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Original Article

Combinatorial targeting of PI3K/AKT pathway with BKM120 increases cisplatin sensitivity and apoptotic response in A549 lung cancer cells

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Abstract

Despite significant advancements in the treatment of non-small cell lung cancer (NSCLC) using conventional therapeutic methods, drug resistance remains a major factor contributing to disease recurrence. In this study, we aimed to explore the potential benefits of combining PI3K inhibition with Cisplatin in the context of NSCLC-derived A549 cells. Human non-small cell lung cancer A549 cells were cultured and treated with BKM120, cisplatin, or their combination. Cell viability was assessed using the MTT assay. Drug interactions were evaluated by calculating combination and dose reduction indices. Cell cycle progression and apoptosis were analyzed by flow cytometry. Quantitative real-time PCR was performed to measure the expression of key genes related to the cell cycle, apoptosis, autophagy, and proteasome function. The effects of autophagy and proteasome inhibition were further examined using chloroquine and bortezomib, respectively. Our findings demonstrated that BKM120 sensitized A549 cells to Cisplatin at lower concentrations. Moreover, we observed that BKM120 enhanced the anti-proliferative effects of Cisplatin by inducing cell cycle arrest in the G1 phase and upregulating the expression of P21 and FOXO4. Moreover, our real-time PCR analysis provided evidence that the combination treatment not only down-regulated Bcl-2 expression but also upregulated BAD and BAX expression in A549 cells, which ultimately led to apoptotic-mediated cell death. In conclusion, this investigation illuminated the role of PI3K inhibition in the chemo-sensitivity of 549 cells and revealed that the combination of BKM120 and Cisplatin may represent a viable therapeutic option for NSCLC.

Keywords: Non-small cell lung cancer (NSCLC), PI3K/AKT pathway, BKM120, Cisplatin, Chemotherapy.

1. Introduction

Lung cancer continues to be the leading cause of mortality globally among different types of cancers [1]. Non-small cell lung cancer (NSCLC) constitutes a significant portion of lung cancer cases and is often detected at advanced stages of the disease [2]. It is worth noting that the mentioned malignancy accounts for roughly 80% of cases with significant metastatic potential [3].

For several years, Cisplatin has been employed as a therapeutic choice for patients with diverse cancer types, such as ovarian, bladder, head and neck, breast, cervical, testicular, esophageal, and lung cancer, among others, for an extensive period of time [4]. In broad terms, this particular chemical compound, which is a constituent of platinum-based anticancer medications, exerts cytotoxic effects by interacting with DNA and forming covalent DNA-platinum adducts. As a result, it induces a DNA damage response within malignant cells [5, 6]. Nevertheless, although Cisplatin combination therapy is currently regarded as one of the most effective treatments for advanced NSCLC, instances of Cisplatin treatment failures, resistance, and recurrence have been reported, indicating the inevitable nature of these phenomena in certain cases [4].

Recently, the involvement of the PI3K/AKT/mTOR signaling pathway has been increasingly acknowledged as a significant contributor to the aggressive nature of lung cancer [7]. Moreover, according to multiple studies, the PI3K/AKT/mTOR signaling pathway exhibits aberrant changes in various cancers, including lung cancer. These alterations contribute to chemotherapeutic resistance and the dysregulation of apoptosis [8]. Researchers are increasingly focusing on the development of therapies that target the PI3K/AKT/mTOR pathway, and new drugs are continuously being discovered. These include the PI3K inhibitor buparlisib (NCT01911325), the AKT inhibitor MK2206 (NCT01294306), the mTOR inhibitor sirolimus, and dual PI3K/AKT inhibitor (Perifosine) (NCT00399789) [9]. Although extensive research has been conducted to explore the effects of different types of PI3K inhibitors on various human cancers, the precise me-

