Free Radical Biology and Medicine 101 (2016) 424–433

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Yap1 and Skn7 genetically interact with Rad51 in response to oxidative stress and DNA double-strand break in *Saccharomyces cerevisiae*

Dae Gwan Yi[a,1], Myung Ju Kim[b,c,1], Ji Eun Choi[b,c], Jihyun Lee[b,c], Joohee Jung[b,c], Won-Ki Huh[a], Woo-Hyun Chung[b,c,*]

[a] Department of Biological Sciences and Institute of Microbiology, Seoul National University, Seoul 08826, Republic of Korea
[b] College of Pharmacy, Duksung Women's University, Seoul 01369, Republic of Korea
[c] Innovative Drug Center, Duksung Women's University, Seoul 01369, Republic of Korea

ARTICLE INFO

Keywords:
Rad51
Double-strand break
Homologous recombination
Yap1
Skn7
Reactive oxygen species
Genome stability

ABSTRACT

Reactive oxygen species (ROS)-mediated DNA adducts as well as DNA strand breaks are highly mutagenic leading to genomic instability and tumorigenesis. DNA damage repair pathways and oxidative stress response signaling have been proposed to be highly associated, but the underlying interaction remains unknown. In this study, we employed mutant strains lacking Rad51, the homolog of *E. coli* RecA recombinase, and Yap1 or Skn7, two major transcription factors responsive to ROS, to examine genetic interactions between double-strand break (DSB) repair proteins and cellular redox regulators in budding yeast *Saccharomyces cerevisiae*. Abnormal expression of *YAP1* or *SKN7* aggravated the mutation rate of *rad51* mutants and their sensitivity to DSB- or ROS-generating reagents. Rad51 deficiency exacerbated genome instability in the presence of increased levels of ROS, and the accumulation of DSB lesions resulted in elevated intracellular ROS levels. Our findings suggest that evident crosstalk between DSB repair pathways and ROS signaling proteins contributes to cell survival and maintenance of genome integrity in response to genotoxic stress.

1. Introduction

The generation of DNA lesions leading to cell death is the fundamental strategy underlying cancer treatment by radiotherapy and certain chemotherapies [1]. Although a substantial proportion of DNA damage that makes cells lethal is attributed to single-strand breaks (SSBs) and double-strand breaks (DSBs), oxidative damages produced by endogenous reactive oxygen species (ROS) and oxidizing chemicals are also significant sources of detrimental base modifications and mutagenesis that might eventually lead to potential tumorigenesis [2–4].

DNA damage repair pathways and oxidative stress signaling have been proposed to be highly associated and exhibit mutual causality. The oxidative DNA adducts resulting from ROS typically includes modified bases, abasic DNA sites and occasional SSBs, all of which are considered non-lethal but highly mutagenic, and are efficiently processed by base excision repair (BER) and to a lesser extent nucleotide excision repair (NER) [4–6]. Conversely, it has also been reported that increased DNA damage per se causes increases in intracellular ROS [7,8]. BER- and NER-defective cells are genetically unstable and highly mutagenic with greatly increased ROS levels [7,9]. DNA damage signaling triggered by non-oxidative alkylating agent is mediated by Yap1, a transcription activator specifically involved in oxidative stress response and redox homeostasis in yeast, suggesting that ROS signaling is interconnected with the DNA damage response [10]. It has been posited, however, that complex and lethal lesions such as DSBs are not easily induced by DNA oxidation unless simultaneous attack of DNA by a very high concentration of hydroxyl radicals causes two neighboring SSBs elaborately in close proximity [4].

It has been reported that the ROS-generating xenobiotic phenytoin increases both the DNA oxidation and homologous recombination (HR) required for DSB repair in a Chinese hamster ovary (CHO) cell line, and that the rate of DNA recombination in yeast cells stimulated by the human leukemogen benzene is diminished by *N*-acetylcysteine, a free radical scavenger, supporting the idea that ROS-induced DNA damage could be recombinogenic [11,12].

