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·论著·

H1N1亚型流感病毒mRNA候选疫苗的构建、表达及鉴定*

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【摘要】 **目的** 从mRNA序列优化和体外转录(IVT)系统优化角度,设计2条基于血凝素(HA)基因的抗H1N1亚型流感病毒mRNA疫苗,分别连接到3种体外转录系统(含不同UTRs序列)中进行候选疫苗抗原的表达及鉴定,并确定候选疫苗所使用的最佳体外转录系统。 **方法** 确保抗原基因(HA)氨基酸序列不变,以2种优化策略对HA基因的密码子进行优化,分别命名为JLH1HA和JLHA,并分别克隆至骨架载体pGEM-T7-Hα(UTRs来自人源α球蛋白)、pGEM-T7-Mα(UTRs来自鼠源α球蛋白)、pGEM-n3(UTRs来自人源β球蛋白)上,通过线性化处理、体外转录、纯化及Cap1加帽处理,获得功能性mRNA,转染A549细胞后通过Western blot和间接免疫荧光试验(IFA)鉴定目的蛋白的表达。 **结果** 成功构建6组mRNA候选疫苗且均能表达抗原蛋白,以pGEM-T7-Hα为骨架载体构建的体外转录系统蛋白表达量较高。 **结论** 抗原的设计具有可行性,筛选出的最优体外转录系统为以pGEM-T7-Hα为骨架载体构建的体外转录系统。密码子优化对蛋白表达量的影响并不显著,可能在动物免疫效果上能够体现。

【关键词】 H1N1亚型流感病毒;mRNA疫苗;血凝素HA;密码子优化;体外转录系统
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Construction, expression and identification of mRNA candidate vaccine of H1N1 subtype influenza virus

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【Abstract】 **Objective** From the perspective of mRNA sequence optimization and in vitro transcription (IVT) system optimization, two mRNA vaccines against H1N1 subtype influenza virus based on hemagglutinin (HA) gene were designed and linked to three in vitro transcription systems (including different UTRs sequences) respectively for the expression and identification of candidate vaccine antigens, and the optimal in vitro transcription system used for candidate vaccines was determined. **Methods** To ensure that the amino acid sequence of antigen gene (HA) remained unchanged, two optimization strategies were used to optimize the codon of HA gene, named JLH1HA and JLHA, respectively. They were cloned into the skeleton vectors pGEM-T7-Hα (UTRs from human α globulin), pGEM-T7-Mα (UTRs from mouse α globulin) and pGEM-n3 (UTRs from human β globulin), respectively. Functional mRNA was obtained by linearization, in vitro transcription, purification and Cap1 capping treatment. A549 cells were transfected with Western blot and indirect immunofluorescence assay (IFA) to identify the expression of target protein. **Results** Six groups of mRNA candidate vaccines were constructed successfully, and all of them could express antigen proteins successfully. Among them, the protein expression of the transcription system constructed with pGEM-T7-Hα as the skeleton vector was the highest. Compared with pGEM-n3, the difference was signficant ($P<0.01$). **Conclusion** The design of antigen was feasible, and the optimal in vitro transcription system was pGEM-T7-Hα skeleton carrier. The effect of codon optimization on protein expression is not significant, which may be reflected in animal immune effect.

【Key words】 H1N1 subtype influenza virus; mRNA vaccine; hemagglutinin HA; codon optimization; *in vitro*

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transcription system

流感病毒呈球形或丝状，属于正黏病毒科，其基因组包含分段的负义单链RNA。根据世界卫生组织的数据，季节性流感每年导致全球300万～500万人感染和29万～65万人死亡[1]。流感病毒有4种主要类型，其中A型、B型和C型可感染人类。2009年2月，墨西哥出现了首例H1N1报告病例。同年4月，世界卫生组织宣布H1N1为具有全球重要性的突发公共卫生事件[2]。2009-2010年，甲型H1N1流感大流行株(pdm09)引起猪流感[3]。A(H1N1) pdm09毒株由于宿主改变而对人类显示出更强的毒性[4]。A型流感病毒的分节段基因组有助于病毒基因重组，有可能出现新的亚型，往往更能有效地避开宿主免疫系统，导致病毒传播增强和大流行暴发。因此，预防和控制H1N1所致的流感具有重要的公共卫生意义。COVID-19 mRNA疫苗在疾病预防方面显示出良好效果，引起了公众的关注。这是有史以来第一次mRNA疫苗获准用于人类。mRNA疫苗的出现改变了疫苗的开发[5-7]。

