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Original Article

The suppression of ATR/Chk1 pathway by Elimusertib ATR inhibitor in triple negative breast cancer cells

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Abstract: Objectives: Genomic instability in cancer cells is based on the aberrant activation of deoxyribonucleic acid (DNA) damage response and repair mechanisms. Targeting Ataxia telangiectasia and Rad3-related (ATR) inhibition in cancer treatment have attracted attention in recent years. In the current study, we aimed for the first time to determine the anti-cancer effects of Elimusertib, an ATR inhibitor, on triple negative breast cancer (TNBC). Methods: The cytotoxic and apoptotic effects of Elimusertib were analyzed by Water-Soluble Tetrazolium 1 (WST-1), Annexin V, cell cycle and acridine orange/propidium iodide staining. Furthermore, Elimusertib induced mitochondrial damage and the intracellular reactive oxygen species were evaluated. Additionally, the inhibition of ATR-Checkpoint kinase 1 (Chk1) DNA damage response and the induction of apoptotic death was analyzed by western blot analysis. Results: Our preliminary findings revealed that Elimusertib significantly decreased the viability of MDA-MB-231 TNBC cells with toxicity in MCF-10A cells ($P<0.05$). Elimusertib caused apoptotic death through gap phase (G0)/growth 1 phase (G1) accumulation, caspase-3 activity and mitochondrial damage. Additionally, Elimusertib significantly suppressed the ATR-based DNA damage response and mediated cell cycle checkpoint. Conclusions: Our findings suggest that Elimusertib suppresses the ATR-based Chk1 pathway in TNBC cells. Therefore, ATR inhibition by Elimusertib could be a potential therapeutic strategy especially in tumor protein p53 (*p53)* mutant TNBC patients.

Keywords: DNA damage response, ATR, cell cycle, apoptosis, triple negative breast cancer

Introduction

Triple negative breast cancer (TNBC) constitutes 15-20% of breast cancer cases with aggressive clinical features. TNBC tends to metastasize easily and has a higher recurrence rate within three years after treatment and a higher mortality rate within five years than other breast cancer subtypes [1, 2]. Surgery and systemic chemotherapy are the main treatment modalities in the treatment of TNBC. However, the heterogeneity and lack of expression of the estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) genes of TNBC tumors cause a low response rate to endocrine therapy and targeted molecular therapies used in the treatment of breast cancers [1-3]. Therefore, targeted innovative treatment strategies are needed in the treatment of patients with TNBC.

In more than 50% of TNBC tumors, tumor protein p53 (p53) signaling is inactive, and loss of Retinoblastoma (RB) results in an inactive growth 1 phase (G1)/Synthesis Phase (S) checkpoint. Oncogene activation and dysfunction of G1/S checkpoint induce the formation of excessive single-strand deoxyribonucleic acid (DNA) breaks and the transition of cells into S phase with accumulated DNA damage. Therefore, Ataxia telangiectasia and Rad3-related protein (ATR)-Checkpoint kinase 1 (Chk1)-based DNA damage response has a promising therapeutic target for the treatment of TNBC patients [4-7]. ATR activity is the main DNA damage pathway and mediates DNA damage response under persistent replication stress. Therefore, the inhibition of the ATR-Chk1 pathway in cancer cells with loss of the G1 checkpoint appears to be an innovative treatment strategy for the treatment of TNBC [8, 9].

To date, clinical phase studies of Berzosertib (M6620, VX-970, VE-822), M4344 (VX-803), Ceralasertib (AZD6738) and Elimusertib (BAY-

1895344) as ATR inhibitors in the treatment of different cancer types are ongoing [10, 11]. Elimusertib is an ATR kinase inhibitor with high antitumor activity as a single agent in preclinical studies of different cancer types, including prostate, colorectal and lymphoma [10, 12]. However, there is no comprehensive study evaluating the therapeutic effects of Elimusertib on the treatment of TNBC and its effect on ATR-based DNA damage response at the molecular level.

In this context, we, for the first time, assessed Elimusertib-induced anti-cancer activity in *p53* mutant TNBC cells and revealed the underlying molecular mechanism of ATR/Chk1-based DNA damage response and cell cycle checkpoint following Elimusertib treatment.

Materials and methods

Cell lines

The *p53* mutant human TNBC cell line MDA-MB-231 and human breast epithelial cell line MCF-10A were purchased from American Type Culture Collection (ATCC) (USA). The cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) (MDA-MB-231) and Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM F-12) (MCF-10A) medium (Gibco; Thermo Fisher Scientific, USA) supplemented with 10% fetal bovine serum (FBS, Gibco) and 1% penicillin/streptomycin (Gibco). DMEM F-12 media was further 100 mg/ml Epidermal growth factor (EGF) (Biovision, San Francisco, CA, USA), 1 mg/ml hydrocortisone (Biovision, San Francisco, CA, USA), 10 mg/ml insulin (Sigma Aldrich, USA). Elimusertib was obtained from Selleck Chemicals (Houston, TX, USA) and dissolved in dimethyl sulfoxide.

Cell viability assay

The cells were seeded at a cell density of 2×10^4 and treated with Elimusertib (1-10 nM) for 24-96 h. Water-Soluble Tetrazolium 1 (WST-1) reagent (Biovision, San Francisco, CA, USA) was added to each well to determine cell viability. The absorbance of the WST-1 reagent was analyzed at 450 nm via a microplate reader (Allsheng, China).

Annexin V and cell cycle assay

The cells were seeded at a cell density of $1\text{-}5\times10^5$ and treated with the most effective concentrations (6 and 8 nM) for 72 and 96 h. After each treatment endpoint, Annexin V & Dead Cell Assay kit (Luminex Corporation, Austin, Texas, USA) was performed. The cells were stained with Muse™ Cell Cycle Kit (Luminex Corporation) for cell cycle analysis. The percentage of apoptotic death and cell cycle phase accumulation was analyzed by the Muse Cell Analyzer (Millipore, Germany).

