定功效的人工合成UTR序列以优化蛋白质翻译^[67,68]. 未来针对不同的治疗需求,为合成治疗性mRNA药物人工设计特定的UTR是可期的.

3 体外合成mRNA时空可控和表达开关的设计

3.1 药物递送材料实现mRNA表达的时空可控性

mRNA为基础的治疗药物主要包含两部分: mRNA及其药物递送材料. 就mRNA基因疗法而言,仅mRNA分子难以穿透靶细胞,且mRNA易在生物体内降解,

导致在全身性给药后药物无法在靶器官中积聚^[69]. 然而,药物递送材料可以帮助将mRNA递送到目标细胞内. 尽管在部分临床应用案例中,将一些裸露的mRNA (naked mRNAs)用于药物治疗(NCT02035956、NCT03480152和NCT03468244等),但是适当的递送方法对实现mRNA的全身给药及进一步扩大其治疗范围是至关重要的.

近年来,研究人员开发了许多适用于mRNA给药的新递送材料,这些递送材料主要分为基于脂质的递送材料、基于聚合物的递送材料、多因素复合递送材料以及其他纳米制剂递送材料等^[9,70]. 可离子化的脂质体和脂质体衍生的材料在低pH下可带正电荷,而在生理pH下变为中性,可降低毒性和免疫原性,同时又维持了药物递送效率^[2].

脂质纳米颗粒(lipid nanoparticles, LNPs)类递送材料已成为最广泛使用的复合mRNA递送材料. LNPs通常包含4个成分: (1) 包裹mRNA的含有叔胺或季胺类的材料,如可电离材料、阳离子脂质或聚合材料等; (2) 与细胞膜中脂质相似的两性离子脂质材料,如1,2-二油酰基-sn-丙三基-3-磷酸乙醇胺(1,2-dioleoyl-sn-glycero-3-phosphoethanolamine, DOPE)等; (3) 稳定LNPs的脂质双层的胆固醇; (4) 改善胶体稳定性并减少蛋白质吸收的LNPs的水合层,如聚乙二醇(polyethylene glycol, PEG)等. 上述四类成分的配比会影响LNPs制剂的功效和耐受性, Weng等人^[9]的综述回顾了mRNA代表性的LNPs递送系统及其组分比率.

致力于mRNA药物研究的生物技术公司针对需求开发了专有的mRNA药物递送系统. CureVac开发了基于鱼精蛋白配制的RNA复合物的RNA疫苗平台,该疫苗平台在临床前动物模型和患者体内均提高了针对癌症和传染病的有利免疫激活^[71]. BioNTech开发的DOTMA(DOTAP)/DOPE脂质复合物通过全身递送的方式可实现递送的mRNA在DCs细胞中优先表达^[72]. BioNTech开发的纳米颗粒RNA-LPX疫苗平台可将嵌合抗原受体(chimeric antigen receptor, CAR)抗原传递到淋巴区室,刺激过继转移的CAR-T细胞,最终可使难以治愈的小鼠模型中的大肿瘤消退^[73]. Moderna开发了一种可生物降解的可电离脂质递送材料用于非人类灵长类动物(non-human primates, NHPs)的mRNA药物递送^[74]. 越来越多的公司及科研机构院校开发新型mRNA递送制剂,并正在进行一些临床前和临床研究^[9,70]. 一些市售的mRNA标准化递送材料也用于实验

室研究,如TransIT[®][75]、*in vivo*-jetPEI[®][76]、LipofectamineTM[77]、StemfectTM[76]和MegaFectinTM[58]等。

药物递送材料可以实现细胞或组织特异性递送。Fornaguera等人[47]通过Michael加成反应对聚氨酯(poly(β -amino esters), PBAEs)上的丙烯酸基团添加了CKKK(Cys+3Lys)寡肽,获得了寡肽修饰的PBAEs(oligopeptide modified PBAEs, OM-PBAEs),该OM-PBAEs能靶向给药APCs细胞。Lou等人[78]报道了一种肽功能化的mRNA多聚物,可通过唾液酸介导的吞噬作用进入DCs细胞,从而实现靶向激活APCs细胞。

