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Research Article

Ataxia-Telangiectasia Mutated (ATM) inhibitor induces antineoplastic effects in brain cancer cells: Evidence from in vitro investigations

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Abstract

Background: Brain cancer is a life-threatening disease that originates from abnormal cells growth uncontrollably in brain tissues. Treatment is challenging because of the delicacy of brain and blood–brain barrier which does not allow to enter the medicines easily. Surgery and existing chemotherapeutic measures are helpful but may not destroy all the cancer cells. Thus, there is need for new and more effective medicines to treat brain tumors safely for subsequent improvement and survival.

Methods: Human neuroblastoma cells IMR-32 were cultured and exposed with commercially available Ataxia-Telangiectasia Mutated (ATM) inhibitor. Effects on viability of cells were evaluated using a colorimetric assay to determine cytotoxic impact of the treatment. Crystal violet and DAPI staining were performed to monitor cellular growth, nuclear morphology and apoptotic changes in the cells. Gene expression levels were determined using real-time PCR (RT-PCR) to quantify changes in the expression of target genes including apoptosis, cell cycle, stress related markers and transcription factors.

Results: Toxicity assay showed reduction in cell viability after treatment with ATM inhibitor. Inhibitory concentration data showed ~7.5μM as the concentration inhibited 50% of the cell viability after 48h exposure. DAPI staining revealed nuclear condensation and fragmentation of DNA content indicating apoptotic changes. Crystal violet staining indicated inhibition of proliferation and colony forming ability of the cells. Apoptosis related genes (CASP3, CASP7, CASP9, BAX, BCL2) were largely induced, while cell cycle (CCND1, CCND2, CDKN1A, CDKN2A, CDKN1B, CDKN2B), stress (GADD34, GADD45A, GADD153) and transcription factors (E2F1, E2F6) were largely inhibited.

Conclusion: Exposure with ATM inhibitor restrained the cell proliferation, induced apoptosis, and suppressed colonization. Transcriptomic levels of the genes were altered by ATM inhibitor treatment. The results indicate potential therapeutic value of ATM inhibitor in controlling brain cancer growth and progression.

Key Words: Brain cancer, Ataxia-Telangiectasia Mutated, Anticancer effects, Apoptosis, Cell cycle

INTRODUCTION

Brain cancer is one of the most aggressive cancers and is therapeutically challenging disease [1]. Despite therapeutic advances, limited long-term survival is a major challenge [2]. Biology of brain tumors along with blood brain barrier restricts efficacy of anticancer drugs [3]. Consequently, there is need to identify new targets and therapeutic strategies to selectively eliminate malignant brain cells.

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In this condition, repositioning of the existing drug and identification of the novel potential molecules is important domain to explore [4]. Ataxia-Telangiectasia Mutated (ATM) is a kinase and regulator of cellular response towards DNA double-strand breaks [5]. ATM orchestrates cell-cycle checkpoints, repair mechanisms after DNA error and apoptosis related events [6, 7]. Aberrant activation of ATM and associated signaling has been involved in development and progression of cancers [8-10]. Furthermore, treatment resistance in cancer treatment including brain tumors is witnessed [11, 12]. The reports indicate that pharmacological inhibition of ATM is promising therapeutic approach to

sensitize cancer cells and impair tumor survival mechanisms [13, 14].

Recent investigations have demonstrated that ATM inhibitors induce antitumor activity via promoting DNA damage, cell-cycle arrest and triggering the apoptotic cell death [15, 16]. In addition, ATM inhibition is involved in overcoming therapeutic resistance while improving efficacy of the conventional anticancer treatments [17, 18]. Thus, combining the ATM inhibition along with existing treatment strategies can be instrumental in controlling the disease burden. However, antineoplastic effects of ATM inhibitors on cancer cells need to be understood at mechanistic levels. Therefore, present study was designed to evaluate antineoplastic potential of a commercially available ATM inhibitor in brain cancer cells. For this, *in vitro* experimental approach was adopted and cellular responses towards ATM inhibition were assessed. The work was aimed to provide insights into the therapeutic potential of targeting ATM signaling for brain cancer treatment.

METHODS

Cell Culture

Standard procedure was adopted to culture the human neuroblastoma cancer cells (IMR-32). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) media supplemented with end concentration of 10% fetal bovine serum. To restrict microbial growth, combination of penicillin (100U/mL) and streptomycin (100µg/mL) was used. Humidified incubator with 37°C temperature and 5% CO_2 was used for cultures. The culture medium was changed regularly (every 2–3 days) and the cells were sub-cultured when reached approximately 80–90% confluency.

ATM Inhibitor

Commercial ATM inhibitor (Selleck Chemicals, S1092) was used in this study. ATM inhibitor was dissolved in dimethyl sulfoxide (DMSO) to prepare a stock solution of 10mM and diluted subsequently in complete culture medium to achieve desired concentrations.

