

Original Article

URI prevents potassium dichromate-induced oxidative stress and cell death in gastric cancer cells

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Abstract: Chromium VI can provoke oxidative stress, DNA damage, cytotoxicity, mutagenesis and carcinogenesis. Aberrantly high level of reactive oxygen species (ROS) has been associated with oxidative stress and subsequent DNA damage. Notably, multiple previous studies have shown the increased level of ROS in chromium (VI) induced oxidative stress, but its effect on cell death and the underlying mechanism remain to be determined. In this study, we aimed to investigate the role of URI, an unconventional prefoldin RBP5 interactor, in potassium dichromate induced oxidative stress and cell death through in vitro loss-of-function studies. We have shown that knockdown of URI in human gastric cancer SGC-7901 cells by URI siRNA enhanced potassium dichromate-induced production of ROS. The level of rH2AX, a marker of DNA damage, was significantly increased, along with a reduced cell viability in URI siRNA treated cells that were also exposed to potassium dichromate. Comet assay showed that URI knockdown increased the tail moment in potassium dichromate-treated SGC-7901 cells. Accordingly, the cell rates of apoptosis and necrosis were also increased in URI knockdown cells treated with potassium dichromate at different concentrations. Together, these results suggest that URI is preventive for the oxidative stress and cell death induced by potassium dichromate, which potentially leads to cancer cell survival and therapeutic resistance.

Keywords: URI, gastric cancer cell, Chromium VI, ROS, oxidative stress, cell death

Introduction

Reactive oxygen species (ROS), including superoxide anion O_2^- , hydrogen peroxide H_2O_2 , single oxygen, and hydroxyl radicals (OH \cdot), are by-products of cellular metabolic pathways [1, 2]. ROS at normal level are important cell signaling molecules that are known to participate in a variety of basal and adaptive physiological responses [3]. Mitochondrion is the primary source of intracellular ROS. Structure and function integrity of mitochondria is a precondition of stabilization of ROS level. Dysfunction of mitochondria is a major cause of elevated ROS, which has been shown to contribute to occurrence and development of multiple diseases, including inflammation, neurodegenerative disorders and cancer [4].

Excessive ROS leading to cancer development or cancer cell death involves a variety of mechanisms. It was previously shown that elevated

ROS may induce cell apoptosis not only through the extrinsic but also the intrinsic pathway, which causes mitochondrial damage and alteration of apoptotic-related proteins [5]. ROS may cause DNA damage and allow accumulation of mutations and thus, increase the risk of cancer development [6]. ROS has also been shown to participate in cancer cell migration, invasion, and metastasis through modulation of multiple signaling pathways and transcription factors (TFs), including AP-1, CXCR4, AKT and PTEN [7]. Notably, a recently defined oncogenic protein, and also a TF, URI, has been associated with a mitochondrial signaling network containing S6K1, URI, PP1g, and BAD, that controls mitochondrial stress-related cell death [8]. URI is known to promote the growth and survival and enhance drug resistance of multiple cancer cells [9-12]. URI has also been shown to maintain DNA integrity in drosophila and to promote liver tumorigenesis in human through inhibition of de novo NAD $^+$ synthesis to cause DNA dam-

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age [13, 14]. However, whether and how URI may influence cell oxidative stress reaction and its associated DNA damage in cancer cells has never been elucidated.

In this study, we investigated the effectiveness of URI on oxidative stress induced by potassium dichromate in SGC-7901 gastric cancer cells. Potassium dichromate (K₂Cr₂O₇) is a common salt of heavy metal Chromium (Cr), i.e. Chromium VI. Cr(VI) has been widely used as an oxidizing agent in various laboratories and industries [15]. Cr(VI) has also been shown to provoke oxidative stress, DNA damage, cytotoxicity, mutagenesis and multiple carcinogenesis [16, 17]. Here, we showed that knocking-down of URI in SGC-7901 cells exposed to potassium dichromate resulted in enhanced oxidative stress and DNA damage, and increased cell death, suggesting a preventive function of URI in cancer cell death.

Materials and methods

Cell culture

The human gastric cancer cell line SGC-7901 was a gift from Professor Wei Zhu at Jiangsu University. SGC-7901 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM, Corning, USA). All cells were supplemented with 10% fetal bovine serum (Gibco, New Zealand) and 1% penicillin/streptomycin (Invitrogen) and cultured at 37°C in a humidified incubator containing 5% CO₂.

siRNA transfection

To knockdown URI expression, a small interfering RNA sequences (siRNA-A) targeting URI was transfected into cells as previously described [9]. siRNA-A and the scrambled control sequences were synthesized by Origene Technologies, Inc. Sequences of siRNA-A (rArGrArGrGrUrArGrArUrArUrGrArCrUrArUrArArUGC) and the scrambled control (rCrGrUrUrArArUrCrGrCrGrUrArUrArUrArUrArCrGrCrGrUAT) are as shown. Transfection of siRNA-A and the scrambled control was performed using siTran 1.0 reagent (Origene) according to the manufacturer's instructions.

Western blot

After 48 h transfection, cells were washed with cold phosphate-buffered saline (PBS) and then collected and lysed in RIPA buffer (Beyotime Biotechnology, CA, China) containing protease

inhibitor cocktail (Kangchen, Shanghai, China). Cells were placed on ice for 30 min, followed by centrifuge at 14000 rpm for 10 min to remove cellular debris. The supernatant was collected and the protein concentration was determined by BCA-assay (Eppendorf, Hamburg, Germany). 50 µg of total protein were subjected to SDS-PAGE and subsequently transferred onto Immobilon-P membranes (Millipore, Billerica, USA), which were then blocked with 5% non-fat milk for 1 h under constant shaking. These membranes were then treated with rabbit anti-human URI antibody or rabbit anti-human β-actin antibody (Santa Cruz Biotechnology, CA, USA) at 4°C overnight. After washing with TBST containing 0.1% Tween 20 three times, the membranes were incubated with horseradish peroxidase conjugated goat anti-rabbit IgG antibody (Fcmacs Biotechnology, CA, China) at room temperature for 1 h followed by detection using an enhanced chemiluminescence system (Minichemi, China). Western blot assay was performed three times and data from representing one set of experiments was shown.

