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Aurora B kinase inhibition intensifies cisplatin cytotoxicity in MCF7 breast cancer cells

Aurora B kinaz inhibisyonu MCF7 meme kanseri hücrelerinde sisplatin sitotoksisitesini yoğunlaştırır

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ABSTRACT

Aim: Cancer, a complex and multifaceted group of diseases, poses a formidable challenge to global health. Characterised by uncontrolled cell growth and proliferation, it manifests in diverse forms, each with unique biological traits. Comprehending the intricate landscape of cancer biology stands as a fundamental cornerstone in the pursuit of tailored therapeutic interventions. This research aimed to explore the impact of inhibiting Aurora B kinase with BI-831266 on the anticancer efficacy of cisplatin in MCF7 cells, contributing to our understanding of potential treatment strategies.

Materials and Methods: Good Cell Culture Practices were conducted in this research, where MCF7 human breast cancer cells were used in order to assess the therapeutic potential of the BI-831266 and cisplatin combination. Regarding functional experiments, we employed in vitro cell proliferation assay, 2D clonogenic survival assay, 3D colony formation assay and wound-healing assay. To elucidate the molecular mechanism underlying the observed functional outcomes, SDS-PAGE and Western blotting experiments were additionally conducted.

Results: Our findings uncovered a synergistic interaction between inhibiting Aurora B kinase and treating MCF7 cancer cells with cisplatin. The combined treatment significantly increased cisplatin's cytotoxicity, hindered cancer cell migration, and influenced apoptotic pathways, as it is evident from changes in key protein expressions.

Conclusion: Our study underscores the importance of directing focus towards Aurora B kinase to amplify therapeutic outcomes of cisplatin in MCF7 breast cancer cells. This research offers valuable insights into potential combination therapies, paving the way for a more efficacious and precisely targeted approach to breast cancer treatment.

Keywords: Aurora B kinase inhibition, cisplatin, breast cancer.

ÖZ

Amaç: *Karmaşık ve çok yönlü bir hastalık grubu olan kanser, küresel sağlık açısından zorlu bir sorun teşkil etmektedir. Kontrolsüz hücre büyümesi ve çoğalması ile karakterize edilen bu hastalık, her biri benzersiz biyolojik özelliklere sahip olan çeşitli formlarda kendini göstermektedir. Kanser biyolojisinin karmaşıklığının anlaşılması, hedefe yönelik terapötik müdahalelerin geliştirilmesi için önemlidir. Bu araştırma, Aurora B kinazın BI-831266 ile inhibe edilmesinin, MCF7 hücrelerinde sisplatinin anti-tümör etkinliği üzerindeki etkisini araştırmayı ve potansiyel tedavi stratejilerini anlamamıza katkıda bulunmayı amaçlamaktadır.*

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Gereç ve Yöntem: *BI-831266 ve sisplatin kombinasyonunun terapötik potansiyelini değerlendirmek amacıyla MCF7 insan meme kanseri hücrelerinin kullanıldığı bu araştırmada İyi Hücre Kültürü Uygulamaları gerçekleştirilmiştir. Fonksiyonel deneylerle ilgili olarak, in vitro hücre proliferasyon analizi, 2D klonojenik sağkalım analizi, 3D koloni formasyon analizi ve yara iyileşme analizi uygulandı. Gözlemlenen fonksiyonel sonuçların altında yatan moleküler mekanizmayı açıklamak için ayrıca SDS-PAGE ve Western blot deneyleri gerçekleştirildi.*

Bulgular: *Bulgularımız, MCF7 kanser hücrelerinde Aurora B kinazın inhibe edilmesi ile sisplatin tedavisi arasında sinerjistik bir etkileşimi ortaya çıkardı. Kombinasyon tedavisinin, önemli protein ifadelerindeki değişikliklerden de anlaşılacağı üzere sisplatinin sitotoksisitesini önemli ölçüde arttırdığı, kanser hücresi göçünü engellediği ve apoptotik yolakları etkinleştirdiği belirlendi.*

Sonuç: *Araştırmamız, MCF7 meme kanseri hücrelerinde sisplatinin terapötik yanıtını arttırmak için Aurora B kinaz aktivitesini hedeflemenin önemini vurgulamaktadır. Çalışmamız, meme kanseri tedavisinde daha etkili ve hedefe yönelik bir yaklaşım sunarak potansiyel kombinasyon tedavilerine değerli katkılar sağlamaktadır.*

Anahtar Sözcükler: *Aurora B kinaz inhibisyonu, sisplatin, meme kanseri.*

INTRODUCTION

Breast cancer ranks as the most frequently diagnosed cancer among women and stands as the leading cause of cancer-related mortality for women on a global scale. Its multifaceted nature necessitates a comprehensive understanding of genetic, environmental, and lifestyle factors influencing its onset and progression (1–3).

Cisplatin is a widely used platinum-based chemotherapy drug in the treatment of various cancers, including testicular, ovarian, bladder, and lung cancers. Its effectiveness stems from its ability to interfere with the DNA replication process, ultimately inducing cell death (4, 5). While it is a well-established and effective treatment for various solid tumours, such as ovarian and testicular cancers, information about its role in breast cancer has been more limited. However, the use of cisplatin in breast cancer remains an area of ongoing investigation, and its inclusion in treatment regimens is not yet considered as a standard application (6, 7).