肝细胞可以接纳多种类型的脂质体和聚合物载药系统,且肝脏是许多分泌蛋白质的生产基地,因此,肝脏靶向输送的mRNA载药系统是产生大量分泌蛋白的一种有效途径[79]。对于许多疾病,肝脏是基于mRNA蛋白替代疗法的首选器官,因为全身给药的mRNA药物主要被网状内皮系统(reticuloendothelial system, RES)捕获至肝脏。此外,载脂蛋白E(apolipoprotein E, apoE)与生物材料(如脂质体和脂质体衍生的材料)之间的相互作用促进了mRNA药物在肝脏中的积累[80]。N-乙酰半乳糖胺(N-acetylgalactosamine, GalNAc)偶联也是一种主要的肝脏靶向给药的策略,并且在临床试验中被应用于越来越多的RNA治疗药物。然而,针对肝脏以外的器官则需要发现新的配体。

对肺mRNA药物的靶向递送,过去主要采用静脉注射和气管内给药的方式。通过静脉注射实现肺靶向递送mRNA药物具有较高的挑战性,因为全身给药的mRNA纳米颗粒大量富集在肝脏和脾脏中[81]。采用被动靶向肺部给药的纳米颗粒的粒径大于7 μm 才可使药物保留在肺泡毛细血管中[82]。肺毛细血管是无孔的,阻碍了大分子从血液到肺组织的自由交换[81]。将肺亲和配体与纳米颗粒偶联可以显著增加肺靶向的mRNA递送[83]。Schrom等人[84]通过将mRNA与预组装的C12-(2-3-2)oligoalkylamines脂质胶束混合制备了肺靶向的脂质体纳米颗粒(pulmonary lipid formulation, PLF)。Kaczmarek等人[85]开发了由PBAEs和PEG组成的混合聚合物脂质纳米颗粒,用于肺靶向mRNA的输送。此后,通过对聚合物结构和纳米颗粒配方的优化,Kaczmarek等人[86]又得到了一种新型高效的肺特异性纳米颗粒用于mRNA的递送。Cheng等人[87]报道了一种用于组织特异性mRNA递送和CRISPR-Cas基因编辑的选择性器官靶向(selective organ targeting, SORT)纳米颗粒。该技术通过添加补充的SORT分子设计专门用于编辑肝外组织

(针对肺、脾脏和肝脏)的SORT脂质纳米颗粒,以选择性编辑与治疗相关的细胞类型,包括上皮细胞、内皮细胞、B细胞、T细胞和肝细胞[87]等。

此外,许多生物材料也被开发出来用于将mRNA递送到特定器官(皮肤[88]、眼睛[89]和心脏[90]等)。对于上述器官,局部给药是主要的给药途径。高通量技术、优化筛选方法和调节细胞信号通路以开发识别组织特异性的高效递送系统也会加速解决器官的靶向给药问题[91,92]。

3.2 针对特定细胞构建表达开关控制mRNA的表达

定量与工程生物学方法的应用大大提高了mRNA药物的效力和安全性,其在推进mRNA疗法用于疫苗开发、免疫肿瘤学和蛋白质替代疗法等领域也已取得重大进展。一个潜在的值得探索的治疗领域是针对特定细胞构建表达开关用以控制mRNA的表达。在肿瘤治疗过程中,mRNA编码有毒蛋白质选择性地在病变细胞中表达,进而诱导细胞死亡,这种特洛伊木马式的治疗策略需要将毒性蛋白的表达严格控制在靶细胞中[93]。

微小RNA(microRNA, miRNA)的作用类似于小干扰RNA(small interfering RNA, siRNA),能够降解mRNA。将该策略应用于癌症的治疗,具有细胞特异性和组织特异性表达的miRNAs可用于抑制非预期受体细胞中mRNA的蛋白表达。miRNA-mRNA调控的保守原则使得这项技术可以跨物种应用。miRNA以细胞选择性的方式控制外源性mRNA的表达,这些调控体外转录mRNA的miRNA被称为miRNA开关(miRNA switches)。

