Direct-capture Perturb-seq for the identification of heretofore approved drugs for the treatment of COVID 19

Duha Al-Awad

EXMD 610

22nd April 2020

1) COVID 19: an introduction

In late 2019, the city of Wuhan, China, experienced an outbreak of a novel coronavirus, SARS-CoV-2, the etiological agents behind the coronavirus disease 19 (COVID 19) pandemic that took the world by surprise. The clinical manifestations of the disease range from asymptomatic infections, mild respiratory symptoms, to severe respiratory disease that could be fatal. Coronaviruses are enveloped single stranded RNA viruses that cause a wide range of zoonotic and human diseases, including the dangerous severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS)[1]. As of the of April 21, 2020, nearly four months after SARS-CoV-2 first appeared, over 2.3 million people have tested positive for the virus, while more than 160,000 people have died[2]. Due to the high transmissibility and mortality rate of COVID 19, there is a pressing need to find curative and safe treatments promptly.

2) Challenges of creating a COVID 19 treatment

SARS-CoV-2 is an emerging virus, therefore, treatments and vaccinations against it are not available yet[1]. Developing and testing new drugs or vaccines against SARS-CoV-2 is a lengthy process. Therefore, several antiviral and non-antiviral drugs such as remdesivir, lopinavir, ritonavir, and the anti-malarial chloroquine and hydroxychloroquin are being evaluated for their effectiveness against SARS-CoV-2 in clinical trials[3]. Drug repurposing of previously approved drugs facilitates the process of finding effective treatments against COVID 19 in a short period of time. However, due to the limited knowledge on SARS-CoV-2, rapid identification of drugs that could be repurposed to treat it becomes a challenge.

3) Hypothesis

Analysis of single-cell CRISPR screens of lung cells infected with SARS-CoV-2 will uncover host factors that are permissive or protective against the viral infection. Identifying these factors will allow the characterization of pathways and gene interactions that are vital for viral replication and disease progression. Searching data bases for safe drugs that work by targeting these gene interactions will shed the light on possible treatment options against SARS-CoV-2.

4) Method

Single-cell CRISPR screening is a combination of pooled CRISPR screening and single-cell RNA sequencing[4]. CRISPR screening is a method used to study gene function by knocking genes out, creating perturbations that manifest as phenotypic changes. The powerful ability of CRISPR technology to create targeted breaks in the genome leading to the inactivation of genes is the essence of this technique. Pooled CRISPR screening refers to the process of simultaneously creating unique perturbations in millions of cells. Each cell receives a single perturbation, however, all the cells are 'pooled' and sequenced together[5]. Single-cell RNA sequencing (scRNA-seq) is a high-throughput method used to sequence single cells' transcriptomes, which encompasses the entire RNA content of a cell. Nevertheless, due to their high biological value and relative ease of isolation, most scRNA-seq platforms are adapted for the isolation of polyadenylated messenger RNA (mRNA) transcripts[6]. Direct-capture Perturb-seq is a modified single-cell CRISPR screening that relies on droplet-based scRNA-seq, where a single cell and a 'capture bead' attached to oligo-dT primers with unique cell barcodes (CBCs) and unique molecular identifiers (UMIs) are encapsulated in an oil droplet. Within the droplet, the cell is lysed and its RNA content is released, allowing the oligo-dT primers to anneal to polyadenylated mRNA transcripts. This process is followed by reverse

transcription (RT) to create cDNA libraries for sequencing. During RT RNA sequences are tagged with CBCs and UMIs at either the 5' or 3' end of the mRNA to allow the identification of their origin during analysis. Capturing nonpolyadenylated guide RNA (gRNA) alongside the cell's mRNA transcripts allows for accurate identification of the genetic perturbation in individual cells and their consequent effect on the transcriptome. To efficiently capture the gRNA received by each cell, guide specific primers are added during RT to capture the 5' end. To capture and tag the 3' end of the gRNA, 'capture sequences' that do not interfere with CRISPR activity are added to the gRNA. Primers tagged with CBCs and UMIs targeting these capture sequences are added to the RT reaction, successfully capturing the 3' end[4].

