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Inhibition of PI3K pathway using BKM120 intensified the chemo-sensitivity of breast cancer cells to arsenic trioxide (ATO)

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ABSTRACT

Although conventional therapeutic approaches have brought remarkable advantages for the treatment of breast cancer (BC), drug-resistance still remains as a leading cause of tumor recurrence in this malignancy. In the present study, we designed experiments to evaluate the therapeutic value of PI3K inhibition in combination with Arsenic trioxide (ATO) in MCF-7 cells. The results of our study manifested that BKM120 sensitized MCF-7 cells to the lower concentrations of ATO. The significant anti-cancer effect of PI3K inhibition became even more evident when we found that BKM120, either as a single agent or in combination with ATO, reduced clonogenic ability of anoikis-resistant stem-like MCF-7 cells. Our findings also showed that BKM120 augmented ATO-induced anti-proliferative effects through inducing G1 arrest and reducing the incorporation of BrdU into the synthesized DNA of drugs-treated cells, which was coupled with c-Myc-mediated suppression of hTERT expression. Moreover, we found that in the presence of PI3K inhibitor, ATO is able to more profoundly induce apoptosis in MCF-7 cells, as revealed by the increment in the percentage of haplodiploid sub-G1 cells and the externalization of phosphatidylserine. Real-time PCR analysis also revealed that probably down-regulation of survivin coupled with up-regulation of forkhead family transcription factors is responsible for the enhancive effect of drugs in this cell line. Conclusively, this study shed lights on the effect of PI3K inhibition in chemo-sensitivity of MCF-7 cells, disclosing that combination of BKM120 and ATO could be a promising therapeutic approach in BC.

1. Introduction

The perplexing etiology of breast cancer along with its heterogeneity made it the second most prevalent cause of cancer death and converted it into one of the most daunting health problems in global public (Movahedi et al., 2012). It was optimistically believed that surgery procedure together with anti-estrogen-based chemotherapy could significantly reduce the number of malignant cells in the patients (Hortobagyi, 1998); however, the recurrence of the disease and tumor metastasis have spurred the expectation that probably other agents could brought advantages for these patients (Masuda, 2017). From the first identification of arsenic trioxide (ATO), a traditional Chinese medicine, the profound impact of this compound on different human diseases has been established (Hoonjan et al., 2018). The therapeutic value of ATO was initially approved for the treatment of relapsed acute promyelocytic leukemia (APL) patients (Fox et al., 2008) and its favorable anti-leukemic property put this agent under the magnifying glass for other malignancies, as well. Soon after, the anti-cancer property of ATO was examined on wide range of solid tumors, such as prostate (Su et al., 2013), lung (Zheng et al., 2015), glioma (Zhou et al., 2015), liver (Cui et al., 2006) and breast cancers (Wang et al., 2015). The history beyond the anti-cancer property of ATO is summarized in Fig.1.

Although endorsed successes of ATO therapy have been observed in wide range of malignancies, its dose-dependent toxicity has limited its clinical use (He et al., 2017; Mohan et al., 2017), proposing that probably applying this agent in combined modality may be more profitable. The recognition of the PI3K over-activation in breast cancer and its fundamental role in the acquisition of chemo-resistant phenotype (Clark et al., 2002) lend compelling weight into the application of PI3K inhibitors in the treatment strategies of breast cancer. Among different members of the selective small molecule inhibitors of PI3K, a

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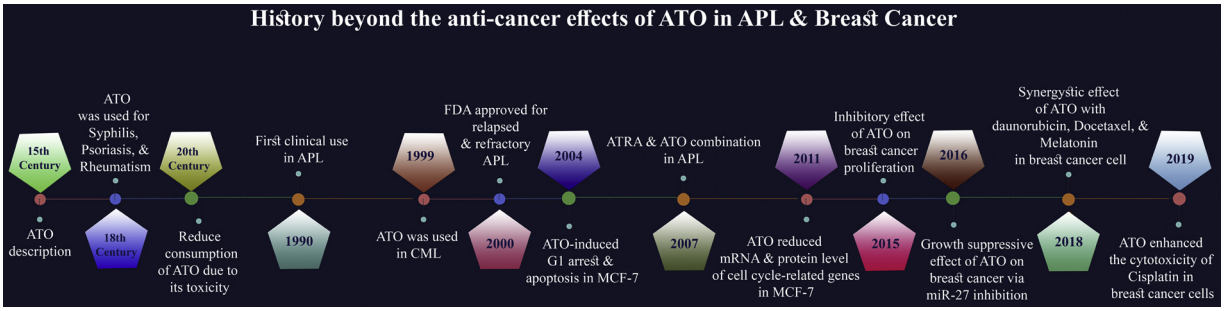

Fig. 1. A glance into the history of ATO in cancer treatment.

well-known orally bioavailable 2,6-dimorpholino pyrimidine derivative (BKM120) has shown to have a broad anti-cancer impact on wide range of human malignancies, including glioma (Li et al., 2017), lung (Vansteenkiste et al., 2015), leukemia (Bashash et al., 2017, 2016), multiple myeloma (Safaroghli-Azar et al., 2019) and breast cancer cells (Geuna et al., 2015) with various molecular alterations. Moreover, its ability to profoundly reinforce the anti-cancer effect of chemotherapeutic drugs, while lowering their toxic concentrations makes BKM120 the most clinically advanced agent in pan-PI3K inhibitors classes (Bashash et al., 2018; Yu et al., 2016). These promising effects together with the safety profile of BKM120 (Bendell et al., 2012) encouraged us to design experiments to evaluate for the first time whether BKM120 could potentiate the anti-cancer effect of ATO in breast cancer cells.

2. Material and methods

2.1. Cell culture and drug treatment

MCF-7 and MDA-MB-468 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 2 mM L-glutamine, 10% heat-inactivated fetal bovine serum, 100 units/ml penicillin, and 100 µg/ml streptomycin in a humidified 5% CO2 at 37 °C. Stock solutions of the pan-PI3K inhibitor BKM120 (50 µM) and PI3K-δ inhibitor CAL-101 (10 mM) (selleckchem, Germany) were made in sterile dimethyl sulfoxide (DMSO, Sigma, USA). For the treatment of breast cancer cell lines, relevant amounts of the agents, in the presence or absence of arsenic trioxide (ATO, Sian Daroo) (with the stock solution of 1 mM), were added into the culture medium to gain the desired concentrations. As negative control, equal volume of DMSO was added in control samples in which the final concentration of DMSO did not exceed than 0.1% of total volume.