Human Aurora kinases (Aurora A, B and C) are a family of serine/threonine protein kinases which have crucial roles in the regulation of cell cycle and the maintenance of genomic stability (8–10). Of those, Aurora kinase B is a key factor of the chromosomal passenger complex (CPC), playing a pivotal role in cytokinesis and chromosome condensation (11, 12). Dysregulation of Aurora kinases has been implicated in various cancers, making them attractive targets for anticancer therapy. Inhibition of Aurora kinases disrupts the cell cycle and induces mitotic defects, ultimately leading to cell death (13–15). Several Aurora kinase-targeted small molecule inhibitors are currently under investigation in preclinical and clinical studies (16), holding promise for the development of novel anti-neoplastic therapies with a focus on disrupting aberrant cell division in cancer cells.

In our study, we identified a synergistic interaction between Aurora B kinase inhibition and cisplatin treatment in MCF7 cancer cells. The inhibition of Aurora B kinase markedly enhanced the cytotoxic effect of cisplatin.. We further revealed that the combined inhibition impaired the migratory capacity of MCF7 cells, suggesting a dual effect of enhancing cisplatin cytotoxicity and impeding cancer cell migration. Additionally, the assessment of apoptotic markers showed significant changes in the expression levels of fundamental proteins, such as cleaved-PARP (cPARP), indicating potential modulation of apoptotic pathways by the combination treatment. These outcomes underscore the significance of targeting Aurora B kinase alongside cisplatin in terms of augmenting therapeutic responses in breast cancer.

MATERIALS AND METHODS

Chemicals

BI-831266 was kindly provided by Boehringer Ingelheim via its open innovation platform opnMe, available at https://opnme.com. Crystal violet and dimethyl sulfoxide (DMSO) were bought from Sigma-Aldrich (#c6158 and #D2438, respectively). Cisplatin was obtained from Santa Cruz (#sc-200896). Primary antibodies used in this study were acquired from various suppliers: anti-cPARP was obtained from Cell Signaling Technology (#9541S), anti-p53 was obtained from Santa Cruz (#sc-126); anti-BCL2 and anti-

proCas3 were obtained from St John's Laboratory (#STJ96943 and #STJ97448, respectively). The HRP(horseradish peroxidase)-linked anti-mouse and anti-rabbit secondary antibodies were sourced from ThermoFisher (#31460 and #32430, respectively). UltraPure™ Low Melting Point Agarose was purchased from ThermoFisher (Invitrogen - 16520050).

Mammalian cell culture techniques

DMEM, FBS, Trypsin-EDTA (0.25%) and dPBS were obtained from ThermoFisher. The antibiotic solution containing penicillin, streptomycin, and amphotericin B was bought from Capricorn (#AAS-B) Dr Alexander Hergovich (Evotec, France) kindly provided human breast cancer cell line MCF7. Unless otherwise stated, MCF7 cells were maintained in DMEM supplemented with 10% FBS. and grown in humidity-saturated incubators at 37°C supplied with 5% CO2. DMSO was used to prepare BI-831266 stock solutions stored at -80°C.

Crystal violet cell proliferation assay

Cells were seeded into 60-mm Petri dishes, treated, and grown for 24 hours before further treatment. At 10 days after seeding, cells were fixed in methanol-acidic acid solution (3:1) for 5 minutes and stained with crystal violet solution (0.5% w/v) for 15 minutes. The plates were gently washed with distilled water to eliminate surplus crystal violet and left to air-dry thoroughly. The percentage of cell proliferation was calculated according to densities of the stained cells acquired by the ImageJ program (NIH) and the data was analysed by comparing experimental groups with control conditions.

Clonogenic survival assays

Clonogenic survival experiment was carried out as indicated in (17). In brief, 1500 MCF7 cells in the exponential phase were seeded into 60-mm Petri dishes and incubated for 24 hours before being subjected to BI831266 treatment with or without cisplatin combination for an additional 24 hours. After 7-12 days incubation, colonies were initially subjected to the methanol and acidic acid (3:1) fixation, then stained with crystal violet solution (0.5% w/v). Colonies were visualised and counted by using an inverted microscope, and a cluster of at least 50 cells was defined as a colony. Plating efficiencies and survival fractions were calculated as described in (18). Three independent experiments were conducted for all clonogenic survival experiments.

Anchorage-independent colony formation assays

The anchorage-independent colony formation assay (aka soft agar) was conducted to investigate the colony-forming ability of MCF7 cells in a three-dimensional environment as described in (19) with some modifications. A 1.2% agarose solution was prepared in complete cell culture medium and 2 mL of the agarose solution was transferred into each well of the culture dish. After allowing the agarose to solidify at 4°C for 30 minutes, 2.5×10^3 cells were mixed with 0.6% agarose solution in 1:1 dilution in order to achieve a final concentration of 0.3% agarose, which was then added on top of the solidified base agar layer. Cells were incubated for at least 3 weeks, colony formation was monitored regularly and fresh medium was added every 3-4 days. Ultimately, colonies underwent staining using a 0.5% w/v crystal violet solution and were subsequently scanned.

Wound-healing assays

The wound-healing assay, a well-established two-dimensional technique, was employed to study collective migration of MCF7 cells, following the procedure outlined in reference (20). Logarithmically growing cells (4×10^5) were seeded in 6-well culture plates. After 24 hours, a straight-line scratch was created in the cell monolayer, and subsequent treatments were applied for 24 hours. Images of the wound area were captured at time points (0th, 6th, 12th, and 24th hours) using an inverted microscope (Motic-AE200). Image J software (NIH) analysed the images, and wound closure was evaluated by comparing 0 and 24-hour images, normalised to the control set as 1. Three independent experiments were conducted for all wound-healing assays.