Abbreviations: DSB, double-strand break; SSB, single-strand break; ROS, reactive oxygen species; HR, homologous recombination; NHEJ, non-homologous end joining; BER, base excision repair; NER, nucleotide excision repair; PQ, paraquat; PHL, phleomycin; HU, hydroxyurea; MMS, methyl methanesulfonate; GFP, green fluorescent protein; H_2DCFDA, 2',7'-dichlorodihydrofluorescein diacetate

* Corresponding author at: College of Pharmacy, Duksung Women's University, Seoul 01369, Republic of Korea.
E-mail address: whchung23@duksung.ac.kr (W.-H. Chung).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.freeradbiomed.2016.11.005
Received 1 April 2016; Received in revised form 17 October 2016; Accepted 4 November 2016
Available online 09 November 2016

Yeast has been used as a prominent model organism to assess the biological consequences of cytotoxicity mediated by ROS and unrepaired DNA damage in higher organisms. HR-mediated DSB repair pathways mediated through Rad52 epistasis group proteins in yeast are highly conserved in all forms of complex life studied to date [13]. Studies delineating the relationships between proteins in pathways of ROS signaling and of HR in yeast would provide useful clues for the identification of novel candidate therapeutic targets in human cancers and for understanding their mechanisms of action [14–16].

A global genetic analysis of synthetic fitness or lethality defect (SFL) interactions in yeast revealed that mutations in five genes required for oxidative stress response (*TSA1*, *SOD1*, *LYS7*, *SKN7*, and *YAP1*) impaired growth of HR pathway mutants; interestingly, all of these genes play a significant role in suppression of mutagenesis [17,18]. Among these, Tsa1, the most potent peroxiredoxin that scavenges H_2O_2, is also the most significant contributor to genome stability with a severe mutator phenotype seen in the *tsa1* mutant [17]. Defects in Rad51-mediated DSB repair by HR and Rad6-mediated postreplicative repair (PRR) cause synthetic lethality in the absence of Tsa1 [19]. Moreover, SOD1 (Superoxide dismutase 1) inhibition has been proposed as a promising approach for selective killing of cancer cells and synthetic lethal interaction between yeast *rad54* and *sod1* has been shown to be conserved within a human colorectal cancer (CRC) context based on the observation that DNA damage resulting from an increase in ROS following SOD1 inhibition persists within *RAD54B*-deficient cells and induces apoptosis [20,21]. Despite the results above, the underlying mechanism of action by which ROS signaling and DSB repair through HR are interconnected remains unclear. Instead of creating *rad51 sod1* and *rad51 tsa1* double mutants that are lethal in aerobic growth conditions, we introduce *RAD51* deletions into mutant strains that lack Yap1 or Skn7, which are two major transcription factors that modulate large oxidative stress response regulons including the *TSA1*, *TRX2*, and *TRR1* genes in yeast [22]. Cells inactive in either Yap1 or Skn7 are hypersensitive to H_2O_2 and superoxide generators such as paraquat [23,24]. Induction of many antioxidant genes by oxidative stress is nearly equally affected by deletion of *YAP1* or *SKN7*, although a *yap1 skn7* double mutant has almost the same phenotype as does either single mutant, with no additional effect of mutation of the second gene [25,26].

Here, we report that Rad51, a crucial player of recombinational repair, acts in concert with Yap1 and Skn7 in cellular responses to genome instability caused by both DNA damage and oxidative stress. Aberrant expression of *YAP1* or *SKN7* aggravates mutation rates and sensitivity of *rad51* mutants to DSB- and ROS-generating reagents. Rad51 deficiency contributes to elevated accumulation of DSB lesions in response to increased ROS levels, and the ensuing genomic instability increases the intracellular ROS levels, leading to relocalization of Yap1 to the nucleus. Collectively, these results suggest a specific crosstalk between DSB repair pathways and ROS signal transducing proteins, contributing to cell survival and maintenance of genome stability in the presence of genotoxic stress.

