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Acute methylmercury exposure induces inactivation of ATR pathway and enhancement of DNA replication stress through degradation of Rad17

Yasunori Fukumoto*, Dongyue Wang, Yu-ki Tanaka, Noriyuki Suzuki, Yasumitsu Ogra

Graduate School of Pharmaceutical Sciences, Chiba University, Chiba 260-8675, Japan

Abstract

Methylmercury (MeHg) is a neurotoxic chemical of significant public health concern. Acute exposure to MeHg causes inhibition of cellular proliferation and DNA replication in cellular and animal models, and impedes S-phase entry and progression in several types of primary and cultured cells. However, the effect of MeHg on the DNA damage response remains unknown. The ATM and ATR pathways are two major components of the DNA damage response that regulate cell cycle progression. Here we report that acute MeHg exposure enhanced DNA replication stress in S phase. MeHg also inhibited the kinase activity of ATR, suggesting that replication forks collapsed in S phase. Moreover, MeHg promoted the degradation of Rad17 as a possible mechanism underlying the inactivation of ATR. These results suggest a novel connection between acute MeHg exposure and the ATR pathway, providing insight into a yet unidentified mechanism of the inactivation of Rad17 and the ATR pathway.

Keywords : Methylmercury, DNA damage response, DNA replication stress, ATM, ATR, Rad17

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Abbreviations : MeHg, methylmercury; ATM, ataxia telangiectasia mutated; ATR, ATM and Rad3-related; DSB, DNA double-strand break; UV, ultraviolet; 9-1-1 complex, Rad9-Hus1-Rad1 complex

Introduction

ATM- and ATR-dependent checkpoint signaling pathways are two major components of the DNA damage response. The kinase activity of ATM is activated by DNA double-strand breaks (DSBs), leading to the phosphorylation of downstream substrates, including Chk2 and Kap1, to promote DSB repair. Various chemically modified DNA, including DSBs, are converted into single-stranded DNAs. Single-stranded DNA regions in the genome, which are also generated by the inhibition of DNA replication through polymerase-helicase uncoupling, activate the ATR pathway, and the ATR pathway stabilizes arrested replication forks. Thus, the inhibition of ATR leads to the collapse of replication forks, resulting in the conversion of the arrested forks into DSBs. ATR phosphorylates Chk1 and downstream substrates to promote cell cycle arrest [1-3].

Rad17 initiates the activation of the ATR pathway. It recognizes a junction between single-stranded DNA and double-stranded DNA to bind damaged chromatin and interacts with the Rad9-Hus1-Rad1 complex (9-1-1 complex) to load the complex

*Correspondence:
Yasunori Fukumoto, Ph.D.
Graduate School of Pharmaceutical Sciences, Chiba University,
1-8-1 Inohana, Chuo-ku, Chiba 260-8675, Japan
Tel & Fax: +81 43 226 2945
E-mail: fukumoto@faculty.chiba-u.jp

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onto the damaged chromatin [4-6]. The 9-1-1 complex recruits TopBP1, and this interaction activates the kinase activity of ATR [7, 8]. The activated checkpoint proteins are inactivated by dephosphorylation and proteasomal degradation; Rad17 [9], Chk1 [10], and Claspin [11-13] have been reported to undergo proteasome-dependent protein degradation. We recently showed that the degradation of Rad17 is promoted by the nuclear translocation of Rad17 through the N-terminal D-boxes [14].

Methylmercury (MeHg) is a neurotoxic chemical of significant public health concern according to the World Health Organization [15, 16]. Acute MeHg exposure causes inhibition of cellular proliferation and DNA replication in cellular models [17-19], hinders DNA replication in the developing brain of a rat model [19], and impedes S-phase progression in murine erythroleukemia cells [20]. The inhibition of S-phase entry due to acute MeHg exposure has also been observed in primary cerebellar granule and cortical precursor cells, with a reduction in cyclin E protein levels [19]. The p21 protein, an inhibitor of cyclin-dependent kinases, has been suggested to be involved in cell cycle arrest after MeHg exposure [21]. The toxicity of MeHg is at least partly attributed to the generation of reactive oxygen species [22], and oxidative DNA damage is a major type of DNA damage targeted by the ATM and ATR pathways. Yet, little is known about the effects of acute MeHg exposure on the ATM and ATR pathways. Here we demonstrate that acute MeHg exposure enhances DNA replication stress in S phase.

