Down Regulation of Long Noncoding RNA MALAT1 and EZH2 Reduces SARS-CoV-2 Entry to Human Lung Cells

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Down Regulation of Long Noncoding RNA MALAT1 and EZH2 Reduces SARS-CoV-2 Entry to Human Lung Cells

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The Master's Thesis
submitted to the Department of Medical Science,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Master of Medical Science

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December 2022

This certifies that the Master's Thesis of

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December 2022

ACKNOWLEDGEMENTS

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마지막으로, 한결같이 묵묵히 지켜봐주시고 항상 지지해주셨던 부모님과 동생에게 감사합니다.

이 밖에도 언급하지 못했지만, 도움주신 많은 실험실 동료분들과 여러 분야에서 도움을 주신 다양한 분들이 있어서 학위과정을 마무리 할 수 있었습니다. 아직 부족한 점이 많지만 언제나 어디서든 더욱 더 발전할 수 있는 사람이 되고자 노력하겠습니다. 감사합니다.

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Long noncoding RNA (lncRNA), longer than 200 nucleotides without the potential for coding protein, has been observed during diverse viral infections to human. It is unclear whether lncRNAs and lncRNA related transcription factors deregulate the SARS-CoV-2 infection in the human host. During SARS-CoV-2 infection *in vitro*, expression level of metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) was significantly downregulated. To identify the role of MALAT1 during SARS-CoV-2 infection, MALAT1 was experimentally downregulated MALAT1 using siRNA system in a human lung cancer, Calu-3. As a result, I found that MALAT1 knock down reduced the SARS-CoV-2 viral replication. Moreover, I attested if inhibited enhancer of zeste 2 (EZH2), the enzymatic subunit of polycomb repressive complex 2 (PRC2) could reduce the SARS-CoV-2 viral replication. Finally, I propose that downregulated TMPRSS2 by EZH2 inhibitor successfully reduces SARS-CoV-2 viral infection. In conclusion, both downregulated MALAT1 and EZH2 inhibition could reduce TMPRSS2 expression, resulting in reduced SARS-CoV-2 infection.

Key words : MALAT1, EZH2, and SARS-CoV-2

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I. INTRODUCTION

COVID-19, caused by the SARS-CoV-2 virus, is a rather heterogeneous disease. The basis of SARS-CoV-2 is in the beta-coronavirus genus and usually causes mild respiratory infections in humans. Due to the SARS-CoV-2 structural and nonstructural contents and its high potential for emergence, virus induced respiratory infections have recently led to deadly endemics in humans, such as SARS and measles. Therefore, disease courses range from mainly asymptomatic and mild courses to more severe and critical courses that might be fatal to patients [1 2 3 4]. One of the significant differences between those courses was the variance of gene set enrichment levels of the patients [5 6]. Among the differentially expressed gene sets, I focused on MALAT1, one of the well-known long noncoding RNA.

Long noncoding RNAs (lncRNAs) are defined as RNA molecules longer than 200 nucleotides. LncRNAs regulate gene expression at the transcriptional or post-transcriptional levels. During the viral infection to a host, both host and viral encoded lncRNA can modulate the expression of host genes which are critical for viral replication, activation of various signal pathways, cytokine and chemokine production, expression of interferons and interferon-stimulated genes (ISGs) [7 8 9]. Interaction of lnc RNA and MALAT1 in SARS-CoV-2 infected cells and the ISGs were also introduced by *in silico* analysis [10].

MALAT1, one of the lncRNAs, was underexpressed in mild COVID-19 patients compared to both healthy controls and severe COVID-19 patients [6]. Therefore, investigation of this lncRNA and host-virus interaction may elucidate understanding of COVID-19. MALAT1 has been reported that it promotes HIV-1 transcription and infection [8]. Its knockdown by CRISPR/Cas9 also reduced the HIV-1 long terminal repeat (LTR)-driven gene transcription and viral replication. More precisely, MALAT1 interacts with chromatin modulator polycomb repressive complex 2 (PRC2). MALAT1 detached the core component of PRC2, enhancer of zeste homolog2 (EZH2) and relieved methylation of histone H3 on lysine 27 (H3K27me3) and its epigenetic regulation of HIV-1 transcription. This lncRNA may also contribute to the number of SARS-CoV-2 viral titers in the human cell as it interacts with PRC2. The possible mechanism that supports MALAT1 expression level to regulate the quantity of virus when the patients are infected is followed.

It has been proved that MALAT1 expression level positively regulates EZH2 [11 12 13 14]. Indeed, MALAT1 binds to EZH2 and enhances EZH2-mediated repression of polycomb-dependent target genes [12].

