Polo-like kinase 1 inhibitor BI2536 causes mitotic catastrophe following activation of the spindle assembly checkpoint in non-small cell lung cancer cells

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Abstract

Polo-like kinase 1 (PLK1), a critical kinase that regulates multiple steps in mitosis, is overexpressed in diverse human cancers; thus many PLK1 inhibitors have been developed as potential cancer therapeutic agents. One of these compounds, the PLK1-specific inhibitor BI2536, has been investigated as a cytotoxic drug in several cancers, including lung cancer; however, the detailed mechanism by which BI2536 induces defects in cell proliferation of non-small cell lung cancer (NSCLC) has not yet been determined. We found that BI2536 treatment resulted in mitotic arrest due to improper formation of the mitotic spindles and mitotic centrosomes. The unattached kinetochores in BI2536-treated NSCLC cells activated the spindle assembly checkpoint (SAC). The prolonged activation of the SAC led to a type of apoptotic cell death referred to as mitotic catastrophe. Finally, BI2536-treated NSCLC cells show a defect in cell proliferation. Overall, these data indicate that PLK1 inhibition via mitotic disruption represents a potential approach for the treatment of NSCLC.

Introduction

Mitosis is a critical cell cycle phase that passes one of each pair of sister chromatids to each daughter cell; thus precise orchestration of mitosis is necessary for the maintenance of chromosomal stability in cells [1]. Aberrant mitotic progression leads to chromosomal mis-segregation and can contribute to cancer development [2–4]. To prevent the steps of mitosis from occurring out of order, cells are equipped with safeguard systems, as follows: the antephase checkpoint, which controls entry into mitosis [5]; the spindle assembly checkpoint (SAC), which senses kinetochores not attached to microtubules [6–8]; and the abscission checkpoint, which prevents the completion of cytokinesis in the presence of lagging chromosomes [9]. Among these, the SAC has been most extensively investigated.

The SAC, also called the mitotic checkpoint, is required for proper chromosome segregation [6–8]. When one or more kinetochores are not attached to microtubules, the SAC is activated to delay the transition from metaphase to anaphase until the cell can correct the aberrant kinetochore–microtubule attachment. In humans, the SAC is dynamically regulated by several key players including monopolop spindle protein 1 (Mps1), budding uninhibited by benzimidazoles 1 (Bub1), Bub3, Bub1-related 1 (BubR1), mitotic arrest-deficient protein 1 (Mad1), Mad2, Aurora B, and Polo-like kinase 1 (PLK1). SAC activation is initiated by the formation of the mitotic checkpoint complex (MCC) composed of Mad2, BubR1, Bub3, and Cdc20. Enrichment of MCC at unattached kinetochores inactivates Cdc20, a co-factor of the anaphase-promoting complex/cyclosome (APC/C) E3 ubiquitin ligase, thereby blocking degradation of cyclin B and securin and delaying the onset of anaphase. Recruitment of SAC proteins and amplification of SAC signaling is also regulated by the Mps1, Bub1, BubR1, Aurora B, and PLK1 kinases [10,11]; thus SAC and its regulatory kinases act as a crucial cell cycle surveillance mechanism that protects the cell against chromosomal instability.

