mRNA stability and translational efficiency regulated by miRNA binding site in UTR

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mRNA stability and translational efficiency regulated by miRNA binding site in UTR

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The Master's Thesis

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Kyu Ho Hwang

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This certifies that the Master's Thesis

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June 2022

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2022년 6월 황규호

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ABSTRACT

mRNA stability and translational efficiency regulated by miRNA binding site in UTR

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(Directed by Professor Jong In Yook, D.D.S., Ph.D.)

Although mRNA vaccine technology is developing rapidly after Covid-19, problems such as low stability and intracellular delivery efficiency are still raised. miRNA is a single-stranded RNA molecule with a length of ~22 nucleotides(nt), which binds to the 5' and 3' untranslated regions of the target mRNA and acts as an important regulator to suppress the expression of the corresponding gene. Recently, there have been many

studies on the regulation of viruses by miRNA, and studies have been reported that, unlike the existing miRNA interactions, miR-17 and let-7 directly bind to RNA viruses to rather stabilize RNA virus proliferation. In addition, the importance of the location of the miRNA binding site targeting the viral RNA was emphasized through studies that the secondary structure of the virus changes after miRNA binds to the viral RNA. Therefore, in this study, we developed a method to quantify the stability of mRNA and changed the binding energy of the site that can directly bind to miRNA, the presence or absence of 5'UTR, 5' capping structure, zipper structure, and hairpin structure. We tried to optimize the structure that can increase the stability and translation efficiency of let-7a mRNA vaccine.

The result is as follows.

1) To check translation efficiency, a reporter assay system using luciferase was established.

2) When there was a miRNA binding site with high miRNA-mRNA binding energy in the 3' UTR site after luciferase, the translation efficiency of luciferase by let-7a was increased.

3) The translation efficiency of let-7a was higher than that of BVDV and CHIKV viral RNAs having let-7f binding sites used as controls.

4) The higher the miRNA-mRNA binding energy, the smaller the decrease in

translation efficiency with time, and the higher the translation efficiency after 48 hours. On the other hand, when miRNA-mRNA was combined as a seed match, translation efficiency was rather reduced.

5) In the presence of a let-7a binding site in the 5' UTR region, the translation efficiency was lower than in the presence of a binding site in the 3'UTR, but the translation efficiency was highest when both the 5'UTR and 3'UTR regions were present. .

6) The 5' capping structure increased translation efficiency, and the higher the binding energy in the zipper structure, the higher the stability of luciferase mRNA.

7) Hairpin structure was not effective in increasing translation efficiency.

From the above results, it was confirmed that the translation efficiency of luciferase was increased when miRNA binding site with high binding energy to let-7a was added to the 5'UTR and 3'UTR regions. Based on this, if a construct that increases the stability of mRNA is developed, it is thought that it will contribute to research on new mRNA vaccines with improved stability.

Key words : miRNA, Let-7a, MBS, Translational efficiency, mRNA stability

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I. INTRODUTION

COVID-19 (coronavirus infection 2019) is a disease caused by a virus called SARS-CoV-2, and according to WHO statistics, it was reported that until recently, more than 500 million people were diagnosed with the coronavirus, and more than 6 million people died. Vaccine technology has advanced rapidly since the novel coronavirus infection (COVID-19), but there is still a sense that it is insufficient to conquer this virus. The production and distribution of vaccines are not keeping up with the rate of virus spread, and it is

difficult to say that the vaccines currently in use are sufficient. Pfizer's and Moderna's mRNA vaccines inject a gene mRNA into the body to produce an antigen, a spike protein, in cells. However, since it has to be distributed in the cold chain at minus 20 degrees Celsius or 70 degrees Celsius, considerable costs are incurred, and there are still many concerns about the continuity of the vaccine once administered.(Crommelin et al. 2021; Lu et al. 2020; Noorimotlagh et al. 2020; Sanami et al. 2020; V'kovski et al. 2021)

microRNA(miRNA) is a small non-coding RNA molecule composed of 21-22 nucleotides, and functions such as RNA silencing and regulation of gene expression after transcription. It is expressed not only in humans but also in plants and viruses, and plays an important role in various physiological phenomena such as development, differentiation, cell death, and cancer. miRNAs interact with multiple target mRNAs by binding to the 5' and 3' untranslated regions (UTRs) adjacent to the mRNA coding sequence. It inhibits translation of target mRNA or promotes deadenylation and subsequent degradation. The interaction between miRNA and target mRNA is dynamic and depends on many factors such as the miRNA's intracellular localization and affinity.(Bushati and Cohen 2007; Fabian, Sonenberg, and Filipowicz 2010; Lee et al. 2009; Leppek, Das, and Barna 2018; Mukhopadhyay and Mussa 2020; O'Brien et al. 2018)

A recent study reported a correlation between miRNA and coronavirus. Numerous studies have revealed that non-coding RNAs encoded by the host and virus play an important role in the pathology and pathogenesis of human viral infection.(Arghiani,

Nissan, and Matin 2021; El-Nabi, Elhiti, and El-Sheekh 2020; Fani et al. 2021; Khan et al. 2020; Lei et al. 2022) While, miRNA downregulate gene expression by binding to the UTR of the target mRNA, in this case, the microRNA anneals to the 5′UTR of the viral genomes and upregulates the viral lifecycle. In the current understandings of the mechanisms by which miR-122 promotes the HCV lifecycle, and its contributions to pathogenesis.(Jopling, Schütz, and Sarnow 2008)

Let-7 was one of the first miRNAs discovered and plays a role in regulating the developmental timing of C. elegans. It is also known to exist in Drosophila and mammals, and its role is known to be evolutionarily conserved, and all let-7 family members can function as tumor suppressors. Replication of pestiviruses, a major threat to the milk and meat industry, relied heavily on the interaction of cellular miR-17 and let-7 with the viral 3'UTR. In contrast to standard miRNA interactions, studies have shown that miR-17 and let-7 binding enhance pestivirus translation and RNA stability.(Liu et al. 2015; Long et al. 2009; Lytle, Yario, and Steitz 2007; Scheel et al. 2016; Wakiyama et al. 2007; Wang et al. 2019)

Therefore, in this study, we developed a method to quantify the stability of mRNA and changed the binding energy of the site that can directly bind to miRNA, the presence or absence of 5'UTR, 5' capping structure, zipper structure, and hairpin structure. As a result, We tried to optimize the structure that can increase the stability and translation efficiency of let-7a mRNA vaccine.