Dual staining of acridine orange/propidium Iodide (AO/PI)

The cells were seeded at a cell density of 5×10^5 cells/well and treated with the most effective concentrations (6 and 8 nM) for 72 and 96 h. The cells were fixed with 4% paraformaldehyde (PFA) and then stained by AO (100 mg/mL) (Sigma Aldrich, USA) and PI solution (1 mg/mL) (Sigma Aldrich, USA). Fluorescent signals were observed by EVOS FL Cell Imaging System (Thermo Fisher Scientific, USA).

Oxidative stress assays and Mitotracker staining

To assess the changes the intracellular level of reactive oxygen species (ROS) and mitochondrial damage, the cells were seeded at a cell density of 5×10^5 cells and treated with the most effective concentrations (6 and 8 nM) for 72 and 96 h. Following treatment, the cells were stained with Muse Oxidative Stress Assay (Luminex Corporation) according to the protocol and analyzed with Muse Cell Analyzer (Millipore). Additionally, the cells were stained with MitoTracker Red CMXRos (Thermo Fisher Scientific) for 30 min at 37°C and observed with EVOS Floid Cell Imaging System (Thermo Fisher Scientific, USA).

Western blot analysis

After treatment with Elimusertib as described above, Radioimmunoprecipitation assay buffer (RIPA buffer) (Santa Cruz, USA) was used for protein isolation. Following isolation, the concentrations of proteins were analyzed by Qubit 4 Fluorometer (Thermo Fisher Scientific, USA). The obtained proteins were separated through Bolt™ 4-12%, Bis-Tris, 1.0 mm, Mini Protein Gels (Thermo Fisher Scientific, USA) and transferred to iBlot™ Transfer Stack nitrocellulose membrane (Thermo Fisher Scientific, USA) by iBlot 2 Gel Transfer Device (Invitrogen). Then, iBind Flex Card (Thermo Fisher Scientific, USA)

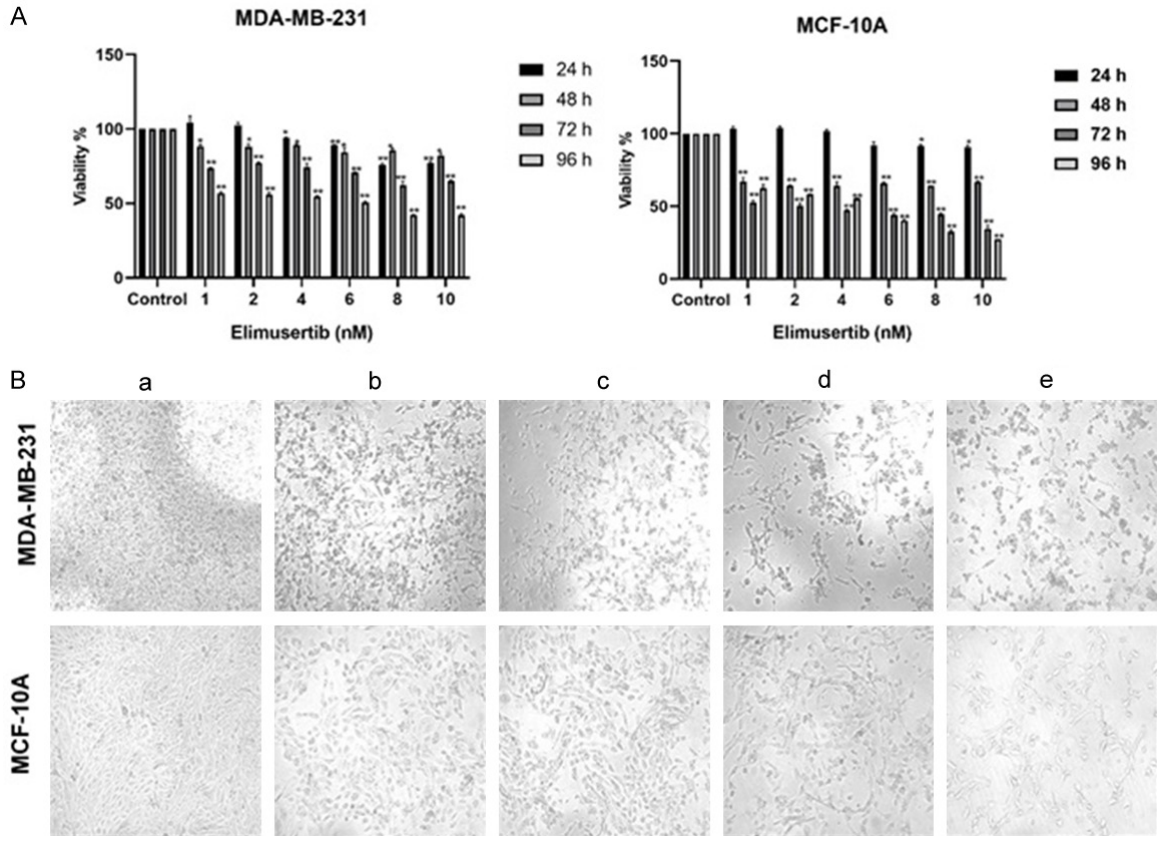

Figure 1. (A) WST-1 assay of MDA-MB-231 and MCF-10A cells after 1-10 nM Elimusertib for 24, 48, 72 and 96 h (P<0.05*, P<0.01**). (B) Representative inverted microscopy images of the cells treated with Elimusertib (a) Control, (b) 6 nM and (c) 8 nM for 72 h, (d) 6 nM and (e) 8 nM for 96 h.