Materials and Methods

Cell culture and plasmids

COS-1 cells were maintained in Dulbecco's modified Eagle medium (DMEM) high glucose (D5796, Sigma-Aldrich) supplemented with 5% bovine serum (16170-078, Gibco) and 1% fetal bovine serum (FB-1290/500, Biosera). Human Rad17 (isoform 1, NCBI NP_579921.1) was cloned in a pcDNA3 vector with an N-terminal flag tag [6].

Antibodies

The following antibodies were used: FLAG (PM020, Medical & Biological Laboratories), Hsc70 (sc7298, Santa Cruz Biotechnology), Chk1 (2360, Cell Signaling Technology), Chk1-pSer345 (2348, Cell Signaling Technology), Chk2-pThr68 (2661, Cell Signaling Technology), Kap1 (A300-274A, Bethyl Laboratories), and Kap1-pSer824 (A300-767A, Bethyl Laboratories).

Effect of MeHg on the ATM pathway

COS-1 cells were seeded at 2×10^5 cells per well in 12-well plates, incubated overnight at 37 °C with 5% CO_2, and treated with 4 mM thymidine for 20 hours. The cells were exposed to 20–40 μM MeHg (M0589, Tokyo Chemical Industry) or 5 mM caffeine for 5 hours in the presence of thymidine, and SDS-lysate was prepared. Mock and thymidine-exposed samples were prepared in duplicate for each experiment.

Effect of MeHg on Chk1

COS-1 cells were seeded at 4×10^5 cells in 35-mm dishes and incubated overnight. The medium was removed, and the cells were irradiated with 30 J/m^2 UV-C (256 nm) at 135 μw/cm^2. The UV-irradiated cells were exposed to 10–40 μM MeHg or 5 mM caffeine for 5 hours, and SDS-lysate was prepared. Samples were prepared in singlicate or duplicate for each experiment.

Effect of MeHg on Rad17

COS-1 cells were seeded at 4×10^5 cells in 35-mm dishes and incubated overnight. Cells were transfected with 1 μg of pcDNA3/flag-Rad17 or pcDNA4-TO vectors with 5 μL Lipofectamine 2000 (11668-019, Invitrogen). Twenty-four hours after transfection, the cells were divided into four wells of a 24-well plate and incubated overnight. The cells were exposed to 10–40 μM MeHg for 6 hours, and SDS-lysate was prepared. Mock-exposed samples were prepared in duplicate for each experiment.

MTS assay

COS-1 cells were seeded at 2×10^4 cells per well in 96-well plates and cultured overnight. The cells were exposed to 20 or 40 μM MeHg for 5 hours. The medium was changed to fresh medium, and 20 μL of CellTiter 96 solution (G3580, Promega) was added to each well. The cells were incubated for 3 hours, and the absorbance at 490 nm was measured using a SpectraMax ABS (Molecular Devices). Cell viability was calculated and presented as mean ± standard deviation.

Results

Acute exposure to MeHg induced caspase-independent cell death in COS-1 cells

We evaluated the effect of acute exposure to MeHg in COS-1 cells, which are derived from the kidney of an African green monkey. This cell line has been immortalized using SV40 large T antigen and is not derived from tumors. The effects of MeHg on cell viability were examined by the MTS assay. The exposure to 20 and 40 μM MeHg decreased cell viability to 63% and 7%, respectively (**Figure 1**). Previous studies have shown that MeHg-induced cell death involves both caspase-dependent and caspase-independent pathways [23, 24]. We examined the effect of caspase inhibitors Z-VAD-FMK and Ac-DEVD-CHO on MeHg-induced cell death. However, these inhibitors did not rescue cell viability (data not shown), suggesting that MeHg-induced cell death is not caspase-dependent under the present experimental conditions.

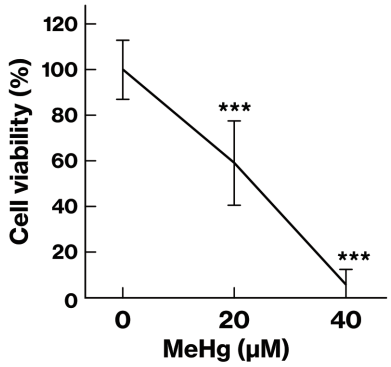

Figure 1. Cell viability analysis by the MTS assay.
COS-1 cells were exposed to 20 or 40 μM MeHg for 5 hours, and cell viability was examined by the MTS assay. The graph represents results from six independent experiments. *P* values were calculated using Student's *t*-test.***, $p < 0.001$.

