Heliyon 10 (2024) e32015

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Synergistic anticancer effects of SMYD2 inhibitor BAY-598 and doxorubicin in non-small cell lung cancer

Jiaqi Meng [a,b], Weichang Yang [b,c], Can Li [a,**], Fengyuan Li [a,b,*]

[a] Department of Pulmonary and Critical Care Medicine, The First Hospital of Nanchang, The Third Affiliated Hospital of Nanchang University, Nanchang, 330008, Jiangxi, China
[b] Medical Department of Graduate School, Nanchang University, Nanchang, 330006, Jiangxi, China
[c] Department of Pulmonary and Critical Care Medicine, The Second Affiliated Hospital of Nanchang University, Nanchang, 330006, Jiangxi, China

ARTICLE INFO

Keywords:
NSCLC
DOX
JAK/STAT signaling
BAY-598

ABSTRACT

Background: Non-small cell lung Cancer (NSCLC) persists as a lethal neoplastic manifestation, exhibiting a diminished 5-year survival rate, partially attributable to chemotherapeutic resistance. This investigative endeavor aimed to elucidate the synergistic antineoplastic effects and underlying mechanisms of the SMYD2 inhibitor BAY-598 and the chemotherapeutic agent doxorubicin (DOX) in NSCLC.

Methods: The human non-small cell lung cancer cell lines A549 and H460 were subjected to treatment regimens involving BAY-598 and/or DOX. Cellular viability, apoptotic events, invasive capacity, and migratory potential were evaluated through the implementation of CCK-8 assays, flow cytometric analyses, and Transwell assays, respectively. Protein expression levels were quantified via Western blot analyses. An in vivo xenograft murine model was established to assess therapeutic efficacy.

Results: BAY-598 and DOX synergistically suppressed the viability, invasiveness, and migratory capabilities of NSCLC cells. Co-treatment Promoting cell apoptosis and cell cycle arrest. Additionally, Furthermore, co-administration significantly inhibited cell migration and invasion. Mechanistic studies revealed coordinately inhibited JAK-STAT signaling upon combination treatment. In vivo study further validated the synergistic antitumor efficacy of BAY-598 and DOX against NSCLC xenografts.

Conclusions: Our findings demonstrate that BAY-598 potentiates the anti-cancer effects of DOX in non-small cell lung cancer cells by modulating the JAK/STAT signaling pathway as a synergistic strategy. The combination holds promise as an emerging therapeutic strategy for NSCLC. Further optimization and validation are warranted to promote its translational potential.

Abbreviations: NSCLC, Non-Small Cell Lung Cancer; DOX, Doxorubicin; SMYD2, SET and MYND domain-containing protein 2; EMT, Epithelial-Mesenchymal Transition.

* Corresponding author. Department of Pulmonary and Critical Care Medicine, The First Hospital of Nanchang, The Third Affiliated Hospital of Nanchang University, Nanchang, 330008, Jiangxi, China.
** Corresponding author.
E-mail addresses: 15072165260@163.com (C. Li), lfy55672023@163.com (F. Li).

https://doi.org/10.1016/j.heliyon.2024.e32015
Received 29 January 2024; Received in revised form 26 May 2024; Accepted 27 May 2024
Available online 29 May 2024

1. Introduction

Pulmonary carcinoma stands as the preeminent etiology of cancer-related mortality on a global scale [1]. The non-small cell lung cancer (NSCLC) subtype constitutes approximately 80 % of all lung neoplasm cases [2,3]. Notwithstanding the advancements in targeted therapeutic modalities for NSCLC, the 5-year survival prognosis persists at a suboptimal level, primarily attributed to the acquisition of chemoresistance [4,5]. Therefore, exploring novel combinatorial therapies that can enhance chemosensitivity and overcome drug resistance is key to improving the survival and life quality of NSCLC patients.

BAY-598 is an inhibitory agent targeting the catalytic activity of the SMYD2 (SET and MYND domain-containing protein 2) [6], SMYD2, a constituent of the SET and MYND-containing lysine methyltransferase (SMYD) family, exerts many array of functional roles by catalyzing methylation events on both histone and non-histone protein substrates [7]. SMYD2 exerts a crucial regulatory function in gene expression modulation through its histone methyltransferase activity. It is a widely recognized lysine methyltransferase enzyme capable of catalyzing methylation at the lysine 4 and 36 residues (H3K4 and H3K36) of histone proteins, in addition to its ability to target non-histone protein substrates. Accumulating evidence suggests that SMYD2 exhibits aberrant upregulation and activation across various malignancies, including pulmonary, hepatic, and pancreatic carcinomas, wherein its dysregulation is intimately associated with the initiation and progression of these neoplastic processes [8–10]. BAY-598 can inhibit its methylation activity by competitively binding to the SAM (S-adenosyl methionine) binding site of SMYD2. Several investigative studies have substantiated the capacity of BAY-598 to impede the proliferative potential of diverse solid tumor malignancies, including hepatocellular carcinoma, pancreatic adenocarcinoma, and glioblastoma multiforme. Specifically, BAY-598 can competitively bind to the SET domain of SMYD2, inhibit SMYD2's methylation modification of chromatin histones, reduce the generation of H3K9me2 and H3K36me2, cause changes in gene expression profiles, and ultimately culminating in the suppression of neoplastic cellular proliferation and metastatic dissemination [11]. This may be an important mechanism for BAY-598 to exert anti-tumor activity.