Furthermore, EZH2 mediates transcription of transmembrane protease, serine 2, TMPRSS2 [15]. TMPRSS2 is a host protease that cleaves S protein of SARS-CoV-2 [16 17]. SARS-CoV-2 uses SARS-CoV-2 entry receptor, ACE2 and the serine protease, TMPRSS2, for S protein priming. Clinically proven serine protease inhibitor Camostat mesylate, which is active against TMPRSS2, partially blocked SARS-CoV-2 entry into CaCo-2 and Vero-TMPRSS2 cell [16]. TMPRSS2 is one of the major therapeutic targets for COVID-19 treatment. Recent research has shown that EZH2 functions as a transcriptional activator and its knockdown showed inhibition of TMPRSS2 expression [15]. Commonly used EZH2 inhibitors, GSK343, GSK126 (SAM-competitive inhibitors) and DZNep (SAH hydrolase inhibitor) [18 19] were experimentally used to see the knock down effect of TMPRSS2 on Western blot. Additionally, by infecting various cells that express

different levels of TMPRSS2, it proved higher TMPRSS2 expressing cell lines would result in higher SARS-CoV-2 titer.

In this study, I investigated the correlation of MALAT1 and SARS-CoV2 and down regulated MALAT1 and EZH2 as a positive regulator of TMPRSS2.

I found that MALAT1 was significantly down regulated during SARS-CoV-2 infection. Upon viral infection, siRNA of MALAT1 and EZH2 inhibitors were treated and observed reduced viral mRNA and progeny of SARS-CoV-2. In both types of treatments, the expression level of TMPRSS2 was reduced, and I propose that SARS-CoV-2 was reduced via down regulated TMPRSS2 level. Thus, these findings provided a potential target lncRNA and chemical inhibitors for controlling SARS-CoV-2 infection with down regulation of virus receptor, TMPRSS2.

II. MATERIALS AND METHODS

1. Cells

Calu-3, HuH-7, HepG2, Vero and Vero E6 cells were used in this study. The cell lines were purchased from Korean Cell Line Bank. Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) (Corning, New York, United States), 100 U/mL penicillin, and 100 μg/mL streptomycin were used to culture. All the cell lines were incubated at 37°C under 5% CO_2.

2. Virus infection and titration

SARS-CoV-2 was provided by Korea Centers for Disease Control (KCDC, Cheongju, South Korea). SARS-CoV-2 replication was produced by transfecting with Vero cells. Virus infectivity titers were measured by plaque-formation unit (PFU) assay.

3. siRNA mediated gene silencing

The sequences of siRNA duplexes were as follows: siMALAT1_1: forward, 5'-GAG CAA AGG AAG UGG CUU ATT-3' and reverse, 5'-UAA GCC ACU UCC UUU GCU CTT-3'. siMALAT1_2: forward, 5'- GCG GAA GCU GAU CUC CAA UTT-3' and reverse, 5'-AUU GGA GAU CAG CUU CCG CTT-3'. siRNAs were transfected into Calu-3 cells by using Lipofectamine RNAiMAX (Invitrogen, Waltham, Massachusetts, United States)

4. Real-time (RT) PCR analysis

Total RNAs in the viral infected cell supernatant were extracted from samples using easy-Blue™ total RNA Extraction Kit (iNtRONbio, Seongnam, South Korea). Luna® Universal Probe One-Step RT-qPCR kit (NEB, Ipswich, Massachusetts, United States) was used to quantify SARS-CoV-2 RdRp. The cellular RNAs were extracted from samples using Accuprep Universal RNA

extraction kit (Bioneer, Daejeon, South Korea). Reverse transcription to cDNA was done by RNA to cDNA EcoDry™ Premix (TAKARAbio, Kusatsu, Japan). qRT-PCR were performed by using QuantStudio 3 real-time PCR System (Applied biosystems, Waltham, Massachusetts, United States) with following conditions: 40 cycles of denaturation (95℃, 30 s), annealing (55℃, 30 s), and extension (72℃, 30 s). The primers were used as follows: *GAPDH*, forward, 5′-GGG AAA TCG TGC GTG ACA T-3′ and reverse, 5′-GTC AGG CAG CTC GTA GCT CTT-3′. RdRp-SARS-CoV-2, forward, 5'-GTG ARA TGG TCA TGT GTG GCG G-3' and reverse, 5'-CAR ATG TTA AAS ACA CTA TTA GCA TA-3'. MALAT1, forward, 5′-CTT CCC TAG GGG ATT TCA GG-3′ and reverse, 5′-GCC CAC AGG AAC AAG TCC TA-3′. ACE2, forward, 5′- GGA CCC AGG AAA TGT TCA GA-3' and reverse, GGC TGC AGA AAG TGA CAT GA-3'. TMPRSS2, forward, 5′- AAT CGG TGT GTT CGC CTC TAC -3' and reverse, 5'- CGT AGT TCT CGT TCC AGT CGT -3'.

5. Virus infection and detection: Plaque assay

After 48 hr of siRNA treatment, the Calu-3 cells were infected with 0.01 MOI of SARS-CoV-2. Another 48 hr later, the viral quantities were counted by plaque assay. Vero E6 cells were seeded in 12 well plates before the day of infection. Cells were infected with SARS-CoV-2 for 1 hr at 37°C. After the infection, viruses were sucked out by aspirator and the infected cells were covered with overlay media containing 1% SeaPlaque™ agarose (Lonza, Basel, Switzerland). At 5 days post infection, cells were fixed overnight with 4% paraformaldehyde (Biosesang, Seongnam, South Korea). The overlay media was sucked out, the remained cells were stained with 0.5% crystal violet (Duksan, Seoul, South Korea) and the plaque were counted after washed out the cells.

