Sigma-1 Receptor (S1R) Modulation with ANAVEX®2-73 as Potential Therapy for COVID-19
The coronavirus disease 2019 (COVID-19), emerged in December 2019, has spread rapidly, with cases now confirmed in multiple countries. As of February 23, 2020, there were 76,936 reported cases in mainland China and 1,875 cases in locations outside mainland China. There have been 2,462 associated deaths worldwide (1).

The impact of viral infection on cellular homeostasis is increasingly recognized as a major contributor to pathological manifestations and the mechanisms by which the virus promotes both viral replication and survival of the infected cell.

Targets and mechanisms of viral infection include the unfolded protein and oxidative stress responses, as well as auto- and mitophagy and ER homeostasis (2).

Thus, in addition to identifying anti-viral agents and vaccines, it would be valuable to test modulators of ER homeostasis as inhibitors of cellular stress in infected cells.

Protection of infected cells by modulators of ER homeostasis could help the immune system to better deal with the viral infection, thereby improving the health status of infected individuals and decreasing mortality.

SARS-CoV-2 Nsp6 Protein Interacts with the Sigma Receptor

- Sigma-1 receptors modulate ER stress and the protein unfolding response

A SARS-CoV-2-Human Protein-Protein Interaction Map Reveals Drug Targets and Potential Drug-Repurposing


Sigma Receptor Identified as a Druggable Target for SARS-CoV-2

A SARS-CoV-2-Human Protein-Protein Interaction Map Reveals Drug Targets and Potential Drug-Repurposing; David E. Gordon, et al.; March 2020; bioRxiv 2020.03.22.002386; doi: https://doi.org/10.1101/2020.03.22.002386
Rationale for ANAVEX®2-73, a validated Sigma-1 Receptor ligand as Treatment candidate for Coronavirus Disease 2019 (COVID-19)

• Very recent proteomic/chemoinformatic analysis (1) identifying drug and clinical molecules that might perturb the viral-human COVID-19 interactome, it gives these potentially therapeutic perturbations a mechanistic context. Among those that may be infection relevant are the inhibition of lysosomal acidification and trafficking with Bafilomycin A1, via modulation of the ER stress and the protein unfolding response pathway by targeting the Sigma-1 receptor
• SARS-CoV-2 Nsp6 protein interacts with the Sigma-1 receptor, which is thought to regulate ER stress response
• Indeed, several of the human proteins in the interactome are targeted by drugs that have emerged phenotypically as candidate therapeutics for treating COVID-19, such as chloroquine, which targets the Sigma-1 receptors at mid-nM concentrations

(1) David E. Gordon et al., A SARS-CoV-2-Human Protein-Protein Interaction Map Reveals Drug Targets and Potential Drug-Repurposing; bioRxiv March 27th 2020; doi: https://doi.org/10.1101/2020.03.22.002386
Coronavirus Infection, ER stress, apoptosis and innate immunity

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The replication of a coronavirus, a family of important animal and human pathogens, is closely associated with the cellular membrane compartments, especially the endoplasmic reticulum (ER). Coronavirus infection of cultured cells was previously shown to cause ER stress and induce the unfolded protein response (UPR), a process that aims to restore ER homeostasis by global translation shutdown and increasing the ER folding capacity. However, under prolonged ER stress, UPR can also induce apoptotic cell death. Accumulating evidence from recent studies has shown that induction of ER stress and UPR may constitute a major aspect of coronavirus-host interaction. Activation of the three branches of UPR modulates a wide variety of signaling pathways, such as mitogen-activated protein kinase (MAPK) kinase activation, autophagy, apoptosis, and innate immune responses. ER stress and UPR activation may therefore contribute significantly to the viral replication and pathogenesis during coronavirus infection. In this review, we summarize the current knowledge on coronavirus-induced ER stress and UPR activation, with emphasis on their cross-talking to apoptotic signaling.

Severe Acute Respiratory Syndrome Coronavirus Envelope Protein Regulates Cell Stress Response and Apoptosis

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Abstract

Severe acute respiratory syndrome coronavirus (SARS-CoV) that lacks the envelope (E) gene (SARS-CoV-2) is attenuated in vitro. To investigate the role of E protein in the attenuation of SARS-CoV, we compared virus infection of cells infected with SARS-CoV with or without E gene was compared. Twenty-five stress response genes were preferentially upregulated during infection in the absence of the E gene. In addition, gene levels involved in stress transduction, transcription, cell metabolism, immune-regulation, inflammation, apoptosis and cell cycle and differentiation were differentially regulated in cells infected with SARS-CoV with and without the E gene. Administration of E protein in mice reduced the stress responses in cells infected with SARS-CoV-2 or with recombinant viral vector, as treated with drugs, such as tunicamycin and thapsigargin that block cell stress due to different mechanisms. In addition, SARS-CoV-2 strain regulated the confounding pathway involved in regulating E gene. Thus, our findings suggest that the E protein is crucial for the attenuation of SARS-CoV-2 and for the regulation of the confounding pathway involved in regulating E gene.
ER Stress Implicated in Viral Infections

