Letter to the Editor

Poly(ADP-ribosyl)ation of Apoptosis Antagonizing Transcription Factor Involved in Hydroquinone-Induced DNA Damage Response*

LING Xiao Xuan[1,2], LIU Jia Xian[1], YUN Lin[1], DU Yu Jun[3], CHEN Shao Qian[4], CHEN Jia Long[5], TANG Huan Wen[1,#], and LIU Lin Hua[1,#]

The molecular mechanism of DNA damage induced by hydroquinone (HQ) remains unclear. Poly(ADP-ribose) polymerase-1 (PARP-1) usually works as a DNA damage sensor, and hence, it is possible that PARP-1 is involved in the DNA damage response induced by HQ. In TK6 cells treated with HQ, PARP activity as well as the expression of apoptosis antagonizing transcription factor (AATF), PARP-1, and phosphorylated H2AX (γ-H2AX) were maximum at 0.5 h, 6 h, 3 h, and 3 h, respectively. To explore the detailed mechanisms underlying the prompt DNA repair reaction, the above indicators were investigated in PARP-1-silenced cells. PARP activity and expression of AATF and PARP-1 decreased to 36%, 32%, and 33%, respectively, in the cells; however, γ-H2AX expression increased to 265%. Co-immunoprecipitation (co-IP) assays were employed to determine whether PARP-1 and AATF formed protein complexes. The interaction between these proteins together with the results from IP assays and confocal microscopy indicated that poly(ADP-ribosyl)ation (PARylation) regulated AATF expression. In conclusion, PARP-1 was involved in the DNA damage repair induced by HQ via increasing the accumulation of AATF through PARylation.

Hydroquinone (HQ) is a ubiquitous chemical in the environment and is widely used in various industries. It is an important component in the manufacture of antioxidants or stabilizers for some materials, antiozonants, agrochemicals, skin-whitening agents, and in the polymer industry[1-2]. However, HQ is a major metabolite derived from benzene and can induce cytotoxicity and genotoxicity[3], including DNA damage; hence, it is commonly used as a substitute of benzene in *in vitro* studies. In the event of DNA damage, DNA repair, called the DNA damage response (DDR), is immediately initiated to maintain genomic stability. HQ-containing genotoxic agents induce DDR, a sophisticated process in which post-translational modifications are of great importance. Poly(ADP-ribose) polymerase-1 (PARP-1) is one of the most well-characterized molecular sensors in DDR[4]; moreover, as the founding member of the PARP family, it is a ubiquitous nuclear enzyme in DDR and involved in regulating processes such as cell differentiation, transcription, and post-translational modification. Post-translational modifications include phosphorylation, acetylation, methylation, ubiquitination, SUMOylation (modification by small ubiquitin-like modifiers), and poly(ADP-ribosyl)ation (PARylation). PARylation refers to the polymerization of linear or branched chains of ADP-ribose, from donor nicotinamide adenine dinucleotide (NAD^+) molecules, on target proteins. PARylation can be mediated by the PARP family and catalyzed by PARP-1. PARylation catalyzed by PARP-1 is the major source of poly(ADP-ribose) (PAR) production[5], and, PAR production is an indicator of PARP activity[6].

doi: 10.3967/bes2016.008

*This work was supported by grants from the National Natural Science Foundation of China (81202231, 81273116, 81430079) and the Science and Technology Program of Guangdong Bureau of Science and Technology, China (2013B021800069).

1. Department of Environmental and Occupational Health, Dongguan Key Laboratory of Environmental Medicine, School of Public Health, Guangdong Medical University, Dongguan 523808, Guangdong, China; 2. School of Public Health, Guangzhou Medical University, Guangzhou 510182, Guangdong, China; 3. Electrocardiogram Department of Cardiovascular Center, Affiliated Hospital of Guangdong Medical University, Zhanjiang 524023, Guangdong, China; 4. Department of Clinical Laboratory, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou 510080, Guangdong, China; 5. Department of Occupational Health and Occupational Medicine, Guangdong Provincial Key Laboratory of Tropical Disease Research, School of Public Health and Tropical Medicine, Southern Medical University, Guangzhou 510515, Guangdong, China

PARP-1 involves in DDR through PARylation, changing local chromatin structure, and modulating several DDR proteins by post-translational modification[7].