血凝素(HA)是诱导针对流感病毒的保护性免疫反应的主要抗原，是关键的疫苗靶点[8]。HA在病毒表面以三聚体糖蛋白的形式表达，与靶细胞上的唾液酸结合以促进病毒进入宿主细胞并介导病毒包膜与晚期内体膜的融合。阻断HA的中和抗体可有效防止病毒进入靶细胞，并可保护宿主免受感染[9]。非编码区(UTR)在调节蛋白质表达以及影响mRNA的降解和翻译速率方面具有重要的细胞功能[10]。此外，5'UTR有助于稳定mRNA，3'UTR的长度对翻译效率有所影响[10]。例如，可以使用来自α和β球蛋白的3'-UTR[11]设计模板序列。本研究为了探究不同来源、不同排布的5 'UTR和3 'UTR序列对mRNA翻译效率的影响，拟构建3种体外转录系统：IVT-pGEM-T7-Hα(UTRs来自人源α球蛋白)、IVT-pGEM-T7-Mα(UTRs来自鼠源α球蛋白)、IVT-pGEM-n3(UTRs来自人源β球蛋白)。

较高的GC含量和较低的U含量不仅有助于提高mRNA稳定性，而且还能降低其免疫原性[12]。增加GC含量的方法包括优化开放阅读框(ORF)的密码子和去除尿苷[13]。此外，假尿苷(Ψ)或1-甲基假尿苷(m1Ψ)取代天然尿苷和胞苷修饰mRNA也能有效降低免疫原性，提高稳定性[14-15]。终止子通过调节mRNA的稳定性在蛋白质表达中发挥关键作用。终止子序列一般含有特征性元件，如富含AT的效率元件和富含A的定位元件(AAG/TAA)[16]，本研究设计选用强启动子TAAA和TAA来探究不同终止子对基因表达的影响。选用A/Jilin/JYT-01/2018(H1N1)亚型流感病毒的HA基因作为抗原基因，通过改变密码子中的GC含量对mRNA进行优化，并设计3种体外转录系统用于提升抗原基因的表达量，筛选最优的表达体系，为流感病毒mRNA疫苗的抗原设计和制备提供新思路，为疫苗动物试验的开展奠定基础。

材料与方法

1 材料

2×Phanta Max Master Mix和同源重组酶2×ClonExpress Mix购自南京诺唯赞生物科技有限公司；限制性核酸内切酶Pac Ⅰ、Nhe Ⅰ、Kpn Ⅰ、Xho Ⅰ、Xma Ⅰ购自美国NEB公司；无内毒素质粒大量提取试剂盒购自天根生化科技(北京)有限公司；T7-Flash ScribeTM Transcription Kit和Script CapTM Cap 1 Capping System购自美国CELLSCRIPT公司；N1-Methylpseudouridine-5,-Triphosphate购自美国Trilink Biotech公司；MEGAclearTM Kit Purification for Large Scale Transcription Reactions，LipofetamineTM 3000 Reagent和PierceTM ECL Western Blotting Substrate购自美国Thermo Fisher公司；质粒pGEM-3Zf-n3、pGEM-3Zf-T7-Mα、pGEM-3Zf-T7-Hα由军事兽医研究所分子病毒学与免疫学实验室保存。

2 方法

2.1 引物的设计与合成 利用CE Design设计特异性引物，由吉林省库美生物科技有限公司合成。引物名称、序列、产物长度见表1，下划线部分为Cla Ⅰ、Pac Ⅰ酶切位点。

2.2 目的基因的扩增与纯化 分别以合成的pVAX1-JLH1HA、pVAX1-HA基因为模板进行PCR扩增。PCR体系(50 μL)：2×Phanta Master Mix 1 μL模板1 μL，上、下游引物各2 μL，2×Phanta Max Buffer 25 μL，加水补足50 μL。PCR反应程序：95 ℃ 30 s；95 ℃ 15 s，70 ℃ 15s，72 ℃ 1 min 30 s，共35个循环；72 ℃延伸5 min。PCR产物4 ℃保存。取PCR扩增产物进行1.2 %琼脂糖凝胶电泳，采用FastPure Gel DNA Extraction Kit试剂盒回收与纯化目的基因片段，按照说明书操作。获得的基因片段分别命名为：Cla Ⅰ-JLH1HA-Pac Ⅰ、Cla Ⅰ-JLHA-Pac Ⅰ。

