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OPINION ARTICLE

NPC1 as a Modulator of Disease Severity and Viral Entry of SARS-CoV-2

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Abstract: The COVID-19 plague is hitting mankind. Several viruses, including SARS-CoV-1, MERS-CoV, EBOV, and SARS-CoV-2, use the endocytic machinery to enter the cell. Genomic variants in *NPC1*, which encodes for the endo-lysosomal Niemann-Pick type C1 protein, restricts the host-range of viruses in bats and susceptibility to infections in humans. Lack of NPC1 and its pharmacological suppression inhibits many viral infections including SARS-CoV-1 and Type I Feline Coronavirus Infection. Antiviral effects of NPC1-inhibiting drugs for COVID-19 treatment should be explored.

Keywords: COVID-19, SARS-Cov-2, Niemann-Pick C1, modifier gene, drug repurposing, virus, infection.

1. INTRODUCTION

The COVID-19 pandemic, caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), has spread worldwide since December 2019. There is little evidence of potential therapies, no randomized clinical trials, although there are currently more than 300 currently proving repurposed or experimental COVID-19 treatments (clinicaltrials.gov).

The SARS-CoV-2 is an enveloped RNA virus that binds via its Spike (S) protein, the angiotensin converting enzyme 2 (ACE2), to enter into the cell, from where it is delivered to the endo/lysosomal (endo/lys) pathway [1]. The transit through these vesicular organelles is shared with several other enveloped viruses including Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-1), Middle East respiratory syndrome coronavirus (MERS-CoV), Ebola virus (EBOV), Human Immunodeficiency Virus (HIV), among others, and is required for completion of the infectious cycle. Therefore, targeting the endo/lys pathway is an attractive common strategy for treating viral infections, including SARS-CoV-2 [2].

2. NPC1 AND SUSCEPTIBILITY TO VIRAL INFECTIONS

Currently, several drugs that impair the endo/lys pathway are being tested, including Arbidol, Amantadine and until recently hydroxychloroquine (HQ) [3,4]. The latter increases the pH of the lysosome, decreasing the activity of many enzymes within this organelle. The effects of HQ on SARS-CoV-2 patients have been controversial, likely due to the broad inhibition of the lysosome, therefore clinical trials have been halted.

A key protein that controls endo/lys vesicular trafficking and cholesterol export from these organelles is the Niemann-Pick type C1 (NPC1) protein. It consists of 13 transmembrane domains that generate three distinct luminal domains. Among them, there is a sterol-sensing domain conserved with other molecules involved in cholesterol metabolism. In order to direct cholesterol out of the endo/lys compartment, NPC1 partners with a soluble lysosomal cholesterol binding protein called NPC2. Loss-of-function variants in either the *NPC1* or *NPC2* genes cause Niemann-Pick type C (NPC) disease, a neuro-visceral lysosomal storage disorder characterized by lysosomal buildup of cholesterol and other lipids [5].

Growing evidence links NPC1 and susceptibility to viral infections. For instance, bat species present with differential susceptibility to Filoviruses (EBOV and Marburg; MARV). Various strains can be infected by EBOV, others by MARV, and some are refractory to either. This variability resides on a few coding variants in the host *npc*1 gene, suggesting that a molecular mechanism needs to be evolved to restrict the host-range of infection of these viruses [6,7]. In humans, missense Single-Nucleotide Variants (SNVs) in the *NPC1* gene were found to influence Filovirus entry into cells [8]. Although none of the alleles in those SNVs fully ablated the infection, they might contribute to differential disease susceptibility. Furthermore, human cells derived from NPC disease patients are not susceptible to infection by EBOV and MARV [9,10].

The mechanism by which NPC1 depletion/inhibition protects against EBOV infections has been explored.

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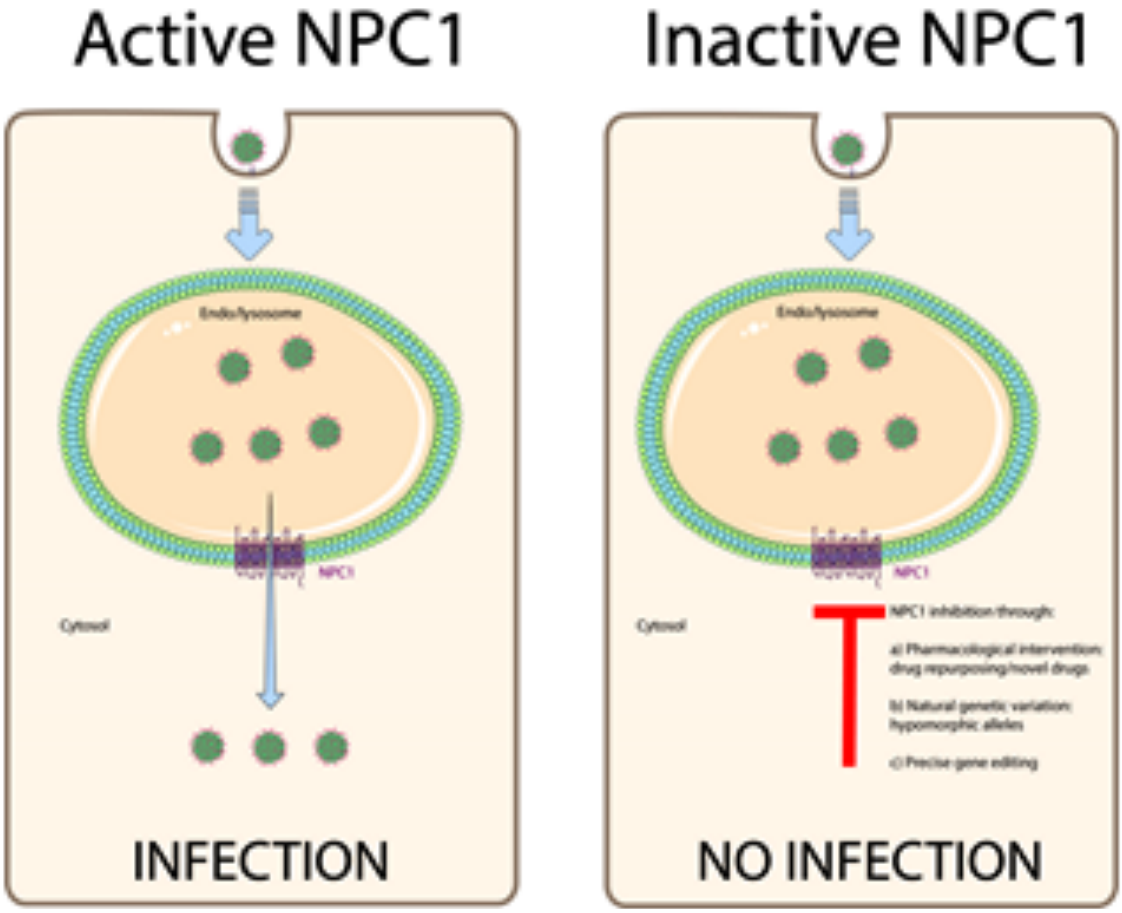