Doxorubicin (DOX) is a first-line chemotherapeutic agent that finds widespread application in the treatment of NSCLC [12,13]. It exhibits potent antineoplastic activity against various malignancies, including breast carcinoma, ovarian carcinoma, gastric adenocarcinoma, and pulmonary carcinoma [13–16]. However, its clinical utility is constrained by its inherent adverse effects and the development of chemoresistance. Consequently, there is an urgent imperative to find a better efficacious and safer therapeutic approach that incorporates low-dose DOX in combination with adjuvant pharmacological agents to circumvent resistance mechanisms and mitigate toxicity.

Although BAY-598 and doxorubicin have shown anti-tumor effects when used alone, the synergistic anticancer mechanism between them in NSCLC treatment is still not completely clear. It is worth noting that similar synergistic effects have also been observed in other types of tumors. Here is a rewritten version using more technical terminology while avoiding repetition:For instance, under specific experimental paradigms, the concomitant administration of these two pharmacological agents can elicit a more pronounced therapeutic response by potentiating apoptotic induction, impeding cell cycle progression, and modulating pivotal signal transduction cascades [17,18]. However, whether the synergistic mechanism of action between DOX and BAY-598 is applicable to NSCLC, and its specific molecular regulatory mechanisms, still require further in-depth research and verification. Consequently, the overarching objective of this investigative endeavor is to elucidate the synergistic antineoplastic mechanisms underlying the combined administration of BAY-598 and doxorubicin in NSCLC. This is aimed at furnishing novel theoretical underpinnings and therapeutic strategies for the management of NSCLC. By comprehensively dissecting the interaction dynamics between these two pharmacological agents, we aspire to unveil more efficacious treatment modalities for patients afflicted with NSCLC, while concurrently providing valuable insights into combination therapeutic approaches for other neoplastic pathologies.

2. Methods

2.1. Cell culture and treatment

In this study, human non-small cell lung cancers lines A549 and H460, procured from the Chinese Academy of Sciences Cell Repository, were employed for the present study. These cell lines were cultured in RPMI-1640 medium enriched with 10 % fetal bovine serum (Gibco, Grand Island, NY) and incubated at 37 °C in a humidified atmosphere containing 5 % CO2. Subculturing procedures were executed upon attainment of 90 % confluency. Cells in the logarithmic growth phase were subjected to drug treatments, and four experimental groups were established: The DMSO group as the negative control, the DOX(MCE, MedChem Express, HY-15142 group (treated with DOX at 30 nM), the BAY-598 (MCE, MedChem Express, HY-19546)group (treated with BAY-598 at 20 nM), and the DOX + BAY-598 group (treated with DOX at 30 nM and BAY-598 at 20 nM). The final DMSO concentration in all treatments was maintained at 0.5 % (v/v). Post 48-h treatment, cells were harvested for subsequent analyses, including proliferation assays, apoptosis assays, migration assays, and protein expression studies using Western blotting.

2.2. CCK-8 assay

To detect the effects of DOX and BAY-598, individually or in combination, on the proliferation of lung cancer cells, we performed experiments using the CCK-8 cell viability assay kit. First, lung cancer cells were seeded into 96-well culture plates at a certain density and allowed to adhere. Subsequently, the cells were treated with varying concentrations of DOX, BAY-598, or a combination of both, along with untreated blank control and vehicle control groups. After 24 and 48 h of drug treatment, CCK-8 reagent was added to each well. This reagent contains a water-soluble compound that can be reduced by dehydrogenases in viable cells to produce a soluble

orange-yellow colored product. The absorbance values at 450 nm were measured using a microplate reader, with the absorbance values being directly proportional to the number of viable cells. By comparing the absorbance values of the experimental groups with the control groups, the cell viability inhibition rates at different drug concentrations and time points were calculated. The CCK-8 assay is a convenient and reproducible method widely used to evaluate the effects of drugs on cell proliferation.