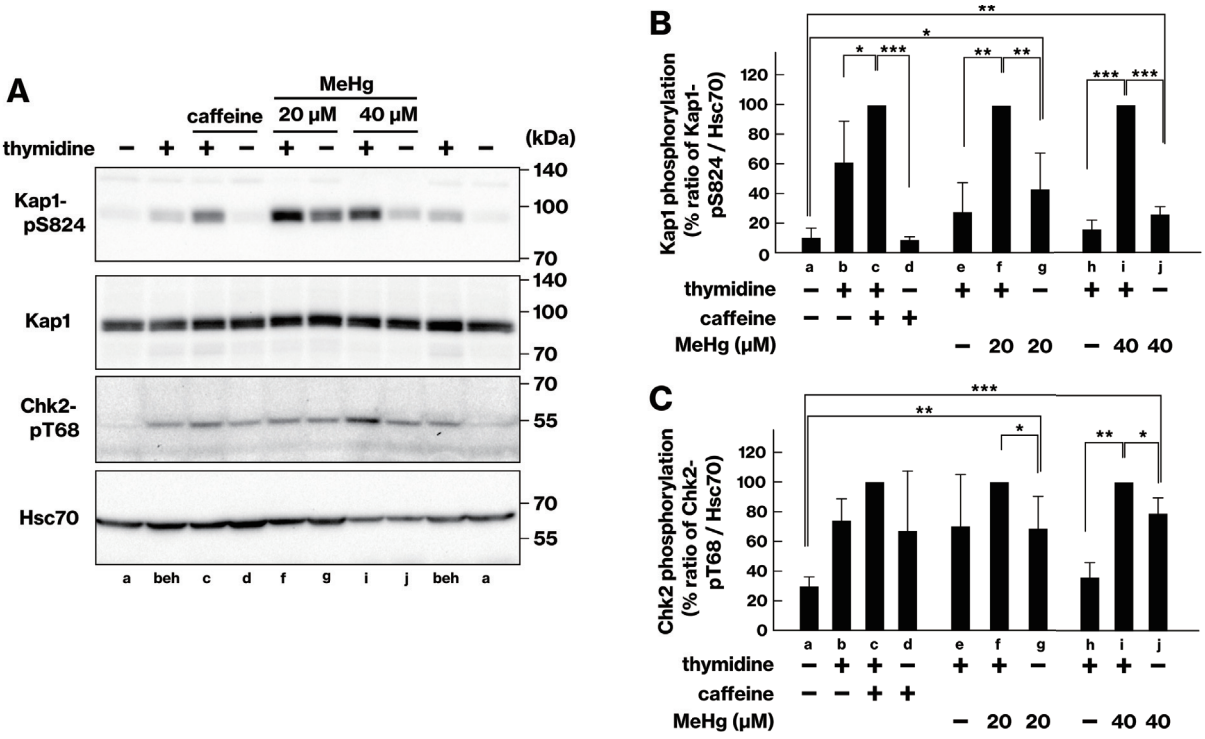

Figure 2. MeHg exposure activated the ATM pathway in the presence of replication stress.
(A) COS-1 cells were exposed to thymidine for 20 hours, followed by exposure to MeHg for 5 hours. SDS-lysate was prepared and probed with the indicated antibodies. Caffeine was used as a positive control.
(B, C) Graphs represent results from more than three independent experiments. *P* values were calculated using a paired *t*-test. *, $p < 0.05$. **, $p < 0.01$. ***, $p < 0.001$.

