Combined use of PI3K and MEK inhibitors synergistically inhibits lung cancer with *EGFR* and *KRAS* mutations

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Abstract. *EGFR* and *KRAS* mutations are the two most common driver mutations in non-small cell lung cancer (NSCLC). Molecular target-based therapy using small molecules such as gefitinib has been used for inhibiting EGFR with good initial responses; however, drug resistance is common when using a mono-targeting strategy. At present, KRAS remains an undruggable target. As such, the development of new drugs targeting the downstream of KRAS and EGFR and their crosstalk pathways is critically needed to effectively treat NSCLC. The present study aimed to elucidate the anticancer effects of PI3K (BKM120) and MEK (PD1056309) inhibitors on NSCLC cell lines with *KRAS* or *EGFR* mutations. Inhibition of the EGFR and KRAS downstream P13K pathway using BKM120 significantly inhibited the growth of NSCLC cell lines with either EGFR or KRAS mutations. In addition, significant cell cycle arrest and induction of apoptosis were observed following BKM120 treatment. Notably, although the A549 and H358 NSCLC cell lines harbor the same KRAS mutation, A549 cells were less sensitive than H358 cells in the response to BKM120 treatment. Similarly, PC-9 and H1650 cells harbor the same EGFR mutation, however, H1650 was less sensitive to BKM120. Different sensitivity between NSCLC cell lines with the same oncogenic mutation suggests that multiple crosstalk pathways exit. Combined usage of BKM120 and PD1056309 synergistically enhanced apoptosis in the A549 cells and mildly enhanced apoptosis in the H1650 and H358 cells, suggesting the crosstalk of the MEK pathway with the P13K/Akt pathways in these cell lines. Overall, our findings suggest that inhibition of EGFR and KRAS downstream with a P13K/Akt inhibitor could be useful for treating NSCLC. However, for NSCLC exhibiting crosstalk with other survival pathways, such as the MEK pathway, combination treatment is required.

Introduction

Lung cancer is the most lethal cancer worldwide, with an incidence of 1.6 million new cases annually and 1.38 million deaths reported in 2008 (1). In 2014, there were an estimated 1,665,540 new cancer cases diagnosed and 585,720 lung cancer-related deaths in the US, and lung cancer continues to be the most common cause of cancer-related mortality in both men and women (2). In China, lung cancer is also the leading cause of cancer-related deaths in both men and women (3). Although tremendous effort has been put into lung cancer research, the 5-year survival rate of lung cancer patients is still only 14%, implying the need for new treatment strategies such as new single effective anticancer drug intervention or new rationale drug combination protocols (4).

EGFR and *KRAS* are the two most common driver mutations in lung cancer (5), and are the major drug targets for NSCLC treatment. Interestingly, the frequency of *EGFR* mutations is particularly higher in Eastern Asia countries when compared with that in Western countries (6). It is known that lung cancer treatment can be achieved by using cytotoxic agents such as platinum compounds, tubulin inhibitors and molecular-targeting agents to interrupt the signaling pathways responsible for cell proliferation and survival, such as the RAS/RAF/MEK/MAPK and the phosphatidylinositol 3-kinase (PI3K)/Akt/mTOR pathways (5). However, the effect of inhibition of single or both pathways on NSCLC cells with

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EGFR or *KRAS* mutations remain unclear. Multiple studies have reported the close association of the P13K/Akt/mTOR pathway with cancer cell survival, differentiation, adhesion, migration and invasion (7). Preclinical studies suggest that targeting the PI3K pathway can be an effective treatment strategy for certain patients with NSCLC (8). Recently, a new P13K/Akt/mTOR inhibitor, BKM120 was developed, which has shown efficacy in inhibiting lung cancer as well as other types of cancer, including squamous and non-squamous carcinoma by suppressing Akt and mTOR and their downstream effectors (8,9).

BKM120 was launched into a phase II clinical trial and was demonstrated to be safe and well-tolerant for patients, with a favorable pharmacokinetic profile, clear evidence of target inhibition and preliminary clinical antitumor activity (8). However, in a study involving a panel of 353 cancer cell lines, BKM120 exhibited preferential inhibition of tumor cells bearing *PIK3CA* mutations, in contrast to either *KRAS* or *PTEN* mutant alone models (10). Thus, it appears that the treatment effect of BKM120 on NSCLC cells with *KRAS* or even *EGFR* mutation is not completely known. Moreover, PD0325901 is one of the currently investigated MEK inhibitors, and is a synthetic organic molecule that selectively binds to and inhibits mitogen-activated protein kinase (MEK) (11). The RAS/MEK pathway plays an important role in cancer development and progression (7), and often demonstrates pathway convergence to the PI3K/Akt/mTOR pathway. Recent studies have demonstrated that co-targeting both pathways can enhance the therapeutic response in cancer patients (12). However, the types of gene mutations have not been verified in these patients showing a positive response. Thus, the precise biomarkers and mechanism of the antitumor effect are less clear (13).

In the present study, we aimed to investigate the *in vitro* cancer inhibitory effect of a newly developed PI3K/Akt/mTOR pathway inhibitor (BKM120) on NSCLC cell lines with either *EGFR* or *KRAS* mutations, the two most commonly found mutations in NSCLC. Both PC-9 and H1650 cells habour *EGFR* mutations while H358 and A549 cells habour *KRAS* mutations. We first examined and compared the effect of the single use of BKM120 on these cell lines, and then subsequently compared a combined treatment effect of BKM120 and the MEK inhibitor PD0325901. Functional assays including apoptosis and cell cycle analysis as well as treatment mechanism were performed so as to evaluate their treatment value.

Materials and methods

Reagents. BKM120 was supplied by Roche Diagnostics Ltd. (Lewes, East Sussex, UK) and was dissolved in dimethyl sulfoxide (DMSO) and stored at -80°C. PD0325901 was purchased from Sigma (St. Louis, MO, USA), dissolved in DMSO and stored at -80°C. Primary antibodies against p-AKT (S473), p-S6 (S235/S236), S6, p-p70s6k, p70s6k, GAPDH, PARP, P-ERK and ERK were purchased from Cell Signaling Technology (Danvers, MA, USA). Primary antibodies against AKT were purchased from Santa Cruz Biotechnology (Dallas, TX, USA). Fluorescein-conjugated goat anti-rabbit and mouse secondary antibodies were purchased from Odyssey (Belfast, ME, USA). Annexin V/PI staining dye was purchased from BD Biosciences (San Jose, CA, USA).
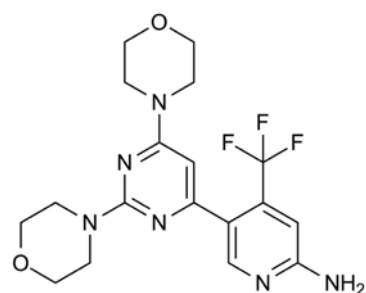

Figure 1. The chemical structure of BKM120.

