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Short communication

Mutation of the mitochondrial large ribosomal RNA can provide pentamidine resistance to *Saccharomyces cerevisiae*

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ABSTRACT

Pentamidine is used to treat several trypanosomal diseases, as well as opportunistic infection by pathogenic fungi. However, the relevant targets of this drug are unknown. We isolated dominant mutations providing pentamidine resistance to *Saccharomyces cerevisiae*, one of which was localized to mitochondrial DNA. Next-generation sequencing revealed alteration of a widely conserved base at the peptidyl transferase center of the mitochondrial 21S ribosomal RNA. Our results provide a potential rationale for the toxicity of this drug to patients, and we discuss whether blockade of mitochondrial translation is the mechanism by which pathogenic fungi or protists are killed by pentamidine.

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1. Introduction

Pentamidine is used to treat infection by trypanosomatids or by the fungus *Pneumocystis jirovecii* (Soeiro et al., 2013). However, the precise molecular target(s) of this drug are unknown (Alsford et al., 2013; Baker et al., 2013; Werbovetz, 2006). Much evidence suggests that pentamidine can affect mitochondrial function in organisms susceptible to killing by pentamidine. For example, pentamidine can rapidly reduce the mitochondrial electrochemical potential ($\Delta\Psi^{mito}$) across the inner membrane of trypanosomid mitochondria (Lanteri et al., 2008; Vercesi and Docampo, 1992). Pentamidine also prompts damage to or loss of the kinetoplast DNA (Hentzer and Kobayasi, 1977; Shapiro and Englund, 1990), which encodes the machinery used for parasite oxidative phosphorylation. In *Saccharomyces cerevisiae*, pentamidine treatment leads to an inhibition of mitochondrial translation (Zhang et al., 2000), and overexpression of Pnt1p, a polypeptide involved in export of mtDNA-encoded protein domains from the mitochondrial matrix (He and Fox, 1999) can provide a measure of pentamidine resistance (Ludewig and Staben, 1994). Importantly, pentamidine causes significant patient toxicity (Soeiro et al., 2013), and a large-scale comparison of several mitochondrial parameters upon treatment of a myoblast cell line with hundreds of different drugs suggested that myopathy associated with pentamidine treatment might be linked to mitochondrial dysfunction (Wagner et al., 2008). By taking a genetic approach to identify potential mitochondrial targets of pentamidine, we discovered that mutation of the mitochondrial ribosome can provide pentamidine resistance.

2. Materials and methods

2.1. Yeast strains and culture conditions

Yeast media and genetic techniques are as described in Adams et al. (1997). YEPGE medium contains 3% glycerol and 3% ethanol. Gene disruptions were performed as detailed in Sikorski and Hieter (1989). Strains were cultured at 30 °C. Pentamidine isothionate (Sigma, St. Louis, MO) was added at the indicated concentrations. Pentamidine in aqueous solution is unstable (Martindale et al., 1972), leading to potential variation in the effective drug concentration during plate assays. We also noted that pentamidine is sensitive to inactivation in molten agar at high temperature, and so one should quickly cool media to which pentamidine has been added to minimize variability in pentamidine efficacy between plate batches. Plates were also protected from exposure to light before use. Pentamidine-resistant mutants were isolated by culture in liquid YEPGE containing pentamidine isothionate for ~9 h before plating at high density to solid medium also containing drug. Where indicated, ethidium bromide (EtBr; Thermo-Fisher Scientific, Waltham, MA) was used at 25 μg/ml to destroy mtDNA. Serial dilution assays were performed as in Garipler et al. (2014). Cytoduction was accomplished by patching *kar1 cyh2* ρ^- strain CDD723 to either *WT* strain CDD725 or *PNT3-1* strain CDD726, followed by spreading the

Abbreviations: mtDNA, mitochondrial DNA; EtBr, ethidium bromide; BAM, binary sequence alignment/map format; rRNA, ribosomal ribonucleic acid.