Fig. (1). Strategies for inhibiting NPC1 function for restricting viral infections. SARS-CoV-2 enters the host cell by binding its Spike protein to the ACE2 receptor and making use of the endo/lys internalization pathway. NPC1 is a key component of the endo/lys machinery and is required for the delivery of cargos into the cytosol. SARS-CoV-2 passes through this compartment to complete its infectious cycle (A). We hypothesize that pharmacological suppression of NPC1 protein as well as hypomorphic alleles of *NPC1* that could be present in different populations and even gene-editing-directed silencing of NPC1 can lead to decreased levels of viral entry into the host cell and thus, ameliorate and even suppress clinical manifestations of the disease (B). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Mice lacking NPC2, which accumulate cholesterol intracellularly, are susceptible to EBOV to the same extent than wild-type controls, while *Npc1*$^{-/-}$ mice are resistant to the virus. *Npc1*$^{+/-}$ mice can be infected but show reduced viral loads. It can be speculated that people heterozygous at this locus may be protected, at least to some extent. *Npc1*$^{-/-}$ mice treated with the cholesterol-reducing agent hydroxypropyl-β-cyclodextrin were resistant to infection by EBOV, indicating that resistance to the virus is independent of cholesterol accumulation, but dependent on NPC1 [11]. Additional research has shown that the interaction between NPC1 and the viral surface glycoprotein (GP) is required for the penetration of viral genomes into the cytoplasm in order to trigger infection [12].

3. NPC1 AS A BROAD THERAPEUTIC TARGET FOR VIRAL INFECTIONS

Complementary antiviral therapeutic strategies targeting NPC1 have been proposed or are under development. Pharmacological interventions aimed at inhibiting NPC1 have shown promising broad antiviral effects (Figure **1**). The use of U18666A, a cationic amphiphile that blocks NPC1 function triggering intracellular cholesterol buildup has shown positive results for a variety of viruses, including Dengue, EBOV, Hepatitis C, and also SARS-CoV-1 and Type I Feline Coronavirus Infection (F-CoV), among many others [10,13-16].

U18666A can be used only for experimentation due to its toxicity. However, multiple lysosomotropic cationic amphiphiles, including several FDA-approved agents, inhibit EBOV entry in an NPC1-dependent fashion [10,17]. The effects of these drugs should be tested as candidate antiviral agents for the same U18666A-susceptible viruses and expanded to others such as SARS-CoV-2. Particular attention should be paid to FDA-approved drugs, including clomiphene, and terconazole —also, some benzylpiperazine adamantane diamide-derivates [9,16]. Imipramine, an FDA-approved antidepressant that mimics an NPC phenotype, inhibits Chikungunya, Zika, West Nile and Dengue virus [18].

An interesting experimental approach was the development of bispecific-antibodies (bsAbs) where mouse antibodies against NPC1 were coupled to a mouse antibody against a conserved surface epitope of GP of the EBOV. The bsAbs neutralized EBOV entry into cells and significantly extended the lifespan of infected mice [19]. Another strategy used modified antisense oligonucleotides against NPC1. With this approach, knockdown of NPC1 significantly reduced EBOV viral loads in human and murine cell lines [20]. These kinds of strategies, or even the use of genome-editing approaches, in combination with other proposed strategies for treating SARS-CoV-1, [21] can potentially be repurposed for SARS-CoV-2 and deserve further exploration.

CONCLUSION

Increasing evidence in animals and humans suggest that NPC1 can serve as a modifier gene of viral entry into the cell. For EBOV, molecular evidence indicates that this process is independent of the stored cholesterol in the cell. Furthermore, complementary approaches aimed at inhibiting NPC1 have shown broad antiviral effects, including for Dengue, EBOV, Hepatitis C, type 1 F-CoV, SARS-CoV-1 [10,13-16]. Therefore, we strongly suggest that these drugs can be repurposed for COVID-19.

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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