Cell proliferation assay

Cell viability was determined using a cell counting kit-8 (CCK-8) assay in accordance with the manufacturer's protocol (Vazyme Biotech, Nanjing, China). In brief, cells were seeded in 96-well plates at a density of 1×10⁴ cells per well and incubated for an additional 12 h after 24 h transfection. 10 µl of CCK-8 kit reagent was added to each well, and the cells were incubated at 37°C for an additional 1 h. The absorbance of cells was measured spectrophotometrically at a wavelength of 450 nm by a microplate reader (Bio-Rad Model 680, Richmond, CA, USA). For potassium dichromate treatment, cells were incubated with desired concentrations of potassium dichromate (Ling Feng Chemical Company, Shanghai, China) for 15 min, after removal of the potassium dichromate, cells were continually incubated for an additional 12 h before CCK8 assay. The mean value of each concentration was based on the data of five replicates and each experiment was repeated in triplicate.

Alkaline comet assay

Cells cultured in 24-well plates were treated with 1 and 10 µM of potassium dichromate for 1 h. Then, the cells were washed with PBS for three times and incubated with fresh DMEM

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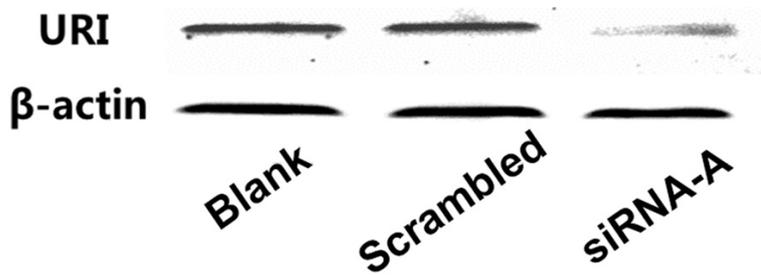


Figure 1. URI expression in gastric cancer cells. Western blot analysis showed that URI significantly down regulated in URI siRNA transfected cells compared with the blank and scramble sequence controls. β -actin was used as an internal control.

medium supplemented with 10% fetal bovine serum for additional 0 min, 30 min, 1 h, 2 h. Cells were then harvested for the comet assay, which was a modified version as previously described [18]. Specifically, hot 1% normal melting point agarose (NMPA) was layered on slide and kept for solidification. Then 75 μ l of low melting point agarose (LMPA) were added and mixed with 10 μ l of cell suspension and allowed 5-10 min for solidification at 4°C. 10^3 - 10^4 cells per slide were analyzed. The slides were immersed in prepared 1% Triton lysis buffer (pH10) for 90 min and placed in the electrophoresis buffer (NaOH, pH>13) for 20 min for unwinding of the DNA. Electrophoresis was performed in the same alkaline solutions for 30 min at 25 v. Slides were neutralized in Tris buffer for 15 min. and stained with gelRed. Each of the slides was imaged by a fluorescent microscope and analysed using CASP (Comet Assay Software Project). A total of 300 cells were analysed per sample. The mean and standard error of the median oliver tail moment (OTM) value from these triplicates were calculated. Data was expressed as mean \pm SEM of three separated experiments, comparison mean between each group using SPSS20.0 software for variance analysis. * p <0.05, *** p <0.01.

Intracellular ROS detection

The specific fluorescent dye DCFH-DA (Beyotime, Nantong, China) was used to detect the intracellular ROS level. DCFH-DA was reportedly to cross the cell membrane and subsequently undergo deacetylation by intracellular esterases. The resulting 2',7'-dichlorodihydrofluorescein (DCFH) is proposed to react with intracellular hydrogen peroxide or other oxidizing ROS to give the fluorescent 2',7'-dichlorofluorescein (DCF). After 48 h transfection of URI, SGC-7901

cells in 6-well plates or 12-well plates were treated with different concentrations of potassium dichromate for designated time. Then, 5 μ M DCFH-DA was added for 15 min. The cells in 6-well plates were washed three times with PBS and collected and subjected to Flow Cytometry (FCM) analysis (BD Accuri™ C6 system). Cells in 12-well plates were fixed with 10% buffered formalin for 10 min.

After washing, images were captured using a fluorescence microscope (Nikon ECLIPSE TE2000-S, Nikon, Kanagawa, Japan). At the same time, PI staining was used to display dead cells. Analyses were performed in triplicate and the results are expressed as mean \pm SEM, *** p <0.01.

Immunofluorescence

Cells after treatment were seeded on coverslips and fixed for 20 minutes in 4% paraformaldehyde on ice. The permeabilization and blocking of nonspecific binding of antibodies were performed by incubation of cells in PBS buffer containing 10 mM HEPES, 3% BSA and 0.1% Triton X-100 at room temperature for 45 minutes. Incubations with primary and secondary antibodies diluted in 1% BSA (PBS buffer) were performed at 4°C overnight or at room temperature for 60 minutes, respectively. The staining of nuclei was carried out by incubation for 10 minutes with DAPI (1 mg/ml in PBS solution) at room temperature. Between all the steps, cells were washed three times for 5 minutes with PBST, and examined under a fluorescence microscope (Nikon ECLIPSE TE2000-S, Nikon, Kanagawa, Japan). Fluorescent secondary antibodies directed to rabbit (Alexa Fluor 488) and rabbit (Alexa Fluor 555) IgG were purchased from Beyotime (Nantong, China).

