Nuclear export inhibitor Selinexor targeting XPO1 enhances coronavirus replication

Masmudur M. Rahman¹, Bereket Estifanos¹², Honor L. Glenn¹, Karen Kibler¹, Yize Li¹³, Bertram Jacobs¹³, Grant McFadden¹³, Brenda G. Hogue¹³

¹ Center for Immunotherapy, Vaccines, and Virotherapy, Biodesign Institute, Arizona State University, Tempe, AZ 85287, USA.
² School of Life Sciences Microbiology Graduate Program, Arizona State University, Tempe, AZ 85287, USA
³ School of Life Sciences, Arizona State University, Tempe, AZ 85287, USA

Corresponding authors:

Masmudur M. Rahman: masmudur.rahman@asu.edu
Brenda G. Hogue: brenda.hogue@asu.edu
Abstract

Nucleocytoplasmic transport of proteins using XPO1 (exportin 1) plays a vital role in cell proliferation and survival. Many viruses also exploit this pathway to promote infection and replication. Thus, inhibiting XPO1-mediated nuclear export with selective inhibitors activates multiple antiviral and anti-inflammatory pathways. The XPO1 inhibitor, Selinexor, is an FDA-approved anticancer drug predicted to have antiviral function against many viruses, including SARS-CoV-2. Unexpectedly, we observed that pretreatment of cultured human cells with Selinexor actually enhanced protein expression and replication of coronaviruses, including SARS-CoV-2. Knockdown of cellular XPO1 protein expression significantly enhanced the replication of coronaviruses in human cells. We further demonstrate that Selinexor treatment reduced the formation of unique cytoplasmic antiviral granules that include RNA helicase DHX9 in the virus-infected cells. These results, for the first time, show that the anti-cancer drug Selinexor enhances the replication of coronaviruses in human cells in vitro and thus should be further explored in vivo for the potential impact on the dual use for anticancer and antiviral therapy.
Introduction

The nucleocytoplasmic transport of proteins and other molecules is a highly regulated cellular process. Many nuclear export (called exportins) and import (called importins) proteins are involved in this process by exploiting nuclear pore complexes that form proteinaceous channels in the nuclear envelope (1, 2). Exportin 1 (XPO1), also known as CRM1 (chromosome region maintenance 1), is one of the major nuclear export proteins, transporting hundreds of cellular cargo proteins involved in diverse cellular processes such as transcription, translation, cellular growth, differentiation, and mediating inflammatory responses that include antiviral pathways (3, 4). Thus, direct inhibition of XPO1 function has been explored as a potential antiviral and anticancer therapeutic target (5-8). Various natural or chemically synthesized small molecules that bind to XPO1 and block the export of XPO1 cargo proteins from the nucleus to the cytoplasm have been developed (9, 10). Collectively these molecules are known as selective inhibitors of nuclear export (SINEs). SINEs have been shown to have anticancer activity against diverse types of human cancers and at least one modified drug version with increased safety profile, called Selinexor, has been approved by the FDA for treating multiple myeloma and diffuse large B-cell lymphoma (11, 12). SINEs are also reported in the literature to have antiviral activity against RNA viruses like influenza and respiratory syncytial virus (RSV) that causes respiratory infections due to the blocking of key cellular processes and virus-mediated hijacking of the nucleocytoplasmic transport process (6, 13, 14).

Coronaviruses are enveloped, positive-sense RNA viruses that are known to infect humans and animals. At least seven coronaviruses are known to infect humans. Coronaviruses 229E, NL63, OC43, and HKU1 infect people around the world to cause the common cold (15). Other coronaviruses, MERS-CoV, SARS-CoV, and SARS-CoV-2, are responsible for major disease outbreaks and cause high mortality (16). SARS-CoV-2, which causes COVID-19 killed millions globally due to the ongoing pandemic (https://coronavirus.jhu.edu/map.html). In nature, bats
known to be primary reservoirs of many coronaviruses, from where viruses spill over to intermediate animals such as palm civet, pangolins, rodents, camels, pigs, cattle where viruses evolve and then spillover to humans, causing mild to severe disease (17, 18). The rapid emergence of different variants of SARS-CoV-2 indicates that the viral genome acquire multiple mutations during replication (19, 20). In this context, it is crucial to study the host factors and compounds that can enhance or reduce replication of SARS-CoV-2 (21-23). It is possible that factors which enhance virus replication can also enhance mutational rates in the viral genome.