Toxicity Assay

The cytotoxic effects of ATM inhibitor on the cancer cells were evaluated. Cells were seeded into 96-well plates at density of 5×10^3 cells per well and incubated overnight. Treatment with different concentrations of ATM inhibitor (1.56, 3.12, 6.25, 12.5, 25.0, 50.0µM) for 48h period was done and afterwards 10µL of MTT solution (5mg/mL) was added in each well and incubated for 3h at 37°C. Following the incubation, culture medium was carefully removed and formazan crystals formed by viable cells were dissolved in 50µL of the solvent (DMSO). In this calorimetric assay, absorbance was measured at 570nm by using a microplate reader. Cell viability was calculated with reference to untreated control cells as per following equation:

$$\text{Cell viability (\%)} = (\text{Absorbance of treated cells} / \text{Absorbance of control cells}) \times 100$$

Half-maximal inhibitory concentration (IC50) value was calculated from dose response curve generated by using nonlinear regression analysis. Experiments were performed in triplicate.

Nuclear Staining

Nuclear changes including blebbing and breakage are associated with apoptosis. Effect of ATM inhibitor was evaluated using 4′,6-diamidino-2-phenylindole (DAPI) staining. The cells were seeded in 6-well plates and allowed to adhere overnight. Following treatment with ATM inhibitor (5 and 15µM) for 48h, the cells were washed with phosphate-buffered saline (PBS) and fixed with cold formaldehyde (4%) and incubation for 15min (4°C). Afterwards, the cells were permeabilized with 0.1% Triton X-100 for 5min and washed with PBS to remove the chemical. The fixed porous cells were stained with DAPI solution (0.5µg/mL) for 2min in the dark at room temperature. Before microscopy, excess of the stain was removed by washing cells two times with PBS. The cells were examined under fluorescence microscope equipped with the required DAPI filter and nuclear morphology including nuclear fragmentation and chromatin condensation was assessed qualitatively by capturing the images from the random fields.

Cell Staining

Cells were cultured and exposed to ATM inhibitor with two concentrations (5 and 15µM). After a period of 48h, the media was removed and cells were washed with PBS. Crystal violet working solution, prepared in PBS (0.5% by volume) was used and the cells were immersed in solution for 15min at room temperature. Afterwards, the plates were carefully rinsed with the running distilled water to remove excess of the stain and allowed to air-dry. Stained cells were examined under light microscope, and the representative images were taken by using digital camera. Cell morphology and the colonies were evaluated for comparative analysis.

RNA Isolation and cDNA Synthesis

The cells were cultured and exposed to two different concentrations of ATM inhibitor (5 and 15µM) for a

period of 48h. Total RNA was extracted from untreated controls and ATM inhibitor treated cancer cells by using commercial column-based kits (Thermo Fisher, K0731). The concentration and purity of RNA was determined by measuring absorbance at 260 and 280 nm with Nanodrop apparatus. RNA samples with A260/A280 ratio of 1.8 to 2.0 were used for cDNA synthesis. For this purpose, 500ng of total RNA per sample was reverse transcribed using a commercial reverse transcription kit (Thermo Fisher, K1622). The synthesized cDNA was stored until further use.

Real-Time PCR

Gene expression analysis was done by using real-time polymerase chain reaction (qRT-PCR) method along with SYBR Green chemistry. Amplification reactions were carried out containing SYBR Green Master Mix, gene-specific optimized primers (Table 1) and cDNA samples. Real-time PCR system (QS3, ABI) was used with thermal cycling conditions of initial denaturation (95°C/5min), followed by 40 cycles of denaturation (95°C/15s), annealing (58–60°C/30s) and extension (72°C/40s). Expression levels of apoptosis (CASP3, CASP7, CASP9, BAX, BCL2), cell cycle (CCND1, CCND2, CDKN1A, CDKN2A, CDKN1B, CDKN2B), transcription factors (E2F1, E2F6) and stress (GADD34, GADD45A, GADD153) related genes were analyzed. Housekeeping gene (GAPDH) was used as internal control to normalize the data. Relative gene expression was determined using 2ΔΔCt method to express fold change differences among the groups.

