ANTICANCER RESEARCH *41*: 4343-4351 (2021)
doi:10.21873/anticanres.15239

Allyl Isothiocyanate Induces DNA Damage and Impairs DNA Repair in Human Breast Cancer MCF-7 Cells

CHING-LUNG LIAO[1], SHU-FEN PENG[2,3], JAW-CHYUN CHEN[4], PO-YUAN CHEN[2], AN-CHENG HUANG[5], JIN-CHERNG LIEN[6], FU-SHIN CHUEH[7], TAI-AN CHIANG[8], PING-PING WU[6*] and KUN-I LIN[9,10*]

[1]*College of Chinese Medicine, School of Post-Baccalaureate Chinese Medicine, China Medical University, Taichung, Taiwan, R.O.C.;*
[2]*Department of Biological Science and Technology, China Medical University, Taichung, Taiwan, R.O.C.;*
[3]*Department of Medical Research, China Medical University Hospital, Taichung, Taiwan, R.O.C.;*
[4]*Department of Medicinal Botany and Health Applications, Da-Yeh University, Changhua, Taiwan, R.O.C.;*
[5]*Department of Nursing, St. Mary's Junior College of Medicine, Nursing and Management, Yilan, Taiwan, R.O.C.;*
[6]*School of Pharmacy, China Medical University, Taichung, Taiwan, R.O.C.;*
[7]*Department of Food Nutrition and Health Biotechnology, Asia University, Taichung, Taiwan, R.O.C.;*
[8]*Department of Medical Technology and Graduate Institute of Biological Science and Technology, College of Medicine and Life Science, Chung-Hwa University of Medical Technology, Tainan, Taiwan, R.O.C.;*
[9]*Department of Obstetrics and Gynecology, Chang Bing Show-Chwan Memorial Hospital, Changhua, Taiwan, R.O.C.;*
[10]*Department of Cosmetic Science, Providence University, Taichung, Taiwan, R.O.C.*

Abstract. *Background/Aim: Ally lisothiocyanate (AITC), a constituent of naturally occurring isothiocyanates (ITCs) found in some Brassica vegetables, has been previously demonstrated to have anti-carcinogenic activity. However, there is no available information showing that AITC induces DNA damage and alters DNA damage repair proteins in human breast cancer MCF-7 cells. Materials and Methods: In the present study, we investigated the effects of AITC on DNA damage and repair responses in human breast cancer MCF-7 cells in vitro. Cell viability was measured by flow cytometric assay. DNA condensation (apoptotic cell death) and DNA fragmentation (laddered DNA) were assayed by DAPI staining and DNA gel electrophoresis assays, respectively. Furthermore, DNA damage (comet tail) was measured by the comet assay. Western blotting was used to measure the expression of DNA damage- and repair-associated proteins. Results: AITC decreased cell viability in a dose-dependent and induced apoptotic cell death (DNA condensation and fragmentation) and DNA damage in MCF-7 cells. AITC increased p-ATM^{Ser1981}, p-ATR^{Ser428}, p53, p-p53^{Ser15}, p-H2A.X^{Ser139}, BRCA1, and PARP at 10-30 μM at 24 and 48 h treatments. However, AITC decreased DNA-PK at 24 and 48 h treatment, and decreased MGMT at 48 h in MCF-7 cells. Conclusion: AITC induced cytotoxic effects (decreased viable cell number) through induction of DNA damage and condensation and altered DNA damage and repair associated proteins in MCF-7 cells in vitro.*

Breast cancer is the most common form of cancer and the most frequently diagnosed cancer in women. It is also the leading cause of cancer-related death among females, and it remains the most prevalent cancer worldwide. Breast cancer has been recognized to be a predominant disease with aging; however, in the developed world about 5-7% of patients are diagnosed with breast cancer below the age of 40 (1). Worldwide, breast cancer accounts for 25% of all cancer cases and 15% of all cancer deaths (2). The risk factors of breast cancer include genetic mutations, reproductive history,

*These Authors contributed equally to this study.

Correspondence to: Ping-Ping Wu, Professor, School of Pharmacy, China Medical University, No.91, Hsueh-Shih Road, Taichung 40402, Taiwan, R.O.C. Tel: +886 422053366 ext. 5108, Fax: +886 422031075, e-mail: ppwu@mail.cmu.edu.tw; Kun-I Lin, Dr., Department of Obstetrics and Gynecology, Chang Bing Show-Chwan Memorial Hospital, No.6, Lugong Road, Lukang Zhen, Changhua County 505, Taiwan, R.O.C. Tel: +886 47812012, e-mail: kunidg107@gmail.com

Key Words: Ally lisothiocyanate (AITC), human breast cancer MCF-7 cells, DNA damage, DNA condensation and fragmentation.

weight gain, alcohol consumption, lack of physical exercise, and exposure to common chemicals and radiation (3, 4). The current treatment for patients with breast cancer includes surgery, radiotherapy, chemotherapy, target therapy; still presenting with increased adverse events (drug resistance and side effects). Therefore, numerous studies have focused on natural products for the treatment of human breast cancer.

One of the feature of cancer cells is genomic instability, which can be combined with both DNA damage and tumor-specific DNA repair defects that are involved in tumorigenesis (5). DNA damage response has been recognized to be a vital cell network for maintaining genome stability. Agents inducing DNA damage may trigger a cascade of molecular changes such as DNA damage signaling (DDS), DNA double-strand breaks (DSBs), or DNA damage response (DDR) for causing cell death or survival (6, 7). In particular, DSBs are recognized to be the most serious factors which may induce cell death (8). DSBs may be triggered *via* cells directly interacting with a damaging agent, reactive oxygen species (ROS), metabolic processes, impaired DNA repair processes, and telomere erosion (9-13). Some chemotherapy agents have been designed to target DNA repair to induce cancer cell apoptosis. Studies should focus on measuring if natural products cause cancer cell DNA damage and/or alter or halt the cellular DNA repair systems.