6. Western blot analysis

Cells were washed with phosphate buffered saline (PBS) (WELGENE INc, Gyeongsan, South Korea) and lysed in 1× RIPA buffer (150 mM NaCl, 1% Triton X-100, 1% deoxycholic acid sodium salt, 0.1% SDS, 50 mM Tris-HCl pH 7.5, and 2 mM EDTA, GenDEPOT, Katy, Texas, United States) containing with protease inhibitor cocktail (GenDEPOT, P3100) and phosphatase inhibitor cocktail (GenDEPOT, Katy, Texas, United States). WCLs were centrifuged at 20,000 × *g* for 10 min at 4°C. Reducing and non-reducing protein sample buffers (100 mM Tris-HCl pH 6.8, 2% SDS, 25% glycerol, 0.1% bromophenol blue, and with/without 5% β-mercaptoethanol) were added to the WCLs followed by heating at 94 °C for 5 min. Proteins were separated by molecular weight via SDS-PAGE or nonreducing SDS-PAGE and then transferred to nitrocellulose membranes (GE Healthcare Life Sciences, Chicago, Illinois, United States). Non-specific binding sites were blocked with 5% w/v skim milk for 1 hr. Anti-β-actin (Cell Signaling Technology, #4967S), anti-phospho-Serine/Theronine (Abcam, ab17464), anti-ACE2 (Abcam, Cambridge, United Kingdom), anti-EZH2 (Abcam, Cambridge, United Kingdom), and anti-TMPRSS2 (Abcam, Cambridge, United Kingdom) antibodies were used. Membranes were washed three times for 10 min with TBS containing 1% Tween 20 and probed with the appropriate horseradish peroxidase (HRP)-conjugated secondary Ab (Jackson ImmunoResearch, West Grove, Pennsylvania, United States) for 1 hr. After washing three times, an enhanced chemiluminescence substrate by TMB solution (GenDEPOT, Katy, Texas, United States).

III. RESULTS

1. qRT-PCR analysis reveals a down-regulation of MALAT1 upon SARS-CoV-2 infection

Since MALAT1 was significantly underexpressed in mild symptom COVID-19 patients[5], I wondered the relevance of host MALAT1 expression level and SARS-CoV-2 replication in the human cells. Furthermore, MALAT1 and viral infection had a strong correlation. MALAT1 was overexpressed when $CD4^+$ T cells were infected with HIV-1[8], and it was underexpressed when Raw264.7 cells were infected with Vesicular stomatitis virus (VSV), Encephalomyocarditis virus (EMCV), and Herpes simplex virus type 1 (HSV-1) [20]. I first examined whether expression of MALAT1 is regulated during SARS-CoV-2 infection *in vitro* by performing qRT-PCR. In different ACE-2 expressing cell lines, expression level of MALAT1 was decreased after 72 hr infection (Figure 1A to C). The time-course of infection also showed expression level of MALAT1 was decreased (Figure 1D), and also by amount of viral infection (Figure 1E). Taken together, these results demonstrate that MALAT1 expression is consistently down-regulated upon SARS-CoV-2 infection *in vitro*.