2.2. MTT assay

To investigate the inhibitory effects of BKM120 on the viability index of breast cancer cells, microculture tetrazolium assay (MTT) was applied. Moreover, to investigate whether inhibition of the PI3K activity could increase cytotoxic effect of ATO, the cells were treated with BKM120 in combination with ATO. The cells (3000/well) were plated in 96-well plates and incubated with the indicated concentrations of the agents up to 72 h. After removing the media, cells were further incubated with MTT solution (5 mg/ml in PBS) at 37 °C for 3 h. The resulting formazan was solubilized with DMSO and the absorption was measured at 570 nm in an ELISA reader.

2.3. Crystal violet staining

About 2×10^3 cells were seeded in 24-well plates and treated with desired concentrations of BKM120 and ATO. After 48 h, cells were fixed with ice-cold methanol and stained with crystal violet (0.5%w/v). Plates were then photographed using an inverted microscope to determine both the morphology change and the number of viable cells.

2.4. Colony formation assay

About 500–1000 cells were seeded in each well of 6-well plates and treated with BKM120 and ATO. After 48 h of incubation, drug-containing media was replaced with drug-free media. Plates were incubated for 10–14 days till each colony contained almost 50 cells. They were fixed with ice-cold methanol and stained with crystal violet solution (0.5–2%w/v). Colonies were counted and the surviving fraction (SF) was calculated as:

SF = Mean colony counts of treated well / Mean colony count of control well.

2.5. Anoikis resistance assay

Wells in a 96-well plate were covered with Poly HEMA solution to stimulate anoikis. About 8×10^3 cells were added into each well and treated with different concentrations of BKM120 and ATO at the same time. After 48 h of incubation, plates were centrifuged at 200 *g* for 10 min and then, the cell viability and metabolic activity were analyzed using MTT assay.

2.6. BrdU cell proliferation assay

The suppressive effect of BKM120-plus-ATO on the growth and proliferation of breast cancer cells was assessed by measuring DNA synthesis rate of the cells using a colorimetric bromodeoxyuridine (BrdU)-based cell proliferation ELISA kit (Roche, Penzberg, Germany) as per manufacturer's recommendations. The experiment process has been described in our previous article (Sheikh-Zeineddini et al., 2019). Initially, MCF-7 cells were seeded into 96-well plate at a density of 5000 cells/well and treated with the indicated concentrations of the drugs. Afterwards, 10 µl/well of BrdU labeling solution was added, and the cells were re-incubated at 37^{oC} for 12 h. Then, cells were then fixed and DNA was denatured using 200 µl of FixDenat solution provided with the kit. After 30 min and discarding Fixodent, 100 µl peroxidase-conjugated anti-BrdU (antiBrdU-POD) antibody was added to each well. Finally, the cells were incubated with tetramethylbenzidine (TMB) for 3 min at room temperature and the reaction product was quantified by measuring the absorbance at 450 nm.

2.7. Median-effect analysis of drug combinations

To investigate whether there is an additive or a synergistic effect between BKM120 and ATO, we computed the values of combination index (CI) using the evaluations of CI value and Isobologram using CalcuSyn Software (Biosoft, Cambridge, UK) according to the classic isobologram equation: CI = D1/Dx1 + D2/Dx2, where Dx1 and Dx2 indicate the individual dose of ATO and BKM120 required to inhibit a given level of viability index and D1 and D2 are the doses of ATO and BKM120 necessary to produce the same effect in combination,

respectively.

2.8. Assessment of apoptosis using flow cytometry

To investigate whether BKM120 as a single agent or in combination with ATO could induce apoptotic cell death, MCF-7 cells were subjected to flow cytometry analysis. The cells were harvested after 48 h treatment with the agents, washed with PBS and resuspended in a total volume of 100 µl of the incubation buffer. Annexin-V Flous (2 µl per sample) was added, and cell suspensions were incubated for 20 min in the dark. Fluorescence was then measured using flow cytometry. Annexin V-positive and PI-negative cells were considered to be in early apoptotic phase and cells having positive staining both for annexin-V and PI were deemed to undergo late apoptosis.

2.9. Flowcytometric analysis of DNA content

Briefly, MCF-7 cells at the concentration of 5×10^5 were seeded into six-well plates and were treated with BKM120 and ATO. After 48 h, the cells were harvested, washed with PBS and fixed with 70% ethanol at −20 °C overnight. Afterward, the cells were treated with 0.5 µg/ml RNase in PBS and incubated at 37 °C for 30 min before staining with 50 µg/ml PI for 30 min. The cellular DNA content was quantified from the peak analysis of flowcytometric DNA histograms, and the data were interpreted using the Windows FlowJo V10 software.

2.10. Quantitative real-time PCR

After treatment of the cells, RNA was extracted by high pure RNA isolation kit (Roche) and quantified by a Nanodrop instrument (Nanodrop ND-1000 Technologies). Thereafter, 1 µg of RNA from each sample was applied for reverse transcription using the revertAid First Strand cDNA synthesis kit (Takara BIO, Japan). The prepared cDNA was subjected to qRT-PCR using SYBR Premix Ex Taq technology (Takara BIO) on a light cycler instrument (Roche). Melting curves were analyzed to validate single PCR product of each primer, and the values for the relative quantification were calculated based on $2^{-\Delta\Delta ct}$ relative expression formula.

2.11. Statistical analysis

Data are expressed as the mean ± S.D. of three independent experiments. All tests were performed in triplicate. The significance of differences between experimental variables was determined by the use of two-tailed Student's *t*-test and by one-way variance analysis. In order to compare the control group and the drug-treated cells, the Dunnett's multiple comparison test was used. A probability level of $P \leq 0.05$ was considered statistically significant.

