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Drug combination therapy has the potential to extend the shelf-life of existing antimicrobial drugs by reducing the rate at which resistance evolves, but the consequences of this therapeutic approach for pathogen fitness are poorly understood. Now, Hill *et al.* evaluate the evolution of resistance in the leading fungal pathogen *Candida albicans* following combination therapy and find that fitness trade-offs occur that may minimize the emergence of resistance to antifungal agents.

The most frequently used class of antifungal drugs are the azoles, which are fungistatic and are therefore prone to selecting for resistance. Resistance to azoles typically involves Hsp90, a molecular chaperone that regulates stress response proteins, such as the protein phosphatase calcineurin. Previously, the authors studied two sets of *C. albicans* mutants (clinical isolates and mutants evolved *in vitro*) that had acquired resistance to both the azole drug fluconazole and an Hsp90 inhibitor (geldanamycin) or an inhibitor of calcineurin (FK506), and in this new study, they investigate the mechanisms and fitness consequences of resistance.

Growth competition experiments revealed that the two sets of resistant strains were generally less fit than their ancestral strains during growth in the absence of the drugs, which indicated that resistance to the drug combination has a fitness cost. Interestingly, unlike the *in vitro*-evolved strains and early-stage clinical isolates, fluconazole resistance in the late-stage clinical isolates was mostly independent of Hsp90 and calcineurin; whole-genome sequencing revealed that the amino acid substitutions A736V in Tac1 (a transcriptional activator of ABC drug transporters), A643V in Upc2 (a protein involved in ergosterol biosynthesis) and R467K in Erg11 (which is also involved in the ergosterol pathway) were responsible for resistance. Thus, these data show that azole resistance can evolve to become independent of mutations in the stress response regulators Hsp90 and calcineurin.

> “resistance to the drug combination is accompanied by fitness trade-offs”

The authors then went on to determine the cost of resistance under several stress conditions that *C. albicans* experiences in the host. Both the *in vitro*-evolved and late-stage clinical isolates were generally less fit than their ancestors in all of the conditions tested, most notably following exposure to the reactive oxygen species (ROS) H_2O_2. Consistent with this, these strains were more susceptible to killing by macrophages, which are involved in the production of ROS in the host.

The ability of *C. albicans* to transition from a yeast form to a filamentous form is central for virulence and occurs following exposure to cues such as Hsp90 inhibitors. The authors found that although most of the resistant strains retained the ability to form filaments in response to geldanamycin, late-stage clinical isolates had lost this virulence trait. This defect was caused by the Tac1 A736V substitution, which led to increased expression of two ABC transporters that are involved in the efflux of geldanamycin; thus, these strains seem to have lost their morphogenesis ability owing to export of the drug out of the cell. Collectively, these data reveal that resistance to the drug combination is accompanied by fitness trade-offs, which manifest as reduced survival in the host environment and impaired virulence.

This study demonstrates the evolutionary constraints imposed by drug combination therapy and indicates that this treatment strategy has the potential to limit the emergence and spread of resistant fungal pathogens.

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ORIGINAL RESEARCH PAPER Hill, J. A. *et al.* Fitness trade-offs associated with the evolution of resistance to antifungal drug combinations. *Cell Rep.* **10**, 809–819 (2015)