- Infection with Severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV) induces ER stress
- SARS-CoV uses the ER as a site for synthesis and processing of viral proteins
- SARS-CoV spike (S) protein specifically modulates the unfolded protein response (UPR) to facilitate viral replication
- Modulation of the UPR by the SARS-CoV S protein also represents a viral strategy to combat the cellular protective response

The three stages that induce ER stress are highlighted with numbered star signs, namely:

1. Formation of double-membrane vesicles (DMVs)
2. Massive production and modification of structural proteins
3. Depletion of ER membrane during budding
In virus-infected cells, three membrane transducers, PERK, ATF6, and IRE1, are differentially activated to enhance ER homeostasis. Arrows represent activation of UPR components by viral infection; lines indicate inhibition of UPR components by viral infection or inhibition between UPR components.
The Sigma-1 Receptor (S1R) – An Overview

- S1Rs are distributed in neurons, astrocytes, microglia, oligodendrocytes, and in non-neural tissues
- S1R is an endoplasmic reticulum (ER) protein located at the mitochondria-associated ER membrane (MAM), which links the ER with the mitochondria
- S1R has been proposed to play a protective role in stressed cells, as over-expression of S1R reduces ER and oxidative stress and mitigates the effect of pro-apoptotic signals (1)
- S1R may control the stability of certain cellular proteins to prevent stress-induced cell death

Modulation of S1R results in a wide range of cellular activities aiming at restoring homeostasis

Sigma-1R Regulation of the Three Signal Pathways in ER-stress Activated UPR

ER chaperone BiP/GRP78 under normal conditions binds all the three ER-stress sensors (PERK: protein kinase RNA like ER-kinase; IRE1α: inositol requiring enzyme 1α; ATF6: activating transcription factor 6). Under ER-stress BiP dissociates from sensors. PERK and IRE1α are phosphorylated and oligomerized, ATF6 is translocated to the Golgi. (Abbreviations: eIF2α: eukaryotic translation initiation factor 2α; XBP1: X-box binding protein 1 (spliced form); TRAF2: TNF-associated factor-2; ATF4: transcriptional activator factor-4; MT: mitochondrion; CHOP: c/EBF homologous protein; Bcl-2: B-cell lymphoma 2; Bax: Bcl-2 like protein 2; Bak: Bcl-2 homologous antagonist killer; JNK: c-Jun terminal amino kinase; ASK1: apoptosis signal regulating kinase). Sig-1R activation increases cell survival by decreasing the activation of PERK, ATF6 and IRE1α as well as by lowering pro-apoptotic responses (CHOP, Bax, Bak) and increasing anti-apoptotic Bcl-2 activity.

Multiple viruses induce ER stress by dysregulating UPR, intrinsic mitochondrial apoptosis and other related cellular processes, in order to ensure survival, replication and pathogenesis.

In the case of HCV infection, the ER-mitochondria MAM coordinates the antiviral innate response and is the primary site for HCV replication.

S1R, located at the MAM, has been proposed to play a protective role in stressed cells, as overexpression of S1R reduces ER and oxidative stress and mitigates the effect of pro-apoptotic signals. Conversely, silencing of S1R leads to ER stress, induction of UPR, oxidative stress, alterations of Ca\(^{2+}\) homeostasis (ER Ca\(^{2+}\) influx into the mitochondria), induction of dysfunctional autophagosomes, as well as increased susceptibility to pro-apoptotic signals.

Given the tight links between ER stress, UPR, mitochondrial apoptosis, MAM and viral replication of HCV and other viruses, it is possible that the S1R could play a role in modulating the cellular response to viral infection and ameliorate pathogenesis.
## Pipeline of Sigma-1 Receptor (S1R) Modulators

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* = Orphan Drug Designation by FDA

Fast Track, Rare Pediatric, Orphan Drug (U.S./EU)
S1R Target Occupancy of ANAVEX®2-73 both in vivo and clinically Established

2D [18F]FTC-146-PET imaging of ANAVEX®2-73: Dose-dependent ANAVEX®2-73 Target Engagement with S1R

Source: Reyes S et al, AAIC 2018; H Hampel et al., AAIC 2018; *Alzheimer’s Disease Cooperative Study Activities of Daily Living 23-item scale (ADCS-ADL)