MeHg exposure activated the ATM pathway in the presence of replication stress

We evaluated the effect of MeHg on the ATM pathway. ATM phosphorylates Kap1-S824 in response to DSBs [25]. Thymidine exposure induces replication stress through the depletion of dCTP [26]. The thymidine-exposed cells showed a significant increase in Kap1 and Chk2 phosphorylation (**Figures 2A–C**), consistent with our previous study [27]. The thymidine-induced increase in Kap1 phosphorylation was further enhanced by caffeine exposure (**Figure 2B, lanes b and c**). Previous studies have reported that caffeine inhibits ATR and ATM *in vitro* but acts as an activator of these kinases *in vivo* [27, 28]. This discrepancy is attributed to the fact that the inhibition of ATR under replication stress results in replication fork collapse to activate ATM (**Figure 5**). MeHg exposure also induced Kap1 and Chk2 phosphorylation (**Figures 2B, C, lanes g and j**). Co-exposure to MeHg and thymidine resulted in significantly higher phosphorylation of Kap1 and Chk2 (**Figures 2B, C, lanes f and i**). In other words, MeHg and thymidine had a synergistic effect on Kap1 and Chk2 phosphorylation (**Figure 2B, lanes f and i**), suggesting that MeHg exposure enhances the kinase activity of ATM. Our anti-Chk2 antibody did not detect the Chk2 protein in COS-1 cells (data not shown). These results raised the possibility that the arrested replication forks due to thymidine exposure were converted into DSBs through replication fork collapse upon MeHg exposure.

MeHg exposure inactivated the ATR-Chk1 pathway

The synergistic effect of MeHg and thymidine raised the possibility that MeHg exposure might have led to the inactivation of the ATR pathway. We examined the effect of MeHg on the ATR pathway following UV irradiation. Chk1-S345 is a well-known ATR-dependent phosphorylation site [1]. As reported previously, UV irradiation induced Chk1 phosphorylation in COS-1 cells, which was suppressed by caffeine exposure (**Figures 3A, B**). These results suggest that the kinase activity of ATR was inhibited by caffeine. Moreover, MeHg exposure decreased Chk1 phosphorylation (**Figures 3C, D**), suggesting that MeHg inhibits ATR-dependent Chk1 phosphorylation and induces replication fork collapse by inhibiting ATR in the presence of replication stress (**Figure 2**).

MeHg exposure promoted degradation of Rad17

We examined the effect of MeHg on the components of the ATR pathway to elucidate the mechanism by which MeHg inactivates ATR-Chk1 signaling. Chk1 has been reported to undergo proteasome-dependent protein degradation [10]; however, MeHg exposure did not decrease the amount of Chk1 (**Figure 3C**). Rad17 is essential for activating and maintaining the ATR-Chk1 pathway. We examined the amount of Rad17 using an exogenous flag-Rad17 protein, as our anti-Rad17 antibody did

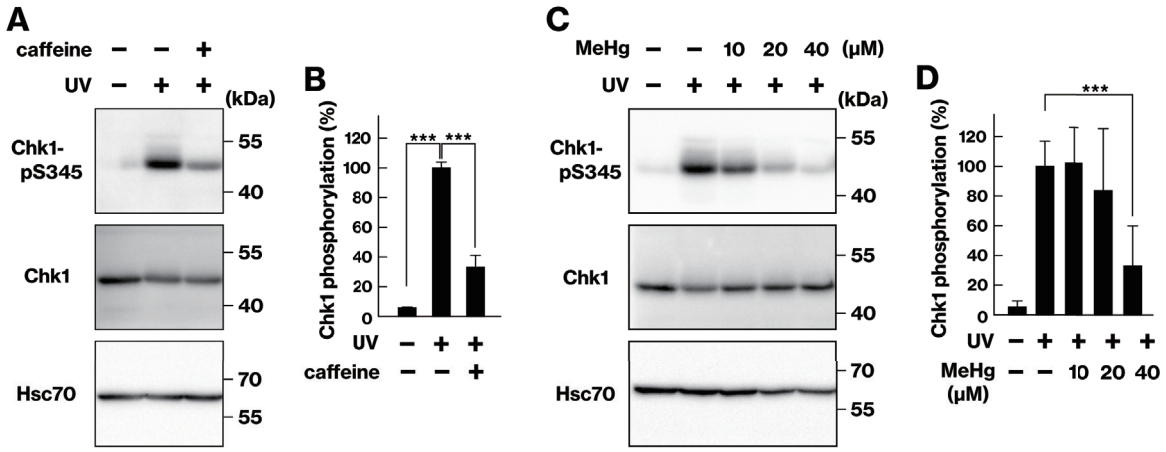

Figure 3. MeHg exposure inactivated the ATR-Chk1 pathway.
COS-1 cells were irradiated with 30 J/m^2 UV-C, and the cells were recovered for 5 hours in the presence of caffeine (A, B) or MeHg (C, D). SDS-lysate was prepared and probed with the indicated antibodies. Graphs represent results from two (B, n = 3) or three (D) independent experiments. Ratios of Chk1-pS345/Hsc70 are shown (B, D). *P* values were calculated using Student's or Welch's *t*-test. ***, $p < 0.001$.