Epidemiological studies have demonstrated that people with a high dietary consumption of cruciferous vegetables such as broccoli, Brussels sprouts, cabbage, cauliflower, and watercress will have lower disease occurrence and elicited chemopreventive effects (14, 15). These vegetables, rich in isothiocyanates (ITCs), have been recognized as nutraceutical agents against cancer development (16-19). AITC, a constituent of naturally occurring ITCs, is derived from the glucosinolate sinigrin, and it presents multiple biological functions, including anti-cancer activities (20-24). It was reported that in human lung cancer NSCLC cells, combination therapy involving AITC followed by radiation treatment leads to increased DNA damage responses and cell killing when compared to single-agent therapy (25). Our earlier studies showed that AITC induced cell apoptosis in human breast cancer MCF-7 cells *via* AIF and Endo G signaling pathways (20). AITC induced apoptotic cell death in many human cancer cells *in vitro* and *in vivo*; however, no report shows if AITC induces DNA damage and impairs DNA repair in human breast cancer cells. Therefore, in the present study, we investigated the effects and mechanisms of AITC on DNA damage and repair in human breast cancer MCF-7 cells *in vitro*.

Materials and Methods

Chemicals and reagents. Allyl isothiocyanate (AITC), dimethyl sulfoxide (DMSO), propidium iodide (PI), and trypsin-EDTA were obtained from Sigma-Aldrich Corp. (St. Louis, MO, USA). Minimum Essential Medium Eagle (MEM) medium, fetal bovine serum (FBS), L-glutamine, penicillin, and streptomycin were obtained from Invitrogen Life Technologies (Carlsbad, CA, USA). Anti-p-ATM^{Ser1981} and -p-H2A.X^{Ser139} were obtained from GeneTex Inc. (Irvine, CA, USA); anti-p-ATR^{Ser428}, -PARP, -BRCA1 and -p-p53^{Ser15} were purchased from Cell Signaling Technology, Inc. (Beverly, MA, USA); anti-MGMT, -p53 and -β-actin were obtained from Sigma-Aldrich Corp.; anti-DNA-PK was got from Calbiochem (San Diego, CA, USA) The goat anti-mouse IgG (secondary antibody) was obtained from Sigma-Aldrich Corp.. In the entire experiment, the AITC was dissolved in DMSO.

Cell culture. MCF-7 human breast cancer cell line was purchased from the Food Industry Research and Development Institute (Hsinchu, Taiwan, ROC). Cells were cultured in 90% Minimum Essential Medium Eagle (MEM) medium contained 10% FBS, 2 mM of L-glutamine, and penicillin-streptomycin (100 Units/ml penicillin and 100 μg/ml streptomycin) onto 75 cm^2 flasks at 37˚C and a 5% CO_2 atmosphere under 90% humidified incubator as previously described (20).

Determination of cell viability by flow cytometric assay. MCF-7 cells (1×10^5 cells/well) were maintained in 12-well culture plates for 24 h and incubated with 0, 10, 20, and 30 μM of AITC for 48 h. After incubation, cells were harvested from each well (each treatment), washed with PBS, and re-suspended in PBS containing 5 μg/ml of PI to measure viable cell number (cell viability) using flow cytometer as cited previously (20).

DNA condensation was measured by 4,6-diamidino-2-phenylindole dihydrochloride (DAPI) staining. MCF-7 cells (1×10^5 cells/well) were maintained in 12-well culture plates for 24 h and incubated with AITC at the final concentrations (0, 10, 20, and 30 μM) for 24 and 48 h. After incubation, cells were washed with PBS and fixed with 4% paraformaldehyde (v/v) in PBS for 15 min. Cells were followed by adding 0.1% Triton X-100 in PBS for 5 min to permeabilize cells. All cells in each treatment were stained with DAPI (2 μg/ml) for 10 min, examined, and photographed using a fluorescence microscope at 200× as described previously (20).

DNA fragmentation was measured by DNA gel electrophoresis. MCF-7 cells (1.5×10^6 cells/dish) were plated in 10-cm dishes and incubated with 0, 10, 20, and 30 μM of AITC for 24 and 48 h. After incubation, cells were harvested, washed with PBS, and lysed in ice-cold lysis buffer. DNA from each treated cells was extracted, quantitated, and electrophoresed on a 1.5% agarose gel. The gel was stained by ethidium bromide and then observed and photographed under UV-box as previously described (20, 26).

DNA damage was measured by comet assay. MCF-7 cells (1×10^5 cells/well) were plated in 12-well culture plates and treated with 0, 10, 20, and 30 μM of AITC for 24 and 48 h. After incubation, cells from each well were performed single-cell electrophoresis (comet assay), examined, and photographed under microscopy. The comets of cells were randomly caltured at a constant depth of the gel from each treatment. The comet tail length was measured and quantified using the Tri Tek Comet ScoreTM software image analysis system (TriTek Corp, Sumerduck, VA, USA) as described previously (20).

DNA damage and repair associated protein expression were examined by western blotting. MCF-7 cells (1.5×10^6 cells/dish) were plated in 10-cm dishes and treated with 0, 10, 20, and 30 μM of AITC for 24 and 48 h. At the end of incubation, cells were collected for protein extraction by RIPA buffer [50 mM Tris-HCI (pH7.4), 125 mM NaCl, 0.1% Triton X-100, and 5 mM EDTA containing both 1% protease inhibitor and 1% phosphatase inhibitor mixture II] (Sigma-Aldrich Corp.) as described previously (26). Proteins from each treatment were quantitated using the Bradford method as described previously (26). Proteins were further performed by western blotting and probed by antibodies anti-p-ATM^{Ser1981}, -p-ATR^{Ser428}, -p53, -p-p53^{Ser15}, -p-H2A.X^{Ser139}, -BRCA1, -DNA-PK, -MGMT, -PARP, and -β-actin, followed with goat anti-mouse IgG coupled to HRP. The membranes that were bound antibodies were detected by chemiluminescence kits (Amersham Biosciences ECLTM, Buckinghamshire, UK) and quantified by Image J as described previously (20, 26).

Statistical analysis. The results (data) were performed at least three independent assays and expressed as the mean values with a standard deviation (SD) (mean±SD) in the figures and were analyzed using one-way ANOVA. $^{**}p<0.01$ or $^{***}p<0.001$ is considered as significant differences between the AITC-treated and untreated (control) groups.