Recently, it was reported that the XPO1 inhibitor Selinexor enhanced the nuclear localization of ACE-2 protein, the primary receptor for SARS-CoV-2 (24). Selinexor treatment thus reduced virus entry and infection in vitro and the viral load in the lungs in a COVID-19 ferret model (24). In addition, Selinexor treatment downregulated the expression of pro-inflammatory cytokines, which are associated with cytokine storm observed in COVID-19 patients (24, 25).

We previously reported that XPO1 inhibitors, including leptomycin B (LMB) and Selinexor, enhanced the replication of oncolytic myxoma virus (MYXV), a member of the leporipoxvirus genus of poxviridae, in diverse types of cultured human cancer cells where intrinsic cellular pathways restrict virus replication (26). In addition, Selinexor treatment reduced the formation of cytoplasmic DHX9 antiviral granules, which are involved in lowering MYXV late protein synthesis and replication. Selinexor also enhanced the nuclear localization of DHX9 and, as predicted, blocked the XPO1-mediated export of DHX9 nuclear transport (26). These findings led us to investigate further the role of Selinexor-mediated inhibition of XPO1 nuclear export pathway on other viruses.

Here, we show that Selinexor treatment unexpectedly enhances the in vitro replication of coronaviruses, including SARS-CoV-2, which replicate in the cytoplasm of infected cells. This enhanced replication of the virus by Selinexor was confirmed in murine and human cell lines. Furthermore, the targeted knockdown of XPO1 using siRNA also enhanced coronavirus
replication, suggesting the pro-coronavirus drug action is indeed related to the expected nuclear export pathway target. Coronavirus infection in human cells results in the unique localization of RNA helicase DHX9 in the cytoplasm, which is blocked with Selinexor treatment. The observation that Selinexor treatment exacerbates coronavirus infection of human cells in vitro should serve as a cautionary note to carefully track SARS-CoV-2 infections in cancer patients when treated with the drug.

Results

Nuclear export inhibitor Selinexor enhances gene expression and replication of coronavirus in murine L2 cells.

Murine L2 cells are naturally infected by the mouse hepatitis virus (MHV). We first monitored and measured the viability of L2 cells in response to treatment with different concentrations of Selinexor (Fig 1A). L2 cell viability was reduced only after treatment with a Selinexor concentration of 5µM or above but had minimal or no effect with a concentration of 1 µM or less. To test the effect of Selinexor on MHV replication, L2 cells were first pretreated with different concentrations of Selinexor for one hour and infected with a GFP-expressing MHV A59 (rA59/SMHV-2-EGFP) to monitor the level of GFP expression in the infected cells (27-29). Infection with this MHV exhibits reduced cell fusion since the virus expresses a less fusogenic form of the spike protein and thus allowed counting the number of GFP positive cells. We observed the enhanced GFP expression in L2 cells pretreated with Selinexor between 10 and 0.01 µM concentration (Fig 1B). We confirmed the enhanced expression of GFP by counting the number of GFP-positive cells (Fig 1C). To assess the number of viruses produced in the presence of Selinexor, L2 cells were treated with different concentration of Selinexor and infected with another GFP expressing MHV A59 (MHVE-GFP) that expresses a highly fusogenic form of spike and readily forms plaques. After 24h post infection, the infected cells and supernatant were collected to titer the progeny virus formation by plaque assay. Virus titration results show a significantly enhanced number of viruses.
in the cells pretreated with Selinexor between 1 and 10 µM concentration (Fig 1D). These results confirm that Selinexor enhances MHV replication and progeny virus formation in naturally permissive L2 cells.

**Selinexor enhances gene expression and replication of coronavirus in human cells.**

To further confirm the effects of Selinexor on MHV replication, we used human cell lines expressing MHV receptor (MHVR) carcinoembryonic antigen-related cell adhesion molecule 1 (CECAM1), HeLa-MHVR (human HeLa cell line expressing MHVR) (30) and A549-CECAM/MHVR (human A549 cell line expressing MHVR) (31). Both cell lines were first treated with different concentrations of Selinexor to test the cell viability (Fig 2A and 3A). Unlike L2 cells, Selinexor concentrations above 1µM significantly reduced viability of the human cells. However, with a concentration of less than 0.1µM, we observed minimal or no reduction in the cell viability.