目前多数mRNA药物递送方法在全身性给药后会倾向于在肝脏中积累并高表达,因此,需要找到消除mRNA药物递送到肝脏或阻断mRNA在肝脏中表达的方法。利用内源性miRNA库,编码有毒蛋白的mRNA在驱动肿瘤细胞凋亡的同时也能避免对肝脏的损伤。例如,含有miRNA靶点的编码凋亡蛋白(caspase或PUMA)的治疗性mRNA可以阻止其在健康肝细胞中的表达,同时触发肝癌细胞的凋亡[93]。

已有研究显示,包含人工miRNA靶向结合位点的mRNA,即包含miRNA开关的mRNA能够成功用于实现合成回路(synthetic circuits)的细胞特异性表达[56],鉴定并消除非分化诱导干细胞[55,94],以及使用miRNA响应的CRISPR-Cas9实现细胞选择性基因组编辑[95]。

然而,也有研究显示, Ψ 、m1 Ψ 和m5C在增加翻译效率的同时也会减弱miRNA开关的功能[96]。核苷酸修

饰能够增加翻译效率并稳定mRNA的高级结构,从而改变RNA与自身或与RNA结合蛋白之间的相互作用^[97].虽然有研究显示了这样一种现象,但是化学修饰核苷酸对miRNA作用的mRNA沉默的影响并未研究透彻.

4 总结

本文探讨了定量与工程生物学在mRNA基因疗法

应用中的最新进展,展示了定量与工程生物学在优化mRNA基因治疗上的潜在应用.尽管某些案例仍处于概念验证阶段,但是各种定量与工程生物学方法或工具的积累可能为拓宽新一代mRNA基因疗法奠定基础.定量与工程生物学创新设计将有助于充分发挥mRNA基因治疗潜力,并在不久的将来转变为基因治疗的主要治疗工具.