3. Results

3.1. Anti-leukemic effects of BKM120 alone and in combination with chemotherapeutic drugs

Intrigued by the constant activation of PI3K signaling pathway in breast cancer cells (López-Knowles et al., 2010), we first set out to investigate the effects of a pan-PI3K inhibitor in two breast cancer cell lines (MCF-7 and MDA-MB-468) by performing MTT assay. As presented in Fig. 2A, PI3K inhibition using BKM-120 induced a considerable decrease in the viability of both breast cancer cells; however, MDA-MB-468 cells were resistant to the lower concentrations (0.25 and 0.5 µM) of BKM120. We also evaluated the cytotoxic effect of Arsenic trioxide (ATO), which has been proved as an effective chemotherapeutic drug in breast cancer (Alzuwaidi and Khalil, 2019; Shi et al., 2017; Zhang et al., 2016), in MCF-7 and MDA-MB-468 cell lines. As presented in Figure 2A, single agent of ATO exerted a dose- and time-dependent growth suppressive effect in breast cancer cells; however 2.5 µM of this drug could not induce significant anti-cancer activity. While over-activation of PI3K signaling pathway has an undeniable role in acquisition of chemo-resistant phenotype (Lee et al., 2004), it was tempting to address whether PI3K inhibition could potentiate anti-cancer assets of chemotherapeutic drugs. The results of the synergistic examinations and calculation of combination index (CI) (Figure 2B) showed that combination of BKM120 and ATO was merely effective in promoting cytotoxicity in MDA-MB-468 cell line, which may be as a result of less sensitivity of the cells to the lower concentrations of BKM120. However, we found that ATO in combination with BKM120 was more effective in inhibiting MCF-7 breast cancer cell survival as compared to either drug alone (Figure 2B). While BKM120 (0.5 µM) and ATO (2.5 µM) individually decreased the viability of MCF-7 cell by approximately 40% and 25%, respectively, the combination of these agents diminished the survival capacity of MCF-7 cells by almost 65% (Figure 2B). We also evaluated the morphological changes of the MCF-7 cells in response to the drugs treatment using crystal violet staining. Of particular interest, we found that suppression of PI3K using BKM120 led to changes in both morphology and number of viable cells either as a single agent or in combination with ATO (Fig. 3). To shed more light on the mechanisms by which PI3K inhibition could enhance the anti-cancer effects of ATO, we therefore expanded our further experiments on this combined-modality strategy in MCF-7 cells.

3.2. BKM120 in combination with ATO inhibits the survival of anoikis-resistant MCF-7 cells

Based on the inhibitory effects of the designated combination treatment on MCF-7 cell growth, it was of particular interest to evaluate whether BKM120-plus-ATO could reduce the survival rate of anoikis-resistant cells. Of note, we found that the combination of BKM120 at the concentrations of 0.25 and 0.5 µM with 2.5 µM of ATO decreased the viability of anoikis-resistant cells by almost 60% and 50%, respectively (Fig. 4A). The profound effect of both drugs on these cells were further confirmed by the results of qRT-PCR analysis revealed that the anti-survival effect was coupled with the up-regulation of Bad mRNA expression up to 1.8-fold which is one of the most important genes considered as an anoikis marker (Figure 4A). Intrigued by the cytotoxic effects of BKM120-plus-ATO on anoikis-resistant cells, we also addressed the effect of this combination on the clonogenic ability of the MCF-7 cells. Our results showed that the colony formation ability of the cells was inhibited more prominently, when MCF-7 cells were treated simultaneously with both drugs (Figure 4B).

3.3. Stimulatory effect of BKM120 on ATO-induced anti-proliferative properties was coupled with suppression of hTERT and c-Myc

It has been demonstrated that the PI3K signaling pathway is closely linked to an extraordinarily diverse group of cellular functions, including cell proliferation and DNA synthesis (Jason and Cui, 2016). To investigate the stimulatory effects of PI3K inhibition on the growth suppressive properties of ATO in MCF-7 cells, BrdU cell proliferation assay was applied. Interestingly, our data demonstrated that inhibition of PI3K not only suppressed the proliferative potential and DNA synthesis rate of the breast cancer cells as a single agent but also has the potential to sensitize the MCF-7 cells to ATO. As presented in Fig. 5, while the single agent of ATO reduced the ability of MCF-7 cells to replicate DNA by 6.4%, the presence of a PI3K inhibitor led to the remarkably decrease in the replicative potential of cells by 33.5%. On account of numerous studies which conceded that human telomerase reverse transcriptase (hTERT) plays an important regulatory role in cell proliferation (Huang et al., 2005), we aimed to investigate the effect of the drug combination on the mRNA expression level of this critical gene. As presented in Figure 5, our results showed that co-treatment of the MCF-7 cells with BKM120 and ATO not only suppressed hTERT

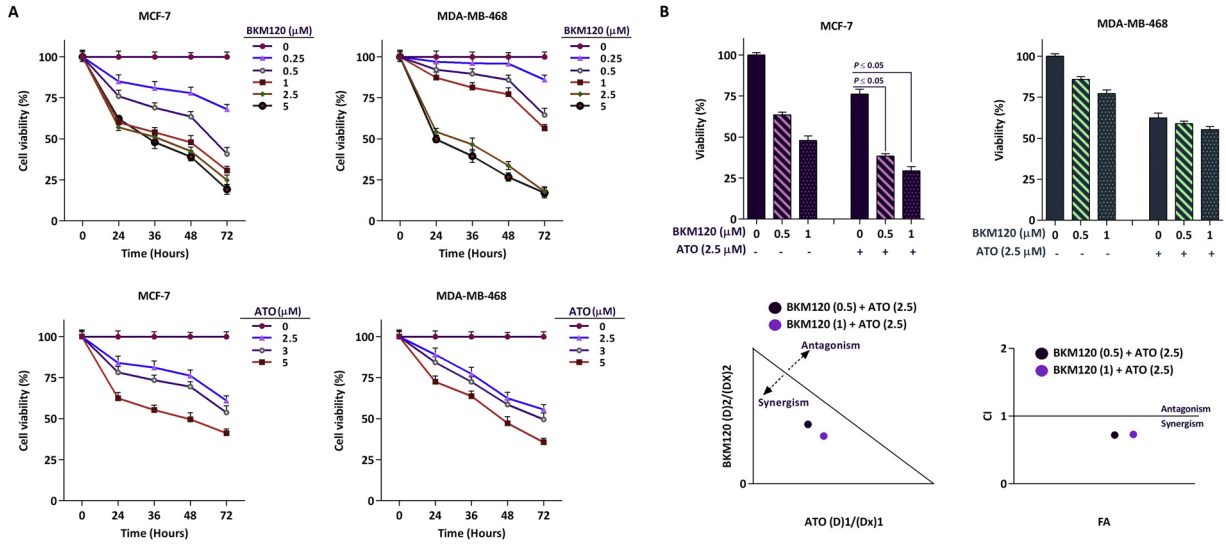

Fig. 2. Synergistic effects of ATO and BKM120 on MCF-7 cells. (A) MCF-7 and MDA-MB-468 cells were treated with different concentrations of BKM120 (0, 0.25. 0.5, 1, 2.5 and 5 μM) and ATO (0, 2.5, 3 and 5 μM) up to 72 h and cell viability was evaluated using MTT assay. B) Simultaneous treatment of MCF-7 cells with BKM120 (0.5 and 1 μM) and ATO (2.5 μM) after 48 h resulted in synergistic outcomes; however, this combination was merely effective in promoting cytotoxicity in MDA-MB-468 cell line. The combination index (CI) was calculated according to the classic isobologram equation. Values are given as mean ± S.D. of three independent experiments.

transcription but also decreased the mRNA expression of c-Myc, as the most predominant positive regulator of hTERT gene transcription. Taken together, our findings indicated that the anti-proliferative effect of PI3K inhibition in ATO-treated MCF-7 cells is probably induced through transcriptional suppression of c-Myc-mediated hTERT expression.