not detect endogenous Rad17. Exposure of COS-1 cells to 20 or 40 μM MeHg decreased the amount of Rad17 to 87% and 37%, respectively (**Figure 4**). We also examined the effect of MeHg on Rad17 K/R359–363A mutant that shows resistance to proteasomal degradation [14]. The amount of Rad17 K/R359–363A mutant was also reduced after MeHg exposure (data not shown). In our previous work, flag-Rad17 wild type and K/R359–363A mutant showed a half-life of 7.9 and 34.7 hours, respectively [14]. Given that MeHg exposure reduced the amount of Rad17 within 5 hours, it is likely that the decrease in Rad17 was due to protein degradation and not the inhibition of transcription or translation. These findings suggest that MeHg exposure inactivates the ATR pathway through Rad17 degradation. Moreover, as the exposure to 10 μM MeHg increased the amount of Rad17 (**Figure 4**), MeHg-induced cellular stress may upregulate Rad17 before degradation.

We also examined the effect of Z-VAD-FMK, a pan-caspase inhibitor, on the MeHg-induced Rad17 degradation. However, Z-VAD-FMK did not prevent Rad17 degradation (data not shown). The mechanism underlying the degradation of Rad17 remains to be elucidated.

Discussion

MeHg is classified by the International Agency for Research on Cancer as a Group 2B compound, indicating its potential carcinogenicity in humans. MeHg exposure has been shown to induce DNA strand breaks [29-33], mutations [34], and chromosomal abnormalities [32] in cellular models. The possible mechanisms include direct oxidation of the DNA and inhibition of DNA repair machinery. In this study, we have demonstrated that acute exposure to MeHg inhibits the ATR pathway (**Figure 3**) and also induces DSBs (**Figure 2**). Our findings suggest that that the inhibition of ATR-dependent DNA damage responses may also be a possible mechanism underlying HeMg-induced DNA strand breaks and mutations.

We examined the effect of acute MeHg exposure on the ATR pathway several hours later. Despite a decrease in the MTS score to 7%, the expression of proteins remained unaffected (**Figures 2–4**). The MTS assay monitors mitochondrial activity, and it has been demonstrated that MeHg inhibits mitochondrial function [35]. We speculate that MeHg suppresses mitochondrial function before the onset of cell death. Previous studies have shown that exposure to MeHg can cause chromosome abnormalities in experimental animals [36, 37]. Under physiological conditions, chronic exposure to MeHg may inhibit the ATR pathway, resulting in the accumulation of chromosome abnormalities observed in experimental animals.

Previous studies have reported that the ATR pathway prevents apoptotic responses upon replication stress and UV irradiation [38, 39]. ATR also plays a direct anti-apoptotic role in mitochondria [40]. Our results show that MeHg exposure leads to Rad17 degradation (**Figure 4**), which inactivates the ATR pathway (**Figure 3**). In the presence of replication stress, the ATR pathway stabilizes stalled replication forks. However, the inactivation of ATR can cause stalled replication forks to collapse, leading to the formation of DSBs, which are the most cytotoxic type of DNA lesion [1, 2] (**Figure 5**). Because exposure to MeHg caused replication stress and DSBs by deactivating ATR (**Figures 2, 3**), the inactivation of ATR and the presence of DSBs contributed to cell death, in addition to other cytotoxic effects of MeHg.

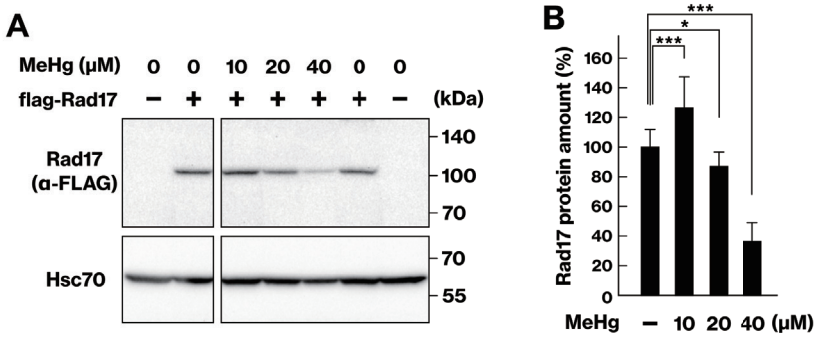

Figure 4. **MeHg exposure promoted Rad17 degradation.**

(A) COS-1 cells were transfected with flag-Rad17. Forty-eight hours after transfection, the cells were exposed to 10–40 μM MeHg for 6 hours. SDS-lysate was prepared and probed with the indicated antibodies. All lanes were obtained from the same blot.