Table 1. Primer Sequences of the genes

Genes	Forward Primer	Reverse Primer
CASP3	GAGGCCGACTTCTTGTATGC	AGCGTCAAAGGAAAAGGACT
CASP7	TTCCACGGTTCCAGGCTATT	AGTTCCTTGGTGAGCATGGA
CASP9	GAAAATGGTGCTGGCTTTGC	TCTTTCTGCTCCCCACCAC
BAX	TCTGACGGCAACTTCAACTG	GGAGGAAGTCCAATGTCCAG
BCL2	GGATAACGGAGGCTGGGATG	TGATGCAAGCTCCCACCAG
CCND1	GGGGGCGTAGCATCATAGTA	GTGGTGGCACGTAAGACACA
CCND2	GTCTCAAAGCTTGCCAGGAG	ATATCCCGCACGTCTGTAGG
CDKN1A	GCTTCATGCCAGCTACTTCC	CTGTGCTCACTTCAGGGTCA
CDKN2A	CCCTCAGAAATGATCGGAAA	CAGCTTGCGATAACCAAAGG
CDKN1B	CCGGCTAACTCTGAGGACAC	TGCAGGTCGCTTCCTTATTC
CDKN2B	GACCGGGAATAACCTTCCAT	AAACCCTGAAAAGCAAACGA
GADD34	GAGACTCCCCTAAAGGCCAG	GGGGCTAAAGGTGGGTTCC
GADD45A	AACGGTGATGGCATCTGAAT	CCCTTGGCATCAGTTTCTGT
GADD153	TCTTGACCCTGCTTCTCTGG	GCTGTGCCACTTTCCTTTCA
E2F1	GGGCTCTAACTGCACTTTCG	AGAAGTGCTCTCACCGTCCT
E2F6	TGCCTTCGCCATGAATCCTT	GGAGCCCATTCCTACATTCTCA
GAPDH	ACGGATTTGGTCGTATTGGG	CGCTCCTGGAAGATGGTGAT

RESULTS

ATM Inhibitor Induced Cytotoxicity in Brain Cancer Cells

Cytotoxic effects by a compound against cancer cells is important functional consideration. In this study, cytotoxic effects of ATM inhibitor in brain cancer cells (IMR-32) were assessed via MTT assay. Exposure with ATM inhibitor reduced the percentage of viable cells in concentration-dependent manner as compared to untreated control cells (Figure 1). Toxic effects were minimal at lower concentrations (up to 3.12μM) while clear inhibition of viability was witnessed at higher concentrations (6.25μM and above). Exposure to these higher concentrations of ATM inhibitor imposed progressive decreases in cellular metabolic activity. This indicated impaired cell survival. In summary, cell viability was reduced to 67, 73, 90 and 92% in response to 6.25, 12.5, 25 and 50μM concentration of ATM inhibitor respectively. Based on calculated data sets, IC50 value was found to be ~7.5μM.

ATM Inhibitor Induced Nuclear Morphology Alterations

Based on cytotoxicity data, we were interested in investigating whether ATM inhibitor induced effects

were associated with any apoptotic events. For this purpose, DAPI staining of the nuclear material was performed to examine nuclear morphology. Untreated cells served as controls and exhibited uniformly stained intact nuclei and nucleic acid architecture. In contrast, ATM inhibitor treatment displayed nuclear condensation, deregulated morphology and chromatin fragmentation in the cells. These indications reflected the potential induction of apoptotic machinery in response to exposure with the selected inhibitor. Furthermore, frequency of the apoptotic nuclei was higher with increasing concentration of ATM inhibitor. Overall, this qualitative data provided evidence of apoptotic events initiated in response to treatment of brain cancer cells with ATM inhibitor (Figure 2).

ATM Inhibitor Induced Changes in Cell Morphology

Crystal violet staining of the cells after exposure with ATM inhibitor revealed notable morphological alterations and growth outlook in the cells. Compared to untreated controls, ATM inhibitor treatment exhibited changes in cellular outlook characterized by cell enlargement, irregular shape, and lesser number of colonies growing in cultured wells. Morphological changes and growth pattern changes became more intense with higher concentration of the inhibitor (Figure 3). Overall, Crystal violet staining showed that ATM inhibitor affected cell morphology and density of the formed colonies which is in line with the toxicity and nuclear staining data.

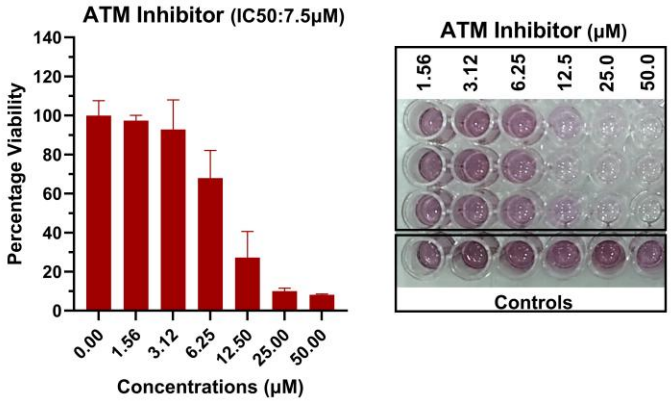

Figure 1. Cell viability of brain cancer cells reduced by ATM inhibitor. MTT assay method was used to assess mitochondrial activity after the treatment which corresponds to viable cells. Visual impact of the treatment resulted into decreased formazan crystals intensity and numerical presentation as percentages of the data are shown.