II. MATRIAL AND METHODS

1. Cell Lines and Cell Culture

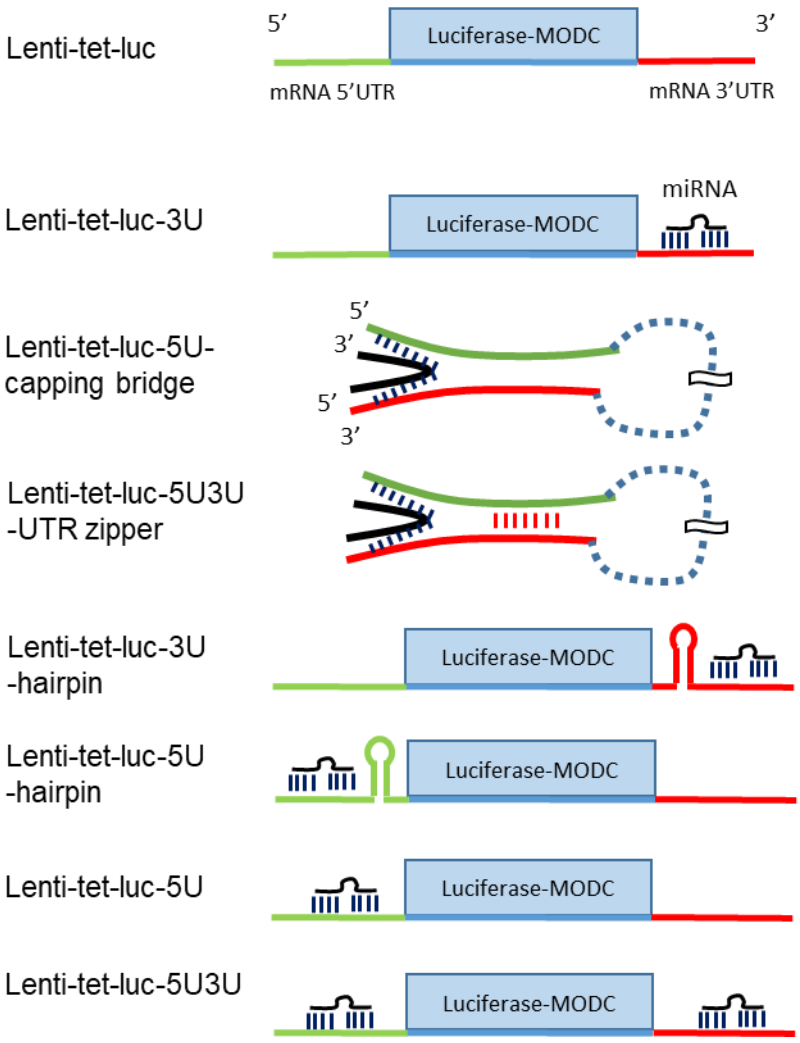
Experiments were carried out in HEK 293 cells, a cell line displaying an epithelial morphology isolated from human embryonic kidneys. HEK 293 cell were grown in DMEM (Dullbecco's Modified Eagle's Medium; BioWhittaker, USA) supplemented with 10% fetal bovine serum (FBS; gibco), 1% penicillin/streptomycin (BioWhittaker). Cells were cultured in a humidified incubator at 37℃ and 5% CO2.

2. DNA constructs

Tetracycline-inducible luciferase expression vectors (lenti-tet-luc) were generated with the pCW57-RFP-P2A-MCS (Addgene #78933) by replacing RFP. By annealing the oligo, the 3'UTR vector was subcloned downstream of luciferase and the 5'UTR vector was subcloned upstream of luciferase. The let-7a miRNA binding site was located in the 3' UTR and 5' UTR, and the 5' capping structure, zipper structure and hairpin structure are summarized (Table 1). To show the interaction with let-7a, we first obtained the sequence of the gene of let-7a from miRbase(https://www.mirbase.org/cgi-bin/mature.pl?mature_acc=MIMAT00000062). Then we made a design that binds to let-