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chanisms by which they reduce the viability of cancer cells are not fully understood [10]. Among the various classes of PI3K inhibitors, BKM120 (Buparlisib), an oral pan-class I PI3K inhibitor, has garnered significant attention due to its remarkable therapeutic effectiveness. It achieves this by specifically targeting the four catalytic isoforms of class I PI3K, namely p110α, p110β, p110δ, and p110γ. The implementation of this agent in preclinical models shows a decrease in PI3K/AKT/mTOR signaling as well as induction of pro-apoptotic, anti-angiogenic and anti-proliferative effects [11]. Therapeutic investigations have been conducted on BKM120 for progressive solid tumors, including prostate, breast, and colorectal cancer, as well as advanced NSCLC [12-17]. Furthermore, the antitumor efficacy of BKM120 has been documented in glioblastoma, multiple myeloma, as well as bone and soft tissue sarcoma [18-20]. Given these, we conducted experiments to assess the synergistic impact of combining BKM120 with reduced concentrations of Cisplatin in A549 cells derived from cancerous lung tissue. Additionally, we investigated the molecular mechanism through which BKM120 can modify the anticancer effects initiated by Cisplatin. These preliminary results provide a foundation for further investigation into the potential synergistic effects of combining BKM120 with Cisplatin in the context of cancer treatment.

2. Materials and methods

2.1. Cell culture and drug treatment

In order to evaluate the efficacy of BKM120 and Cisplatin in a lung cancer cell line, A549 cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (Invitrogen), 2 mM l-glutamine (Invitrogen), and antibiotics. The cells were then incubated in a humidified incubator. For the experimental treatments, the cells were exposed to either Cisplatin (MYLAN) or BKM120 (Selleckchem, Germany) alone, or a combination of both compounds. Additionally, the cells were treated with chloroquine (CQ) (Sigma Germany) and Bortezomib (BTZ) (Selleckchem, Germany), to evaluate the effects of autophagy and proteasome. As a control, an equivalent volume of dimethyl sulfoxide (DMSO) as a solvent was administered to the cells at a final concentration of 0.1%.

2.2. Microculture tetrazolium test for detection of metabolic activity

To evaluate the impact of BKM120, Cisplatin, CQ, and BTZ on the metabolic function of A549 cells, we seeded the cells in 96-well plates at a concentration of 7×10^3 cells. After a 12-hour incubation period, the cells were treated with the specified drugs either individually or in combination. Following a 24 and 48-hour incubation, 100 µl of MTT solution was added to each well to assess metabolic activity. The plates were then incubated at 37 °C for 3 hours, followed by centrifugation. Next, the media were replaced with 100 µl of sterile DMSO. Finally, the dissolved formazan was measured for optical densitometry at a wavelength of 570 nm using an enzyme-linked immunosorbent assay (ELISA).

2.3. Combination index (CI) and dose reduction index (DRI) determination

Combination index (CI) and dose reduction index (DRI), which are quantitative measures used to assess the efficacy and interaction of multiple agents when used in combination for therapeutic purposes, were assessed as previously stated [21]. It is worth noting that CI values below 1, equal to 1, and above 1 correspond to synergism, an additive effect, and antagonism between the agents, respectively.

2.4. Analysis of cell cycle distribution

The effects of BKM120 and Cisplatin on the progression of the cell cycle were examined using flow cytometry. Following a 48-hour exposure to the drugs, approximately 1×10^6 A549 cells were collected and underwent two rounds of rinsing with cold PBS. Subsequently, the cells were fixed in a solution consisting of 70% ethanol. For the purpose of staining DNA and RNA degradation, propidium iodide (PI) and RNase were added to each sample, respectively. Using flow cytometric histograms, the quantity of cells in every single phase of the cell cycle was determined. Additionally, FlowJo V10 for Windows was the tool used for the result interpretation.

2.5. Apoptosis assessment by flow cytometry

In 24-well plates, A549 cells were cultured and then exposed to BKM120 (1 µM), Cisplatin (5 µg/ml), and in combination for 48 h. The harvested cells were washed with PBS, and 100 µl of incubation buffer was added to each sample. Subsequently, the cells were resuspended and incubated with Annexin V-Fluos solution in the dark for 20 minutes. Flow cytometry and the Windows FlowJo software were used to quantify the annexin-V and annexin-V/PI positive cells percentage.