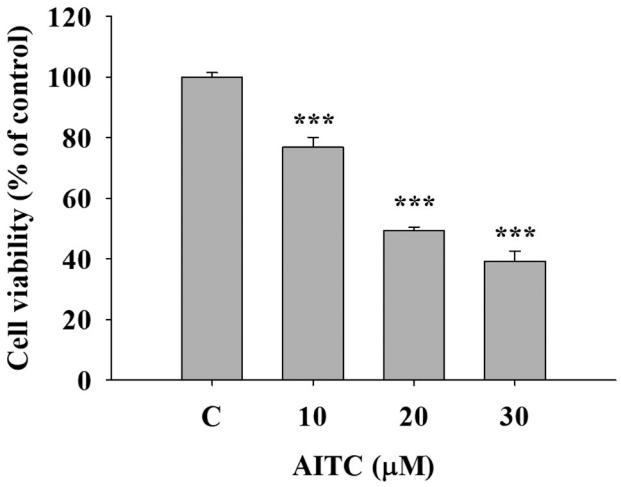

Figure 1. *AITC decreased cell viability (total viable cell number) in MCF-7 cells. Cells were incubated with 0, 10, 20, and 30 μM of AITC for 48 h and collected for measuring total viable cell numbers by flow cytometry as described in Materials and Methods. Experiments were performed in triplicate and data are represent as mean±SD. ***p<0.001 was significantly different between AITC-treated cells and control groups.*

Results

AITC reduced cell viability of MCF-7 cells. To confirm whether or not AITC induced DNA damage in MCF-7 cells, we examined the cytotoxic effects of AITC on total cell viability. MCF-7 cells were incubated with or without AITC (0, 10, 20, and 30 μM) for 48 h. After treatment, cells were harvested and the percentage of viable cell numbers was measured by flow cytometric assay, and results were presented in Figure 1. Results indicated that increased doses of AITC (10-30 μM) decreased the viable cell number (cell viability) from 77% to 39% compared to the untreated (control) group. These effects are dose-dependent (Figure 1).

AITC induced DNA condensation (apoptotic cell death) in MCF-7 cells. To confirm whether AITC decreased cell viability in MCF-7 cells was mediated by induction of DNA condensation (one of the marks of apoptotic cell death), MCF-7 cells were incubated with AITC (0, 10, 20, and 30 μM) for 24 and 48 h. After treatment, cells were stained with DAPI solution to monitor nucleus morphology under fluorescence microscopy, and results are presented in Figure 2. Results indicated that increased doses of AITC (10-30 μM) led to increases in DAPI staining intensity when compared to the control group in MCF-7 cells for 24 and 48 h.

AITC induced DNA fragmentation in MCF-7 cells. DNA fragmentation (laddered DNA) was examined by DNA agarose gel electrophoresis in MCF-7 cells. Cells were incubated with AITC (0, 10, 20, and 30 μM) for 24 and 48 h and then harvested to extract DNA for agarose gel electrophoresis. Results are presented in Figure 3. The typical ladder pattern of oligonucleosomal fragments (DNA ladders) was shown after AITC (10, 20, and 30 μM) treatments in MCF-7 cells, which indicated the development of apoptotic cell death (DNA fragmentation is one of the markers of cell apoptosis) occurring in MCF-7 cells.

AITC induced DNA damage in MCF-7 cells. To further confirm whether or not AITC decreased viable cell number was mediated by induction of DNA damage in MCF-7 cells, cells were incubated with AITC (0, 10, 20, and 30 μM) for 24 and 48 h. After treatment, cellular DNA damage was measured by the comet assay and the results are presented in Figure 4. Results indicated that increased doses of AITC (10-30 μM) led to a significantly increased comet tail length than that of control groups in MCF-7 cells (Figure 4). The observations also showed AITC induced DNA damage (the higher of comet tail, the higher of DNA damage) at 24 and 48 h treatment and these effects are dose-dependent.

AITC affected the expression of DNA damage and repair associated proteins in MCF-7 cells. For further investigating the molecular mechanism involved in AITC-induced DNA damage in MCF-7 cells, cells were harvested after exposure to AITC for protein extraction. Then total proteins were quantitated and their expression was determined by western blotting. Results were shown in Figure 5. AITC increased the levels of p-ATM^{Ser1981}, p-ATR^{Ser428}, p53, and p-p53^{Ser15} at

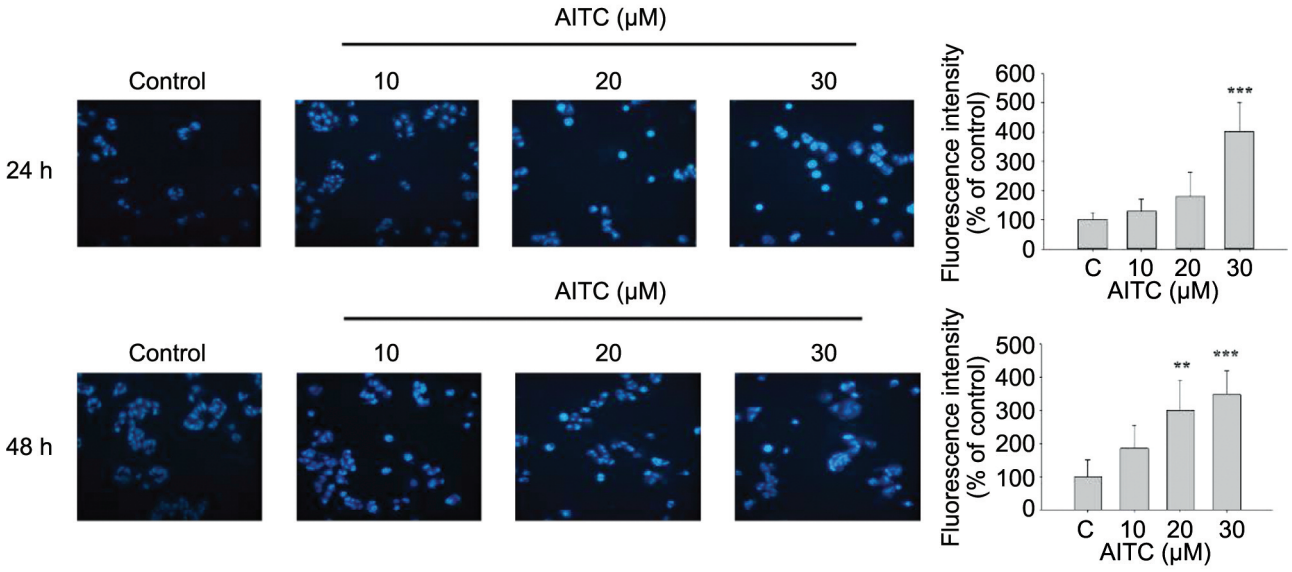

Figure 2. *AITC induced chromatin condensation (apoptotic cell death) in MCF-7 cells. Cells were incubated with 0, 10, 20, and 30 μM of AITC for 24 and 48 h. Cells were fixed, stained, examined, and photographed using a fluorescence microscope at 200×. Cells were measured the DAPI intensity of fluorescence as described in Materials and Methods. Data represent are mean±SD. **p<0.01 and ***p<0.001 were significantly different between AITC-treated cells and control groups.*

10-30 μM (Figure 5A) and p-H2A.X^{Ser139}, BRCA1, and active PARP (Figure 5B) at 24 and 48 h treatment. However, AITC increased MGMT at 24 h treatment, but decreased it at 48 h treatment; furthermore, AITC decreased DNA-PK at 24 and 48 h treatment (Figure 5B). Based on these observations, AITC induced DNA damage for cell death, therefore affecting associated protein expression in MCF-7 cells.