2. Materials and methods

2.1. Yeast strains, plasmids and growth media

All of the strains used in this study are isogenic derivatives of *S. cerevisiae* BY4741 (*MAT*a *his3*Δ1 *leu2*Δ0 *lys2*Δ0 *ura3*Δ0) obtained from the Yeast Knockout (YKO) collection (YSC1053 glycerol stock, Thermo Scientific) unless noted otherwise. The strains with C-terminally GFP-fused proteins were constructed by oligonucleotide-directed in-frame tagging method as previously described [27]. The genotypes of all strains are listed in Supplementary Table 1. Yeast cell cultures and treatments with DNA damaging agents, ROS-generating reagents, and antioxidants were all performed in standard rich YEPD media or minimal SD media supplemented with the required amino acids. All cultures were incubated at 30 °C. Overexpression plasmids of the wild-type *YAP1* and *SKN7* genes were created using pRS426, which includes an *ADH1* promoter [28]. The ORF regions of C-terminally TAP-tagged *YAP1* and *SKN7* were PCR-amplified and cloned into the *Spe*I/*Xho*I and *Hin*dIII/*Xho*I sites of pRS426ADH, respectively, and transformed into WT yeast and each mutant strain. Overexpression of Yap1 and Skn7 was confirmed by Western blot analysis.

2.2. Drug sensitivity analysis

The drug sensitivity of yeast cells was measured via spotting assays. For these assays, cells were grown overnight at 30 °C, and the culture was re-inoculated into fresh media and grown in 5 ml YEPD or SD liquid media to reach approximately $3–4\times10^7$ cells/ml, then diluted 10-fold serially and spotted in rows onto YEPD or SD plates containing the selected chemicals. The plates were incubated for 2–3 days at 30 °C and then photographed. For experiments involving exposure to the ROS/DSB-inducing agents, paraquat (PQ), hydrogen peroxide (H_2O_2), phleomycin (PHL), hydroxyurea (HU), or methyl methanesulfonate (MMS) were included in the plates before spotting.

2.3. Measurement of cell viability and mutation frequency

Yeast cells were inoculated into 5 ml of YEPD media and grown overnight at 30 °C. On the following day, cells were diluted into 5 ml of fresh media to an optical density at 600 nm (OD_{600}) of ~0.2 and incubated with shaking for 6 h. PHL or H_2O_2 was added at the indicated final concentrations, followed by incubation with shaking for 2 h. The number of cells was estimated for each population using a hemocytometer, and the viability and mutation rate of cells were determined. To determine viability under several conditions, a colony forming unit (CFU) assay was used. One hundred cells from different cultures were plated onto three YEPD plates. The plates were incubated at 30 °C for 2–3 days and then colonies were counted. The rate of spontaneous mutations as a result of genome instability caused by drug treatment was determined by a forward-mutation assay that detects mutations in the *CAN1* gene [29]. The yeast *CAN1* gene encodes an arginine permease that is normally required for arginine uptake but is also able to transport canavanine, a toxic arginine analog. Cells with loss-of-function mutations in the *CAN1* gene locus can form colonies on canavanine-containing SD plates. Yeast cells were treated with PHL or H_2O_2 for 4 h at the indicated concentrations and then were grown on plates with or without 60 μg/ml canavanine. Spontaneous mutation rates were determined by counting CFUs after incubation for 3–4 days at 30 °C. All rates represent the average of three independent experiments and error bars indicate the standard deviation.

2.4. Fluorescence microscopy

Fluorescence microscopy was carried out on a Nikon Eclipse Ti inverted microscope. Image analysis was performed using NIS-Elements AR3.1 microscopy software (Nikon) in order to determine the percentage of cells with subnuclear foci or predominately nuclear fluorescence. To detect nuclear foci of Rad52-GFP or Yap1-GFP, yeast cells were treated with PHL or H_2O_2, and then further incubated with shaking for the indicated time periods in YEPD before being photographed. At least 100 cells were counted at least three times for each measurement.

2.5. Measurement of intracellular ROS level

Yeast cells were grown to mid-log phase and diluted to an OD_{600} of ~0.2. After treatments with MMS, PHL, or H_2O_2 for 2 h, H_2DCFDA (2′,7′-dichlorodihydrofluorescein diacetate) (excitation/emission: 492–495/517–527 nm, ThermoFisher Scientific) a cell-permeant indicator of ROS detection, was added at a final concentration of 5 μg/ml,