Western blotting experiments

Western blot analysis, following the method described in reference (17), was conducted as follows: cell pellets were suspended in standard lysis buffer (SLB), incubated on ice for at least 60 minutes, and then centrifuged. The resulting soluble protein fractions were mixed with Laemmli SDS sample buffer and subjected to five-minute-heat at 95°C. 12% SDS-PAGE was used to separate proteins, which were then transferred to a PVDF membrane, and blocked with 5% skim milk in TBS-T. The membranes were then incubated with specific antibodies overnight, followed by probing with HRP-conjugated secondary antibodies and exposure to ECL substrates for chemiluminescent detection. Densitometry analysis was performed using the ImageJ program (NIH), and three

technical replicates were conducted for all Western blotting experiments. SLB: 20 mM Tris-HCl, 150 mM NaCl, 10% glycerol, 1% NP-40, 0.5 mM EGTA, 20 mM beta-glycerophosphate, 5 mM EDTA, 1 mM Na3VO4, 50 mM NaF, 0.5 mM PMSF, 1 mM DTT, 1 mM benzamidine, 1 mM leupeptin, pH 8.0; TBT-T: 50 mM Tris, 150 mM NaCl, 0.5% Tween-20, pH 7.5.

Statistical analysis

GraphPad Prism software (GraphPad, CA, USA) was used to generate graphics and study statistical analyses and results were plotted using the means ± SEM. Statistical significance of mean differences was assessed through a one-tailed unpaired Student's t-test. Differences were considered statistically significant when p-values were below 0.05 (*), 0.01 (**), 0.001 (***) or 0.0001 (****) for all experiments.

RESULTS

BI-831266 inhibits the proliferation of MCF7 cancer cells

In our study, we initially assessed the impact of BI-831266 on the proliferation of MCF7 breast cancer cells, widely employed in breast cancer research (21). Notably, BI-831266 suppressed cell proliferation, causing nearly 50% eradication at a concentration of 10 nM (Figure-1A, B). Subsequently, we investigated the IC50 values for BI-831266 and cisplatin in terms of clonogenic survival in MCF7 cells. Both compounds significantly inhibited clonogenic survival, with IC50 values of 7.67 nM for BI-831266 and 2.89 µg/mL for cisplatin (Figure-2A, B and Figure-3A, B). Overall, these findings demonstrate the anticancer effect of BI-831266 in MCF7 cells.

cancer cell survival, primarily through apoptosis.

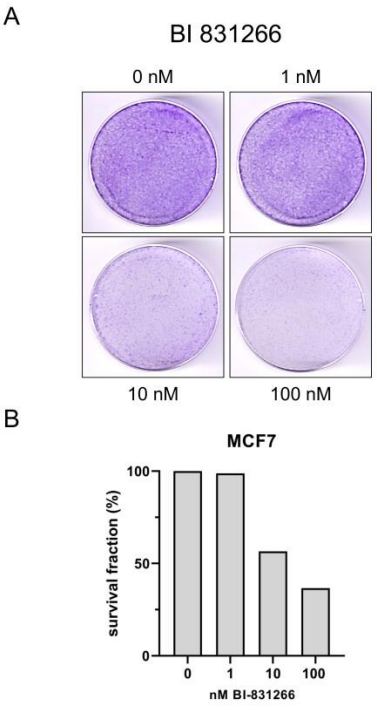

Figure-1. Treatment with BI-831266 restricts the proliferation of MCF7 cancer cells. (A) MCF7 cells were seeded into 60-mm petri dishes and treated with BI-831266 (0 - 1 - 10 - 100 nM) for 72 h. After 10 days of seeding, cells were fixed using a methanol-acidic acid solution and then subjected to staining with a 0.5% w/v crystal violet solution. (B) The ImageJ program (NIH) was utilised to determine the percentage of cell proliferation based on the densities of the stained cells, and the data were analysed by comparing experimental groups with control conditions.

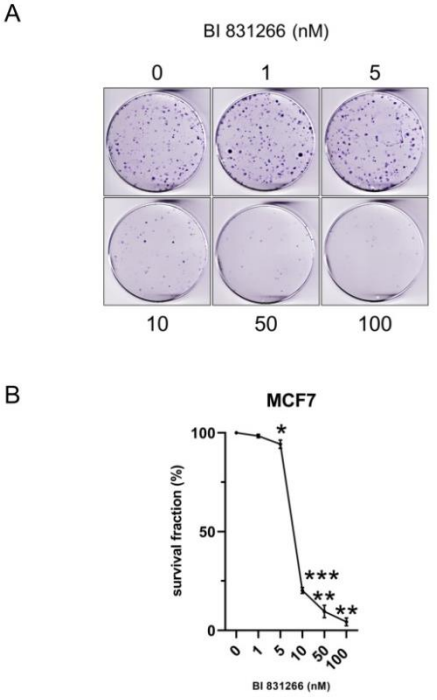

Figure-2. BI-831266 treatment impedes the clonogenic survival of MCF7 cancer cells. (A) A consistent number of MCF7 cells were seeded in 60-mm petri dishes and allowed to adhere for 24 hours before undergoing treatment with 0 - 1 - 5 - 10 - 50 - 100 nM BI831266 for 24 hours. Following an incubation period of 7-12 days, colonies were fixed with a methanol and acidic acid solution and subsequently stained with 0.5% w/v crystal violet. (B) Colony visualization and counting were performed using an inverted microscope, defining a cluster of at least 50 cells as a colony. Plating efficiencies and survival fractions were calculated according to the method outlined in reference (18) (n=3).

