

The big challenge of SARS-CoV-2 latency: testes as reservoir

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Abstract

In the efforts to explain COVID-19 pathophysiology, studies are being carried out on the correspondence between the expression of SARS-CoV-2 cell receptors and viral sequences. ACE2, CD147 and TMPRSS2 receptors expression could indicate poorly explored potential infection targets. For the genomic analysis of SARS-CoV-2 receptors, using BioGPS information was decided, which is a portal that centralizes genetic annotation resources, in combination with that of The Human Protein Atlas, the largest portal of human transcriptome and proteome data. We also reviewed the most recent articles on the subject. RNA and viral receptor proteins expression was observed in numerous anatomical sites, which partially coincides with the information reported in the literature. High expression in testicular cells markedly stood out, and it would be therefore important ruling out whether this anatomical site is a SARS-CoV-2 reservoir; otherwise, germ cell damage, as it is observed in infections with other RNA viruses, should be determined.

KEY WORDS: SARS. COVID-19. SARS-CoV-2 cell receptors. RNA virus.

El gran desafío de la latencia de SARS-CoV-2: el testículo como reservorio

Resumen

En el afán por explicar la fisiopatogenia de COVID-19 se están realizando estudios en torno a la correspondencia entre la expresión de receptores celulares de SARS-CoV-2 y las secuencias virales. La expresión de los receptores ACE2, CD147 y TMPRSS2 podría indicar blancos de infección poco explorados. Para el análisis genómico de los receptores de SARS-CoV-2 se optó por utilizar la información del BioGPS, un portal que centraliza los recursos de anotación genética, en combinación con la de The Human Protein Atlas, el portal más grande de datos del transcriptoma y proteoma humanos. También se revisaron los artículos más recientemente respecto al tema. En numerosos sitios anatómicos se observó la expresión de ARN y proteínas de los receptores del virus, que coinciden parcialmente con la información reportada en la literatura. Resaltó la alta expresión en las células de los testículos, por lo que sería importante descartar si este sitio anatómico es un reservorio de SARS-CoV-2; de no ser así, determinar el daño en las células germinales, tal como sucede en infecciones por otros virus ARN.

PALABRAS CLAVE: SARS. COVID-19. Receptores celulares de SARS-CoV-2. Virus ARN.

Introduction

In December 2019, in Wuhan, China, a type of pneumonia associated with a new coronavirus that causes severe acute respiratory syndrome emerged;

it was named SARS-CoV-2.¹ By May 2020, more than 7,000 publications had been generated, including more than 150 articles on molecular aspects (according to the National Center for Biotechnology Information of the United States),² which shows a clear

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Date of reception: 15-05-2020

Date of acceptance: 02-06-2020

DOI: 10.24875/GMM.M20000403

Gac Med Mex. 2020;156:324-329

Contents available at PubMed

www.gacetamedicademexico.com

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interaction between clinical practice and biomedical research to find possible solutions to the health problem represented by the pandemic caused by this virus.

SARS-CoV-2 is a betacoronavirus that infects human and vertebrate epithelial cells. It has a positive single-stranded RNA of approximately 29,900 nucleotide bases covered by a lipid membrane, with more than 80 % genomic similarity to SARS-CoV-1.³ In addition, it shows similarities to the sequences of coronaviruses that infect the bat and the pangolin, as well as to those of the human immunodeficiency virus; in other words, it is a chimera virus of natural divergent evolution.^{4,5} These viruses are known to code for 23 proteins, among which the most widely studied are structural N-protein (nucleocapsid), S-protein (spike, crucial for its entrance to the cell, virulence and tropism), M-protein (membrane) and envelope protein.⁶

Usefulness and transcriptional complexity of SARS-CoV-2 mutations

Like other RNA viruses, SARS-CoV-2 mutation rate is high, which represents a real challenge for vaccine development and is associated with severe clinical presentations and geographic differences. For example, the mortality rate has been reported to be different in China (5.6 %) than outside that country (15.2 %), which could be attributed to genomic differences combined with other factors such as isolation strategies and herd immunity.⁷