Apoptosis assay

Apoptosis assay was performed using the FITC Annexin V apoptosis detection kit (BD Pharmingen™, CA, and USA) according to manufacturer's instructions. In brief, at 48 h after transfection, cells were subjected to corresponding treatment and collected and washed twice with cold PBS, and then re-suspended in 500 μ l Annexin V binding buffer at a concentration of 1×10^6 cells/ml. 100 μ l of the cell suspension

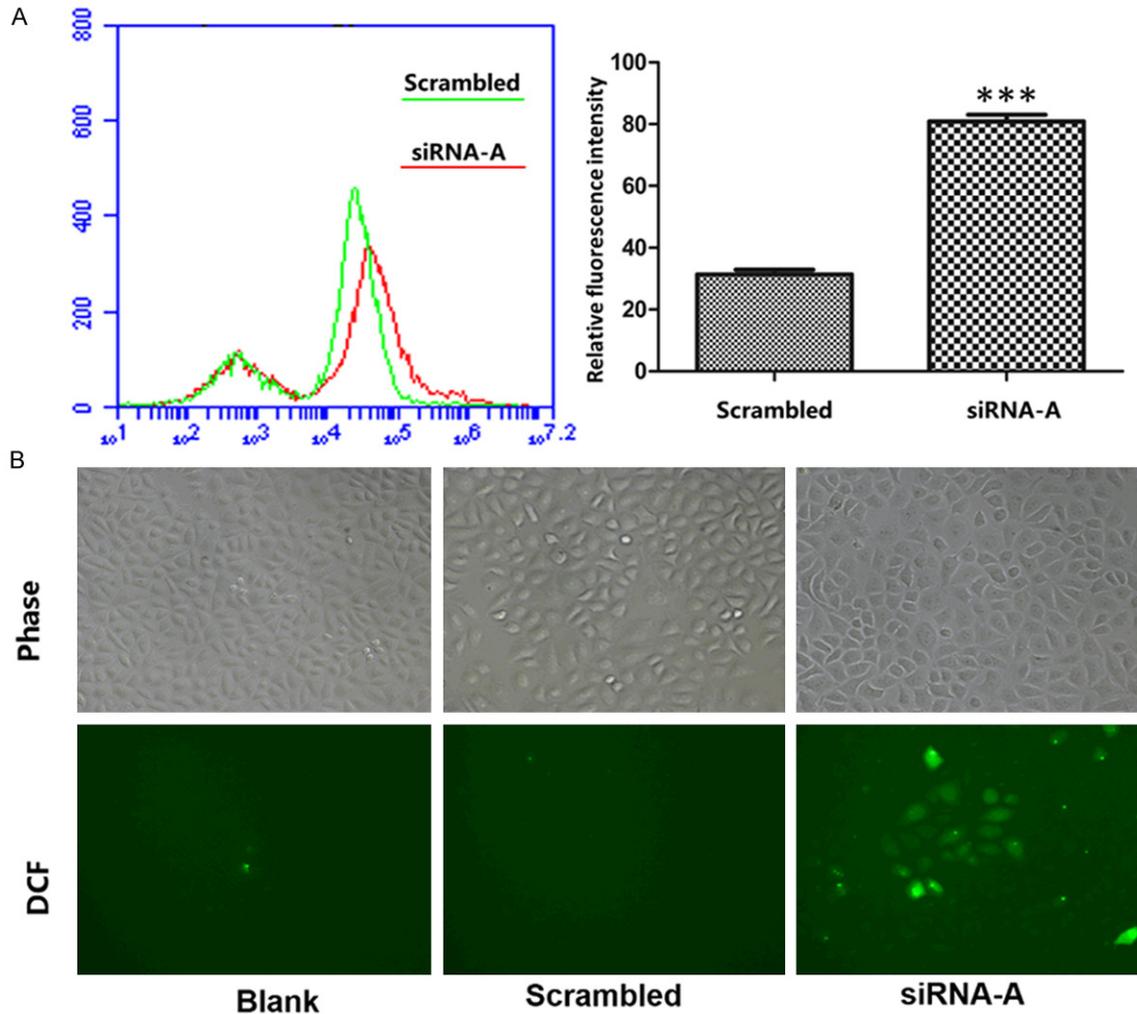


Figure 2. Detection of ROS production after potassium dichromate treatment A. Measurement of ROS fluorescence intensity (A) and DCF (B) of SGC-7901 cells by FCM after 10 μ M potassium dichromate treatment for 30 min. Fluorescent images were captured using a fluorescent microscope (bottom panel). The corresponding phase micrographs are shown in the upper panel. The cells were stained by DCFH-DA and SGC-7901 cells were transfected with URI siRNA-A/control for 48 hours before treated with potassium dichromate. Analyses were performed in triplicate and the results are expressed as mean SEM, *** p <0.01.

(1×10^5 cells) was transferred to a 5 ml culture tube and add 5 μ l each of Annexin V-FITC and propidium iodide (PI) with gentle vortex. The cells were kept in dark for 10 min at RT (25°C) before adding 400 μ l of 1x binding buffer to each tube and subjected to flow cytometry analysis (FCM) (BD AccuriTM C6 system).

Statistical analysis

All data were presented as the mean \pm SEM. The comparison of mean between two groups using t-test. Differences in three groups were analyzed by one-way ANOVA using SPSS20.0 software. $P < 0.05$ was considered statistically significant.

Results

URI expression after siRNA-A transfection in SGC-7901 cells

Western blot result showed the URI expression was significantly reduced after transfection of URI siRNA-A (Figure 1) compared with scramble sequence control and blank control.