参考文献

- Gurdon J B, Lane C D, Woodland H R, et al. Use of frog eggs and oocytes for the study of messenger RNA and its translation in living cells. *Nature*, 1971, 233: 177–182
- Hajj K A, Whitehead K A. Tools for translation: Non-viral materials for therapeutic mRNA delivery. *Nat Rev Mater*, 2017, 2: 17056
- Weissman D. mRNA transcript therapy. *Expert Rev Vaccines*, 2015, 14: 265–281
- Zhang F, Parayath N N, Enc C I, et al. Genetic programming of macrophages to perform anti-tumor functions using targeted mRNA nanocarriers. *Nat Commun*, 2019, 10: 3974
- Pardi N, Hogan M J, Pelc R S, et al. Zika virus protection by a single low-dose nucleoside-modified mRNA vaccination. *Nature*, 2017, 543: 248–251
- Van Hoecke L, Verbeke R, De Vlieger D, et al. mRNA encoding a bispecific single domain antibody construct protects against influenza A virus infection in mice. *Mol Ther Nucleic Acids*, 2020, 20: 777–787
- Ramaswamy S, Tonnu N, Tachikawa K, et al. Systemic delivery of factor IX messenger RNA for protein replacement therapy. *Proc Natl Acad Sci USA*, 2017, 114: E1941–E1950
- Zhu X, Yin L, Theisen M, et al. Systemic mRNA therapy for the treatment of fabry disease: Preclinical studies in wild-type mice, fabry mouse model, and wild-type non-human primates. *Am J Human Genet*, 2019, 104: 625–637
- Weng Y, Li C, Yang T, et al. The challenge and prospect of mRNA therapeutics landscape. *Biotech Adv*, 2020, 40: 107534
- Leppek K, Das R, Barna M. Functional 5' UTR mRNA structures in eukaryotic translation regulation and how to find them. *Nat Rev Mol Cell Biol*, 2018, 19: 158–174
- Ramanathan A, Robb G B, Chan S H. mRNA capping: Biological functions and applications. *Nucleic Acids Res*, 2016, 44: 7511–7526
- Pasquinelli A E, Dahlberg J E, Lund E. Reverse 5' caps in RNAs made *in vitro* by phage RNA polymerases. *RNA*, 1995, 1: 957–967
- Stepinski J, Waddell C, Stolarski R, et al. Synthesis and properties of mRNAs containing the novel “anti-reverse” cap analogs 7-methyl(3'-O-methyl)GpppG and 7-methyl(3'-deoxy)GpppG. *RNA*, 2001, 7: 1486–1495
- Kuhn A N, Diken M, Kreiter S, et al. Phosphorothioate cap analogs increase stability and translational efficiency of RNA vaccines in immature dendritic cells and induce superior immune responses *in vivo*. *Gene Ther*, 2010, 17: 961–971
- Kowalska J, Lewdorowicz M, Zuberek J, et al. Synthesis and characterization of mRNA cap analogs containing phosphorothioate substitutions that bind tightly to eIF4E and are resistant to the decapping pyrophosphatase DcpS. *RNA*, 2008, 14: 1119–1131
- Leung D W, Amarasinghe G K. When your cap matters: Structural insights into self vs non-self recognition of 5' RNA by immunomodulatory host proteins. *Curr Opin Struct Biol*, 2016, 36: 133–141
- Kumar P, Sweeney T R, Skabkin M A, et al. Inhibition of translation by IFIT family members is determined by their ability to interact selectively with the 5'-terminal regions of cap0-, cap1- and 5'ppp-mRNAs. *Nucleic Acids Res*, 2014, 42: 3228–3245
- Schuberth-Wagner C, Ludwig J, Bruder A K, et al. A conserved histidine in the RNA sensor RIG-I controls immune tolerance to N1-2'-O-methylated self RNA. *Immunity*, 2015, 43: 41–51
- Mignone F, Gissi C, Liuni S, et al. Untranslated regions of mRNAs. *Genome Biol*, 2002, 3: reviews0004.0001
- Rabani M, Pieper L, Chew G L, et al. A massively parallel reporter assay of 3' UTR sequences identifies *in vivo* rules for mRNA degradation. *Mol Cell*, 2017, 68: 1083–1094.e5
- Ciulli Mattioli C, Rom A, Franke V, et al. Alternative 3' UTRs direct localization of functionally diverse protein isoforms in neuronal compartments. *Nucleic Acids Res*, 2019, 47: 2560–2573
- Chen S, Wang R, Zheng D, et al. The mRNA export receptor NXF1 coordinates transcriptional dynamics, alternative polyadenylation, and mRNA export. *Mol Cell*, 2019, 74: 118–131.e7
- Evfratov S A, Osterman I A, Komarova E S, et al. Application of sorting and next generation sequencing to study 5'-UTR influence on translation efficiency in *Escherichia coli*. *Nucleic Acids Res*, 2017, 45: 3487–3502