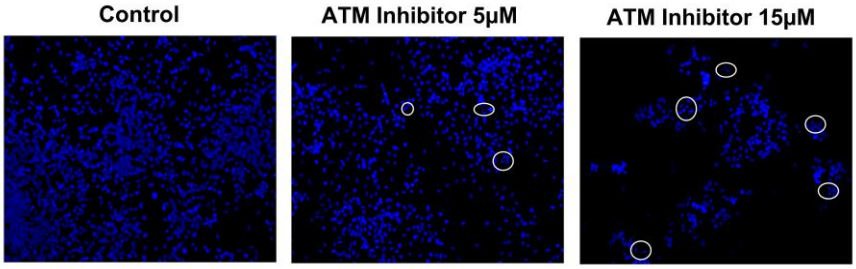

Figure 2. Brain cancer cells were treated with ATM inhibitor and after a period of 48h, the cells were stained with DAPI. Nuclear morphology was captured which showed condensation and fragmentation of nuclear material indicating initiation of apoptotic machinery in the cells. The changes in nuclear outlooks are reflected in white circles as representative areas.

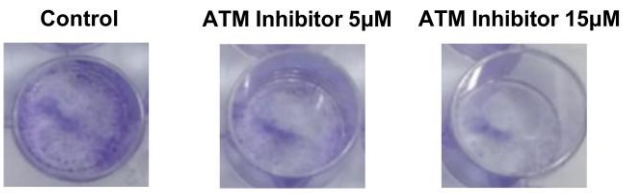

Figure 3. Cells were exposed to ATM inhibitor and stained with crystal violet stain to monitor cell morphology and growth patterns. After the exposure, cell densities were reduced and cellular outlook were altered including deregulated shapes.

ATM Inhibitor Modulated Expression of Genes

To elucidate molecular mechanisms underlying ATM inhibitor mediated antineoplastic effects in brain cancer cells, expression levels of major signaling pathways including apoptosis, cell cycle and stress were examined. For this, important genes related to apoptosis (CASP3, CASP7, CASP9, BAX, BCL2), cell cycle (CCND1, CCND2, CDKN1A, CDKN2A, CDKN1B, CDKN2B), cell stress (GADD34, GADD45A, GADD153) and transcription factors (E2F1, E2F6) were measured at transcriptome levels using real-time PCR method as discussed in method section. Exposure with ATM inhibitor deregulated the expression levels of selected genes, although there were marked variations among the outcomes.

In apoptosis related genes, there was overall induction in proapoptotic genes including members of caspase family and BAX gene, while antiapoptotic gene (BCL2) was inhibited in response to ATM inhibitor exposure. Thus, overall, the regulations favor the apoptotic machinery in the brain cancer cells after treatment with ATM inhibitor (Figure 4). To be precise, in response to ATM inhibitor concentrations (5 and 15μM), there was induction of 1.2 and 1.7fold in CASP3 gene. Similarly, CASP7 expression increases up to 1.2 and 1.3fold respectively. Transcriptome level of BAX also induced to 1.2 and 1.3fold after exposure with the inhibitor. CASP9 was the only pro-apoptotic gene which deviated from this fashion and was inhibited to -1.3 and -1.5fold after treatment. Anti-apoptotic gene (BCL2) was down regulated to -2.8 and -1.6fold in response to ATM inhibitor exposure.

Cell cycle related genes were changed in terms of expression at mRNA levels in a variable fashion. Two cell cycle promoting genes (CCND1 and CCND2) were selected, while four cell cycle regulator genes (CDKN1A, CDKN2A, CDKN1B, CDKN2B) were opted in this study. Cell cycle promoting genes were inhibited after exposure with ATM inhibitor in brain cancer cells. In these, CCND1 was inhibited to -1.3 and -1.1fold, while CCND2 was inhibited to -2.1 and -3.4fold in the cells (Figure 5). Among cell cycle regulator genes, CDKN1A was inhibited to -2.3fold at lower concentration of ATM inhibitor (5μM), while it was induced to 1.3fold ay higher concentration of the compound (15μM). CDKN2A showed the opposite trend in deregulation as the gene was induced at lower ATM inhibitor concentration (1.5fold, 5μM). Other two cell cycle inhibitor genes (CDKN1B, CDKN2B) were consistently and moderately inhibited (up to -1.6fold) after the exposure with ATM inhibitor.

Among the cell stress related genes, two selected candidates (GADD34 and GADD45A) were inhibited in response to ATM inhibitor, while the third gene (GADD153) showed differential response. To be precise, GADD34 gene was inhibited maximum up to -1.1fold while GADD45A was inhibited to -4.4fold in brain cancer cells after treatment with ATM inhibitor. GADD153 was induced by 1.3fold at lower concentrations of ATM inhibitor (5μM) and inhibited to -1.2fold at higher concentration (15μM). Selected transcription factors (E2F1, E2F6) were consistently downregulated in brain cancer cells after exposure with ATM inhibitor. Among two transcription factors, E2F1 was inhibited (up to -1.3fold) while E2F6 was more effectively inhibited (up to -2.1fold). Overall expression modifications in cell stress related genes and transcription factors are shown in Figure 6.

