Expression of Genes Related to Oxidative Stress in Yeast Treated with Ionizing Radiation and *N*-acetyl –L-cysteine

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1. Introduction

Ionizing radiation (IR) induces water radiolysis, which generates highly reactive hydroxyl radicals [1]. Reactive oxygen species (ROS) cause apoptosis and cell damage including DNA strand breaks (DSBs), base damage, protein damage and lipid-hydroperoxide. Detoxifying enzymes are immediately triggered for ROS scavenging [2]. Yeast contains two forms of superoxide dismutase (SOD). SOD1 as a cytosolic copper-zinc superoxide dismutase is located in the cytoplasm and cytosol. SOD2 as a manganese-containing enzyme is act in mitochondria matrix and mitochondrion. These enzymes scavenge superoxide radicals by catalyzing the conversion of two of these radicals into hydrogen peroxide and molecular oxygen. The hydrogen peroxide formed by superoxide dismutase and by other processes is scavenged by catalase, a ubiquitous heme protein that catalyzes the dismutation of hydrogen peroxide into water and molecular oxygen. Yeast contains two catalases. Catalase A (CTA1) and Cytosolic catalase T (CTT1) is located in peroxisome and cytoplasm, respectively. Yeast has two glutathione (GSH) peroxidases, which are GPX1 and GPX2. GPX1 and GPX2 are component of cellular component and cytoplasm, respectively. The biochemical function of GSH peroxidase is to reduce lipid-hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water. Otherwise, chemicals and materials help ROS detoxification against oxidative damage. *N*-acetyl-L-cysteine (NAC) having a thiol, a precursor for glutathione (GSH), is known as one of the antioxidants [3]. In this study, we examined the effect of NAC through gene expressions related to protective enzyme against oxidative stress in yeast.

2. Methods and Results

Saccharomyces cerevisiae W303-1A strain was used in this study. Yeast was cultured in the YPDA medium at 30℃ for 48 hours used. NAC of 5mM to 20mM concentration was treated for 2 hours in prior. Yeast was exposed to 100Gy gamma irradiation. Total RAN was extracted from the whole yeast using TRIzol. The amount of RNA was determined by spectrophotometry at 260nm. After conversion of total RNA into CDNA, real-time PCR was performed.

2.1 Transcriptional expression of SOD1 *and* SOD2

It has been reported that IR cause oxidative stress. Detoxification enzymes are immediately triggered for ROS scavenging against oxidative stress. To determine the transcriptional expression level of SOD in yeast treated with 100Gy gamma rays and NAC of 5 to 20mM, real-time PCR was performed. In the NAC-treated group, without irradiation, transcriptional expression of SOD1 and SOD2 was not induced compared with the control group, while in radiation-treated group without NAC pretreatment, expression of SOD1 and SOD2 was highly induced compared with the non-irradiated control (Fig. 1A and B). Also, real-time PCR result shows that the transcriptional expression of SOD1 and SOD2 dramatically decreased as the concentration of NAC increased. In results, NAC acted as an antioxidant to remove ROS whild radiation induced transcriptional expression of SOD in yeast.

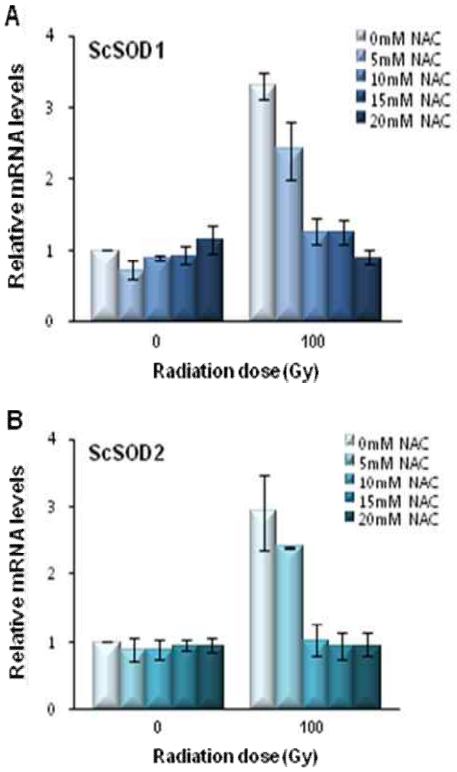

Fig. 1. Superoxide dismutase gene expression in response to ionizing radiation and NAC. SOD1 (A) and SOD2 (B) expression in yeast treated with 100Gy gamma rays and NAC of 5 to 20mM.

2.2Transcriptional expression of CTA1 *and* CTT1

The hydrogen peroxide formed by superoxide dismutase is scavenged by catalase. Catalase catalyzes the dismutation of hydrogen peroxide into water and molecular oxygen. To identify the transcriptional expression level of catalase genes, real-time PCR was performed after treatment with 100Gy gamma rays and NAC of 5 to 20mM. In the NAC treated group without irradiated ionizing, expression of CTA1 was not induced whereas expression of CTT1 increased with NAC concentration (Fig. 2A and B). In the irradiated group, transcriptional expression of both of CTA1 and CTT1 decreased with NAC concentrations. These results indicate that the NAC reduced ROS levels. Accordingly, hydrogen peroxides were formed at lower levels. The reduced transcriptional expression level of catalase genes may be responsible for the reduced ROS and hydrogen peroxide.