The virus mutates to adapt to the host and thus it evolves. Thirteen genome variations contained in open reading frame (ORF) 8, ORF1ab, S, ORF3a and N regions have been identified; positions 28144 (ORF8) and 8782 (ORF1a) have mutation rates of 30.53 % and 29.47 %, respectively.⁸ RNA-dependent RNA polymerases, especially at position 14408, are associated with the appearance of emerging multiresistant viral phenotypes.⁹ Knowing the mutations will allow the development of targeted drugs, such as favipiravir, remdesivir, ribavirin for other diseases caused by RNA viruses.¹⁰

SARS-CoV-2 functional architecture is extremely complex. It is suggested that this virus' ORFs might serve as accessory proteins to modulate viral replication. Discontinuous transcription from its transcriptome and epitranscriptome generates new genomic fusions, causing more than 40 modifications that contribute to its pathogenicity, viral cycle, successful host immune response evasion and survival.⁹

First SARS-CoV-2 infection target and detection tests

SARS-CoV-2 infection main targets have been reported to be nasopharyngeal tract epithelial cells.¹ Therefore, the approved technique for molecular diagnosis of this virus is viral load estimation by polymerase chain reaction (PCR) in a nasopharyngeal tract sample (which can also be obtained from feces or blood) obtained with a swabbing technique, according to the Berlin protocol published on January 17, 2020.¹¹ This type of tests, while being "fast", require time, since viral RNA purification is essential and the interval for diagnosis is at least eight hours. To date, the United States Food and Drug Administration has approved various molecular tests.¹²

On the other hand, there are faster serological tests that detect high concentrations of IgG and IgM antibodies; unfortunately, most of them have low sensitivity and specificity. Since COVID-19 symptoms are similar to those of other respiratory diseases, it is essential to rule out infections with other RNA viruses.

The application of diagnostic tests and clinical data allow early, late and convalescent or recovered cases to be identified. Especially in early cases, viral load should be estimated, in addition to applying a second test to define the probability of community infection. Once the time window elapses, antibody production is intensified (approximately at second week) and serological tests can be carried out for disease control.¹³⁻¹⁵

Cellular proteins as receptors for entry of the virus into the host

Evidence indicates that SARS-CoV-2 molecular targets are cells that express angiotensin-converting enzyme 2 (ACE2, Figure 1) –involved in lowering blood pressure by catalyzing the cleavage of angiotensin II into angiotensin–,¹⁶ membrane molecule CD147¹⁷ (Figure 2) –which stimulates fibroblasts for the production of matrix metalloproteinases and is associated with spermatogenesis and embryo implantation– and serine protease TMPRSS2 (Figure 3), regulated by androgenic hormones, among other proteins.¹⁸ The most widely studied receptor is ACE2, which acts as a receptor for the glycoproteins of the virus' spike.¹⁹ Previous data suggest SARS-CoV-2 tropism towards cells that express its target cellular proteins.