To assess whether Selinexor enhances MHV replication in the human cell lines, both were pretreated with different concentrations of Selinexor for one hour and infected with a GFP-expressing MHV, rA59/SMHV-2-EGFP to monitor GFP expression in the infected cells. We observed enhanced GFP expression when pretreated with Selinexor at a concentration of 0.01 µM or more (Fig 2B). Enhanced GFP expression was further confirmed by counting the number of GFP-positive cells (Fig 2C and 3B). To assess the amount of virus produced in the presence of Selinexor, HeLa-MHVR (Fig 2D) and A549-MHVR (Fig 3C) cells were treated with different concentration of the drug and infected with MHVE-GFP. Again, like L2 cells, we observed significantly enhanced virus production in both the human cell lines when pretreated with Selinexor between 1 and 0.01 µM concentration (Fig 2D and 3C).

**XPO1 knockdown enhances coronavirus gene expression and replication**

Since inhibition of the XPO1-mediated nuclear export pathway using Selinexor enhanced coronavirus replication, we further extended this observation by direct knockdown of XPO1 using siRNA. After transfection of XPO1 siRNA or a non-targeting control siRNA (NT-siRNA) in A549-MHVR cells, the cells were infected with different MOIs of the GFP expressing MHV, rA59/SMHV-2-EGFP.
2-EGFP or MHVE-GFP. An increase in GFP-expressing cells was observed only in the XPO1 knockdown cells (Fig 4A). Furthermore, the enhanced number of GFP-positive cells in the XPO1 knockdown cells compared to controls was confirmed by counting the number of GFP-positive cells (Fig 4C). The level of XPO1 protein knockdown using siRNA was confirmed by western blot analysis (Fig 4B). To quantify the number of progeny virions in the infected cells and supernatant, the cells were infected with MHVE-GFP and samples were collected 24h post-infection. Virus titration shows that significantly increased virus production in the XPO1 knockdown cells compared to the NT-siRNA control or cells infected with the virus alone (Fig 4D), indicating that the effect of Selinexor is mediated by XPO1.

**Selinexor enhances gene expression and replication of SARS-CoV-2.**

We next tested whether Selinexor enhances the replication of SARS-CoV-2 in human cells. We used a human A549 cell line expressing the ACE2 receptor (A549^{ACE2}) and a SARS-CoV-2 virus expressing GFP (SARS-CoV-2-GFP) (32). The A549^{ACE2} cell line showed a similar level of sensitivity to different concentrations of Selinexor like A549-MHVR (data not shown). To assess whether Selinexor enhances SARS-CoV-2 gene expression and replication, A549^{ACE2} cells were first pretreated with 0.01µM of Selinexor for one hour and infected with SARS-CoV-2-GFP. After 24h, cells were collected and fixed for counting the number of GFP-positive cells. A significantly increased number of GFP-positive cells were observed in the treated infected cells compared to the untreated cells (Fig 5A). In another experiment, the infected cells and supernatant were collected to titer the progeny virus formation. Virus titration showed significantly increased virus production in the treated cells (Fig 5B). These results indicate that, like MHV, Selinexor enhances the replication of SARS-CoV-2 in human cells.

**Selinexor treatment inhibited the unique cytoplasmic localization of DHX9 in the coronavirus-infected human cells to enhance viral gene expression.**

RNA helicase DHX9 forms cytoplasmic antiviral granules to restrict the cytoplasmic replication of poxviruses such as MYXV and orthopoxvirus vaccinia virus (VACV) in human cells (33). We
tested whether the nuclear localization of DHX9 is changed during the cytoplasmic replication of coronavirus. In uninfected A549-MHVR, as expected, DHX9 is mainly localized in the nucleus (Fig 6A, top panels). However, when infected with MHV, DHX9 was detected also in the cytoplasm of infected cells, mainly in the perinuclear region (Fig 6A, middle panels). We then tested whether Selinexor treatment had any effect on the cytoplasmic localization of DHX9 during coronavirus infection. Selinexor treatment completely blocked the cytoplasmic localization of DHX9 in the coronavirus-infected cells (Fig 6A, bottom panels). Based on these observations, in a separate experiment we infected A549-MHVR cells with MHV and tittered progeny virus in the supernatant and cells separately in the presence or absence of Selinexor. Virus titration demonstrated that cells treated with Selinexor have more virus in the cells than the supernatant (Fig 6B). These results suggest that the newly produced viruses from the Selinexor treatment are mainly localized within the infected cells.