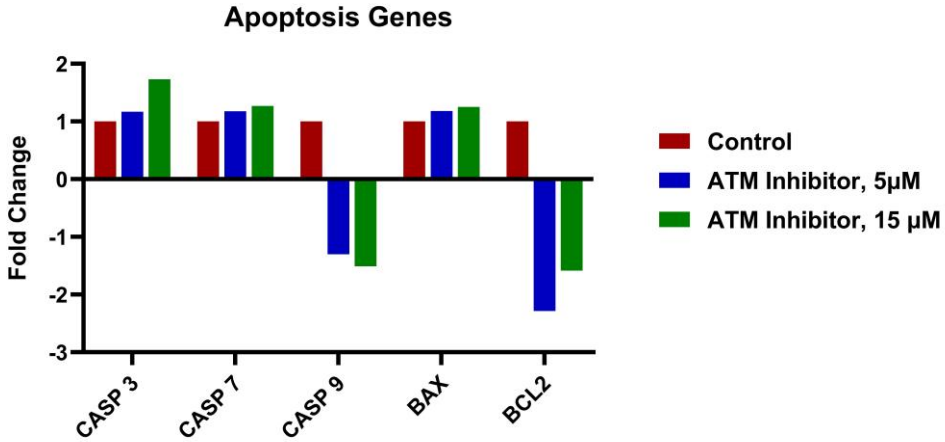

Figure 4. Expression of apoptosis related genes in brain cancer cells identified with real time PCR method after exposure with ATM inhibitor for 48h. The levels were compared with untreated control cells and fold changes were determined with Livak method.

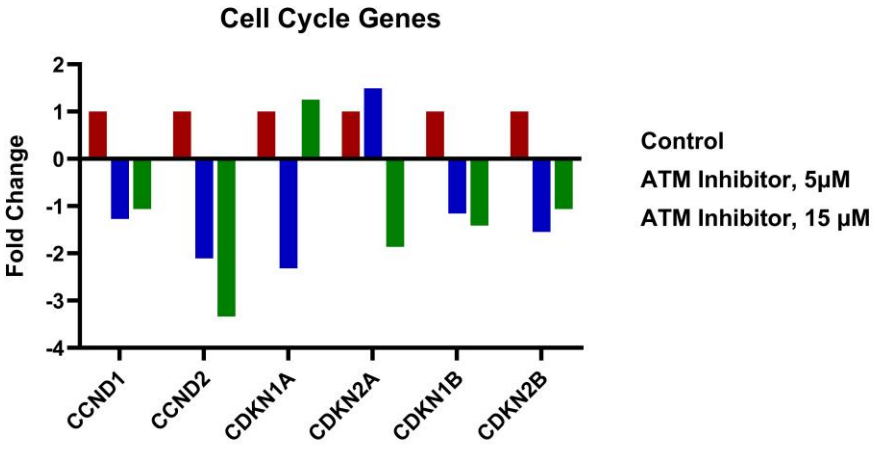

Figure 5. Expression of cell cycle related genes was identified with real time PCR method. Brain cancer cells were exposed to the ATM inhibitor for 48h. The expression levels were identified and compared with untreated control cells. Fold changes were determined with Livak method.

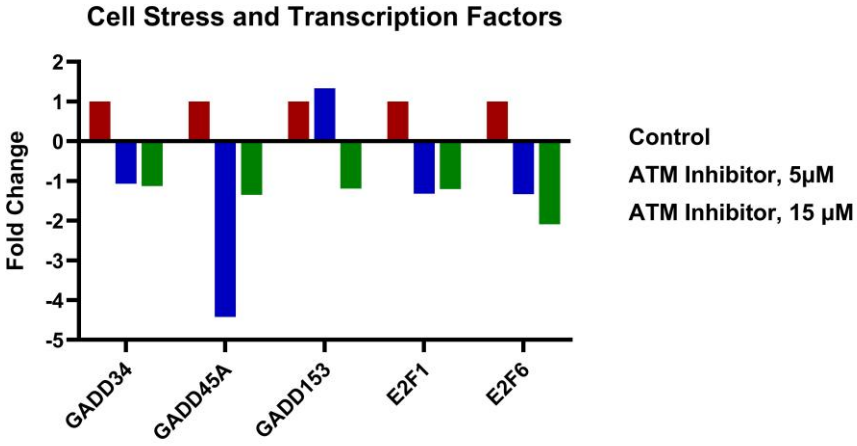

Figure 6. Expression of cell stress genes and transcription factors in brain cancer cells identified with real time PCR method. The cells were exposed with ATM inhibitor for 48h, and the transcriptome levels were compared with untreated control cells. Fold changes were determined with Livak method.