表1 引物信息
Table 1 Primer information

引物名称 Primer	引物序列 Primer sequence(5′-3′)	产物长度 Product length (bp)
Cla Ⅰ-n3-JLH1HA/JLHA-F	CAGCAAGATTAAGCCATCGATGCCACCATGAAGGCCATCC	
Pac Ⅰ-n3-JLH1HA-R	GCAAGAAAGCGAGCTTTAATTAATTTAGATACAGATGCGACACTGCA	1702
Pac Ⅰ-n3-JLHA-R	GCAAGAAAGCGAGCTTTAATTAATTAGATACAGATCCTGCACTGCAGG	1701
Cla Ⅰ-T7-Hα/Mα-JLH1HA/JLHA-F	AGAAAAAATATAAGAATCGATGCCACCATGAAGGCCATCC	
Pac Ⅰ-T7-Mα-JLH1HA-R	CCCGCAAGGCAGCTTAATTAATTTAGATACAGATGCGACACTGCA	1702
Pac Ⅰ-T7-Mα-JLHA-R	CCCGCAGAAGGCAGCTTAATTAATTAGATACAGATCCTGCACTGCAGG	1701
Pac Ⅰ-T7-Hα-JLH1HA-R	CACCGAGGCTCCAGCTTAATTAATTTAGATACAGATGCGACACTGCA	1702
Pac Ⅰ-T7-Hα-JLHA-R	CACCGAGGCTCCAGCTTAATTAATTAGATACAGATCCTGCACTGCAGG	1701

2.3 重组质粒构建与鉴定 将上述纯化产物与载体骨架进行连接，构建重组质粒 pGEM-n3-JLH1HA、pGEM-n3-JLHA、pGEM-T7-Mα-JLH1HA、pGEM-T7-Mα-JLHA、pGEM-T7-Hα-JLH1HA、pGEM-T7-Hα-JLHA。重组体系：线性化载体 2 μL，纯化产物 8 μL，2×ClonExpress Mix 10 μL。反应条件：50 ℃ 30 min。将连接产物进行转化、培养及单菌落鉴定。鉴定正确后于 LB 培养基扩增培养（含氨苄青霉素），提取质粒进行双酶切鉴定，由吉林省库美生物科技有限公司进行测序鉴定。

2.4 重组质粒线性化处理 按照无内毒素大提试剂盒说明书制备质粒并进行酶切处理。单酶切体系：限制性内切酶 Xho Ⅰ 4 μL，重组质粒（pGEM-n3-JLH1HA、pGEM-n3-JLHA、pGEM-T7-Mα-JLH1HA、pGEM-T7-Mα-JLHA、pGEM-T7-Mα-JLH1HA、pGEM-T7-Mα-JLHA）16 μg，10×CutSmart Buffer 5 μL，加水补至 50 μL。反应条件：37 ℃ 3 h。

2.5 体外转录、纯化及加帽反应 将上述线性化产物进行体外转录，使用 T7-Fla-sh ScribeTM Transcription Kit，配制体外转录体系时用 N1-Methylpseudourid-ine-5′-Triphosphate（Trilink Biotech）替换 UTP。采用 MEGAclearTM Kit Purification for Large Scale Transcription Reactions 试剂盒进行纯化，采用 Script C-apTM Cap 1 Capping System 进行加帽反应，最终获得产物记作 Cap-mRNA。具体操作均参照试剂盒说明书。

2.6 Cap-mRNA 体外转染细胞试验 将 A549 细胞以 1×10^5 个/孔接种于 24 孔板，当细胞密度达到 80% 时，将转染试剂 LipofetamineTM 3000 Reagent 和 Cap-mRNA 按照体积与质量比为 1 μL∶2 μg 的比例混匀，室温静置 15 min 后滴入孔中，放入温箱继续培养 24 h，收取细胞。

2.7 Cap-mRNA 功能验证

2.7.1 Western blot 验证 当 pGEM-n3-JLH1HA、pGEM-n3-JLHA、pGEM-T7-Mα -JLH1HA、pGEM-T7-Mα-JLHA、pGEM-T7-Hα-JLH1HA、pGEM-T7-Hα-JLHA 的 Cap-mRNA 转染 A549 细胞 24 h 后，使用 RIPA 细胞裂解液（100∶1 加入蛋白酶抑制剂 PMSF）充分裂解细胞并转入 1.5 ml EP 管中，加入 5×SDS Loading Buffer，变性后收集样品进行 10% SDS-PAGE 电泳。采用湿转法将凝胶中的蛋白转印到 NC 膜上，用 5% 脱脂乳室温封闭 2 h；加入兔源 Influenza A Virus Hemagglutinin/HA 抗体，室温孵育 2 h，用 TBST 洗 3 次，8 min/次；加入 HRP 标记的山羊抗兔 Ig（H+L）抗体，室温孵育 30 min，洗涤后显色并拍照。