(B) The graph represents results from more than three independent experiments. Rad17 protein amount is shown as the ratio of FLAG/Hsc70. *P* values were calculated using Student's *t*-test. *, $p < 0.05$. ***, $p < 0.001$.

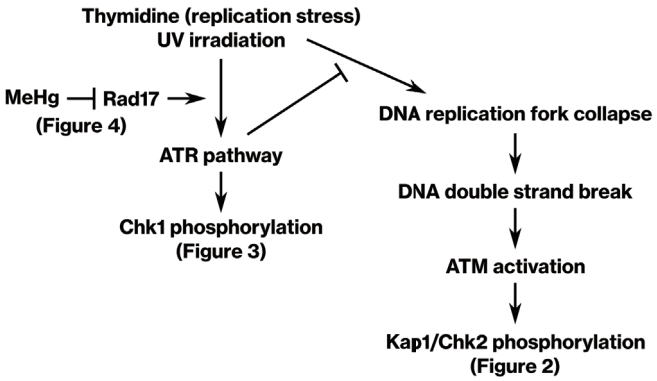

Figure 5. MeHg exposure inactivates the ATR pathway, leading to the induction of DSBs.
Thymidine exposure, which induces replication stress, and UV irradiation activate the ATR pathway, leading to the stabilization of stalled replication forks. If the ATR pathway is inactivated, the stalled replication forks collapse and are converted into DSBs, which activates the ATM pathway. In the diagram depicted, exposure to MeHg degrades Rad17, which in turn prevents ATR activation.

Rad17 is an important activator and regulator of the ATR pathway. Our results revealed a novel connection between acute MeHg exposure and the ATR pathway. Cell cycle progression resumes after DNA damage repair, which is not a spontaneous but an actively regulated process that involves the inactivation of DNA damage checkpoints. Our findings also suggest the presence of an unidentified mechanism of Rad17 degradation leading to the inactivation of the ATR pathway.

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CRediT Author Statement

Y. F.: conceptualization, methodology, formal analysis, investigation, resources, writing—original draft, visualization, project administration, funding acquisition. D. W.: formal analysis, investigation, visualization. Y. T. and N. S.: resources, supervision, funding acquisition. Y. O.: resources, writing—review and editing, supervision, project administration, funding acquisition.

References

[1] Cimprich KA, Cortez D. ATR: an essential regulator of genome integrity. *Nat Rev Mol Cell Biol.* 2008;9(8):616-27.

[2] Saldivar JC, Cortez D, Cimprich KA. The essential kinase ATR: ensuring faithful duplication of a challenging genome. *Nat Rev Mol Cell Biol.* 2017;18(10):622-36.

[3] Blackford AN, Jackson SP. ATM, ATR, and DNA-PK: The Trinity at the Heart of the DNA Damage Response. *Mol Cell.* 2017;66(6):801-17.

[4] Zou L, Cortez D, Elledge SJ. Regulation of ATR substrate selection by Rad17-dependent loading of Rad9 complexes onto chromatin. *Genes Dev.* 2002;16(2):198-208.

[5] Bermudez VP, Lindsey-Boltz LA, Cesare AJ, Maniwa Y, Griffith JD, Hurwitz J, et al. Loading of the human 9-1-1 checkpoint complex onto DNA by the checkpoint clamp loader hRad17-replication factor C complex in vitro. *Proc Natl Acad Sci U S A.* 2003;100(4):1633-8.

[6] Fukumoto Y, Ikeuchi M, Nakayama Y, Yamaguchi N. The KYxxL motif in Rad17 protein is essential for the interaction with the 9-1-1 complex. *Biochem Biophys Res Commun.* 2016;477(4):982-7.