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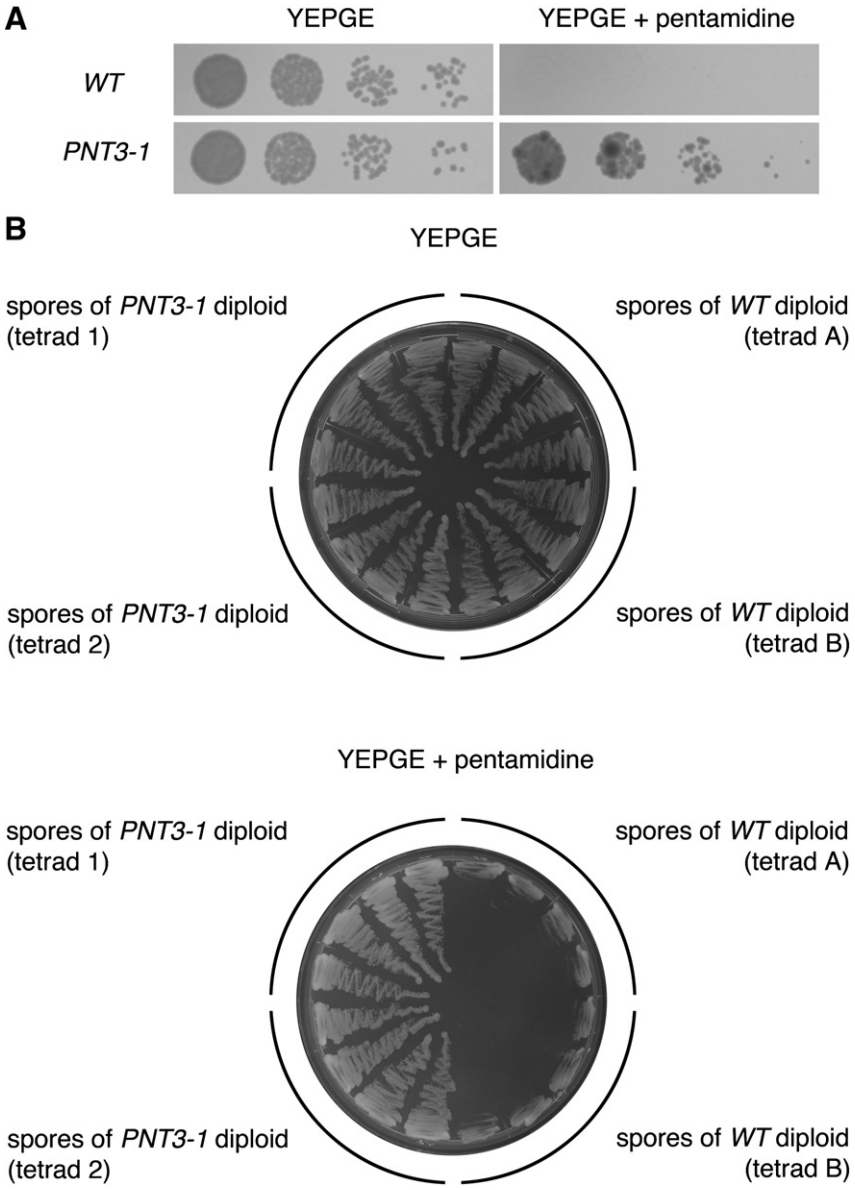

Fig. 1. An allele providing pentamidine resistance is inherited in a non-Mendelian fashion. (A) *PNT3-1* provides dominant pentamidine resistance on non-fermentable medium. Diploid strains BMA64 (*WT*) and CDD693 (*PNT3-1*) were cultured in YEPD medium, then subjected to serial dilution and either spotted upon YEPGE medium and incubated for 2 d or, rather, spotted upon YEPGE to which pentamidine was added at a concentration of 10 μg/ml, then incubated for 7 d. (B) All four spores produced from a *PNT3-1* diploid are pentamidine-resistant. Diploid strains BMA64 (*WT*) and CDD693 (*PNT3-1*) were sporulated. Resultant haploid progeny proliferating on YEPD medium were struck to YEPGE and incubated for 2 d or struck to YEPGE containing pentamidine added to 10 μg/ml and incubated for 3 d.

mated cells on YEPD at a density at which single colonies were apparent, then replica-plating to YEPGE medium containing 3 μg/ml cycloheximide (Sigma-Aldrich, St. Louis, MO) to select for cytoductants. Cytoduction was further confirmed by examining nuclear-background-specific markers. Genotypes of all strains used in the course of this study, along with details of their construction and oligonucleotide sequences, are provided in Supplementary Table 1.

2.2. Genomic DNA isolation and next-generation sequencing

Genomic DNAs from strain BMA64, strain CDD693, and a pool of wild-type haploids derived from strain CDD682 were isolated from saturated cultures essentially as in Looke et al. (2011). Paired-end library preparation was performed at the European Molecular Biology Laboratory Genomics Core Facility (Heidelberg, Germany) using the Illumina protocol for preparation of genomic DNA sequencing libraries. Paired-end library fragments averaging 300 bp (standard deviation of 25 bp) were sequenced on the Illumina MiSeq platform to a read length of 150 bp. Sequencing reads have been deposited into the Sequence Read Archive of the National Center for Biotechnology Information under accession number PRJNA246119. Bioinformatic analysis of genomic sequence to identify single nucleotide polymorphisms was performed as in Mutlu et al. (2014), and VarScan2 output is provided as Supplementary Table 2.

3. Results and discussion

The recovery of dominant mutations providing drug resistance to *S. cerevisiae* is one avenue that may successfully reveal either the protein or the pathway targeted by that drug (Heitman et al., 1991). Among numerous scenarios for how alteration of a gene product might provide dominant resistance to a chemical agent, a mutation might block drug access to the catalytic site of an enzyme, thereby preserving functionality. Under these circumstances, localizing the mutation would directly

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