was used for incubation with primary (anti-mouse ATR (1:1000), anti-mouse H2A histone family member X (γH2AX) (1:500), anti-rabbit p-Chk1 (1:1000), anti-rabbit p-Cdc2/Cyclin-dependent kinase 1 (Cdk1) (pTyr15) (0.2 μg/mL), anti-rabbit cyclin B1 (1:500), anti-mouse-p-Rb (1:250), anti-mouse cyclin D (1:250), anti-mouse Poly (ADP-ribose) polymerase (PARP) (1:1000), anti-rabbit caspase-9 (1:1000), anti-rabbit caspase 3 (1:1000), anti-rabbit glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:1000) and secondary antibodies (Goat anti-mouse and anti-rabbit Immunoglobulin G (IgG) (H+L) HRP) and the incubation was performed with an iBind Flex Western Device (Thermo Fisher Scientific, USA) for at least 3 h. The antibodies were prepared with an iBind Flex Solution Kit (Thermo Fisher Scientific, USA). SuperSignal West Pico PLUS chemiluminescent substrate was used for chemiluminescent detection and observed by a chemiluminescent system (Syngene, USA).

Statistical analysis

All results were expressed as the mean ± SD. Statistical analyses, Student's t-test or one-way Analysis of Variance (ANOVA) by Post-Hoc Tukey test, were performed using GraphPad Prism 8 (GraphPad Software Inc., California San Diego, USA). P<0.05 was considered significant (P<0.05*, P<0.01**).

Results

Identifying the effective concentrations of Elimusertib for TNBC cells

To assess the cytotoxicity of Elimusertib, WST-1 analysis was performed. Our findings demonstrated that Elimusertib exerted considerable cytotoxic effects on MDA-MB-231 cells in a dose and time-dependent manner in **Figure 1A** and **1B**. Whereas the proliferation of MDA-MB-231 cells was 70.8 ± 0.2% and 62.2 ± 2.5% at 6 and 8 nM for 72 h, respectively (P<0.05),

the viability of these cells reduced to 50.8 ± 0.9% and 42.2 ± 0.3%, respectively for 96 h (**Figure 1A**). On the other hand, Elimusertib induced toxicity in MCF-10A cells. The survival of MCF-10A cells decreased to 44.0 ± 1.2% and 44.5 ± 0.9% at 6 and 8 nM for 72 h, respectively ($P<0.05$), whereas a remarkable reduction (59.8 ± 0.8% and 68.5 ± 1.4%, respectively) was observed in the proliferation of MCF-10A cells for 96 h. The IC_{50} concentrations of Elimusertib were 11.08 ± 1.46 nM and 6.26 ± 0.25 nM for 72 h and 96 h, respectively, in MDA-MB-231 cells. Therefore, Elimusertib exerted more anti-cancer activity in MDA-MB-231 cells at 6 and 8 nM for 72 and 96 h and we selected these concentrations and incubation times for further analysis.

Evaluation of Elimusertib-induced apoptotic effects on TNBC cells

To evaluate Elimusertib-induced apoptotic death, we performed Annexin V, cell cycle analysis and AO/PI staining. In **Figure 2A**, Elimusertib treatment significantly increased the percentage of apoptotic death in a dose and time-dependent manner. Whereas the rate of total apoptotic death increased to 25.1 ± 2.2% and 28.1 ± 2.2%, respectively, following treatment of 6 and 8 nM Elimusertib for 72 h, a significant increase (40.9 ± 1.7% and 61.7 ± 1.2%, respectively) was detected for 96 h in MDA-MB-231 cells ($P<0.01$, **Figure 2B**). On the other hand, Elimusertib caused apoptotic death in MCF-10A cells (59.5 ± 0.8% and 63.7 ± 0.7%, respectively, for 96 h). The obtained findings were supported by AO/PI staining. We observed DNA fragmentation and apoptotic bodies after Elimusertib treatment for especially 96 h in MDA-MB-231 cells. On the other hand, Elimusertib induced nuclear rupture in MCF-10A cells (**Figure 2C**).

Furthermore, the cell cycle assay results indicated that Elimusertib treatment caused gap phase (G0)/G1 accumulation in MDA-MB-231 cells (**Figure 3**). The percentage of MDA-MB-231 cells in the G0/G1 phase considerably increased from 57.7 ± 0.6% to 78.0 ± 1.4% and 72.1 ± 1.3% at 6 and 8 nM, respectively, for 96 h ($P<0.01$), while Elimusertib treatment resulted in increased the accumulation of the cells at G0/G1 phase (75.2 ± 0.3% and 75.7 ± 0.3%, respectively) compared with control (54.1 ± 0.6%) in MCF-10A cells (**Figure 3**). Thus, Elimusertib treatment caused significant apoptotic cell death in TNBC cells with, leading to toxicity in MCF-10A cells.

Assessment of intracellular ROS levels and mitochondrial damage

Oxidative stress through increased ROS levels in cells induces apoptosis and mitochondrial damage in the cells. Upon treatment with 6 and 8 nM Elimusertib for 96 h, the intracellular ROS positive cell amount considerably increased to 42.0 ± 1.4% and 50.4 ± 2.2%, respectively, in MDA-MB-231 cells ($P<0.01$, **Figure 4A** and **4B**). Furthermore, the intracellular amount of ROS positive cell percentage was 48.4 ± 0.6% and 49.3 ± 0.4% at 6 and 8 nM Elimusertib, respectively, in MCF-10A cells (**Figure 4A** and **4B**). Additionally, mitochondria with low fluorescence intensity extending to all areas of the cells were observed in the control cells (**Figure 4C**). Following treatment with Elimusertib, increased mitochondrial fluorescence intensity was observed in a dose and time-dependently. Furthermore, numerous rounded and fragmented perinuclear mitochondria were observed, particularly in MDA-MB-231 and MCF-10A cells treated with 8 nM Elimusertib for 96 h (**Figure 4C**). Thus, Elimusertib treatment could increase the intracellular amount of ROS and damage in mitochondria.

