A PiggyBac Transposon-based Genetic Screening System in Haploid Human Fungal Pathogen Candida Albicans

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Fungal infections by drug-resistant Candida albicans pose a global public health threat. However, the pathogen's diploid genome greatly hinders genome-wide investigations of resistance mechanisms. Here, we developed a highly efficient piggyBac transposon-mediated mutagenesis system using stable haploid C. albicans to conduct genome-wide genetic screens. We discovered that fen1 and fen12 null mutants exhibited resistance to fluconazole, a first-line antifungal drug. Fen1 and Fen12 synthesize very-long-chain fatty acids as precursors of sphingolipids. Mass-spectrometry analyses demonstrated dramatic changes in cellular sphingolipid composition in both mutants, especially an increase of several mannosylinositolphosphoceramides with shorter fatty-acid chains by thousands of fold. Treatment with fluconazole induced similar changes in wild-type cells, suggesting a natural response mechanism. Furthermore, the resistance relies on a robust upregulation of sphingolipid biosynthesis genes. Our study demonstrates the superb power of a new technology for whole-genome studies of C. albicans and reveals a previously unknown antifungal resistance mechanism.