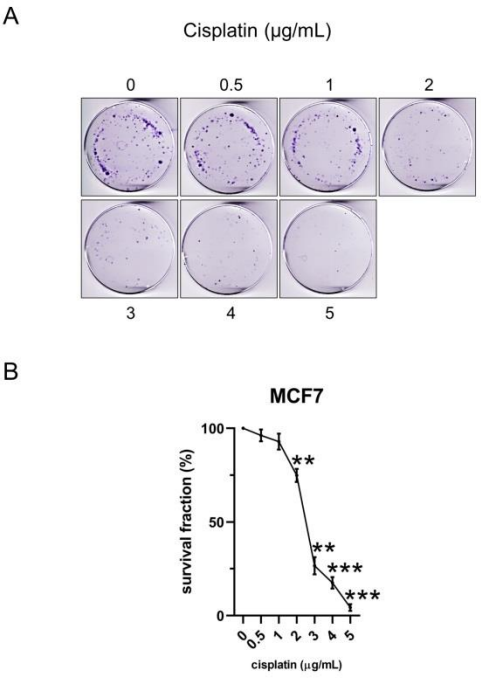

Figure-3. Cisplatin treatment inhibits the clonogenic survival of MCF7 cancer cells. (A) A consistent number of MCF7 cells were seeded in 60-mm petri dishes and allowed to adhere for 24 hours before undergoing treatment with 0 - 0.5 - 1 - 2 - 3 - 4 - 5 µg/mL cisplatin for 1 hour. Following an incubation period of 7-12 days, colonies were fixed with a methanol and acidic acid solution and subsequently stained with 0.5% w/v crystal violet.

(B) Colony visualization and counting were performed using an inverted microscope, defining a cluster of at least 50 cells as a colony. Plating efficiencies and survival fractions were calculated according to the method outlined in reference (18) (n=3).

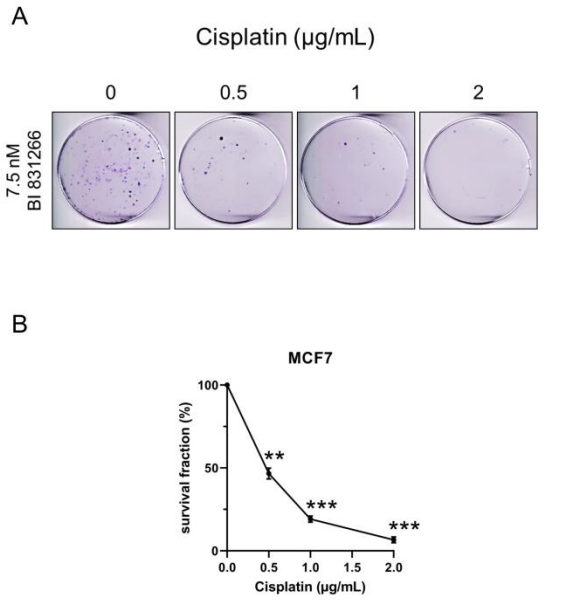

Figure-4. BI-831266 treatment potentiates the cytotoxicity of cisplatin in MCF7 cancer cells. (A) A consistent number of MCF7 cells were seeded in 60-mm petri dishes and allowed to adhere for 24 hours before undergoing treatment with 7.5 nM BI-831266 for 23 hours then combined with 0 - 0.5 - 1 - 1.5 - 2 µg/mL cisplatin for 1 hour. Following an incubation period of 7-12 days, colonies were fixed with a methanol and acidic acid solution and subsequently stained with 0.5% w/v crystal violet.

(B) Colony visualization and counting were performed using an inverted microscope, defining a cluster of at least 50 cells as a colony. Plating efficiencies and survival fractions were calculated according to the method outlined in reference (18) (n=3).

BI-831266 enhances the cytotoxic effects of cisplatin in MCF7 cancer cells

In our combination experiments, we systematically administered fixed or escalating doses of BI-831266 followed by fixed or escalating doses of cisplatin to MCF7 cells. Employing this systematic approach (22) allowed for a comprehensive exploration of potential synergistic or antagonistic interactions between the drugs. Initially, cells were treated with 7.5 nM BI 831266, a concentration corresponding to its IC50 value, for 23 hours, combined with escalating doses of cisplatin (0, 0.5, 1, and 2 µg/mL) for an additional one-hour period. The combined administration of BI-831266 with cisplatin significantly impeded cancer cell survival (Figure-4A), with 7.5 nM BI-831266 in combination with 1 and 2 µg/mL cisplatin eradicating over 75% and 90% of cells, respectively (Figure-4B).

Employing an alternate method, cells were exposed to escalating doses of BI-831266 (0, 1, 5, and 10 nM) for 23 hours, alongside a one-hour treatment with 1.5 µg/mL cisplatin. While results were not as pronounced as prior, BI-831266 significantly influenced cisplatin response in cancer cells (Figure-5A, B). Notably, treatment sequence, concentrations, and durations likely impacted outcomes, suggesting the initial approach induced further cell sensitization.

To confirm our findings, we conducted an anchorage-independent growth assay, simulating in vivo conditions. Cells in agarose were treated with 7.5 nM BI-831266 and/or 0.5 µg/mL cisplatin for at least 3 weeks. Figure-6 shows a significant reduction in colony quantity and size with combination treatment, affirming enhanced anti-neoplastic effects of cisplatin through Aurora B kinase inhibition.