- 24 Dacheux E, Malys N, Meng X, et al. Translation initiation events on structured eukaryotic mRNAs generate gene expression noise. *Nucleic Acids Res*, 2017, 45: 6981–6992
- 25 Asrani K H, Farelli J D, Stahley M R, et al. Optimization of mRNA untranslated regions for improved expression of therapeutic mRNA. *RNA Biol*, 2018, 15: 756–762
- 26 Otsuka H, Fukao A, Funakami Y, et al. Emerging evidence of translational control by AU-rich element-binding proteins. *Front Genet*, 2019, 10: 332
- 27 Chaudhury A, Cheema S, Fachini J M, et al. CELF1 is a central node in post-transcriptional regulatory programmes underlying EMT. *Nat Commun*, 2016, 7: 13362
- 28 Ropero P, González F A, Nieto J M, et al. C>A substitution in NT 46 of the 3' UTR region (the α complex protected region) of the alpha-1 globin gene: A non-deletional mutation or polymorphism? *J Clin Pathol*, 2020, 73: 14–16
- 29 Zarghampoor F, Azarpira N, Khatami S R, et al. Improved translation efficiency of therapeutic mRNA. *Gene*, 2019, 707: 231–238
- 30 Jiang Y, Xu X S, Russell J E. A nucleolin-binding 3' untranslated region element stabilizes β -globin mRNA *in vivo*. *Mol Cell Biol*, 2006, 26: 2419–2429
- 31 van der Velden A W, Voorma H O, Thomas A A M. Vector design for optimal protein expression. *Biotechniques*, 2001, 31: 572–582
- 32 Heil F. Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. *Science*, 2004, 303: 1526–1529
- 33 Nallagatla S R, Hwang J, Toroney R, et al. 5'-triphosphate-dependent activation of PKR by RNAs with short stem-loops. *Science*, 2007, 318: 1455–1458
- 34 Rehwinkel J, Tan C P, Goubau D, et al. RIG-I detects viral genomic RNA during negative-strand RNA virus infection. *Cell*, 2010, 140: 397–408
- 35 Nicholson A L, Pasquinelli A E. Tales of detailed poly(A) tails. *Trends Cell Biol*, 2019, 29: 191–200
- 36 Holtkamp S, Kreiter S, Selmi A, et al. Modification of antigen-encoding RNA increases stability, translational efficacy, and T-cell stimulatory capacity of dendritic cells. *Blood*, 2006, 108: 4009–4017
- 37 Boccaletto P, Machnicka M A, Purta E, et al. MODOMICS: A database of RNA modification pathways. 2017 update. *Nucleic Acids Res*, 2018, 46: D303–D307
- 38 Thess A, Grund S, Mui B L, et al. Sequence-engineered mRNA without chemical nucleoside modifications enables an effective protein therapy in large animals. *Mol Ther*, 2015, 23: 1456–1464
- 39 Karikó K, Muramatsu H, Welsh F A, et al. Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vector with increased translational capacity and biological stability. *Mol Ther*, 2008, 16: 1833–1840
- 40 Karikó K, Buckstein M, Ni H, et al. Suppression of RNA recognition by Toll-like receptors: The impact of nucleoside modification and the evolutionary origin of RNA. *Immunity*, 2005, 23: 165–175
- 41 Van Hoecke L, Roose K. How mRNA therapeutics are entering the monoclonal antibody field. *J Transl Med*, 2019, 17: 54
- 42 Goubau D, Schlee M, Deddouche S, et al. Antiviral immunity via RIG-I-mediated recognition of RNA bearing 5'-diphosphates. *Nature*, 2014, 514: 372–375
- 43 Uzri D, Gehrke L. Nucleotide sequences and modifications that determine RIG-I/RNA binding and signaling activities. *J Virol*, 2009, 83: 4174–4184
- 44 Peisley A, Wu B, Yao H, et al. RIG-I forms signaling-competent filaments in an ATP-dependent, ubiquitin-independent manner. *Mol Cell*, 2013, 51: 573–583
- 45 Durbin A F, Wang C, Marcotrigiano J, et al. RNAs containing modified nucleotides fail to trigger RIG-I conformational changes for innate immune signaling. *mBio*, 2016, 7: e00833-16
- 46 Nallagatla S R, Bevilacqua P C. Nucleoside modifications modulate activation of the protein kinase PKR in an RNA structure-specific manner. *RNA*, 2008, 14: 1201–1213
- 47 Fornaguera C, Guerra-Rebollo M, Ángel Lázaro M, et al. mRNA delivery system for targeting antigen-presenting cells *in vivo*. *Adv Healthc Mater*, 2018, 7: e1800335
- 48 Karikó K, Muramatsu H, Ludwig J, et al. Generating the optimal mRNA for therapy: HPLC purification eliminates immune activation and improves translation of nucleoside-modified, protein-encoding mRNA. *Nucleic Acids Res*, 2011, 39: e142
- 49 Yoshioka N, Gros E, Li H R, et al. Efficient generation of human iPSCs by a synthetic self-replicative RNA. *Cell Stem Cell*, 2013, 13: 246–254
- 50 Michel T, Golombek S, Steinle H, et al. Efficient reduction of synthetic mRNA induced immune activation by simultaneous delivery of B18R encoding mRNA. *J Biol Eng*, 2019, 13: 40
- 51 Liu Y, Chin J M, Choo E L, et al. Messenger RNA translation enhancement by immune evasion proteins: A comparative study between EKB (vaccinia virus) and NS1 (influenza A virus). *Sci Rep*, 2019, 9: 11972
- 52 Xiong Q, Lee G Y, Ding J, et al. Biomedical applications of mRNA nanomedicine. *Nano Res*, 2018, 11: 5281–5309
- 53 Andries O, Mc Cafferty S, De Smedt S C, et al. N1-methylpseudouridine-incorporated mRNA outperforms pseudouridine-incorporated mRNA by