Effects of Elimusertib on ATR/Chk1 pathway in TNBC cells

The inhibition of ATR/Chk1 pathway by Elimusertib was evaluated by western blot analysis (**Figure 5**). Elimusertib treatment decreased ATR, p-Chk1 and p-Cdc2 protein levels in MDA-MB-231 and MCF-10A cells compared to the control group with the upregulation of γ-H2AX and Cyclin B protein levels due to increased DNA breaks. Additionally, the protein level of p-Rb, which is involved in the transition in G0/G1 phase, decreased in these cells treated with Elimusertib with the upregulation of the level of Cyclin D protein for especially 96 h. Furthermore, the total PARP, Caspase-9 and Caspase-3 proteins in cells reduced in time and dose-dependently in these cells. Thus, Elimusertib effectively inhibited ATR based DNA damage response pathway and induced apoptosis in TNBC cells.

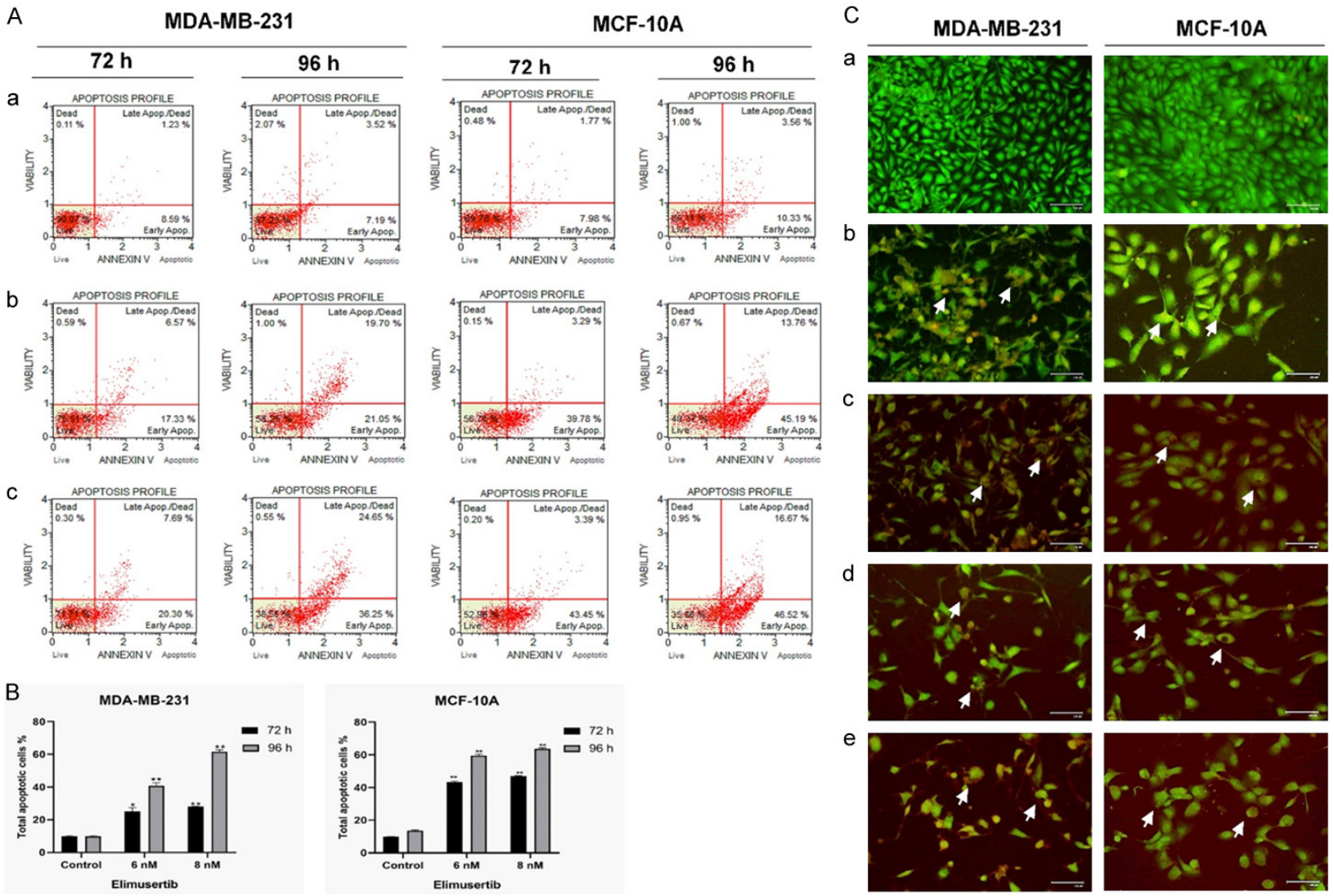

Figure 2. (A) Histograms of Annexin V staining results. Comparison of Elimusertib treatment for two different incubation times. (a) Control, (b) 6 nM and (c) 8 nM Elimusertib. (B) Statistical comparison of MDA-MB-231 and MCF-10A cells treated with 6 and 8 nM Elimusertib for 72 and 96 h ($P<0.05$*, $P<0.01$**). (C) Morphologies of the cells treated with Elimusertib (a) Control, (b) 6 nM and (c) 8 nM for 72 h, (d) 6 nM and (e) 8 nM for 96 h stained by AO/PI. (The scale bar is 100 μm).