The serine/threonine kinase PLK1 governs several mitotic steps, including entry into mitosis, centrosome maturation, bipolar spindle assembly, activation of APC/C by phosphorylation of early mitotic inhibitor 1 (Emi1), chromosome segregation, and mitotic exit [12–14]. PLK1 expression starts to rise in G2 and peaks at mitosis [15,16]. PLK1 is localized at centrosomes in G2/prophase, centrosomes and kinetochores in prometaphase, spindle poles and both aligned and unaligned kinetochores in metaphase, spindle microtubules in anaphase, the central spindle in telophase, and the cleavage furrow and midbody during cytokinesis [15,17,18], suggesting that it plays roles...
in mitotic cell cycle progression. Indeed, overexpression or deletion of PLK1 disturbs mitotic progression and leads to the formation of aberrant chromosomes [19,20]. In the presence of unattached kinetochores, PLK1 is not essential for SAC activation in human cells, but high PLK1 activity on unattached kinetochores is required to promote the accumulation of SAC proteins at kinetochores and attach kinetochores to microtubules via phosphorylation of BubR1 [21,22]. PLK1 is overexpressed in a wide range of cancers, including breast, ovarian, endometrial, prostate, gastric, colorectal, pancreatic, head and neck, papillary thyroid, and non-small cell lung cancers, oropharyngeal and esophageal carcinoma, melanoma, glioblastoma, and non-Hodgkin lymphoma [23–25]. Therefore, PLK1 is a promising target for cancer therapy; indeed, many PLK1 inhibitors such as BI2536, BI6727 (Volasertib), GS-461364, HN-214, ON01910.Na, NMS-P937, TKM-080301 have been developed and tested in clinical phase I or II trials [24–29]. BI2536, an ATP-competitive PLK1 kinase inhibitor, has been well characterized in several cancers [30,31], and two different studies have demonstrated the cytotoxic effect of BI2536 in human non-small cell lung cancer (NSCLC) [31,32]. Furthermore, the effects of BI2536 in NSCLC patients, as a monotherapy or combination therapy, have been evaluated in clinical trials [33,34]. However, although clinical trials of BI2536 have been launched, the molecular mechanism by which BI2536 exerts cytotoxicity in NSCLC cells has not been determined. In this study, we found that BI2536 causes mitotic catastrophe through prolonged SAC activation, suggesting that BI2536 could be therapeutically effective against NSCLC.

Materials and methods

Cell culture and drug treatment

A549 and A427 non-small cell lung cancer cells were obtained from the American Type Culture Collection (ATCC) and maintained in Dulbecco’s modified eagle medium (DMEM, Welgene Inc). NCI-H1299 cells were obtained from the Korean Cell Line Bank (KCLB) and maintained in Roswell Park Memorial Institute (RPMI) 1640. All media were supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin G sodium, 100 μg/ml streptomycin sulfate, and 0.25 μg/ml amphotericin B and then were incubated at 37°C in 5% CO2 incubator. Cells were treated with BI2536 (Selleck Chemicals) for indicated times.

Cell viability assay

A549, A427 and NCI-H1299 cells (5 × 10^3 cells per well) were plated in a 96-well plate, and then treated with various concentrations of BI2536 for 24 hr. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was then added to each well, and the plate was incubated at 37°C for 4 hr to allow formation of MTT formazan crystals. After the culture medium was removed, the formazan crystals were dissolved using dimethyl sulfoxide. The absorbance was measured with a test well plate, and then treated with various concentrations of BI2536 for 24 hr. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was then added to each well, and the plate was incubated at 37°C for 4 hr to allow formation of MTT formazan crystals. After the culture medium was removed, the formazan crystals were dissolved using dimethyl sulfoxide. The absorbance was measured with a test well plate, and then treated with various concentrations of BI2536 for 24 hr and then permeabilized with 0.5% Triton X-100 in PBS on ice for 15 min. The cells were incubated with anti-H3-pS10 (Upstate, 06–570) antibody for 2 hr, and then incubated with secondary antibody at room temperature in the dark for 1 hr. Cells were incubated with RNase A at 37°C for 30 min and then with propidium iodide (PI) at 37°C in the dark for another 30 min. Cell cycle phases were analyzed by flow cytometry.

Bi2536 reduces cell proliferation in NSCLC cells. (A) A549, A427, and NCI-H1299 cells were treated with various concentrations of BI2536 for 24 hr and their viability was determined using the MTT assay. (B) A549, A427, and NCI-H1299 cells treated with 50 nM BI2536 were seeded in plates and colony-forming activity was determined after 10 days.