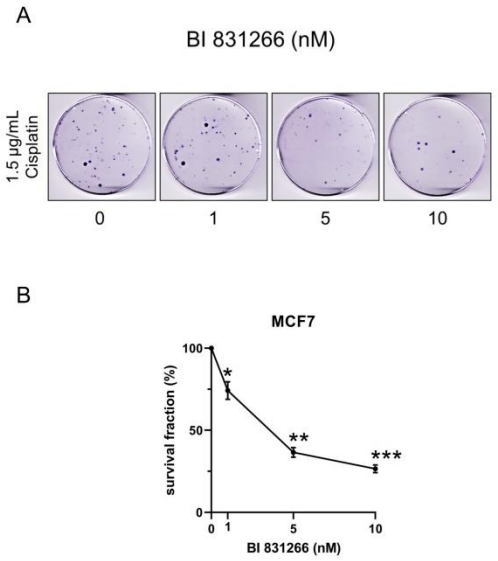

Figure-5. BI-831266 treatment increases the anticancer potential of cisplatin in MCF7 cancer cells.
(A) A consistent number of MCF7 cells were seeded in 60-mm petri dishes and allowed to adhere for 24 hours before undergoing treatment with 0 - 1 - 5 - 10 nM BI-831266 for 23 hours then combined with 1.5 µg/mL cisplatin for 1 hour. Following an incubation period of 7-12 days, colonies were fixed with a methanol and acidic acid solution and subsequently stained with 0.5% w/v crystal violet.
(B) Colony visualization and counting were performed using an inverted microscope, defining a cluster of at least 50 cells as a colony. Plating efficiencies and survival fractions were calculated according to the method outlined in reference (18) (n=3).

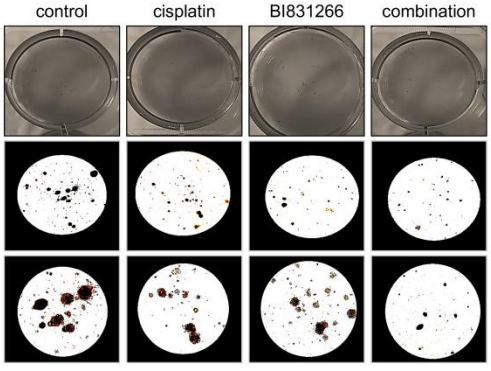

Figure-6. The BI-831266 and cisplatin combination reduces MCF7 tumour formation. A fixed percentage of agarose solution in complete cell culture medium was added to each well of a culture dish. After solidification at 4°C for 30 minutes, a pre-determined number of cells were mixed with the agarose solution and added on top of the solidified base agar layer. Cells were incubated for a minimum of 3 weeks with regular monitoring and fresh medium with the indicated drugs added every 3-4 days. Colonies were then stained with a 0.5% w/v crystal violet solution and scanned.

The combination of BI-831266 and cisplatin reduces the migratory capability of MCF7 cells

To assess the impact of the BI-831266 and cisplatin combination on MCF7 cell migration, we utilized in vitro wound-healing assays, a widely employed tool in cell biology and cancer research (23). Cells were treated with or without 7.5 nM BI-831266 and/or 0.5 µg/mL cisplatin 24 hours post-seeding, with microscopic images captured at the 6th, 12th, and 24th hours. The combination treatment demonstrated a noteworthy effect on cell migration, as evidenced by the fraction of wound area closure, surpassing the effects of individual treatments (Figure-7A, B). Collectively, these findings highlight the significant influence of the combination treatment on MCF7 cell migration.

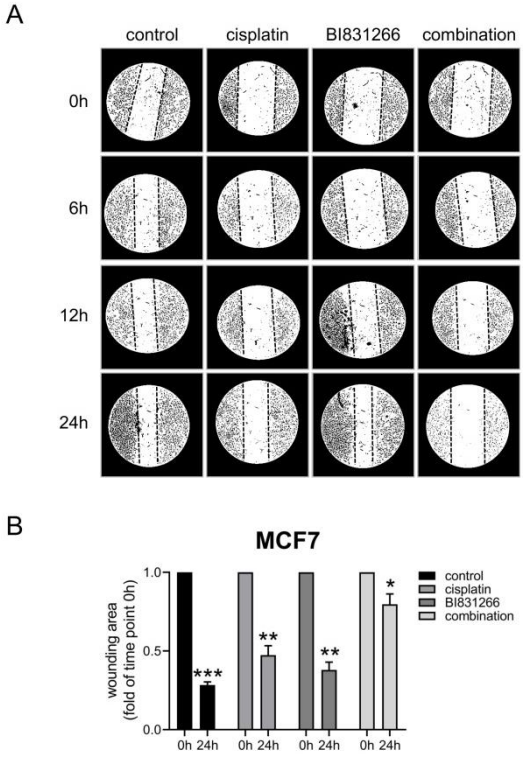

Figure-7. The BI-831266 treatment combined with cisplatin lowers the migration capacity of MCF7 cancer cells. Logarithmically growing cells were seeded in 6-well culture plates. After 24 hours, a straight-line scratch was introduced in the cell monolayer, and subsequent treatments were administered for 24 hours. Images of the wound area were captured at various time points (0, 6, 12, and 24 hours) using an inverted microscope.
(B) Image J software (NIH) analysed the images, and wound closure was assessed by comparing 0 and 24-hour images, normalised to the control set as 1 (n=3).