2.7.2 间接免疫荧光法验证 6 组 Cap-mRNA 转染 A549 细胞 24 h 后弃去细胞悬液并使用多聚甲醛固定液固定 10 min，PBST 洗涤 2 次，5 min/次；使用 3% BSA 封闭液室温作用 30 min，PBST 洗涤 2 次，5 min/次；加入兔源 Influenza A Virus Hemagglutinin/HA 抗体，37 ℃孵育 1 h（1∶800 稀释），PBST 洗涤 3 次，5 min/次；加入 Cy3 标记山羊抗兔 Ig（H+L）抗体，37 ℃孵育 30 min，PBST 洗涤 3 次，5 min/次；加入 DAPI 染色液进行细胞核染色，室温放置 3～5min，PBST 洗涤 2 次，5 min/次，荧光显微镜下观察结果。

结 果

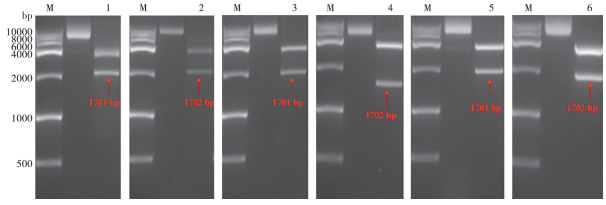
1 重组质粒的鉴定

6 种重组质粒经 PCR 扩增后进行 1.2% 琼脂糖凝胶电泳，均获得符合预期大小的目的基因片段（Cla Ⅰ-pGEM-n3-JLH1HA-Pac Ⅰ、Cla Ⅰ-pGEM-T7-Mα-JLH1HA-Pac Ⅰ、Cla Ⅰ-pGEM-T7-Hα-JLH1HA-Pac Ⅰ 均为 1702 bp，Cla Ⅰ-pGEM-n3-JLHA-Pac Ⅰ、Cla Ⅰ-pGEM-T7-Mα-JLHA-Pac Ⅰ、Cla Ⅰ-pGEM-T7-Hα-JLHA-Pac Ⅰ 均为 1 701 bp）。Cla Ⅰ和 Pac Ⅰ双酶切重组质粒，得到与目的基因大小相符的片段和载体片段（图 1）。重组质粒交由吉林省库美生物科技有限公司测序，结果均正确。

2 重组质粒的线性化处理

经 Xho Ⅰ单酶切处理后得到重组质粒线性化产物（图 2），片段大小与预期相符（pGEM-n3-JLH1HA：5 373 bp；pGEM-n3-JLHA：5 374 bp；pGEM-T7-Hα-JLH1HA：5 145 bp；pGEM-T7-Hα-JLHA：5 146 bp；

pGEM-T7-Mα-JLH1HA：5 155 bp；pGEM-T7-Mα-JLHA：5 156 bp）。

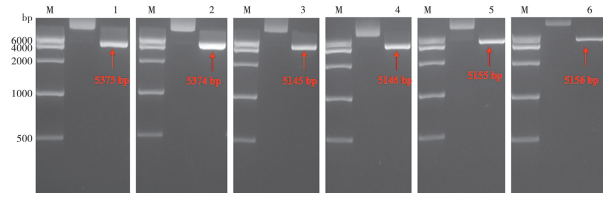


M DNA标志物 1 pGEM-T7-Hα-JLHA双酶切 2 pGEM-T7-Hα-JLH1HA双酶切 3 pGEM-T7-Mα-JLHA双酶切 4 pGEM-T7-Mα-JLH1HA双酶切 5 pGEM-n3-JLHA双酶切 6 pGEM-n3-JLH1HA双酶切

图1 重组质粒双酶切鉴定

M DNA maker 1 Double digestion pGEM-T7-Hα-JLHA 2 Double digestion pGEM-T7-Hα-JLH1HA 3 Double digestion pGEM-T7-Mα-JLHA 4 Double digestion pGEM-T7-Mα-JLH1HA 5 Double digestion pGEM-n3-JLHA 6 Double digestion pGEM-n3-JLH1HA

Fig. 1 Identification of recombinant plasmid by double enzyme digestion



M DNA标志物 1 pGEM-n3-JLHA Xho Ⅰ单酶切 2 pGEM-n3-JLH1HA Xho Ⅰ单酶切 3 pGEM-T7-Mα-JLHA Xho Ⅰ单酶切 4 pGEM-T7-Mα-JLH1HA-Xho Ⅰ单酶 5 pGEM-T7-Hα-JLHA Xho Ⅰ单酶切 6 pGEM-T7-Hα-JLH1HA Xho Ⅰ单酶切

