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Eradicate Coronavirus by blocking replication, counteracting its defense system

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Abstract

SIRT1 inhibitors can reduce replication of many viruses with certain similar characteristics to those of Coronaviruses, while p53 protein is another important factor in down-regulation of growth. There are some molecules that inhibit Sirtuin 1 and 2, in addition to activate p53 protein, by means of regulation of the interactions used by Coronaviruses as self-defense mechanism. By blocking virus growth and continuous replication, associating the already tested Antiviral medicines, Covid-19 could be eradicated.

Keywords: Covid-19, Coronavirus replication, SIRT1 inhibitors, p53, HIF, autophagy, lysosomal

1. Introduction

How Coronavirus enters in the host

To date, it is known that the Novel Coronavirus penetrates into the host via the enzyme converting receptor for angiotensin II (ACE2), by binding to glycoprotein S, with some differences from what happens with Sars-Cov.[1]

From here, you can draw up a list of interventions to reduce the expression of ACE2 activity, in order to avoid (in whole or in part) the entry of the virus into the subject under attack, but not only. It is also possible to reduce the ability of the virus to reproduce and lower its endoribonucleic activity. So, let us see what can be done.

ACE2 inhibitors: direct and indirect way

We assume that ACE inhibitors (*Lisinopril*, *Quinapril*, *Captopril*) don't reduce ACE2 activity, despite being structurally related and traceable in the same organs, ACE2 has opposite biological effects (vasodilatation, bronchodilatation, activity to compensate for physical stresses, as some particular conditions, like hypoxia); some direct ACE2 antagonists are:

- The small molecule MLN-4760-B and its isomeric MILN-4760, much more selective and effective;
- DX600 peptide (and DX-512) having a nanomolar affinity for ACE2, much more than for ACE

(almost null), competitive and non-competitive inhibition.

The effectiveness and selectivity between these two elements are concentration-related, but at the concentration of 10μM, MLN-4760 was found to have a better activity profile.[2]

SIRT1 inhibitors as ACE2 and virus replication suppressor

There is also the possibility to act indirectly, through a Sirtuine group protein, 7 enzymes NAD+ dependent, known as SIRT1.
Expression of ACE2 activity varies with binding to SIRT1 protein, so inhibiting SIRT1, we'll lower ACE2 activity;[3]

Sirtuine are located in the cellular nucleus (SIRT1, SIRT6 and SIRT7), in the cytoplasm (SIRT2), or in the mitochondria (SIRT3, SIRT4 and SIRT5) and are involved in many cellular functions, such as metabolism, the cell cycle, apoptosis, DNA repair, etc. Recent studies have found that sirtuine may also have enzymatic functions. They are sensors of changes in the intra- and extracellular environment, generally involved in maintaining human health, but are also implicated in some viruses replication.

2. Method of Investigation

Examples of virus replication reduced by Sirtuines inhibitors

- Think, for example, of the Tat protein of the HIV virus: its activity is regulated by SIRT1, whose activity of deacetylase, vice versa is inhibited by the protein Tat. The transcription of HIV is regulated by SIRT1, by means of Tat deacetylase. SIRT1 preserves the defenses of the virus, throughout its evolution, allowing the recycling of the protein tat, which binds to TAR, and the continuous prolongation of the mRNA transcription;[4]

- SIRT1 inhibition (and sometimes even SIRT2) by minor changes to siRNA, is known to cause the decrease in replication of additional viruses: flu strains, VSV (Vesicular Stomatitis Virus), KSHV (Kaposi's Sarcoma-associated Herpesvirus), Hepatitis B, HCMV, adenovirus, polyomavirus and in some diseases counteract: some types of cancer and Huntington's chorea, sometimes in combination with antivirals, for the best results;

- Among the various other viruses that respond by slowing their growth, there is just Mers-Cov. It has been observed that SIR2 (SIRT1 is the human correspondent) acts as the proviral of Mers-Cov in the yeast, due to interactions between ORF4a and eukaryotic cells. YDL042C/SIR2 yeast gene is a suppressor of ORF4a function. When SIRT1 is inhibited by either chemical or genetic manipulation, there is a reduction of MERS-CoV replication. Moreover, ORF4a inhibited SIRT1-mediated modulation of NF-κB signaling, demonstrating a functional link between ORF4a and SIRT1 in mammalian cells. It been identified a functional link between the MERS-CoV ORF4a proteins and the YDL042C/SIR2 yeast gene.[5]