The BI-831266 and cisplatin combination promotes an apoptotic response of MCF7 cancer cells

To explore the mechanisms inhibiting neoplastic growth in MCF7 cells with BI-831266 and cisplatin, we conducted experiments focusing on apoptotic cell death. Western blotting included control (DMSO-treated), cisplatin alone (20 µg/mL), BI-831266 alone (10 nM and 50 nM), and two combination groups (20 µg/mL cisplatin + 10 nM or 50 nM BI-831266). Cells underwent treatment either with BI-831266 for 24 hours, cisplatin for one hour, or a combination treatment with BI-831266 for 23 hours followed by cisplatin for an additional one-hour period. Consistent with previous findings (24, 25), a one-hour acute treatment with cisplatin led to an increase in cPARP activation compared to the control (Figure-8A, B). Intriguingly, a 24-hour BI-831266 treatment significantly increased cPARP activation in a dose-dependent manner, doubling its levels (Figure-8A, B), suggesting its impact on mitosis and cellular stress contributes to apoptotic pathway activation through PARP cleavage. In comparison to both the control and individual treatments, the co-administration of cisplatin with a higher BI-831266 concentration resulted in a more pronounced induction of cPARP activation, nearly tripling in magnitude (Figure-8A, B), offering a potential explanation for the enhanced effectiveness of the combination treatment in inhibiting the cancerogenic growth of MCF7 cells.

In our investigation, we focused on the role of p53, a pivotal regulator of apoptosis, in response to cellular stress or DNA damage. Treatment with BI-831266 alone or in combination with cisplatin led to a significant increase in p53 levels (Figure-9). Surprisingly, the combination treatment did not induce a higher level of p53 compared to BI-831266 alone (Figure-9). Despite p53's known role in apoptosis and the activation of cPARP being indicative of apoptosis (26), our findings suggest that alternative pathways, independent of p53, may contribute to PARP cleavage (27, 28).

BCL-2 inhibits apoptosis by preserving mitochondrial membrane integrity and suppressing the release of pro-apoptotic factors (29–31). In line with the observations regarding cPARP, our experiments demonstrated a significant reduction in BCL-2 protein levels following treatment with BI-831266 alone or in combination with cisplatin; nevertheless, there was no significant distinction between the effects of BI-831266 alone and the combined treatments (Figure-10A, B).

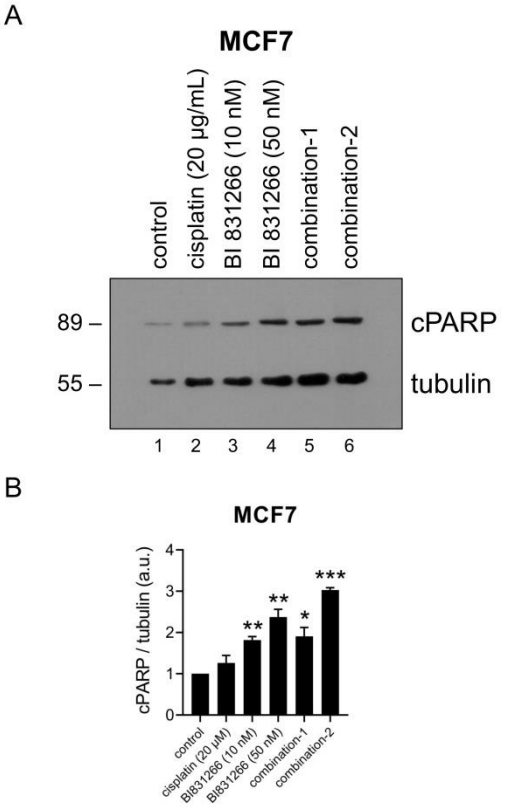

Figure-8. The BI-831266 treatment alone or in combination with cisplatin induces apoptotic cell death through PARP cleavage.

(A) Western blotting with indicated antibodies of MCF7 cell lysates from cells treated with or without the indicated drugs. The experiments were performed with distinct treatment groups, including DMSO control, cisplatin alone (20 µg/mL), BI-831266 alone (10 nM), BI-831266 alone (50 nM), combination-1 (20 µg/mL cisplatin + 10 nM BI 831266) and combination-2 (20 µg/mL cisplatin + 50 nM BI 831266). Cells underwent treatment either with BI-831266 for 24 hours, cisplatin for one hour, or a combination treatment with BI-831266 for 23 hours followed by cisplatin for an additional one-hour period. After 24 hour-treatment cells were collected and subjected to Western blotting analysis.

(B) Histograms showing the expression profile of cPARP protein, obtained by densitometric quantification of Western blots represented in A. Arbitrary units were normalised to the expression of the corresponding total protein (n=3).

Pro-Caspase 3, an inactive precursor of the apoptosis-executing enzyme Caspase 3, undergoes cleavage activation crucial for initiating programmed cell death (32). Our study

revealed that BI-831266 treatment induced apoptotic activation comparable to cisplatin, evident through pro-Caspase 3 cleavage (Figure-11A, B). Interestingly, no significant difference in pro-Caspase 3 protein levels was observed between single-agent and combination treatments (Figure-11A,B). In summary, our findings highlight that Aurora B kinase inhibition enhances the anticancer activity of cisplatin by diminishing

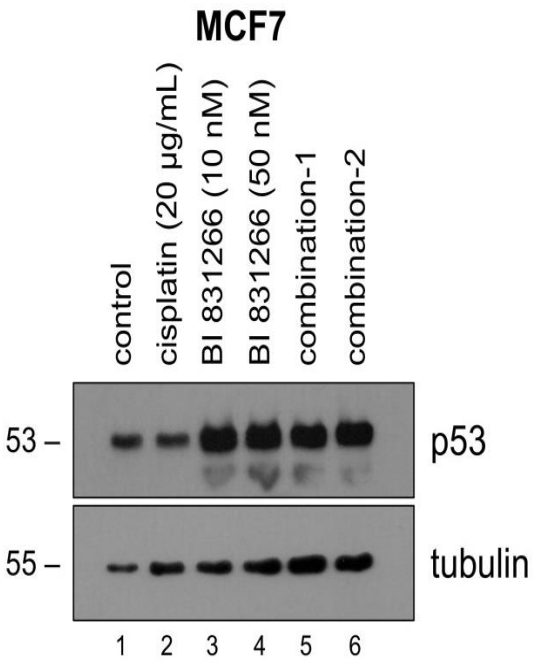

Figure-9. The BI-831266 treatment increases p53 protein levels in MCF7 cancer cells.