2.3. IC50 and combination index calculation

Using the nonlinear regression analysis tool in GraphPad Prism 8.0 software, we fitted the dose-response data to a four-parameter logistic equation, from which the half-maximal inhibitory concentrations (IC50) of the different drugs were calculated. This four-parameter logistic model can accurately depict the mathematical relationship between drug concentration and cell viability inhibition.

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) \ / \ (1 + \widehat{10}((\text{LogIC50} \ - \ X) \times \text{HillSlope}))$$

For combination treatments, A549 and H460 cells were exposed to both DOX and BAY-598 for 48 h. The synergistic effects of the drugs were analyzed using the Chou-Talalay method and CompuSyn software, which calculated the Combination Index (CI). CI values indicated the nature of the interaction: $CI = 1$ for additive effects, $CI < 1$ for synergistic effects, and $CI > 1$ for antagonistic effects.

2.4. Transwell assay

To evaluate the effects of DOX and BAY-598, individually or in combination, on the migratory and invasive capabilities of lung cancer cells, we conducted migration and invasion assays. Initially, a defined number of lung cancer cells were seeded into the upper chambers and allowed to adhere thoroughly before being treated with various concentrations of the drugs, along with untreated blank and vehicle control groups. The upper and lower chambers were separated by a porous membrane pre-coated with an extracellular matrix protein(BD Biosciences, Franklin Lakes, NJ), mimicking the in vivo extracellular matrix environment. After a specific duration of drug exposure, cells that migrated through the porous membrane and invaded the matrix layer were fixed and stained. Finally, the numbers of migrated and invaded cells in each group were quantified through microscopic imaging systems and image analysis software.

2.5. Colony formation assay

To evaluate the effects of the drugs on the long-term proliferative capacity of lung cancer cells, we employed a colony formation assay to analyze tumor cell clonogenic efficiency. Initially, a defined number of lung cancer cells were evenly seeded into 6-well culture plates. After the cells had completely adhered, they were treated with varying concentration gradients of DOX, BAY-598, or their combination, along with appropriate blank and vehicle control groups. Following approximately 7 days of drug exposure, surviving single cells that had formed clones were rendered visible as colonies through specialized fixation and staining procedures. Subsequently, ImageJ software was utilized to enumerate and analyze the colonies in each well, yielding the clonogenic efficiency under different drug treatment conditions. This assay reflects the unlimited proliferative potential of cells and serves as a robust tool for assessing the long-term inhibitory effects of anticancer drugs.

2.6. Apoptosis and cell cycle analysis

To investigate the mechanisms of cytotoxicity induced by the drugs, we employed flow cytometry to analyze the apoptotic rates and cell cycle distributions of lung cancer cells under different drug treatment conditions. At specific time points after exposure to DOX, BAY-598, or their combination, A549, H460 cells were subjected to dual staining with Annexin V (a phospholipid-binding protein) and a nucleic acid dye. Annexin V binds to phospholipids on the outer leaflet of the cell membrane, while the nucleic acid dye penetrates the nuclei of late apoptotic or necrotic cells that have lost membrane integrity. Following appropriate staining reactions, the cell suspension samples were subjected to multiparametric detection and quantitative analysis using a flow cytometer. Based on the distinct fluorescence signal patterns of the dyes, the cell populations could be categorized into viable, early apoptotic, late apoptotic, and necrotic subsets, thereby allowing the calculation of apoptotic rates for each treatment group. Concurrently, we also evaluated the impact of the drugs on cell cycle distribution. After cell fixation and DNA staining, flow cytometric analysis enabled the determination of the DNA content in individual cells, thereby identifying their respective cell cycle phases (G0/G1, S, or G2/M). By analyzing the changes in the proportions of cells in different phases, we could elucidate whether the drugs induced cell cycle arrest, consequently inhibiting the proliferation of tumor cells.

2.7. Western blot analysis

Total protein was extracted from control and various treatment groups of A549 and H460 cells. Protein concentration in each group was measured using the BCA method (Boster Biological Technology) and adjusted to the same level. Equal amounts of protein samples were separated by SDS-PAGE gel electrophoresis and transferred to PVDF membranes for Western blot analysis. The membranes were blocked with 5 % BSA at room temperature, then incubated with primary antibodies (1:15,000, Promega) overnight at 4 °C After washing the membranes with TBST, secondary antibodies were applied at room temperature for 1 h. Following additional washes,

protein expression levels were detected using ECL chemiluminescence reagents, and the results were visualized through exposure, scanning, and imaging. Actin or GAPDH served as housekeeping proteins for relative quantitative analysis of protein expression across different groups. The antibodies used in this study included: anti-p-mTOR, anti-mTOR, anti-p-ERK1/2, anti-ERK1/2, anti-p-STAT3, anti-STAT3, and anti-CDK2 from Cell Signaling Technology. Anti-BCL2, anti-BAX, anti-β-actin, anti-p-JAK2, anti-JAK2, anti-Vimentin, anti-N-cadherin, and anti-E-cadherin were purchased from Abcam (MA, United States).

