Development of a yeast bioassay to screen for Nirmatrelvir drug resistance mutations in the SARS-CoV-2 protease 3CLpro.

Name of Authors: Óscar Barbero-Úriz, Gema González-Rubio, María Molina, Víctor J. Cid, Teresa Fernández-Acero.

Name of Institution: Departamento de Microbiología y Parasitología, Facultad de Farmacia, Universidad Complutense de Madrid.

e-mail: oscaralb@ucm.es, teresafe@ucm.es

SARS-CoV-2 is a single-stranded RNA + polarity virus that belongs to coronavirus family. Its genome is organized into 14 ORFs, 2 of which lead to 2 polyproteins (pp1a and pp1ab) that, in turn, lead to 16 non-structural proteins. To being able to progress in its life cycle, these polyproteins must be processed, for which the virus has 2 cysteine proteases, 3CLpro (nsp5 or Mpro) and PLpro (nsp3). This processing is essential for viral replication, which means that these proteases can be a potential drug target.

We have developed a bioassay in the model yeast *Saccharomyces cerevisiae* based on the growth inhibition caused by 3CLpro overexpression. Thus, when we expose our yeast to a 3CLpro protease inhibitor, it is able to restore its growth. This bioassay has been tested successfully with several compounds, including Nirmatrelvir, a compound commercialized by Pfizer in combination with Ritonavir as a drug against SARS-CoV-2. We observed that the treatment of yeasts expressing a resistant variant to Nirmatrelvir of 3CLpro lead to an incomplete restoration of its growth when compared to wild-type 3CLpro. Thus, we have adapted this system to carry out the search for resistance mutations that could arise due to the use of Nirmatrelvir in clinical practice. As a way to mimic viral evolution during infection, we have done a randomized mutagenesis on 3CLpro followed by gap-repair in yeast. By this method, we have obtained 1190 clones on which we have carried out activity assays. 825 clones that maintain full protease activity have been tested for resistance to Nirmatrelvir, looking for 3CLpro clones that can´t be fully inhibited by this compound, unlike the wild-type 3CLpro. We have obtained 267 clones that behave like resistant to Nirmatrelvir that are currently under retesting before sequencing.

Our screening will provide useful information about new variants that could arise in the clinical through the use of Nirmatrelvir. This structural and functional knowledge will help to design effective 3CLpro inhibitors which avoid the selection of resistant variants.

Keywords:

SARS-CoV-2, 3CLpro, yeast bioassay, Nirmatrelvir, drug resistance.

Bibiography:

1. Decottignies A, Grant AM, Nichols JW, de Wet H, McIntosh DB, Goffeau A. ATPase and multidrug transport activities of the overexpressed yeast ABC protein Yor1p. J Biol Chem. 1998 May 15;273(20):12612-22. doi: 10.1074/jbc.273.20.12612. PMID: 9575223.
2. Jochmans D, Liu C, Donckers K, Stoycheva A, Boland S, Stevens SK et al. The Substitutions L50F, E166A, and L167F in SARS-CoV-2 3CLpro Are Selected by a Protease Inhibitor In Vitro and Confer Resistance To Nirmatrelvir. mBio. 2023 Feb 28;14(1):e0281522. doi: 10.1128/mbio.02815-22. Epub 2023 Jan 10. PMID: 36625640; PMCID: PMC9973015.
3. Zhou Y, Gammeltoft KA, Ryberg LA, Pham LV, Tjørnelund HD, Binderup A, et al. Nirmatrelvir-resistant SARS-CoV-2 variants with high fitness in an infectious cell culture system. Sci Adv. 2022 Dec 21;8(51):eadd7197. doi: 10.1126/sciadv.add7197. Epub 2022 Dec 21. PMID: 36542720; PMCID: PMC9770952.

Acknowledgment:

The work of PhD student Óscar Barbero is funded by a research trainee contract from the Complutense University of Madrid.

This research is funded by the research project PR27/21 (Ayudas para la realización de proyectos de I+D para jóvenes doctores) of the Complutense University of Madrid and the Community of Madrid.