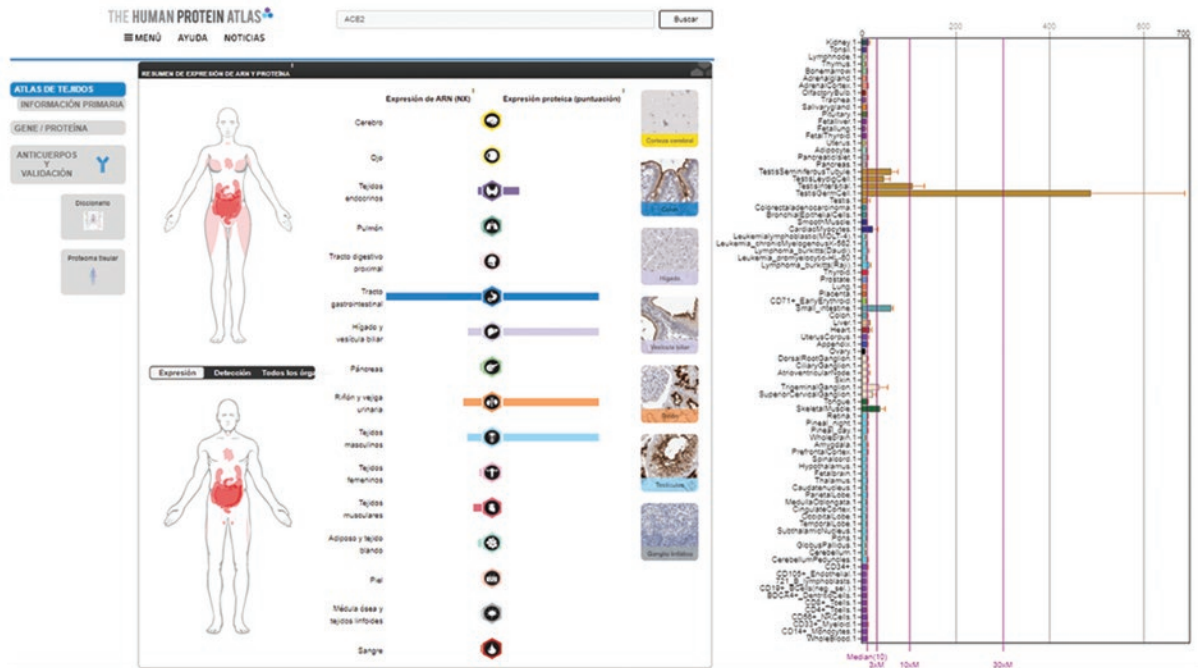


Figure 1. Anatomical sites of ACE2 protein expression that constitute potential targets for interaction with SARS-CoV-2 on each gender. The icons indicate anatomical sites of RNA expression and protein expression. ACE2 tissue expression is also exemplified by immunohistochemistry. The bar graph on the right shows relative expression in different types of cell, and overexpression in testicular cells (ochre colored bars) is highlighted. Source: The Human Protein Atlas.

Accessible database information

A search in different databases was carried out to determine other potential infection targets. The Human Protein Atlas²⁰ was chosen, where all human proteins in cells, tissues and organs are mapped by integrating experimental results obtained from various genomic and histological tools; as well as BioGPS, which brings together information related to gene expression in different sites of the human body.²¹ In an *in silico* analysis, the expression of ACE2 and CD147 was observed on various anatomical sites (Figures 1 to 3); ACE2 was observed in respiratory tract epithelial cells.¹⁸⁻²²

Receptor expression in specific cells: the testes

A recent study showed that ACE2 expression is higher in a small population of type II alveolar cells, which are involved with pneumonia clinical presentation; mononuclear cell alteration, small thrombosed vessels, megakaryocyte activation and platelet aggregates were also observed.²³ Other investigations show that deterioration is general and that it also involves the urinary system,²⁴ the central nervous system neurotropic

mechanism²⁵ and enterocytes,²⁶ which shows that these cells possess viral genomes and express the aforementioned receptors. In addition to the above, CD147 expression shows that numerous organs and tissues might be susceptible to SARS-CoV-2 infection. The high expression of these cellular proteins in the testes stands out. No viral sequences were detected in the only report on the search for SARS-CoV-2 in testicles analyzed on autopsies; however, it should be noted that the search was performed using *in situ* hybridization, a technique that is not comparable with PCR.²⁷

Similarities of SARS-CoV-2 and its latency with other RNA viruses

Parotiditis (mumps) RNA virus infection is very similar to that of COVID-19: it affects epithelial cells (where viral load increases and causes viremia), it is transmitted by droplets of saliva from the carrier, either by direct or indirect contact with fomites and biofluids, and shows tropism towards cells of the salivary glands, of the central nervous system, testicles and ovaries; a parotiditis sequel is orchitis, which is associated with infertility.²⁸

Therefore, we should wonder whether the prodromal symptoms of influenza, other respiratory diseases (such