Discussion

Considerable evidence has shown that dietary fruits and phytochemicals can prevent cancer development. One of the effects of dietary fruits is their antioxidant activities on cervical cancer (27). Overexpression of ROS leads to cell death when cells are exposed to chemicals or carcinogens. ROS have high reactivity to cause lipid peroxidation and oxidative damage to DNA and proteins (28). However, some anti-cancer drugs can induce DNA damage in cancer cells. Damage to cellular DNA may trigger a cascade of multifaceted molecular events for cells to undergo either apoptosis or senescence or enable accessibility of the DNA repair machinery to the damage site (29, 30). Cells involve several systems that monitor their DNA integrity, detect DNA lesions, cell-cycle checkpoints, and regulate DNA repair pathways for cell survival after exposure to chemical agents (31). Activation of DNA damage response has been applied for achieving anti-tumor effects of chemotherapy and radiotherapy. Therefore, measuring DNA damage may be the way for examining chemicals or agents and whether or not they induce cytotoxic effects on cancer or normal cells.

In this study, we investigated the cytotoxic effects of AITC in MCF-7 cells. Cells were treated with AITC for 48 h at final concentrations of 0, 10, 20, and 30 μM, and the total viable cell numbers (cell viability) were measured and results are presented in Figure 1. Data indicated that AITC induced cytotoxic effects in MCF-7 cells and these effects are dose-dependent. Similar results were found in other human cancer cell lines such as prostate cancer cells (32) and bladder cancer cells (33). However, AITC did have an inhibitory effect on breast cancer MCF-7 cells, but it did not inhibit human breast cancer MDA-MB-231 cells (34). Furthermore, there is no report showing that AITC induced DNA damage in MCF-7 cells. Thus, we further investigated whether or not AITC decreased viable MCF-7 cells *via* induction of DNA damage.

For further investigation, AITC decreased the viable cell number of MCF-7 cells by inducing DNA damage. Cells were treated with 0, 10, 20, and 30 μM of AITC and assayed by DAPI staining and results are presented in Figure 2. Results showed that AITC induced chromatin condensation and these effects are dose-dependent. Similar results were also reported in human brain cancer cells (35). We also used DNA gel electrophoresis to confirm that AITC induced DNA fragmentation (laddered DNA) (Figure 3), which is one of the characters of apoptotic cell death (cell apoptosis). Some of the anti-cancer drugs induced cancer cell death *via* induced DNA fragmentation (36). Thus, these findings are in

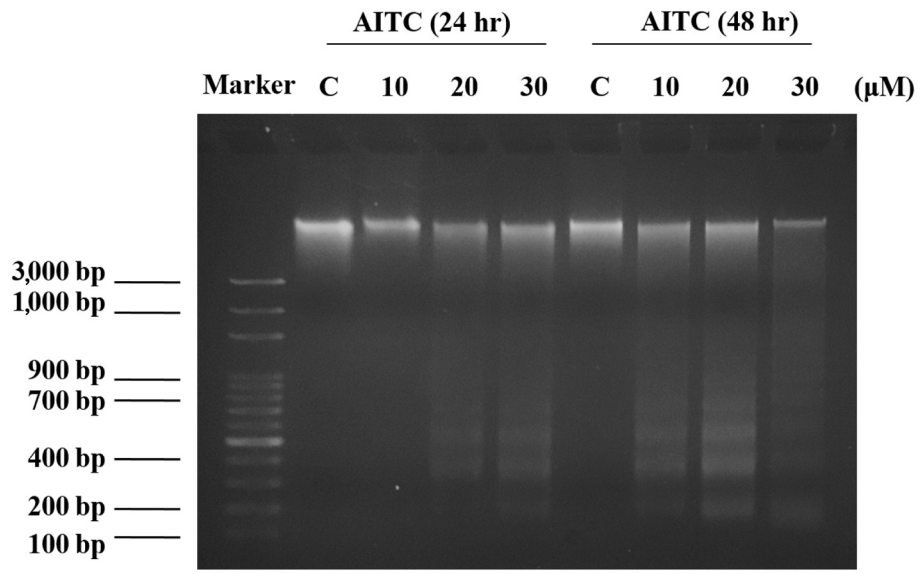

Figure 3. *AITC induced DNA fragmentation (apoptotic cell death) in MCF-7 cells. Cells were incubated with 0, 10, 20, and 30 μM of AITC for 24 and 48 h. Cells were collected and lysed, and then their DNA was extracted for agarose gel electrophoresis as described in Materials and Methods.*