Cell lines and cell culture. The human NSCLC cell lines A549, H1650, H358, PC-9 and normal lung fibroblast cell line CCD19 were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). Both A549 and H358 cell lines have *KRAS* gene mutations (14), while H1650 and PC-9 cells have an in-frame deletion on exon 19 (Del E746-A750) of *EGFR* (15); PC-9 is sensitive to gefitinib while H1650 is resistant to gefitinib. All NSCLC cell lines were grown in monolayer culture in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin from Gibco and were cultured at 37°C in a humidified atmosphere containing 5% CO_2. The normal lung fibroblast CCD19 cells were grown in monolayer culture in Dulbecco's modified Eagle's medium (DMEM).

Cytotoxicity assay. Cell viability was assessed using a standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. All NSCLC cells and the normal lung fibroblast cell line CCD19 were seeded in 96-well plates at a density of $5x10^3$/well overnight. The cells were then treated with a range of concentrations of BKM120 for 72 h or with DMSO as vehicle control. Each dosage was repeated in triplicate. After a 72-h treatment, 10 μl MTT (5 mg/ml) solution was added to each well, and the plates were placed back into the incubator for 4 h, and then 100 μl of the resolved solution (10% SDS and 0.1 mM HCl) was added and the plates were incubated for another 4 h to dissolve the formazan crystals. Finally, the colorimetric intensity of the plates was measured at 570 nm (absorbance) and 650 nm (reference) using a Tecan microplate reader (Tecan US, Inc., Morrisville, NC, USA). The cell viability was calculated as the percent change in absorbance of the treated cells divided by the absorbance of the untreated cells.

Cell cycle and apoptosis assay using flow cytometry. All NSCLC cell lines were plated on a 6-well plate with a cell density of $2x10^5$ cells, and were serum-starved overnight. The cells were then treated with different concentrations of BKM120 or co-treated with the MEK inhibitor (PD0325901) for 24 h. After treatment, the cells were harvested by trypsin digestion and collected by centrifugation. For apoptosis analysis, the cells were then washed twice with ice-cold 1X PBS and stained with 5 μl of propodium iodide (PI, 1 mg/ml) and 5 μl Annexin V fluorescein dye at room temperature in the dark for 15 min. The cells were then resuspended in 400 μl of Annexin-binding buffer (BD Biosciences). The percentage of apoptotic cells was quantitatively determined using a BD FACSAria III flow
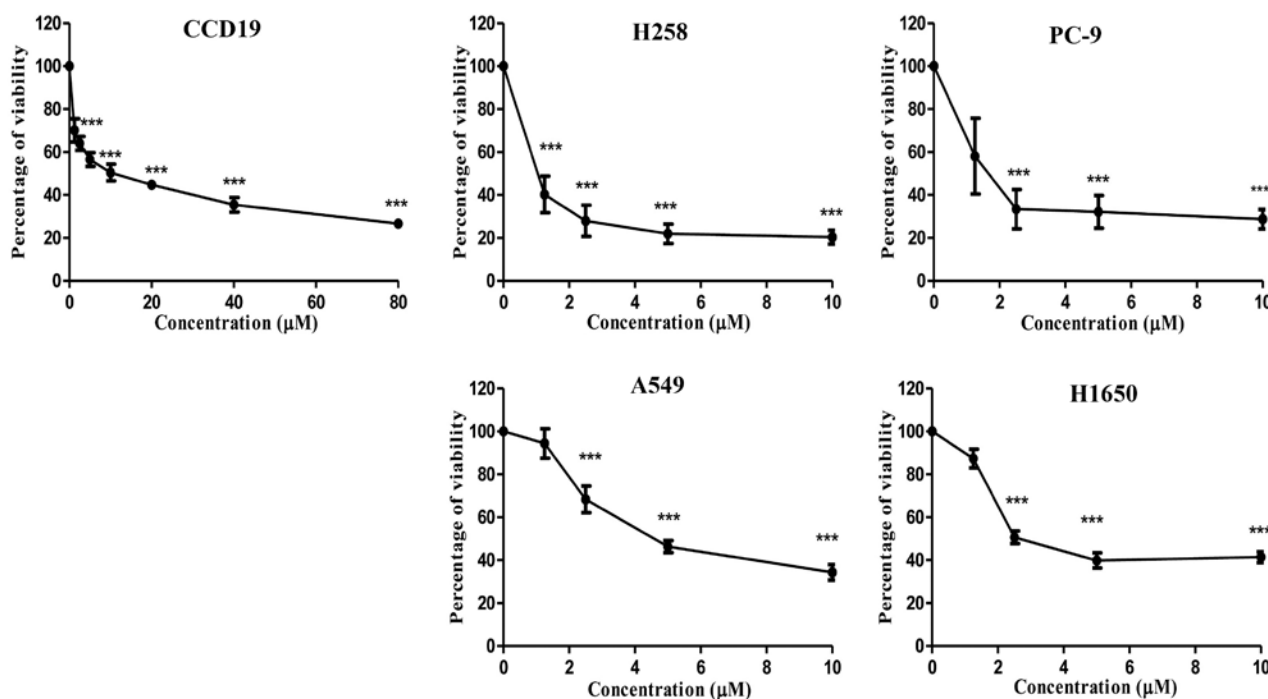
Figure 2. The cytotoxic effects of BKM120 on a panel of NSCLC cell lines and one normal lung fibroblast cell line CCD19. MTT assay analysis of the cell viability of H358, PC-9, A549, H1650 and CCD19 after 72 h of BKM120 treatment. Results are expressed as mean ± SD, n=3 (***P<0.001).

cytometer (BD Biosciences). For cell cycle analysis, the cells were stained with 5 μl of PI (1 mg/ml) alone in the presence of methanol for fixation. Then, the percentages of cells at the S, G1 and G2 phases were quantitatively measured using a BD FACSAria III flow cytometer (BD Biosciences).

Western blot analysis. Preparation of the whole-cell protein lysates and western blot analysis were carried out as follows. After drug treatments, the cells were lysed in RIPA buffer (150 mmol/l NaCl, 50 mmol/l Tris-HCl pH 8.0, 1% Triton X-100, 0.1% SDS and 1% deoxycholate) with a complete protease inhibitor cocktail from Roche for 10 min on ice. The concentration of the total protein extract was determined using a Bio-Rad DC™ protein assay kit (Bio-Rad Laboratories, Philadelphia, PA, USA). Equal amounts of total protein (30 μg) were resuspended in loading buffer, boiled at 100°C for 5 min, and separated by SDS-PAGE, and transferred to NC membranes (Millipore, Billerica, MA, USA). The membranes were then blocked with 5% milk without fat in TBST for 1 h at room temperature.