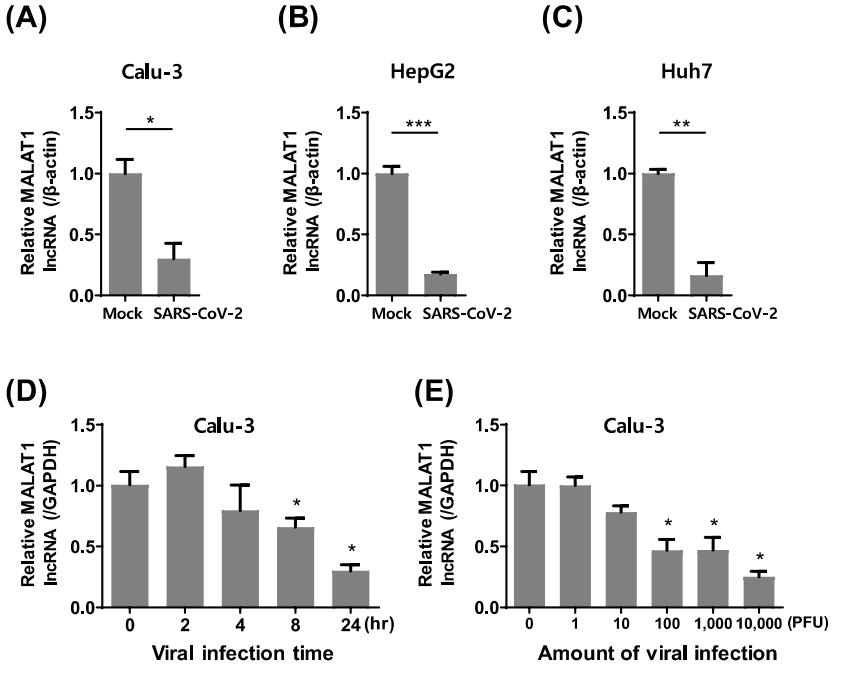

Figure 1. MALAT1 expression level during SARS-CoV-2 infection. (A-C) Calu-3, HepG2 and Huh7 were infected with SARS-CoV-2 by 10 MOI for 6 hr. RNA of whole cells were collected and relative MALAT1 levels were analyzed by qRT-PCR. mRNA level of MALAT1 in Calu-3 cells were analyzed by qRT-PCR after infection with SARS-CoV-2 by 0.01 MOI in different time course (D), and after 6 hr of infection with different SARS-CoV-2 PFU. (E) Data are shown as mean +SEM of n=3 biological replicates (A-E). ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ were considered as significant difference as determined by an unpaired *t*-test.

2. siMALAT1 treatment results efficient SARS-CoV-2 reduction

To determine the role of MALAT1 in SARS-CoV-2 infection, I knocked down the MALAT1 gene in Calu-3 cells by two types of siRNA targeting MALAT1 (siMALAT1) transfection with lipofectamine RNAiMAX. Effectivity of siMALAT1 on Calu-3 was confirmed by qRT-PCR (Figure 2A). I used siMALAT1 to experimentally test whether regulated MALAT1 can affect SARS-CoV-2 replication in host cells. First, two sequences of siMALAT1 were treated on human lung cancer cells, Calu-3 for 72 hr and SARS-CoV-2 were infected by 0.01 MOI. At 24 hr post-infection, supernatants were diluted and re-infected in Vero E6 to perform plaque assay (Figure 2B). The plaques of SARS-CoV-2 were significantly reduced on siMALAT1 treated groups (Figure 2C). Along with the plaque data, the relative mRNA of SARS-CoV-2 was also reduced in siMALAT1 treated groups (Figure 2D).

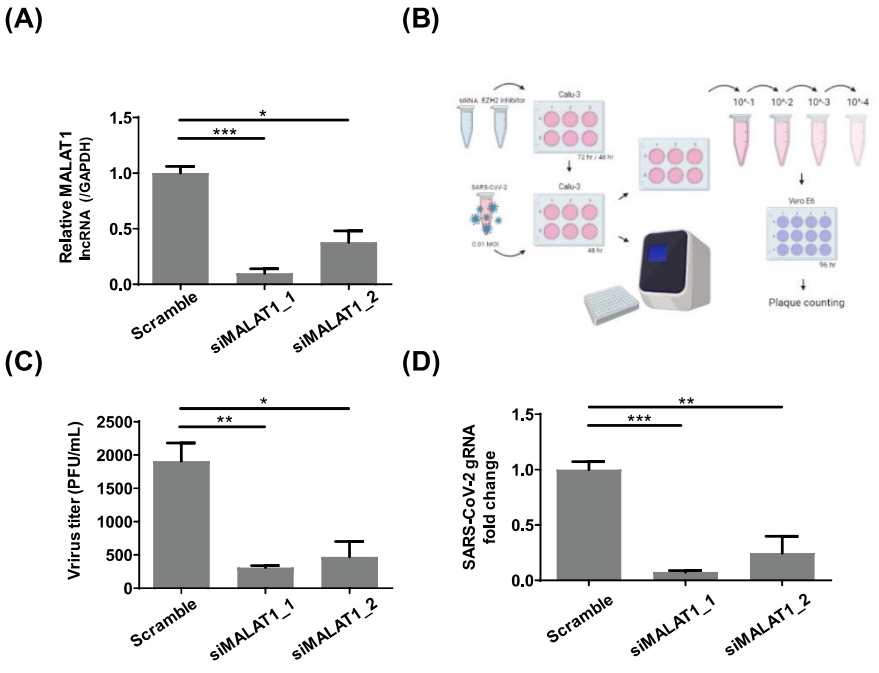

Figure 2. Reduced SARS-CoV-2 viral progeny and mRNA after siMALAT1 treatment. (A) Calu-3 cells were transfected with scramble, siMALAT1_1, and siMALAT1_2 respectively. And the expression of MALAT1 in transfected cells was assayed by qRT-PCR. (B) Schematic illustration of viral titration. (C) After siRNAs were treated in Calu-3 for 72 hr, the Calu-3 cells were infected with SARS-CoV-2 at MOI 0.01. At two days post-infection, infectious viral progeny was quantified in the supernatants by plaque assay in Vero E6 cells. (D) From the taken Calu-3 supernatants, mRNA of SARS-CoV-2 gRNA was relatively analyzed by qRT-PCR. Data are shown as mean +SEM of n=3 biological replicates (A,C,D). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ were considered as significant difference as determined by an unpaired *t*-test.

3. EZH2 inhibitor treatment results efficient reduction of SARS-CoV-2

Previously reported that MALAT1 expression level could regulate the expression level of enhancer of zeste homolog2 (EZH2)[12] . Therefore, I wondered if downstream of MALAT1, EZH2 could also regulate SARS-CoV-2. In agreement with the previous finding, I observed a strong relation between MALAT1 level and EZH2 protein expression level when siMALAT1 was treated (Figure 3A, B). mRNA levels of EZH2 were also followed by mRNA levels of MALAT1 (Figure 3C).