3.4. BKM120 induced G1 arrest in ATO-treated MCF-7 cells

In the light of the anti-proliferative effects of BKM120 and given the fundamental role of the PI3K signaling pathway in the regulation of the cell cycle (Fingar et al., 2004), the effect of BKM120 on cell cycle distribution of MCF-7 cells was examined using PI staining. As shown in Fig. 6, we found that BKM120 not only decreased the percentages of the cancerous cells in S and G2/M phases of the cell cycle but also induced a robust G1 arrest. Intriguingly, meaningful synergistic results obtained when we exposed the cells to BKM120 alongside ATO compared to either agent alone. Noteworthy, an increased percentage of hypodiploid sub-G1 cells was measured from 15% in ATO-treated cells to 27.9% in combination treatment series (Figure 6), shedding light on the pro-apoptotic potential of drug combination in MCF-7 cells. On the basis of these findings, it was of great interest to evaluate the transcriptional activity of the p21 and p27 as the critical regulators of the cell cycle transition from G1 to S phase (Liu and Lozano, 2005). Interestingly, we

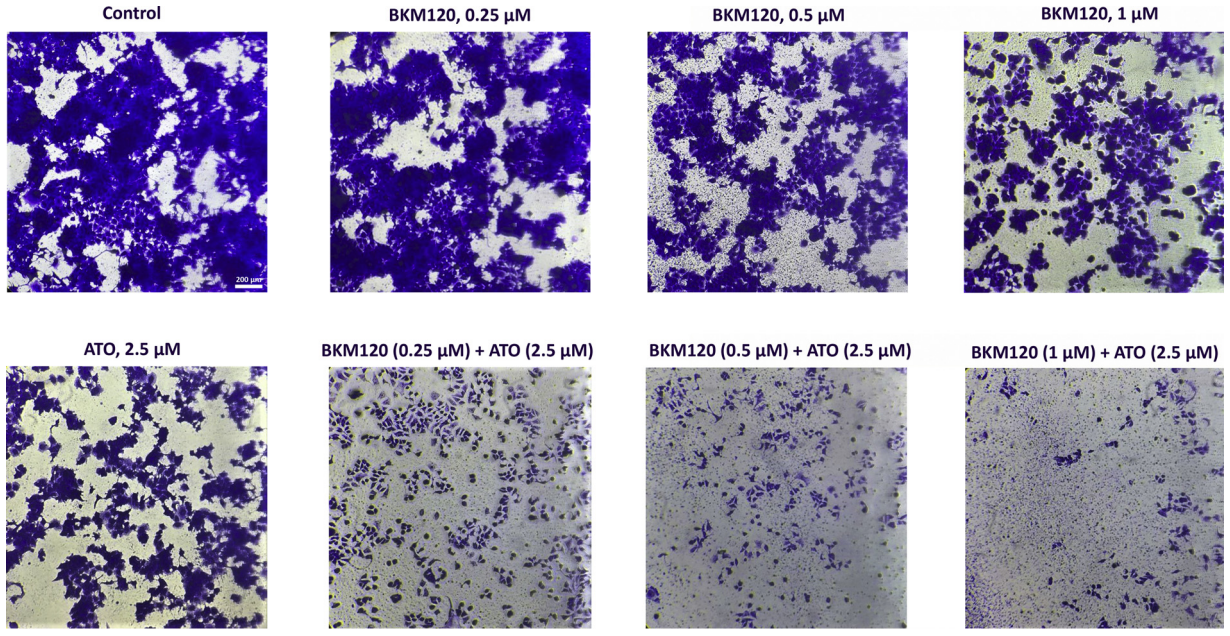

Fig. 3. BKM120 induced morphological changes in MCF-7 cells either as a single agent or in combination with ATO. The cytotoxicity and morphological changes induced by BKM120 alone or in co-treatment with ATO were visualized in MCF-7 cells after 48 h using crystal violet staining assay. The scale bar is 200 μm. Suppression of PI3K using BKM120 resulted in a conspicuous change in both morphology and number of viable cells either as a single agent or in combination with ATO.