- providing enhanced protein expression and reduced immunogenicity in mammalian cell lines and mice. *J Control Release*, 2015, 217: 337–344
- 54 Warren L, Manos P D, Ahfeldt T, et al. Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. *Cell Stem Cell*, 2010, 7: 618–630
- 55 Miki K, Endo K, Takahashi S, et al. Efficient detection and purification of cell populations using synthetic microRNA switches. *Cell Stem Cell*, 2015, 16: 699–711
- 56 Wroblewska L, Kitada T, Endo K, et al. Mammalian synthetic circuits with RNA binding proteins for RNA-only delivery. *Nat Biotechnol*, 2015, 33: 839–841
- 57 Zangi L, Lui K O, von Gise A, et al. Modified mRNA directs the fate of heart progenitor cells and induces vascular regeneration after myocardial infarction. *Nat Biotechnol*, 2013, 31: 898–907
- 58 Kormann M S D, Hasenpusch G, Aneja M K, et al. Expression of therapeutic proteins after delivery of chemically modified mRNA in mice. *Nat Biotechnol*, 2011, 29: 154–157
- 59 Mahiny A J, Dewerth A, Mays L E, et al. *In vivo* genome editing using nuclease-encoding mRNA corrects SP-B deficiency. *Nat Biotechnol*, 2015, 33: 584–586
- 60 Hinnebusch A G, Ivanov I P, Sonenberg N. Translational control by 5'-untranslated regions of eukaryotic mRNAs. *Science*, 2016, 352: 1413–1416
- 61 Ross J, Sullivan T D. Half-lives of beta and gamma globin messenger RNAs and of protein synthetic capacity in cultured human reticulocytes. *Blood*, 1985, 66: 1149–1154
- 62 Volloch V, Housman D. Stability of globin mRNA in terminally differentiating murine erythroleukemia cells. *Cell*, 1981, 23: 509–514
- 63 Sahin U, Karikó K, Türeci Ö. mRNA-based therapeutics—Developing a new class of drugs. *Nat Rev Drug Discov*, 2014, 13: 759–780
- 64 Chen C Y A, Shyu A B. AU-rich elements: Characterization and importance in mRNA degradation. *Trends Biochem Sci*, 1995, 20: 465–470
- 65 Sample P J, Wang B, Reid D W, et al. Human 5' UTR design and variant effect prediction from a massively parallel translation assay. *Nat Biotechnol*, 2019, 37: 803–809
- 66 Grillo G, Turi A, Licciulli F, et al. UTRdb and UTRsite (RELEASE 2010): A collection of sequences and regulatory motifs of the untranslated regions of eukaryotic mRNAs. *Nucleic Acids Res*, 2010, 38: D75–D80
- 67 Cuperus J T, Groves B, Kuchina A, et al. Deep learning of the regulatory grammar of yeast 5' untranslated regions from 500,000 random sequences. *Genome Res*, 2017, 27: 2015–2024
- 68 Chen X S, Brown C M. Computational identification of new structured *cis*-regulatory elements in the 3'-untranslated region of human protein coding genes. *Nucleic Acids Res*, 2012, 40: 8862–8873
- 69 Cullis P R, Hope M J. Lipid nanoparticle systems for enabling gene therapies. *Mol Ther*, 2017, 25: 1467–1475
- 70 Kowalski P S, Rudra A, Miao L, et al. Delivering the messenger: Advances in technologies for therapeutic mRNA delivery. *Mol Ther*, 2019, 27: 710–728
- 71 Alberer M, Gnad-Vogt U, Hong H S, et al. Safety and immunogenicity of a mRNA rabies vaccine in healthy adults: An open-label, non-randomised, prospective, first-in-human phase 1 clinical trial. *Lancet*, 2017, 390: 1511–1520
- 72 Kranz L M, Diken M, Haas H, et al. Systemic RNA delivery to dendritic cells exploits antiviral defence for cancer immunotherapy. *Nature*, 2016, 534: 396–401
- 73 Reinhard K, Rengstl B, Oehm P, et al. An RNA vaccine drives expansion and efficacy of claudin-CAR-T cells against solid tumors. *Science*, 2020, 367: 446–453
- 74 Sabnis S, Kumarasinghe E S, Salerno T, et al. A novel amino lipid series for mRNA delivery: Improved endosomal escape and sustained pharmacology and safety in non-human primates. *Mol Ther*, 2018, 26: 1509–1519
- 75 Stadler C R, Bähr-Mahmud H, Celik L, et al. Elimination of large tumors in mice by mRNA-encoded bispecific antibodies. *Nat Med*, 2017, 23: 815–817
- 76 Chen R, Zhang H, Yan J, et al. Scaffold-mediated delivery for non-viral mRNA vaccines. *Gene Ther*, 2018, 25: 556–567
- 77 Zhang H, De Smedt S C, Remaut K. Fluorescence correlation spectroscopy to find the critical balance between extracellular association and intracellular dissociation of mRNA complexes. *Acta Biomater*, 2018, 75: 358–370
- 78 Lou B, De Koker S, Lau C Y J, et al. mRNA polyplexes with post-conjugated GALA peptides efficiently target, transfect, and activate antigen presenting cells. *Bioconjugate Chem*, 2019, 30: 461–475
- 79 Truong B, Allegri G, Liu X B, et al. Lipid nanoparticle-targeted mRNA therapy as a treatment for the inherited metabolic liver disorder arginase deficiency. *Proc Natl Acad Sci USA*, 2019, 116: 21150–21159
- 80 Trepote Z, Lichtenegger E, Plank C, et al. Delivery of mRNA therapeutics for the treatment of hepatic diseases. *Mol Ther*, 2019, 27: 794–802
- 81 Sahu I, Haque A K M A, Weidensee B, et al. Recent developments in mRNA-based protein supplementation therapy to target lung diseases. *Mol Ther*, 2019, 27: 803–823
- 82 Azarmi S, Roa W H, Löbenberg R. Targeted delivery of nanoparticles for the treatment of lung diseases. *Adv Drug Deliver Rev*, 2008, 60: 863–875

