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Insight into the selectivity of arsenic trioxide for acute promyelocytic leukemia cells by characterizing *Saccharomyces cerevisiae* deletion strains that are sensitive or resistant to the metalloid

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Abstract

The genome-wide set of *Saccharomyces cerevisiae* deletion strains provides the opportunity to analyze how other organisms may respond to toxic agents. Since arsenic trioxide selectively kills human acute promyelocytic leukemia (APL) cells by a poorly understood mechanism we screened the yeast deletion strains for sensitivity or resistance. In addition to confirming mutants previously identified as sensitive to sodium arsenite, a large number of additional genes, and cellular processes, were required for arsenic trioxide tolerance. Of the 4546 mutants, 7.6% were more sensitive to arsenic trioxide than the wild type, while 1.5% was more resistant. IC_{50} values for all sensitive and resistant mutants were determined. Prominent as sensitive was that missing the MAP kinase, Hog1. The most resistant lacked the plasma-membrane glycerol and arsenite transporter, Fps1. Hog1 and Fps1 control the response to osmotic stress in yeast by regulating glycerol production and plasma membrane flux, respectively. We therefore tested whether APL cells have impaired osmoregulation. The APL cell line NB4 did not produce glycerol in response to osmotic stress and underwent apoptotic cell death. Moreover, the glycerol content of NB4 and differentiated NB4 cells correlated with the level of arsenic trioxide uptake and the sensitivity of the cells. Additionally, NB4 cells accumulated more arsenic trioxide than non-APL cells and were more sensitive. These findings demonstrate the usefulness of the *S. cerevisiae* deletion set and show that the selectivity of arsenic trioxide for APL cells relates, at least in part, to impaired osmoregulation and control of uptake of the drug.

Keywords: Yeast; Arsenic trioxide; Acute promyelocytic leukemia; Osmoregulation

1. Introduction

The availability of the genome-wide set of *Saccharomyces cerevisiae* deletion strains has provided the opportunity to identify how cells respond to and survive environmental changes including responses to toxic

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compounds. Since there is a strong degree of conservation of cellular processes across all eukaryotic organisms, the *S. cerevisiae* deletion strain set affords the opportunity to identify how cells of other eukaryotes, including human, may respond. Here we show that the deletion set can be used to identify the basis for drug selectivity in human cells.

The clinical course of APL has changed over the last few decades from one that was often fatal to one of the more treatable subtypes of acute myeloid leukemia. Besides improved supportive care this has mainly been achieved through the introduction of all-trans retinoic acid (ATRA) therapy that targets the underlying molecular lesion and leads to differentiation of leukemic blasts into mature granulocytes (Gazitt & Akay, 2005; Miller, Schipper, Lee, Singer, & Waxman, 2002). In relapsed patients and those refractory to ATRA, arsenic trioxide (As_2O_3) is considered the treatment of choice (Reiter, Lengfelder, & Grimwade, 2004). As_2O_3 is a trivalent arsenical that selectively kills APL cells.

Several studies have shed light on how As_2O_3 induces the death of APL cells, including perturbation of the cytoskeleton (Ramirez, Eastmond, Laclette, & Ostrosky-Wegman, 1997), cleavage of DNA strands (Li & Rossman, 1989) and induction of reactive oxygen species (Grad et al., 2001). The key question, though, is why does this drug selectivity kill APL cells and not other cells? One theory is that APL cells have low oxidant buffering capacity and are, therefore, more sensitive to the reactive oxygen species triggered by As_2O_3 exposure (Yang, Kuo, Chen, & Chen, 1999). Indeed, the growth inhibitory and apoptotic effects of As_2O_3 are inversely proportional to cellular glutathione content (Dai, Weinberg, Waxman, & Jing, 1999), which is a buffer of reactive oxygen species. However, we have observed that endothelial cells have an even lower oxidant buffering capacity than APL cells but are more resistant to As_2O_3 in vitro (Dilda et al., 2005; unpublished observations) and do not appear to be targeted by this compound in vivo.

To better understand how As_2O_3 perturbs cellular functions we screened the *S. cerevisiae* deletion strain set (Winzeler et al., 1999) for sensitivity or resistance to the drug. The large number of deletion strains (4546) makes it very likely that mutations affecting a component or components of an important cellular process will show a phenotype of sensitivity or resistance. The idea was that genes whose loss conferred sensitivity to the drug would reflect mechanisms of cell death, while genes whose loss conferred resistance would suggest mechanisms of selectivity.

In accordance with published studies in mammalian cells, yeast mutants lacking genes required for cytoskeleton stability, response to oxidative stress and heavy metal tolerance (including glutathione synthesis) and DNA repair were prominent in the sensitive list. An unexpected strain in the sensitive list was that missing the kinase, Hog1, and the most resistant strain was that missing the plasma membrane glycerol and arsenite transporter, Fps1. Hog1 and Fps1 control the response to osmotic stress in yeast. Extension of these findings in yeast to APL cells led us to conclude that the selectivity of As_2O_3 for APL relates to impaired osmoregulation and control of uptake of the drug.

2. Materials and methods

As_2O_3 - A 1 M solution of As_2O_3 was made by dissolving solid (Sigma, St. Louis, MO) in 3 M NaOH prepared in deoxygenated water. The solution was diluted 10-fold in deoxygenated water, the pH adjusted to 7.0 using HCl and stored at 4 °C in an airtight container until use. The concentration of active trivalent arsenical was measured by titrating with dimercaptopropanol and calculating the remaining free thiols with 5,5′-dithiobis(2-nitrobenzoic acid) (Donoghue, Yam, Jiang, & Hogg, 2000).

Yeast strains and growth conditions—the *S. cerevisiae* deletion strains used in this study are derivatives of the diploid wild-type strain BY4743 (MATα/MATa; *his3*Δ1/*his3*Δ1; *leu2*Δ0/*leu2*Δ0; *met15*Δ0/*MET15*; *LYS2*/*lys2*Δ0; *ura3*Δ0/*ura3*Δ0; EUROSCARF, Frankfurt Germany) which were homozygous for the relevant gene deletion. The construction of the yeast genome deletion library was described previously (Winzeler et al., 1999). The wild-type strain and deletion strains (4546 mutants) were grown in liquid YEPD medium (2%, w/v glucose; 2%, w/v bacto peptone and 1% yeast extract) for 3 days and inoculated in to YEPD medium without or with As_2O_3 (0.2, 0.7 or 1.2 mM) using a 96-pin replicator. Plates were incubated at 30 °C and cell density (A_{600}) measured at 24 and 48 h using a 96-well Thermomax Plus spectrophotometer (Molecular Devices, Palo Alto, CA). Strains that exhibited a significant difference (lower or higher) in cell yield relative to the wild-type after 24 or 48 h were re-screened using As_2O_3 concentrations of 0.01–4 mM, and the concentration responsible for half-maximal inhibition of growth (IC_{50}) determined. Deletion mutants exhibiting significantly lower or higher IC_{50} compared to the wild-type were classified as sensitive or resistant, respectively. IC_{50} values are the mean of 2–5 individual determinations obtained via at least two independent experiments.