[7] Kumagai A, Lee J, Yoo HY, Dunphy WG. TopBP1 activates the ATR-ATRIP complex. *Cell.* 2006;124(5):943-55.

[8] Delacroix S, Wagner JM, Kobayashi M, Yamamoto K, Karnitz LM. The Rad9-Hus1-Rad1 (9-1-1) clamp activates checkpoint signaling via TopBP1. *Genes Dev.* 2007;21(12):1472-7.

[9] Zhang L, Park CH, Wu J, Kim H, Liu W, Fujita T, et al. Proteolysis of Rad17 by Cdh1/APC regulates checkpoint termination and recovery from genotoxic stress. *EMBO J.* 2010;29(10):1726-37.

[10] Zhang YW, Otterness DM, Chiang GG, Xie W, Liu YC, Mercurio F, et al. Genotoxic stress targets human Chk1 for degradation by the ubiquitin-proteasome pathway. *Mol Cell.* 2005;19(5):607-18.

[11] Mailand N, Bekker-Jensen S, Bartek J, Lukas J. Destruction of Claspin by SCFbetaTrCP restrains Chk1 activation and facilitates recovery from genotoxic stress. *Mol Cell.* 2006;23(3):307-18.

[12] Mamely I, van Vugt MA, Smits VA, Semple JI, Lemmens B, Perrakis A, et al. Polo-like kinase-1 controls proteasome-dependent degradation of Claspin during checkpoint recovery. *Curr Biol.* 2006;16(19):1950-5.

[13] Peschiaroli A, Dorrello NV, Guardavaccaro D, Venere M, Halazonetis T, Sherman NE, et al. SCFbetaTrCP-mediated degradation of Claspin regulates recovery from the DNA replication checkpoint response. *Mol Cell.* 2006;23(3):319-29.

[14] Fukumoto Y, Ikeuchi M, Qu L, Hoshino T, Yamaguchi N, Nakayama Y, et al. Nuclear translocation promotes proteasomal degradation of human Rad17 protein through the N-terminal destruction boxes. *J Biol Chem.* 2021;297(2):100831.

[15] Mercury and health. [Available from: https://www.who.int/news-room/fact-sheets/detail/mercury-and-health]

[16] Lee JY, Hwang GW, Naganuma A, Satoh M. Methylmercury toxic mechanism related to protein degradation and chemokine transcription. *Environ Health Prev Med.* 2020;25(1):30.

[17] Frenkel GD, Randles K. Specific stimulation of alpha-amanitin-sensitive RNA synthesis in isolated HeLa nuclei by methyl mercury. *J Biol Chem.* 1982;257(11):6275-9.

[18] Gruenwedel DW, Cruikshank MK. Effect of methylmercury (II) on the synthesis of deoxyribonucleic acid, ribonucleic acid and protein in HeLa S3 cells. *Biochem Pharmacol.* 1979;28(5):651-5.

[19] Burke K, Cheng Y, Li B, Petrov A, Joshi P, Berman RF, et al. Methylmercury elicits rapid inhibition of cell proliferation in the developing brain and decreases cell cycle regulator, cyclin E. *Neurotoxicology.* 2006;27(6):970-81.

[20] Zucker RM, Elstein KH, Easterling RE, Massaro EJ. Flow cytometric analysis of the mechanism of methylmercury cytotoxicity. *Am J Pathol.* 1990;137(5):1187-98.

[21] Mendoza MA, Ponce RA, Ou YC, Faustman EM. p21(WAF1/CIP1) inhibits cell cycle progression but not G2/M-phase transition following methylmercury exposure. *Toxicol Appl Pharmacol.* 2002;178(2):117-25.

[22] Reardon AM, Bhat HK. Methylmercury neurotoxicity: Role of oxidative stress. *Toxicological & Environmental Chemistry.* 2007;89(3):535-54.

[23] Ceccatelli S, Dare E, Moors M. Methylmercury-induced neurotoxicity and apoptosis. *Chem Biol Interact.* 2010;188(2):301-8.

[24] Novo JP, Martins B, Raposo RS, Pereira FC, Oria RB, Malva JO, et al. Cellular and Molecular Mechanisms Mediating Methylmercury Neurotoxicity and Neuroinflammation. *Int J Mol Sci.* 2021;22(6).

