Effects of Ionizing Radiation and Glutathione Precursor on Antioxidant Enzyme and Cell Survival in Yeast

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1. Introduction

A wide range of cellular lesions can be induced by ionizing radiation. Lesions include growth inhibition, DNA damage, and even cell death by direct energy deposit or by production of reactive oxygen species [1]. Cells react to such an induced oxidative stress through scavenging the generated reactive oxygen species to reduce oxidative damage. Antioxidant enzymes such as glutathione peroxidase, catalase, and superoxide dismutase are immediately triggered for reactive oxygen species. *N*-acetyl-L-cysteine (NAC), a precursor of glutathione, is one of the antioxidants [2, 3]. The effect of NAC as an antioxidant and/or a cell rescue agent was investigated in the present study. Glutathione (GSH) is the most abundant intracellular thiol, which involves in antioxidant defense via direct interaction with ROS or via activities of detoxication enzymes like glutathione peroxidases (GPx). NAC flowed in the cell is converted to cysteine by deacetylation, that is supplied to the depleted GSH by oxidative stress. NAC prevents the depletion of GSH by radiation, increases the production of GSH, and improves enzymes activity such as GPx and alkaline phosphatase. Cell growth and survivorship and transcriptional level of glutathione gene are analyzed in two yeast strains exposed to combined treatment of NAC with gamma-rays.

2. Materials and Methods

Saccharomyces cerevisiae strain W303-1A and BY4741 were used in this study [4]. Growth kinetics was measured by spectrophotometer analysis.

Pre-cultures were inoculated into a fresh YPDA liquid medium in the presence of various concentrations (1~30 mM) of NAC and cultured at 30 ℃ with shaking at 180 rpm. Yeast cells were cultured in the YPDA medium at 30 ℃ for 48 hours, and treated with 0 to 20mM of NAC for 2 hours.

The cells remained in the YPDA liquid media during exposure to gamma-radiation (0~400 Gy) from a ^{60}Co source (7.4 PBq of capacity; KAERI) at room temperature.

Total RNA was extracted from the yeast cells using TRIZOL reagents. The amount of RNA was determined by spectrophotometry at 260 nm, and its integrity was assessed by analyzing the ribosomal RNA bands after gel electrophoresis.

The yeast cells were harvested by centrifugation and homogenized using acid-washed bead with ice-cold MES buffer for determination of glutathione (GSH) levels. GSH levels (µM/ mg protein) were assayed with Cayman Kits according to the manufacturer's instructions and determined by the colorimetric method.

3. Results and Discussions

Grow of the cell treated with NAC

The growth of the cells treated with NAC showed similar was similar patterns until they reach the log phase at 24 h, but it reduced with an increased concentration of NAC (Fig. 1). The relative cell number of yeast was 95.1 % and 89.6 % on presence of 10 mM and 20 mM NAC, respectively. The relative cell number of yeast treated with 30 mM NAC was reduced 11 % than that of the non-treated control. Therefore, the concentration of 30 mM or less affect to growth of yeast cells at stationary phase than exponential phase.

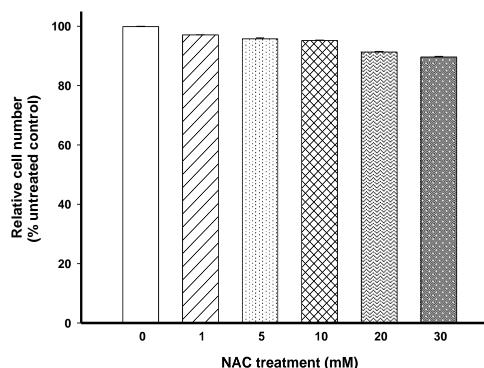

Fig. 1. Relative cell abundance after 72 hours culture in the presence of NAC.

Strains BY4741 and W303-1A were exposed to total doses of 10, 30, 50, 100, 200, 300, and 400 Gy (Fig. 2). The strain W303-1A proved more sensitive to ionizing radiation than the BY4741. The cell survival of the strain W303-1A was 59.2% and 35.8% after 10 and 30 Gy gamma-rays irradiation, respectively. The half lethal dose for the strain W303-1A was about 20 Gy.

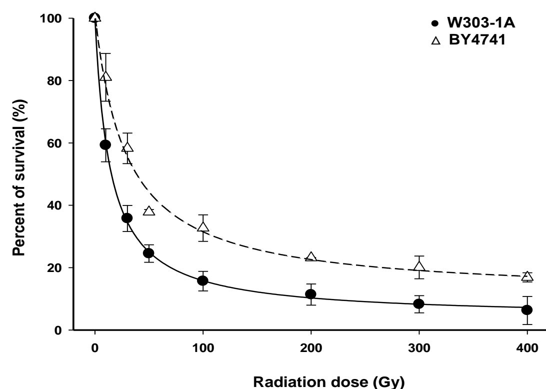

Fig. 2. Cell viability of the two strains after irradiation.

Transcriptional expression of GSH enzyme genes

Ionizing radiation produces hydrogen peroxide via radiolysis of water molecules which is scavenged by GSH peroxidase added glutathione [5]. Yeast has three glutathione peroxidase (GPx) proteins. Among them, GPX1 is a cellular component. The transcriptional expression of *GPX1* gene after irradiation with 100 Gy was higher than the expression of the non-irradiated cells. Under the condition of non-irradiation, the transcriptional expression of *GPX1* was gradually induced as the concentrations of NAC increased.

These results indicate the GSH supply through deacetylation of NAC to cysteine [6,7]. The transcriptional expression of *GPX1* gene was induced by NAC as proved by the experiment. After irradiation, transcriptional expression of the gene decreased with NAC concentrations. These results indicated that NAC induced expression of GSH peroxidase genes in non-irradiated condition. NAC became the cause of the decreased expression of *GPXs* in the irradiated group.

4. Conclusions

The effect of NAC on cell growth was measured during 72 hours. The cell growth was hampered by higher concentrations of NAC at stationary phase. NAC, however, didn't affect the cell division at the exponential phase. The survival of the cells decreased with radiation dose. The cell viability of the strain W303-1A was reduced significantly at the low dose (10 and 30 Gy). By comparison, the strain W303-1A was more sensitive to radiation with having a half lethal dose (LD_{50}) of about 20 Gy. The quantitative RT-PCR analysis showed that the transcriptional expression of antioxidant enzyme gene G*PX1* increased after irradiation while the expression of the gene decreased by the combined treatment of NAC with 100 Gy radiation. The present study shows that NAC can directly scavenge ROS against oxidative stress *in vivo*. In conclusion, NAC can prevent radiation-induced oxidative stress by increasing antioxidant gene expression. Thus it is a useful antioxidant against ROS.

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