URI knockdown enhances potassium dichromate-induced production of ROS

We determined the effects of potassium dichromate treatment on the intracellular ROS level in URI knockdown cells by applying FACS-based

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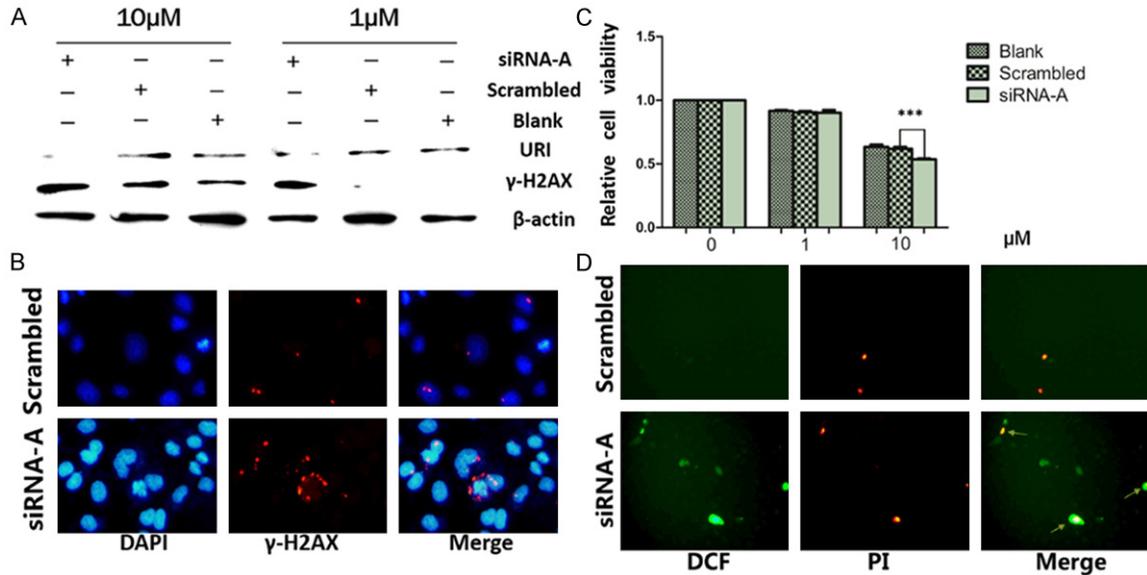


Figure 3. ROS induces DNA damage and cell death in SGC-7901 cells (A) Nuclear extracts from SGC-7901 cells transfected with URIsRNA and controls and with 10 μM potassium dichromate treatment for 30 mins were subjected to western blot analysis to examine P-H2AX expression. (B) Immunofluorescence showed the level of P-H2AX (red dots). (C) At 36 hours after URI siRNA transfection, SGC-7901 cells were exposed to different doses of potassium dichromate for 15 min and incubated for 12 hours in a carbon dioxide incubator, then cell viability was determined by CCK-8 assay at 450 nm. URI knockdown decreased cell viability. (D) ROS levels in cells as indicated by DCF and the stained Propidium Iodide (PI) showed the dead cells. The arrows represent ROS induced dead cells. Analyses were performed in triplicate and the results are expressed as mean ± SEM, ***p<0.01.

assay and fluorescence method. The intensity of DCF fluorescence reflects the level of ROS. The FACS diagrams showed that URI knockdown leads to increased ROS in cells treated with 10 μM potassium dichromate, while no significant change in cells treated with 1 μM potassium dichromate (Figure 2A and data not shown). Fluorescent images showed that ROS was also increased in URI knockdown cells treated with 10 μM potassium dichromate (Figure 2B). These results suggested that URI may attenuate oxidative stress induced by potassium dichromate in SGC-7901 cells.

URI knockdown enhanced γH2AX expression and cytotoxicity in cells treated with potassium dichromate

γH2AX, which usually couples with DNA damage responses (DDR), has been widely regarded as a marker of DNA damage and utilized in pre-clinical drug development and clinical studies [19, 20]. To test whether URI is involved in regulation of DNA damage induced by potassium dichromate in SGC-7901 cells, we measured γH2AX level in cells treated with 1 and 10 μM of potassium dichromate respectively for 30 min. The γH2AX expression of the URI siRNA

cells was significantly higher than the control group (Figure 3A). The immunofluorescence assay also confirmed that γH2AX was significantly increased in URI siRNA cells treated with 10 μM potassium dichromate (Figure 3B), indicating that DNA damage was more severe after URI knockdown. To determine if URI affect the proliferation of cells treated with potassium dichromate, we detected the cell viability by CCK-8 cell viability assay. The result showed that with 10 μM potassium dichromate treatment, the cell viability was significantly reduced in URI siRNA-A transfected cells compared with control groups (Figure 3C).

DNA damage and repair evaluated by comet assay

In order to further verify the correlation between URI and DNA damage we performed single cell gel-electrophoresis (alkaline comet assay), a more powerful and direct assay used to determine DNA breaks. The tail moment level, which is a product of tail length and DNA content in the tail, is positively correlated with the level of DNA damage in a cell [21]. The OTM (Olive Tail Moment) = (Tail. mean - Head. mean) × (Tail % DNA) value correspond with the degree of DNA