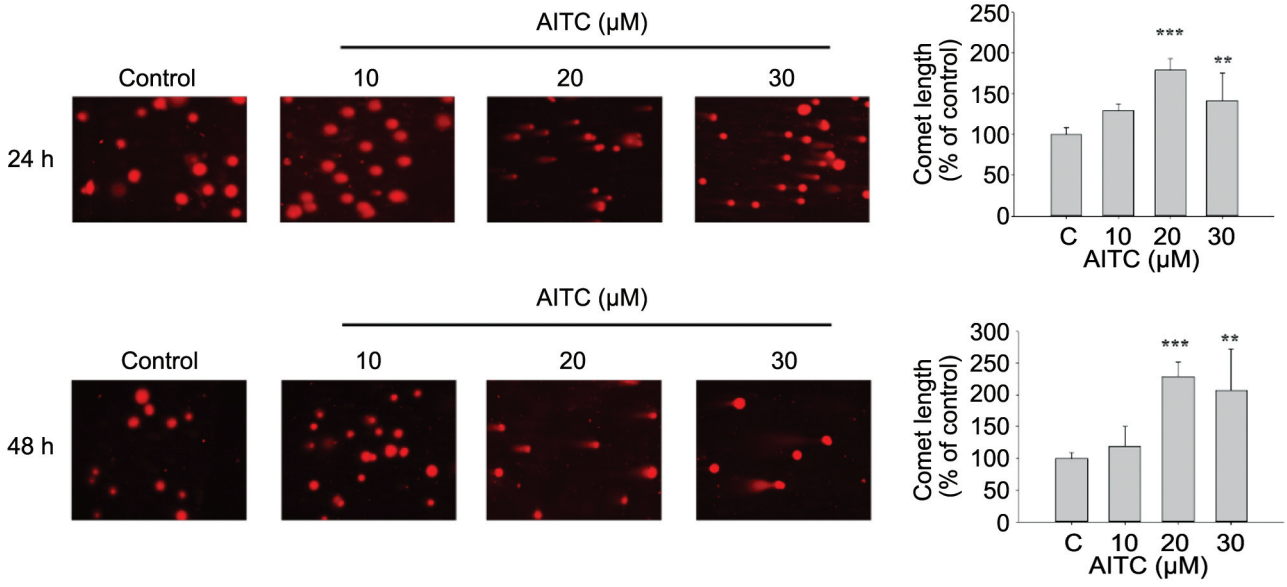

Figure 4. *AITC induced DNA damage in MCF-7 cells. Cells were incubated with 0, 10, 20, and 30 μM of AITC for 24 and 48 h. Cells were investigated by the comet assay and measured the comet tail length at 24 and 48 h as described in Materials and Methods. Data are represent as mean±SD. **p<0.01 and ***p<0.001 were significantly different between AITC-treated cells and control groups.*

agreement with our earlier report noted that AITC induced cell apoptosis in MCF-7 cells (20).

There is no report showing that AITC induced DNA damage in MCF-7 cells; thus, we used the comet assay to determine DNA damage in MCF-7 cells after exposure to AITC at 24 and 48 h and results indicated AITC induced DNA damage in MCF-7 cells (Figure 4). The protocol of the comet assay (single-cell gel electrophoresis) has been shown

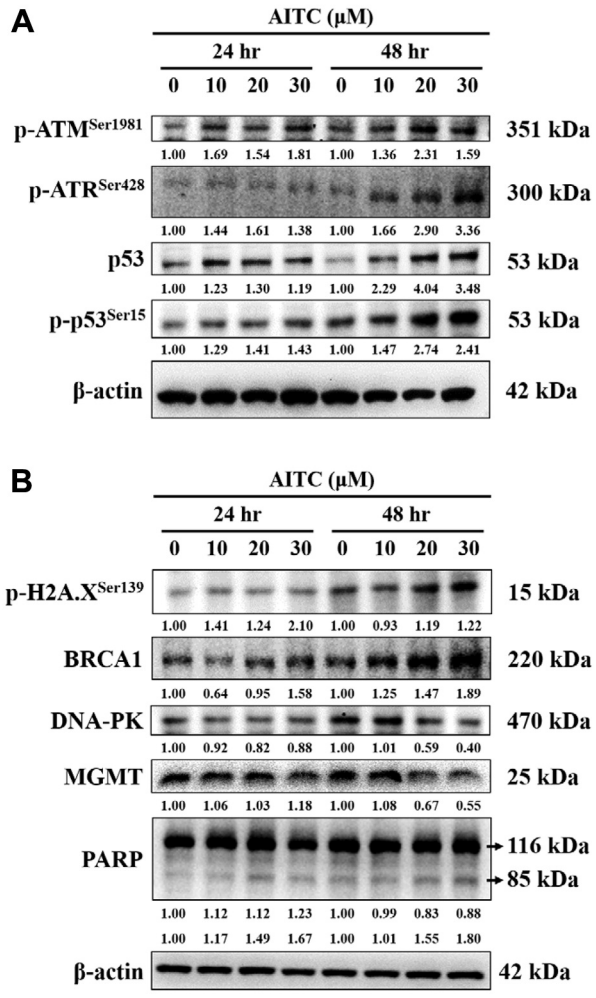

Figure 5. *AITC affected the expressions of DNA damage and repair associated proteins in MCF-7 cells. Cells were incubated with 0, 10, 20, and 30 μM of AITC for 24 and 48 h. Cells were collected for western blotting and the resultant blots were used to probe to anti-p-ATM^{Ser1981}, -p-ATR^{Ser428}, -p53, p-p53^{Ser15} (A); -p-H2A.X^{Ser139}, -BRCA1, -DNA-PK, -MGMT, and -PARP (B) as described in Materials and Methods. β-actin was used as an internal control.*

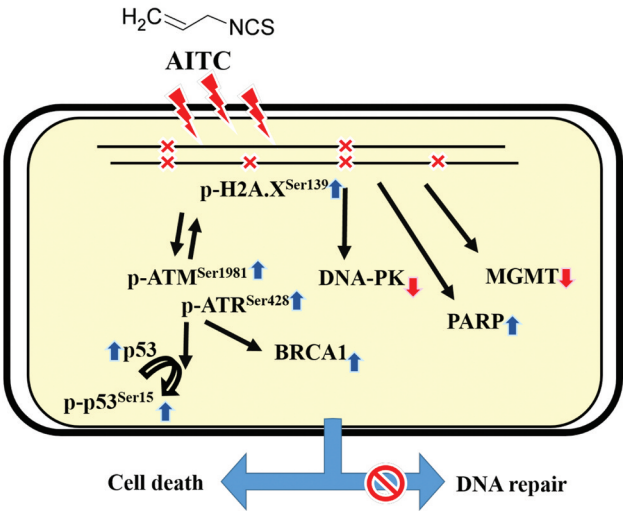

Figure 6. *Proposal of possible signaling pathways for AITC-induced DNA damage and altered repair associated protein expression in MCF-7 cells in vitro.*

to allow the assessment of oxidation of purine and pyrimidine bases (37) and to evaluate cell DNA damage (38) due to it being a simple, sensitive, and low-cost protocol (39). The higher doses of AITC induced a longer comet tail than that of the untreated (control) group. The comet tail length has been recognized to be the most sensitive parameter responding to DNA damage.