Western blotting

BI2536 or nocodazole-treated A549, A427 and NCI-H1299 cells were lysed using NETN buffer (100 mM NaCl, 1 mM EDTA, 20 mM Tris–HCl pH 8.0, 0.5 mM Nonidet P-40, 50 mM β-glycerophosphate, 10 mM NaF and 1 mM Na3VO4) with protease inhibitor (Millipore, 353140) on ice for 10 min. After centrifugation at 12,000 x g for 5 min, the supernatant was saved as a crude cell extract. The crude cell extracts were boiled in the Laemmli buffer and then loaded onto a SDS–polyacrylamide gel. The antibodies used for Western blotting are as follows: Aurora A (Cell Signaling, 4718), Aurora B (Cell Signaling, 3094), Aurora A-pT288/Aurora B-pT232/Aurora C-pT198 (Cell Signaling, 11316; Sigma, T6557) antibodies, the cells were fixed with cold methanol at room temperature for 5 min and then rehydrated in PBS three times. The cells were post-fixed with paraformaldehyde and permeabilized as described above. The nuclei were counterstained with Hoechst 33342. After a final wash with PBS, coverslips were mounted with antifade solution containing para-phenylenediamine and glycerol in PBS. The staining was determined using fluorescence microscope (Eclipse 80i, Nikon) or laser-scanning confocal microscope (LSM700, Carl Zeiss).

Immunofluorescence staining

A549, A427 or NCI-H1299 cells were grown on coverslips and treated with BI2536. The cells were fixed with 3% paraformaldehyde solution at room temperature for 10 min and then permeabilized with 0.5% Triton X-100 at room temperature for 5 min. The cells were incubated with antibody against Aurora A (BD Biosciences, 610938), Aurora A (Santa Cruz, sc-25426), PLK1 (Santa Cruz, sc-55504), BubR1 (BD Biosciences, 612503), Mad2 (Pierce, PA5-21594), or CREST (Immunovision, HCT-0108) at 37°C for 20 min and then incubated with corresponding secondary antibody at 37°C for 20 min. For the staining with α-tubulin (Abcam, 18251) and γ-tubulin (Abcam, 11316; Sigma, T6557) antibodies, the cells were fixed with cold methanol at room temperature for 5 min and then rehydrated in PBS three times. The cells were post-fixed with paraformaldehyde and permeabilized as described above. The nuclei were counterstained with Hoechst 33342. After a final wash with PBS, coverslips were mounted with antifade solution containing para-phenylenediamine and glycerol in PBS. The staining was determined using fluorescence microscope (Eclipse 80i, Nikon) or laser-scanning confocal microscope (LSM700, Carl Zeiss).
sc-5546), and PARP-1 (Santa Cruz, sc-7150). BubR1-pS670 antibody was generated by immunizing rabbit with specific peptide.

**Annexin V/propidium iodide (PI) staining**

A549, A427 and NCI-H1299 cells were treated with BI2536, and then both floating and adherent cells were collected. The Annexin V-FITC apoptosis detection kit I (BD Pharmingen, BD556547) was used to detect apoptotic cells. Briefly, cells were washed twice with PBS, and then stained for 15 min at room temperature in the dark with Annexin V-FITC and PI in binding buffer. Early apoptotic (Annexin V-positive, PI-negative) and late (Annexin V-positive and PI-positive) apoptotic cells were counted using flow cytometry.

**Time-lapse microscopy**

TSIN-H2B-RFP lentiviral construct was kindly provided by Dr. P. J. Galardy (Mayo Clinic). Lentivirus was prepared by transfection of HETK293T cells with TSiN-H2B-RFP, psPAX2, and pMD2.G. A549 cells were infected with lentivirus encoding H2B-RFP in the presence of 8 μg/ml polybrene. Images were acquired using a Cell Observer (Cell Observer Living Cells, Carl Zeiss) equipped with a camera. Frames were recorded every 5 or 7 min for a total of 42 hr. Cell morphology was visualized on a phase-contrast microscope, and RFP was detected by fluorescence. More than 50 cells that are undergoing mitosis in DMSO-treated cells or prometaphase-arrest in BI2536-treated cells were monitored in three independent experiments. In BI2536-treated cells, the number of cells that were arrested at prometaphase, apoptotic, or bypassed cytokinesis was counted.

**Statistical analysis**

All experiments were done more than three times. Each value is expressed as means ± standard deviations. Two-tailed Student’s t-test was used for statistical analysis and statistical difference was considered significant when $P < 0.05$.