(A) Western blotting with indicated antibodies of MCF7 cell lysates from cells treated with or without the indicated drugs. The experiments were performed with distinct treatment groups, including DMSO control, cisplatin alone (20 µg/mL), BI-831266 alone (10 nM), BI-831266 alone (50 nM), combination-1 (20 µg/mL cisplatin + 10 nM BI 831266) and combination-2 (20 µg/mL cisplatin + 50 nM BI 831266). Cells underwent treatment either with BI-831266 for 24 hours, cisplatin for one hour, or a combination treatment with BI-831266 for 23 hours followed by cisplatin for an additional one-hour period. After 24 hour-treatment cells were collected and subjected to Western blotting analysis (n=3). Since there was no clear difference between single and combination treatments, we did not perform the densitometric analysis of the bands.

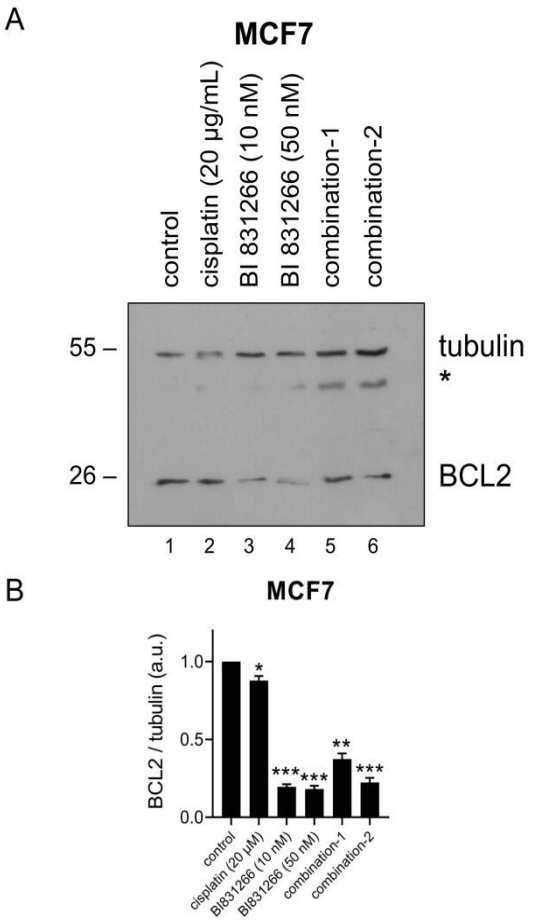

Figure-10. The BI-831266 and cisplatin treatment diminishes the level of anti-apoptotic protein BCL2.

(A) Western blotting with indicated antibodies of MCF7 cell lysates from cells treated with or without the indicated drugs. The experiments were performed with distinct treatment groups, including DMSO control, cisplatin alone (20 µg/mL), BI-831266 alone (10 nM), BI-831266 alone (50 nM), combination-1 (20 µg/mL cisplatin + 10 nM BI 831266) and combination-2 (20 µg/mL cisplatin + 50 nM BI 831266). Cells underwent treatment either with BI-831266 for 24 hours, cisplatin for one hour, or a combination treatment with BI-831266 for 23 hours followed by cisplatin for an additional one-hour period. After 24 hour-treatment cells were collected and subjected to Western blotting analysis.

(B) Histograms showing the expression profile of BCL2 protein, obtained by densitometric quantification of Western blots represented in A. Arbitrary units were normalised to the expression of the corresponding total protein (n=3). *unspecific band.

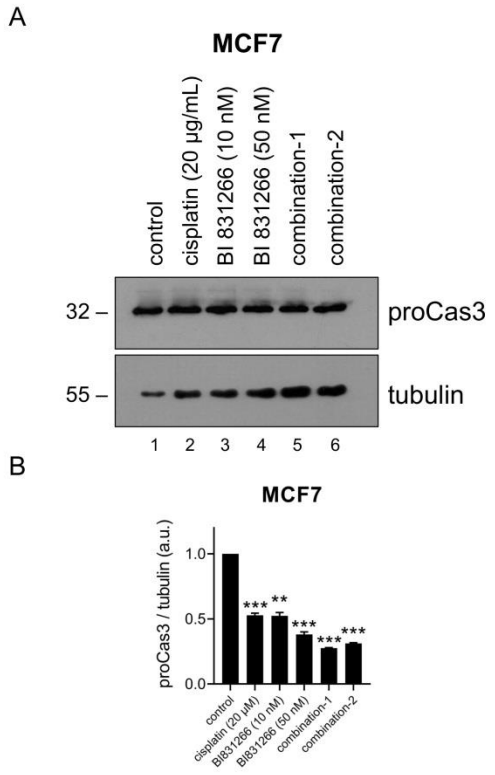

Figure-11. The combination of BI-831266 and cisplatin decreases the level of pro-Caspase 3. (A) Western blotting with indicated antibodies of MCF7 cell lysates from cells treated with or without the indicated drugs. The experiments were performed with distinct treatment groups, including DMSO control, cisplatin alone (20 µg/mL), BI-831266 alone (10 nM), BI-831266 alone (50 nM), combination-1 (20 µg/mL cisplatin + 10 nM BI 831266) and combination-2 (20 µg/mL cisplatin + 50 nM BI 831266). Cells underwent treatment either with BI-831266 for 24 hours, cisplatin for one hour, or a combination treatment with BI-831266 for 23 hours followed by cisplatin for an additional one-hour period. After 24 hour-treatment cells were collected and subjected to Western blotting analysis.
(B) Histograms showing the expression profile of BCL2 protein, obtained by densitometric quantification of Western blots represented in A. Arbitrary units were normalised to the expression of the corresponding total protein (n=3).