We further investigated induction of DNA damage in MCF-7 cells after exposure to AITC which may affect the expression of DNA damage and repair associated proteins *in vitro*. Therefore, after MCF-7 cells were treated with AITC (0, 10, 20, and 30 μM) for 24 and 48 h, cells were collected. Total proteins were quantitated for western blotting and results are presented in Figure 5. Figure 5A indicates that AITC increased the levels of phosphorylated ataxia-telangiectasia-mutated (p-ATM), phosphorylated-Rad3-related (p-ATR), p53, and p-p53 in MCF-7 cells at both treatment times and these effects were dose-dependent. When DNA damage occurs, it will lead to an increase in p53 activation and p53 is linked to DNA-damage-response signaling pathway (40). After DNA double-strand breaks were induced in the cells, they would respond to this damage through ATM, ATR, and DNA-dependent protein kinase (DNA-PK) which would help maintain cell survival (41). DNA-PK, ATM, and ATR have been known to share specific substrates and have some overlapping functions. ATM, ATR, and DNA-PK must be tightly regulated to prevent aberrant activation. Tumor cells rely heavily on ATR for survival; thus, ATR has been focused on the target for anti-cancer therapy. Our results from Figure 5B indicated AITC decreased DNA-PK protein expression in MCF-7 cells in the present study.

AITC increased the expressions of p-H2A.X^{Ser139}, BRCA1, and PARP at 10-30 μM at 24 and 48 h treatment in MCF-7 cells (Figure 5B). BRCA1, p-H2A.X^{Ser139}, and PARP are involved in DNA damage repair systems (42). Moreover, phosphorylation of H2A.X is also a highly specific and sensitive molecular marker for DNA damage response (43). The p-H2A.X^{Ser139} activates ATM, which may phosphorylate p53 and histone H2A.X (γH2AX) (43) to activate ATM kinase in a positive feedback loop (44). The

loss of ATM exhibits a higher predisposition to breast cancer (45). BRCA1 is phosphorylated on multiple residues to respond to DNA damage (46, 47) and the absence of BRCA1 results in increased genomic alterations (48). DNA repair pathways involved in the reaction of DNA damage (49, 50). Results from Figure 5B also showed that AITC decreased DNA-PK at 24 and 48 h treatment and decreased MGMT at 48 h treatment in MCF-7 cells (Figure 5B). DNA-PK and p53 have been reported to be a sensor complex that detects DNA replication disruption (51). DNA-PK is activated by dsDNA breaks (41, 52); thus, it may repair other proteins involved in DNA damage repair systems, such as PARP, which was increased in our case (Figure 5B). PARP plays an impart role in DNA damage and repair responses and genome stability (53), which act as DNA damage sensors and then the activated PARP contributes to cell survival during DNA damage (45).

In conclusion, our findings suggest that AITC decreased the viable cell number (cell viability) of MCF-7 cells *via* DNA damage induction. Western blotting results indicated that AITC altered DNA repair associated protein expression, such as p-ATM^{Ser1981}, p-ATR^{Ser428}, p53, p-p53^{Ser15}, BRCA1, p-H2A.X^{Ser139}, DNA-PK, MGMT, and PARP in MCF-7 cells *in vitro* (Figure 6). Thus, AITC induced DNA damage and altered repair associated protein expression for relevant signaling pathways in MCF-7 cells.

Conflicts of Interest

The Authors confirm that there are no conflicts of interest in regard to this study.

Authors' Contributions

Study conception and design: CLL, PPW, and KIL. Acquisition of data: CLL, JCC, PYC, and ACH. Analysis and interpretation of data: CLL, JCL, FSC, and TAC. Drafting of article: CLL, PPW, and KIL. Critical revision: CLL and KIL. All Authors discussed the results and commented on the article.

Acknowledgements

This study was supported by the Research Funds of China Medical University (CMU104-TC-04) and Chang Bing Show Chwan Memorial Hospital (RD106068), Changhua, Taiwan. Experiments and data analysis were performed in part through the use of the Medical Research Core Facilities Center, Office of Research & Development at China Medical University, Taichung, Taiwan.

References

1 Brinton LA, Sherman ME, Carreon JD and Anderson WF: Recent trends in breast cancer among younger women in the United States. J Natl Cancer Inst *100(22)*: 1643-1648, 2008. PMID: 19001605. DOI: 10.1093/jnci/djn344

2 Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A: Global cancer statistics, 2012. CA Cancer J Clin *65(2)*: 87-108, 2015. PMID: 25651787. DOI: 10.3322/caac.21262

3 Breast Cancer and the Environment. Prioritizing Prevention, Interagency Breast Cancer and Environment Research Coordinating Committee (IBCERCC), 2013. Available at: https://www.niehs.nih.gov/about/assets/docs/breast_cancer_and_the_environment_prioritizing_prevention_508.pdf [Last accessed on July 15, 2021]

4 Colditz GA and Bohlke K: Priorities for the primary prevention of breast cancer. CA Cancer J Clin *64(3)*: 186-194, 2014. PMID: 24647877. DOI: 10.3322/caac.21225

5 Hanahan D and Weinberg RA: Hallmarks of cancer: the next generation. Cell *144(5)*: 646-674, 2011. PMID: 21376230. DOI: 10.1016/j.cell.2011.02.013

6 Chatterjee N and Walker GC: Mechanisms of DNA damage, repair, and mutagenesis. Environ Mol Mutagen *58(5)*: 235-263, 2017. PMID: 28485537. DOI: 10.1002/em.22087

7 Rastogi RP, Richa, Kumar A, Tyagi MB and Sinha RP: Molecular mechanisms of ultraviolet radiation-induced DNA damage and repair. J Nucleic Acids *2010*: 592980, 2010. PMID: 21209706. DOI: 10.4061/2010/592980

8 Papamichos-Chronakis M and Peterson CL: Chromatin and the genome integrity network. Nat Rev Genet *14(1)*: 62-75, 2013. PMID: 23247436. DOI: 10.1038/nrg3345

9 Mehta A and Haber JE: Sources of DNA double-strand breaks and models of recombinational DNA repair. Cold Spring Harb Perspect Biol *6(9)*: a016428, 2014. PMID: 25104768. DOI: 10.1101/cshperspect.a016428

