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Effect of Seven siRNAs in a Vero E6 Cell Line Culture and Discovered Promising siRNAs Complementary to the Spike Protein

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Introduction

The duplex RNA directed strand (active strand) is loaded into an RNA-Induced Silencing Complex (RISC), which is designed to degrade or inhibit messenger RNA's translational activity. 13 Perfect complementary between the viral mRNA's 30 Un Translated Region (UTR) and the small host miRNA seed sequence (2-7 base pair) is enough to cause cleavage, but imperfect complementary can cause viral mRNA translation to be hampered. It is well understood that miRNAs have a function in the immunological process and are involved in the control of the immune system *via* immune cell activation. 16 studies looked at the role of miRNA as an antiviral agent in the treatment of a variety of disorders, including the human immunodeficiency virus-1, 17 herpes simplex viruses, 18 dengue fever, 19 influenza viruses, and 15 hepatitis C virus.

It is critical to use antiviral agents to prevent and treat the highly pathogenic coronavirus. As a result, new biological tactics for the treatment of viral diseases are strongly recommended. The expression change of host miRNAs in epithelial cells has a role in the pathogenesis of chronic and severe acute respiratory tract infections. Our latest research has demonstrated how viral RNA stabilizes monomeric and demerit subunits of the protein complex through appropriate interactions that have been reproduced by screening chemicals from a variety of drug-approved databases with high inhibitory potential against viral mRNA. 1 for the SARS-2 (Severe Acute Respiratory Syndromecoronavirus-2) genome, our work estimated any probable human miRNA targets. It also emphasizes the connection between host miRNAs and the COVID-19 viral genome, which will aid in better understanding the role of host pathogens.

miRNAs Hybridization Prediction

The RNA hybrid web server was utilized to look into the hybridization of viral (COVID-19) pre-miRNAs with potential human mature miRNAs. The RNA hybrid method was utilized to determine if the target host miRNA and viral pre-miRNA hybridized well. RNA hybrid is a method for calculating the Minimal Free Energy (MFE) of long and short RNA hybridization, which is widely used to calculate miRNA targets.

As a result, the length of the seed sequence, complementarity, and supplementary base pairing might be expected to be necessary and critical for the stabilization of the hybridized viral pre-miRNA structure. Sharma et al. estimated 22 possible miRNA from 5 SARS-CoV-2 genomes linked to 12 host miRNAs in a recent study. The presence of G-U pairings in the seed sequence of viral 30pre-miRNAs is known to reduce the target structure's stability. Our hybridization results revealed that the seed sequences of 30 of all five viral pre-miRNAs have a minimal amount of G-U pairs, which greatly contribute to the top 5 host miRNAs binding with viral pre-miRNAs with overall higher stabilities. Most miRNAs' secondary structure has a major transcriptional role in modifying miRNA-mRNA associations, resulting in varied degrees of gene susceptibility for different miRNAs.

The high specificity of siRNA is a big problem, especially when it is being used to produce gene therapy against a catastrophic pandemic like COVID-19, where non-specific silencing could result in fatal consequences. Although several studies have been undertaken on the use of siRNA to examine gene silencing in a closely related coronavirus strain of SARS-CoV, studies on SARS-CoV-2 are almost non-existent. In a study on SARS-CoV, Wu et al. and colleagues investigated the effect of seven siRNAs in a Vero E6 cell line culture and discovered promising siRNAs complementary to the spike protein sequence and the 3 translated regions capable of limiting viral multiplication. Shi et al. focused on SARS-CoV structural proteins such as envelope, membrane, and nucleocapsid of SARS-CoV by siRNA therapy in Vero E6 cell line culture and obtained 3 siRNAs which can reduce the expression of proteins by 80%. A recent computational analysis of SARS-CoV-2 sequences revealed potential siRNA target sequences which can be utilized to develop siRNA for effective silencing of potential proteins of SARS-CoV-2.

However, targeting the SARS-CoV-2 leader sequence can provide extraordinary results by blocking the virus's overall reproduction process in the host body. Previous *in vitro* studies on cell lines cloned with the SARS-CoV leader sequence and virus-infected cell cultures demonstrated the enormous potential of siRNA targeting the leader sequence rather than the virus's structural proteins. As a result, the current study focuses on the in silico prediction of high-potential siRNA that can successfully target the leader sequence of the SARS-CoV-2 virus and be used to produce effective gene therapy against the highly victimizing COVID-19 pandemic disease. The findings of our siRNA prediction by si revealed four powerful siRNAs with seed duplex Tm values of less than 21.5°C with no off-target sequence corresponding to the guide strands of siRNA.

Although metabolic acidosis has been linked to a variety of pathophysiological diseases, and its vaso relaxation effects have been thoroughly documented in several animal and culture models, the molecular mechanisms underlying acidosis-induced vasorelaxation remain unknown. The vascular response to diverse pathologic situations has been studied extensively using mesenteric artery models. Previous research and reports have revealed that the vascular responses of goat and human mesenteric arteries to numerous stimuli, including acidic stress, are very comparable. We used a goat mesenteric artery model to analyse the proteome profile of acid stress-induced vasorelaxation in order to better identify the signalling molecules responsible for altered vasoreactivity in response to acidic pH.

2D-GE using 7 cm IPG strips and micro gels, LC-MS/MS, and MALDI TOF MS were used to compare the vascular proteomes at acidic pH. Actin, transgelin, WD repeat-containing protein 1, desmin, tropomyosin, ATP synthase, Hsp27, aldehyde dehydrogenase, pyruvate kinase, and vitamin K epoxide reductase complex component 1-like protein were among the unique proteins found by mass spectrometry. Three of the five protein locations identified as actin were increased by more than twofold. Under acid stress, ATP synthase was similarly increased (2.14-fold). Transgelin, desmin, and WD repeat-containing protein 1 were also increased actin-associated proteins. Isometric contraction tests demonstrated that acidosis reduced both receptor-mediated (histamine) and non-receptor-mediated (KCl) vasocontraction, whereas acetylcholine-induced vasorelaxation was increased. Overall, the altered vasoreactivity under acidosis observed in the functional studies could possibly be attributed to the increase in expression of actin and ATP synthase β.