DISCUSSION

Brain cancer is indeed a lethal condition and finding new therapeutic modalities is required for effective treatment. With this notion, we investigated potential anticancer effects of ATM inhibitor at functional and molecular levels in the brain cancer cell cultures. The study demonstrated that the inhibition of ATM kinase obtained via commercial inhibitor imposed significant antitumor activity in IMR-32 brain cancer cells. The effects were reflected via induction of cytotoxicity, apoptosis, morphological alterations and modulation of genes at mRNA levels associated with apoptosis, cell cycle, cellular stress and transcriptional activity. Collectively, findings suggested that ATM signaling is important for survival and its inhibition interferes with multiple cellular processes required for tumor growth.

As base line effect, MTT assay revealed concentration dependent reduction in the cell viability after ATM inhibitor treatment. With an IC50 value of ~7.5 μM, ATM inhibition effectively reduced the metabolic activity followed by lower survival of IMR-32 cells. As we know, ATM is regulator of DNA damage response and coordinates the DNA repair and cell-cycle checkpoints to maintain genomic stability [19]. Cancer cells often experience replication stress and DNA damage because of the rapid proliferation. This leads to greater dependency of cancer cells on ATM mediated mechanisms. Considering this, inhibition of ATM limits the ability of malignant cells to repair the DNA lesions leading to genomic instability and cell death. MTT data showed that concentration-dependent decline in cell viability supports the growing concept that ATM is a promising therapeutic target. Cytotoxic effects imposed via ATM inhibitor in this study were also in line with already reported studies in other malignancies [20-22].

Morphological examination in cells further supported the cytotoxic effects induced by ATM inhibition. DAPI staining showed classical apoptotic features which included chromatin condensation and nuclear fragmentation. The effects became more prominent with higher concentrations of inhibitor. These changes represent the established hallmarks of apoptosis and indicate that reduced cell viability observed during cytotoxicity assay was largely attributed to apoptosis machinery. Similar in line, crystal violet staining highlighted the changes in cellular morphology which included lesser cell density, disruption in colony formation and increased numbers of detached cells observed during the procedures. These observations in response to ATM inhibition have already been reported and support our data [23, 24].

Together, observations indicated loss of cell integrity and proliferation, which indicates that ATM inhibition induces apoptotic cell death in the brain cancer cells.

Functional outcomes forced us to look for the changes in expression of key genes associated with apoptosis, cell cycle and cell stress. Expression data of apoptosis related genes supported the activation of apoptotic signaling in response to ATM inhibition. Upregulation of CASP3, CASP7, and BAX along with suppression of anti-apoptotic BCL2 shifted the balance towards initiation of apoptosis. Similar kinds of effects in response to ATM signaling are indicated by other researchers [25, 26].

At mechanistic levels, high expression of BAX leads to permeabilization of mitochondrial membrane and facilitate activation of intrinsic apoptotic pathway. Likewise, elevated CASP7 is associated with activation of mitochondrial mediated apoptotic cascade, while induction of CASP3 is a sign of execution phase of apoptosis. At same time, downregulation of BCL2 promotes apoptosis by reducing protection of mitochondrial membrane permeabilization [27]. All these coordinated events provide evidence of ATM inhibition mediated activation of apoptotic machinery in brain cancer cells.

ATM inhibition also led to alterations in genes related to cell-cycle progression. Downregulation of the key genes like CCND1 and particularly CCND2 tells the story about suppression of cyclin-mediated cell cycle transition. Cyclin D proteins regulate cell cycle progression through G1 phase by activating the associated cyclin-dependent kinases and inhibition of these genes is frequently associated with reduced cell growth [28]. ATM inhibition mediated down regulation of cyclins may have led to the limited cellular proliferation. Among the cell cycle inhibitory factors, CDKN1A/2A and CDKN1B/2B hold a central position [29]. In this study, after ATM inhibition, CDKN1A showed a biphasic regulation as it was suppressed at lower inhibitor concentration and induced at higher concentration. CDKN2A displayed opposite response as compared to CDKN1A. Differential regulation reflects the concentration dependent regulation of distinct checkpoints and/or adaptive cellular responses. In contrast to this, CDKN1B/2B showed consistent and moderate downregulation. The data indicates that biological functions of these cell cycle inhibitors may follow context dependent regulations and highlight the complexity of cell cycle mechanisms.

Cell stress genes display distinct patterns of regulation in this study. Down regulation of GADD34 and GADD45A suggests that ATM inhibition hinders stress adaptation pathways. As we know, these genes participate in DNA repair, genomic stability and cell cycle arrest under genotoxic stress [30, 31]. Downregulation of these genes may have impaired cellular repair capacity, increased DNA damage and lead to apoptosis. On other hand, GADD153 demonstrated biphasic regulation as observed by moderate induction at lower inhibitor concentration and suppression at higher concentration. This indicates an initial adaptive stress response which may have been overwhelmed as ATM inhibition increases.