Next, I examined the potential antiviral efficacies of different EZH2 inhibitors, GSK343, GSK126, and DZNep (EZH2 inhibitors). Before that, cell viability after EZH2 inhibitors treatment was evaluated using cell counting kit-8 assays in Calu-3 cells (Figure 3D).

To test the antiviral efficacy of EZH2 inhibitors, I pretreated Calu-3 cells with various concentrations of inhibitors and infected them at MOI 0.01. After washing out the infectious media, I changed to fresh media containing the same concentration of inhibitors. After 48 hr post infection, I examined infectious viral particles that were measured by the plaque-forming unit (PFU) assay. Supernatants from the SARS-CoV-2 infected Calu-3 cells were taken and re-infected on the Vero E6. Surprisingly, the infectious viral particles indicate significant inhibition of viral progeny release (Figure 3E). Next, RNA was isolated and the abundance of virus was quantified by qRT-PCR. After treatment of all the three inhibitors, viral mRNA was reduced by control groups. In line with plaque reduction, GSK343 has shown the best antiviral effect among the inhibitors (Figure 3F). Taken together, I propose EZH2 inhibitors as a novel inhibitor with a potent antiviral efficacy against SARS-CoV-2.

All the inhibitors were produced to target EZH2 methyltransferase activity. Notably, GSK343 and GSK 126 were SAM-competitive inhibitors and DZNep was SAH hydrolase inhibitor which globally inhibits histone methylation [21].

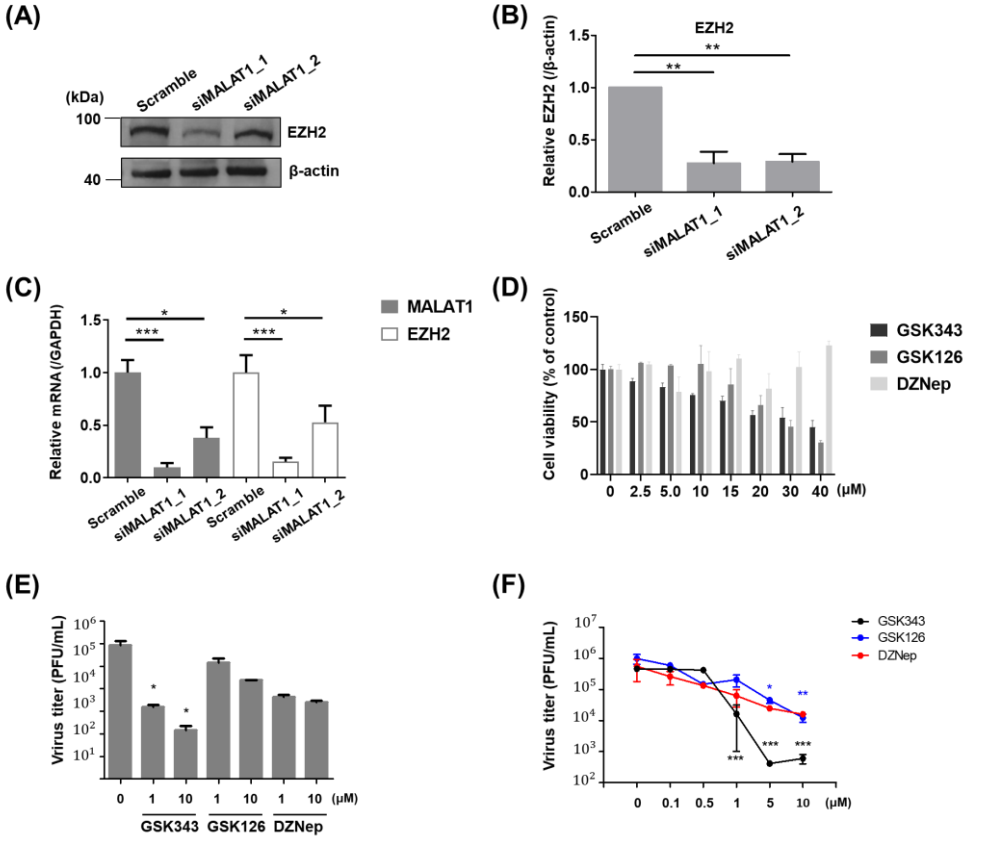

Figure 3. Reduced SARS-CoV-2 viral progeny and mRNA after EZH2 inhibitor treatment (A-B) Western blot analysis of scramble, siMALAT1_1 and siMALAT1_2 treated Calu-3 cells immunoblotted using EZH2 and β-actin. (C) Relative mRNA of MALAT1 and EZH2 after treatment of siRNAs by qRT-PCR. (D) CCK-8 analysis of cell viability after treatment different concentration of GSK343, GSK126 and DZNep for 48hr. (E) Calu-3 cells were infected with SARS-CoV-2 at MOI 0.01. At two days post-infection, infectious viral progeny was quantified in the supernatants by plaque assay in Vero E6 cells. (F) mRNA of SARS-CoV-2 was analyzed from the taken Calu-3 supernatants by qRT-PCR and PFU values were obtained by normalizing with ct value of known PFU virus stock. Data are shown as mean +SEM of n=3 biological replicates (B-E) and ±

SEM of n=6 (F). Results were representative of at least three independent experiments (A). ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ were considered as significant difference as determined by an unpaired *t*-test.