2.6. Analysis of mRNA expression using quantitative real-time PCR after RNA extraction and cDNA synthesis

Total RNA was extracted from A549 cells using the RNA Isolation Kit sourced from Roche, Mannheim, Germany. The extracted RNA was quantified using Nanodrop to ensure adequate quantity. Subsequently, cDNAs corresponding to each isolated RNA sample were synthesized using the cDNA synthesis kit from Takara Bio, Shiga, Japan. To examine the impact of BKM120 and Cisplatin on the expression of genes associated with proliferation, apoptosis, and metastasis, quantitative real-time PCR (qRT-PCR) was conducted on the generated cDNAs. The fold change values were determined using the $2^{-\Delta\Delta CT}$ relative expression formula.

2.7. Statistical analysis

Data were collected from three separate experiments and analyzed in triplicate compared to untreated control cells. All the available data were analyzed using GraphPad Prism Software, utilizing a two-tailed Student's t-test and one-way analysis of variance (ANOVA). The Dennett multiple comparison test was employed to compare the drug-treated and control groups. The data are presented as the mean ± standard deviation (S.D.), and statistical significance was indicated by a probability level of $P \leq 0.05$.

3. Results

3.1. Synergistic effects of BKM120 in combination with Cisplatin in A549 cell line

Given the crucial role of the PI3K signaling pathway in the development of a chemotherapy-resistant phenotype, it was tempting to examine the impact of PI3K inhibition

on anticancer function of chemotherapy medications. First of all, to determine the optimal time and concentration by which BKM120 induces its anticancer effect, A549 cells were treated with different concentrations of this agent. As illustrated in Figure 1A, viability of A542 cells decreased in a time and concentration-dependent manner after 24- and 48-hour treatment with BKM12O and the calculated IC50 was approximately 3.95 µM. The data collected from synergistic experiments and the calculation of combination index (CI) and dose reduction index (DRI) demonstrated that Cisplatin combined with BKM120 was more efficient in inhibition of A549 cell survival in comparison to solo administration of each agent. As displayed in Figure 1B, the synergistic effect of BKM120 with Cisplatin was verified when A549 cells were exposed to both drugs. As presented in Figure 1C, all the points were in synergistic area when combination index (CI) was calculated by Isobologram equation.

3.2. Synergistic effects of BKM120 and Cisplatin were mediated through G1 phase arrest

Promising effects of BKM120 on anti-cancerous activity of Cisplatin piqued our interest in evaluating the antiproliferative property of this agent on A549 cells. For this purpose, PI staining and flow cytometry were utilized to examine the alteration in cell cycle distribution following the exposure of A549 cells to different concentrations of BKM120. As anticipated, PI3K inhibition considerably affected the number of cells in different stages of the cell cycle, where a notable elevation in the percentage of G1 phase was observed. As shown in Figure 2A, the population of cells in G1 phase rose from 65% in control cells to 80% in combinatorial treatment. The expression of P21 as one of the most important proteins in cell cycle regulation was analyzed using qRT-PCR. Consistent with the flow cytometry data, our results showed a marked increase in P21 expression following the combined treatment. Additionally, we evaluated the expression of the FOXO4 gene, a key regulator involved in oxidative stress signaling and cell cycle control. After 48 hours of treatment, a significant upregulation of FOXO4 mRNA was observed in the combination group. Following the 48 hours of treatment of cells, the mRNA expression of FOXO4 demonstrated a remarkable increase in combinatorial modality (Fig. 2B).

