Effects of *N*-acetyl-L-cysteine in two Yeast Strains

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Introduction

Saccharomyces cerevisiae, a simple eukaryotic cell, has been widely used as a model for all eukaryotes including humans for the study of fundamental cellular processes such as DNA replication, DNA recombination, cell cycle, cell division and metabolism. Numerous laboratory strains are used in yeast research. Most of the mutants have been derived from the two widely used laboratory strains W303-1A and BY4741. While BY4741 is a derivative of S288C, used in the systematic sequencing of the *S. cerevisiae* genome, strains with a W303 background serve in many physiological and biochemical studies [1]. It was found in a recent study that W303-1A contains a mutant allele of *YBP1*, *ybp1-1*, encoding four amino acid substitutions, that results in increased peroxide sensitivity [2]. Mutation of *ybp1-1* is not a complete loss of function allele as it is more resistant to peroxides than the knock-out mutant. Ybp1 is required for oxidation of specific cysteine residues of the transcription factor Yap1p resulting in the nuclear localization of Yap1p in response to stress. Ionizing radiation (IR) can produce highly reactive hydroxyl radicals through the decomposition of cellular water, such as superoxide anion radical, hydrogen peroxide, and hydroxyl radical [3]. These reactive oxygen species (ROS) can cause wide-ranging cellular damage, including DNA double-strand breaks (DSBs), lipid peroxidation, and protein modification [4]. Also, ROS produced by IR cause oxidative stress. Detoxification enzymes are activated for ROS scavenging against oxidative stress [5]. Antioxidants are used for detoxification of ROS and reduction of oxidative damage. NAC, one of the antioxidants, is a precursor for glutathione (GSH) [6]. The aim of the present study was to compare the differences in radiosensitivity-associated cell viability between the two strains. Effect of NAC against IR on cell protection was investigated, as well.

Materials and Methods

Saccharomyces cerevisiae W303-1A and BY4741 strains were used in this study (Table 1). Cell viability was measured by means of CFU (colony forming unit). Yeast was cultured in the YPDA liquid medium at 30°C for 48 hours. Yeast cells were diluted in the YPDA media to an $A_{600} = 10^{-4}$. NAC of 0~20 mM was treated for 2 hours in prior. Yeast cells were exposed to gamma-radiation from a ^{60}Co gamma irradiator (7.4 PBq of capacity; AECL, Canada at the Korea Atomic Energy Research Institute) at room temperature. Yeast cells received radiation doses of 10, 30, 50, 100, 200, 300, and 400 Gy. After irradiation, 100 uL of yeast cells were spread on the YPDA solid plates. Plates were incubated at 30°C for 48 hr.

Table 1. Yeast strains used in this study

Strain	Genotype	Reference
W303-1A	MATa, *leu2-3/112 trp1-1 can1-100 ura3-1 ade 2-1 his3-11/15, ybp1-1*	Wallis et al. 1989
BY4741	MATa, his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	EUROSCARF

Results

• Effect of NAC on the Cell Viability after Irradiation

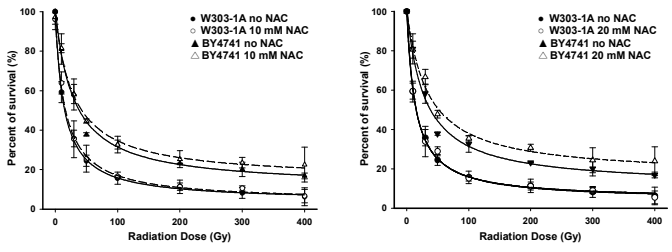

Fig. 1. Comparison of the relative cell viability of W303-1A and BY4741 cells treated with 10 mM (A) and 20 mM (B) of NAC and gamma rays.

Results

• Gene expression of antioxidant enzymes

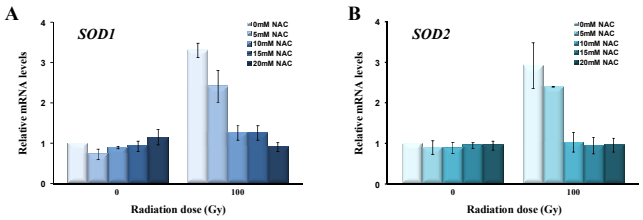

Fig. 3. Superoxide dismutase gene expression in response to ionizing radiation and NAC. *SOD1* (A) and *SOD2* (B) expression in yeast treated with NAC of 5 to 20mM and 100Gy gamma rays.

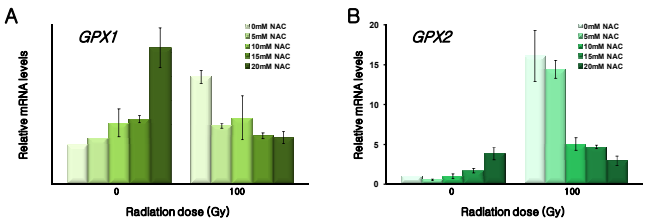

Fig. 4. Glutathione peroxidase gene expression in response to ionizing radiation and NAC. *GPX1* (A) and *GPX2* (B) expression in yeast treated with and NAC of 5 to 20mM and 100Gy gamma rays.

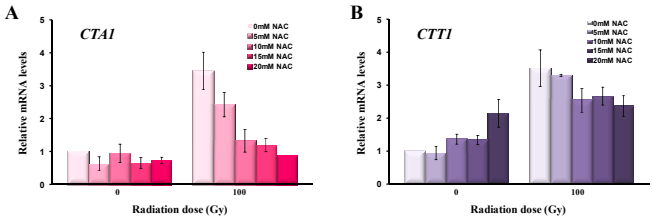

Fig. 5. Catalase gene expression in response to ionizing radiation and NAC. *CTA1* (A) and *CTT1* (B) expression in yeast treated with NAC of 5 to 20mM and 100Gy gamma rays.

Conclusions

1. The gene expression of antioxidant enzymes was induced by ionizing radiation, and by NAC, as well.
2. The high concentration of NAC reduced the cell division rate over 3 days of period in W303-1A.
3. NAC could not protect cells against IR-induced death in radiosensitive strain W303-1A, while it resulted in a slight amelioration of cell death in BY4741.
4. The gene expression of antioxidant enzymes in the yeast cells irradiated with 100 Gy decreased with NAC concentrations.
5. NAC is amenable to further study regarding cell rescues and ROS scavenging in vivo.

References

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