Discussion

The nucleocytoplasmic transport process mediated by XPO1 plays a vital role in the export of hundreds of proteins from the nucleus. The proteins are involved in diverse cellular processes such as cell proliferation, cell cycle progression, and apoptosis (1, 5). Thus, the XPO1-mediated export pathway is targeted by viruses at various stages of their lifecycle to regulate cellular proteins and the appropriate localization of viral proteins (6, 34). Apart from viruses, this nuclear export pathway is also crucial for anticancer therapy due to the export of tumor suppressor proteins by XPO1 to the cytoplasm (7, 10). Therefore, XPO1 inhibitors are developed as potential antiviral and anticancer agents. In the nucleus, XPO1 binds to cargo proteins in the presence of RanGTP via an NES (nuclear export sequence) composed of a cluster of leucine (L)-rich or hydrophobic amino acids. After transiting to the cytoplasm, the hydrolysis of RanGTP by RanGAP (a GTPase) disassembles the trimeric complex, and XPO1 reenters the nucleus. The cysteine residue within the hydrophobic NES-binding region at position 528 is the prime target for most
XPO1 inhibitors, including leptomycin B (LMB). LMB isolated from Streptomyces was the first specific inhibitor of XPO1 (35). However, the clinical development was discontinued due to severe cell toxicity (36). The irreversible binding of LMB with CRM1 caused long-term inhibition of CRM1-mediated nuclear export, and possible other off-target activity resulting in cellular toxicity (36). Synthetic derivatives of LMB with less toxicity due to the reversible binding with CRM1 have been clinically tested in human. The FDA has approved one such XPO1/CRM1 inhibitor called Selinexor for treating hematological cancers (11). XPO1 inhibitors have shown antiviral activity against many viruses, such as influenza, RSV, and recently SARS-CoV-2 (13, 34, 37, 38).

We recently reported that, unlike RNA viruses, LMB or Selinexor enhances the replication of oncolytic MYXV, a leporipoxvirus developed for cancer treatment (26). In human cancer cells, Selinexor enhanced MYXV replication only at a low concentration that had minimal or no toxicity to the cells. However, a higher concentration of Selinexor that caused cellular toxicity also reduced virus replication. Based on our observation that Selinexor can enhance cytoplasmic replication of a poxvirus and a recent report that Selinexor inhibits SARS-CoV-2 replication, we first tested the effect of Selinexor on the replication of a mouse coronavirus MHV using murine L2 cells. To our surprise, we observed that pretreatment of L2 cells with concentrations of Selinexor that had minimal or no toxicity to the cells significantly increased the reporter GFP expression from a recombinant MHV. Furthermore, when we titrated the virus from the infected cells, we also observed increased progeny virus production in the L2 cells. Since our previous observation with MYXV used human cell lines, we tested whether Selinexor similarly affects MHV in human cells. Human HeLa and A549 cells expressing the MHV receptor murine CECAM1 were used (30). Again, increased GFP expression and MHV replication were observed when cells were pretreated with Selinexor concentrations that had minimal or no effect on the cell viability. These results confirm that the impact of Selinexor on MHV replication is independent of the cell type.
Since XPO1 is the only known cellular target of Selinexor, we previously reported that XPO1 knockdown using siRNA enhanced the replication of MYXV in human cancer cells (26). Here, we used the same approach to confirm that the Selinexor-mediated enhancement of MHV replication is through XPO1/CRM1. Using siRNA, we transiently knocked down XPO1 in both A549-MHVR and HeLa-MHVR cell lines. Under these conditions, we again observed a significant increase in GFP expression and MHV replication when infected at low MOI. These results encouraged us to test whether the optimized lower concentration of Selinexor enhances SARS-CoV-2 replication in human cells. In this case, we used A549 cells expressing the SARS-CoV-2 ACE2 receptor. Using a recombinant SARS-CoV-2 that expresses GFP, we show that with a Selinexor concentration of 0.01 µM, there is an increase in GFP expression, which also correlated with increased virus titer. These results indicate that in human cells, both MHV and SARS-CoV-2 replication is enhanced by Selinexor treatment. In a recent study, it was shown that in Vero E6 cells, Selinexor treatment reduced the SARS-CoV-2 plaque number and virus titer by more than two logs with >10nM Selinexor (24). However, on our hand, Selinexor-mediated enhancement of virus replication was not observed in Vero-E6 cells (data not shown). This is consistent with our observation that MYXV replication is also not enhanced by Selinexor in Vero-E6 cells (data not shown). Therefore, we can speculate that nonhuman primates and human cells respond to Selinexor differently against these tested viruses.