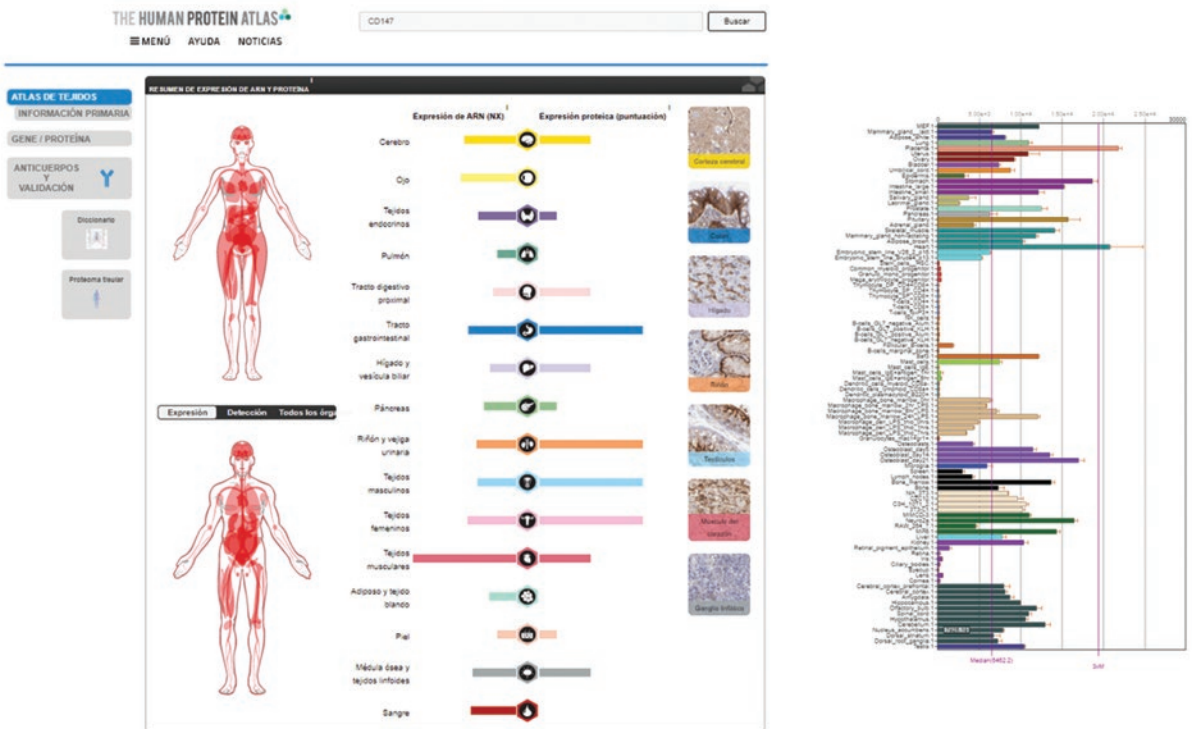


Figure 2. Anatomical sites of CD147 expression that represent potential targets for interaction with the SARS-CoV-2 virus. The bar graph on the right shows relative expression of different cell types in most part of the body. Source: The Human Protein Atlas.