2.8. Xenograft model

Evaluation of in vivo efficacy using a nude mouse xenograft model. A549 cell suspension (3 × 10^6 cells in 200 μL) was

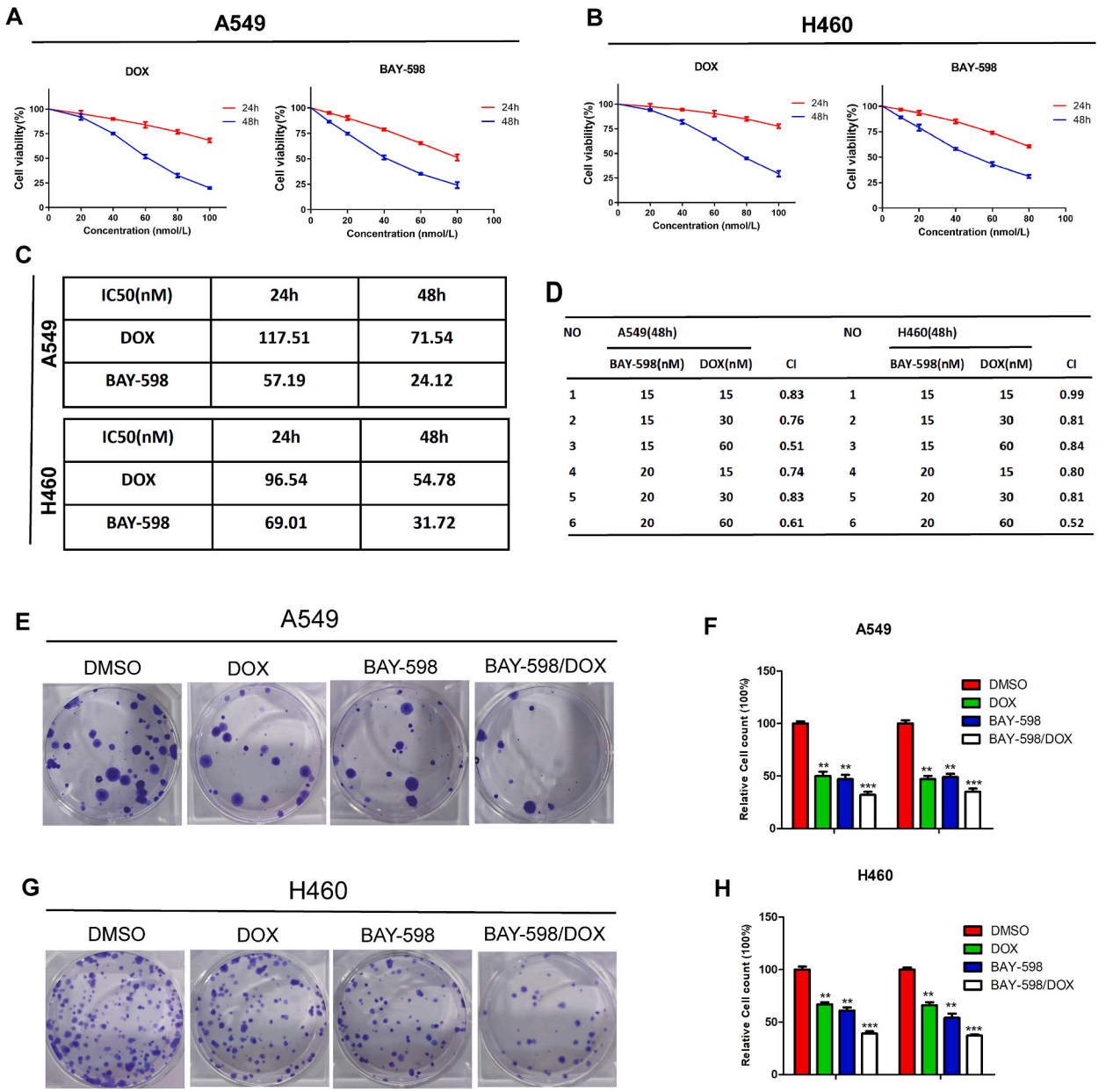

Fig. 1. Effects of DOX alone, BAY-598 alone, and their combination on the viability of A549 cells and H460 cells. (A, B). The effects of either DOX or BAY-598 on lung cancer cells are dose-dependent and time dependent. CCK-8 was used to detect cell proliferation in A549 and H460 cells after they were treated separately with various doses of BAY-598 or DOX for 24 h, 48 h. (C). The drug concentrations of DOX and BAY-598 required to reach half maximal inhibitory concentration (IC50) at different time points. (D). The combination index (CI) of DOX and BAY-598 was calculated for the lung cancer cells. (E–H). Representative images and bar graphs of DOX and BAY-598 synergistically inhibiting clone formation of A549 and H460 cells. Data are means ± SD (n = 3). (*P < 0.05, **P < 0.01, ***P < 0.001, compared to DMSO).

subcutaneously injected into the scapular region of female nude mice. When tumor volume reached approximately 200 mm^3, the mice were randomly divided into four groups: control group (saline), DOX group (intraperitoneal injection of 2 mg/kg every five days), BAY-598 group (intraperitoneal injection of 5 mg/kg every two days), and combination treatment group (DOX and BAY-598 administered at the same doses). Tumor size was measured weekly. After 32 days, the mice were sacrificed, and the tumors were excised and weighed. All experimental procedures were approved by the Ethics Committee of the Third Affiliated Hospital of Nanchang University.

2.9. Statistical analysis

All experimental data were replicated a minimum of three times and presented as the mean ± standard deviation. The Student's t-