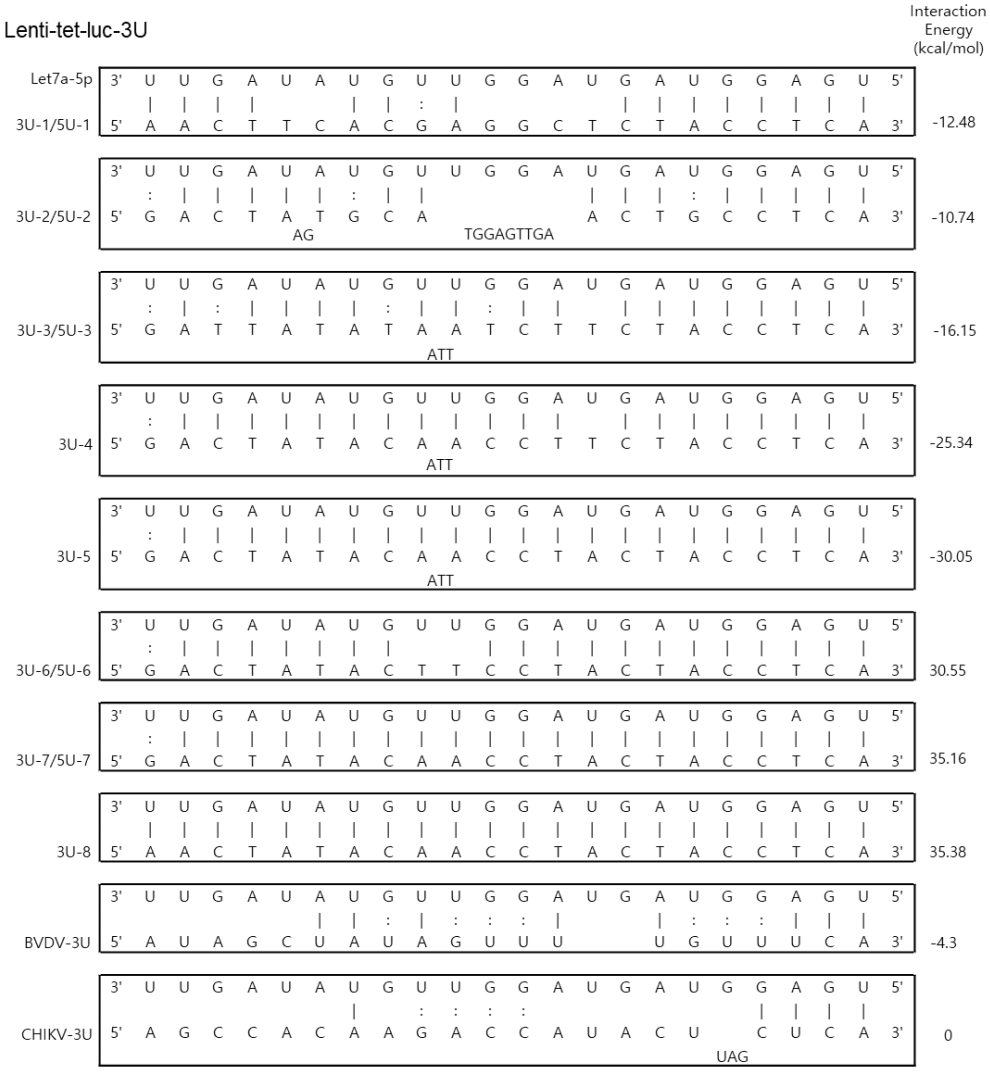
7a, and calculated the interaction energy with let-7a using the Freiburg RNA tool IntaRNA-RNA-RNA interaction(http://rna.informatik.uni-freiburg.de/IntaRNA/). The sequence and interaction energy of each binding site were summarized (Table 2). All vectors were verified by DNA sequencing.

Table 1. Basic scheme of constructs

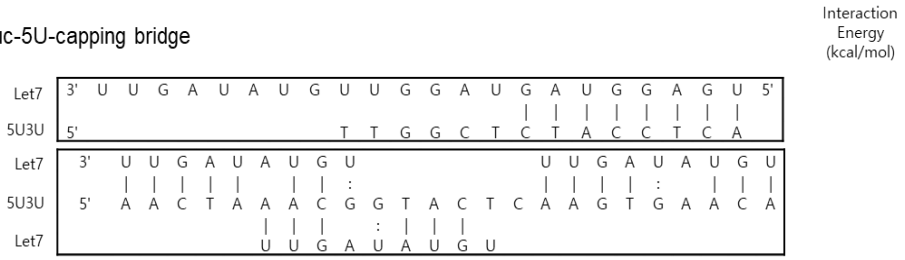


Basic scheme		
Luciferase-Modc control vector		
3U miR interaction site	Artificial binding site	3U-1, -2, -3, -4, -5, -6, -7, -8
	Viral RNA	BVDV, CHIKV
5U interaction capping bridge		5’ capping bridge
5U-3U interaction and UTR zipper		UTR zipper, zMax-1, zMax-2
3U hairpin		TAR from HIV-1
5U hairpin		hairpin from c-jun
5U miR interaction site	Artificial binding site	5U-1,2,3,6,7
5U, 3U miR interaction site	Artificial binding site	5U-7 / 3U-3,6,7

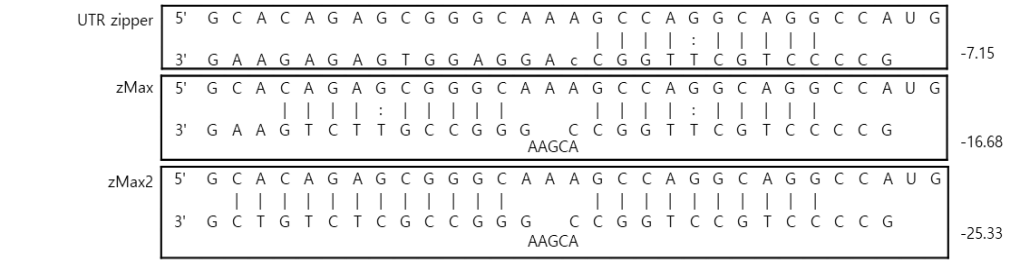
Table 2. Sequence & interaction energy of miRNA binding site



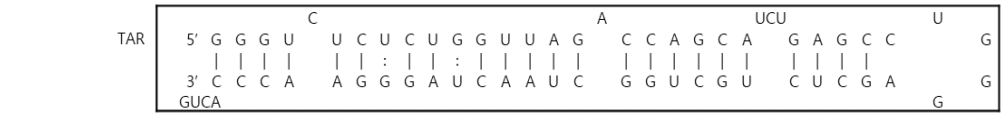
Lenti-tet-luc-5U-capping bridge



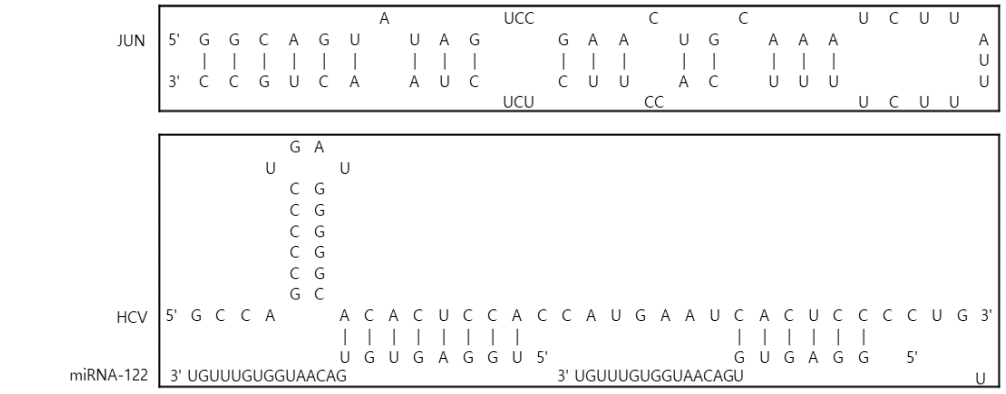
Lenti-tet-luc-5U3U-UTR zipper



Lenti-tet-luc-3U-hairpin



Lenti-tet-luc-5U-hairpin



3. Cell transfection, chemical reagent

HEK 293 cells were transfected with luciferase vector 30ng and Renilla 1ng in 6 wells by Lipofectamine 2000 according to the manufacturer's protocol (Invitrogen, 11668-019). The transfected cells are subcultured into 4x10^5 24 wells and treated with Doxycycline (0, 5ug/ml) for 16 hours to activate the Tet-on/off system. Samples were prepared for each hour by changing to Tet free FBS media (0, 6, 24, 48 hours).