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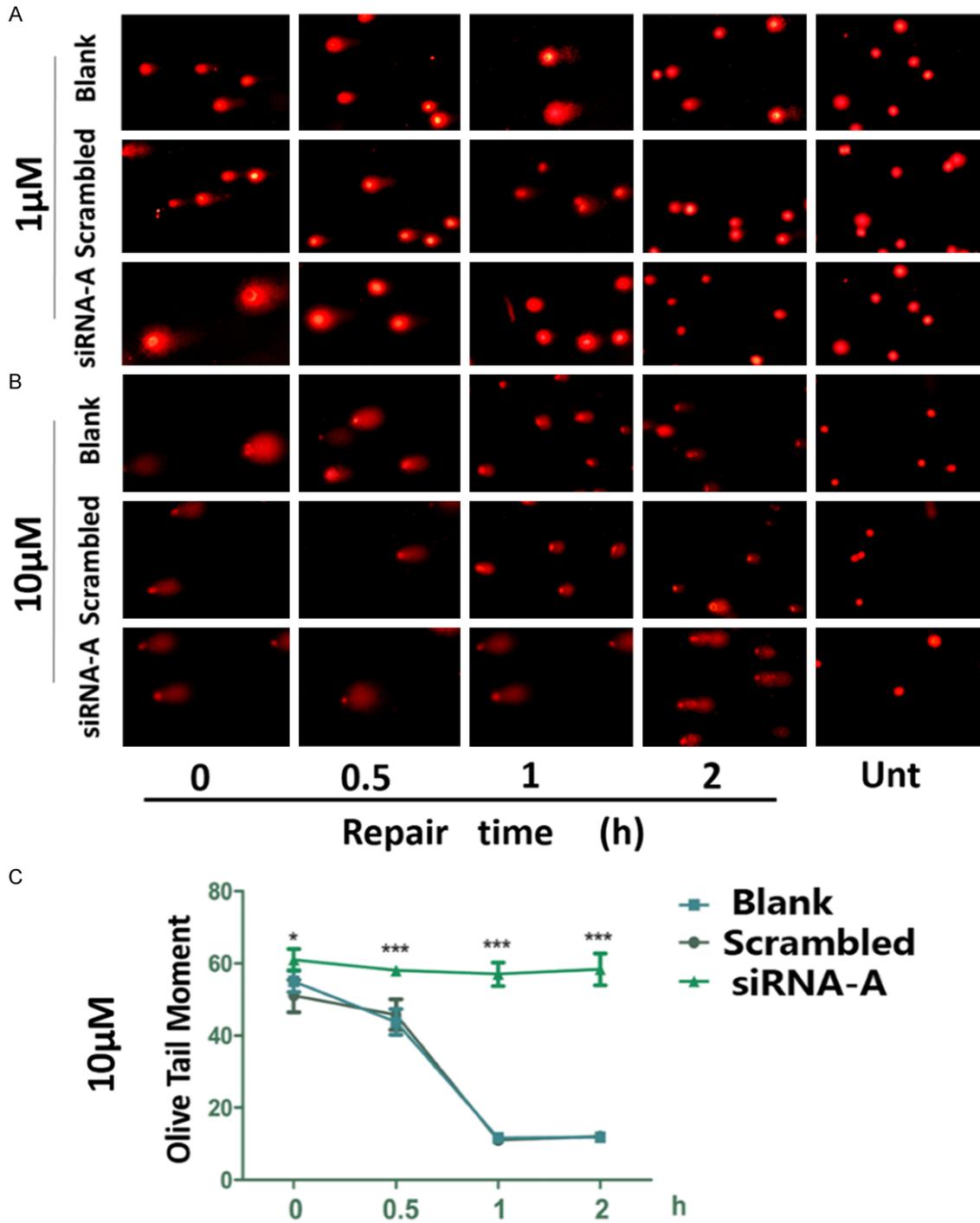


Figure 4. URI is involved in ROS induced DNA damage and repair in SGC-7901 cells (A, B) After treatment with 1 μM (A) or 10 μM (B) potassium dichromate for 1 h, SGC-7901 cells were incubated with complete medium (without chromium salt) for different time points (0 min, 30 min, 1 h, and 2 h respectively). Comet assay was then performed to determine DNA damage and repair as illustrated. (C) OTM levels of SGC-7901 cells after treatment with 10 μM potassium dichromate in URI siRNA and control groups. OTM levels show DNA fragmentation in damaged cells, while changing of OTM levels indicated the repair ability of damaged DNA. Analyses were performed in triplicate and the results are expressed as mean ± SEM, *p<0.05, ***p<0.01.

damage. **Figure 4** shows the comet assay result for cells exposed to potassium dichromate for

60 min and immediately after the exposure for 0.5, 1 and 2 h respectively. The OTM of high

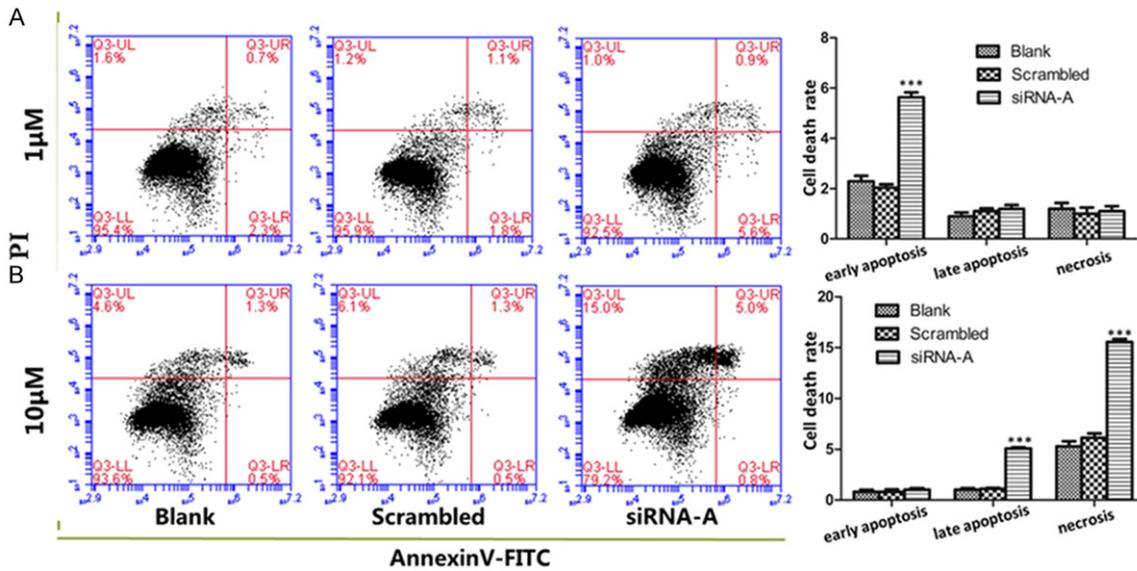


Figure 5. Cell apoptosis and necrosis in SGC-7901 induced by potassium dichromate. (A) Apoptosis assay was performed when the cells were stained for 20 min in dark by annexin V-FITC apoptosis detection reagent after cells pretreated with 1 μM potassium dichromate for 30 min. (B) Apoptosis assay was performed when the cells were stained for 20 min in dark by annexin V-FITC apoptosis detection reagent after cells pretreated with 10 μM potassium dichromate for 30 min. Cells in the lower right, upper right and upper left were considered as early apoptotic, late apoptotic and necrotic cells respectively. Analyses were performed in triplicate and the results are expressed as mean ± SEM, ***p<0.01. SGC-7901 were at 48 hours after URI siRNA transfection before treated with potassium dichromate.