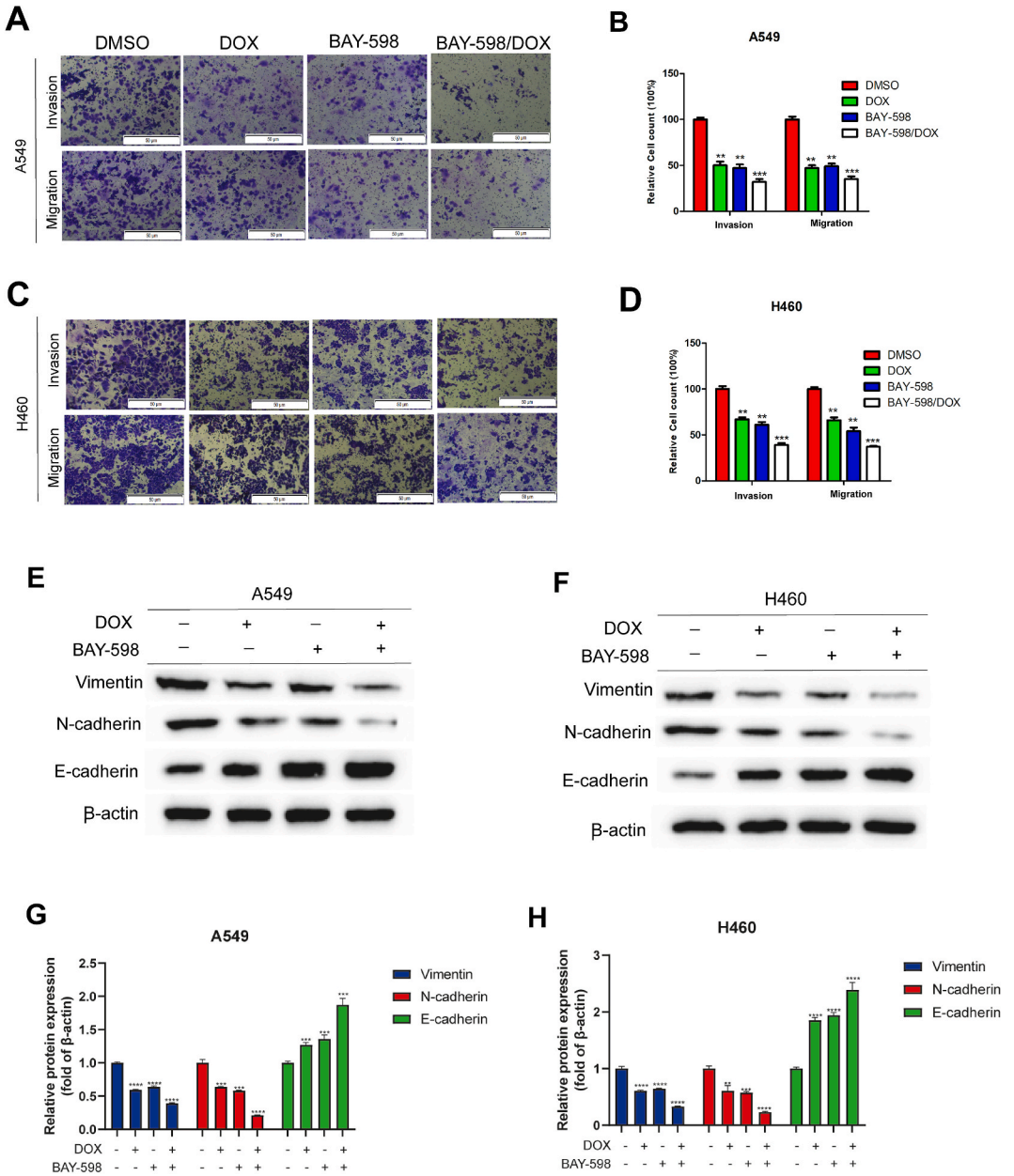

Fig. 2. Co-treatment with DOX and BAY-598 synergistically inhibited the invasion and migration of A549 cells and H460 cells. (A–D). In Transwell assays, the combination of DOX and BAY-598 synergistically inhibited the invasion and migration of A549/H460 cells. (E–H). Western blot analysis of the expression of epithelial-mesenchymal transition and invasion related proteins under treatment with DOX or BAY-598. β-actin was used as a loading control. The protein densities were analyzed with ImageJ. (*P < 0.05, **P < 0.01, ***P < 0.001).

test was employed to analyze the differences between two groups. $P < 0.05$ were considered statistically significant.

3. Results

3.1. Impact of BAY-598, DOX, and their combination on non-small cell lung cancer cell viability

We employed the CCK8 assay to determine the impact of varying concentrations of BAY-598 and DOX on A549 and H460 cells.

