Poster

CRISPR to cure RNA viruses

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ABSTRACT

The versatility of editing technologies based on CRISPR/Cas has led to a revolution in the field of biomedical research, specially in gene therapy, where it is allowed generating genetic modifications to solve multiple congenital defects. Even more interesting is the use of CRISPR systems that using RNA endonucleases such as CAS13d, which have the advantage of allowing regulation of gene expression instead of eliminating it completly. Moreover, beyond the application as a treatment for genetic disorders, the current situation generated worldwide by SARS-CoV-2 and the consequent race to achieve an effective cure has led to set out the viability of using these systems against RNA virus (Uddin, Rudin, and Sen 2020; Yan et al. 2018).

This has led us to create a biological model infection by RNA virus such as the infection of S. cerevisiae by a Totiviridae family virus, the L-A virus. In this process, the expression of a M satellite of the virus like an antifungal exotoxin results in the prevalence of the infected strain (the "killer" strain) over other nearby strains or species. This fact can generate serious problems in industry, where infected yeasts can displace other strains that carry desirable phenotypes (Magliani et al. 1997).

For solving this problem, first we proceed adjusting the system, so we designed an experiment in which it was intended to probe the CRISPR/Cas13d activity through an observable phenotype. So we created strains carrying a yellow fluorescent protein (YFP) and CRISPR/Cas13d system with guides that directed against the YFP transcript. As expected, the strains with anti-YFP guides had lower fluorescent levels than those ones that did not.

These results, which seek demostrate the functionality of CRISPR/Cas13d systems, are meant to be complemented with a quantification of changes in gene expression (using qPCR) of the YFP. In addition, this quantification will be made with the CAN1 gene in another experiment using strains with anti-CAN1 guides.

Finally, we'll try to do a similar procedure using strains that integrate guides directed against the M satellite.

REFERENCES

Magliani, W et al. 1997. "Yeast Killer Systems." Clinical Microbiology Reviews 10(3): 369–400.

Uddin, Fathema, Charles M. Rudin, and Triparna Sen. 2020. "CRISPR Gene Therapy: Applications, Limitations, and Implications for the Future." Frontiers in Oncology 10: 1387. /pmc/articles/PMC7427626/ (February 18, 2021).

Yan, Winston X. et al. 2018. "Cas13d Is a Compact RNA-Targeting Type VI CRISPR Effector Positively Modulated by a WYL-Domain-Containing Accessory Protein." Molecular Cell 70(2): 327–339.e5. https://pubmed.ncbi.nlm.nih.gov/29551514/ (February 18, 2021).