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**Fig. 2.** BI2536 induces mitotic arrest of NSCLC cells. A549, A427, and NCI-H1299 cells were treated with 50 nM BI2536 for 16 hr, and then the cell cycle distribution was analyzed by flow cytometry. (A) Cell cycle phases were analyzed by PI staining. (B) The mitotic fraction of BI2536-treated cells was identified by staining of histone H3 phosphorylation at S10 (H3-pS10). Each cell cycle phase and levels of H3-pS10 are represented as means ± standard deviation ($n = 3$). Asterisks (*) represent statistically significant differences ($P < 0.05$, Student’s t-test).
Results

**BI2536-treated NSCLC cells show a defect in cell proliferation**

To investigate the effect of BI2536 in NSCLC cells, we monitored the cell viability of A549, A427 and NCI-H1299 cells treated with BI2536. Cell growth was significantly reduced in the presence of BI2536 at concentrations above 0.05 μM (Fig. 1A); however, cell viability did not decrease further at concentrations higher than 50 nM. Next, to investigate the prolonged effect of BI2536 treatment on cell proliferation, we performed clonogenic assay. BI2536-treated cells showed significantly low colony-forming activity (Fig. 1B). These data demonstrate that BI2536 inhibits the growth of NSCLC cells.

**BI2536-treated NSCLC cells are arrested at mitosis**

Next, we sought to determine how BI2536 causes the observed proliferation defect. Because PLK1 inhibitors disrupt mitotic progression, we analyzed the distribution of cell cycle phases in BI2536-treated A549, A427 and NCI-H1299 cells. The G2/M population was significantly elevated in BI2536-treated cells, suggesting a potential increase in the number of mitotic cells (Fig. 2A). To confirm the arrest at mitosis, we monitored phosphorylation of histone H3 S10 (H3-pS10), a mitotic marker. The level of H3-pS10 was significantly higher in BI2536-treated cells than in control cells (Fig. 2B). Together, these results demonstrate that BI2536-induced mitotic arrest results in a defect in cell cycle progression in NSCLC cells.

**BI2536-treated NSCLC cells contain abnormal mitotic spindles and mitotic centrosomes**

The findings described above demonstrate that PLK1-depleted cells are arrested at mitosis due to a failure to organize the formation of mitotic spindles and mitotic centrosomes [20]. To further investigate this phenomenon, A549, A427 and NCI-H1299 cells treated with BI2536 were stained for α-tubulin and γ-tubulin to identify the mitotic spindles and centrosomes, respectively. As expected, most cells treated with BI2536 were arrested at prometaphase, as determined by chromosome morphology (Fig. 3A). In addition, monopolar spindles, rather than bipolar spindles, were formed in BI2536-treated cells (Fig. 3A); consistent with this, γ-tubulin stained in a circular arrangement, possibly surrounding monopoles (Fig. 3A). However, the intensity of γ-tubulin around the monopoles was low compared to control cells, indicating that the association of γ-tubulin with centrosome was weak. Next, we visualized kinetochore–microtubule attachment by staining CREST as a centromeric marker and α-tubulin as a mitotic spindle marker. BI2536-treated cells also failed to attach microtubules to kinetochores, whereas control cells contained properly attached bipolar spindles (Fig. 3B). These findings suggest that BI2536 treatment induces mitotic arrest by preventing kinetochore–microtubule attachment, which requires PLK1 activity.