The following primary antibodies were used: rabbit poly-clonal antibodies against GAPDH (1:1,000), phosphatase and p70s6k (1:1,000), phosphatase and S6, phosphatase and ERK, polyADP ribose polymerase (PARP) (1:1,000) and phospha-tase AKT (1:1,000), which were purchased from Cell Signaling Technology. The mouse monoclonal antibody against t-AKT (1:1,000) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Primary antibodies were incubated overnight at 4°C. After washing the membrane with TBST for three times (5 min/time), the secondary fluorescent antibody (Odyssey) was added to the membrane at a 1:10,000 dilution at room temperature for 1 h. Actin or GAPDH was used as the loading control and

for normalization. The signal intensity of the membranes was detected using an LI-COR Odyssey scanner (Belfast, ME, USA)

Statistical analysis. All data are expressed as the mean ± SD of three individual experiments. Differences between groups were determined by one way analysis of variance (ANOVA), followed by Bonferroni's test to compare all pairs of columns. Results were considered to be statistically significant at P<0.05.

Results

BKM120 exhibits different degrees of sensitivity in the different NSCLC cell lines and the normal lung fibroblast cell line. To examine the cytotoxicity of BKM120 on different NSCLC cell lines with different types of mutations, four NSCLC cell lines were treated with BKM120 at 0, 1.25, 2.5, 5 and 10 μM, respectively for 72 h. In addition, the normal lung fibroblast cell line was treated with BKM120 at 0, 1.25, 2.5, 5, 10, 20, 40 and 80 μM, respectively for 72 h. Susequently, the percentage of viable cells was determined by MTT assay. Fig. 1 shows the chemical structure of BKM120. Fig. 2 shows the percentage of viability represented as dose-response curves for H358, A549, PC-9, H1650 and CCD19 cells after 72 h of BKM120 treatment.

Table I summarizes the mutation profiles and the IC$_{50}$ values of BKM120 for the four NSCLC cell lines and one normal lung fibroblast cell line. As shown in Table I, among the four cell lines, H358 and PC-9 were more sensitive to BKM120, with an IC$_{50}$ value of 1.15±0.16 and 2.09±0.50 μM, respectively, while A549 and H1650 were less sensitive, with an IC$_{50}$ value of 5.18±0.53 and 6.72±1.42 μM, respectively. Both H1650 and PC-9 cells harbor the same type of EGFR-activating mutation, which is exon 19 deletion mutation (16). However, H1650 is

Table I. The mutational profile, drug sensitivity and IC_{50} value of BKM120 for four NSCLC cell lines and one normal lung fibroblast cell line (CDD19).

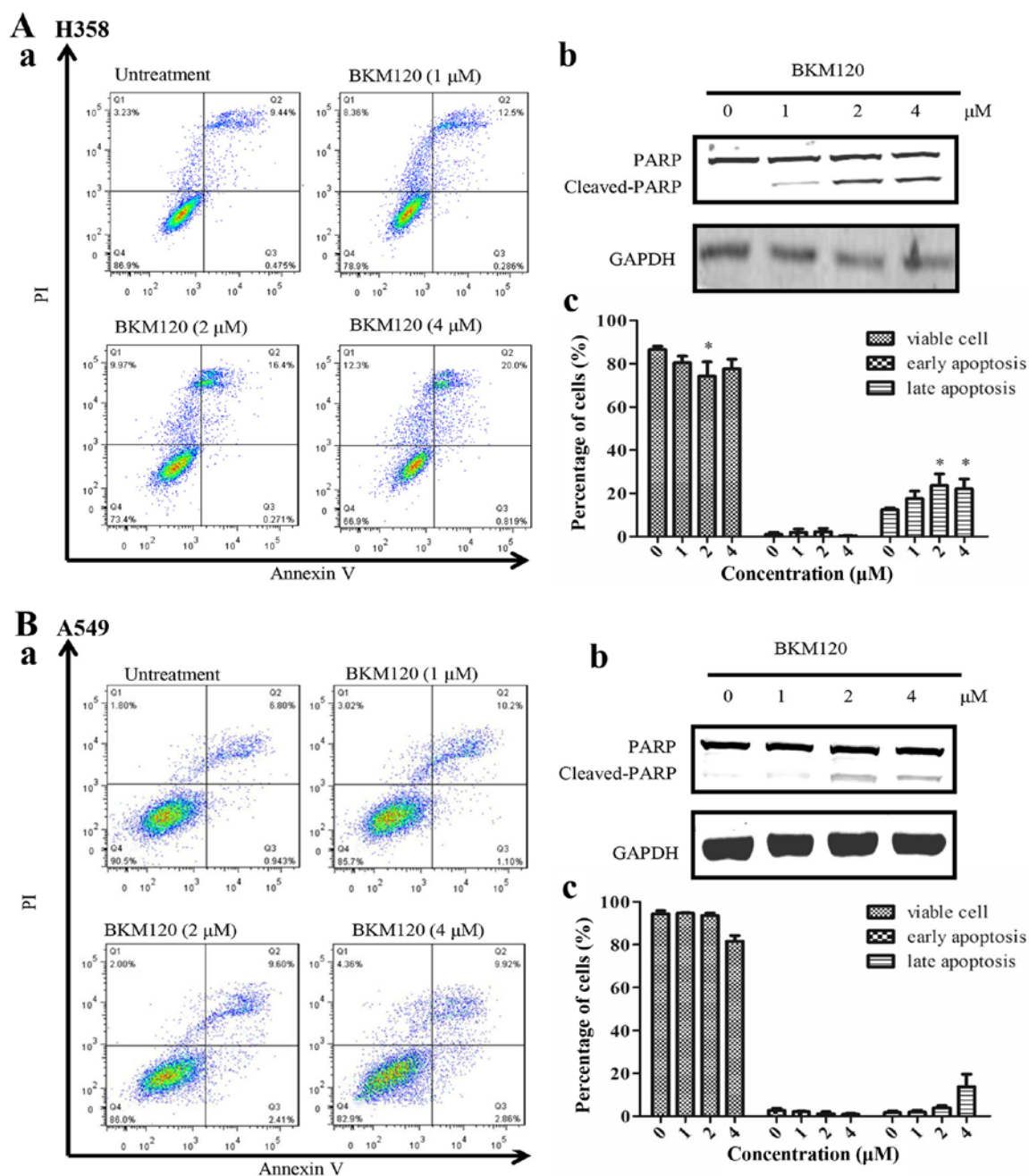| Cell line	KRAS mutation	EGFR mutation	Gefitinib sensitivity	BKM120 sensitivity	IC_{50} values to BKM120 (μM)
H358	G12C	Wild-type	N/A	Sensitive	1.15±0.16
PC-9	N/A	delE746-A750	Sensitive	Sensitive	2.09±0.50
A549	G12S	Wild-type	N/A	Resistant	5.18±0.53
H1650	N/A	delE746-A750	Resistant	Resistant	6.72±1.42
CCD19	N/A	N/A	N/A	Resistant	9.74±2.16