3.3. BKM120 enhanced the efficacy of Cisplatin through alteration of Bcl2 family members in A549 cells

The results of Annexin V-PI analysis demonstrated a significant increase in Annexin-V and Annexin-V/PI double-positive cells following exposure to our combination treatment (Fig. 2C). Notably, the combination therapy of BKM120 and Cisplatin exhibited a more pronounced apoptotic effect, as evidenced by a substantially higher proportion of apoptotic cell death in cells concurrently treated with Cisplatin and BKM120. Furthermore, our findings revealed a modest upregulation in the gene expression of two pro-apoptotic members of the Bcl-2 family, namely BAX and BAD, upon treatment with BKM120 alone. However, when BKM120 was combined with Cisplatin, a remarkable approximately four-fold enhancement in the expression levels of these genes was observed compared to treatment with either agent individually. We investigated the impact of BKM120, alone and in combination with Cisplatin, on the expression of the anti-apoptotic gene Bcl-2. qRT-PCR analysis revealed that the combination of BKM120 and Cisplatin led to a reduction of Bcl-2 expression to less than half compared to the control group (Fig. 2D).

3.4. Autophagy acts as a defensive mechanism to evade the effects of the combination treatment

The analysis of autophagy-related genes, SIRT and C-myc, revealed that both BKM120 alone and in combination with cisplatin increased the expression of these genes.

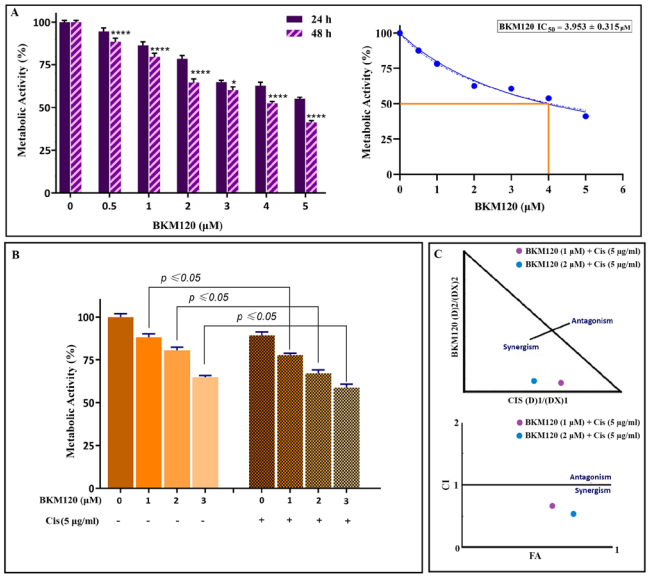

Fig. 1. The suppressive impact of BKM120 on the metabolic activity of A549, either alone or in combination with Cisplatin. (A) A549 cells were exposed to varying concentrations of BKM120 for 24 and 48 hours, and the metabolic activity of the cells was assessed using the MTT assay. The IC50 value for BKM120 was determined to be approximately 3.95 µM. **(B)** When A549 cells were subjected to combination treatment, a synergistic interaction between BKM120 and Cisplatin was observed. Values are given as mean ± S.D. of three independent experiments. * P ≤ 0.05 represented significant changes from the untreated control.

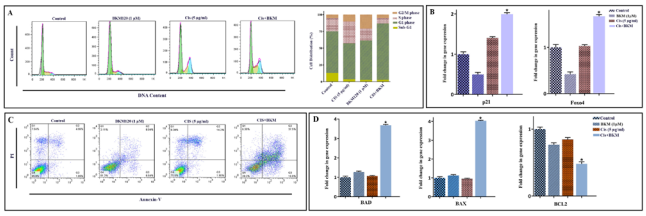

Fig. 2. (A) Combination of BKM120 and Cisplatin-induced arrest in the G1 phase of the cell cycle. **(B)** The data derived from qRT-PCR after 48 h revealed a significant increase in the mRNA expression level of the P21 and FOXO4 genes in BKM120-plus-Cisplatin-treated A549 cells. **(C)** Following the 48 h incubation of A549 cells with BKM120-plus-Cisplatin, the percentages of Annexin-V-positive cells were elevated significantly in comparison to either agent alone. **(D)** Using qRT-PCR, the expression levels of anti-apoptotic and pro-apoptotic genes were evaluated. Unlike Bcl-2, the expression levels of BAD and BAX were markedly increased after simultaneous treatment with BKM120 and Cisplatin. Values are given as mean ± S.D. of three independent experiments. * P ≤ 0.05 represents significant changes from untreated control.