**BI2536-treated NSCLC cells activate the spindle assembly checkpoint**

Once a cell possesses unattached kinetochores, it activates the SAC to delay mitotic progression until all chromosomes have attached their sister kinetochores to the mitotic spindle. BI2536 induces the formation of unattached kinetochores (Fig. 3B); consequently, BI2536-treated A549, A427 and NCI-H1299 cells exhibited elevated phosphorylation of Aurora B (T232) and PLK1 (T210), as well as higher levels of total Aurora A, Aurora B, and PLK1 (Fig. 4A). Autophosphorylation of Aurora B-T232 and phosphorylation of PLK1-T210 by Aurora A indicate that both Aurora B and Aurora A are activated in the presence of BI2536 [35,36]. Furthermore, phosphorylation of PLK1-T210 is dramatically reduced in Aurora B-deficient human cells [37], suggesting that the high level of PLK1-pT210 supports activation of both Aurora A and Aurora B in BI2536-treated cells. Phosphorylation of BubR1-S670 is partially mediated by CDK1 [38] or Mps1 [39], and is sensitive to loss of microtubule attachment, whereas phosphorylation of BubR1 S676 is mediated by PLK1 [22] and is sensitive to loss of kinetochore tension. In three different NSCLC cell lines, the level of BubR1-pS670 was...
significantly elevated in the presence of BI2536, suggesting that the unattached microtubules induced by BI2536 cause activation of CDK1 or Mps1 (Fig. 4A). As shown in Fig. 4B, BubR1 exhibits an electrophoretic mobility shift in cells treated with nocodazole, a microtubule depolymerizer that activates PLK1, leading to PLK1-dependent phosphorylation of BubR1 at S676, T792, and T1008 [22,40]. However, the shifted band of BubR1 did not appear in any of the BI2536-treated cell lines, confirming that BI2536 inhibits PLK1 enzymatic activity (Fig. 4A and B). In addition, the effect of BI2536 on PLK1 activity was investigated in cells treated with nocodazole as an inducer of SAC (Fig. 4C). Treatment with nocodazole or BI2536 induced similar upregulation of Aurora A, Aurora B and PLK1. The levels of cyclin B, one of APC/C substrates, were also upregulated, indicating that the activation of SAC delays anaphase onset. However, the shifted band of BubR1 in nocodazole-treated cells disappeared when cells were reco-treated with BI2536, indicating that BI2536 inhibits PLK1 activity and blocks hyperphosphorylation of BubR1. After BI2536 treatment, the total BubR1 level was elevated in A549 cells, but not in A427 or NCI-H1299 cells (Fig. 4A); the reason for this difference remains to be determined.

In BI2536-treated A549 cells, Aurora A was localized to the centrosome, and Aurora B, PLK1, and BubR1 were localized to unattached kinetochores (Fig. 5A–D). This observation demonstrates that PLK1 activity is not required for the recruitment of Aurora A, Aurora B, PLK1, or BubR1. However, Mad2 did not accumulate on unattached kinetochores in most BI2536-treated cells, although a few cells still exhibited localization of Mad2 (Fig. 5E); thus PLK1 activity is necessary for the recruitment of some, not all, MCC components to unattached kinetochores. APC/C can be inhibited not only synergistically by Mad2 and BubR1 [41,42], but also in a Mad2-independent manner [43–45], although the latter’s case causes weak inhibition. Overall, these results demonstrate that the induction of unattached kinetochores by BI2536 partially, but not fully, activates the SAC and blocks mitotic progression.

**BI2536-treated A549 cells undergo mitotic catastrophe**

To investigate how BI2536-induced mitotic delay influences cell survival, we examined the effect of BI2536 on cell death. BI2536-treated A549, A427 and NCI-H1299 cells contained cleaved PARP-1, indicating that the cells underwent apoptosis, possibly in a caspase-dependent manner (Fig. 6A). Second, the proportion of Annexin V-positive cells was significantly elevated, and the proportion of PI-positive cells was slightly (but not significantly) elevated, after BI2536 treatment of A549 and NCI-H1299 cells for 40 hr or A427 cells for 16 hr (Fig. 6B). This finding suggests that BI2536 treatment induces cell death through apoptosis, rather than necrosis. In addition, we performed live-cell imaging by time-lapse fluorescence video microscopy to confirm that the prolonged mitotic delay resulted in cell death. Chromosome morphology was identified by transfection of RFP-fused H2B. DMSO-treated A549 cells showed the normal mitotic division resulting in the formation of two daughter cells (Fig. 7A and C). During 42 hr-treatment with BI2536, most A549 cells were arrested at prometaphase (Fig. 7B and D). Of the arrested cells, 51.1% eventually manifested highly condense chromatin and membrane blebbing, indicating that they underwent mitotic catastrophe; the remaining 16.6% were still arrested, but 32.3% overcame the arrest, progressed into anaphase, and then bypassed cytokinesis, resulting in polyploidy (Fig. 7D). The latter cells undergoing mitotic exit via mitotic
Fig. 5. BI2536 activates spindle assembly checkpoint in A549 cells. (A–E) The localization of Aurora A, Aurora B, PLK1, BubR1 and Mad2 in A549 cells treated with 50 nM BI2536 for 16 hr was determined by staining with the indicated antibodies.
slippage might result from partial activation of SAC due to the defect in Mad2 recruitment [46,47]. These cells also exhibited chromatin fragmentation and membrane blebbing after entering interphase. Taken together, these data demonstrate that BI2536 causes cell death by inducing a mitotic defect in NSCLC cells.