Figure 3. The apoptotic effect induced by BKM120 in four NSCLC cell lines (H358, A549, PC-9 and H1650). Flow cytometric analysis of (A) H358 and (B) A549 cell apoptosis induced by BKM120 (0, 1, 2 and 4 μM). The percentage of cells in early or late apoptosis are presented in the lower right and upper right quadrants. Western blot analysis of PARP (full length 110 kDa, and cleaved 89 kDa) activation in (A-b) H358 and (B-b) A549 cells was investigated after BKM120 administration. GAPDH served as the loading control. The percentage of early and late apoptotic cells was calculated: (A-c) H358 and (B-c) A549 cells. Results are expressed as mean ± SD (n=3, *p<0.05,)
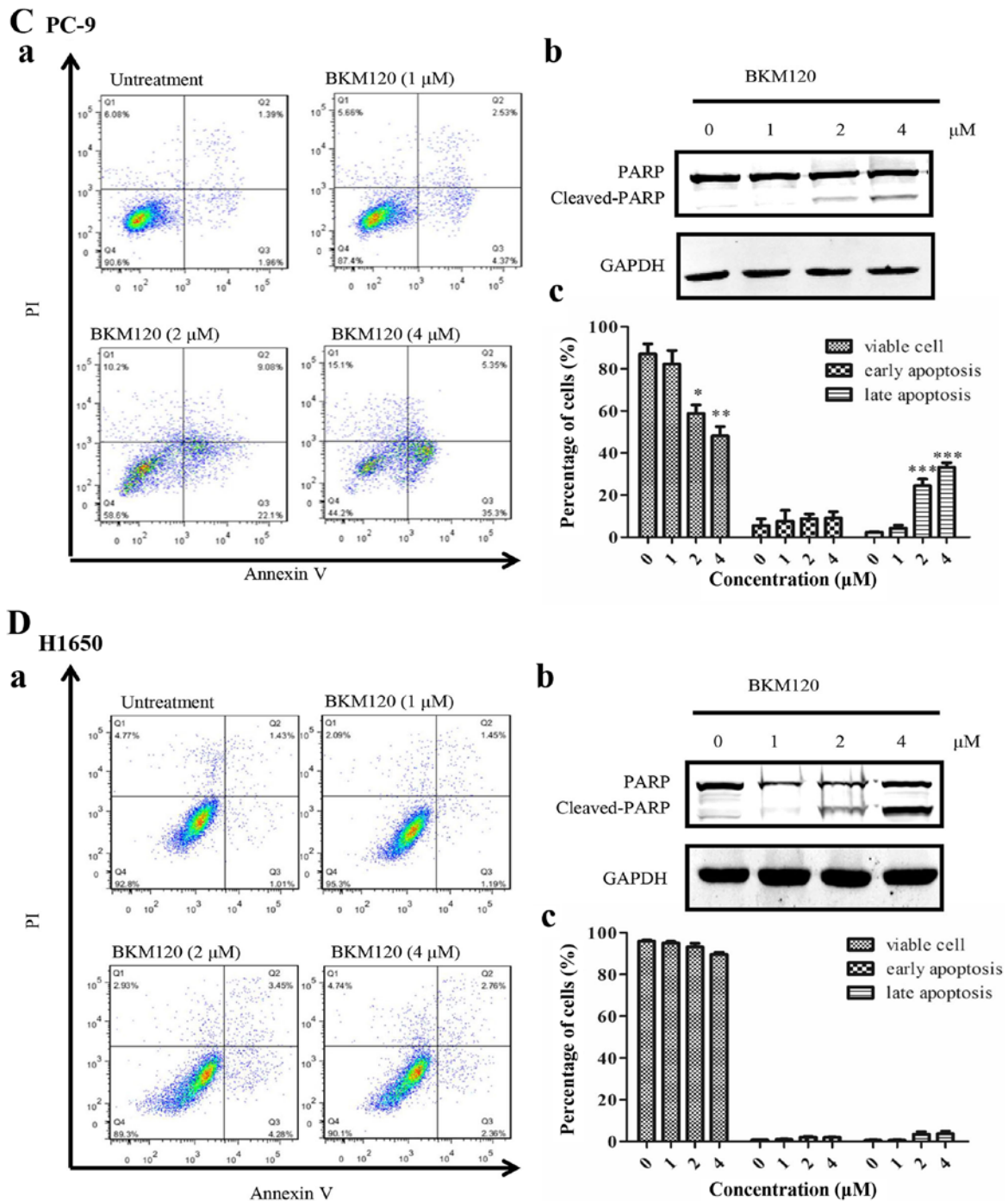
Figure 3. Continued. The apoptosis effect induced by BKM120 in four NSCLC cell lines (H358, A549, PC-9 and H1650). Flow cytometric analysis of (C) PC-9 and (D) H1650 cell apoptosis induced by BKM120 (0, 1, 2 and 4 μM). Percentage of cells in early or late apoptosis is presented in the lower right and upper right quadrants. Western blot analysis of PARP (full length 110 kDa, and cleaved 89 kDa) activation in (C-b) PC-9 and (D-b) H1650 cells was investigated after BKM120 administration. GAPDH served as the loading control. The percentage of early and late apoptotic cell was calculated: (C-c) PC-9 and (D-c) H1650 cells. Results are expressed as mean ± SD (n=3, *P<0.05, **P<0.01, ***P<0.001).

reported to be resistant to gefitinib and PC-9 is sensitive to gefitinib (17). Notably, the IC_{50} value of BKM120 in PC-9 cells was approximately one-third of that of the H1650 cells, indicating that the EGFR auto-activating pathway may have a crosstalk pathway in addition to the P13 kinase pathway in H1650 cells which may contribute to the attenuation of BKM120 sensitivity. Similarly, both the H358 and A549 cells contain *KRAS* mutations (18), yet H358 was much more sensitive to BKM120 than A549, indicating that the KRAS/

Raf signaling pathway may also have potential crosstalk in addition to the PI3 kinase pathway in A549 cells. In addition, the toxicity of BKM120 was further tested in a normal lung fibroblast cell line (CCD19), and the IC_{50} value of BKM120 for the CCD19 cell line was 9.7±2.16 μM. Although the fold different was not marked, the IC_{50} of BKM120 for CCD19 was still the highest among all tested cell lines.