and low level can be induced respectively by 10 μM and 1 μM potassium dichromate. URI siRNA, compared with scramble sequence control and blank control, could increase the OTM of SGC-7901 cells and maintain OTM at a high level from 30 minutes to 2 hours when induced with 10 μM potassium dichromate, but no significant change in 1 μM potassium dichromate (Figure 4A, 4B). The reduced comet length of the cell can represent the repair of DNA damage [21, 22]. The result showed that OTM was gradually reduced from 30 minutes to 2 hours in control groups suggesting that URI knockdown inhibits DNA damage undergoing repair (Figure 4C).

URI knockdown induces apoptosis and necrosis of cells exposed to potassium dichromate

Along with the protective role of URI on DNA damage caused by potassium dichromate, we examined whether URI knockdown affected apoptosis of cells induced by potassium dichromate. We applied FACS-based Annexin V/PI staining assay to assess the effects of URI knockdown on death of SGC-7901 cells. Treating with low concentrations of potassium dichromate (1 μM), URI knockdown significantly increased the proportion of early-stage apop-

totic cells (Annexin V+/PI-) (Figure 5A), while late-stage apoptosis (annexin V+/PI+ cells) and necrosis (annexin V-/PI+ cells) were significantly increased when treated with high concentration of potassium dichromate (10 μM) (Figure 5B). These results suggested that URI prohibited cell death via inhibition of DNA damage.

To explore whether AIF was involved in the cell death. Immunofluorescence assay was conducted and increased mitochondrial AIF expression was detected in URI siRNA transfected cells that were exposed to 10 μM/ml potassium dichromate for 30 min, 1 h, and 2 h respectively compared with corresponding scramble controls (Figure 6). These results provided evidence that URI modulates necrosis through AIF release.

Discussion

In this work, we investigated the effect of URI on potassium dichromate-induced oxidative stress in SGC-7901 cells. We found that URI knockdown can enhance potassium dichromate-induced production of ROS and DNA damage. Consistent with the increase of ROS, we observed that URI RNAi increased apoptosis and necrosis, which appears to be due to

