Available online at www.sciencedirect.com

ScienceDirect

Journal of Genetics and Genomics 42 (2015) 671−684

JGG

ORIGINAL RESEARCH

Deletion of Mitochondrial Porin Alleviates Stress Sensitivity in the Yeast Model of Shwachman-Diamond Syndrome

Waruenada Kanprasoet [a,1], Laran T. Jensen [b,1], Suwimon Sriprach [a], Kanate Thitiananpakorn [c], Khanti Rattanapornsompong [b], Amornrat Naranuntarat Jensen [a,*]

[a] *Department of Pathobiology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand*
[b] *Department of Biochemistry, Faculty of Science, Mahidol University, Bangkok 10400, Thailand*
[c] *Toxicology Graduate Program, Faculty of Science, Mahidol University, Bangkok 10400, Thailand*

Received 10 June 2015; revised 13 September 2015; accepted 14 September 2015
Available online 25 September 2015

ABSTRACT

Shwachman-Diamond syndrome (SDS) is a multi-system disorder characterized by bone marrow failure, pancreatic insufficiency, skeletal abnormalities, and increased risk of leukemic transformation. Most patients with SDS contain mutations in the Shwachman-Bodian-Diamond syndrome gene (*SBDS*), encoding a highly conserved protein that has been implicated in ribosome biogenesis. Emerging evidence also suggests a distinct role of SBDS beyond protein translation. Using the yeast model of SDS, we examined the underlying mechanisms that cause cells lacking Sdo1p, the yeast SBDS ortholog, to exhibit reduced tolerance to various stress conditions. Our analysis indicates that the environmental stress response (ESR), heat shock response (HSR), and endoplasmic reticulum unfolded protein response (UPR) of *sdo1*Δ cells are functional and that defects in these pathways do not produce the phenotypes observed in *sdo1*Δ yeast. Depletion of mitochondrial DNA (mtDNA) was observed in *sdo1*Δ cells, and this is a probable cause of the mitochondrial insufficiency in SDS. Prior disruption of *POR1*, encoding the mitochondrial voltage dependent anion channel (VDAC), abrogated the effects of *SDO1* deletion and substantially restored resistance to environmental stressors and protected against damage to mtDNA. Conversely, wild-type cells over-expressing *POR1* exhibited growth impairment and increased stress sensitivity similar to that seen in *sdo1*Δ cells. Overall, our results suggest that specific VDAC inhibitors may have therapeutic benefits for SDS patients.

KEYWORDS: Shwachman-Diamond syndrome; Oxidative stress; Yeast; Porin; Mitochondria

INTRODUCTION

Shwachman-Diamond syndrome (SDS) is an autosomal recessive disorder characterized by bone marrow failure, exocrine pancreatic dysfunction, skeletal abnormalities, and an increased risk of malignant transformation (Bodian et al., 1964; Shwachman et al., 1964; Mack et al., 1996; Smith et al., 1996; Ginzberg et al., 2000; Dror and Freedman, 2002; Makitie et al., 2004). Most SDS patients are deficient for myeloid-lineage cells and are susceptible to infections (Aggett et al., 1980; Ginzberg et al., 1999; Donadieu et al., 2005). Despite the fact that SDS is a rare genetic disorder (frequency of 1:77,000), it is one of the most common causes of inherited exocrine pancreatic dysfunction and bone marrow failure (Ginzberg et al., 2000; Goobie et al., 2001; Federman and Sakamoto, 2005). Mutations in *SBDS* (Shwachman-Bodian-Diamond syndrome), encoding the evolutionary conserved protein SBDS, account for approximately 90% of all SDS patients (Boocock et al., 2003). Studies in mammalian and yeast systems have demonstrated that SBDS and the yeast ortholog Sdo1p are required for biogenesis of the

* Corresponding author. Tel: +66 2 201 5579.
E-mail address: amornrat.nar@mahidol.ac.th (A.N. Jensen).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.jgg.2015.09.004

60S ribosomal subunit (Menne et al., 2007; Moore et al., 2010; Finch et al., 2011; Wong et al., 2011; Sezgin et al., 2013).

In addition to their role in ribosome biogenesis, SBDS and Sdo1p have been implicated in other cellular processes. These include the response to endoplasmic reticulum (ER) stress and DNA damaging agents (Ball et al., 2009) as well as limiting the generation of reactive oxygen species (ROS) (Ambekar et al., 2010). Recent evidence has also revealed that yeast cells lacking Sdo1p are unable to grow on the respiratory media, suggesting a defect in mitochondrial function (Henson et al., 2013). Similarly, mitochondrial membrane potential and oxygen consumption were decreased significantly in mammalian cells with reduced *SBDS* expression (Henson et al., 2013). It is possible that the involvement of SBDS/Sdo1p in functions such as stress response and mitochondrial function may be due to indirect effects from impaired translation; however, these functions appear independent from their role in ribosome maturation (Ball et al., 2009). Even with the advances in the understanding of the SBDS/Sdo1p proteins, their precise biological role and the relation of SBDS mutations with the disease outcome have not been clearly elucidated.