4. Luciferase reporter assay

The prepared 24 wells were measured for luciferase reporters using the Luciferase Assay system (Promega) according to the manufacturer's protocol. 20ul of Luciferase assay reagent was put into 20ul of the prepared sample, and after 1s measurement, 20ul of mixed Stop&glow was put in and Renilla was measured. Luciferase reporter measurements were corrected with Renilla measurements.

5. Quantitative real time PCR

RNA was prepared using Trizol (Invitrogen) according to the manufacturer's protocol. cDNA was prepared at 1ug RNA using CycleScript RT PreMix (dT20, BIONEER) under the following PCR conditions: 20℃ for 10 minutes, 45℃ for 60 minutes, 95℃ for 5 minutes, and thereafter at 4℃. Quantitative real-time RT-PCR was performed in a 20ul

reaction volume containing 10ul of Applied Biosystems 2X SYBR Green Mix, 1ul of each primer and 1ul cDNA. Real-time PCR was performed using an Applied Biosystems STEP One detection system. Reactions occurred at 95 ℃ for 10 min in the holding phase, 95 ℃ for 15 s, and 60 ℃ for 40 cycles in the cycling phase for 1 min and then at 95 ℃ for 15 s. The qPCR primer was Luciferase, Ampicillin resistant: Luciferase forward primer, 5'-cgaaggttgtggatctggata-3' ; Reverse primer, 5'-cgcttccggattgtttacata-3', Ampicillin resistant forward primer, 5'-ccagaaacgctggtgaaagta-3'; Reverse primer, 5'-ggcgaaaactctcaaggatct-3' The expression of the ΔCt value of each sample was calculated by normalizing it to the Amp-Registant value in the triplicate experiment.

6. Translational efficiency

Translational efficiency at each time was obtained by dividing the normalized luciferase reporter (translation level) by the normalized luciferase qPCR (transcript level) value.

III. RESULTS

1. Increased translation efficiency of luciferase by let-7a

Experiment with Lenti-tet-luc as a control vector and 3U-1,2,3 vector containing let-7a miRNA binding site(MBS) in 3' UTR to increase mRNA stability and translational efficiency in the presence of let-7a miRNA binding site (MBS) proceeded. As a result of the experiment, the transcripts level of the control group was the highest (Figure 1a). On the other hand, the translation level was high in 3U-3, and the translational efficiency obtained from these values was also high in 3U-3. Among the three experimental groups, the interaction energy of 3U-3 with let-7a was the highest, and the translation level and efficiency were increased (Figure 1b, c).

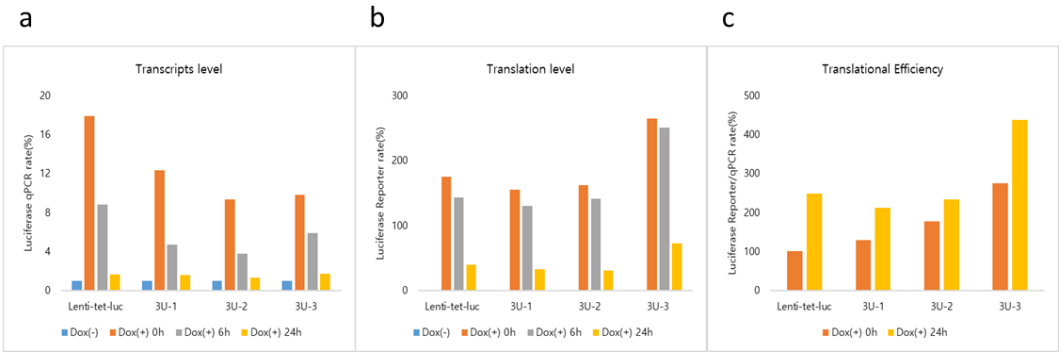


Figure 1. The role of 3'UTR MBS on luciferase translational efficiency

In the Lenti-tet-luc vector, the experiment was carried out with 3U-1,2,3 in which the let 7a miRNA interaction site was placed in 3'UTR. (a) 3U-1,2,3 had a lower transcript level than the control Lenti-tet-luc. (b, c) 3U-3 with the highest interaction energy had the highest translation level and translational efficiency at Dox(+) 0hr, and maintained well up to Dox(+) 24h.

2. Let-7a increased translation efficiency than viral RNA

Bovine viral diarrhea virus (BVDV) and chikungunya virus (CHIKV) are known to interact with let-7f. Experiments were conducted with the viral group of BVDV and CHIKV vectors containing the let-7f interaction site of each virus and 3U-3 as a positive control. In the Viral group, the transcripts level of CHIKV was high (Figure 2a), and the translation level was similar to that of the control. Both viral groups showed lower translational efficiency than 3U-3, so let-7a MBS increased translational efficiency more effectively. (Figure 2b, c)

