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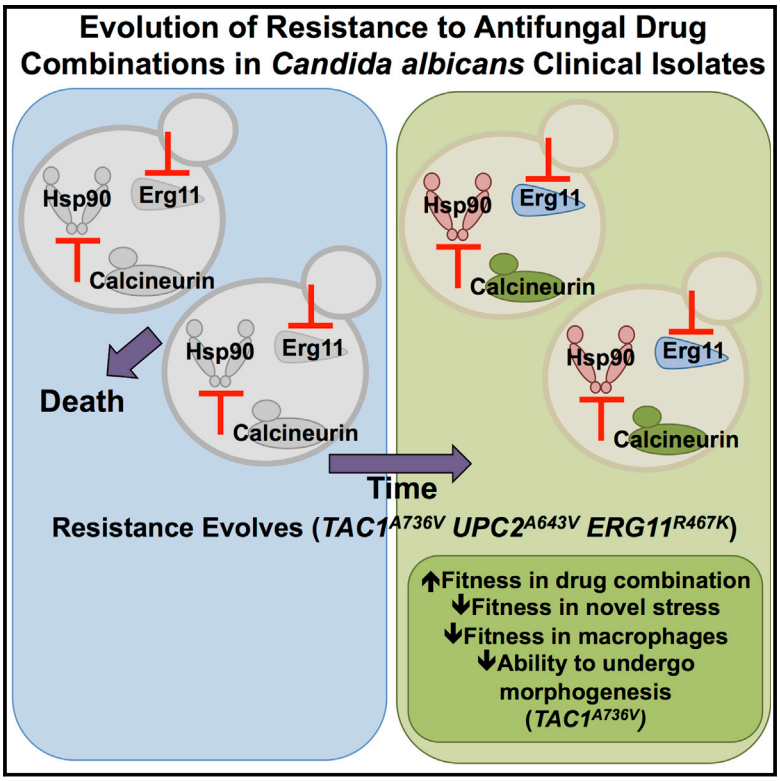
Cell Reports

Fitness Trade-Offs Associated with the Evolution of Resistance to Antifungal Drug Combinations

Graphical Abstract



Highlights

- Adaptation to antifungal drug combinations is associated with fitness trade-offs
- *TAC1*^{A736V}, *UPC2*^{A643V}, and *ERG11*^{R467K} confer resistance to azole and Hsp90 inhibitors
- *TAC1*^{A736V} blocks morphogenesis in response to Hsp90 inhibitors
- Azole resistance can evolve independence from stress response regulators in the host

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In Brief

Drug combinations are emerging as a promising strategy for managing difficult-to-treat fungal infections, but little is known about the fitness consequences of resistance. Hill et al. determine that resistance to drug combinations in *Candida albicans* causes fitness trade-offs that may minimize the evolution and persistence of resistance.

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Fitness Trade-Offs Associated with the Evolution of Resistance to Antifungal Drug Combinations

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SUMMARY

The evolution of drug resistance threatens human health worldwide. An emerging strategy to mitigate drug resistance is combination therapy. The fate of multidrug-resistant pathogens depends on their fitness relative to susceptible counterparts, yet the fitness consequences of multidrug resistance remain enigmatic. Here, we dissect fitness consequences of the evolution of resistance to antifungal drug combinations in the leading human fungal pathogen, *Candida albicans*. We focus on the most widely deployed antifungals, the azoles, and inhibitors of the molecular chaperone Hsp90 and protein phosphatase calcineurin, which regulate cellular stress responses required for azole resistance. We find trade-offs such that adaptation to drug combinations is associated with reduced fitness in distinct environments, including those relevant to the human host. We identify mutations associated with fitness trade-offs in clinical isolates and that influence morphogenesis, a key virulence trait. Thus, we delineate evolutionary constraints that may minimize the evolution of resistance to antifungal combinations.

INTRODUCTION

The evolution of drug resistance is a looming public health crisis. Imprudent use of antimicrobials in agriculture and medicine compromises the effectiveness of our arsenal of drugs, as the rate at which new antimicrobials are developed does not keep pace with the rapid emergence of resistance (Laxminarayan et al., 2013). Resistance complicates medical outcomes, imposes significant financial burdens, and threatens a post-antibiotic era of medicine (Andersson and Hughes, 2011). Thus, reducing the spread of drug resistance is of critical importance.

Drug-resistance mechanisms provide a clear fitness advantage for the pathogen in the presence of drug, but their maintenance in the absence of drug selection is contingent on minimal fitness costs of resistance (Gagneux et al., 2006; MacLean et al., 2010). Resistance mechanisms often alter key cellular functions; thus, resistance is frequently associated with a cost in the absence of drug. Epidemiological models and experimental evidence suggest that this can slow the spread of resistance (Andersson and Hughes, 2010), yet drug resistance is not necessarily costly. For example, an isochromosome (i5L) in the fungal pathogen *Candida albicans* confers resistance to the antifungal azoles and is not deleterious in the absence of drug (Selmecki et al., 2009). Understanding whether drug resistance is costly in clinically relevant environments is crucial to evaluating whether resistance will persist.

Drug-combination therapy is a promising strategy to decrease the rate of drug resistance. Combination therapy can enhance the utility of antimicrobials by hindering the evolution of resistance, such as by targeting cellular processes required for resistance. An example is to cripple cellular stress response regulators when treating human pathogens like *C. albicans*. *C. albicans* is the fourth most common cause of hospital-acquired infection, and systemic *C. albicans* infections are recalcitrant to treatment, with mortality rates approaching 40% (Pfaller and Diekema, 2010). The most frequently deployed class of antifungals is the azoles; however, they are fungistatic and vulnerable to resistance (Anderson, 2005). Resistance is often contingent on the molecular chaperone Hsp90, which regulates key stress response proteins, including the protein phosphatase calcineurin (Cowen and Lindquist, 2005; Singh et al., 2009). Combination therapy with an inhibitor of Hsp90 or calcineurin and an azole provides a powerful strategy for rendering azole-resistant *C. albicans* infections responsive to treatment (Cowen, 2009). However, we must anticipate that resistance to drug combinations will evolve. We recently established an experimental evolution approach to track the evolution of resistance to drug combinations, and while most lineages went extinct, a minority was able to evolve resistance to the azole fluconazole (FL) in combination with the Hsp90 inhibitor geldanamycin (GdA) or the calcineurin inhibitor FK506. The fitness consequences of the evolution of resistance to drug combinations remain enigmatic.

Several questions emerge about the fitness consequences of resistance to drug combinations. Is resistance to drug combinations costly in the absence of drug or in the presence of a single drug? Does resistance to these drug combinations, particularly ones that target stress response regulators, confer cross-resistance to novel stress environments? Or does resistance to drug combinations create a trade-off in other environments? To address these questions, we take advantage of two distinct sets of strains of FL-resistant *C. albicans* that evolved resistance to FL and an inhibitor of