4. Down regulation of MALAT1 and EZH2 levels reduced TMPRSS2 level in the host cell

EZH2 acts as an epigenetic regulator of tumor suppressor genes and upregulates oncogenes, promoting cancer cell survival, proliferation, epithelial to mesenchymal and invasion [9]. Moreover, from previous research, EZH2 has revealed its potential role as a transcription activator of Androgen Signaling. One of the androgen signal proteins that EZH2 could upregulate is TMPRSS2 [12], also known as SARS-CoV-2 receptor. In order to assess whether regulation of MALAT1 could manage the expression level of TMPRSS2, I used siRNA of MALAT1 and I observed a strong reduction of TMPRSS2 expression in protein level (Figure 4a). Reduction of TMPRSS2 was also confirmed by qRT-PCR (Figure 4B).

In agreement with previous findings that regulated EZH2 with shRNA system [12], I used EZH2 inhibitors to demonstrate the knock down effect of EZH2 and following TMPRSS2. After treatment of GSK343 and GSK126, EZH2 expression level was downregulated in a concentration-dependent manner. Using DZNep also showed downregulated EZH2 level but not in a concentration-dependent manner. As I expected, TMPRSS2 would be downregulated if EZH2 was reduced, GSK343 and GSK 126 groups had significant reduction of TMPRSS2 and DZNep also showed reduction of TMPRSS2 (Figure 4C). Reduction of TMPRSS2 was also confirmed by qRT-PCR (Figure 4D). Schematic illustration showing the process of siMALAT1 and EZH2 inhibitors treatment is described (Figure 5).

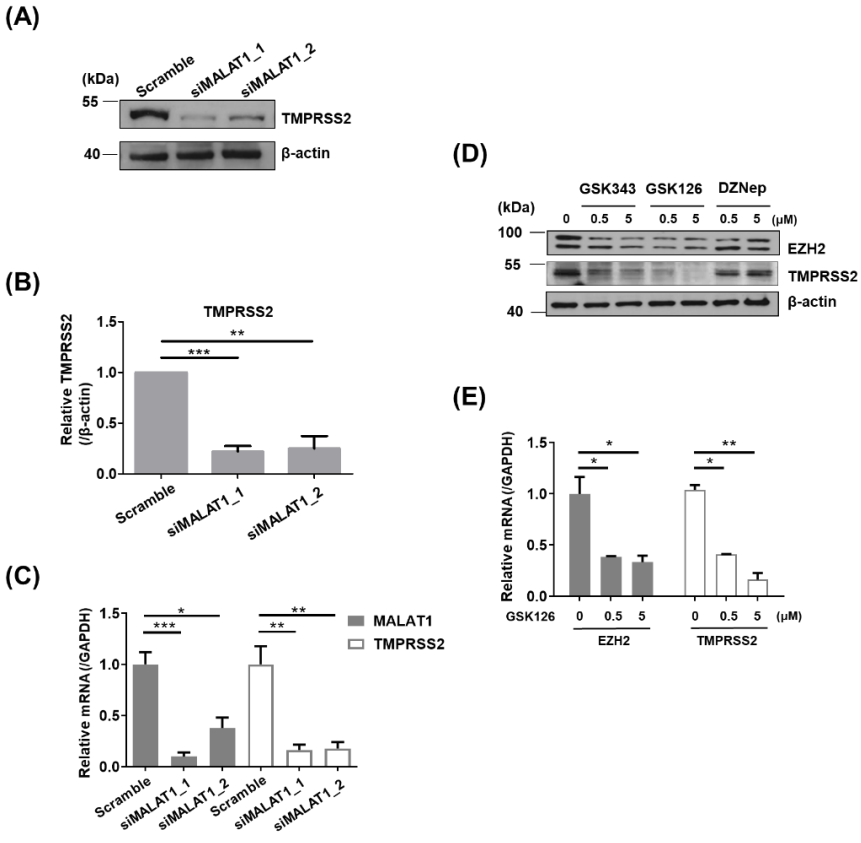

Figure 4. Regulation of TMPRSS2 level in host cell by down regulation of MALAT1 and EZH2 (A-B) Western blot analysis of scramble, siMALAT1_1 and siMALAT1_2 treated Calu-3 cells immunoblotted using TMPRSS2 and β-actin. (C) Relative mRNA of MALAT1 and TMPRSS2 after treatment of siRNAs by qRT-PCR. (D) Western blot analysis of GSK343, GSK126, and DZNep treated Calu-3 cells immunoblotted using EZH2, TMPRSS2, and β-actin. (D) Relative mRNA of EZH2 and TMPRSS2 after treatment of GSK126 (0.5 or 5 uM). Data are shown as mean +SEM of n=3 biological replicates (B,C,E). Results were representative of at least three independent experiments (A,D). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ were considered as significant difference as determined by an unpaired *t* test.