MTT assay results showed that BKM120 decreased the cell viability of H358, PC9, A549, H1650 and CCD19 cells
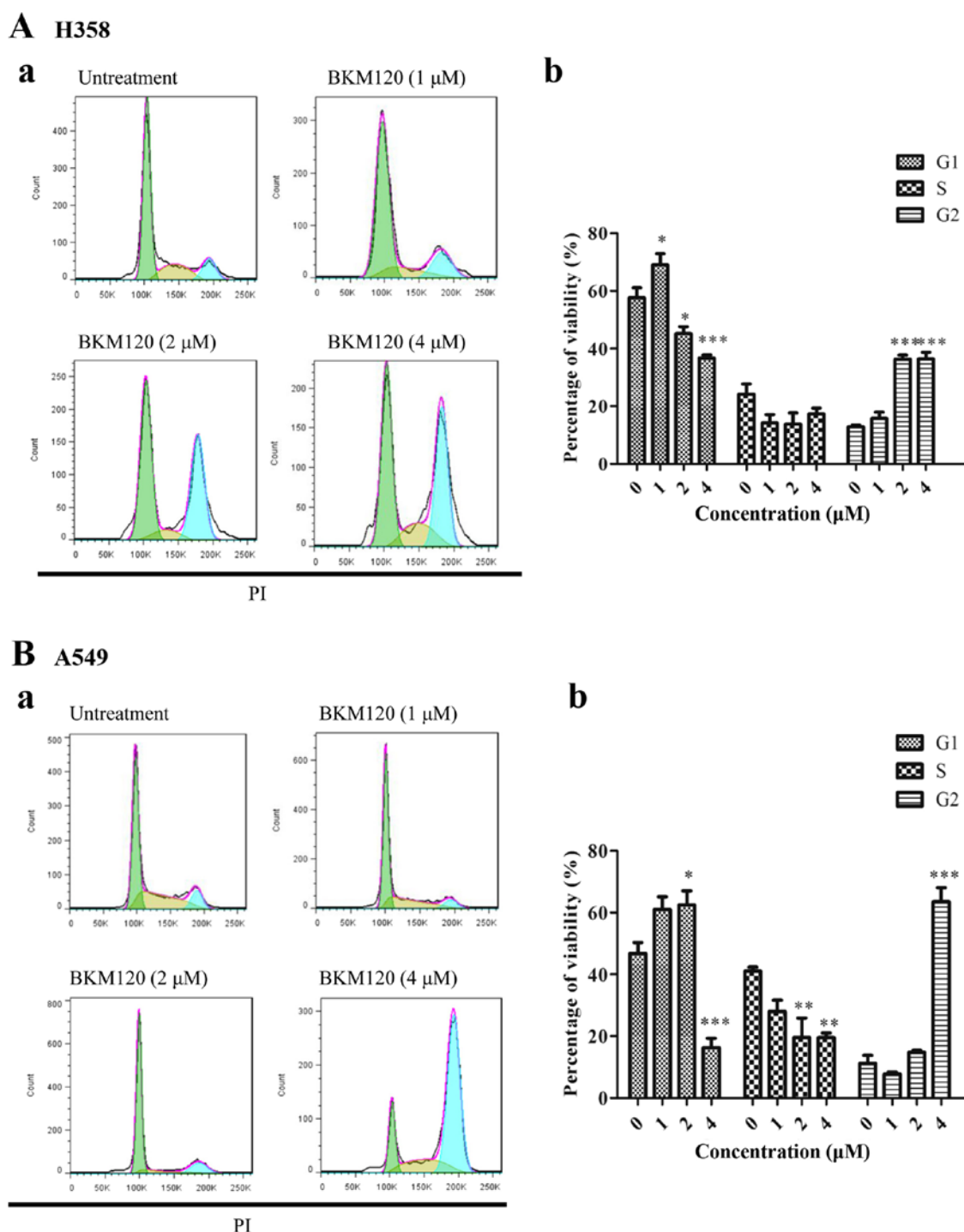
Figure 4. The effect of BMK120 on the cell cycle arrest in four NSCLC cell lines (H358, A549, PC-9 and H1650). Flow cytometric analysis of the cell cycle in (A-a) H358 and (B-a) A549 cells after treatment with different concentrations of BKM120 for 24 h was studied. Percentages of (A-b) H358 and (B-b) A549 cells at the G1, S and G2 phases were statistically analyzed. Each experiment was repeated at least three times. Results are expressed as mean ± SD (n=3, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$).

after a 72-h treatment. BKM120 inhibited H358, PC-9, H1650 and CCD19 cell growth in a dose-dependent manner.

BKM120 significantly induces apoptosis in the H358 and PC-9 BKM120-sensitive cell lines. Previous studies have demonstrated that BKM120 induces apoptosis in some NSCLC cell lines at the concentration range of 2 to 4 μM (18). To test the biological effect of BKM120, a similar dosage range was taken into reference to treat the cells, and the level of apoptosis was determined.

As shown in Fig. 3, flow cytometric analysis showed that when BKM120 was used at or below 1 μM, no significant increase in apoptosis was observed in all four cell lines. However, when BKM120 were used at or above 2 μM, it induced a significant level of late stage apoptosis in both the H358 and PC-9 cells. However, both A549 and H1650 cells exhibited no significant apoptotic effect by BKM120 at all dosages.