To understand the possible mechanisms of how Selinexor enhances coronavirus replication, we studied the localization of RNA helicase DHX9 in the MHV-infected human cells. DHX9 is primarily localized in the nucleus. However, due to its involvement with diverse cellular processes such as transcription and translation, it shuttles between the nuclear and cytosolic compartments. We reported that in the MYXV-infected human cancer cells, DHX9 was exported from the nucleus to form the antiviral granules in the cytoplasm of infected human cancer cells (33). These antiviral granules reduced virus late protein synthesis and reduced progeny virus formation. DHX9 is
associated with the replication of many RNA and DNA viruses such as human immunodeficiency virus HIV-1 (39), hepatitis C Virus (40), influenza A (41), hepatitis E (42), cytomegalovirus (43), chikungunya virus (44, 45). In the case of MHV, we observed that in the infected cells, the majority of DHX9 remains still localized in the nucleus; additionally, DHX9 also accumulated in the perinuclear region of the infected cell cytoplasm. When cells were pretreated with Selinexor, localization of DHX9 in the cytoplasm of infected cells was not observed. Selinexor treatment also enhanced the viral GFP expression compared to the MHV infection alone. This localization of DHX9 in MHV infected cells is different from the distinct antiviral granules observed with MYXV (33). This could be due to the differences in how these two classes of viruses replicate in the cytoplasm. Additionally, direct knockdown of DHX9 using siRNA in human cells didn’t significantly enhance MHV replication (data not shown), suggesting that other cellular proteins might be involved in this process. For the majority of RNA viruses that have been tested, DHX9 is known as the cellular factor required for optimum virus replication (46). However, our results indicate that DHX9, at least in these cells, is not needed for coronavirus replication; rather, it has an antiviral role in this case. In future the role of DHX9 in coronavirus replication must be further explored.

Several SARS-CoV proteins such as ORF3b, ORF9b and nucleocapsid have been reported to be localize in the nucleus and use the XPO1-mediated export pathway (47). However, a direct impact on virus infection and replication has not been reported. Here, we show that inhibition of XPO1 with Selinexor or direct knockdown of XPO1 enhances the gene expression and replication of mouse hepatitis coronavirus and SARS-CoV-2.

Selinexor is currently approved for treating selected hematological malignancies such as multiple myeloma and diffuse large B-cell lymphoma (11, 48). Multiple clinical trials are presently undergoing to use Selinexor as a monotherapy or in combination with other treatments against diverse types of malignancies (49, 50). A Phase 2 placebo-controlled trial of oral Selinexor in COVID-19 patients did not show significant differences in clinical outcomes (NCT04349098).
Although one published report suggests that Selinexor reduces replication of SARS-CoV-2 and symptoms in animal models, our results indicate that Selinexor enhances replication of both MHV and SARS-CoV-2. Future studies should focus on how Selinexor and nuclear transportation pathway regulate the replication of coronavirus.

Materials and methods

Cell lines

L2 murine fibroblast cell line and African green monkey kidney Vero cells E6 (obtained from ATCC) were cultured and maintained using Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, 100 U/ml of penicillin, and 100 µg/ml streptomycin. HeLa-MHVR cell line (human HeLa cell line expressing mouse coronavirus receptor mCECAM1) was provided by Tom Gallagher at Loyola University and maintained using DMEM media with 10% FBS, HEPES buffer, 100 U/ml of penicillin, 100 µg/ml streptomycin, MEM non-essential amino acids, and sodium pyruvate. A549-MHVR (human A549 cell line expressing mouse coronavirus receptor mCECAM1) and A549ACE2 (human A549 cell line expressing ACE2 receptor) was provided by Susan Weiss at the Perelman School of Medicine at the University of Pennsylvania and maintained with RPMI1640 media supplemented with 10% FBS, 100 U/ml of penicillin, and 100 µg/ml streptomycin (31).

Viruses and viral replication assay

Mouse hepatitis coronavirus MHV A59 that expresses GFP was used for all the assays. rA59/SMVH-2-EGFP is a recombinant MHV A59 virus that expresses the spike of MHV-2 in place of the WT protein and GFP (27-29). MHVE-GFP was constructed using a MHV A59 reverse genetics system essentially as previously described (51-54). The coding sequence for the MHV envelope (E) protein was fused to the GFP gene with an intervening tetra glycine linker. The construct was cloned into ORF 4a/b in the G subclone of the MHV infectious clone using SbfI and
EcoRV restriction sites. The construct was designed to maintain the transcription regulatory sequences for both ORF 4 and ORF5. Virus was recovered after assembling the full-length genomic cDNA and transcription of full-length genomic RNA as previously described (51-54). Following electroporation into baby hamster kidney grown in 17CI1 mouse cells virus was recovered and passage 1 stock was grown in mouse 17CI1 cells and titered in L2 cells by plaque assay. The SARS-CoV-2-GFP virus was provided by Ralph Baric at the University of North Carolina Chapel Hill (32).