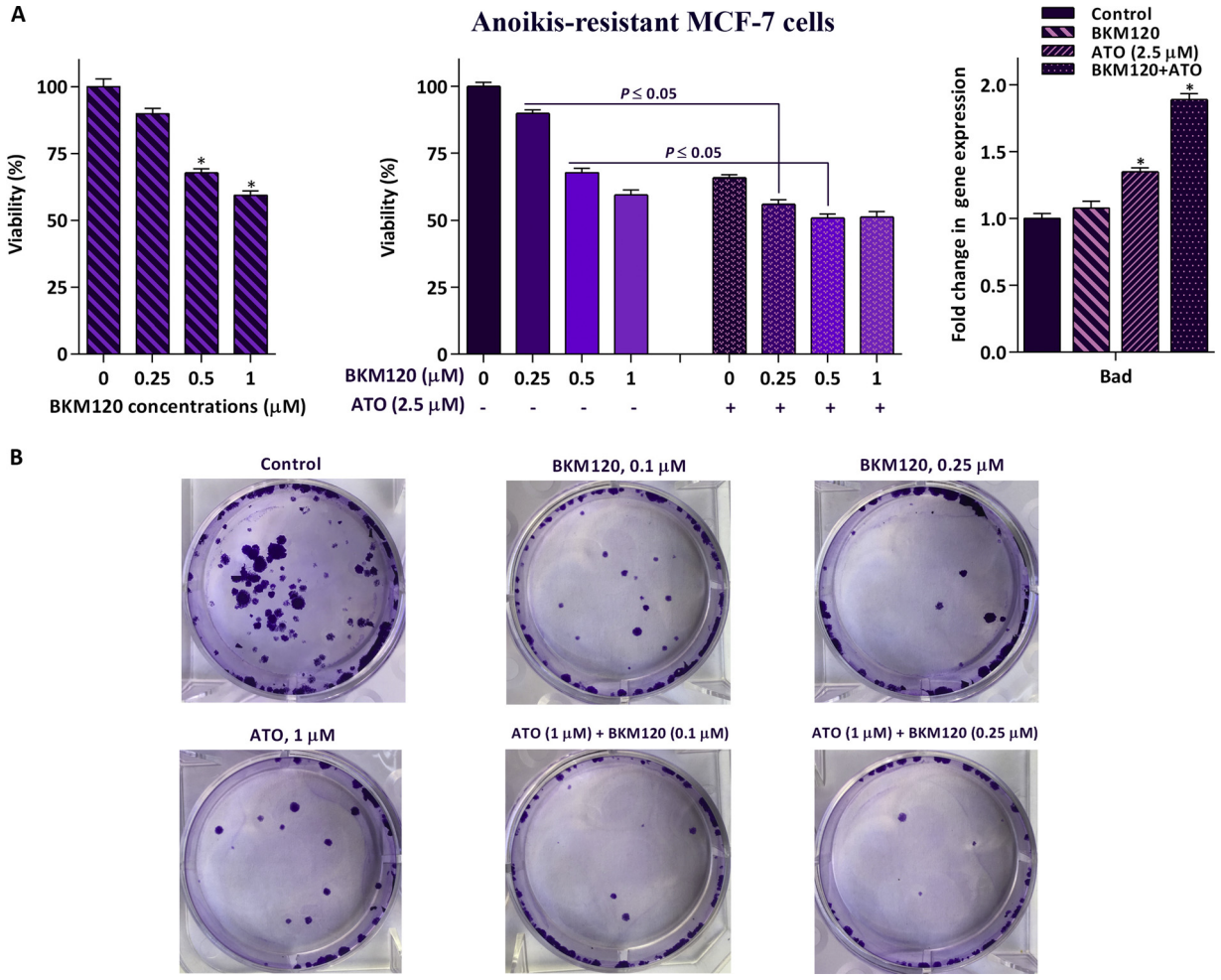

Fig. 4. Synergistic effects of ATO and BKM120 on anoikis-resistant MCF-7 cells. A)The results of MTT assay in an anoikis-inducing condition unraveled that BKM120 could suppress the survival of anoikis-resistant MCF-7 cells, in a concentration-dependent manner after 48 h treatment. Moreover, significant synergistic outcomes were evaluated after exposing anoikis-resistant MCF-7 to BKM120 (0.25 and 0.5 μM) and ATO (2.5 μM). In harmony, the data derived from qRT-PCR after 48 h, revealed that this synergy was coupled with the up-regulation of BAD mRNA expression. B) Clonogenic ability of MCF-7 cells was examined using colony formation assay. Values are given as mean ± S.D. of three independent experiments. *, $P \leq 0.05$ represent significant changes from untreated control.

found a considerable increase in the mRNA expression level of the aforementioned genes in ATO-treated MCF-7 cells which was in consistent with results achieved from cell cycle analysis (Figure 6).

3.5. BKM120 augmented ATO-induced apoptosis through alteration of apoptosis-related genes

To determine whether the cytotoxic effects induced in drugs-treated cells were likely because of the induction of apoptosis, the binding of

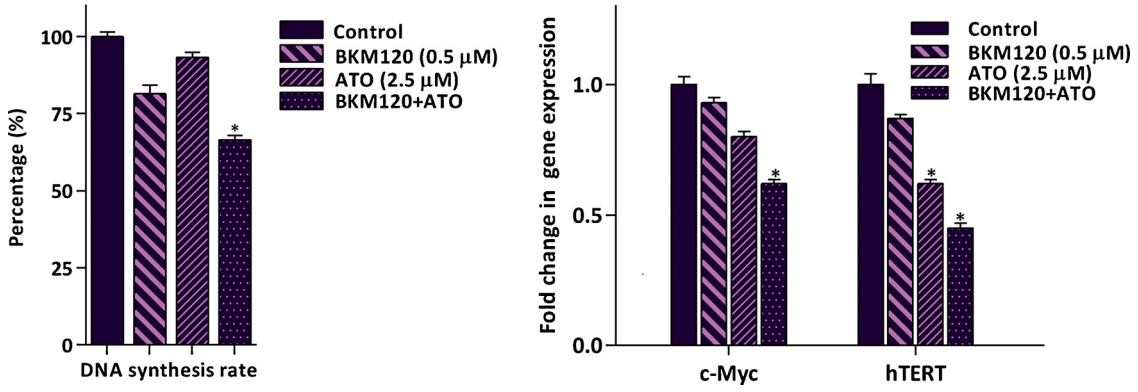

Fig. 5. BKM120 and ATO induced anti-proliferative effects through suppression of c-Myc and hTERT. A)After 48 h treatment of the MCF-7 cells with BKM120 and ATO, we evaluated the DNA synthesis rate using BrdU incorporation assay. B) The results derived from qRT-PCR revealed that BKM-120-plus-ATO-induced anti-proliferative activity was coupled with transcriptional suppression of c-Myc-mediated hTERT expression. Values are given as mean ± S.D. of three independent experiments. *, $P \leq 0.05$ represent significant changes from untreated control.