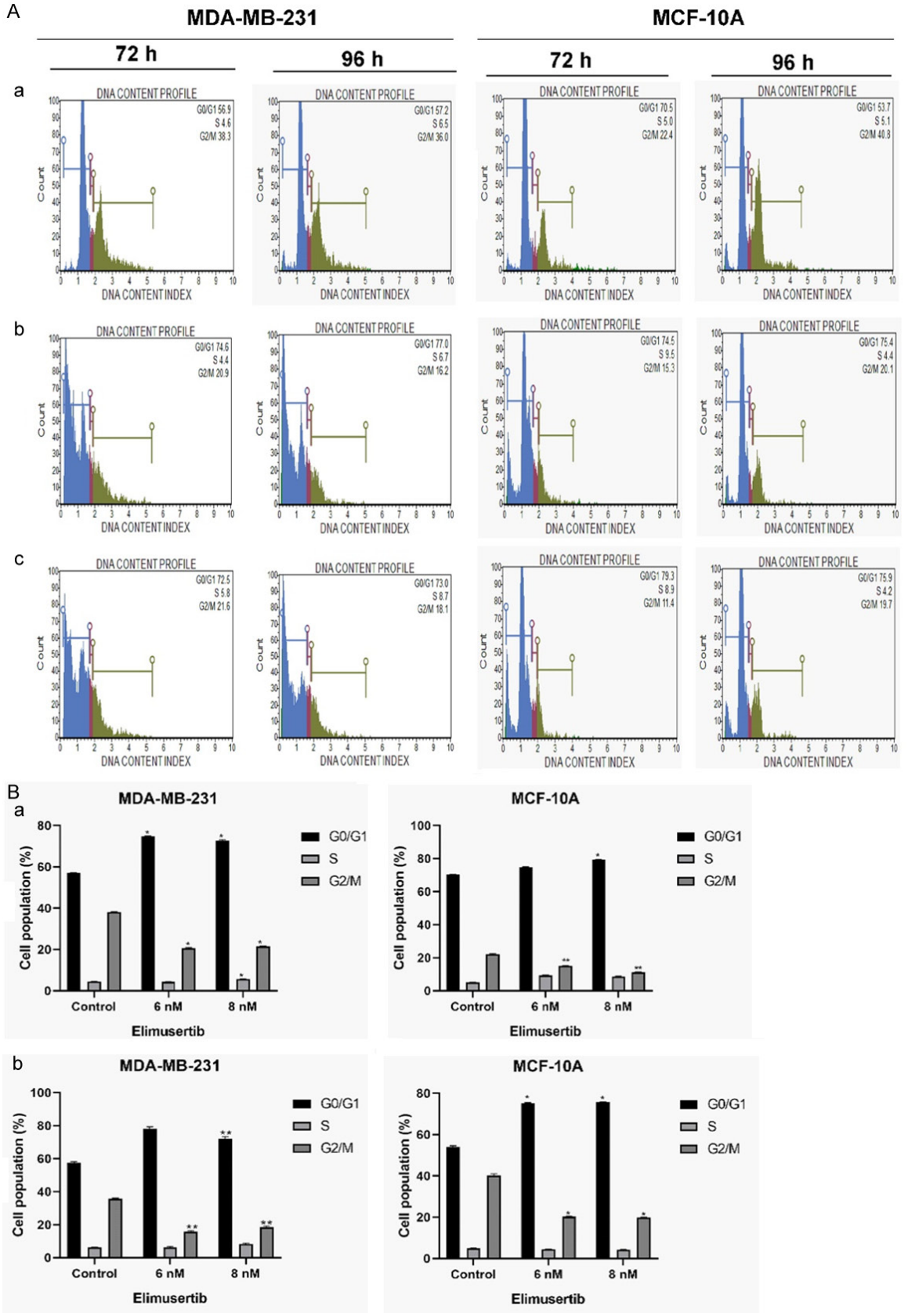

Figure 3. The impact of Elimusertib on cell cycle phase. (A) Histograms of cell cycle results. (a) Control, (b) 6 nM and (c) 8 nM Elimusertib. (B) Statistical comparison of MDA-MB-231 and MCF-10A cells treated with 6 and 8 nM Elimusertib for (a) 72 and (b) 96 h ($P<0.05$*, $P<0.01$**).