Consistent downregulation of two transcription factors (E2F1 and E2F6) upholds suppression of proliferative signaling. Members of E2F family regulate multiple genes involved in cell cycle, DNA synthesis and DNA repair [32]. Thus, reduced expression of E2F members contributes to decreased proliferation via limiting the transcription of genes associated with cell cycle progression. These changes also complemented the observed suppression of viability and expression of cell cycle genes (cyclin). The data indicates that ATM inhibition also restrains proliferative signaling at the level of transcriptional activity.

Taking together, functional and molecular data suggest that ATM inhibition interferes with several biological processes and interconnected signaling networks. ATM inhibition appears to fabricate a coordinated antitumor activity involving induction of apoptosis, inhibition of cell cycle and deregulating the associated genes. Despite these encouraging findings, limitations of this study should be acknowledged as the experiments were performed using only one brain cancer cell line, which limits the generalization across molecular subtypes of the brain tumors. However, to summarize, present study demonstrates the fact that pharmacological inhibition of ATM meaningfully suppresses growth of IMR-32 cancer cells via apoptosis, cellular morphology disruption and modulating multiple genes. Further preclinical studies are needed to establish therapeutic potential of ATM inhibitors as anticancer treatments.

REFERENCES

1. Pellerino A, Caccese M, Padovan M, Cerretti G, Lombardi G. Epidemiology, risk factors, and prognostic factors of gliomas. Clinical and Translational Imaging. 2022 Oct;10(5):467-75.
2. Aldape K, Brindle KM, Chesler L, Chopra R, Gajjar A, Gilbert MR, Gottardo N, Gutmann DH, Hargrave D, Holland EC, Jones DT. Challenges to curing primary brain tumours. Nature reviews Clinical oncology. 2019 Aug;16(8):509-20.
3. Arvanitis CD, Ferraro GB, Jain RK. The blood–brain barrier and blood–tumour barrier in brain tumours and metastases. Nature Reviews Cancer. 2020 Jan;20(1):26-41.
4. Adasme MF, Parisi D, Sveshnikova A, Schroeder M. Structure-based drug repositioning: potential and limits. InSeminars in cancer biology 2021 Jan 1 (Vol. 68, pp. 192-198). Academic Press.
5. Lee JH, Paull TT. Cellular functions of the protein kinase ATM and their relevance to human disease. Nature Reviews Molecular Cell Biology. 2021 Dec;22(12):796-814.
6. Wagh V, Joshi P, Jariyal H, Chauhan N. ATM and ATR checkpoint kinase pathways: a concise review. Advances in Human Biology. 2020 May 1;10(2):51-9.
7. Balmus G, Pilger D, Coates J, Demir M, Sczaniecka-Clift M, Barros AC, Woods M, Fu B, Yang F, Chen E, Ostermaier M. ATM orchestrates the DNA-damage response to counter toxic non-homologous end-joining at broken replication forks. Nature communications. 2019 Jan 8;10(1):87.
8. Stucci LS, Internò V, Tucci M, Perrone M, Mannavola F, Palmirotta R, Porta C. The ATM gene in breast cancer: its relevance in clinical practice. Genes. 2021 May 13;12(5):727.
9. Varadhan V, Manikandan MS, Nagarajan A, Palaniyandi T, Ravi M, Sankareswaran SK, Baskar G, Wahab MR, Surendran H. Ataxia-Telangiectasia Mutated (ATM) gene signaling pathways in human cancers and their therapeutic implications. Pathology-Research and Practice. 2024 Aug 1;260:155447.
10. Ueno S, Sudo T, Hirasawa A. ATM: functions of ATM kinase and its relevance to hereditary tumors. International journal of molecular sciences. 2022 Jan 4;23(1):523.