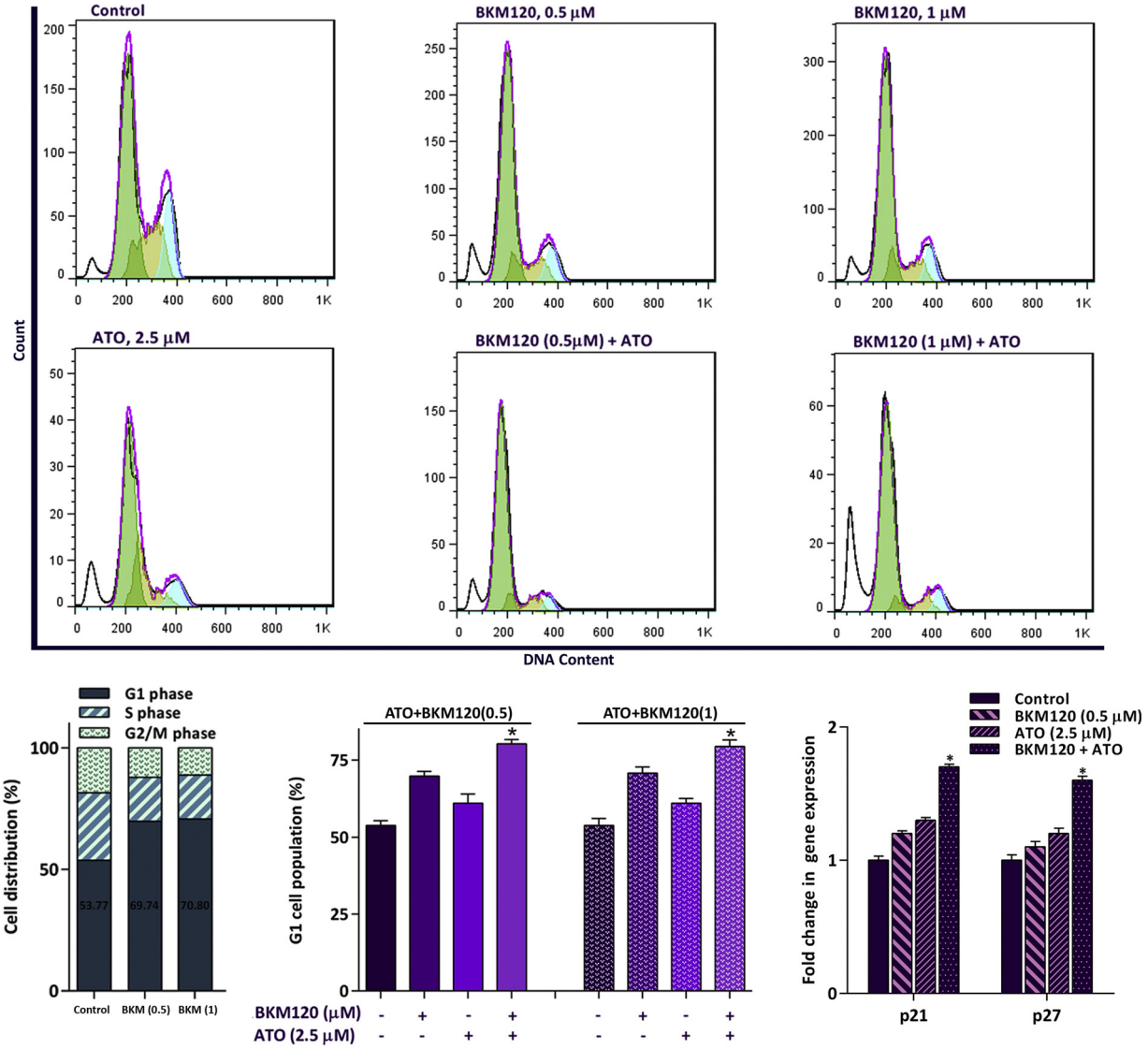

Fig. 6. Combination of BKM120 and ATO induced G1 cell cycle arrest in MCF-7 cells. The effect of BKM120 on cell cycle distribution of MCF-7 cells was examined using PI staining after 48 h. The data derived from qRT-PCR after 48 h revealed considerable increase in the mRNA expression level of the p21 and p27 genes in ATO-treated MCF-7 cells. Values are given as mean ± S.D. of three independent experiments. *, $P \leq 0.05$ represent significant changes from untreated control.

annexin-V combined with PI were analyzed by flow cytometry. Along with the elevated cell population in sub-G1, analysis of annexin-V staining assay revealed that single agent of BKM120 considerably increased Annexin-V/PI cell population in MCF-7 cells from 5.85% in control group to 33.6% in treated cells (1 μM) (Fig. 7A). Noteworthy, as depicted in this figure, the apoptotic effect of BKM120 in MCF-7 was even more conspicuous in combinational treatment, as the percentage of apoptotic/necrotic cells was robustly higher in the cultures of the cells co-treated with ATO and BKM120. To investigate whether the stimulatory effect of BKM120 on ATO-induced cytotoxicity in breast cancer cells is a general feature in PI3K inhibition, we examined the effects of ATO in combination with CAL-101, as a highly selective inhibitor of PI3K p110-δ isoform. In agreement with the results of BKM120, it became evident that PI3K inhibition using CAL-101 sensitized MCF-7 cells to ATO (Figure 7B). To delve into the molecular mechanisms by which BKM120 boosted the anti-cancerous assets of ATO in MCF-7 cells, we aimed to analyze the mRNA expression levels of apoptosis-related genes using real-time PCR. Our results illustrated that while ATO at the concentration of 2.5 μM had minimal effects on the mRNA expression level of the anti-apoptotic genes, its combination with BKM120 notably decreased the transcriptional expression of death repressor genes, including survivin, cIAP-1, Pin1 and Bcl-2 (Figure 7A). Moreover, we found that combinational treatment of MCF-7 cells with BKM120 and ATO could result in a meaningful increase in the mRNA expression level of pro-apoptotic genes like FOXO-3a, FOXO-4, Bax, Bad and Bid (Figure 7A).

4. Discussion

Identifying the constant activation of the PI3K signaling pathway in the development of chemo-resistance characteristic in breast cancer (Berns et al., 2007; Burris, 2013; Clark et al., 2002) rang the alarming bells that probably PI3K inhibition could be a promising approach for the treatment of this malignancy. Of note, the results of the current study showed that abrogation of PI3K, either using pan-PI3K inhibitor BKM120 or p110-δ isoform inhibitor CAL-101, considerably decreased the viability of both ER-positive MCF-7 and triple negative MDA-MB-468 cells; however, as compared to CAL-101, BKM120 evidently induced more pronounced anti-cancer effect in breast cancer cells. This finding was in corroboration with the previous studies indicating that breast cancer cells could activate other p110 isoforms as a compensatory way to bypass PI3K inhibition; suggesting that pan-PI3K inhibitors