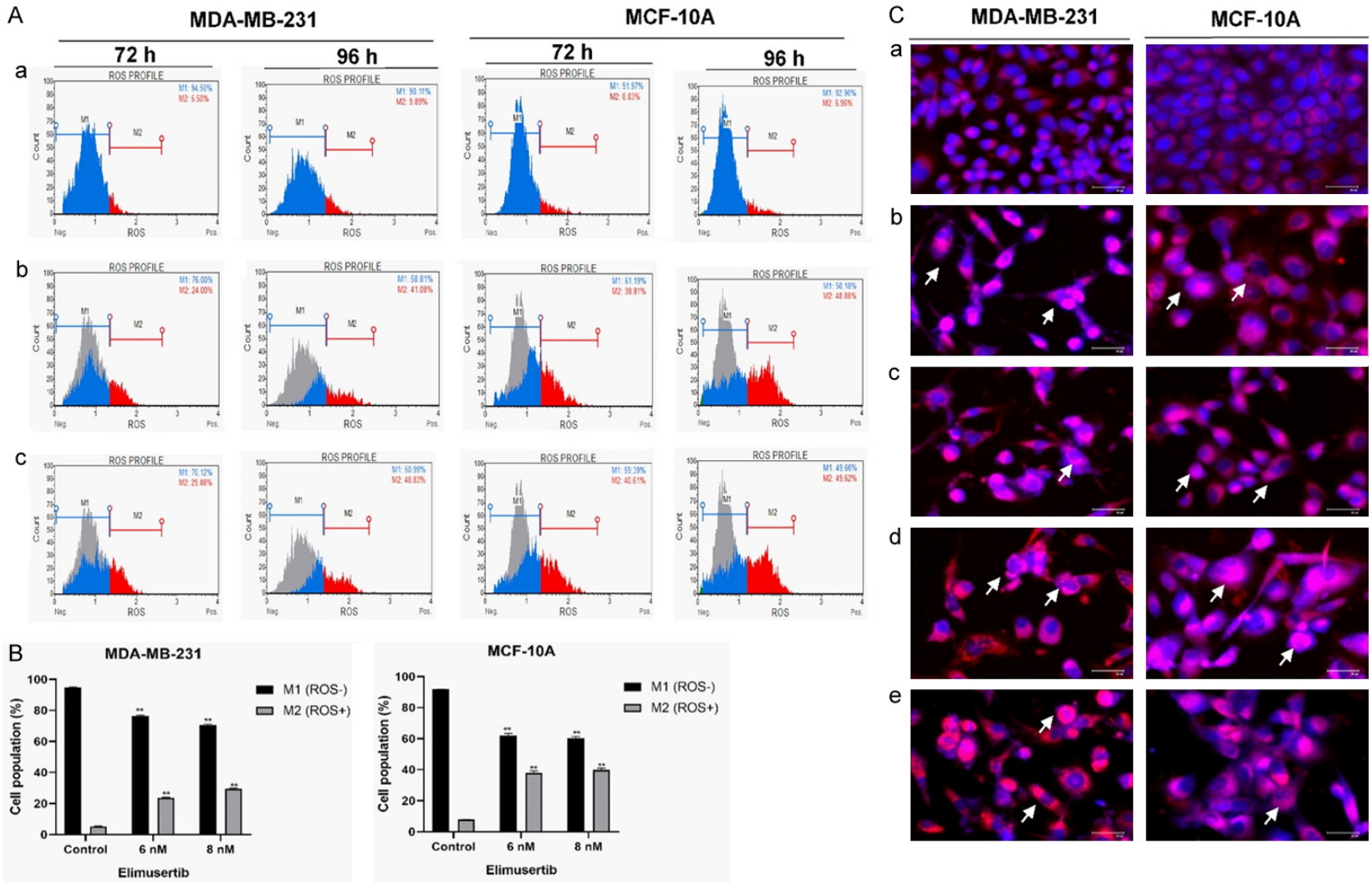

Figure 4. (A) Histograms of ROS staining results. Comparison of Elimusertib treatment for two different incubation times. (a) Control, (b) 6 nM and (c) 8 nM Elimusertib. (B) Statistical comparison of MDA-MB-231 and MCF-10A cells treated with 6 and 8 nM Elimusertib for 96 h ($P<0.01$**). (C) Morphologies of the cells treated with Elimusertib (a) Control, (b) 6 nM and (c) 8 nM for 72 h, (d) 6 nM and (e) 8 nM for 96 h stained by Mitotracker dye. (The scale bar is 50 μm).

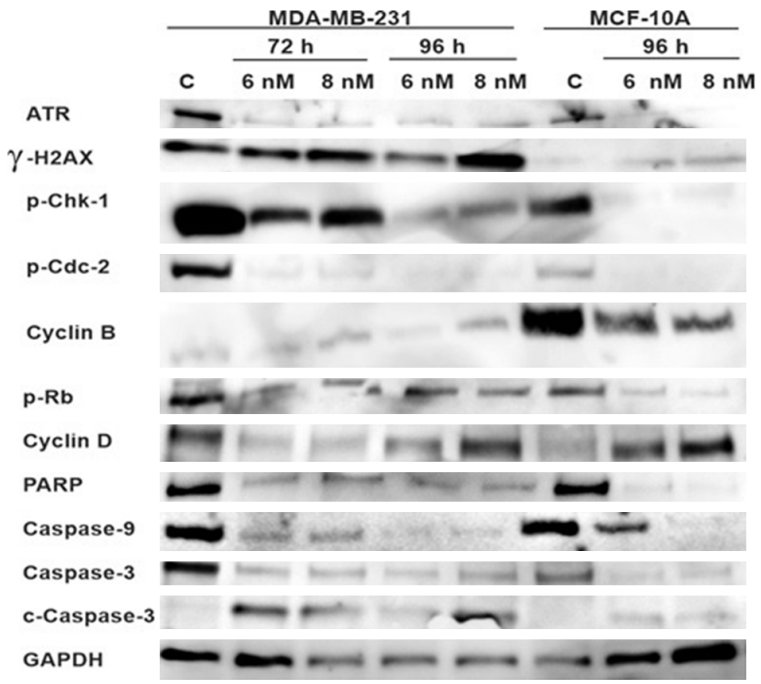

Figure 5. Western blot assay for ATR/Chk1 pathway and its associated proteins in cell cycle and apoptosis on MDA-MB-231 cells and MCF-10A cells treated with 6 and 8 nM Elimusertib for 72 and 96 h.

Discussion

Herein, we, for the first time, assessed the anticancer effect of Elimusertib on TNBC cells at the molecular level. Our results showed that Elimusertib caused apoptosis through G0/G1 arrest, increased ROS level, mitochondrial damage and decreased total PARP, Caspase-9 and Caspase-3 protein levels in TNBC cells in a dose and time-dependently. Furthermore, Elimusertib treatment suppressed ATR/Chk1 pathway through decreased ATR, p-Chk1, p-Cdc2 and p-Rb levels and caused DNA breaks due to increased γH2AX, Cyclin B and Cyclin D protein expression levels. On the other hand, apoptotic death, increase in G0/G1 phase arrest, decrease in the expression levels of ATR, p-Chk1, p-Cdc2 and p-Rb proteins, and increased expression levels of Cyclin B and Cyclin D proteins were detected in MCF-10A cells due to the toxic effect of Elimusertib.