- 83 Parhiz H, Shuvaev V V, Pardi N, et al. PECAM-1 directed re-targeting of exogenous mRNA providing two orders of magnitude enhancement of vascular delivery and expression in lungs independent of apolipoprotein E-mediated uptake. *J Control Release*, 2018, 291: 106–115
- 84 Schrom E, Huber M, Aneja M, et al. Translation of angiotensin-converting enzyme 2 upon liver- and lung-targeted delivery of optimized chemically modified mRNA. *Mol Ther Nucleic Acids*, 2017, 7: 350–365
- 85 Kaczmarek J C, Patel A K, Kauffman K J, et al. Polymer-lipid nanoparticles for systemic delivery of mRNA to the lungs. *Angew Chem Int Ed*, 2016, 55: 13808–13812
- 86 Kaczmarek J C, Kauffman K J, Fenton O S, et al. Optimization of a degradable polymer-lipid nanoparticle for potent systemic delivery of mRNA to the lung endothelium and immune cells. *Nano Lett*, 2018, 18: 6449–6454
- 87 Cheng Q, Wei T, Farbiak L, et al. Selective organ targeting (SORT) nanoparticles for tissue-specific mRNA delivery and CRISPR-Cas gene editing. *Nat Nanotechnol*, 2020, 15: 313–320
- 88 Lescan M, Perl R M, Golombek S, et al. *De novo* synthesis of elastin by exogenous delivery of synthetic modified mRNA into skin and elastin-deficient cells. *Mol Ther Nucleic Acids*, 2018, 11: 475–484
- 89 Patel S, Ryals R C, Weller K K, et al. Lipid nanoparticles for delivery of messenger RNA to the back of the eye. *J Control Release*, 2019, 303: 91–100
- 90 Magadam A, Kaur K, Zangi L. mRNA-based protein replacement therapy for the heart. *Mol Ther*, 2019, 27: 785–793
- 91 Sago C D, Lokugamage M P, Paunovska K, et al. High-throughput *in vivo* screen of functional mRNA delivery identifies nanoparticles for endothelial cell gene editing. *Proc Natl Acad Sci USA*, 2018, 115: E9944–E9952
- 92 Patel S, Ashwanikumar N, Robinson E, et al. Boosting intracellular delivery of lipid nanoparticle-encapsulated mRNA. *Nano Lett*, 2017, 17: 5711–5718
- 93 Jain R, Frederick J P, Huang E Y, et al. microRNAs enable mRNA therapeutics to selectively program cancer cells to self-destruct. *Nucleic Acid Therapeutics*, 2018, 28: 285–296
- 94 Parr C J C, Katayama S, Miki K, et al. microRNA-302 switch to identify and eliminate undifferentiated human pluripotent stem cells. *Sci Rep*, 2016, 6: 32532
- 95 Hirosawa M, Fujita Y, Parr C J C, et al. Cell-type-specific genome editing with a microRNA-responsive CRISPR-Cas9 switch. *Nucleic Acids Res*, 2017, 45: e118
- 96 Lockhart J, Canfield J, Mong E F, et al. Nucleotide modification alters microRNA-dependent silencing of microRNA switches. *Mol Ther-Nucleic Acids*, 2019, 14: 339–350
- 97 Helm M. Post-transcriptional nucleotide modification and alternative folding of RNA. *Nucleic Acids Res*, 2006, 34: 721–733