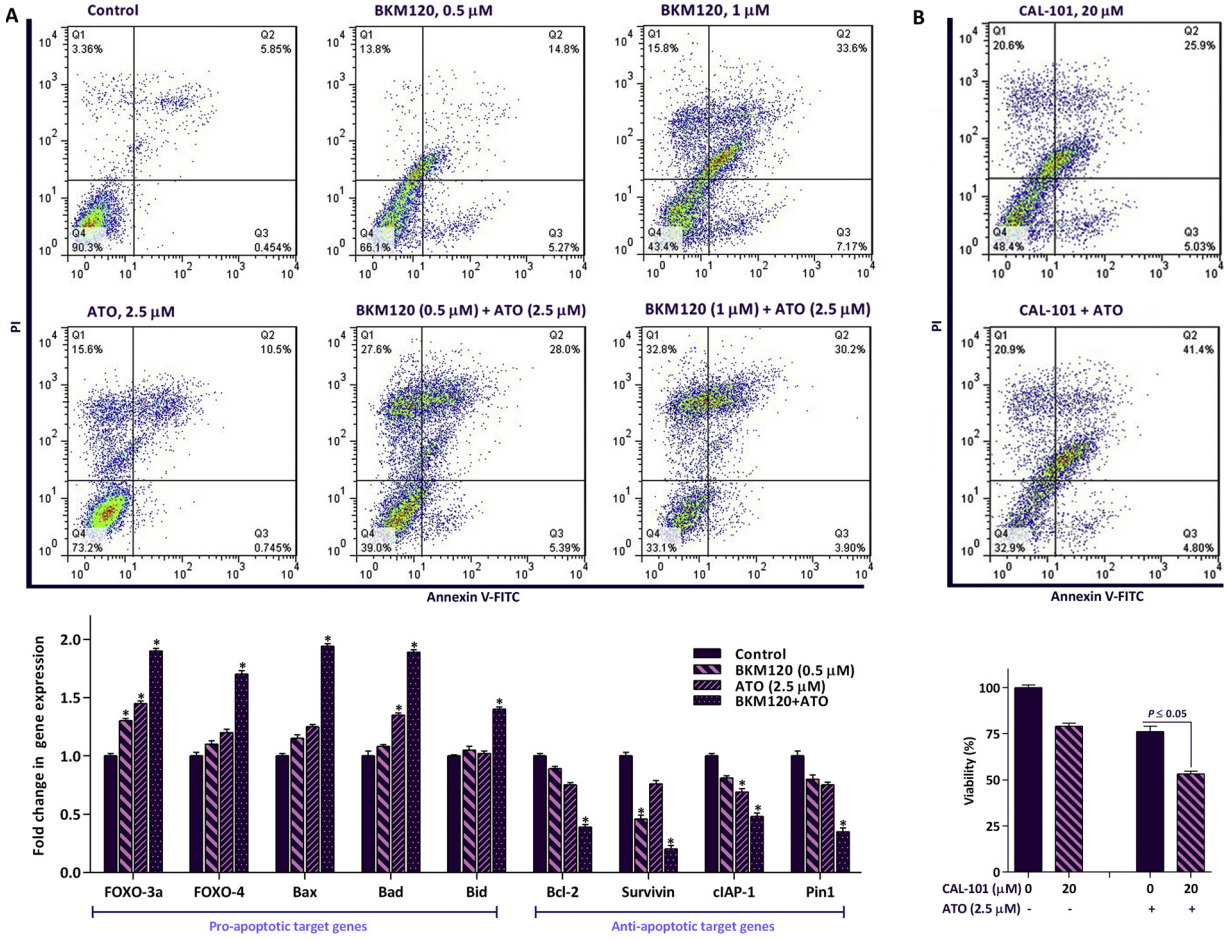

Fig. 7. Combination of BKM120 and ATO increased apoptosis in MCF-7 cells through alteration of apoptosis-related genes. A) After 48 h incubation of MCF-7 cells with BKM120 in combination with ATO, the percentages of Annexin-V-positive cells were increased remarkably as compared to the either agent alone. The expression levels of anti-apoptotic and pro-apoptotic genes were examined using qRT-PCR after normalizing the cycle thresholds (Ct) of each triplicate against their corresponding ABL after 48 h. B) We examined the effects of ATO in combination with CAL-101 using flow cytometry and MTT assay. Values are given as mean ± S.D. of three independent experiments. *, $P \leq 0.05$ represents significant changes from untreated control.

may be the best choice for the treatment of breast cancer (Patsouris et al., 2019; Robert et al., 2017).

The entrance of the PI3K inhibitors into the treatment protocol of breast cancer open up the possibility of other drugs to be engaged in the therapeutic approaches. Being a state-of-the-art candidate for the treatment of acute promyelocytic leukemia (Kiguchi, 2018), arsenic trioxide (ATO) currently find its way into the treatment strategies of a wide range of solid tumors, such as glioblastoma (Ghaffari et al., 2016), lung (Huang and Zeng, 2019), and gastric (Chi et al., 2018) cancers. Moreover, the ability of this agent in collaborating with other anti-cancer drugs such as ATRA (Huynh et al., 2019), telomerase inhibitors (Asghari-Kia et al., 2017; Bashash et al., 2013), melatonin (Nooshinfar et al., 2016), and chemotherapeutic drugs (Nakaoka et al., 2014; Zhang et al., 2015) has reaped applauses for the application of this agent in cancer treatment. Although ATO is a highly effective anti-cancerous drug widely used to treat several types of cancer, the appropriate effects are achieved in high doses which are not clinically achievable without the risk of various side effects (Chow et al., 2004). Given this and in an effort to enhance the effectiveness of the breast cancer treatment, we designed experiments to evaluate the effects of ATO in combination with BKM120 in human breast cancer cells. Our synergistic experiments revealed that BKM120 could potentiate the anti-cancer effect of the lower concentration of ATO (2.5 μM) in MCF-7 cells. The significant effect of BKM120 on breast cancer cells became even more evident when we tested its anti-cancer effect on anoikis-resistant stem-like breast cancer cells which possess the ability to invade distant sites and cause metastasis. We found that BKM120, either as a single agent or in combination with ATO, remarkably reduced the clonogenic ability of this cell line, proposing that probably inhibition of all PI3K isoforms could exert a potent anti-metastatic effect in breast cancer cells. This finding was in accordance with the results of Moro et al. who introduced the PI3K signaling pathway as a leading mechanism responsible for the formation of anoikis-resistance cells in prostate cancer epithelial cells (Moro et al., 2009).