In preclinical studies, the therapeutic effect of different ATR inhibitors (VE-821, AZD6738, M4344, M6620) on different cancer types (prostate, ovarian, glioblastoma, breast, neuroblastoma, osteosarcoma, etc.) has been identified [13-15]. On the other hand, there is a limited number of studies investigating the efficacy of Elimusertib in cancer treatment, *in vitro* and *in vivo*. In the study of Wegner et al. (2020), the half-maximal inhibitory concentration (IC_{50}) value of Elimusertib is in the range of 9-490 nmol/L in several cancer cell lines and the majority of cancer cells carrying mutations in the Ataxia Telangiectasia Mutated (ATM) pathway are more sensitive to Elimusertib [10]. Tang et al. (2021) state that ATR inhibitors (Elimusertib or VX-970) are more effective than olaparib PARP inhibitor in RM-9, RM-1, RM-1-BM, C4-2b and PC-3 prostate cancer cells and ATR inhibitors cause G1 phase arrest by rendering cell accumulation in the G2/Mitosis (M) phase caused by olaparib [16]. Furthermore, Elimusertib causes apoptosis, cleavage caspase-3 and PARP and double-strand DNA breaks in neuroblastoma and cancer cell lines [17, 18]. In this context, we assessed Elimusertib-induced apoptotic death and its effect on ATR/Chk1 pathway in TNBC cells for the first time. In the current study, Elimusertib induced both early and late apoptotic cell death through G0/G1 arrest, DNA fragmentation and formation of apoptotic bodies in TNBC cells. However, Elimusertib treatment resulted in significant toxicity in MCF-10A cells. Tu et al. (2018) note that VX-970 ATR inhibitor does not increase the toxicity caused by radiotherapy in MCF-10A cells [14]. Therefore, further innovative formulations or combination therapy strategies are needed to reduce the toxicity of Elimusertib in control cell lines.

ATR inhibitors cause retention in the G0/G1, S and G2/M phases of the cell cycle in different cancer types. Two mechanisms in the cell cycle regulate the delay in the G0 or G1 phase in cancer cells. (i) Mutations in the Rb/cyclin-dependent kinase inhibitor 2A (p16) pathway enable the proliferation of cancer cells in a growth factor-independent manner. (ii) Mutations in the p53 pathway prevent the cell cycle at G1 phase after DNA damage. These changes allow

cancer cells to spend more time in the S phase and to transition from the G0/G1 phase to the S phase with more DNA damage than normal cells [19]. TNBC is characterized by a high frequency of *p53* mutations, and increased loss of murine double minute 2 (MDM2) and Rb1 [5, 6]. These changes mediate the dependence on the ATR-based G2/M checkpoint and consequently mitotic catastrophe and early mitosis. Tu et al. (2018) state that the accumulation of cells in the G2/M phase induced by radiotherapy significantly decreased, especially in MDA-MB-231 cells compared to other TNBC cells [14]. In the current study, cancer cells with loss of G1 checkpoint are more sensitive to ATR inhibitors. In this context, Elimusertib treatment resulted in G0/G1 arrest in *p53* mutant MDA-MB-231 cells.

Furthermore, Elimusertib significantly increased the intracellular ROS level and mitochondrial damage. Loss of ATM/ATR and DNA-dependent protein kinase (DNA-PK) sensor proteins, which are involved in recognition of single strand breaks in DNA, cause increased ROS levels [20-22]. Additionally, the loss of ATM causes an increase in abnormal mitochondria and thus an increase in ROS. In ATM-deficient cells, the amount of ROS increases due to the Nuclear factor erythroid 2-related factor 2 (NRF2) defect, which regulates the expression of antioxidant proteins under cellular stress [23]. Moreover, ATR is required for oxidative stress induced by hydrogen peroxide (H_2O_2) to initiate γH2AX accumulation, DNA damage response, and ATR-dependent Chk1 phosphorylation [24]. Therefore, therapeutic strategies based on mediating ROS levels and ATR/Chk1 DNA damage response pathway will be further investigated for the treatment of TNBC.

Finally, the efficacy of ATR inhibitors in the DNA damage response has been clarified at the molecular level. In the study of Suzuki et al. (2022), the combination of ATR inhibitor and 5-fluorouracil (5-FU) causes a decrease in p-Chk1 protein expression with an increase in γH2AX and c-Caspase-3 protein levels compared to the drug alone [15]. Tang et al. (2021) state that Elimusertib suppresses p-ATR/total ATR, p-Chk1/Total Chk1, Cyclin B and Cdk1 protein levels in mouse RM-1BM and human C4-2b and PC-3 prostate cancer cells [16]. In our study, Elimusertib decreased ATR, p-Chk1, p-Cdc2 and Cyclin B1 protein levels in TNBC cells and effectively suppressed ATR-based DNA damage response and our results supported these studies. However, further *in vivo* studies should performed for the validation of ATR/Chk1 pathway inactivation by ATR inhibitors.

Conclusion

Consequently, our results suggest that Elimusertib as an ATR inhibitor is effective for the treatment of TNBC cells and causes apoptotic death through G0/G1 phase arrest, increased ROS level and DNA breaks, decreased PARP, Caspase-9 and Caspase-3 protein expression levels, and mitochondrial damage. Additionally, Elimusertib effectively suppressed ATR-based DNA damage response in TNBC cells. Therefore, ATR inhibition may be synthetically lethal in *p53* mutant TNBC cells and may be an innovative treatment strategy in TNBC patients with *p53* mutations. However, further studies are required to investigate the combined effect of Elimusertib and radiotherapy or inhibitors associated with DNA damage response due to the toxicity of Elimusertib. Additionally, further studies will be conducted on the relationship between signaling pathways involved in the development of TNBC and the inhibition of ATR-based DNA damage response at the molecular level.

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Disclosure of conflict of interest

None.