图2 重组质粒单酶切鉴定

M DNA maker 1 pGEM-n3-JLHA-Xho Ⅰ 2 pGEM-n3-JLH1HA-Xho Ⅰ 3 pGEM-T7-Mα-JLHA-Xho Ⅰ 4 pGEM-T7-Mα-JLH1HA-Xho Ⅰ 5 pGEM-T7-Hα-JLHA-Xho Ⅰ 6 pGEM-T7-Hα-JLH1HA-Xho Ⅰ

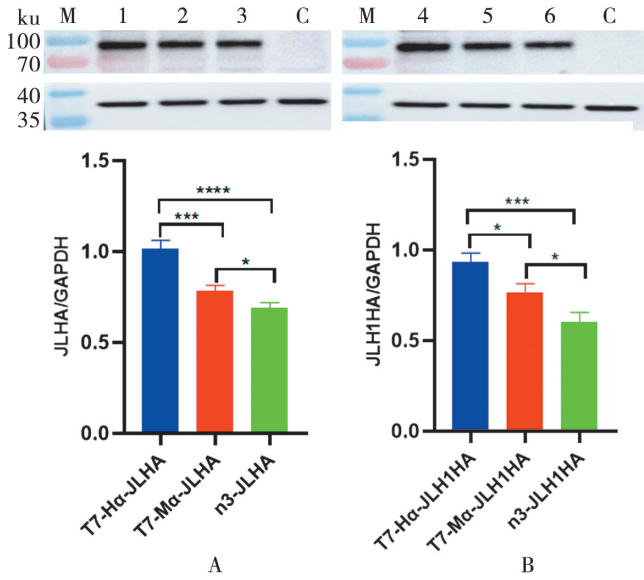
Fig. 2 Single restriction endonuclease digestion of recombinant plasmid

3 Western blot验证6组Cap-mRNA的表达

在6组Cap-mRNA中，分别以兔源Influenza A Virus Hemagglutinin/HA、内参GAPDH抗体为一抗，HRP标记的山羊抗兔IgG抗体为二抗进行Western blot鉴定，结果如图3。Cap-mRNA转染A549细胞24 h后均可检测到对应的目的蛋白。3种载体表达的目的蛋白含量不同，以pGEM-T7-Hα载体蛋白表达量较高，pGEM-T7-Mα载体蛋白表达量次之，pGEM-n3载体蛋白表达量偏低。其中，内参蛋白相对分子质量为37×10^3，目的蛋白理论值为63.49×10^3，实际结果较理论值偏高。

4 IFA验证目的蛋白的表达

重组质粒Cap-mRNA转染A549细胞36 h后固定细胞，以兔源Influenza A Virus Hemagglutinin/HA抗体为一抗，Cy3标记的山羊抗兔Ig(H+L)抗体为二抗进行间接免疫荧光验试验，结果如图4。mRNA转染细胞后pGEM-T7-Hα、pGEM-T7-Mα、pGEM-n33种载体均检测到有目的蛋白的表达，荧光显微镜下显示红色荧光，而对照组未检测到荧光。



M 蛋白分子质量标准 1 pGEM-T7-Hα-JLHA 2 pGEM-T7-Mα-JLHA 3 pGEM-n3-JLHA C 对照 4 pGEM-T7-Hα-JLH1HA 5 pGEM-T7-Mα-JLH1HA 6 pGEM-n3-JLH1HA 注：组间比较，[a] $P<0.05$；[b] $P<0.01$。

图3 表达蛋白的Western blot检测(A)与反应条带的灰度分析(B)

M Protein maker 1 pGEM-T7-Hα-JLHA 2 pGEM-T7-Mα-JLHA 3 pGEM-n3-JLHA C Control 4 pGEM-T7-Hα-JLH1HA 5 pGEM-T7-Mα-JLH1HA 6 pGEM-n3-JLH1HA Notes：Comparison between groups，[a] $P<0.05$；[b] $P<0.01$.