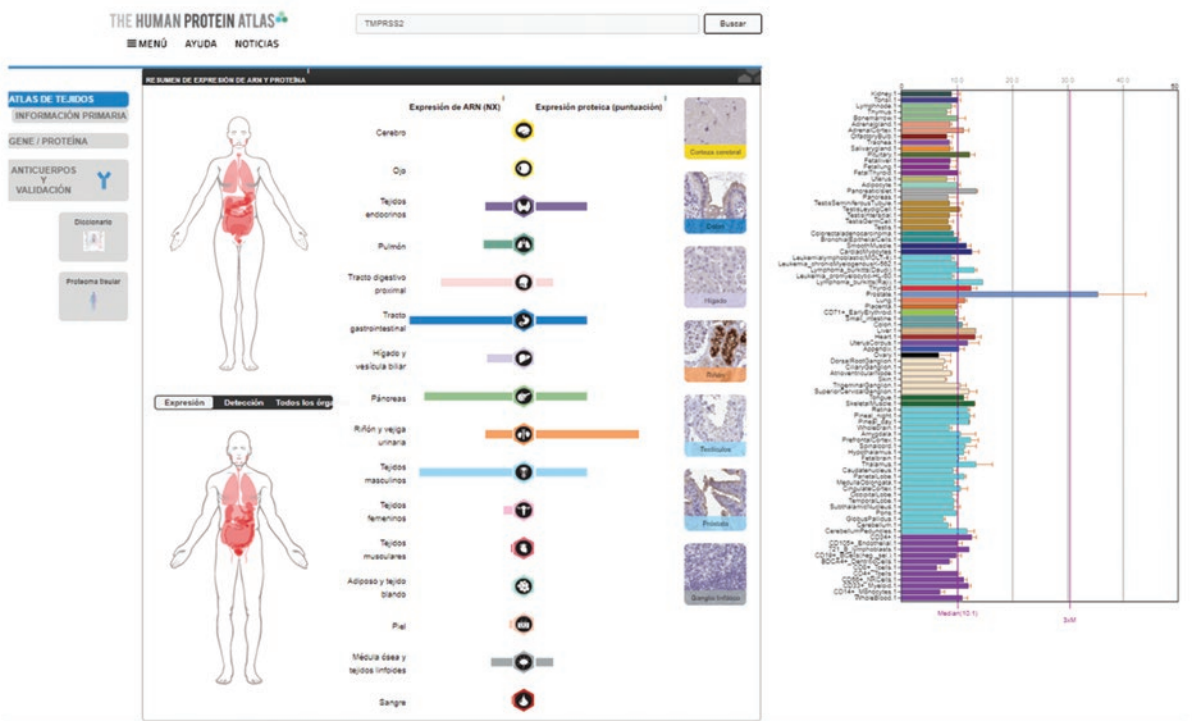


Figure 3. Anatomical sites of TMPRSS2 cellular protein expression as potential targets for interaction with the SARS-CoV-2 virus. The bar graph on the right shows different cell types relative expression in most part of the body, and higher expression in the prostate (blue bar) is highlighted. Source: The Human Protein Atlas.

as mumps) and those of SARS-CoV-2 infection are just a coincidence. In this context, ACE2 high expression in the testes could be correlated with SARS-CoV-2 infection latency; however, there is no clear evidence on this association. Therefore, we consider the need to investigate ACE2 expression in testicular tissue on autopsies of individuals who die from COVID, as well as SARS-CoV-2 sequences detection by PCR, in order to determine if chronic or latent infection existed. A sequel of viral infections (with DNA and RNA viruses) is latency, as in hepatitis²⁹ and herpes.³⁰ In COVID-19 patients who did not show symptoms, viral RNA was not identified with commercial tests practiced during follow-up (more than four weeks);³¹ however, with Sherlock's technology, whose detection limit is 100 copies/mL, in nasal and anal samples, 75 % of positive results were obtained for S gene and 41.6 % for ORF genes.³²

The referred data indicate SARS-CoV-2 sequences persistence for at least two months in the respiratory and digestive tracts (latent infection) and that anal swab can be another follow-up test, hence the importance of innovating and combining more sensitive and specific clinical and molecular resources, in order to rule out false positive and false negative results to the extent possible.³³ So far, information on latent SARS-CoV-2³⁴ infection and its consequences is insufficient.

It would be desirable that COVID-19 recovered patients, as well as intensive care and cardiopulmonary resuscitation personnel (without a parotiditis history) who directly interact with individuals with SARS-CoV-2 infection could undergo molecular studies (including spermatobioscopy), in order to rule out sperm damage and absence of latent infection. Therefore, it is urgent for more evidence on the subject to be available. During the writing of this document, a report in this regard was published.³⁵

Conclusions

Given the comorbidities of the Mexican population, SARS-CoV-2 infection represents a huge challenge and a "Pandora box" and, therefore, the possibility of new pathophysiogenic mechanisms should not be ruled out. SARS-CoV-2 involvement could be suggested if there was coincidence of infection in testicular cells that express this virus' receptors.

Acknowledgements

The authors thank the authorities and the Instituto Mexicano del Seguro Social Health Research

Coordination for their support during the quarantine period. To Tomás Hernández, for fulfilling his duty.

Conflict of interests

The authors declare that they have no conflicts of interest.

Funding

The authors did not receive any sponsoring to carry out this article.

Ethical disclosures

Protection of people and animals The authors declare that no experiments were performed on humans or animals for this research.

Confidentiality of data The authors declare that no patient data appear in this article.

Right to privacy and informed consent The authors declare that no patient data appear in this article.

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