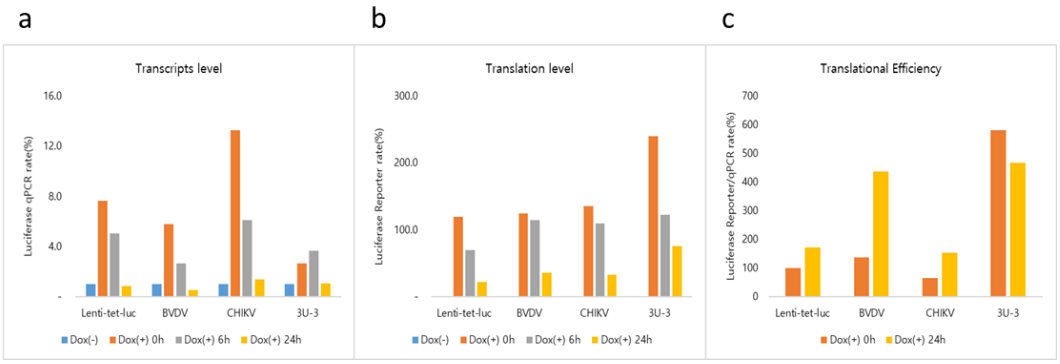


Figure 2. The comparison with Viral RNA 3'UTR

When 3U-3, which improved translation efficiency well, was compared with the known viral RNA group, (A) the transcript level was higher in the viral RNA group, but (B,C) translation level and efficiency were higher in 3U-3.

3. Change in translation efficiency up to 48 hours according to binding energy

By proceeding in the direction of increasing the interaction energy with let-7a MBS, 3U-4,5 with higher interaction energy than 3U-3 was produced. As a result, in 3U-3, the translational efficiency decreased significantly from 0 to 24 hours of Dox(+) in 3U-3, but the decrease diminished after 24 hours in 3U-4 and 5, and in particular, it increased in 3U-5 seemed. (Figure 3c) Thus, as the interaction energy increased, the translational efficiency increased, and it was observed that it became more prominent as time passed. Based on this, we wanted to increase the interaction energy and report the results up to 48 hours of Dox(+). As a result of the experiment, high translation level was shown in 3U-6,7,8 with increased interaction energy, and translational efficiency was very high in 3U-6,7, which was particularly stable at 48 hours. (Figure 4b, c) 3U-8, which completely

matched the binding sequence, had a higher translation level but higher transcripts level, so that the translational efficiency was lower than that of 3U-6,7. Through this, when let-7a MBS with high interaction energy was present in 3' UTR, mRNA stability and translational efficiency were increased, and the stability was also increased.

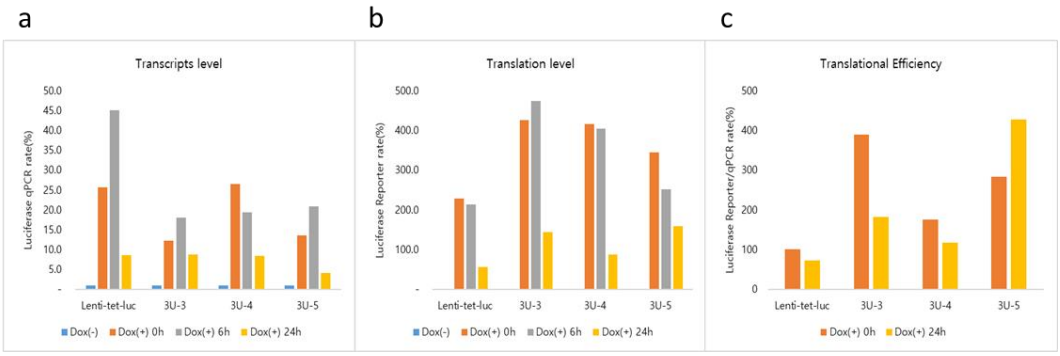


Figure 3. Effect of 3'UTR-miR interaction site on luciferase stability

(a) When the interaction energy with let-7a miRNA was changed in the direction to increase further, 3U-3 and 3U-4,5 were compared with higher interaction energy (b, c) 3U-3 showed a significant decrease in translational efficiency at Dox(+) 24 hr, but the decrease diminished in 3U-4, and 3U-5 showed an increase in translational efficiency at Dox(+) 24 hr.

Figure 4. Effect of 3'UTR-miR interaction site on luciferase stability

(a) 3U-6,7 with higher interaction energy and 3U-8 with the same sequence as let-7a were prepared, and 3U-1~8 and Viral RNA groups were tested together by extending Dox(+) 48hr. (b, c)3U-6, 7, 8 showed a very high translation level among the whole set, and among them, 3U-6, 7 showed high translation efficiency and was stable even after Dox(+) 48hr. 3U-8 showed lower translational efficiency than 3U-6,7.

4. Translation efficiency according to the location of the MBS

In the vector containing the let-7a miRNA binding site in the 5' UTR, the overall translation level was very low, and the translational efficiency was also low. (Figure 5b, c) We tried to find a sequence with high mRNA stability and translational efficiency in the presence of let-7a MBS in both 5'UTR and 3'UTR. A vector containing 5U-7, which has the highest translation level in 5U, was produced in 3U-3, 6, and 7, which showed a high translational efficiency, and the experiment was conducted. The translation level and efficiency of 5U-7/3U-3 were increased compared to 3U-3, and especially at Dox(+) 24hr, it showed a very high value.(Figure 6b, c) The translation level of 5U-7/3U-6 was increased compared to 3U-6, and the translational efficiency was greatly increased. It was confirmed that translational efficiency was significantly increased when let-7a MBS 5U-7 in 5'UTR and 3U-3 and 3U-6 in 3'UTR were coexisted.

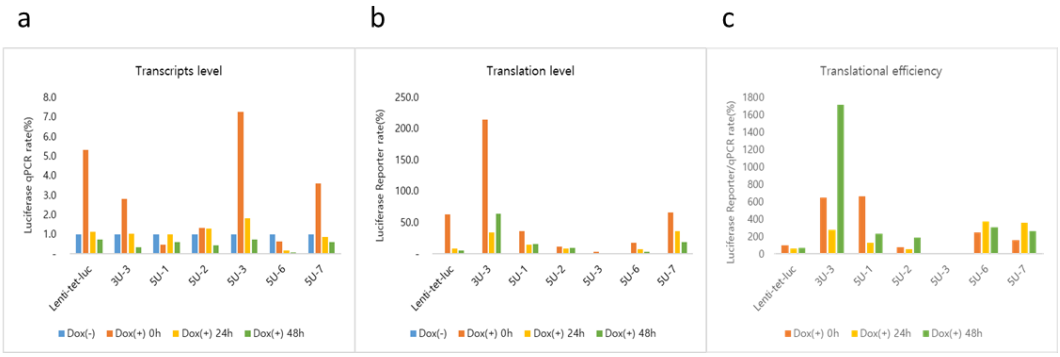


Figure 5. Effect of 5'UTR-miR interaction site on luciferase stability

5U group in which let-7a MBS was added to 5'UTR and 3U-3 as a positive control. (a) 5U-3 showed high transcipts level, but (b, c) 5U group had low translation level and low efficiency. The translation level showed the highest value in 5U-7.