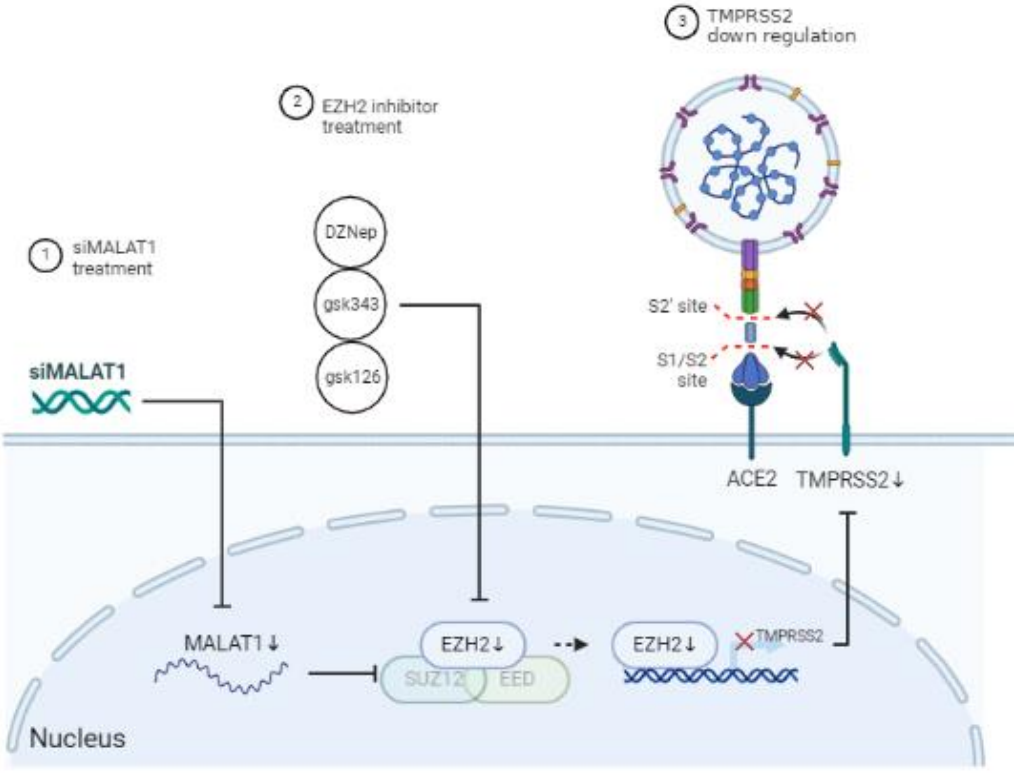

Figure 5. Schematic illustration showing process of siMALAT1 and EZH2 inhibitors treatment.

①,③ Expression level of TMPRSS2 and SARS-CoV-2 reduction was observed when siMALAT1 was treated

②,③ Expression level of TMPRRS2 and SARS-CoV-2 reduction was observed when EZH2 inhibitors were treated.

IV. DISCUSSION

Here, I showed the antiviral effect against SARS-CoV-2 via down regulation of MALAT1 and EZH2. While the molecular mechanism behind MALAT1 and EZH2 in the viral life cycle is yet to be clearly delineated, it is likely that MALAT1 and EZH2 engaged in Viral Entry using TMPRSS2. Recent *in silico* studies suggest a possible correlation of SARS-CoV-2 and host expression levels of MALAT1 and EZH2 [10,22]. The patients of the mild symptom groups showed significant expression levels of MALAT1 compared to severe symptom groups. A possible explanation may be the IFN level of the patients were significantly low in the mild symptom group.

As the first line of defense against viral infection, the production of type 1 interferons plays a central role in activating the expression of hundreds of IFN-stimulated genes (ISGs) for establishing an “antiviral state” to restrict viral replication within infected cells [23]. Insufficient production of IFNs causes chronic viral infection, while excessive amounts of IFNs also induce autoimmune inflammation. Thus, it is important to precisely regulate the production of IFNs both in acute and chronic diseases. IFN expression is heavily inhibited by SARS-CoV-2 but down regulation of MALAT1 and EZH2 increases IFN level [24-26 27,28].

Reports about the transcript of MALAT1 gene, also known as NEAT2, provided evidence of its association with metastasis in early-stage non-small cell lung cancer patients. Subsequent studies reported that MALAT1 is extremely abundant and widely conserved among 33 mammalian species. From the previous report, MALAT1 was a highly abundant nuclear transcript localized to the nuclear speckles, but also exported to the cytoplasm as a form of mascRNA [29].