10 Woodbine L, Brunton H, Goodarzi AA, Shibata A and Jeggo PA: Endogenously induced DNA double strand breaks arise in heterochromatic DNA regions and require ataxia telangiectasia mutated and Artemis for their repair. Nucleic Acids Res *39(16)*: 6986-6997, 2011. PMID: 21596788. DOI: 10.1093/nar/gkr331

11 Khadaroo B, Teixeira MT, Luciano P, Eckert-Boulet N, Germann SM, Simon MN, Gallina I, Abdallah P, Gilson E, Géli V and Lisby M: The DNA damage response at eroded telomeres and tethering to the nuclear pore complex. Nat Cell Biol *11(8)*: 980-987, 2009. PMID: 19597487. DOI: 10.1038/ncb1910

12 Ma W, Westmoreland JW, Gordenin DA and Resnick MA: Alkylation base damage is converted into repairable double-strand breaks and complex intermediates in G2 cells lacking AP endonuclease. PLoS Genet *7(4)*: e1002059, 2011. PMID: 21552545. DOI: 10.1371/journal.pgen.1002059

13 Zeman MK and Cimprich KA: Causes and consequences of replication stress. Nat Cell Biol *16(1)*: 2-9, 2014. PMID: 24366029. DOI: 10.1038/ncb2897

14 Herr I and Büchler MW: Dietary constituents of broccoli and other cruciferous vegetables: implications for prevention and therapy of cancer. Cancer Treat Rev *36(5)*: 377-383, 2010. PMID: 20172656. DOI: 10.1016/j.ctrv.2010.01.002

15 Pan JH, Abernathy B, Kim YJ, Lee JH, Kim JH, Shin EC and Kim JK: Cruciferous vegetables and colorectal cancer prevention through microRNA regulation: A review. Crit Rev Food Sci Nutr *58(12)*: 2026-2038, 2018. PMID: 28394633. DOI: 10.1080/10408398.2017.1300134

16 Abbaoui B, Lucas CR, Riedl KM, Clinton SK and Mortazavi A: Cruciferous vegetables, isothiocyanates, and bladder cancer prevention. Mol Nutr Food Res *62(18)*: e1800079, 2018. PMID: 30079608. DOI: 10.1002/mnfr.201800079

17 Kumar G, Tuli HS, Mittal S, Shandilya JK, Tiwari A and Sandhu SS: Isothiocyanates: a class of bioactive metabolites with chemopreventive potential. Tumour Biol *36(6)*: 4005-4016, 2015. PMID: 25835976. DOI: 10.1007/s13277-015-3391-5

18 Mitsiogianni M, Mantso T, Trafalis DT, Vasantha Rupasinghe HP, Zoumpourlis V, Franco R, Botaitis S, Pappa A and Panayiotidis MI: Allyl isothiocyanate regulates lysine acetylation and methylation marks in an experimental model of malignant melanoma. Eur J Nutr *59(2)*: 557-569, 2020. PMID: 30762097. DOI: 10.1007/s00394-019-01925-6

19 Vanduchova A, Anzenbacher P and Anzenbacherova E: Isothiocyanate from Broccoli, Sulforaphane, and Its Properties. J Med Food *22(2)*: 121-126, 2019. PMID: 30372361. DOI: 10.1089/jmf.2018.0024

20 Bo P, Lien JC, Chen YY, Yu FS, Lu HF, Yu CS, Chou YC, Yu CC and Chung JG: Allyl isothiocyanate induces cell toxicity by multiple pathways in human breast cancer cells. Am J Chin Med *44(2)*: 415-437, 2016. PMID: 27080949. DOI: 10.1142/S019 2415X16500245

21 Jiang Z, Liu X, Chang K, Liu X and Xiong J: Allyl isothiocyanate inhibits the proliferation of renal carcinoma cell line GRC-1 by inducing an imbalance between Bcl2 and Bax. Med Sci Monit *22*: 4283-4288, 2016. PMID: 27834342. DOI: 10.12659/MSM.897315

22 Qin G, Li P and Xue Z: Effect of allyl isothiocyanate on the viability and apoptosis of the human cervical cancer HeLa cell line *in vitro*. Oncol Lett *15(6)*: 8756-8760, 2018. PMID: 29805614. DOI: 10.3892/ol.2018.8428

23 Rakariyatham K, Yang X, Gao Z, Song M, Han Y, Chen X and Xiao H: Synergistic chemopreventive effect of allyl isothiocyanate and sulforaphane on non-small cell lung carcinoma cells. Food Funct *10(2)*: 893-902, 2019. PMID: 30694275. DOI: 10.1039/c8fo01914b

24 Zhang Y: Allyl isothiocyanate as a cancer chemopreventive phytochemical. Mol Nutr Food Res *54(1)*: 127-135, 2010. PMID: 19960458. DOI: 10.1002/mnfr.200900323

25 Tripathi K, Hussein UK, Anupalli R, Barnett R, Bachaboina L, Scalici J, Rocconi RP, Owen LB, Piazza GA and Palle K: Allyl isothiocyanate induces replication-associated DNA damage response in NSCLC cells and sensitizes to ionizing radiation. Oncotarget *6(7)*: 5237-5252, 2015. PMID: 25742788. DOI: 10.18632/oncotarget.3026

26 Kuo JH, Shih TY, Lin JP, Lai KC, Lin ML, Yang MD and Chung JG: Cantharidin induces DNA damage and inhibits DNA repair-associated protein expressions in TSGH8301 human bladder cancer cell. Anticancer Res *35(2)*: 795-804, 2015. PMID: 25667459.

27 Ono A, Koshiyama M, Nakagawa M, Watanabe Y, Ikuta E, Seki K and Oowaki M: The preventive effect of dietary antioxidants on cervical cancer development. Medicina (Kaunas) *56(11)*: 604, 2020. PMID: 33182663. DOI: 10.3390/medicina56110604

28 Beckman KB and Ames BN: Oxidative decay of DNA. J Biol Chem *272(32)*: 19633-19636, 1997. PMID: 9289489. DOI: 10.1074/jbc.272.32.19633

29 Helt CE, Cliby WA, Keng PC, Bambara RA and O'Reilly MA: Ataxia telangiectasia mutated (ATM) and ATM and Rad3-related protein exhibit selective target specificities in response to different forms of DNA damage. J Biol Chem *280(2)*: 1186-1192, 2005. PMID: 15533933. DOI: 10.1074/jbc.M410873200

