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To uncover the key cellular pathways associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infectivity, Daniloski and coworkers used CRISPR-based whole-genome screening. Their results could propose new or repositioned drugs for the ongoing fight against COVID-19.

The COVID-19 pandemic has led to a major worldwide health crisis with multiple sociological, economical and medical aspects [1]. Multiple lines of investigation have addressed virus detection, different COVID-19 therapeutic and prevention strategies, and the pathophysiology associated with this emerging infectious disease. Major advances have been made in understanding SARS-CoV-2 pathogenesis, host–virus interactions, and immunological and genetic aspects of COVID-19. Genetic studies based on large-scale GWAS analysis revealed a multiple gene locus at 3p 34.2 and the ABO blood group locus at 9q34.2, pointing towards a large set of factors that predispose to severe COVID-19 [2]. As a complement to such a global strategy, we recently raised the hypothesis that genetic polymorphisms (SNPs) may modulate the expression of the SARS-CoV-2 cellular target (ACE2) and the two main proteases regulating its cell-membrane processing [3]. This was based on a large public database and allowed us to define further genetic risk factors for developing COVID-19 [3].

Genetics was also the basis of a study recently published in Cell, entitled ‘Identification of required host factors for SARS-CoV-2 infection in human cells’ [4]. This work aimed to identify potential new SARS-CoV-2 targets based on genome-wide loss-of-function clustered regularly interspaced short palindromic repeats (CRISPR) screens to systematically knock out components of the human genome [4]. The investigators specifically utilized human lung carcinoma cells sensitive to SARS-CoV-2 infection. The products of the top-ranked genes, whose loss significantly reduces viral infection, clustered into protein complexes including vacuolar ATPases, endosomal trafficking complexes (Retromer, Commander, Arp2/3), and phosphoinositide 3-kinase (PI3K).

Different molecular strategies were used to validate their findings, including knock-out by CRISPR, gene suppression using RNAi, and drug inhibition. A particular focus on ACE2 resulted in the identification of a gene, RAB7A, that had a marked influence on ACE2 trafficking to the cell membrane, likely by sequestering ACE2 in endosomal vesicles. Interestingly, the cholesterol biosynthesis pathway was reported to be a negative regulator of SARS-CoV-2. In our opinion, the main genome-scale loss-of-function discovery was the involvement of the PI3K pathway – confirmed by the finding that most inhibitors of PI3K catalytic subunit type 3 (PI3KC3) reduce SARS-CoV-2 viral load by a factor of 100. Interestingly, tamoxifen, a well-known anticancer drug that modulates the estrogen receptor, also produced a substantial reduction in SARS-CoV-2 viral load.

Since the discovery of the CRISPR/Cas9 system by Emmanuelle Charpentier and Jennifer Doudna, who were awarded the Nobel prize in 2020, this system has been applied in a wide range of situations. Compared to RNAi technology, gene silencing using CRISPR performs better because the target genes can be completely deleted. Genome-wide loss-of-function screens have been used to uncover host dependency factors for infection by several viruses, including HIV, West Nile virus, dengue virus, and Zika virus [5,6]. Four recent studies used CRISPR screening to investigate genes important for SARS-CoV-2 infection [4,7–9]. Among these, which used only two cell types (VERO-TMPRSS2 and A549-ACE2), it is puzzling that there was little overlap in the genes necessary for SARS-CoV-2 infectivity other than ACE2 itself. In addition, ACE2 levels were evaluated either by cytometry or by mRNA quantification, making comparison between the studies difficult. Furthermore, the apparent lack of reproducibility may raise concerns about technical issues (such as different CRISPR libraries and variations in guide representation) and biological differences (e.g., different cell types, different host species, cell culture heterogeneity, confluence, even serum conditions) [4,7–9].

This highlights not only the urgent need for reproducibility between studies in different laboratories but also the need for different cellular models [10] and animal models (Figure 1). In this context, two approaches could be envisaged, one based on a new CRISPR system (CRISPRa/i) that would target the promoters not only of coding genes but also of non-coding genes, and thus more accurately identify the regulators of SARS-CoV-2 load and replication. The second approach would involve the development of in vivo models for SARS-CoV-2 infection (3D reconstituted models or mice) to further analyze viral infection through deep sequencing and/or single cell-based technologies [10].

Overall, the study from Daniloski and co-workers brings a better understanding of how host genes impact on SARS-CoV-2 virus entry and should open up new perspectives for therapeutic applications that could accelerate the recovery of COVID-19 patients.
Figure 1. Dissecting Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Infection and Therapeutic Tools. Abbreviations: CRISPR, clustered regularly interspaced short palindromic repeats; IFN, interferon.