MALAT1 involved in the regulation of antiviral innate immune responses needs to be further studied. MALAT1 is abundant in the nucleus but significantly down-regulated after viral infection. MALAT1 acts as a negative regulator of antiviral type 1 production. MALAT1 expression is down-regulated, IRF3 activation is induced and type 1 IFN production is aberrantly activated [24]. EZH2 was first found to be one of the most upregulated genes in aggressive PCa [30]. Recent study reported that EZH2 inhibitor induced an antiviral state and suppressed RNA (Zika virus) virus [20]. Interferon-alpha (IFN-α) and interleukin-8 mRNAs were significantly upregulated in GSK126-treated cells. Understanding MALAT1 and EZH2 in cellular immune systems against SARS-CoV-2 can inform further intervention strategies.

While one of the possible antiviral effects of down regulated MALAT1 and EZH2 is inducing antiviral state, MALAT1 and EZH2 can also inhibit cell entry of SARS-CoV-2 by down regulation of TMPRSS2 expression. EZH2 has been reported to be capable of stimulating or repressing gene expression beyond PRC2 and H3K27me3 [31]. Furthermore, EZH2 as a transcription activator, TMPRSS2, Androgen Receptor (AR) can be activated and repressed when EZH2 siRNA was treated [15]. Interaction of EZH2 and TMPRSS2 protein were further analyzed in CHIP-seq data [32]. In 90% of prostate cancers overexpressing ERG, TMPRSS2 and ERG exist in a fusion form [33] and ERG is methylated by EZH2 at a specific lysine residue (K362) causing enhanced activity of ERG [32].

Interestingly, IFN-α activates transcription of endothelial plasminogen activator inhibitor (SERPINE1) in Calu-3 cells [34] and Serprin E1 inhibits plasminogen activator also including TMPRSS2 [35]. Down regulation of MALAT1 and EZH2 both activate IFN-α[24-26] [27,28] and potentially they may inhibit TMPRSS2. However, direct inhibition of TMPRSS2 by IFN- α activation has not been studied in depth.

Since the outbreak of SARS-CoV-2, plenty of variants have appeared. Efficacy of first-generation COVID-19 vaccines was gradually reduced as spikes have been modified frequently. Moreover, understanding viral escape from host systems and blocking the mechanism may help reducing viral infection. Overall, I demonstrate that inhibiting MALAT1 with siRNA and inhibiting EZH2 with EZH2 inhibitors down regulate TMPRSS2 activity. Therefore, SARS-CoV-2 entry was potentially blocked to the host cell resulting in reduced SARS-CoV-2 replication (Figure 5). Consistently, reduced SARS-CoV-2 was observed after treating siMALAT1 and EZH2 inhibitors. In particular, I demonstrate robust antiviral effects against SARS-CoV-2 using EZH2 inhibitors by down regulating TMPRSS2 making it excellent treatment candidate against SARS-CoV-2.

V. CONCLUSION

1. Consistent down-regulation of MALAT1 upon SARS-CoV-2 infection was confirmed and suggested relation of MALAT1 and SARS-CoV-2.
2. SARS-CoV-2 reduction was observed by siMALAT1 treatment.
3. More efficient reduction of SARS-CoV-2 was observed by EZH2 inhibitors treatment
4. Potential cause of SARS-CoV-2 reduction was due reduced SARS-CoV-2 receptor, TMPRSS2

Consistently, reduced SARS-CoV-2 were observed after treating siMALAT1 and EZH2 inhibitors. In particular, I demonstrate robust antiviral effects against SARS-CoV-2 using EZH2 inhibitors by down regulating TMPRSS2 making it excellent treatment candidate against SARS-CoV-2.

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ABSTRACT (IN KOREAN)

긴 비암호화 RNA MALAT1과 EZH2 억제로 인한 사람 폐 세포에서의 SARS-CoV-2 감소

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이후정

긴 비암호화 RNA (lncRNA)는 200개의 뉴클레오타이드보다 길고, 단백질 변환이 되지 않는 RNA이다. 이러한 lncRNA가 최근 사람의 바이러스 감염에 다양한 역할을 할 수 있다는 보고가 되어 많은 주목을 받고 있다. 하지만 lncRNA가 SARS-CoV-2 감염 중, 사람세포에 어떤 역할을 하는지 잘 밝혀진 바가 없으며 SARS-CoV-2 감염 중, 특정 환자군에서 MALAT1이라는 lncRNA가 유의미하게 증가되어 있어 SARS-CoV-2 감염에서 MALAT1의 역할을 알아보고자 한다. siRNA 시스템을 이용하여 MALAT1을 감소 조절하고 SARS-CoV-2 감염의 억제를 확인하였다. 또한, EZH2란 MALAT1과 다양한 상호작용을 하는 전사인자를 억제하였을 때 SARS-CoV-2 감염이 감소하는 것을 확인하였다. 마지막으로, MALAT1과 EZH2의 감소 조절이 TMPRSS2라는 사람세포 내의 SARS-CoV-2 수용체를 억제할 수 있다는 것을 확인하였다. MALAT1과 EZH2 의 감소 조절을 새로운 SARS-CoV-2의 치료법으로 제시하고자 한다.

핵심되는 말 : MALAT1, EZH2 그리고 SARS-CoV-2