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References

[1] Chang-Qing Y, Jie L, Shi-Qi Z, Kun Z, Zi-Qian G, Ran X, Hui-Meng L, Ren-Bin Z, Gang Z, Da-Chuan Y and Chen-Yan Z. Recent treatment prog-

ress of triple negative breast cancer. Prog Biophys Mol Biol 2020; 151: 40-53.
[2] Bou Zerdan M, Ghorayeb T, Saliba F, Allam S, Bou Zerdan M, Yaghi M, Bilani N, Jaafar R and Nahleh Z. Triple negative breast cancer: updates on classification and treatment in 2021. Cancers (Basel) 2022; 14: 1253.
[3] Shen M, Pan H, Chen Y, Xu YH, Yang W and Wu Z. A review of current progress in triple-negative breast cancer therapy. Open Med 2020; 15: 1143-1149.
[4] Italiano A. Atr inhibition as an attractive therapeutic resource against cancer. Cancer Discov 2021; 11: 14-16.
[5] Synnott NC, Murray A, McGowan PM, Kiely M, Kiely PA, O'Donovan N, O'Connor DP, Gallagher WM, Crown J and Duffy MJ. Mutant p53: a novel target for the treatment of patients with triple-negative breast cancer? Int J Cancer 2017; 140: 234-246.
[6] Witkiewicz AK, Chung S, Brough R, Vail P, Franco J, Lord CJ and Knudsen ES. Targeting the vulnerability of RB tumor suppressor loss in triple-negative breast cancer. Cell Rep 2018; 22: 1185-1199.
[7] Jin J, Tao Z, Cao J, Li T and Hu X. DNA damage response inhibitors: an avenue for TNBC treatment. Biochim Biophys Acta Rev Cancer 2021; 1875: 188521.
[8] Lodovichi S, Cervelli T, Pellicioli A and Galli A. Inhibition of dna repair in cancer therapy: toward a multi-target approach. Int J Mol Sci 2020; 21: 6684.
[9] Smith HL, Southgate H, Tweddle DA and Curtin NJ. DNA damage checkpoint kinases in cancer. Expert Rev Mol Med 2020; 22: e2.
[10] Wengner AM, Siemeister G, Lucking U, Lefranc J, Wortmann L, Lienau P, Bader B, Bomer U, Moosmayer D, Eberspacher U, Golfier S, Schatz CA, Baumgart SJ, Haendler B, Lejeune P, Schlicker A, von Nussbaum F, Brands M, Ziegelbauer K and Mumberg D. The novel ATR inhibitor BAY 1895344 is efficacious as monotherapy and combined with DNA damage-inducing or repair-compromising therapies in preclinical cancer models. Mol Cancer Ther 2020; 19: 26-38.
[11] Wengner AM, Scholz A and Haendler B. Targeting DNA damage response in prostate and breast cancer. Int J Mol Sci 2020; 21: 8273.
[12] Barnieh FM, Loadman PM and Falconer RA. Progress towards a clinically-successful ATR inhibitor for cancer therapy. Curr Res Pharmacol Drug Discov 2021; 2: 100017.
[13] Jo U, Senatorov IS, Zimmermann A, Saha LK, Murai Y, Kim SH, Rajapakse VN, Elloumi F, Takahashi N, Schultz CW, Thomas A, Zenke FT and Pommier Y. Novel and highly potent ATR inhibitor M4344 kills cancer cells with replication stress, and enhances the chemotherapeutic activity of widely used DNA damaging agents. Mol Cancer Ther 2021; 20: 1431-1441.
[14] Tu X, Kahila MM, Zhou Q, Yu J, Kalari KR, Wang L, Harmsen WS, Yuan J, Boughey JC, Goetz MP, Sarkaria JN, Lou Z and Mutter RW. ATR inhibition is a promising radiosensitizing strategy for triple-negative breast cancer. Mol Cancer Ther 2018; 17: 2462-2472.
[15] Suzuki T, Hirokawa T, Maeda A, Harata S, Watanabe K, Yanagita T, Ushigome H, Nakai N, Maeda Y, Shiga K, Ogawa R, Mitsui A, Kimura M, Matsuo Y, Takahashi H and Takiguchi S. ATR inhibitor AZD6738 increases the sensitivity of colorectal cancer cells to 5-fluorouracil by inhibiting repair of DNA damage. Oncol Rep 2022; 47: 78.
[16] Tang Z, Pilié PG, Geng C, Manyam GC, Yang G, Park S, Wang D, Peng S, Wu C, Peng G, Yap TA, Corn PG, Broom BM and Thompson TC. ATR inhibition induces CDK1-SPOP signaling and enhances anti-PD-L1 cytotoxicity in prostate cancer. Clin Cancer Res 2021; 27: 4898-4909.
[17] Szydzik J, Lind DE, Arefin B, Kurhe Y, Umapathy G, Siaw JT, Claeys A, Gabre JL, Van den Eynden J, Hallberg B and Palmer RH. ATR inhibition enables complete tumour regression in ALK-driven NB mouse models. Nat Commun 2021; 12: 6813.
[18] Sule A, Van Doorn J, Sundaram RK, Ganesa S, Vasquez JC and Bindra RS. Targeting IDH1/2 mutant cancers with combinations of ATR and PARP inhibitors. NAR Cancer 2021; 3: zcab018.
[19] Hanahan D and Weinberg RA. The hallmarks of cancer. Cell 2000; 100: 57-70.
[20] Blackford AN and Jackson SP. ATM, ATR, and DNA-PK: the trinity at the heart of the DNA damage response. Mol Cell 2017; 66: 801-817.
[21] Srinivas US, Tan BWQ, Vellayappan BA and Jeyasekharan AD. ROS and the DNA damage response in cancer. Redox Biol 2019; 25: 101084.
[22] Sullivan KD, Palaniappan VV and Espinosa JM. ATM regulates cell fate choice upon p53 activation by modulating mitochondrial turnover and ROS levels. Cell Cycle 2015; 14: 56-63.
[23] Zhou S, Ye W, Shao Q, Zhang M and Liang J. Nrf2 is a potential therapeutic target in radioresistance in human cancer. Crit Rev Oncol Hematol 2013; 88: 706-715.
[24] Katsube T, Mori M, Tsuji H, Shiomi T, Wang B, Liu Q, Nenoi M and Onoda M. Most hydrogen peroxide-induced histone H2AX phosphorylation is mediated by ATR and is not dependent on DNA double-strand breaks. J Biochem 2014; 156: 85-95.