- VSV-SARS-St19 infection is mediated by SARS-CoV-S protein in an ACE2-dependent manner. VSV-SARS-St19 will be useful for analyzing the function of SARS-CoV-S protein and for developing rapid methods of detecting neutralizing antibodies specific for SARS-CoV infection.
SIRT1 inhibitors stop the growth of VSV, since that cells' apoptotic response is reduced in cells affected by VSV.[6]

HIF-1α Stabilization Promotes Covid-19 Replication and "Cytokine Storms"

SIRT1 stabilizes HIF-1α via direct binding and deacetylation, while SIRT1 depletion or inhibition led to reduced hypoxic HIF-1α accumulation, accompanied by an increase in HIF-1α acetylation. SIRT1-mediated accumulation of HIF-1α protein led to increased

expression of HIF-1α target genes, including VEGF, GLUT1 and MMP2.
HIF-1α stabilization requires SIRT1 activation.[7]

Blood monocytes from severe COVID-19 patients present high expression of HIF-1α in comparison to healthy controls.
The target genes are involved in the glucose transport and glycolytic pathway, were increased in CoV-2-infected monocytes. Stabilization of HIF-1α increased the expression of ACE2, IL-1β, TNF-α, IL-6, and IFN α, β, and λ in CoV-2-infected monocytes.
Reduced respiration in LPS-activated macrophages is known to increase mitochondrial ROS (mtROS) production, which are strong inducers of HIF-1α.[8]

Coronavirus self-defense mechanism

INTERACTION WITH SARS-UNIQUE DOMAIN (SUD) AND PAPAIN-LIKE PROTEASE (PLPRO) AGAINST P53

The strategy developed from Sars-Cov and other coronaviruses against host immune recognition system is expressed by a Sars-Unique Domain (SUD) that interacts with his partner cellular E3 ubiquitin ligase ring-finger and CHY zinc-finger domain-containing 1 (RCHY1) and with papain-like protease (PLPRO). The consequence is a down-regulation of p53, involved in reduction of virus replication. The SARS-CoV papain-like protease is encoded next to SUD within nonstructural protein 3. A SUD–PLPRO fusion interacts with RCHY1 more intensively and causes stronger p53 degradation than SARS-CoV PLPRO alone.[9]

In mammals, SIRT1 has been shown to deacetylate and thereby deactivate the p53 protein.

How p53 is very important in Coronavirus replication

P53 protein reduce coronaviruses replication, because normally activates genes that are involved in the cell's non-specific antiviral defense system. In cells which p53 is too lower, the rate of coronavirus replication is several orders of magnitude higher than that observed in cells in which the p53 is present.[9]

Autophagy and lysosomotropic agents

Hypothetic useful drugs in Covid-19 fight also interfere with lysosomal activity and autophagy, interact with membrane stability and alter signalling pathways and transcriptional activity, which can result in inhibition of cytokine production and modulation of certain co-stimulatory molecules. This because Entry of CoVs into the host cells is mainly mediated by the endocytic pathway, meanwhile the autophagy has also been implicated in the viral replication in the cells, a process partly related to the formation of DMV in the host cell.[10]

3. Conclusions

Hypothetic perfect molecule in the halt of replication

There are some small molecules inhibitors of sirtuin 1 and activators of p53 protein, which protect it from mdm2-mediated degradation with little effect on p53 synthesis. Histone deacetylase inhibitors can also indirectly affect the function of some E3 ubiquitin ligases.
In addition, these compounds are autophagy inhibitors and lysosomotropic agents.

References

1 Yun Chen, Yao Guo, Yihang Pan, Zhizhuang Joe Zhao. Structure analysis of the receptor binding of 2019-nCoV. Biochemical and Biophysical Research Communications 525, 135–140 Elsevier BV, 2020. Link

2 Wenhui Li, Chengsheng Zhang, Jianhua Sui, Jens H Kuhn, Michael J Moore, Shiwen Luo, Swee-Kee Wong, I-Chueh Huang, Keming Xu, Natalya Vasilieva, Akikazu Murakami, Yaqing He, Wayne A Marasco, Yi Guan, Hyeryun Choe, Michael Farzan. Receptor and viral determinants of SARS-coronavirus adaptation to human ACE2. The EMBO Journal 24, 1634–1643 Wiley, 2005.

