Dynamic duo takes down fungal villains

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Fungal pathogens are notoriously difficult to treat because of antifungal drug toxicity and drug resistance, and the limited armamentarium (1). Some drug classes inhibit fungal growth but do not efficiently eliminate pathogens, and there is a pressing need for advances in antifungal therapy. The problem is especially acute in the immunocompromised. In a recent issue of PNAS, Cowen et al. (2) describe how harnessing heat shock protein 90 (Hsp90) dramatically enhances the efficacy of available antifungal drugs and provides a powerful new avenue for eradicating fungal infections.

Hsp90 is an essential chaperone responsible for folding and maturation of client proteins. These include signal transducers with unstable conformations that only acquire their active structure in response to specific signals. Moreover, Hsp90 acts as a capacitor for evolution and buffers the effects of mutations, allowing their gradual accumulation (3, 4). When stress conditions overwhelm Hsp90, otherwise silent polymorphisms are expressed and can be selected if they provide a survival advantage. For example, Hsp90 potentiates the emergence of antifungal resistance and its inhibition reduces *Candida albicans* resistance to azoles and enhances action of caspofungin (CS) against *Aspergilli* (5, 6). These observations first implicated Hsp90 as a novel antifungal target and set the stage for Cowen et al. (2) to test synergistic effects of Hsp90 inhibitors and conventional antifungal drugs.

In their study, Cowen et al. (2) tested several Hsp90 inhibitors combined with antifungal drugs of different mode of actions against evolutionarily divergent fungi in 2 complementary pathogenicity models. The Hsp90 inhibitors are geldanamycin (GdA) and analogs already in clinical trials as anticancer agents. 17-(Allylamino)-17-demethoxygeldanamycin (17-AAG) and 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (17-DMAG) were tested in association with 2 commonly used antifungal drugs, fluconazole (FL; an ergosterol biosynthesis inhibitor) and CS (inhibits 1,3-β-glucan synthase to block cell wall biosynthesis). The efficacy of combinatorial therapy against *C. albicans* and *Aspergillus fumigatus*, fungal pathogens associated with high mortality rates, was evaluated in *Galleria mellonella* and murine models. *G. mellonella* larvae serve as a facile, inexpensive, and ethically convenient model for fungal pathogenicity and allow evaluation of host immune response to infection (7). Studies using different fungal pathogens in the *G. mellonella* system show a significant correlation between virulence in larvae and mice, and the efficacy of antifungal therapies in this invertebrate corresponds well to results in humans (7).

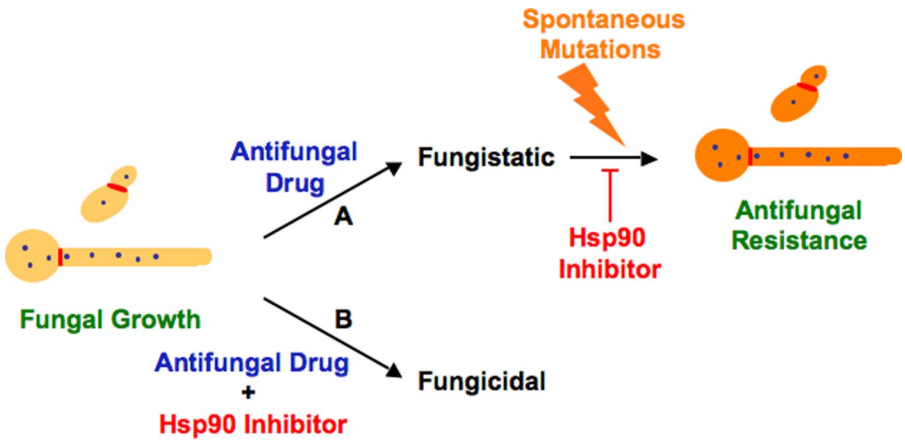

Fig. 1. Hsp90 inhibitor modes of action in antifungal therapy. Combination therapy with an Hsp90 inhibitor and a fungistatic agent restrains development of antifungal resistance (*A*) and potentiates the activity of available antifungal drugs (*B*).

In all scenarios tested by Cowen et al. (2), inhibition of Hsp90 improved the response to antifungal drugs. Hsp90 inhibitors converted FL fungistatic activity against *C. albicans* to fungicidal, and this synergistic interaction rescued *G. mellonella* larvae from lethal infection. Similar effects were observed when *A. fumigatus* was treated with the GdA–CS combination in vitro and in *G. mellonella*. In a murine model of disseminated candidiasis, genetic abrogation of *C. albicans HSP90* resulted in clearance of the fungal infection upon FL treatment. These findings support inhibition of Hsp90 in combination with fungistatic agents as a novel, efficacious broad-spectrum therapy against fungal pathogens (Fig. 1). Because Hsp90 is important for development of antifungal resistance (5), inhibiting Hsp90 early in infection has the potential to restrain phenotypic variation that might be selected and result in emergence of resistant isolates (Fig. 1). Blocking the evolvability of antifungal resistance is an entirely new mode of action, which has not been applied therapeutically and merits further exploration.