Notably, c-Myc and SIRT expression levels were elevated by approximately three- and four-fold, respectively, compared to the control group. Notably, c-Myc and SIRT gene expression levels increased by approximately three-fold and four-fold, respectively, compared to the control group (Fig. 3A). In order to elucidate the involvement of autophagy in A549 cancer cells, the impact of chloroquine, an autophagy inhibitor, on the metabolic activity of these cells was examined at varying concentrations and time intervals of 24 and 48 hours. As depicted in Figure 3B, the inhibition of the autophagy system in cells treated with BKM120 and Cisplatin resulted in a significant reduction in the metabolic activity of A549 cells compared to the control group.

3.5. The effect of BKM120-Cisplatin on A549 cells was enhanced through suppression of proteasome

The qRT-PCR findings indicated that the expression of EGFR, a commonly altered oncogene in solid cancers, was increased in cells treated with single agents of BKM120 and Cisplatin. However, in the combination treatment group, the expression of EGFR was reduced, although this reduction was not statistically significant (Fig. 3C). Previous studies have reported that the upregulation of EGFR can act as a compensatory mechanism for cancer cells to evade treatment, and this process may involve the NF-κB pathway [22]. Moreover, there is solid evidence supporting the effectiveness of the proteasome inhibitor bortezomib (BTZ) in inhibiting both classical and alternative NF-κB signaling [23]. Based on this understanding, we formulated a hypothesis that the NF-κB pathway may function as a protective mechanism, thereby reducing the anticancer effects of our combination treatment. Interestingly, when we used BTZ to inhibit NF-κB pathway signaling, we observed a substantial enhancement in the efficacy of BKM120-Cisplatin on A549 cells (Fig. 3D).

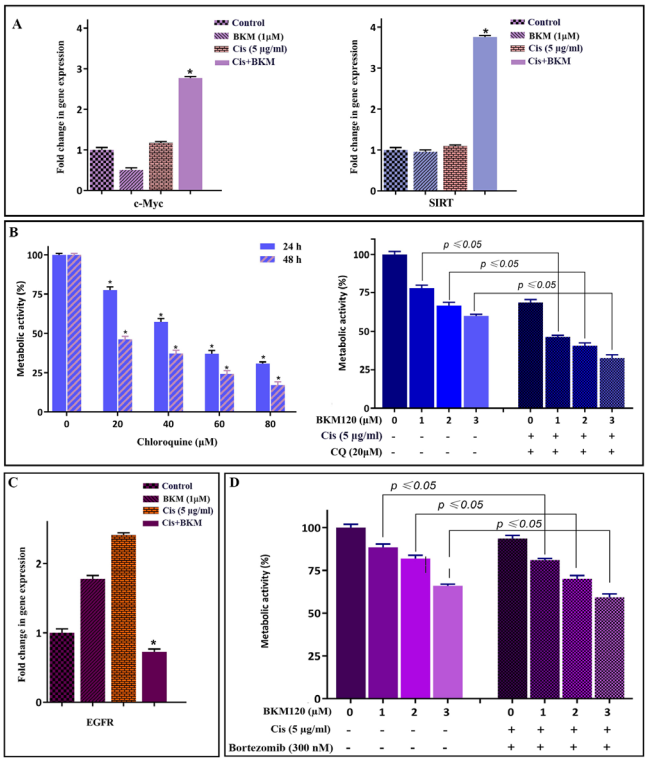

Fig. 3. The effect of autophagy and proteasome suppression on the cytotoxicity of BKM120-plus-Cisplatin on A549 cells. (A) The analysis of qRT-PCR indicated that the expression of SIRT and c-Myc was significantly increased in combinatorial modality as compared to either drug alone. **(B)** Simultaneous treatment of A549 cells with a non-cytotoxic concentration of CQ (20 μM) combined with BKM120-Cisplatin resulted in a cytotoxicity enhancement. **(C)** qRT-PCR results demonstrated no significant alteration in the expression of EGFR. **(D)** As presented, a significant increase in metabolic activity of A549 cells was seen when Bortezomib was added to the medium. Values are given as mean ± S.D. of three independent experiments. * P ≤ 0.05 represented significant changes from the untreated control.