Discussion

The results of this study demonstrate that BI2536 arrests NSCLC cells at prometaphase, causing them to activate the SAC. Ultimately, this aberrant mitotic progression results in mitotic catastrophe; thus BI2536 is a promising anti-cancer drug for the treatment of NSCLC.

Inhibition of PLK1 activity by BI2536 leads to mitotic arrest, indicating that PLK1 activity is not required for SAC activation. In BI2536-treated cells, PLK1 is phosphorylated on T210 in the T-loop, possibly by Aurora A and Aurora B. This phosphorylation is required for activation of PLK1 [48], which, in turn, phosphorylates BubR1-S676 at kinetochores under no tension [22,40]; however, because BI2536 binds the kinase domain of PLK1 and inhibits its activity [26,49,50], it might reduce phosphorylation of BubR1-S676, which is required for stable kinetochore–microtubule attachment. In addition to BubR1-S676, several other residues (T680, T792, and T1008) have been identified as sites for PLK1-dependent phosphorylation, which also stimulates chromosome alignment [40,51]. This is consistent with the previous finding that the phosphorylation of BubR1 induced by nocodazole treatment was inhibited in the presence of BI2536, indicating that phosphorylation of BubR1 requires PLK1 activity [30]. Therefore, the suppression of PLK1 activity by BI2536 maintains the loss of kinetochore tension, resulting in prolonged SAC activation.

Our findings regarding the recruitment of mitotic components differ from those of previous reports. First, several reports have shown that BI2536-treated HeLa cells [30,52], ZK-Thiazolidinone (PLK1 inhibitor)-treated HeLa S3 cells [53], and PLK1 siRNA-transfected U2OS [54] or HeLa S3 cells [53] exhibit weak or barely detectable staining for centrosomal γ-tubulin, indicating that PLK1 plays a role in centrosome maturation via recruitment of the γ-tubulin-ring complex (γ-TuRC). However, although we tested to use two different γ-tubulin antibodies (Abcam, 11316; Sigma, T6557), both antibodies still weakly detected γ-tubulin surrounding monopoles. Second, PLK1 accumulated normally in BI2536-treated A549 cells, in contrast to the impaired accumulation of PLK1 on unattached kinetochores in BI2536-treated HeLa cells [30,55]. Third, recruitment of Mad2 to unattached kinetochores is normal in HeLa cells [30], but defective in A549 cells in the presence of BI2536. Our observation is supported by the finding that PLK1 depletion [21,56] or a PLK1-dependent phosphorylation site mutant of BubR1 [57,58] leads to Mad2 dissociation from mitotically arrested chromosomes. The absence of Mad2 is still enough to inhibit APC/C because BubR1 alone can inhibit the activation of Cdc20 [43]. At least in our study, partial activation of SAC due to the absence of Mad2 in the unattached kinetochore is sufficient to delay the onset of anaphase and it might still ultimately lead to cell death. Overall, however, the recruitment of mitotic components to centrosomes and unattached kinetochores remains controversial. These discrepancies might
be the result of differences among cell lines in sensitivity to BI2536 or the threshold for SAC activation; alternatively, they may be a consequence of epitope differences in the antibodies used in various studies.