Fig. 3 Western blot analysis of expression protein (A) and gray scale analysis of reaction strip (B)

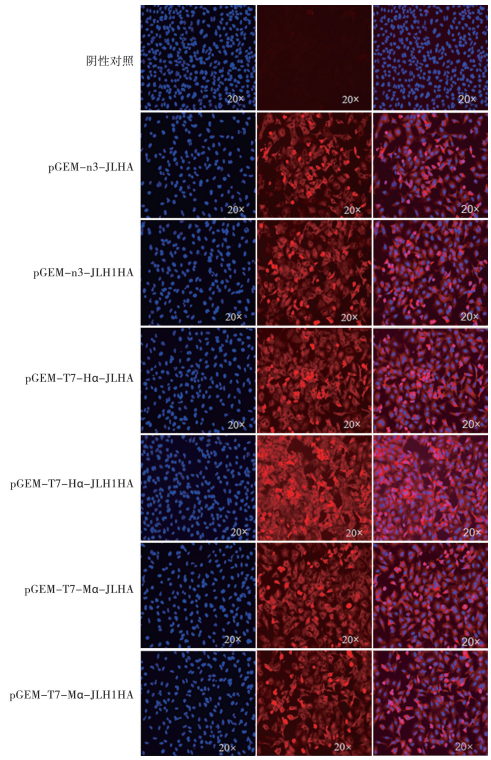


图4 间接免疫荧光法验证目的蛋白的表达

Fig. 4 Parallel verification of indirect immun of luorescence

讨 论

甲型流感病毒可感染各种禽类和哺乳动物[17-24]，重组和点突变事件促进了流感病毒基因组的进化。因此，新的亚型和基因型不断出现，这可能会在免疫缺陷人群中引发大流行[25-27]，严重危害人类健康和社会经济发展。接种疫苗是预防和控制流感大流行最有效的手段。随着近年来新冠疫情的爆发，mRNA疫苗研发成为高度关注的领域之一，带动了mRNA药物行业的发展。与传统疫苗相比，mRNA疫苗具有突出的优势：生产快速。当突发传染病爆发时可在短时间内提供大批量的供应。

3′UTR是mRNA分子的细胞内动力学的关键调节因子之一，有研究表明3′UTR元件的长度与其稳定性呈负相关：具有较长3′UTR的mRNA具有较短的半衰期[28]，而具有较短3′UTR的mRNA翻译效率较低[29]。mRNA治疗中常用的3′UTR来源于α和β球蛋白[30]，在患血友病的红细胞和人类网织红细胞中被证实具有增加mRNA半衰期的能力[31]。5′UTR是基因表达最终产物的关键调控因子可以增强或抑制mRNA的翻译状态[32]。本研究从5′UTR和3′UTR入手设计了3种载体，发现不同载体构建的转录体系表达量存在显著差异，以UTR为人源α球蛋白的载体表达蛋白含量最高。有研究检测到富含鸟苷酸或胞嘧啶(富含G或C)的基因的mRNA水平增加可能是由两种机制引起的：mRNA的合成增加或mRNA的降解减少[13]。文献[33-35]报道mRNA水平的提高导致密码子优化(富含GC)基因的蛋白质水平的提高。而本研究中两种GC含量不同的基因序列蛋白表达水平有待通过进一步设计免疫试验来验证。

本研究构建了6组含疫苗抗原基因的重组质粒。通过体外转录、纯化、加帽反应获得6组功能性mRNA，转染A549细胞通过Western blot和间接免疫荧光试验证明均能表达目的蛋白，且pGEM-T7-Hα、pGEM-T7-Mα、pGEM-n3 3种载体的蛋白表达量存在差异，以pGEM-T7-Hα载体表达效果较好。由此证明了本研究抗原设计的可行性，为mRNA疫苗的抗原设计提供了新思路，为疫苗的动物实验及免疫效果评价奠定了基础。

【参考文献】

[1] Yu Y, Tazeem, Xu Z, et al. Design and synthesis of heteroaromatic-based benzenesulfonamide derivatives as potent inhibitors of H5N1 influenza A virus[J]. Medchemcomm, 2019(101): 89-100.

[2] Oliveira CM, Soares VJ, Rechenmacher C, et al. From H1N1 to COVID-19: What we have seen in children with hemoglobinopathies[J]. Clinics (Sao Paulo), 2022(77): 100004.

[3] Lubna S, Chinta S, Burra P, et al. New substitutions on NS1 protein from influenza A (H1N1) virus: Bioinformatics analyses of Indian strains isolated from 2009 to 2020[J]. Health Sci Rep, 2022(53): e626.

[4] Zhong X, Madrahimov A, Helika T, et al. Molecular phylogeny and evolutionary dynamics of influenza A nonstructural (NS) gene[J]. Infect Genet Evol, 2014(22): 192-200.

