Dissecting the mechanism of action of 3,5-dihydroxy-4-isopropyl-trans-stilbene

Duha Alkhder,[1] Fabrizio Alberti,[2] Nick Waterfield,[1] Irene Stefanini[1,3]

[1] Warwick Medical School, University of Warwick, Coventry CV4 7AL, UK

[2] School of Life Sciences and Department of Chemistry, University of Warwick, Coventry CV4 7AL, UK

[3] Dipartimento di Scienze della Vita e Biologia dei Sistemi, University of Turin, 10124 Turin, Italy

duha.alkhder@warwick.ac.uk

3,5-Dihydroxy-4-isopropyl-trans-stilbene (IPS) is a stilbene natural product made by *Photorhabdus spp*., which are bacteria associated with entomopathogenic *Heterorhabditis spp.* nematodes. IPS has diverse biological and pharmacological properties, including antibacterial, antifungal, insecticidal, nematocidal, antioxidant and anticancer activities [1-3]. IPS has recently been approved as a treatment for psoriasis in China and Japan (under the commercial names of Benvitimod and Tapinarof, respectively). However, its mechanism of action remains unknown. The aim of this work is to investigate the mechanism of action of IPS in yeast and filamentous fungi.

Previous analyses carried out by our group the group using a heterozygous *S. cerevisiae* deletion collection, identified 19 genes as being involved in pathways that may be potential targets of IPS. Interestingly, 7 of these identified genes are essential for the yeast survival, making them promising antifungal target candidates. To confirm the effect of IPS on products of these gene candidates, heterozygous recombinant strains were constructed by deleting the target genes through the integration of a KanMX cassette (conferring resistance to geneticin) in place of one of the two alleles. We found that yeast *dis3*/*dis3::KanMX* mutant strain is more sensitive to IPS compared to the wild type *S. cerevisiae* W303 and mutant strains for the other potential targets. This suggests that Dis3 – responsible for RNA processing and degradation in both the nucleus and the cytoplasm – is a target of IPS.

In order to study the mechanism of action of IPS in filamentous fungi, we overexpressed in *Aspergillus oryzae* the native ortholog of the yeast *dis3* gene, given that the deletion of a potentially essential gene in this haploid strain may have been lethal. The analysis of the mutant strain is currently underway.

Further high-throughput molecular analyses, e.g. proteomic analysis and/or RNAseq will be used to identify the exact pathways affected by IPS in both yeast and filamentous fungi. In future work we will compare our data with the work done in the Waterfield lab on the mode of action of IPS in *Staphylococcus aureus*[4] We will determine if IPS has the same or different targets in the eukaryote and prokaryote.

References: 1. Li J. *et al.* (1995) *Appl Environ Microbiol* 61: 4329-4333. 2. Shi D. *et al.* (2017) *J Agric Food Chem* 65: 60-65. 3. Kumar S.N. *et al.* (2014) *Ann Microbiol* 64: 209-218. 4. Hapeshi A. *et al*. (2019) *Microbiology* 165: 516-526.