5) Experimental procedure

A pool of lentiviruses each packaged with gRNA and Cas9, CRISPR associated protein 9, is used to stably transfect normal human epithelial lung cells, such as BEAS-2B. Transfected cells are then allowed to grow for a couple of days to allow the expression and activation of CRISPR machinery. Under biosafety level 3 with strict adherence to laboratory safety guidelines, SARS-CoV-2 will be cultured in VeroE6/TMPRSS2 cell lines which are supportive for the viral growth[7]. Pre-evaluated titers of SARS-CoV-2 are then used to infect the transfected normal lung cell line for different durations. Uninfected, but transfected, lung cells are used as a control for cell function in the absence of viral infection. Cells are then mechanically scraped or enzymatically digested yielding a suspension of single cells. The cell suspension is then introduced to a microfluidic droplet-based platform that will isolate a single capture bead and a single cell in a reaction droplet where cell lysis will free RNA transcripts. mRNA captured on beads is then isolated, washed, and RT is performed to generate cDNA. The generated cDNA will be amplified using PCR, after which, a cDNA library is created. Generated cDNA libraries are sequenced using a sequencing platform. Bioinformatic tools are then used to align and normalize sequencing data. Perturbations are assigned to cell identities and gene interactions are mapped out. Lastly, drug targets are identified, and drugs with desired action are selected.

6) Anticipated results and potential pitfalls

The use of CRISPR screens allowed us to gain a better understating of host host-virus interaction[8]. By combining CRISPR screens with in scRNA-seq in direct-capture Perturb-seq, we expect that the high throughput sequencing data generated from this experiment will reveal a list of candidate genes that are vital for SARA-Cov-2 infection and replication. These pro-viral genes could be involved directly in viral entry, replication, or packaging. Identifying pro-viral genes will mitigate the search for commercially available drugs that target those genes to be used as therapeutics to combat COVID 19.

Although extremely promising, critical parameters regarding this study need to be addressed. Despite their rarity, off-site CRISPR cutting may cause undesirable effect, such as an unexpected gain-of-function. Direct-capture Perturb-seq is a new technique that requires the use of custom gRNA, meaning that a limited gRNA library is currently available. Although possible, requesting additional custom gRNA will increase the duration and cost of the experiment. Collectively, these factors compel the selection of gene candidates that are known to play a role in host-pathogen interactions, limiting the scope of the study. Moreover, The large amount of complex data generated from this experiment need to be analyzed by a highly skilled bioinformatician. To analyze the data in a timely manner, a team of bioinformatician is needed. Last, the detailed mode-of-action for a fair number of commercially available drugs is not well elicited, meaning that a large proportion of drugs that could potentially work against SARS-CoV-2 will be overlooked.

References:

1 He, F., Deng, Y. & Li, W. Coronavirus disease 2019: What we know? *J Med Virol*, doi:10.1002/jmv.25766 (2020).

2 WHO. Coronavirus disease 2019 (COVID-19): situation report, 92. (2020).

3 Kupferschmidt, K. & Cohen, J. (American Association for the Advancement of Science, 2020).

4 Replogle, J. M. *et al.* Combinatorial single-cell CRISPR screens by direct guide RNA capture and targeted sequencing. *Nature Biotechnology*, 1-8 (2020).

5 Doench, J. G. Am I ready for CRISPR? A user's guide to genetic screens. *Nature Reviews Genetics* **19**, 67 (2018).

6 Olsen, T. K. & Baryawno, N. Introduction to single-cell RNA sequencing. *Current protocols in molecular biology* **122**, e57 (2018).

7 Matsuyama, S. *et al.* Enhanced isolation of SARS-CoV-2 by TMPRSS2-expressing cells. *Proceedings of the National Academy of Sciences* **117**, 7001-7003, doi:10.1073/pnas.2002589117 (2020).

8 McDougall, W. M., Perreira, J. M., Reynolds, E. C. & Brass, A. L. CRISPR genetic screens to discover host–virus interactions. *Current opinion in virology* **29**, 87-100 (2018).