11. Ferri A, Stagni V, Barilà D. Targeting the DNA damage response to overcome cancer drug resistance in glioblastoma. International Journal of Molecular Sciences. 2020 Jul 11;21(14):4910.
12. Lavin MF, Yeo AJ. Clinical potential of ATM inhibitors. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 2020 May 1;821:111695.
13. Ampolini EA, Jimenez-Sainz J, Long DT. The development of ATM inhibitors in cancer therapy. Targeted Oncology. 2025 Mar;20(2):281-97.
14. Al-Zoubi RM, Garada K, Al Huneidi R, Baddar ZM, Zarour AA, Elaarag M, Al-Zoubi SR, Al-Qudimat AR, Al Zoubi MS, Agouni A, Alrumaihi K. ATM inhibitors in cancer radiotherapy: Mechanisms, clinical development, and future directions. European Journal of Medicinal Chemistry. 2025 Dec 15;300:118137.
15. Fernandez A, Artola M, Leon S, Otegui N, Jimeno A, Serrano D, Calvo A. Cancer vulnerabilities through targeting the ATR/Chk1 and ATM/Chk2 axes in the context of DNA damage. Cells. 2025 May 20;14(10):748.
16. Scotto L, Serrano XJ, Zullo K, Kinahan C, Deng C, Sawas A, Bates S, O'Connor OA. ATM inhibition overcomes resistance to histone deacetylase inhibitor due to p21 induction and cell cycle arrest. Oncotarget. 2020 Sep 15;11(37):3432.
17. Zhang Q, Green MD, Lang X, Lazarus J, Parsels JD, Wei S, Parsels LA, Shi J, Ramnath N, Wahl DR, Pasca di Magliano M. Inhibition of ATM increases interferon signaling and sensitizes pancreatic cancer to immune checkpoint blockade therapy. Cancer research. 2019 Aug 1;79(15):3940-51.
18. Phan LM, Rezaeian AH. ATM: main features, signaling pathways, and its diverse roles in DNA damage response, tumor suppression, and cancer development. Genes. 2021 May 30;12(6):845.
19. Yan S, Sorrell M, Berman Z. Functional interplay between ATM/ATR-mediated DNA damage response and DNA repair pathways in oxidative stress. Cellular and Molecular Life Sciences. 2014 Oct;71(20):3951-67.
20. Terlizzi C, De Rosa V, Iommelli F, Pezone A, Altobelli GG, Maddalena M, Dimitrov J, De Rosa C, Della Corte CM, Avvedimento VE, Del Vecchio S. ATM inhibition blocks glucose metabolism and amplifies the sensitivity of resistant lung cancer cell lines to oncogene driver inhibitors. Cancer & Metabolism. 2023 Nov 6;11(1):20.
21. Tozaki Y, Aoki H, Kato R, Toriuchi K, Arame S, Inoue Y, Hayashi H, Kubota E, Kataoka H, Aoyama M. The combination of ATM and Chk1 inhibitors induces synthetic lethality in colorectal cancer cells. Cancers. 2023 Jan 25;15(3):735.
22. Zhang Q, Green MD, Lang X, Lazarus J, Parsels JD, Wei S, Parsels LA, Shi J, Ramnath N, Wahl DR, Pasca di Magliano M. Inhibition of ATM increases interferon signaling and sensitizes pancreatic cancer to immune checkpoint blockade therapy. Cancer research. 2019 Aug 1;79(15):3940-51.
23. Sahu RP, Batra S, Srivastava SK. Activation of ATM/Chk1 by curcumin causes cell cycle arrest and apoptosis in human pancreatic cancer cells. British journal of cancer. 2009 May;100(9):1425-33.
24. Li Y, Yang DQ. The ATM inhibitor KU-55933 suppresses cell proliferation and induces apoptosis by blocking Akt in cancer cells with overactivated Akt. Molecular cancer therapeutics. 2010 Jan 1;9(1):113-25.
25. Pusapati RV, Rounbehler RJ, Hong S, Powers JT, Yan M, Kiguchi K, McArthur MJ, Wong PK, Johnson DG. ATM promotes apoptosis and suppresses tumorigenesis in response to Myc. Proceedings of the National Academy of Sciences. 2006 Jan 31;103(5):1446-51.
26. Yi HK, Chang SH, Lee DY, Kim JS, Hwang RH. Induced apoptosis of cancer cells by antisense ATM gene therapy. Journal of the Korean Cancer Association. 2000 Apr 1;32(2):339-49.
27. Reed JC. Mechanisms of apoptosis. The American journal of pathology. 2000 Nov 1;157(5):1415-30.
28. Pellarin I, Dall'Acqua A, Favero A, Segatto I, Rossi V, Crestan N, Karimbayli J, Belletti B, Baldassarre G. Cyclin-dependent protein kinases and cell cycle regulation in biology and disease. Signal transduction and targeted therapy. 2025 Jan 13;10(1):11.
29. Li B, Li A, You Z, Xu J, Zhu S. Epigenetic silencing of CDKN1A and CDKN2B by SNHG1 promotes the cell cycle, migration and epithelial-mesenchymal transition progression of hepatocellular carcinoma. Cell Death & Disease. 2020 Oct 2;11(10):823.
30. Siafakas AR, Richardson DR. Growth arrest and DNA damage-45 alpha (GADD45α). The international journal of biochemistry & cell biology. 2009 May 1;41(5):986-9.
31. Hollander MC, Zhan Q, Bae I, Fornace AJ. Mammalian GADD34, an apoptosis-and DNA

damage-inducible gene. Journal of Biological Chemistry. 1997 May 23;272(21):13731-7.
32. Ren B, Cam H, Takahashi Y, Volkert T, Terragni J, Young RA, Dynlacht BD. E2F integrates cell cycle progression with DNA repair, replication, and G2/M checkpoints. Genes & development. 2002 Jan 15;16(2):245-56.

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