补充材料

表S1 mRNA疗法的临床代表案例

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Summary for “定量与工程生物学在 mRNA 基因疗法中的应用”

Application of quantitative and engineering biology in mRNA gene therapy

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As a transient vector of gene information, messenger RNA (mRNA) is a multifunctional, flexible and safe gene therapy. However, due to the general instability and immunogenicity of mRNA molecules, mRNA gene therapy has not been popularized at first. Over the past few decades, the cross-integration of quantitative and engineering biology has led to a gradual shift from the concept validation phase to the clinical treatment phase. In this paper, we discussed the application of quantitative and engineering biology in mRNA gene therapy from the functional structure of mRNA, design and technical innovation of *in vitro* synthetic mRNA. Effective engineering approaches will help to establish a precise mRNA gene therapy platform for a variety of indications and genetic backgrounds. Although mRNA therapy is still in the pre-clinical phase in most indications, the accumulation of various engineering and quantitative bio-design methods may broaden the path for next-generation mRNA gene therapy.

mRNA is an intermediate of genetic information and a template for the biological expression of proteins. Therefore, exogenous mRNA can be introduced into target cells to express the protein of interest. In the early 1970s, Gurdon first verified the above concept in *Xenopus oocytes* using microinjection. The advantages of mRNA-based gene therapy methods are: First, mRNA therapy has a broader application. mRNA does not need to enter the nucleus to express proteins in the cytoplasm to achieve its function. Therefore, mRNA can be played in cells that divide slowly or do not divide role. Second, mRNA therapy has higher safety, due to its transient expression and the extremely low possibility of genome integration, thereby reducing the two main risks associated with gene therapy (host genome integration and mutations in key regions). Third, the half-life and expression abundance of mRNA synthesized *in vitro* can be determined by the structure and component design, so that the pharmacokinetics of mRNA drugs can be better controlled. This has created favorable conditions for the pharmaceutical good manufacturing practice (GMP) of mRNA synthetic drugs. In addition, therapeutic proteins synthesized using the natural mechanism of cells have natural post-translational modifications and suitable protein folding effects, which are more advantageous than recombinant proteins synthesized *in vitro*. In recent years, mRNA, as a therapeutic method, has received increasing attention in the field of gene therapy. Currently, *in vitro*-transcribed (IVT) mRNA has been used in pre-clinical and clinical trials, including cancer treatment, vaccine development, and protein replacement drug development. Compared with cancer and vaccine treatment, most protein replacement therapies using mRNA are still in preclinical development stage.

quantitative biology, synthetic biology, chemical modified mRNA, gene therapy

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