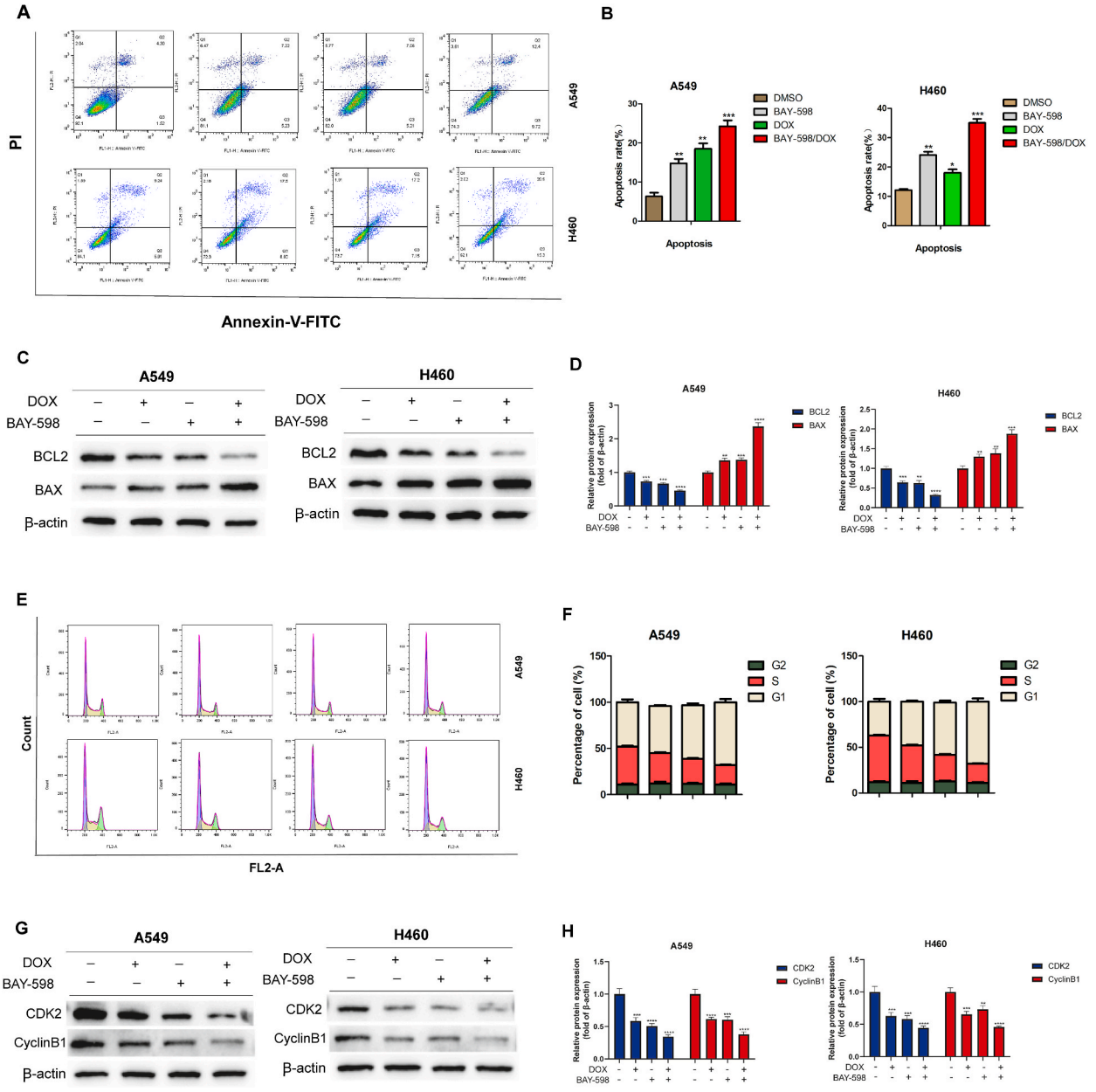

Fig. 3. DOX and BAY-598 synergistically induce apoptosis and inhibit cell cycle progression in lung cancer cells. (A, B). Flow cytometry was used to assess the apoptosis of A549/H460 cells. FITC and PI reagents were used to treat lung cancer cells that had been infected with DOX and BAY-598 for 48 h. The percentage of apoptosis in various drug treatment groups was measured. (C, D). The expression of BAX and BCL2 protein in DMSO, DOX and/or BAY-598. (E, F). The cell cycle distribution was analyzed by flow cytometry. The percentage of lung cancer cells in the G1, S, and G2 phases in the bar chart indicates that DOX/BAY-598 cooperatively arrests the cell cycle in the G2 phase. (G, H). Western blot analysis of cyclin-related proteins was performed in A549 and H460 cells. β-actin was used as a loading control. The protein densities were analyzed with ImageJ. (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$).

Results showed that both BAY-598 and DOX inhibited the proliferation of these cells in a time- and dose-dependent manner. After 24 h of treatment, the 50 % inhibitory concentration (IC50) for BAY-598 was 57.19 nM in A549 cells and 69.01 nM in H460 cells. After 48 h, these values decreased to 24.12 nM for A549 cells and 31.72 nM for H460 cells (Fig. 1A–C). Similarly, the IC50 for DOX in A549 cells was 117.51 nM at 24 h and 71.54 nM at 48 h (Fig. 1C). In H460 cells, the IC50 values were 96.54 nM at 24 h and 54.78 nM at 48 h