3.6. The combination of BKM120 and Cisplatin resulted in a decrease in the expression of genes associated with metastasis

To conduct a more comprehensive analysis, we aimed to assess the expression of genes associated with metastasis and cellular migration in each treatment group. To achieve this, we utilized qRT-PCR to evaluate the expression of matrix metalloproteinases (MMPs), which are key factors involved in cell invasion and metastasis [24]. According to our findings, the expression of both MMP2 and MMP9 exhibited a substantial decrease in the BKM120-Cisplatin treatment group. In order to gain further insights into the anti-invasive effects of our combination treatment, we also examined the expression of other genes that play a vital role in cancer cell invasion. Following a 48-hour drug treatment with BKM120, we assessed the mRNA expression level of COL1A1, which belongs to the collagen family and is associated with metastasis and cell survival in lung cancer [25]. The results clearly demonstrated a significant reduction in the expression of this gene when the drugs were used in combination. Additionally, considering the findings from previous studies that indicated a positive relationship between the PI3K/Akt pathway and VEGF expression in a mouse model of lung carcinoma, we also examined the expression of the VEGF gene [26]. Interestingly, the expression of VEGF was significantly increased in cells treated with BKM120 alone. However, when Cisplatin was added to the cell culture medium, the expression of VEGF gene was significantly reduced (Fig. 4).

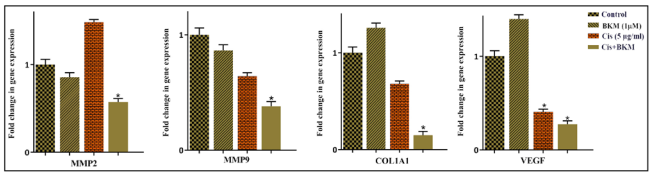

Fig. 4. The effects of BKM120 combined with Cisplatin on the mRNA levels of MMP2, MMP9, COL1A1 and VEGF. Values are given as mean ± S.D. of three independent experiments. * P ≤ 0.05 represented significant changes from the untreated control.

4. Discussion

Currently, lung cancer, particularly non-small cell lung cancer (NSCLC), accounts for 80% of all cases and remains a significant health concern, posing a considerable risk to individuals [3]. Nevertheless, the conventional chemotherapy approach for NSCLC shows unsatisfactory outcomes, and various factors contribute to treatment failure and subsequent mortality of patients [27]. Increasing the dosage of chemotherapy has limited therapeutic

benefits and tends to amplify the adverse effects associated with the treatment, making it an ineffective strategy [28]. Combining and simultaneously administering multiple agents has been demonstrated as an effective approach for treating various types of cancers, including metastatic or drug-resistant malignancies [29]. Previous studies have indicated that the synergistic use of two or more chemotherapeutic drugs can effectively eliminate cancer cells while minimizing systemic side effects at lower doses [30-32]. Following multiple cycles of chemotherapy, patients diagnosed with lung cancer often develop resistance to Cisplatin, a commonly employed treatment for this type of cancer [4]. In order to mitigate complications such as severe renal injury, several other chemotherapeutic agents have been combined with Cisplatin, allowing for a reduction in the overall dosage of chemotherapy administered [33, 34].

Considerable progress has been achieved in the field of small-molecule inhibitors aimed at targeting different classes of PI3K. Ongoing studies and clinical trials are actively investigating new drugs that disrupt components of the PI3K/AKT pathway [35]. Thus far, a range of inhibitors targeting the PI3K/AKT signaling pathway have been created, encompassing isoform-specific, dual, or pan inhibitors [36]. BKM120 (Buparlisib), a pan-class I PI3K inhibitor, has gained significant interest as a selective small-molecule inhibitor due to its strong anti-tumoral effects. Currently, the clinical effectiveness of BKM120 is being assessed in the treatment of diverse adult cancers, such as prostate, breast, advanced non-small cell lung cancers, and advanced solid tumors [37-40]. In the present study, we examined the impact of BKM120 on the anticancer effects induced by Cisplatin in A549 cells derived from NSCLC.