Viral titers in different cell lines were determined using a viral replication assay. The cells were seeded in 24 well plate (2x10^5 cells/well). The next day, the cells were treated for 1h with different concentrations of Selinexor diluted in the appropriate media used for growth of the specific cell lines. Virus was added to the cells (volume calculated based on different multiplicities of infection and incubated for 1h at 37°C in the presence of Selinexor. After 1h, the unbound virus was removed, cells were washed with DPBS (Dulbecco’s phosphate-buffered saline) and media without Selinexor was added for further incubation. Both cells and media were collected at different time points and stored at -80°C freezer until processing. Samples were subjected to three freeze/thaw cycles before titration. Afterwards, different dilutions were prepared in the appropriate media and plated on cell lines and fluorescent foci were counted after 24h using a fluorescent microscope. All assays and dilutions were performed in triplicate. For SARS-CoV-2, all infections and virus manipulations were conducted at biosafety level 3 (BSL-3) in the Biodesign Institute using appropriate and IBC-approved personal protective equipment and protocols. For plaque assay, samples were serially diluted 10-fold and absorbed on Vero cells at 37°C for 1h. Cells were overlaid with media plus 0.7% agarose and incubated for two days at 37°C. Cells were fixed with 4% paraformaldehyde and subsequently stained with 1% crystal violet for counting the plaques.

Reagents and Antibodies
Rabbit polyclonal antibodies for DHX9 and XPO1 and mouse monoclonal antibody against β-actin were purchased from Thermo Fisher Scientific. HRP-conjugated goat anti-rabbit and anti-mouse IgG antibodies were purchased from Jackson Immuno Research Laboratories. All the secondary antibodies conjugated to Alexa Fluor 488, 594, 568 and 647 were purchased from Thermo Fisher Scientific. Selinexor (KPT330) was purchased from Apex Bio (Tokyo, Japan).

**Immunofluorescence**

Cells (5x10^5-1x10^6/dish) were seeded onto glass bottom 35 mm petri dishes overnight. The next day cells were treated with Selinexor or mock treated and infected with the virus. At the indicated time points after treatment cells were washed with PBS three times, fixed with 2% paraformaldehyde in PBS for 12 min at room temperature, washed with PBS three times, and permeabilized in 0.1% Triton X-100 in PBS for 90 sec at room temperature. Fixed cells were washed with PBS 3 times and then blocked with 3% BSA in PBS for 30 min at 37°C. Samples were then incubated with primary antibody (1:300 dilution) for 30 min at 37°C, washed with PBS six times, and incubated with secondary antibodies conjugated to different Alexa Fluors. After washing again with PBS six times, samples were mounted on glass slides with Vecta Shield (Vectorlabs) containing DAPI (4′,6-diamidino-2-phenylindole) to stain DNA in the nuclei and viral factory. Fluorescence images were captured using confocal microscopy.

**siRNA transfection**

ON-TARGETplus SMART pool siRNAs for exportin 1/XPO1 and a non-targeting control (NT siRNA) were purchased from Dharmacon (Horizon Discovery). In 24 well plate cells were seeded with 40–50% confluence, left overnight for adherence, and then transfected with siRNAs (40 nM) using Lipofectamine RNAiMAX (Invitrogen) transfection reagent. After 48 h of transfection, the cells were infected with different MOIs of virus for 1h, washed to remove the unbound virus, and incubated with complete media. At the indicated time points, cells were
either observed by fluorescence microscopy to monitor and record the expression of fluorescent proteins or harvested and processed for titration of progeny virions. For detection of proteins, Western blot analysis was performed from the total proteins as described before (55).

**Cell viability assay**

To assess the viability of different cell lines after Selinexor treatment, 10,000 cells were seeded into each well of a 96-well plate. The next day, cells were treated with different concentrations of Selinexor. A minimum of four to five wells were used for each treatment condition, and untreated cells (mock) served as controls. Cell viability at different time points was assessed using MTS reagents (Promega) according to manufacturer instructions.

**Statistical analysis**

Statistical analyses were performed using GraphPad Prism software. Values are represented as mean ± SD for at least two or three independent experiments. A paired two-tailed Student’s t-test was used to determine the significance between the two groups. *P* values are reported as follows: no significant (ns) *P* > 0.05, * *P* < 0.05, ** *P* < 0.01, *** *P* < 0.001, **** *P* < 0.0001.