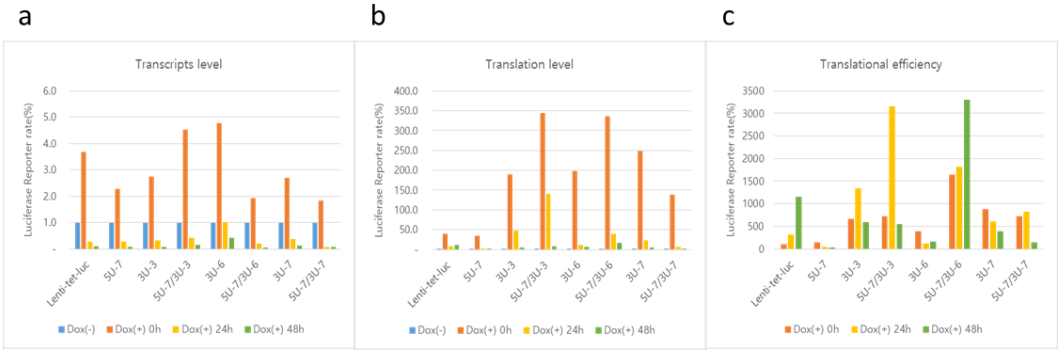


Figure 6. Effect of 5' UTR, 3' UTR-miR interaction site on luciferase stability

(a) 5' UTR and 3' UTR were compared with let-7a MBS. (b, c) The translation level and efficiency of 5U-7/3U-3 were higher than those of 3U-3, and in particular, the value in Dox(+) 24hr was higher. In 5U-7/3U-6, the transctipt level was decreased compared to 3U-6, but the translation level was increased and the efficiency was greatly increased. In

5U-7/3U-7, both transcripts and translation levels were decreased compared to 3U-7.

5. Stability of luciferase mRNA in 5' capping and zipper structures

In the 5' capping bridge structure and in the zipper structure, the transcript level was decreased, the translation level was the same as the control in the 5' capping bridge, and the zipper was slightly decreased.(Fig. 7a, b) Translational efficiency showed a high value in the 5' capping bridge structure. (Figure 7c) By increasing the interaction energy of the zipper structure, zMax and zMax2 were fabricated. As a result of the experiment, zMax efficiency decreased significantly at Dox(+) 24hr, but zMax2 increased at Dox(+) 24hr. (Figure 8b, c) In zMax2, which has higher interaction energy than zMax, stability and efficiency were higher.

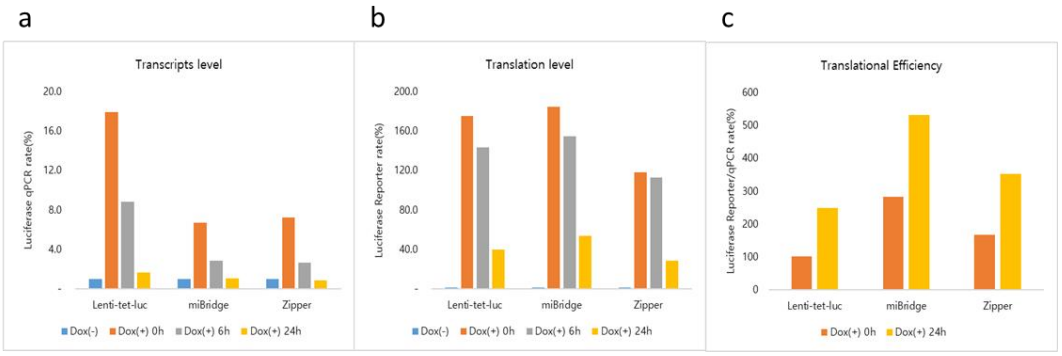


Figure 7. Gene Regulation by 5'Cap mediated UTR-miR interaction

(a) As a result of experimentation with the 5' capping bridge structure and zipper structure, both 5' capping bridge and zipper groups showed low transcipts levels. (b, c) The 5' capping bridge showed high translational efficiency, and the zipper showed a

slightly increased value. In this zipper structure, the interaction energy was further increased to produce zMax, zMax2 vectors.

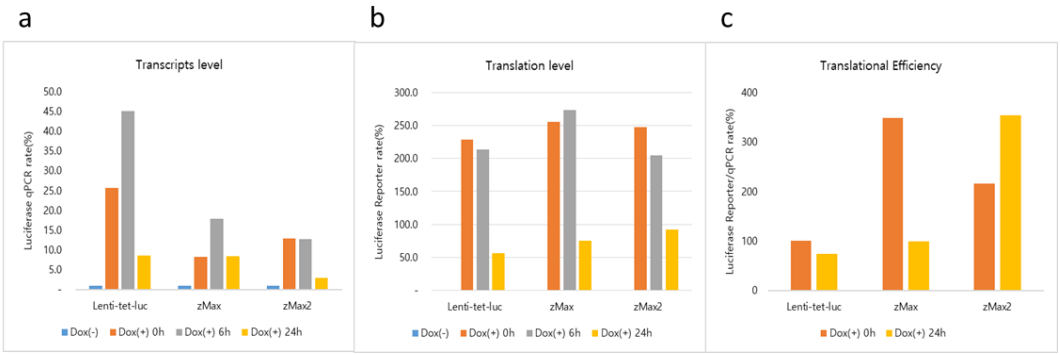


Figure 8. Gene Regulation by 5'-3' UTR-miR interaction and UTR zipper

(a) When the translational efficiencies of zMax and zMax2 with increased interaction energy in the Zipper structure were compared, both groups showed low transcipts levels. (b, c) zMax decreased from 0hr to 24hr of Dox(+), whereas zMax2 showed a further increase. In the structure with higher interaction energy, the stability of the mRNA was increased.

