Regulation of DNA repair machinery in *Paracoccidioides brasiliensis* during stress induction

Alison F.A. Chaves¹, Marina V. Navarro¹, Daniele G. Castilho¹, Juliana C. Calado¹, Palloma M. Conceição², Beatriz F. de Castro², Wagner L. Batista^{1,2}

¹ Department of Microbiology, Immunology and Parasitology, Universidade Federal de São Paulo, São Paulo, Brazil
² Department of Pharmaceutical Sciences, Universidade Federal de São Paulo, Diadema, São Paulo, Brazil
Correspondence author: felipealison@gmail.com

INTRODUCTION

P. brasiliensis fungus performs the pathogenic switch from mycelium to yeast at 37°C, the human body temperature and the pathogenic switch involves a series of transcription changes, mainly in virulence related genes and oxidative stress responsive genes. Reactive oxygen species are well known threatening to DNA integrity and the capacity of the fungus to cope with the oxidative stress imposed by the immune cells from the human host is essential to the infection and disease progression. Homologous recombination (HR) and non-homologous end joining (NHEJ) are the major players in the DNA repair processes. To assess the regulation of the DNA repair machinery from *P. brasiliensis* under different stress conditions, we performed quantitative PCR from the *P. brasiliensis* genes related to HR (*RAD51* and *RAD52*) and NHEJ (*KU70*) pathways under different H₂O₂ concentration (2.5, 5 or 10 mM), heat shock (42°C for 30 min or 60 min), and osmotic stress (50 or 300 mM NaCl). Additionally, we used a NHEJ inhibitor (SCR7 pyrazine) to treat the *P. brasiliensis* yeast cells. The cells were submitted to microdilution assay with the inhibitor to determine the non-cytotoxic drug concentration and them we performed the inhibition assay under the conditions above mentioned. *P. brasiliensis* fungus is able to cope with the oxidative stress as reported elsewhere. Here we report that this fungus has a DNA repair machinery responsive to diverse types of stress. When NHEJ inhibitor was added to the medium and cultures treated with increasing H₂O₂ concentrations, the fungus shown an unexpected response. After NHEJ inhibition the fungus was able to survive despite the 10 mM H₂O₂ concentration. This effect may guard some relation with the activation of HR-related genes to repair the fungus DNA, while NHEJ would be a secondary player at this event. When the fungus was treated with increasing H₂O₂ concentrations we observed a dose dependent up-regulation of the HR and NHEJ related genes. *KU70* (NHEJ-related gene) increased its expression when 10 mM H₂O₂ was added to cultures. At the same condition, *RAD51* and *RAD52* (HR-related genes) increased 5 and near to 30-fold, respectively. When yeast cells were exposed to 42°C during 60 min, we observed a more modest 2-fold increase in *KU70* expression, suggesting that the DNA repair genes are less sensitive to this type of stress. No differences were observed regarding the cell morphology (mycelium or yeast). Finally, *KU70* is up-regulated under osmotic stress (300 mM NaCl) what led us to conjecture a role to this NHEJ-related gene in the Hog1 pathway. *P. brasiliensis* is a high complex fungus with an intricate repertoire of responses to the stress imposed to immune cells from the host. It repertoire includes, but not limited to, a sophisticated DNA repair machinery able to cope with the oxidative stress. Our findings reveals that *KU70*, *RAD51*, and *RAD52* genes are up-regulated by the oxidative stress, suggesting that DNA is actually under threatening at these conditions. *P. brasiliensis* expressed all the genes important for the repair via homologous recombination and non-homologous end joining. In *P. brasiliensis*, the DNA repair machinery is regulated by both Hog1 and Ras pathways and, finally but not less important, genes involved with homologous recombination have the expression induced during oxidative stress.

RESULTS