In this study, we utilized a yeast *Saccharomyces cerevisiae* model of SDS to investigate the biological impacts due to impaired Sdo1p function as well as to explore the underlying mechanism of the cellular defects in Sdo1p depleted cells. We report herein that cells lacking Sdo1p display a significant increase in sensitivity to various types of stress and display elevated protein oxidation following exposure to hydrogen peroxide (H_2O_2). In addition, loss of Sdo1p appears to promote mitochondrial DNA (mtDNA) damage, as mtDNA is absent in *sdo1*Δ cells. Defects associated with *sdo1*Δ cells were prevented by prior disruption of *POR1*, encoding a mitochondrial voltage dependent anion channel (VDAC). In addition, over-expression of *POR1* in wild-type yeast produced many of the features of *sdo1*Δ cells. These findings suggest that in addition to ribosome maturation, Sdo1p has an important role in limiting mitochondrial stress and protecting mtDNA from damage, perhaps through controlling the permeability of the outer mitochondrial membrane (OMM).

RESULTS

Yeast cells lacking Sdo1p display increased sensitivity to various types of stress

Previous studies have shown that *sdo1*Δ cells exhibit a slow growth phenotype and fail to grow under conditions of heat stress (Menne et al., 2007; Vitiello et al., 2010). In addition, *SBDS* depleted HEK293 cells are hypersensitive to agents that induce ER stress and DNA damage (Ball et al., 2009). This suggests that Sdo1p may participate in the cellular response to stress insults. In order to better understand the role of Sdo1p in cellular stress responses, growth under various types of stressors was monitored in *SDO1* deleted cells. As shown in Fig. 1, the growth of wild-type cells was not significantly altered under stress conditions including 37°C (high temperature stress), 4 mmol/L H_2O_2 (oxidative stress), 20 mmol/L β-mercaptoethanol (β-ME, ER stress), 550 mmol/L NaCl (saline stress), and 10% ethanol (ethanol stress). Loss of *SDO1* resulted in a significant decrease in tolerance to heat stress, H_2O_2 and ethanol. In contrast, *sdo1*Δ cells exhibited only a slight reduction in growth when challenged with β-ME and NaCl. It appears that Sdo1p has a potential role in the cellular stress response, especially to heat, oxidants and ethanol.

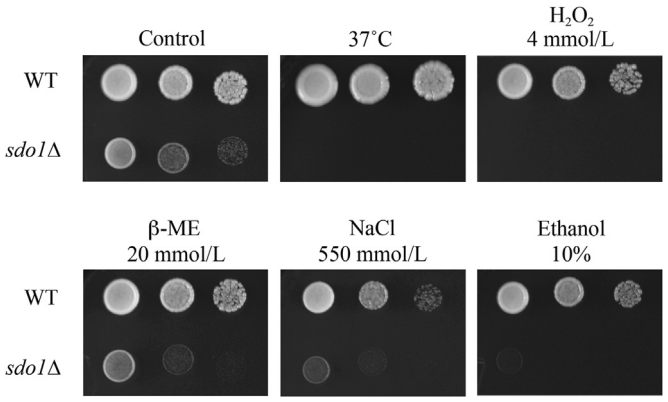

Fig. 1. Yeast *sdo1*Δ cells are sensitive to several environmental stressors. Sensitivity of WT (wild-type) and *sdo1*Δ cells was monitored by spotting 10^4, 10^3, and 10^2 cells onto solid YPD medium alone (control) or supplemented with the indicated concentrations of stressors and incubated at 25°C for 3 days. For heat stress, cells were incubated at 37°C. The *sdo1*Δ strain displayed enhanced sensitivity to heat stress, H_2O_2, and ethanol. Growth of *sdo1*Δ cells was only slightly reduced when challenged with β-ME and NaCl.

Stress sensitivity of *sdo1*Δ cells is not observed in other mutants with reduced polysome formation

The involvement of Sdo1p in ribosome biogenesis and translation (Menne et al., 2007; Moore et al., 2010) suggested that impaired activity of these processes may promote sensitivity to several stresses. To test the role of translational efficiency on stress sensitivity, we examined several mutant strains (*dbp3*Δ, *dbp7*Δ, *dom34*Δ, and *yar1*Δ) that exhibit reduced number of polysomes (Weaver et al., 1997; Daugeron and Linder, 1998; Bhattacharya et al., 2010; Koch et al., 2012). Deletion of *DBP3*, *DOM34*, and *YAR1* involved in precursor rRNA processing, dissociating inactive ribosomes, and nuclear export of 40S ribosome subunit protein, respectively (Weaver et al., 1997; Koch et al., 2012; Guydosh and Green, 2014), displayed stress sensitivity similar to wild-type cells (Fig. 2). Deletion of *DBP7*, required for 60S ribosomal subunit assembly (Daugeron and Linder, 1998), resulted in a slight increase in sensitivity to H_2O_2 and ethanol; however, *dbp7*Δ cells were more resistant than the *sdo1*Δ strain to these stresses (Fig. 2). Reduced translational efficiency does not appear to necessarily promote stress sensitivity. However, ribosome biogenesis defects in the mutants examined may be less severe than those present in the *sdo1*Δ strain. Thus, it remains a possibility that altered translational efficiency may contribute to stress sensitivity in *sdo1*Δ cells, although other factors may also be involved in the sensitivity of *sdo1*Δ cells to environmental stressors.