3 Nicola E. Clarke, Nikolai D. Belyaev, Daniel W. Lambert, Anthony J. Turner. Epigenetic regulation of angiotensin-converting enzyme 2 (ACE2) by SIRT1 under conditions of cell energy stress. Clinical Science 126, 507–516 Portland Press Ltd., 2013.

4 Emre Koyuncu, Hanna G. Budayeva, Yana V. Miteva, Dante P. Ricci, Thomas J. Silhavy, Thomas Shenk, Ileana M. Cristea. Sirtuins Are Evolutionarily Conserved Viral Restriction Factors. mBio 5 American Society for Microbiology, 2014.

5 Stuart Weston, Krystal L. Matthews, Rachel Lent, Alexandra Vlk, Rob Haupt, Tami Kingsbury, Matthew B. Frieman. A Yeast Suppressor Screen Used To Identify Mammalian SIRT1 as a Proviral Factor for Middle East Respiratory Syndrome Coronavirus Replication. Journal of Virology 93 American Society for Microbiology, 2019.

6 Shuetsu Fukushi, Rie Watanabe, Fumihiro Taguchi. Pseudotyped Vesicular Stomatitis Virus for Analysis of Virus Entry Mediated by SARS Coronavirus Spike Proteins. 331–338 In Methods in Molecular Biology. Humana Press, 2008.

7 Joo HY, Yun M, Jeong J, et al. SIRT1 deacetylates and stabilizes hypoxia-inducible factor-1α (HIF-1α) via direct interactions during hypoxia. Biochem Biophys Res Commun. 2015;462(4):294-300.

8 Ana Campos Codo, Gustavo Gastão Davanzo, Lauar de Brito Monteiro, Gabriela Fabiano de Souza, Stéfanie Primon Muraro, João Victor Virgilio-da-Silva, Juliana Silveira Prodonoff, Victor Corasolla Carregari, Carlos Alberto Oliveira de Biagi Junior, Fernanda Crunfli, Jeffersson Leandro Jimenez Restrepo, Pedro Henrique Vendramini, Guilherme Reis-de-Oliveira, Karina Bispo dos Santos, Daniel A. Toledo-Teixeira, Pierina Lorencini Parise, Matheus Cavalheiro Martini, Rafael Elias Marques, Helison R. Carmo, Alexandre Borin, Laís Durço Coimbra, Vinícius O. Boldrini, Natalia S. Brunetti, Andre S. Vieira, Eli Mansour, Raisa G. Ulaf, Ana F. Bernardes, Thyago A. Nunes, Luciana C. Ribeiro, Andre C. Palma, Marcus V. Agrela, Maria Luiza Moretti, Andrei C. Sposito, Fabrício Bíscaro Pereira, Licio Augusto Velloso, Marco Aurélio Ramirez Vinolo, André Damasio, José Luiz Proença-Módena, Robson Francisco Carvalho, Marcelo A. Mori, Daniel Martins-de-Souza, Helder I. Nakaya, Alessandro S. Farias, Pedro M. Moraes-Vieira, Elevated Glucose Levels Favor SARS-CoV-2 Infection and Monocyte Response through a HIF-1α/Glycolysis-Dependent Axis, Cell Metabolism, 2020.

9 Yue Ma-Lauer, Javier Carbajo-Lozoya, Marco Y. Hein, Marcel A. Müller, Wen Deng, Jian Lei, Benjamin Meyer, Yuri Kusov, Brigitte von Brunn, Dev Raj Bairad, Sabine Hünten, Christian Drosten, Heiko Hermeking, Heinrich Leonhardt, Matthias Mann, Rolf Hilgenfeld, Albrecht von Brunn. p53 down-regulates SARS coronavirus replication and is targeted by the SARS-unique domain and PLpro via E3 ubiquitin ligase RCHY1. Proceedings of the National Academy of Sciences 113, E5192–E5201 Proceedings of the National Academy of Sciences, 2016.

10 Yang, N., Shen, H.-M.Targeting the Endocytic Pathway and Autophagy Process as a Novel Therapeutic Strategy in COVID-19. International Journal of Biological Sciences, 2020.