The remarkable anti-cancer effect of ATO is mediated through different mechanisms, including caspase-3 activation, modulation of the intracellular glutathione redox system and induction of mitotic arrest due to inhibition of microtubuline formation (Ghaffari et al., 2012). It is also suggested that ATO could exert cytotoxic effect in breast cancer through either induction of apoptosis (Alzuwaidi and Khalil, 2019) or modulating the expression of microRNAs, such as let-7a (Shi et al., 2017). Interestingly, our results delineated that BKM120 augments ATO-induced anti-proliferative effects, at least partly, through the induction of G1 cell-cycle arrest as a result of up regulated p21 and p27 expression level. Being popular as the most important regulator of DNA replication and growth kinetics, hTERT is now believed to participate in other intracellular processes including modulation of pro- and anti-apoptotic target genes expression (Del Bufalo et al., 2005). Noteworthy, our results illustrated that co-treatment of the MCF-7 cells with BKM120-plus-ATO not only suppressed hTERT transcription and its associated gene (c-Myc), but also elevated the percentage of both apoptotic and necrotic breast cancer cells by shifting the expression

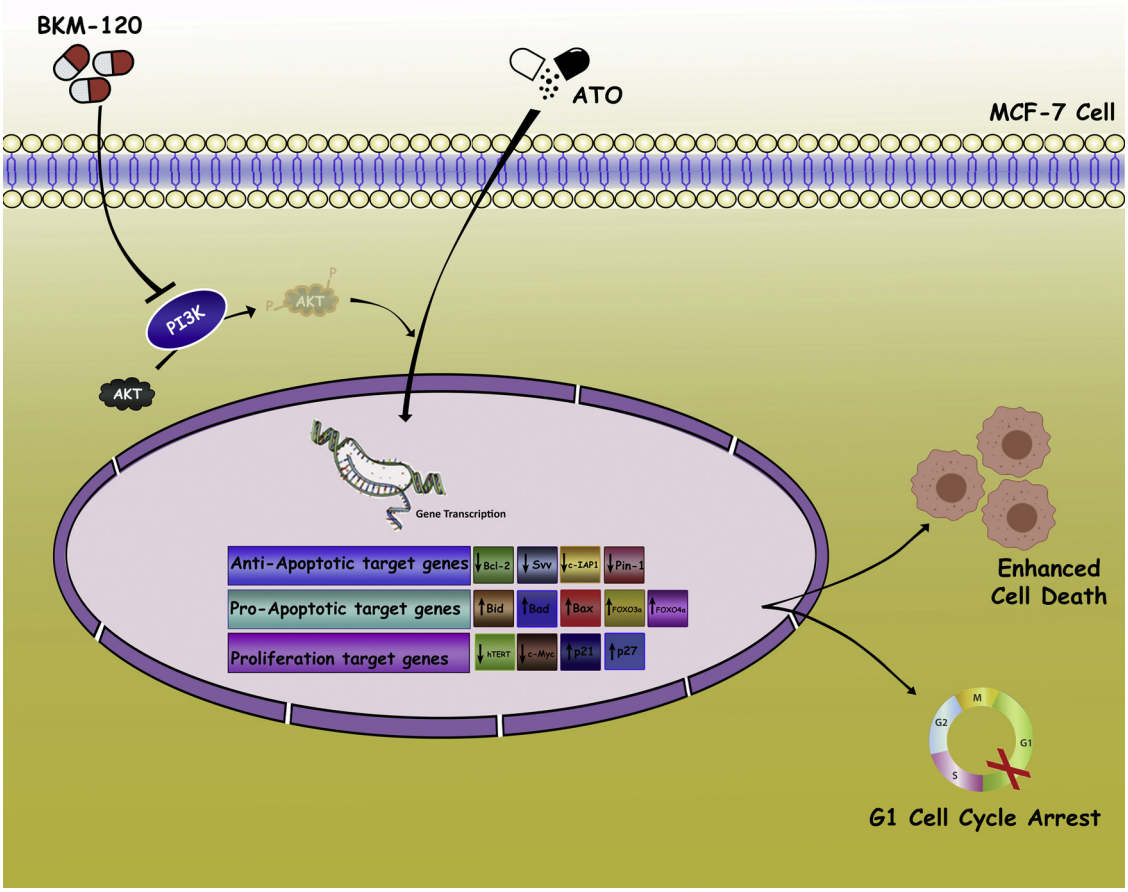

Fig. 8. Schematic representation proposed for the plausible mechanisms of action of BKM120 and ATO in MCF-7 cells. Abrogation of the PI3K signaling pathway, using BKM120 potentiated the cytotoxic effect of ATO on breast cancer-derived MCF-7 cells. As presented, co-treatment of cells with both agents not only induced apoptosis through alteration in the expression level of pro- and anti-apoptotic related genes, but also halted the transition of cells from G1 phase of cell cycle through replicative potential of cells via c-Myc-mediated suppression of hTERT.

levels of apoptosis-related genes. Given to the previous disclosure that ATO could promote cell death through both apoptosis and necrosis mechanisms (Selvaraj et al., 2013), we postulated that suppression of the PI3K signaling pathway has the capacity to potentiate the effect of ATO on both of these pathways (Fig. 8).

Although it is early to hazard a conjecture on the mechanism by which ATO-plus-BKM120 decreased survival of MCF-7 cells, a possible candidate would be survivin, a key cellular transcription factor that engages in unique crosstalk with mitosis and apoptosis (Mita et al., 2008). It has been reported that the transcriptional activity of survivin, which has an association with the mRNA expression levels of several apoptotic target genes of forkhead family transcription factors (Luo et al., 2007), could decrease overall survival and increase risk of recurrence and metastasis in breast carcinomas (Gritsko et al., 2006; Kennedy et al., 2003; Marioni et al., 2006). Of particular interest, we found that combination treatment of MCF-7 cells with ATO and BKM120 not only suppressed the mRNA expression level of survivin, but also increased the mRNA expression levels of FOXO3a and FOXO4.

Taken together, the results of the present study showed that inhibition of the PI3K signaling pathway enhanced the anti-cancer effect of the lower concentrations of ATO in breast cancer cells. Moreover, we found that induced p21, subsequent G1 cell cycle arrest and transcriptional suppression of survivin expression may contribute in the enhanced growth suppressive effect of drugs combination. Due to the suggested safety profile of BKM120 in clinical trials, our study suggests that pan-PI3K inhibition in combination with ATO might be a promising adjuvant therapy and may confer advantages for breast cancer treatment. However, to ascertain the safety and the efficacy of this combination therapy for the treatment of breast cancer patients, further in vivo investigations are warranted in the near future.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.biocel.2019.105615.

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