6. Hairpin structure is not effective in increasing translation efficiency.

In c-Jun and HIV-1 TAR hairpin structure vectors, the translation level of Jun hairpin was very low, and transcipts level and translation level of TAR hairpin were high, but there was no significant increase in translational efficiency. The hairpin structure was not effective in increasing translation efficiency. (Figure 9)

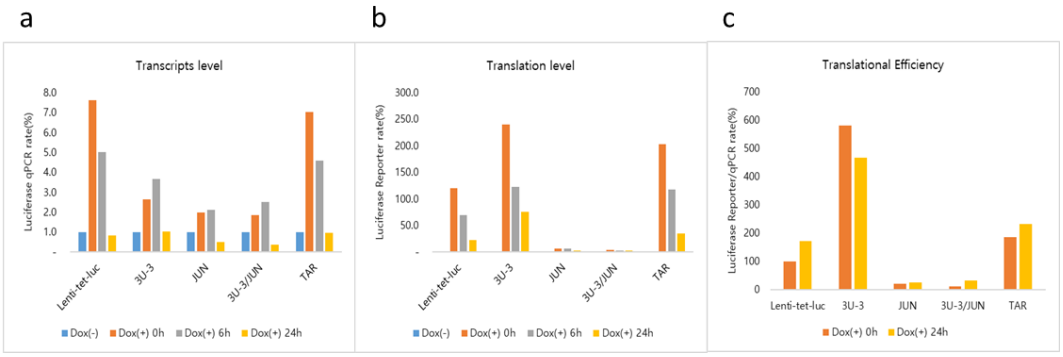


Figure 9. Identification of UTR hairpin structure on luciferase stability

(a) As a result of the experiment with c-jun, HIV-1 TAR hairpin structure, JUN had low transcripts level, and TAR showed the same transcripts level as control. (b, c) JUN showed very low translation level and translational efficiency, and TAR had high translation level, but there was no significant increase in translational efficiency.

IV. DISCUSSION

Although it was only known that mRNA is transcribed from DNA in the central dogma and transmits genetic information, recently it has emerged as an innovative technology for the treatment of numerous diseases. As Pfizer and Moderna's mRNA vaccines for COVID-19, mRNA vaccines are highly likely to become mainstream in future vaccine development, but RNA is easily broken and cold chains must be maintained and stability is low. But compared to existing vaccines, mRNA vaccines have many advantages. There is no risk of infection and the possibility of causing mutations is also eliminated. By simply changing the mRNA information, various antigens can be easily combined, thereby inducing a wide range of immune responses. In particular, in the industrial aspect, mRNA vaccines can be synthesized only by chemical reaction in vitro, which takes much less time and is easy to mass-produce. In addition, by changing the mRNA base sequence, various vaccines can be produced using the same facility and process, so production management is efficient.

In a recent researches, a correlation between miRNA and coronavirus was reported. It has been reported that miRNA let-7b suppresses the replication of coronavirus by targeting the S and M proteins of the coronavirus, and attempts are being made to use miRNA as a biomarker of the virus.(Xie et al. 2021) However, from the perspective of the mRNA vaccine, it can be considered that miRNA can inhibit vaccine activity.

There has been a study of ceRNA that indirectly regulates mRNA expression through

normal mRNA competition with respect to limited intracellular miRNA and RNA binding molecules. Based on this, it has been reported that non-coding RNA having a complementary sequence capable of binding to miRNA competes with the target mRNA of miRNA and acts like a sponge, sequestering all miRNAs and inhibiting the function of miRNA.(Dai et al. 2015; Ebert, Neilson, and Sharp 2007; Ebert and Sharp 2010; Franco-Zorrilla et al. 2007; Li et al. 2022; Subramanian 2014; Thomson and Dinger 2016)

Unlike the existing miRNA interactions, studies have been reported that miR-17 and let-7 directly bind to RNA viruses and rather stabilize RNA virus proliferation. In addition, the importance of the location of the miRNA binding site targeting the viral RNA was emphasized through studies that the secondary structure of the virus changes after miRNA binds to the viral RNA. Also in this study, it was confirmed that the translational efficiency of luciferase was increased when MBS, which has high binding energy to let-7a, was added to the 5'UTR and 3'UTR regions, before and after luciferase. It was observed that the aspect of mRNA stability varies depending on the shape of miRNA and mRNA structures, and through this, as shown in (Figure 10), RNA stability can increase or decrease depending on the structure to which miRNA binds. If further research to increase mRNA stability through the miRNA sponge system is conducted, it will be of great help to the development of vaccine technology in the future.

