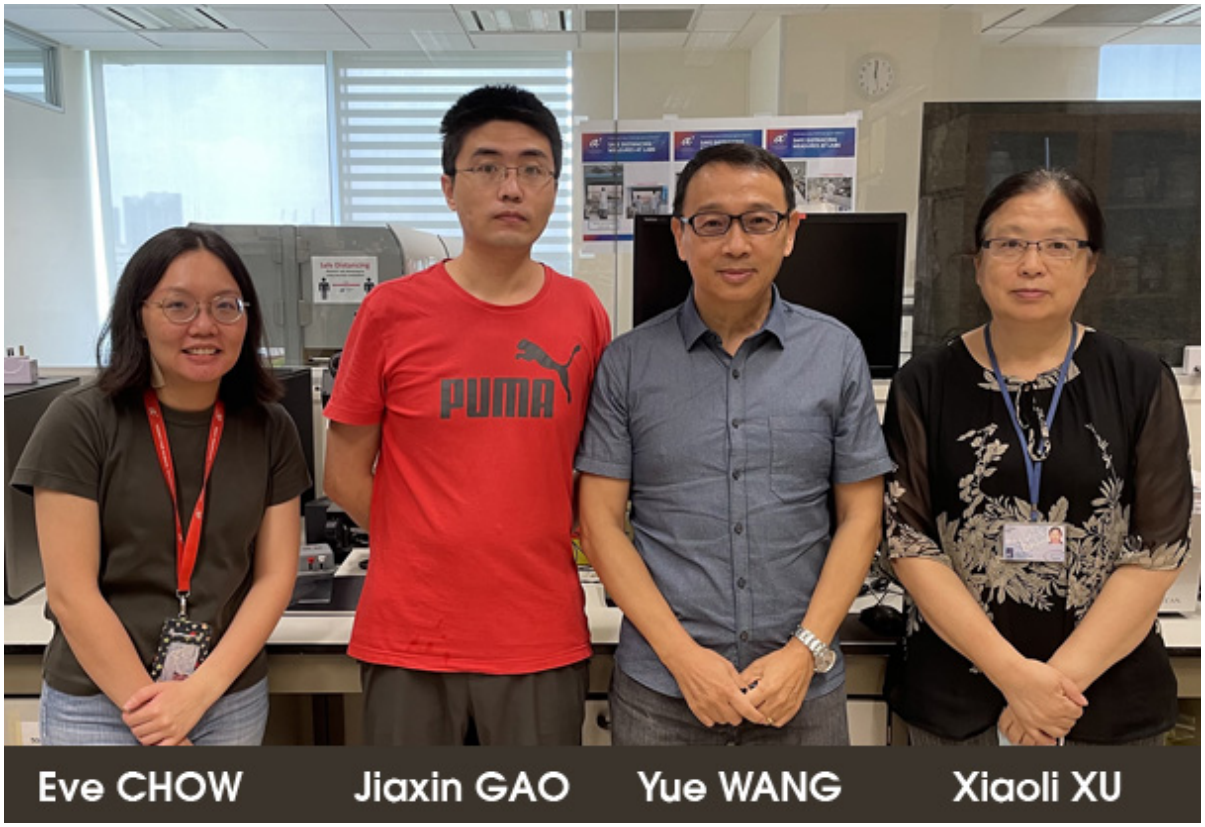
LncRNA DINOR is a virulence factor and global regulator of stress responses in *Candida auris*

Wednesday, 23 June 2021



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Published in *Nature Microbiology* on 3rd June 2021
DOI: 10.1038/s41564-021-00915-x

Abstract

The emergent fungal pathogen *Candida auris* exhibits high resistance to antifungal drugs and environmental stresses, impeding treatment and decontamination. The fungal factors mediating this stress tolerance are largely unknown. Here, we performed *piggyBac* transposon-mediated genome-wide mutagenesis and genetic screening in *C. auris*, and identified a mutant that grew constitutively in the filamentous form. Mapping the transposon insertion site revealed the disruption of a long noncoding RNA, named *DINOR* for DNA damage-inducible noncoding RNA. Deletion of *DINOR* caused DNA damage and an upregulation of genes involved in morphogenesis, DNA damage, and DNA replication. The DNA checkpoint kinase Rad53 was hyperphosphorylated in *dinor*Δ mutants, and deletion of *RAD53* abolished DNA damage-induced filamentation. DNA-alkylating agents, which cause similar filamentous growth, induced *DINOR* expression, suggesting a role for *DINOR* in maintaining genome integrity. Upregulation of *DINOR* also occurred during exposure to the antifungal drugs caspofungin and amphotericin B, macrophages, H_2O_2, and SDS, indicating that *DINOR* orchestrates multiple stress responses. Consistently, *dinor*Δ mutants displayed increased sensitivity to these stresses and were attenuated for virulence in mice. Moreover, genome-wide genetic interaction studies revealed links between the function of *DINOR* and TOR signaling, an evolutionarily conserved pathway that regulates the stress response. Identification of the mechanism(s) by which *DINOR* regulates stress responses in *C. auris* may in future provide opportunities for the development of therapeutics.