DISCUSSION

Breast cancer, a pervasive malignancy, epitomizes the intricate interplay of molecular factors shaping its pathogenesis. Globally, breast cancer ranks as the most common cancer among women, with an alarming incidence (3). Analysis of publicly accessible databases additionally indicated a substantial correlation between heightened Aurora kinase B expression and diminished survival rates among individuals diagnosed with breast cancer (33). Furthermore, the expression of Aurora kinase B has been recognized as a prognostic indicator in glioblastoma (34), gastric cancer (35), and oral cancer (36). Elevated expression of Aurora kinase B was also observed in prostate cancer tissues in comparison to healthy controls (37).

The fundamental roles played by Aurora kinases, coupled with their abnormal expression observed in various tumour types, have led to the exploration of several small molecule inhibitors as potential cancer treatments. Among others, BI-831266 is a powerful and specifically targeted low-molecular-weight inhibitor designed to act on Aurora kinase B. Preclinical investigations indicate that BI-831266 impedes the growth of cell lines associated with prostate cancer, human non-small cell lung cancer, and pancreatic cancer. Moreover, in murine xenograft tumour (HCT116) models, BxPC3 pancreatic adenocarcinoma, and NCI-H460 NSCLC, a constant 24-hour infusion of BI-831266 demonstrated tumour regression and inhibited growth, as reported in internal data from Boehringer Ingelheim (38). To the best of our knowledge, no preclinical study has been undertaken to explore the anti-neoplastic role of BI 831266 in breast cancer cell lines. In our research, we discovered a synergistic effect between Aurora B kinase inhibition and cisplatin treatment in MCF7 cancer cells. Specifically, we revealed that the inhibition of Aurora B kinase significantly enhanced the cytotoxicity of cisplatin. Furthermore, we found that the combination of Aurora B kinase inhibition and cisplatin impaired the migratory capacity of MCF7 cells. This finding indicates a potential dual effect, not only enhancing the cytotoxicity of cisplatin but also hampering the migratory ability of cancer cells. Finally, our investigation included an assessment of the protein expression levels of key apoptotic markers in MCF7 cells subjected to cisplatin treatment, both in the presence and absence of Aurora B kinase inhibition. Our findings revealed significant alterations in the expression levels of crucial apoptotic proteins including cPARP, suggesting that the combination of Aurora B kinase inhibition and cisplatin treatment may modulate apoptotic pathways. These findings highlight the significance of targeting Aurora kinase B in conjunction with cisplatin to enhance the therapeutic response in MCF7 breast cancer cells.

Cancer cells often develop resistance to single-agent therapies over time. Therefore, the combination of small molecule inhibitors with chemotherapy agents represents a multifaceted and effective approach to combat the complexity of cancer, addressing both the heterogeneity of tumours and the challenges associated with drug resistance. Larsen et al. found barasertib to selectively impede the growth of fulvestrant-resistant T47D breast cancer cell lines (39). Barasertib prompted the degradation of Aurora kinase B, resulting in mitotic errors, and initiated apoptotic cell death. This was substantiated by the accumulation of SubG1 cells and PARP cleavage observed in the fulvestrant-resistant cells (39). Our experiments also revealed that treatment with BI-831266 as a single agent robustly restrained the growth of MCF7 cancer cells and induced apoptosis, as indicated by the activity of cleaved-PARP. Further studies will reveal which apoptotic pathways are activated upon the BI 831266-dependent Aurora kinase B inhibition. As mentioned earlier, we found that BI-831266 treatment significantly increases the anticancer potential of cisplatin in MCF7 cells. Subsequent investigations will elucidate whether BI-831266 exhibits efficacy in targeting cisplatin-resistant MCF7 breast cancer cells. Understanding its effectiveness in resistant cells could pave the way for the development of more robust and adaptable therapeutic strategies for breast cancer patients, especially those with limited responsiveness to conventional treatments. Testing the effect of a compound in just one cell line may provide valuable initial insights into its biological activity and potential therapeutic impact within a specific context. However, relying solely on results from a single cell line has limitations. Different cell lines can exhibit diverse genetic backgrounds, phenotypic characteristics, and responses to treatments due to their unique origins and genetic alterations. Therefore, while our initial testing in a single cell line can offer valuable insights, comprehensive and reliable conclusions are further required to evaluate BI 831266's effects across a range of relevant cell lines to ensure broader applicability and relevance to diverse biological contexts.

In conclusion, by targeting Aurora B kinase alongside cisplatin, our study aligns with the ongoing efforts to address key hallmarks such as sustained proliferative signalling and evading growth suppressors, offering promising insights into more effective and targeted strategies for breast cancer treatment.

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Declaration of interest statement

We received BI831266 compound gratis from Boehringer Ingelheim, aiding our research. Notably, the company had no role in experiment design, execution, analysis, or interpretation. The authors declare no other conflicts of interest.

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