Initially, we conducted preliminary experiments to assess the impact of BKM120 on the metabolic activity of A549 cells. The measured IC50 value of BKM120, which represents the concentration required to inhibit 50% of cell growth, was determined to be 3.95 µM, which was consistent with previous studies that have examined the effects of BKM120 on various cancer cell lines [41, 42]. The findings from our combinatorial experiments yielded intriguing results, indicating a synergistic interaction between BKM120 and Cisplatin. The combination index (CI) values were consistently below 1, suggesting a significant synergistic effect between the two agents and the concentration of 1 µM of BKM120 was chosen for further experiments. Given the fact that the PI3K/AKT pathway plays a crucial role in regulating various aspects of the cell cycle, including progression, we conducted a comprehensive analysis to investigate how the combination of BKM120 and Cisplatin impacts cell cycle distribution. When cells were treated with BKM120 or Cisplatin as single agents, there were no significant changes in the percentage of cells in each cell cycle phase compared to the control group. However, in the combinatorial treatment group, a remarkable increase in the percentage of cells in the G1 phase of the cell cycle was observed, which indicates that BKM120 enhances the efficacy of Cisplatin, at least in part, through G1 cell cycle arrest. Similarly, the inhibition of PI3K in breast cancer cells could also develop synergistic effects for BKM120 via inducing G1 cell cycle arrest [43]. The concurrent upregulation of P21 and FOXO4, which was proven by the results of qRT-PCR, suggests a coordinated response of the cell cycle and apoptosis pathways to our combination treatment. Various lines of evidence indicate that these molecular alterations play a role in disrupting cell cycle progression and facilitating apoptotic cell death [44, 45]. Consistent with this, the flow cytometric analysis results revealed a significant increase in the proportion of both early and late apoptotic cells in the combination treatment compared to each individual agent alone. Of particular interest, we found that combination of BKM120 and Cisplatin not only reduced the mRNA expression level of BCL2 but also elevated the expression of the death-promoting genes such as BAD and BAX. In accordance with this, Alipour et al. provided evidence that in MCF7 cells derived from breast cancer, BKM120 augmented the anti-survival effects of arsenic trioxide (ATO) by influencing the expression of both antiapoptotic and proapoptotic genes, which results in a shift in the balance between these genes, ultimately contributing to the observed effects [46]. The upregulation of autophagy-related genes, including c-Myc and SIRT1, led us to hypothesize that A549 cells employ the autophagy system to evade the effects of our combination treatment. To explore this hypothesis, we assessed the impact of autophagy inhibition using chloroquine on the efficacy of BKM120-Cisplatin. The results indicated that inhibiting autophagy enhanced the anti-cancer effects of our combination treatment. This suggests that autophagy may act as a compensatory mechanism that diminishes the effectiveness of BKM120-Cisplatin. The underlying reason for selection of SIRT for autophagy assessment is that numerous transcription factors, such as FOXO1, can be deacetylated by SIRT1 to regulate autophagy [47]. Interestingly, previous studies indicate that increased expression of c.Myc can induce an escalation in SIRT1 protein levels, which can indirectly affect the autophagy [48]. Furthermore, the application of Bortezomib (BTZ), a proteasome inhibitor, resulted in heightened sensitivity to BKM120-Cisplatin. This observation suggests that the