Although PARP cleavage was shown in all four cell lines at 2 μM of BKM120 treatment, still, no significant level of
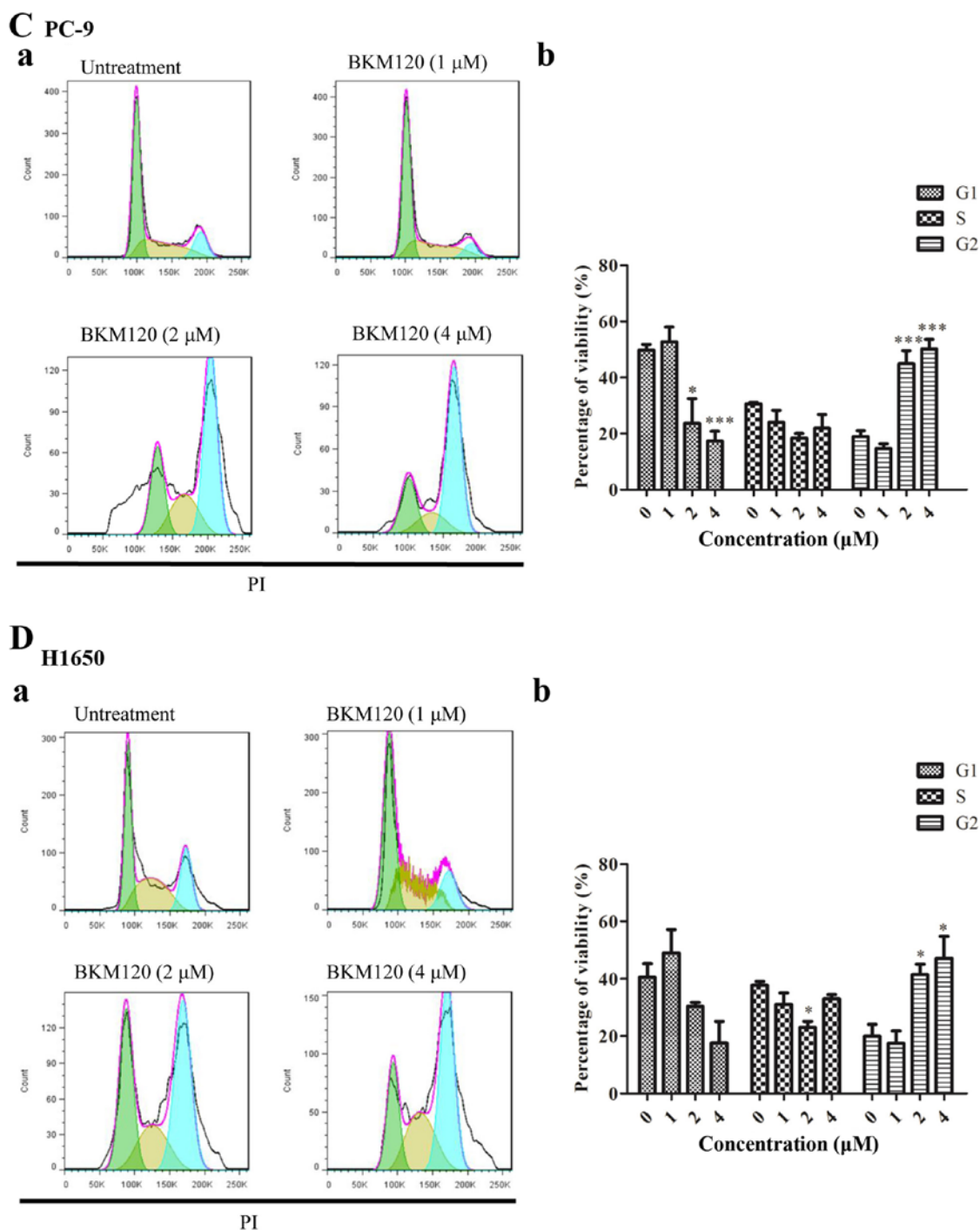
Figure 4. Continued. The effect of BMK120 on the cell cycle arrest in four NSCLC cell lines (H358, A549, PC-9 and H1650). Flow cytometric analysis of cell cycle in (C-a) PC-9 and (D-a) H1650 cells after treatment with different concentrations of BKM120 for 24 h was studied. Percentages of (C-b) PC-9 and (D-b) H1650 cells at the G1, S and G2 phases were statistically analyzed. Each experiment was repeated at least three times. Results are expressed as mean ± SD (n=3, *P<0.05, **P<0.01, ***P<0.001).

apoptosis could be detected in the A549 and H1650 cells. This may be due to the fact that PARP cleavage is only an indicator of active caspase activity, but it may not eventually lead to apoptotic effects.

BKM120 induces cell cycle arrest at G2 phase. Previous studies demonstrated that BKM120 induced cell cycle arrest at G1 phase in three *KRAS*-mutated lung cancer cell lines, Calu-1, H157 and A549 (18). In order to test whether a similar effect

was shown in our NSCLC cell lines with *EGFR* and *KRAS* mutations, we examined the cell cycle by using flow cytometry. Fig. 4 shows that BKM120 inhibited the growth of H358, PC-9 and H1650 NSCLC cells at the concentration starting at 2 μM, while BKM120 enhanced G2 arrest. For A549 cells, cell cycle arrest was only shown at a high dose (4 μM).

BKM120 inhibits the mTOR signaling pathway. EGFR and KRAS were found to be hyperactivated in certain types of
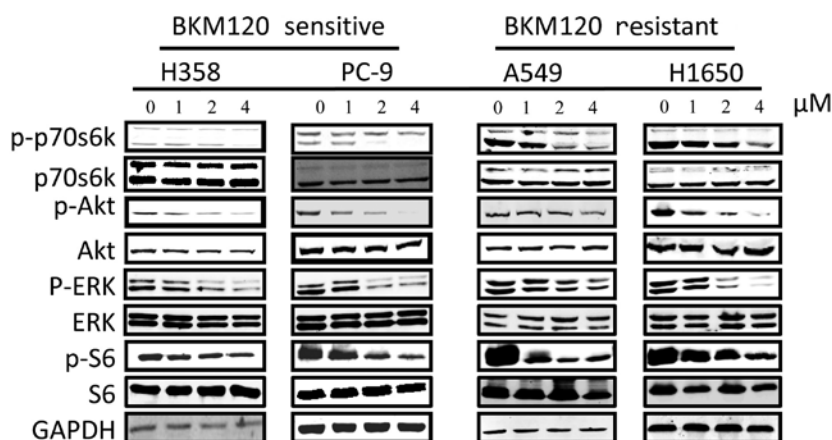
Figure 5. Western blot analysis of the effect of BKM120 on the mTOR and PI3K signalling pathways in different NSCLC cell lines. The four NCSLC cell lines were treated with BKM120 for 24 h. The levels of phospho(p)-ERK, p-p70s6k, p-AKT, p-S6, AKT, ERK, p70s6k, S6, and GAPDH were determined by western blot analysis. Total ERK1/2 (42/44 kDa), total p70s6k (70/85 kDa), total S6 (32 kDa) and GAPDH (37 kDa) served as loading controls. Each experiment was repeated at least three times.
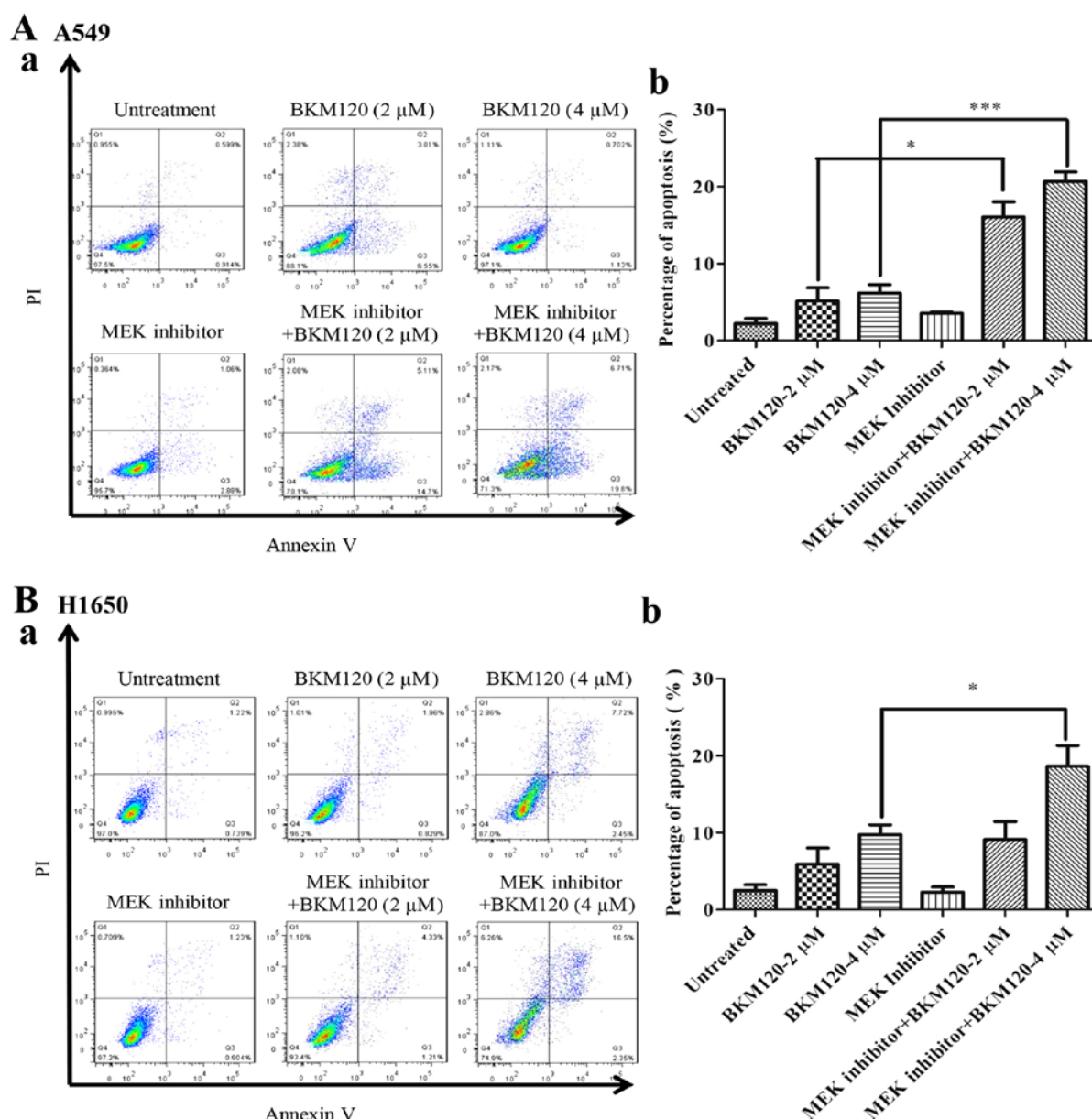