[5] Tanzilli A, Pace A, Ciliberto G, et al. Ire study: safety and efficacy of the BNT162b2 mRNA COVID-19 vaccine in patients with brain tumors[J]. Neurol Sci, 2022(43): 3519-3522

[6] Walter EB, Talaat KR, Sabharwal C, et al. Evaluation of the BNT162b2 Covid-19 Vaccine in Children 5 to 11 Years of Age[J]. N Engl J Med, 2022(3861): 35-46.

[7] Du Y, Chen L, Shi Y. Safety, immunogenicity, and efficacy of COVID-19 vaccines in adolescents, children, and infants: A systematic review and meta-analysis[J]. Front Public Health, 2022(10): 829176.

[8] Arunachalam AB, Post P, Rudin D. Unique features of a recombinant haemagglutinin influenza vaccine that influence vaccine performance[J]. NPJ Vaccines, 2021(61): 144.

[9] Cowling BJ, Lim W, Perera R, et al. Influenza hemagglutination-inhibition antibody titer as a mediator of vaccine-induced protection for influenza B[J]. Clin Infect Dis, 2019(6810): 1713-1717.

[10] Deng Z, Tian Y, Song J, An G and Yang P. mRNA Vaccines: The Dawn of a New Era of Cancer Immunotherapy[J]. Front Immunol, 2022(13): 887125.

[11] Orlandini AG, Poleganov MA, C Rechner, et al. Improving mRNA-Based therapeutic gene delivery by expression-augmenting 3' UTRs identified by cellular library screening[J]. Mol Ther, 2019(274): 824-836.

[12] Kudla G, Lipinski L, Caffinn F, et al. High guanine and cytosine content increases mRNA levels in mammalian cells[J]. PLoS Biol, 2006(46): e180.

[13] Mauger D, Cabral B, Presnyak V, et al. mRNA structure regulates protein expression through changes in functional half-life[J]. Proc Natl Acad Sci, 2019(11648): 24075-24083.

[14] Durbin A, Wang C. RNAs Containing modified nucleotides fail to trigger RIG-I conformational changes for innate immune signaling[J]. mBio, 2016: 75

[15] Andriesn O, Smedt SD, Weiss R, et al. N(1)-methylpseudouridine-incorporated mRNA outperforms pseudouridine-incorporated mRNA by providing enhanced protein expression and reduced immunogenicity in mammalian cell lines and mice[J]. J Con Rel 2015(217): 337-344.

[16] Kumarn P, Sahoo D, Sharma D. The identification of novel promoters and terminators for protein expression and metabolic engineering applications in [J]. Metabolic Engineering Communications, 2021(12): e00160.

[17 Chauhan R, Gordon M. Review of genome sequencing technologies in molecular characterization of influenza A viruses in swine[J]. J Vet Diagn Invest, 2022(342): 177-189.

[18] Chauhan RP, Gordon ML. A systematic review analyzing the prevalence and circulation of influenza viruses in swine

population worldwide[J]. Pathogens, 2020: 95.
[29] Chauhan R, Gordon M. A systematic review of influenza A virus prevalence and transmission dynamics in backyard swine populations globally[J]. PHM, 2022(81): 10.
[20] Ciminski K, Pfaff F, Beer M, et al. Bats reveal the true power of influenza A virus adaptability[J]. PLoS pathogens, 2020(164): e1008384.
[21] Liu J, Li Z, Cui Y, et al. Emergence of an Eurasian avian-like swine influenza A (H1N1) virus from mink in China[J]. Vet Microbiol, 2020(240): 108509.
[22] Smith G, Vijaykrishna D, Bahl J, et al. Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza A epidemic [J]. Nature, 2009(4597250): 1122-1125.
[23] Venter M, Treurnicht F, Buys A, et al. Risk of human infections with highly pathogenic H5N2 and low pathogenic H7N1 avian influenza strains during outbreaks in ostriches in South Africa [J]. JID, 2017(216): S512-S519.
[24] Zhao P, Sun L, Xiong J, et al. Semiaquatic mammals might be intermediate hosts to spread avian influenza viruses from avian to human[J]. Sci Rep, 2019(91): 11641.
[25] Bertram S, Glowacka I, Steffen I, et al. Novel insights into proteolytic cleavage of influenza virus hemagglutinin[J]. Rev Med Virol, 2010(205): 298-310.
[26] Neumann G, Noda T, Kawaoka Y. Emergence and pandemic potential of swine-origin H1N1 influenza virus[J]. Nature, 2009 (4597249): 931-939.
[27] Parrish C, Kawaoka Y. The origins of new pandemic viruses: the acquisition of new host ranges by canine parvovirus and influenza A viruses[J]. Ann Rev of Microbio, 2005(59): 553-586.
[28] Schwanhausser B, Busse D, Li N, et al. Corrigendum: Global quantification of mammalian gene expression control[J]. Nature, 2013(4957439): 126-127.
[29] Tanguay R, Gallie D. Translational efficiency is regulated by the length of the 3' untranslated region[J]. MCB, 1996(161): 146-156.
[30] Peixeiro I, Silva A, Romao L. Control of human beta-globin mRNA stability and its impact on beta-thalassemia phenotype [J]. Haematologica, 2011(966): 905-913.
[31] Waggoner S, Liebhaber S. Regulation of alpha-globin mRNA stability[J]. Exp Biol Med (Maywood, N. J.), 2003(2284): 387-395.
[32] Sendoel A, Dunn J, Rodriguez E, et al. Translation from unconventional 5' start sites drives tumour initiation[J]. Nature, 2017(5417638): 494-499.
[33] Graf M, Bojak A, Deml L, et al. Concerted action of multiple cis-acting sequences is required for Rev dependence of late human immunodeficiency virus type 1 gene expression[J]. J Virol, 2000 (7422): 10822-10826.
[34] Kotsopoulou E, Kim V, Kingsman A, et al. A Rev-independent human immunodeficiency virus type 1 (HIV-1)-based vector that exploits a codon-optimized HIV-1 gag-pol gene [J]. J VIROL, 2000(7410): 4839-4852.
[35] Nguyen K, llano M, Akari H, et al. Codon optimization of the HIV-1 vpu and vif genes stabilizes their mRNA and allows for highly efficient Rev-independent expression[J]. Virology, 2004 (3192): 163-175.