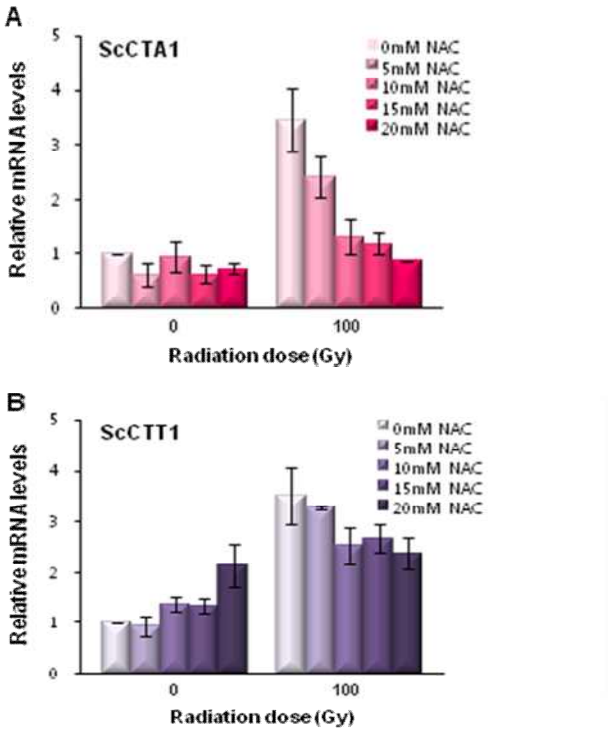

Fig. 2. Catalase gene expression in response to ionizing radiation and NAC. CTA1 (A) and CTT1 (B) expression in yeast treated with 100Gy gamma rays and NAC of 5 to 20mM.

2.3 Transcriptional expression of GPX1 *and* GPX2

The hydrogen peroxide is scavenged by GSH peroxidase added glutathione. To investigate the transcriptional expression level of GSH peroxidase genes, real-time PCR was performed using the total RNA extracted from yeast treated with 100Gy gamma rays and NAC of 5 to 20mM. The transcriptional expression of GPX1 and GPX2 in 100Gy gamma ray irradiated condition was higher than expression of non-irradiated condition (Fig. 3A and B). Under the condition of non-irradiation, the expression of GPX1 and GPX2 was gradually induced as the concentrations of NAC increased. After irradiation, transcriptional expression of GPX1 and GPX2 decreased with increasing NAC concentrations. These results indicate that NAC induced expression of GSH peroxidase genes in non-irradiated condition. NAC become the cause of decreased expression of GPXs in the irradiated group.

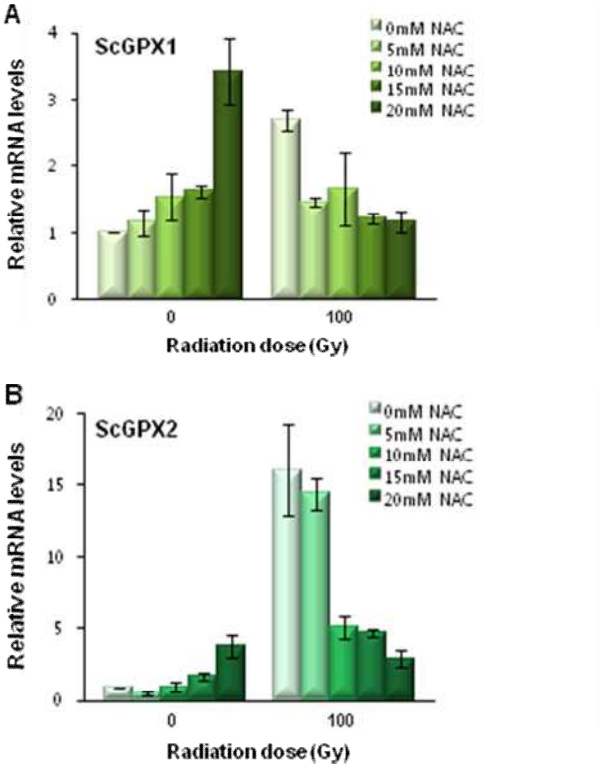

Fig. 3. Glutathione peroxidase gene expression in response to ionizing radiation and NAC. GPX1 (A) and GPX2 (B) expression in yeast treated with 100Gy gamma rays and NAC of 5 to 20mM.

3. Conclusions

The present study shows that the possible role of NAC against oxidative stress is an ROS scavenger. Gene expression of most protective enzymes was induced after irradiation of ionizing radiation. In conclusion, NAC can prevent radiation-induced oxidative stress and is a useful antioxidant against ROS.

REFERENCES

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