According to the Tufts Center for the Study of Drug Development in Boston, up to 1.8 billion dollars and 18 years can be required to develop a new drug. Therefore, using a combination of marketed drugs as a novel treatment can accelerate therapeutic advance. Combinatorial therapies to enhance efficacy of individual drugs have been widely and successfully used to treat cancer and bacteria. The principle is simple: inhibiting multiple pathways simultaneously results in combinatorial blockage of cell viability and limits exposure to toxic side effects. Hence, simultaneous administration of available drugs is likely to provide several advantages to conventional antifungal therapy: broader spectrum and potency, decreased emergence of resistance, and reduction in the dosage of toxic agents such as amphotericin B (AmB). However, this therapeutic modality has not been implemented in clinical practice with the exception of cryptococcal meningitis, often treated with a flucytosine–AmB combination (8). The results of Cowen et al. (2) should accelerate further clinical applications of antifungal combination therapy.

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Although modulation of Hsp90 function in combination with fungistatic drugs provides a mechanistically appealing antifungal strategy, the identification of fungal-specific Hsp90 inhibitors may be required for this combinatorial therapy to be implemented clinically. For instance, despite the fact that Cowen et al. (2) used concentrations of inhibitors that were well tolerated in clinical trials as antineoplastic agents, Hsp90 inhibition in mice during a systemic fungal infection had detrimental effects. It is not surprising that harnessing an essential chaperone as Hsp90 could have deleterious side effects. In this case the lethality might be explained by the fact that Hsp90 inhibition is immunosuppressive (9) and may render mice more susceptible to invasive candidiasis. Although these results confirm that mouse studies are often complex, they reaffirm the value of the *G. mellonella* system as a way station between in vitro and animal tests. The fact that genetic regulation of Hsp90 enhanced FL efficacy in mice with disseminated candidiasis provides a key proof of principle that drugs that target Hsp90, particularly fungal-specific ones, have vast potential as novel antifungal agents.

An alternative strategy to potentially target fungal Hsp90 involves a human recombinant antibody Mycograb® (MY), which binds a conserved Hsp90 epitope and is efficacious against different *Candida* species and synergistic with AmB in vitro and in vivo (10). Clinical studies using the MY–AmB combination to treat patients with invasive candidiasis increased fungal clearance and reduced mortality (11). Because MY lacks the Fc component, its antifungal activity cannot be explained by Fc-mediated recruitment of white blood cells or complement. The strains developed by Cowen et al. (2) in which Hsp90 can be genetically manipulated provide an approach to establishing unequivocally the target specificity of MY.

Cowen et al. provide a foundation from which to exploit Hsp90 for antifungal therapy.

Likewise, mechanistic insights into how Hsp90 inhibitors potentiate antifungal activity are critical. A key mediator of Hsp90-dependent azole resistance is the client protein calcineurin, a serine-threonine Ca^{2+} activated phosphatase (5, 12). Calcineurin is required for *C. albicans* to tolerate azoles, and simultaneous inhibition of ergosterol biosynthesis and calcineurin has synergistic fungicidal activity (13–15). Possible clinical applications of such combinatorial therapy involve topical formulations to avoid the immunosuppressive effects of calcineurin inhibitors. An azole–calcineurin inhibitor combination is synergistic against both *C. albicans* keratitis in a murine corneal infection model (16) and *Trichophyton mentagrophytes* in an *ex vivo* human skin infection model (17). Moreover, Uppuluri et al. (18) showed that calcineurin is involved in the resistance of *C. albicans* biofilms to FL and that biofilms can be treated by a FL–calcineurin inhibitor combination but not with either drug alone. Hence, blocking the signaling network activated by calcineurin, like inhibition of Hsp90, has synergistic effects with azoles, raising the possibility of triple combination therapy.

Although calcineurin activity is likely to contribute to the fungicidal effects of Hsp90 inhibitors, given a vast repertoire of client proteins, it is possible other targets also participate. 17-AAG has been reported to induce apoptosis in colon-carcinoma-derived cell lines (19), and an interesting speculation is that harnessing Hsp90 could induce apoptotic-like cell death in fungi. Several fungi have been reported to undergo apoptotic-like cell death in response to diverse stimuli, including antifungal treatment (20). Additional studies for understanding fungal apoptotic pathways should further the discovery of much needed new antifungal therapies.

In conclusion, Cowen et al. (2) provide a foundation from which to exploit Hsp90 for antifungal therapy. Considering all of the actions exerted by Hsp90 inhibitors, frank antifungal activity, synergism with 2 different classes of commonly-used existing agents, broad activity across the fungal kingdom, and the ability to block or reverse drug resistance, they hold great promise in the ongoing battle to stay one step ahead of fungal maladies.

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