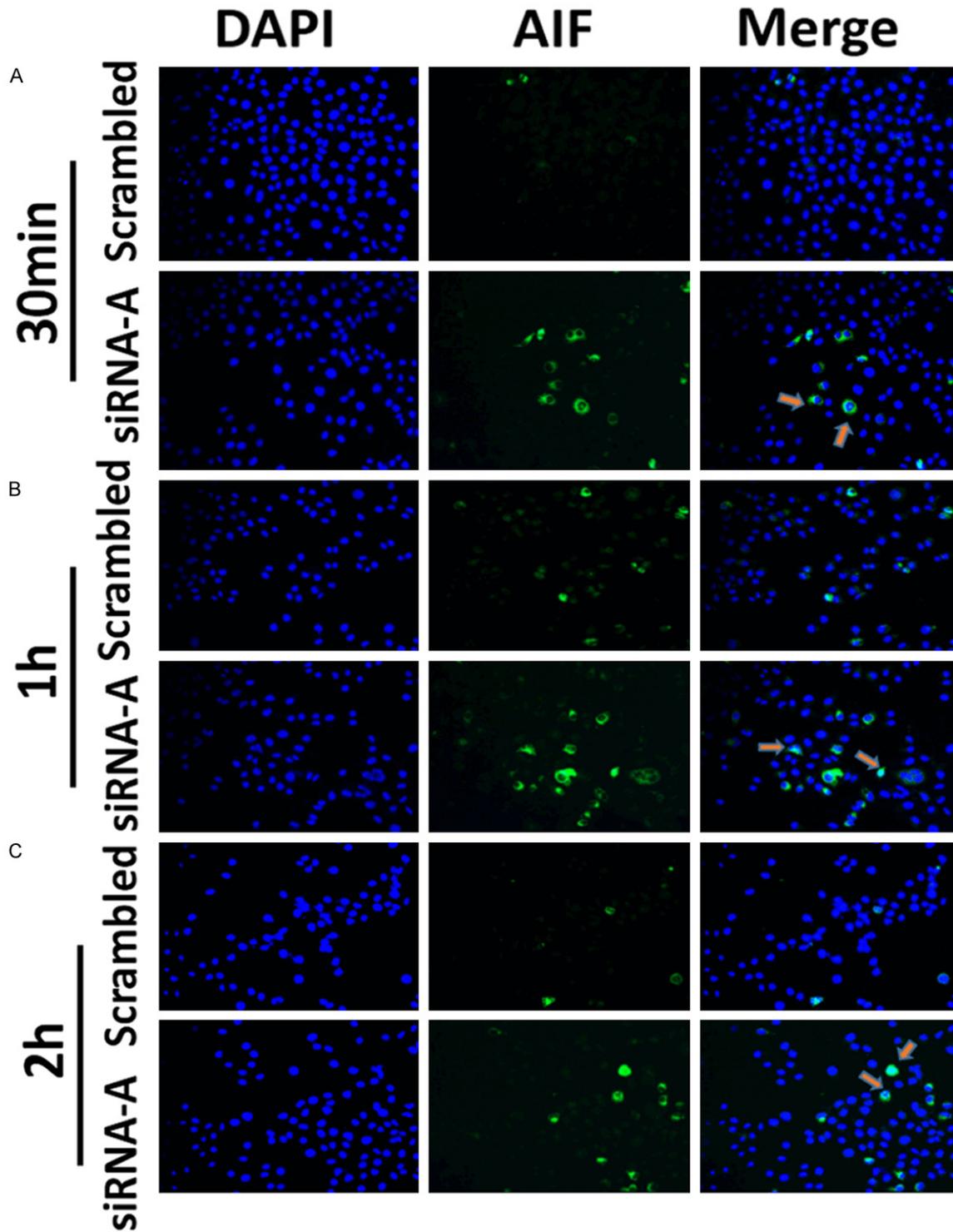


Figure 6. URI knockdown promotes the release of mitochondrial AIF. Immunofluorescence assay vividly indicated the levels of mitochondrial AIF, after the cells were exposed to 10 μ M/ml potassium dichromate for different period of time (30 min, 1 h, 2 h). SGC cells were transfected with URI siRNA and controls for 48 hours before treated with potassium dichromate.

DNA damage induced by ROS, as well as inhibition of DNA repair. URI may maintain DNA integ-

rity by inhibiting cellular processes of oxidative stress. It was previously shown that URI in-

creases S6K1 activity-dependent survival through inhibition of phosphatase PP1g. URI/PP1g complexes maintain the mitochondrial threshold for apoptosis in accordance to nutrient availability [11]. As a downstream effector of mTOR/S6K1 signalling pathway, URI depletion enhances cancer cell apoptosis [13]. There are also lines of evidence which support that URI in different species (*C. elegans*, *Drosophila* etc.) plays essential roles in maintaining DNA/genomic stability and DNA integrity [23]. It has been demonstrated that Ionization radiation can induce mitochondrial dysfunction to cause persistent oxidative stress and DNA damage, which contributes to genomic instability [24, 25]. Interestingly, we have previously shown that upon exposure to γ -irradiation, URI (RMP) expression in human hepatocellular carcinoma SMMC-7721 cells was significantly increased, suggesting that URI is a radiation-sensitive factor [9]. Hence, we surmise that URI protection of gastric cancer cells from potassium dichromate-induced oxidative stress and DNA damage is possibly via regulation of mitochondrial function.

Hexavalent chromium compounds have been examined for genotoxicity and mutagenicity in a large number of genotoxicity assays. Chromium toxicity has been an established cause for lung cancer [26, 27] and has also been associated with increased risks of stomach cancer. The exact molecular mechanisms of Cr (VI) causing carcinogenesis remain to be determined [19]. It has previously been proposed that Chromium genotoxicity could be a double-edged sword [26]. The cellular effects of Cr (VI) include the formation of Cr-DNA adducts, genomic damage and mutagenesis, reactive oxygen species production, and alteration of survival signalling pathways. When exposed to a certain level of Cr (VI) capable of eliciting some manifestation of toxicity, a cell will undergo a transient checkpoint arrest and damage repair. If the damage is irreparable, the cell will undergo terminal growth arrest or apoptosis. However, a small population of cells with Cr (VI) exposure may have acquired an intrinsic mechanism(s) of death resistance through dysregulated DNA repair mechanisms and/or dysregulated survival, and eventually lead to tumor cell formation [28]. It is a basic fact that tumor cells possess intrinsic death-resistance to chemotherapeutics. This is true for URI as it has been

shown in multiple studies to maintain tumor cell survival via drug resistance [10-13]. In addition, abnormally elevated mitochondrial oxidative stress can promote the release of the mitochondrial protein apoptosis inducing factor (AIF), which tends to cause caspase independent cell death [29, 30]. We observed increased AIF expression in potassium dichromate treated SGC-7901 cells by knocking-down of URI. This implies that URI inhibits cell apoptosis at least partially through the AIF pathway.

In summary, we have observed enhanced oxidative stress, increased ROS induced DNA damage in gastric cancer cells treated with potassium dichromate, suggesting URI as a promoter for cancer cell survival. Although still inconclusive, our results provide a new viewpoint between URI and potassium dichromate-induced oxidative stress. URI may be involved in chromium-mediated carcinogenesis through mitochondrial or other relevant pathways.

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Disclosure of conflict of interest

None.

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References

- [1] Le Bras M, Clement MV, Pervaiz S and Brenner C. Reactive oxygen species and the mitochondrial signaling pathway of cell death. *Histol Histopathol* 2005; 20: 205-219.
- [2] Kongara S and Karantza V. The interplay between autophagy and ROS in tumorigenesis. *Front Oncol* 2012; 2: 171.
- [3] Shadel GS and Horvath TL. Mitochondrial ROS Signaling in Organismal Homeostasis. *Cell* 2015; 163: 560-569.
- [4] Thanan R, Oikawa S, Hiraku Y, Ohnishi S, Ma N, Pinlaor S, Yongvanit P, Kawanishi S and Mura-