Apoptosis antagonizing transcription factor (AATF), a human RNA polymerase binding protein, has been implicated to play a major role in cell cycle control, apoptosis, and regulation of transcription. AATF has also been found to be involved in DDR, in which it is promptly activated by DNA damage and accumulates to mediate efficient repair of DNA damage through post-translational modifications, including phosphorylation and PARylation[8]. In addition, phosphorylated histone H2AX (γ-H2AX) is also a well-known indicator of DNA damage.

On the basis of the above theories, we hypothesized that there are interactions between PARP-1 and AATF in response to DNA damage induced by HQ. PARP-1-silenced TK6 cells were used to investigate the possible underlying molecular mechanism. The influence of HQ on DDR-related proteins and the interaction between PARP-1 and AATF proteins were investigated.

The TK6 lymphoblastoid cell line was kindly provided by Prof. ZHANG LiShi (Sichuan University). Cell properties, culture conditions, and chemical treatment methods have been described in our previous reports[9]. Cells plated in 6-cm dishes were treated with 10.0 μmol/L HQ for 0, 0.5, 1, 2, 3, 4, 5, and 6 h. PARP-1-silenced TK6 cells with a stable expression of PARP-1-shRNA (shPARP-1) were provided by Prof. TANG[10]. Empty vector TK6 cells (shNC) were used as controls.

Data from three individual experiments were described as mean±SD values. One-way ANOVA was used for comparing mean values in multiple groups. *SNK-t* test was used for multiple comparisons. Statistical analysis was performed using SPSS software (version 15.0) and statistical significance was considered at $P<0.05$.

DNA is under assault frequently, such as the exposure to toxicants existing in natural and occupational environment and food. It is widely accepted that HQ induces several cascade changes, including oxidative cell stress, apoptosis, and DNA damage, while many studies have shown that DNA damage induced by HQ is intensified in PARP-1-deficient cells[11]. However, the detailed mechanisms of how PARP-1 is involved in DDR induced by HQ remain unclear, which we attempted to determine in our study. γ-H2AX is a surrogate indicator of genomic DNA damage[12], and the expression of γ-H2AX in TK6 cells (treated with 10.0 μmol/L HQ for 0.5, 1, 2, 3, 4, 5, and 6 h) was detected by western blotting. After HQ treatment, the expression of γ-H2AX increased immediately within 0.5 h and peaked at 3 h, and then decreased gradually (Figure 1A and 1B), which showed that HQ could induce DNA damage within a very short time. However, DDR was also initiated immediately such that after 3 h, the speed at which DDR happened was faster than that at which DNA damage occurred.

It is expected that high DDR efficiency is dependent on DDR-related proteins. Thus, the expression of DDR molecules (PARP-1, PAR, and AATF) was also detected by western blotting. After HQ treatment, the expression of AATF increased immediately within 0.5 h, peaked at 3 h, before becoming more stable (Figure 1A and 1C). However, the expression of PARP-1 decreased to the lowest level within 3 h and then gradually increased (Figure 1A and 1D). PAR production was increased to the maximum within 0.5 h, and then decreased gradually to the lowest level at 6 h (Figure 1A and 1E). HQ dramatically activated PARP-1 and its activity was enhanced. Moreover, as the major executor of PARylation[5], PARP-1 decreased owing to its automodification for the PARylation of target proteins. Accordingly, we demonstrated that PAR production reached the maximum within 0.5 h; however, PARP-1 expression decreased immediately within 0.5 h. These results further support that PARP activity is enhanced in DDR induced by HQ. AATF has been found to be involved in DDR; most of the AATF functions are regulated by post translational modifications, which affect the accumulation of AATF and make it efficiently respond to DNA damage. For example, AATF phosphorylation and accumulation contributed to the maintenance of the G2/M checkpoint and affected the expression of p53, thereby enhancing the DDR capacity. Besides phosphorylation, PARylation also enhanced the stabilization of AATF protein during DDR[8]. Interestingly, we found that the accumulation of AATF was positively correlated with PARP activity, indicating the possible role of PARylation in efficient DDR.

To understand the potential links among AATF, PARP-1, and PARylation in DDR induced by HQ, PARP-1-silenced TK6 cells were utilized for further investigation. Compared with the control cells, these cells showed 75% reduced expression of PARP-1 protein ($P<0.05$), indicating that PARP-1 was efficiently silenced (Figure 2A and 2B). In