[25] Ziv Y, Bielopolski D, Galanty Y, Lukas C, Taya Y, Schultz DC, et al. Chromatin relaxation in response to DNA double-strand breaks is modulated by a novel ATM- and KAP-1 dependent pathway. *Nat Cell Biol.* 2006;8(8):870-6.

[26] Bjursell G, Reichard P. Effects of thymidine on deoxyribonucleoside triphosphate pools and deoxyribonucleic acid synthesis in Chinese hamster ovary cells. *J Biol Chem.* 1973;248(11):3904-9.

[27] Fukumoto Y, Miura T, Morii M, Kubota S, Honda T, Kubota S, et al. v-Src inhibits the interaction between Rad17 and Rad9 and induces replication fork collapse. *Biochem Biophys Res Commun.* 2014;450(1):885-90.

[28] Cortez D. Caffeine inhibits checkpoint responses without inhibiting the ataxia-telangiectasia-mutated (ATM) and ATM- and Rad3-related (ATR) protein kinases. *J Biol Chem.* 2003;278(39):37139-45.

[29] Williams MV, Winters T, Waddell KS. In vivo effects of mercury (II) on deoxyuridine triphosphate nucleotidohydrolase, DNA polymerase (alpha, beta), and uracil-DNA glycosylase activities in cultured human cells: relationship to DNA damage, DNA repair, and cytotoxicity. *Mol Pharmacol.* 1987;31(2):200-7.

[30] Ben-Ozer EY, Rosenspire AJ, McCabe MJ, Jr., Worth RG, Kindzelskii AL, Warra NS, et al. Mercuric chloride damages cellular DNA by a non-apoptotic mechanism. *Mutat Res.* 2000;470(1):19-27.

[31] Ryu TH, An KG, Kim JK. Genotoxicity in earthworm after combined treatment of ionising radiation and mercury. *Radiat Prot Dosimetry.* 2014;159(1-4):111-7.

[32] Attia SM, Ahmad SF, Nadeem A, Attia MSM, Ansari MA, Al-Hamamah MA, et al. Multiple exposure to methylmercury aggravates DNA damage in the BTBR T (+) Itpr3 (tf)/J autistic mouse model: The role of DNA repair efficiency. *Toxicology.* 2022;477:153277.

[33] Pieper I, Wehe CA, Bornhorst J, Ebert F, Leffers L, Holtkamp M, et al. Mechanisms of Hg species induced toxicity in cultured human astrocytes: genotoxicity and DNA-damage response. *Metallomics.* 2014;6(3):662-71.

[34] Wang X, Yan M, Zhao L, Wu Q, Wu C, Chang X, et al. Low-Dose Methylmercury-Induced Apoptosis and Mitochondrial DNA Mutation in Human Embryonic Neural Progenitor Cells. *Oxid Med Cell Longev.* 2016;2016:5137042.

[35] Carratu MR, Signorile A. Methyl Mercury Injury to CNS: Mitochondria at the Core of the Matter? *Open Access J of Tox.* 2015;1(1):555551.

[36] Ghosh AK, Sen S, Sharma A, Talukder G. Effect of chlorophyllin on mercuric chloride-induced clastogenicity in mice. *Food Chem Toxicol.* 1991;29(11):777-9.

[37] Miller CT, Zawidzka Z, Nagy E, Charbonneau SM. Indicators of genetic toxicity in leucocytes and granulocytic precursors after chronic methylmercury ingestion by cats. *Bull Environ Contam Toxicol.* 1979;21(3):296-303.

[38] Myers K, Gagou ME, Zuazua-Villar P, Rodriguez R, Meuth M. ATR and Chk1 suppress a caspase-3-dependent apoptotic response following DNA replication stress. *PLoS Genet.* 2009;5(1):e1000324.

[39] Heffernan TP, Kawasumi M, Blasina A, Anderes K, Conney AH, Nghiem P. ATR-Chk1 pathway inhibition promotes apoptosis after UV treatment in primary human keratinocytes: potential basis for the UV protective effects of caffeine. *J Invest Dermatol.* 2009;129(7):1805-15.

[40] Hilton BA, Li Z, Musich PR, Wang H, Cartwright BM, Serrano M, et al. ATR Plays a Direct Antiapoptotic Role at Mitochondria, which Is Regulated by Prolyl Isomerase *Pin1. Mol Cell.* 2015;60(1):35-46.