URI and chromium VI induced oxidative stress and cell death

- ta M. Oxidative stress and its significant roles in neurodegenerative diseases and cancer. *Int J Mol Sci* 2015; 16: 193-217.
- [5] Ozben T. Oxidative stress and apoptosis: impact on cancer therapy. *J Pharmaceut Sci* 2007; 96: 2181-2196.
- [6] Mantovani A, Allavena P, Sica A and Balkwill F. Cancer-related inflammation. *Nature* 2008; 454: 436-444.
- [7] Gupta SC, Hevia D, Patchva S, Park B, Koh W, Aggarwal BB. Upsides and downsides of reactive oxygen species for cancer: the roles of reactive oxygen species in tumorigenesis, prevention, and therapy. *Antioxid Redox Signal* 2012; 16: 1295-1322.
- [8] Djouder N, Metzler SC, Schmidt A, Wirbelauer C, Gstaiger M, Abersold R, Hess D and Krek W. S6K1-mediated disassembly of mitochondrial URI/PP1 γ complexes activates a negative feedback program that counters S6K1 survival signaling. *Mol Cell* 2007; 28: 28-40.
- [9] Yang H, Gu J, Zheng Q, Li M, Lian X, Miao J, Jiang J and Wei W. RPB5-mediating protein is required for the proliferation of hepatocellular carcinoma cells. *J Biol Chem* 2011; 286: 11865-11874.
- [10] Theurillat JP, Metzler SC, Henzi N, Djouder N, Helbling M, Zimmermann AK, Jacob F, Soltermann A, Caduff R, Heinzelmann-Schwarz V, Moch H and Krek W. URI is an oncogene amplified in ovarian cancer cells and is required for their survival. *Cancer Cell* 2011; 19: 317-332.
- [11] Fan JL, Zhang J, Dong LW, Fu WJ, Du J, Shi HG, Jiang H, Ye F, Xi H, Zhang CY, Hou J and Wang HY. URI regulates tumorigenicity and chemotherapeutic resistance of multiple myeloma by modulating IL-6 transcription. *Cell Death Dis* 2014; 5: e1126.
- [12] Gu J, Liang Y, Qiao L, Lu Y, Hu X, Luo D, Li N, Zhang L, Chen Y, Du J and Zheng Q. URI expression in cervical cancer cells is associated with higher invasion capacity and resistance to cisplatin. *Am J Cancer Res* 2015; 5: 1353-1367.
- [13] Kirchner J, Vissi E, Gross S, Szoor B, Rudenko A, Alphey L and White-Cooper H. Drosophila Uri, a PP1 α binding protein, is essential for viability, maintenance of DNA integrity and normal transcriptional activity. *BMC Mol Biol* 2008; 9: 36.
- [14] Tummala KS, Gomes AL, Yilmaz M, Grana O, Bakiri L, Ruppen I, Ximenez-Embun P, Sheshapannavar V, Rodriguez-Justo M, Pisano DG, Wagner EF and Djouder N. Inhibition of de novo NAD(+) synthesis by oncogenic URI causes liver tumorigenesis through DNA damage. *Cancer Cell* 2014; 26: 826-839.
- [15] De Celis R, Pedron-Nuevo N and Feria-Velasco A. Toxicology of male reproduction in animals and humans. *Arch Androl* 1996; 37: 201-218.
- [16] Bagchi D, Stohs SJ, Downs BW, Bagchi M and Preuss HG. Cytotoxicity and oxidative mechanisms of different forms of chromium. *Toxicology* 2002; 180: 5-22.
- [17] Welling R, Beaumont JJ, Petersen SJ, Alexeeff GV and Steinmaus C. Chromium VI and stomach cancer: a meta-analysis of the current epidemiological evidence. *Occup Environ Med* 2015; 72: 151-159.
- [18] Olive PL, Chan AP and Cu CS. Comparison between the DNA precipitation and alkali unwinding assays for detecting DNA strand breaks and cross-links. *Cancer Res* 1988; 48: 6444-6449.
- [19] Banath JP and Olive PL. Expression of phosphorylated histone H2AX as a surrogate of cell killing by drugs that create DNA double-strand breaks. *Cancer Res* 2003; 63: 4347-4350.
- [20] Dickey JS, Redon CE, Nakamura AJ, Baird BJ, Sedelnikova OA and Bonner WM. H2AX: functional roles and potential applications. *Chromosoma* 2009; 118: 683-692.
- [21] Olive PL, Banath JP and Durand RE. Heterogeneity in radiation-induced DNA damage and repair in tumor and normal cells measured using the "comet" assay. *Radiat Res* 1990; 122: 86-94.
- [22] Trzeciak A, Kowalik J, Malecka-Panas E, Drzewoski J, Wojewodzka M, Iwanenko T and Blasiak J. Genotoxicity of chromium in human gastric mucosa cells and peripheral blood lymphocytes evaluated by the single cell gel electrophoresis (comet assay). *Med Sci Monit* 2000; 6: 24-29.
- [23] Parusel CT, Kritikou EA, Hengartner MO, Krek W and Gotta M. URI-1 is required for DNA stability in *C. elegans*. *Development* 2006; 133: 621-629.
- [24] Szumiel I. Ionizing radiation-induced oxidative stress, epigenetic changes and genomic instability: the pivotal role of mitochondria. *Int J Radiat Biol* 2015; 91: 1-12.
- [25] Yoshida T, Goto S, Kawakatsu M, Urata Y and Li TS. Mitochondrial dysfunction, a probable cause of persistent oxidative stress after exposure to ionizing radiation. *Free Radic Res* 2012; 46: 147-153.
- [26] Proctor DM, Suh M, Campleman SL and Thompson CM. Assessment of the mode of action for hexavalent chromium-induced lung cancer following inhalation exposures. *Toxicology* 2014; 325: 160-179.
- [27] Urbano AM, Ferreira LM and Alpoim MC. Molecular and cellular mechanisms of hexavalent chromium-induced lung cancer: an updated

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- perspective. *Curr Drug Metab* 2012; 13: 284-305.
- [28] Nickens KP, Patierno SR and Ceryak S. Chromium genotoxicity: A double-edged sword. *Chem Biol Interact* 2010; 188: 276-288.
- [29] Fouquerel E and Sobol RW. ARTD1 (PARP1) activation and NAD(+) in DNA repair and cell death. *DNA Repair (Amst)* 2014; 23: 27-32.
- [30] Natarajan SK and Becker DF. Role of apoptosis-inducing factor, proline dehydrogenase, and NADPH oxidase in apoptosis and oxidative stress. *Cell Health Cytoskeleton* 2012; 2012: 11-27.