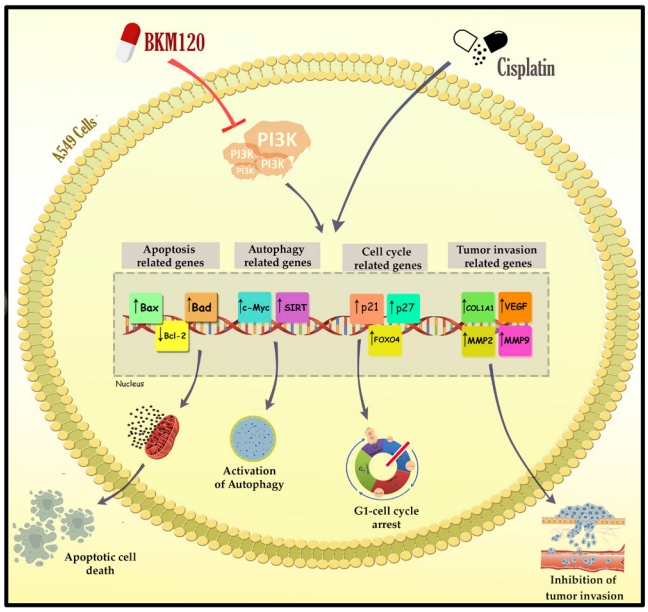

Fig. 5. Schematic representation illustrating the plausible mechanisms by which BKM120-Cispaltin induce anti-cancer effects in NSCLC-derived A549 cells. Abrogation of PI3K/AKT signaling using small molecule BKM120 in combination with Cisplatin leads to induction of apoptosis, G1 cell cycle arrest, activation of autophagy and suppression of tumor invasion..

NF-kB signaling pathway may play a contributory role in the cellular response to the combination of these drugs. To investigate the effects of our combination treatment on the invasive capacity of A549 cells, we examined the mRNA levels of various factors associated with cancer cell invasion, including matrix metalloproteinases (MMPs) such as MMP2 and MMP9, vascular endothelial growth factor (VEGF), and collagen [49]. Our results revealed a significant decrease in the expression of these factors following the combinatorial treatment, which indicates that the simultaneous use of BKM120 and Cisplatin enhances their anti-metastatic potential. The exact molecular mechanism underlying the anticancer activity of our drug combination in A549 cells has been summarized in Figure 5.

Taken together, our in vitro findings further substantiate the beneficial effects of PI3K inhibition in enhancing chemotherapy efficacy in lung cancer cells. Additionally, our results underscore the potential counteractive roles of autophagy and proteasome activity, which may reduce the overall anti-cancer effectiveness of this combination therapy. Considering the favorable safety profile of BKM120 demonstrated in clinical trials, combining Cisplatin with pan-PI3K inhibition holds promise as a valuable adjuvant therapy for breast cancer treatment. However, comprehensive evaluation through clinical trials and in vivo studies is essential to confirm the efficiency and safety of this combination approach.

In summary, our study provides compelling evidence that PI3K inhibition with BKM120 potentiates the antitumor effects of cisplatin in NSCLC-derived A549 cells through synergistic induction of cell cycle arrest and apoptosis. Importantly, we demonstrated that modulation of apoptotic and cell cycle regulators underlies this enhanced efficacy, while autophagy and proteasome activity may serve as escape mechanisms limiting the full therapeutic benefit. These findings not only support the use of PI3K inhibitors in combinatorial chemotherapy regimens for lung cancer but also highlight the significance of targeting cellular stress responses to further improve treatment outcomes in NSCLC. Further preclinical and clinical validation of this combinatorial approach is warranted to pave the way toward more effective therapeutic strategies for patients with resistant or advanced lung cancer.

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Declarations
Conflicts of interest
The authors declare that they have no conflict of interest.

Availability of data and materials
All data generated or analyzed during this study are included in this published article and its supplementary information files. Further details are available from the corresponding author upon reasonable request.

Authors' contributions
Sara Razi and Fattah Sotoodehnejadnematalahi: Conceptualization, Methodology, Investigation, Data curation. Sepideh Chodary Khameneh: Formal analysis, Validation, Visualization, Writing—original draft preparation. Amir-Mohammad Yousefi and Mahdi Kohansal Vajari: Software, Resources, Investigation, Review & Editing. Davood Bashash: Project administration, Supervision, Writing—review and editing, Correspondence. All authors have read and approved the final manuscript.

Use of artificial intelligence tools
Some sentences in this manuscript were revised using artificial intelligence language models to enhance clarity and readability. All final content decisions and intellectual contributions remain solely the responsibility of the authors.

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