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**Figure Legends**

**Figure 1**: Selinexor enhances the replication of MHV in L2 cells. A) Effect of Selinexor on L2 cells viability. Cells were plated in 96 well plate and treated with different concentration of Selinexor and cell viability was measured after 48h. B-D) Effect of Selinexor on MHV virus gene expression and replication. Cells were plated in 24 well plates, treated with the indicated concentration of Selinexor for 1h and infected with rA59/S<sub>MHV-2</sub>-EGFP or MHVE-GFP virus with a MOI of 0.01. B) Fluorescence images were taken 24h post infection with rA59/S<sub>MHV-2</sub>-EGFP; C) percent of GFP positive cells were counted using Countess II cell counter at different time points after infection with rA59/S<sub>MHV-2</sub>-EGFP; D) MHVE-GFP virus replication was measured by plaque assay from the total number of viruses in the cells and supernatant 24h post infection.

**Figure 2**: Selinexor enhances the replication of MHV in human HeLa-MHVR cells. A) Effect of Selinexor on the viability of HeLa-MHVR cells. Cells were plated in 96 well plate and treated with different concentration of Selinexor and cell viability was measured after 48h. B-D) Effect of Selinexor on MHV virus replication in HeLa-MHVR cells. Cells were plated in 24 well plates, treated with indicated concentration of Selinexor for 1h and infected with rA59/S<sub>MHV-2</sub>-EGFP or MHVE-GFP virus with a MOI of 0.01. B) Fluorescence images were taken 24h post infection with rA59/S<sub>MHV-2</sub>-EGFP; C) percent of GFP positive cells at 24h and 48h post infection with rA59/S<sub>MHV-2</sub>-EGFP.
2-EGFP were counted using Countess II cell counter; D) Total number of viruses in the cells and supernatant was determined by plaque assay 24h post infection of cells with MHVE-GFP.

Figure 3: Selinexor enhances the replication of MHV in human A549-MHVR cells. A) Effect of Selinexor on the viability of A549-MHVR cells. Cells were plated in 96 well plate and treated with different concentration of Selinexor and cell viability was measured after 48h. B-C) Effect of Selinexor on MHV virus replication in A549-MHVR cells. Cells were plated in 24 well plates, treated with indicated concentration of Selinexor for 1h and infected with rA59/S_MHV-2-EGFP or MHVE-GFP virus with a MOI of 0.01. B) percent of GFP positive cells at 24h and 48h post infection with rA59/S_MHV-2-EGFP were counted using Countess II cell counter; C) Total number of viruses in the cells and supernatant was determined by plaque assay 24h post infection of cells with MHVE-GFP.

Figure 4: XPO1 knock down enhances the replication of MHV in human A549-CECAM cells. Cells were plated in 24 well plate, transfected with control non-targeting siRNA (NT-siRNA) or XPO1 siRNA for 48h and infected with rA59/S_MHV-2-EGFP or MHVE-GFP for another 24h. A) Images were taken using a fluorescence microscope 24h post infection with MHVE-GFP; B) Knock down of XPO1 was confirmed with Western blot analysis using anti-XPO1 antibody. Actin was used as total protein loading control. C) percent of GFP positive cells after infection with rA59/S_MHV-2-EGFP were counted using Countess II cell counter. D) Total number of viruses in the cells and supernatant was determined by plaque assay 24h post infection of cells with MHVE-GFP.

Figure 5: Selinexor enhances the replication of SARS-CoV-2 in human A549ACE2 cells. Cells were plated in 12 well plates, treated with indicated concentration of Selinexor for 1h and infected with SARS-CoV-2 virus. A) percent of GFP positive cells were counted using Countess II cell counter after fixation of cells. B) Total number of progeny virus formation was measured by plaque assay using Vero E6 cells.
Figure 6: Selinexor blocks the cytoplasmic localization of DHX9 in the MHV infected cell. A) HeLa-MHVR cells were seeded on glass bottom 35mm petri dishes and left over-night to adhere. Next day cells were mock infected or infected with rA59/S_{MHV-2-EGFP} (MOI = 0.01) in the presence or absence of selinexor. After 24h cells were fixed and stained with antibodies against DHX9. Nuclei were stained with DAPI. B) Titration of progeny virus in the infected cells and supernatant. Cells were plated in 24 well plates, treated with indicated concentration of selinexor for 1h and infected with MHVE-GFP virus with a MOI of 0.1. 24h post infection the cells and supernatants were collected separately. Number of viruses in the cells and supernatants were measured by plaque assay.