Figure:

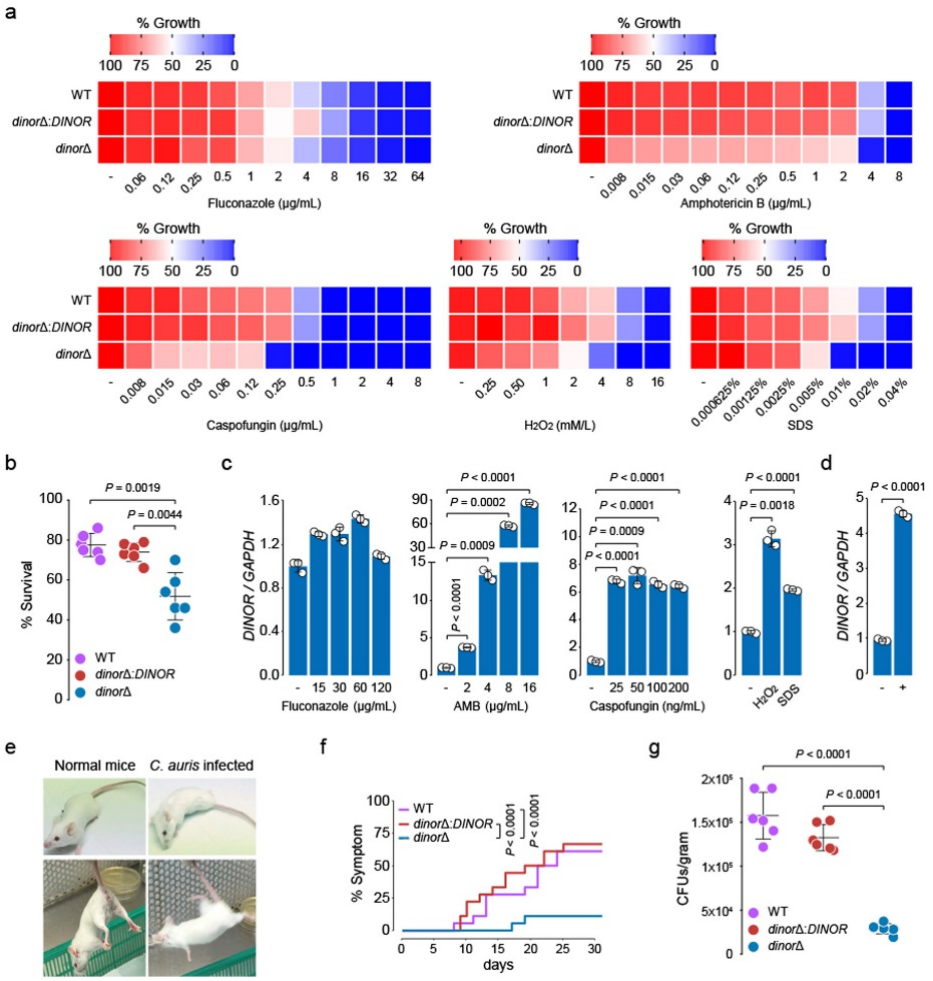

Figure legend: *DINOR* is required for *C. auris* response to antifungal drug, H_2O_2, and SDS and pathogenicity in mice.

a, Antifungal drugs, H_2O_2, and SDS susceptibility assays. WT, *dinor*Δ, and *dinor*Δ:*DINOR* cells were inoculated into YPD containing 2-fold serially diluted antifungal drugs, H_2O_2, or SDS and incubated at 37 °C for 48 h or 24 h. Growth was determined by taking OD_{600} and expressed as the relative growth with the no-drug well of each strain set as 100%.

b, Survival rates of WT, *dinor*Δ, and *dinor*Δ:*DINOR* cells after exposure to macrophages. Cells were co-cultured with RAW264.7 macrophages for 4 h. Fungal survival was measured by counting CFUs. Error bars, s.d. from the mean of three independent experiments.

c, qPCR analysis of *DINOR* expression in WT cells in the presence or absence of antifungal drugs, H_2O_2, or SDS. WT cells were treated with or without antifungal drugs, H_2O_2, or SDS before RNA extraction. *DINOR* expression levels were normalized against that of *GAPDH.* The level of the untreated sample was set as 1. Error bars, s.d. from the mean of three independent experiments.

d, qPCR analysis of *DINOR* expression in WT cells after exposure to macrophages. WT cells were co-cultured with RAW264.7 macrophages for 4 h at 37 °C for RNA extraction. *DINOR* expression levels were normalized against that of *GAPDH*, and that of the untreated sample was set as 1. Error bars, s.d. from the mean of three independent experiments.

e, Appearance of head tilting and body spinning in mice injected with *C. auris*.

f, Symptom curves of mice injected with WT, *dinor*Δ, and *dinor*Δ:*DINOR* cells. Each mouse (n = 18) was injected with 1×10^7 cells of a strain.

g, Fungal burdens in the kidney of mice injected with WT, *dinor*Δ, and *dinor*Δ:*DINOR* cells. Three mice were used for each strain. Mice were sacrificed for fungal burden analysis at 48 h post-infection. Error bars, s.d. from the mean of six technical replicates.