30 Nakamura AJ, Rao VA, Pommier Y and Bonner WM: The complexity of phosphorylated H2AX foci formation and DNA repair assembly at DNA double-strand breaks. Cell Cycle *9(2)*: 389-397, 2010. PMID: 20046100. DOI: 10.4161/cc.9.2.10475

31 Giglia-Mari G, Zotter A and Vermeulen W: DNA damage response. Cold Spring Harb Perspect Biol *3(1)*: a000745, 2011. PMID: 20980439. DOI: 10.1101/cshperspect.a000745

32 Xiao D, Srivastava SK, Lew KL, Zeng Y, Hershberger P, Johnson CS, Trump DL and Singh SV: Allyl isothiocyanate, a constituent of cruciferous vegetables, inhibits proliferation of human prostate cancer cells by causing G2/M arrest and inducing apoptosis. Carcinogenesis *24(5)*: 891-897, 2003. PMID: 12771033. DOI: 10.1093/carcin/bgg023

33 Sávio AL, da Silva GN and Salvadori DM: Inhibition of bladder cancer cell proliferation by allyl isothiocyanate (mustard essential oil). Mutat Res *771*: 29-35, 2015. PMID: 25771977. DOI: 10.1016/j.mrfmmm.2014.11.004

34 Savio AL, da Silva GN, de Camargo EA and Salvadori DM: Cell cycle kinetics, apoptosis rates, DNA damage and TP53 gene expression in bladder cancer cells treated with allyl isothiocyanate (mustard essential oil). Mutat Res *762*: 40-46, 2014. PMID: 24625788. DOI: 10.1016/j.mrfmmm.2014.02.006

35 Sayeed MA, Bracci M, Ciarapica V, Malavolta M, Provinciali M, Pieragostini E, Gaetani S, Monaco F, Lucarini G, Rapisarda V, Di Primio R and Santarelli L: Allyl isothiocyanate exhibits no anticancer activity in MDA-MB-231 breast cancer cells. Int J Mol Sci *19(1)*: 145, 2018. PMID: 29300316. DOI: 10.3390/ijms19010145

36 Rahbar Saadat Y, Saeidi N, Zununi Vahed S, Barzegari A and Barar J: An update to DNA ladder assay for apoptosis detection. Bioimpacts *5(1)*: 25-28, 2015. PMID: 25901294. DOI: 10.15171/bi.2015.01

37 Duthie SJ, Ma A, Ross MA and Collins AR: Antioxidant supplementation decreases oxidative DNA damage in human lymphocytes. Cancer Res *56(6)*: 1291-1295, 1996. PMID: 8640816.

38 Kuchařová M, Hronek M, Rybáková K, Zadák Z, Štětina R, Josková V and Patková A: Comet assay and its use for evaluating oxidative DNA damage in some pathological states. Physiol Res *68(1)*: 1-15, 2019. PMID: 30433808. DOI: 10.33549/physiolres.933901

39 Collins AR: The comet assay for DNA damage and repair: principles, applications, and limitations. Mol Biotechnol *26(3)*: 249-261, 2004. PMID: 15004294. DOI: 10.1385/MB:26:3:249

40 Shiloh Y and Ziv Y: The ATM protein kinase: regulating the cellular response to genotoxic stress, and more. Nat Rev Mol Cell Biol *14(4)*: 197-210, 2013. PMID: 23847781.

41 Blackford AN and Jackson SP: ATM, ATR, and DNA-PK: The Trinity at the heart of the DNA damage response. Mol Cell *66(6)*: 801-817, 2017. PMID: 28622525. DOI: 10.1016/j.molcel.2017.05.015

42 Harper JW and Elledge SJ: The DNA damage response: ten years after. Mol Cell *28(5)*: 739-745, 2007. PMID: 18082599. DOI: 10.1016/j.molcel.2007.11.015

43 Mah LJ, El-Osta A and Karagiannis TC: gammaH2AX: a sensitive molecular marker of DNA damage and repair. Leukemia *24(4)*: 679-686, 2010. PMID: 20130602. DOI: 10.1038/leu.2010.6

44 Friedberg EC: DNA damage and repair. Nature *421(6921)*: 436-440, 2003. PMID: 12540918. DOI: 10.1038/nature01408

45 Swift M, Reitnauer PJ, Morrell D and Chase CL: Breast and other cancers in families with ataxia-telangiectasia. N Engl J

Med *316(21)*: 1289-1294, 1987. PMID: 3574400. DOI: 10.1056/NEJM198705213162101

46 Cortez D, Wang Y, Qin J and Elledge SJ: Requirement of ATM-dependent phosphorylation of brca1 in the DNA damage response to double-strand breaks. Science *286(5442)*: 1162-1166, 1999. PMID: 10550055. DOI: 10.1126/science.286.5442.1162

47 Sung P and Klein H: Mechanism of homologous recombination: mediators and helicases take on regulatory functions. Nat Rev Mol Cell Biol *7(10)*: 739-750, 2006. PMID: 16926856. DOI: 10.1038/nrm2008

48 Takaoka M and Miki Y: BRCA1 gene: function and deficiency. Int J Clin Oncol *23(1)*: 36-44, 2018. PMID: 28884397. DOI: 10.1007/s10147-017-1182-2

49 Ciccia A and Elledge SJ: The DNA damage response: making it safe to play with knives. Mol Cell *40(2)*: 179-204, 2010. PMID: 20965415. DOI: 10.1016/j.molcel.2010.09.019

50 Jackson SP and Bartek J: The DNA-damage response in human biology and disease. Nature *461(7267)*: 1071-1078, 2009. PMID: 19847258. DOI: 10.1038/nature08467

51 Achanta G, Pelicano H, Feng L, Plunkett W and Huang P: Interaction of p53 and DNA-PK in response to nucleoside analogues: potential role as a sensor complex for DNA damage. Cancer Res *61(24)*: 8723-8729, 2001. PMID: 11751391.

52 Jette N and Lees-Miller SP: The DNA-dependent protein kinase: A multifunctional protein kinase with roles in DNA double strand break repair and mitosis. Prog Biophys Mol Biol *117(2-3)*: 194-205, 2015. PMID: 25550082. DOI: 10.1016/j.pbiomolbio.2014.12.003

53 D'Amours D, Desnoyers S, D'Silva I and Poirier GG: Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions. Biochem J *342 (Pt 2)*: 249-268, 1999. PMID: 10455009.

Received April 16, 2021
Revised June 24, 2021
Accepted July 16, 2021