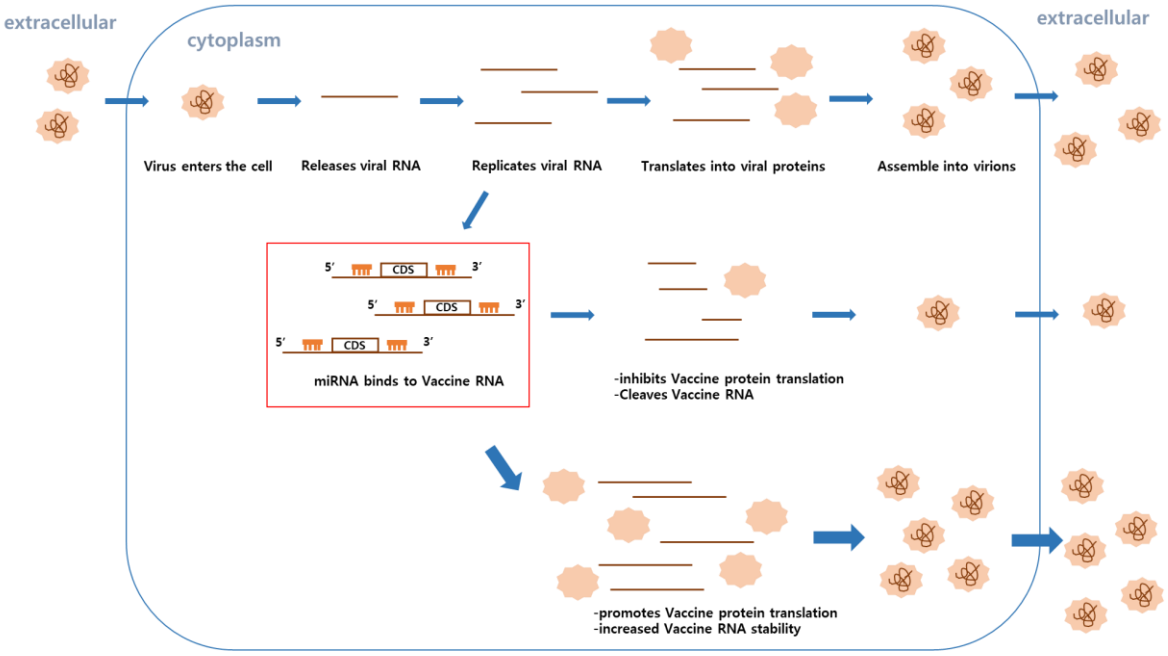


Figure 10. The upregulation of mRNA stability by miRNA-MBS binding.

V. CONCLUSION

1) When there is a miRNA binding site with high miRNA-mRNA binding energy in the 3' UTR site after luciferase, the translation efficiency of luciferase by let-7a increases.

2) The translation efficiency of let-7a is higher than that of BVDV and CHIKV viral RNA having a let-7f binding site used as a control.

3) The higher the miRNA-RNA binding energy, the smaller the decrease in translation efficiency, and the higher the translation efficiency after 48 hours. But when 100% binding was rather reduced.

4) Let-7a binding sites are added to the 5' UTR site in front of luciferase, the translation efficiency is lower than when a let-7a binding site is inserted to the 3'UTR site. When binding sites are added to both the 5'UTR and 3'UTR regions, the translation efficiency is increased compared to when only the 3'UTR is inserted.

5) The 5' capping structure increases translation efficiency, and the higher the binding energy in the zipper structure, the higher the stability of luciferase mRNA.

6) Hairpin structure is not effective in increasing translation efficiency.

From the above results, it was confirmed that the translation efficiency of luciferase was increased when MBS with high binding energy to let-7a was added to the 5'UTR and

3'UTR sites before and after luciferase. Based on this, if a construct that increases the stability of mRNA is developed, it is thought that it will contribute to research on new mRNA vaccines.

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ABSTRACT (IN KOREAN)

UTR의 miRNA 결합 부위에 의해 조절되는

mRNA 안정성 및 번역 효율성

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Covid-19 이후 mRNA 백신 기술은 급격히 발전하고 있지만, 아직 낮은 안정성과 세포 내로의 전달 효율 등의 문제점이 제기되고 있다. miRNA는 ~22 nucleotide(nt)의 길이를 갖는 단일 가닥 RNA 분자로서, 표적 mRNA의 5', 3' 비번역부위에 결합하여 해당 유전자의 발현을 억제하는 중요한 조절자 역할을 한다. 최근 miRNA에 의한 바이러스의 조절에 관한 많은 연구가 있으며, 기존

의 miRNA 상호작용과는 달리 miR-17 및 let-7가 RNA 바이러스에 직접 결합하여 RNA 바이러스 증식을 오히려 안정화시킨다는 연구가 보고되었다. 또한 miRNA가 바이러스 RNA에 결합한 후, 바이러스의 2차 구조가 변화한다는 연구 등을 통해 바이러스 RNA를 표적으로 하는 miRNA 결합 부위 위치의 중요성이 강조되었다. 따라서 본 연구에서는 mRNA의 안정성을 정량화할 수 있는 방법을 개발하고, miRNA와 직접적으로 결합할 수 있는 부위 (miRNA binding site, MBS)의 결합에너지, 5'UTR의 유무, 5' -Capping 구조, Zipper 구조 및 hairpin 구조 등을 변화시켜, let-7a에 의한 mRNA 백신의 안정성과 번역효율성을 증가시킬 수 있는 구조를 최적화하고자 하였다.

결과는 다음과 같다.

1) 번역효율성 확인을 위해 luciferase를 이용한 reporter assay system을 확보하였다.

2) Luciferase 뒤인 3' UTR 부위에 miRNA-mRNA 결합에너지가 높은 miRNA 결합부위가 있을 때, let-7a에 의한 luciferase의 번역효율성이 증가하였다.

3) 대조군으로 사용된 let-7f의 결합부위를 지닌 BVDV, CHIKV 바이러스 RNA 보다 let-7a의 번역효율성이 높았다.

4) miRNA-mRNA 결합에너지가 높을수록 시간에 따른 번역효율성의 감소폭이 적어지며, 48시간 후의 번역효율성이 높아진다. 반면, miRNA-mRNA 가 seed match로 결합 시에는 오히려 변역효율성이 감소되었다.

5) 5' UTR 부위에 let-7a 결합부위가 존재시, 3'UTR에 결합부위가 있을 때보다 번역효율이 떨어지지만, 5'UTR 과 3'UTR 부위 양쪽에 존재 시에 번역효율성이 가장 높았다.

6) 5' Capping 구조는 변역효율성을 증가시키고, zipper 구조에서 결합에너지가 높을수록 luciferase mRNA의 안정성이 더 높았다.

7) Hairpin 구조는 번역효율성 증가에 효과적이지 않았다.

이상의 결과에서, let-7a와의 결합에너지가 높은 MBS를 5'UTR 및 3' UTR 부위에 넣었을 때, luciferase의 번역 효율성이 증가하는 것을 확인하였다. 이를 바탕으로 mRNA의 백신 안정성을 증가시키는 구조체가 개발된다면 안정성이

개선된 새로운 mRNA 백신 연구에 기여를 할 것이라 사료된다.

핵심어 : miRNA, Let-7a, miRNA 결합부위, 번역 효율성, mRNA 안정성