References:


Figure 1: Selinexor enhances the replication of MHV in L2 cells. A) Effect of Selinexor on L2 cells viability. Cells were plated in 96 well plate and treated with different concentration of Selinexor and cell viability was measured after 48h. B-D) Effect of Selinexor on MHV virus gene expression and replication. Cells were plated in 24 well plates, treated with the indicated concentration of Selinexor for 1h and infected with rA59/S_{MHV-2-EGFP} or MHVE-GFP virus with a MOI of 0.01. B) Fluorescence images were taken 24h post infection with rA59/S_{MHV-2-EGFP}; C) percent of GFP positive cells were counted using Countess II cell counter at different time points after infection with rA59/S_{MHV-2-EGFP}; D) MHVE-GFP virus replication was measured by plaque assay from the total number of viruses in the cells and supernatant 24h post infection.
Figure 2: Selinexor enhances the replication of MHV in human HeLa-MHVR cells. A) Effect of Selinexor on the viability of HeLa-MHVR cells. Cells were plated in 96 well plate and treated with different concentration of Selinexor and cell viability was measured after 48h. B-D) Effect of Selinexor on MHV virus replication in HeLa-MHVR cells. Cells were plated in 24 well plates, treated with indicated concentration of Selinexor for 1h and infected with rA59/S_{MHV-2}-EGFP or MHVE-GFP virus with a MOI of 0.01. B) Fluorescence images were taken 24h post infection with rA59/S_{MHV-2}-EGFP; C) percent of GFP positive cells at 24h and 48h post infection with rA59/S_{MHV-2}-EGFP were counted using Countess II cell counter; D) Total number of viruses in the cells and supernatant was determined by plaque assay 24h post infection of cells with MHVE-GFP.
Figure 3: Selinexor enhances the replication of MHV in human A549-MHVR cells. A) Effect of Selinexor on the viability of A549-MHVR cells. Cells were plated in 96 well plate and treated with different concentration of Selinexor and cell viability was measured after 48h. B-C) Effect of Selinexor on MHV virus replication in A549-MHVR cells. Cells were plated in 24 well plates, treated with indicated concentration of Selinexor for 1h and infected with rA59/S_{MHV-2}^−EGFP or MHVE-GFP virus with a MOI of 0.01. B) percent of GFP positive cells at 24h and 48h post infection with rA59/S_{MHV-2}^−EGFP were counted using Countess II cell counter; C) Total number of viruses in the cells and supernatant was determined by plaque assay 24h post infection of cells with MHVE-GFP.
Figure 4: XPO1 knock down enhances the replication of MHV in human A549-CECAM cells. Cells were plated in 24 well plate, transfected with control non-targeting siRNA (NT-siRNA) or XPO1 siRNA for 48h and infected with rA59/S_{MHV-2}-EGFP or MHVE-GFP for another 24h. A) Images were taken using a fluorescence microscope 24h post infection with MHVE-GFP; B) Knock down of XPO1 was confirmed with Western blot analysis using anti-XPO1 antibody. Actin was used as total protein loading control. C) percent of GFP positive cells after infection with rA59/S_{MHV-2}-EGFP were counted using Countess II cell counter. D) Total number of viruses in the cells and supernatant was determined by plaque assay 24h post infection of cells with MHVE-GFP.
Figure 5: Selinexor enhances the replication of SARS-CoV-2 in human A549\textsuperscript{ACE2} cells. Cells were plated in 12 well plates, treated with indicated concentration of Selinexor for 1h and infected with SARS-CoV-2 virus. A) percent of GFP positive cells were counted using Countess II cell counter after fixation of cells. B) Total number of progeny virus formation was measured by plaque assay using Vero E6 cells.
Figure 6: Selinexor blocks the cytoplasmic localization of DHX9 in the MHV infected cell. 
A) HeLa-MHVR cells were seeded on glass bottom 35mm petri dishes and left over-night to adhere. Next day cells were mock infected or infected with rA59/S\textsubscript{MHVs-2-EGFP} (MOI = 0.01) in the presence or absence of selinexor. After 24h cells were fixed and stained with antibodies against DHX9. Nuclei were stained with DAPI. B) Titration of progeny virus in the infected cells and supernatant. Cells were plated in 24 well plates, treated with indicated concentration of selinexor for 1h and infected with MHVE-GFP virus with a MOI of 0.1. 24h post infection the cells and supernatants were collected separately. Number of viruses in the cells and supernatants were measured by plaque assay.