Figure 6. Effect of the combined treatment of BKM120 and MEK inhibitor (PD0325901). Single and combined treatment effects on (A-a) A549 and (B-a) H1650 cells. (A-b and B-b) Results are expressed as mean ± SD (n=3, *P<0.05, **P<0.01, ***P<0.001).
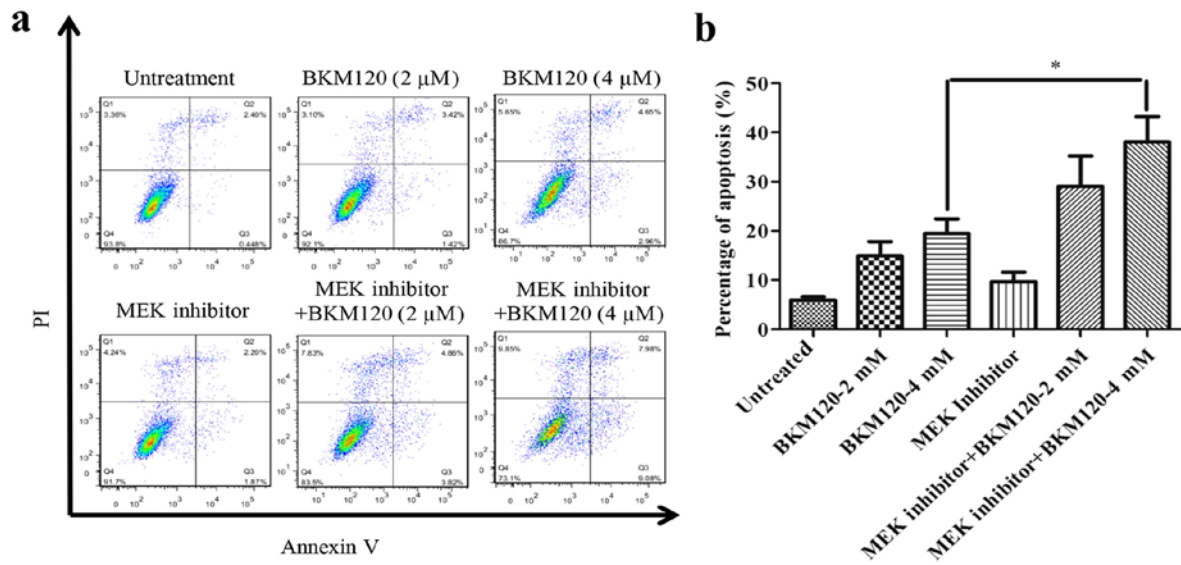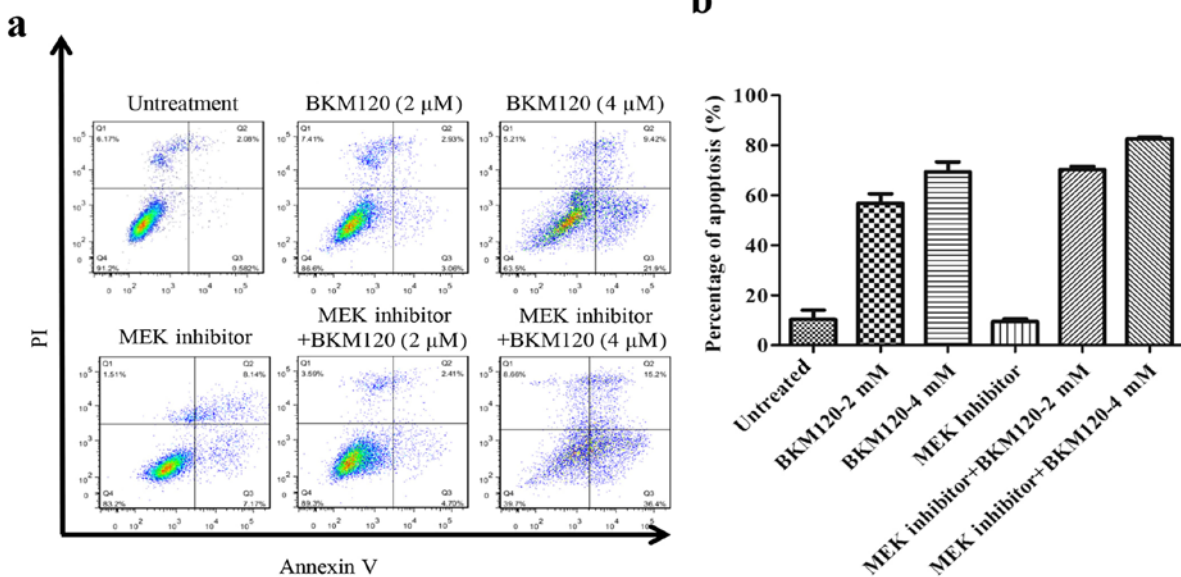
Figure 6. Continued. Effect of the combined treatment of BKM120 and MEK inhibitor (PD0325901). Single and combined treatment effect on (C-a) H358 and (D-a) PC-9 cells. (C-b and D-b) Results are expressed as mean ± SD (n=3, *P<0.05).