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[15] Hirschinger J, Munoz MC, Hingrat Y, et al. Exposure to and Circulation of Avian Influenza and Newcastle Disease Viruses in Peridomestic Wild Birds in the United Arab Emirates[J]. J Wildl Dis, 2020, 56(2): 437-442.
[16] Cappelle J, Caron J, Gil P, et al. Empirical analysis suggests continuous and homogeneous circulation of Newcastle disease virus in a wide range of wild bird species in Africa[J]. Epidemiol Infect, 2015, 143(6): 1292-1303.
[17] Bansal N, Singh R, Chaudhary D, et al. Prevalence of Newcastle Disease Virus in Wild and Migratory Birds in Haryana, India[J]. Avian Dis, 2022, 66(2): 141-147.
[18] Wang JJ, Lv Y, Zhao YL, et al. Pathogenic Surveillance and Phylogenetic Analyses of Class-Ⅰ Newcastle Disease Viruses from 2011 to 2015[J]. CNKI JTP, 2017, 33(2): 211-215.
[19] Trogu T, Canziani S, Salvato S, et al. Survey on the presence of viruses of economic and zoonotic importance in avifauna in Northern Italy[J]. Microorganisms, 2021, 9(9): 1957.
[20] European Food Safety Authority, Opinion of the Scientific Panel on Animal Health and Welfare (AHAW) to review Newcastle disease focussing on vaccination worldwide in order to determine its optimal use for disease control purposes[J]. EFSA, 2007, 5 (5): 477.
[21] Wang JJ, Yu XH, Jiang WM, et al. Pathogenic Surveillance and Phylogenetic Analysis on Class Ⅰ Newcastle Disease Virus during 2016 to 2019 in China[J]. CNKI JTP, 2020, 37(11): 1-5.
[22] Xiang B, Chen L, Cai J, et al. Insights into genomic epidemiology, evolution, and transmission dynamics of genotype VII of class ii newcastle disease virus in China[J]. Pathogens, 2020, 9(10): 837.
[23] Xiang B, Han L, Gao P, et al. Spillover of Newcastle disease viruses from poultry to wild birds in Guangdong province, southern China[J]. Infect Genet Evol, 2017(55): 199-204.
[24] Snoeck CJ, Adeyanju AT, Owoade AA, et al. Genetic diversity of newcastle disease virus in wild birds and pigeons in West Africa [J]. Appl Environ Microbiol, 2013, 79(24): 7867-7874.
[25] de Graaf JF, van Nieuwkoop S, de Meulder D, et al. Assessment of the virulence for chickens of Newcastle Disease virus with an engineered multi-basic cleavage site in the fusion protein and disrupted V protein gene [J]. Vet Microbiol, 2022 (269): 109437.
[26] Alexander DJ, Gordon Memorial Lecture. Newcastle disease[J]. Br Poult Sci, 2001, 42(1): 5-22.

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