Our Annexin V/PI staining assay revealed that A549 (K-Ras mut, p53 wt) and A427 (K-Ras mut, p53 wt) were more sensitive to BI2536 than NCI-H1299 (K-Ras wt, N-Ras mut, p53 mut). This observation suggests a positive correlation between the presence of a K-Ras mutation and sensitivity to PLK1 suppression. These results are consistent with a previous finding that colorectal cancer cells harboring a K-Ras mutation exhibited a higher rate of cell death than wild-type cells following transfection with PLK1 shRNA or BI2536 treatment [59]. However, one cannot firmly conclude from these data that K-Ras-mutated cancer cells are sensitive to PLK1 suppression because other oncogenes or tumor suppressors involved in Ras signaling must also be considered [60]. Moreover, the relationship between p53 status and sensitivity to PLK1 inhibitors remains controversial [32]. However, although there was the difference in the level of apoptosis among three different cell lines (Fig. 6B), no significant difference was observed in cytotoxicity and survival assay (Fig. 1). It indicates that BI2536-treated cells might undergo other types of cell death as well as apoptosis. In light of these issues, the difference in sensitivity to BI2536 among NSCLC cell lines should be investigated in greater detail.

PLK1 is overexpressed and associated with poor prognosis in NSCLC [61–64], and thus several attempts have been made to develop

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**Fig. 7.** BI2536 leads to mitotic catastrophe in A549 cells. (A–D) H2B-RFP-transduced A549 cells were treated with DMSO or 50 nM BI2536. Cells were monitored using time-lapse fluorescence microscopy for 42 hr. Mitotic cells in DMSO-treated cells and apoptotic cells in BI2536-treated cells were indicated by arrows. (D) The cells undergoing either mitotic catastrophe or mitotic slippage were counted in BI2536-treated cells. The number of cells represents the average of three independent experiments.
a strategy for treatment of NSCLC by suppressing the expression or activity of this kinase. For example, PLK1 depletion using anti-sense oligonucleotide, siRNA, or shRNA leads to cell death in NSCLC cells including A549 [62,64–67] and SPC-A1 [63], and reduced tumor growth and metastasis in an A549 xenograft model [64,68,69]. In addition, PLK1 inhibitors such as volasertib, GSK461364, HMN-176, and ON01910Na also exhibit cytotoxic effects in NSCLC cell lines or xenograft models [70]. Because BI2536 is the first-in-class prototype PLK1 inhibitor, its anti-proliferative effect on diverse cancer cells has been tested and confirmed by several groups. In regard to lung cancer, BI2536 exerts cytotoxicity against NSCLC cells such as A549 (adenocarcinoma, p53 wt), NCI-H460 (large cell carcinoma, p53 wt), and NCI-H520 (squamous cell carcinoma, p53 mut), irrespective of their p53 status [31,32]. In addition, BI2536 also inhibits tumor growth in vivo in xenograft models using A549 or NCI-H460 cells [31]. Together, these observations show that PLK1 suppression is an effective target for the treatment of advanced lung tumors. Whereas previous studies in lung cancer cells were only able to demonstrate a reduction in cancer cell growth, the results described here show that BI2536 leads to prolonged activation of SAC and, ultimately, to mitotic catastrophe in several NSCLC cell lines.

Mitotic catastrophe is a type of cell death that occurs after mitotic arrest or the formation of aneuploidy due to dysregulated mitosis [71,72]. In this study, most BI2536-induced arrested cells underwent cell death during mitosis. In addition, the remaining cells exited mitosis, but also underwent cell death shortly after entering interphase. Therefore, because it is capable of inducing mitotic catastrophe, BI2536 represents a potential oncosuppressive drug for...
the treatment of NSCLC; however, BI2536 has some side effects, the most common of which are neutropenia and gastrointestinal disturbance [33,34]. Therefore, effective anti-cancer therapy could be accomplished by combining BI2536 with conventional drugs such as cisplatin and paclitaxel, which also disrupt mitotic progression.

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Conflict of interest

The authors declare that they have no conflict of interest.

References


H. Huang, T.J. Yen, BubR1 is an effector of multiple mitotic kinases that specifies kinetochore: microtubule attachments and checkpoint, Cell Cycle 8 (2009) 1164–1167.