cancer, such as lung and breast (19), while the PI3K signaling pathway is the downstream of EGFR and KRAS, which may play an important role in the regulation of cell survival and proliferation. Therefore, we determined whether BKM120 can inhibit the mTOR signaling pathway in our cell lines by examining the phosphorylation level of the mTOR signaling pathway proteins, such as AKT, ribosomal S6 and p70s6k. After treatment of the cells with BKM120 for 24 h, we determined the phosphorylation levels of several key proteins involved in this pathway. As shown in Fig. 5, western blot results showed that BKM120 reduced the levels of phosphorylation of AKT, p70S6K and S6K in a dose-dependent manner in all the NSCLC cell lines, suggesting involvement of the mTOR pathway. The level of ERK phosphorylation suppres-

sion was more prominent in the H358 than that in the A549 cells although both cell lines are KRAS mutants. However, there were no significant differences in the phosphorylation of ERK in the H1650 and PC-9 cells following BKM120 treatment.

Combination usage of PI3K and MEK inhibitors synergistically enhance the apoptosis in BKM120-resistant A549 cells. Since A549 and H1650 cells are relatively less sensitive to BKM120 treatment, we further examined whether co-targeting the PI3K pathway by BKM120 and the MEK pathway by PD0325901 could synergistically enhance the cancer inhibiting effect. Previously, PD0325901 was used at a concentration of 100 nM in combination with BKM120 in the

KRAS mutant human and murine CRC cells (20). Therefore, we took 100 nM as a reference concentration and used it in combination with BKM120 on A549, PC-9, H358 and H1650 cells. Fig. 6 demonstrates that single use of either one of these drugs did not induce significant levels of apoptosis in both the A549 and H1650 cells; however, when both drugs were used in combination, the levels of apoptosis increased synergistically in the A549 cells, mildly in the H1650 and H358 cells but apoptosis was not increased in the PC-9 cells.

Discussion

The PI3K pathway has been regarded as an important drug target for cancer treatment, including NSCLC (8). RAD001 and BEZ235 as well as several rapamycin analogues are the current mTOR inhibitors undergoing development designed for cancer with rapamycin resistance (21). RAD001 and BEZ235 both increased AKT phosphorylation in various lung cancer cell lines such as A549 and H157 (18). BKM120, similar to RAD001, is also a PI3K inhibitor, which has been developed and is also in clinical trial for various types of cancer (22).

In our previous study, we found that BKM120 effectively inhibited the growth of human NSCLC cell lines with different types of gene mutations (18). It was also suggested that BKM120 was more sensitive to cell lines with PIK3CA mutation (18). In the present study, we examined the effect of BKM120 in four NSCLC cell lines with *EGFR* or *KRAS* mutations. We found that H358 and PC-9 cells were sensitive to BKM120 treatment. Although similar mutations are present in H358 and PC-9 cells, these cell lines were less sensitive to BKM120, indicating that the presence of EGFR and KRAS mutations are not the only criteria to determine whether single BKM120 treatment is enough. Further mTOR and MEK activation screening is needed to decide whether combinational therapy should be used. Similar to RAD001, our data showed that BKM120 decreased AKT phosphorylation in all four cell lines. Using human NSCLC cell lines with different mutations, we demonstrated that BKM120 effectively inhibited the growth of NSCLC cell lines with a different degree of sensitivity. In a recent study, it was suggested that BKM120 could induce G1 arrest at a low concentration in A549, H157 and Calu-1 cells, which contain KRAS mutation (18). In addition, another study reported that BKM120 could induce G2 phase cell cycle arrest in T-cell acute lymphoblastic leukemia (23). Here, we further showed that inhibition of the PI3K pathway resulted in both a decrease in proliferation in the four lung cancer cell lines and an increase in apoptosis in the H358 and PC-9 cells, which were both sensitive to BKM120. It was reported that BKM120 arrests cancer cells at the G1 phase at low concentrations (≤2 μM); our data indicated that BKM120 also induced G1 arrest but at a lower concentration. However, after treating the cells with BKM120 at higher concentrations (>2 μM), it alternatively induced G2 arrest.

A previous study reported that co-treatment of an MEK inhibitor and BKM120 in NSCLC cell lines with *KRAS* mutations significantly inhibited the growth of tumors both *in vitro* and *in vivo* (24). In the clinic, drug decisions are usually based on a genetic mutation test to govern the use of drugs common in personalized therapy (25). For patients with *EGFR* mutations and who are less sensitive to gefitinib, the clinical outcome is poor (26); therefore, more new drug options are required. In addition, although *KRAS* mutations were identified in the 1970s, this target remains undruggable (27). Out data indicated that for NSCLC patients with *EGFR* or *KRAS* mutations, inhibition of the PI3K pathway alone or in combination with the MEK inhibitor may suppress tumor growth, and was even effective in the gefitinib-resistance H1650 cell line. Recent studies also suggest that combinational use of PI3K inhibitor with other agents have demonstrated greater efficacy than monotherapy (20). When we used BKM120 alone at a high dose (4 μM), the PC-9 cell line with EGFR or the H358 cell line with KRAS mutation demonstrated inhibition of cell growth and induction of apoptosis, while the gefitinib resistant cancer cell lines H1650 and A549 both were less sensitive to BKM120 than the other cell lines. However, combined drug treatment demonstrated good cancer inhibition, indicating the need for more rational combinational therapy for gefitinib-resistant patients in the future.

In the present study, we found that following BKM120 treatment, AKT and ERK were activated as shown by the increased phosphorylation levels in the lung cancer cells. This is further supported by previous studies on the PI3K pathway which found that single treatment with a PI3K inhibitor is not sufficient as it induces at least one signaling mediator in the alternate pathway. Overall, BKM120 alone or in combination with PD0325901 was demonstrated to be useful in NSCLC with *KRAS* and *EGFR* mutations. More valid biomarkers must be identified for deciding on the use of single or combinational therapy in the future.

Acknowledgements

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