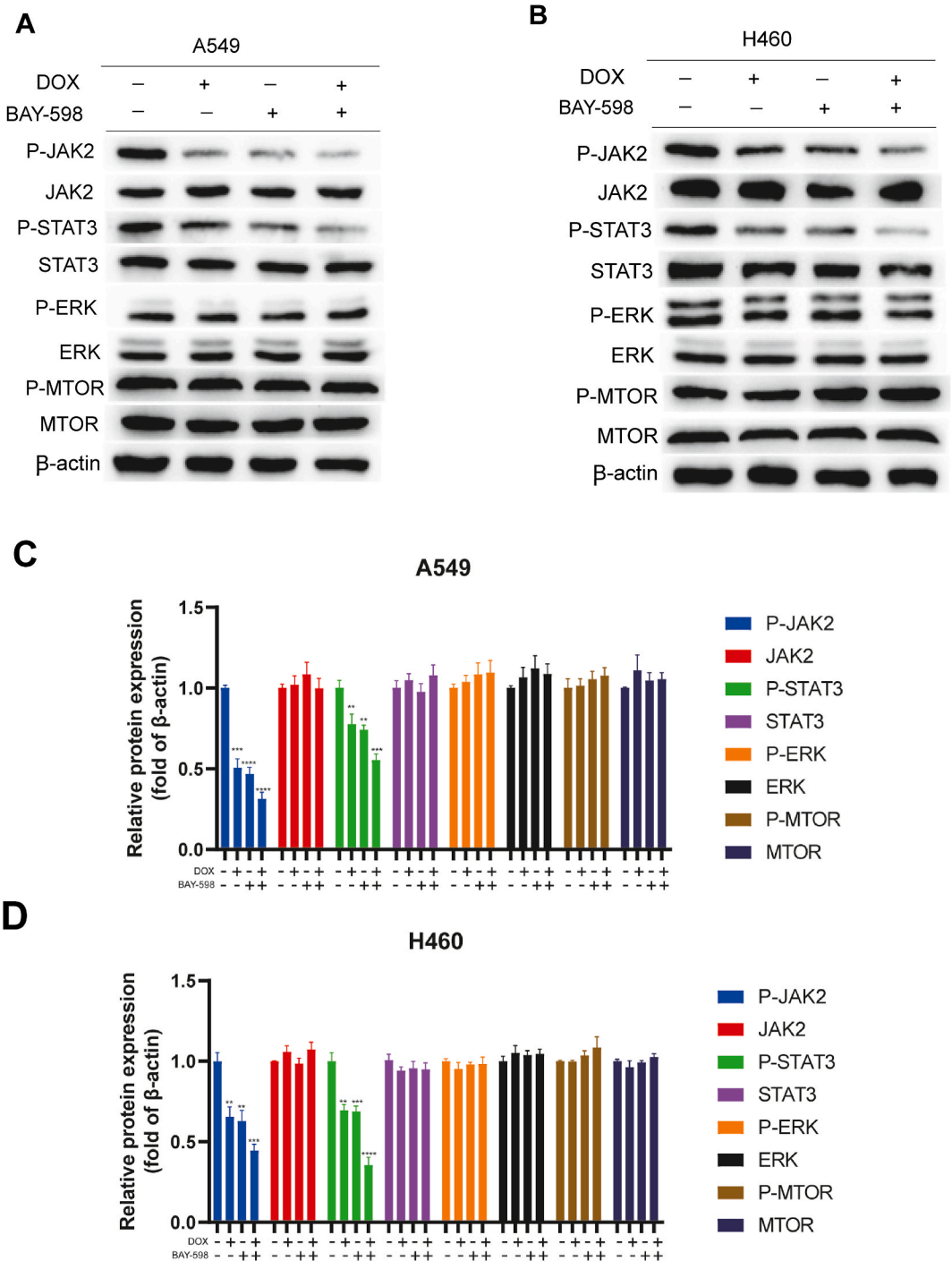

Fig. 4. Co-treatment with BAY-598 and DOX inhibits lung cancer cell growth through the NFκB and JAK-STAT signaling pathway. (A). Western blotting showing expression levels of P-p65, p65, P-STAT3, STAT3, P-ERK, ERK, P-MTOR, MTOR in A549 cells. (B). Western blotting showing expression levels of P-p65, p65, P-STAT3, STAT3, P-ERK, ERK, P-MTOR, MTOR in H460 cells. (C, D) Pathway-related Protein Quantification, The protein densities were analyzed with ImageJ. (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001).

(Fig. 1C). We also evaluated the cytotoxicity of BAY-598 on normal cell lines 293 T and HBE. As indicated in Supplementary Figs. 1A and 1B, BAY-598 showed minimal impact on these normal cells at concentrations effective against cancer cells.

To investigate if BAY-598 could potentiate the effect of DOX, we treated A549 and H460 cells with different concentrations of BAY-598 (15 or 20 nM) in combination with DOX (15 nM, 30 nM, or 60 nM) for 48 h. The data revealed a significant synergistic reduction in cell viability when both drugs were used together compared to each drug alone (Supplementary Figs. 1C,D, and Fig. 1D). Specifically, the combination of BAY-598 (20 nM) and DOX (30 nM) inhibited A549 cell growth by approximately 50 %, with a Combination Index (CI) of less than 1, signifying a synergistic effect. Notably, at these concentrations, BAY-598 and DOX had negligible effects on the viability of normal cell lines 293 T and HBE, underscoring the specificity and enhanced efficacy of the drug combination. These results led us to select BAY-598 (20 nM) and DOX (30 nM) for further experiments.

3.2. BAY-598 and DOX collaboratively suppress invasion and migration of non-small cell lung cancer cells

We probed the effects of BAY-598 and DOX on the proliferation of NSCLC cells. Utilizing a colony formation evaluation, we witnessed that the combined application of BAY-598 and DOX remarkably suppressed the multiplication of NSCLC cells (Fig. 1E–H). Further, we utilized a Transwell assay to assess their impact on NSCLC cell invasion and migration (Fig. 2A–D). Both BAY-598 and DOX individually reduced cell invasion and migration significantly, with their combination showing an even stronger inhibitory effect.

Epithelial-mesenchymal transition (EMT) is essential for cancer metastasis. Therefore, we analyzed EMT-related protein levels via western blotting. The combined treatment with BAY-598 and DOX significantly lowered the expression of Vimentin and N-cadherin while elevating E-cadherin levels. These findings indicate that the BAY-598 and DOX combination synergistically hinders the EMT process in NSCLC cells (Fig. 2E–H).

3.3. BAY-598 and DOX jointly trigger apoptosis and hinder cell cycle progression in non-small cell lung cancer cells

Next, we further investigated the inhibitory effects of DOX and BAY-598 on the proliferation of NSCLC cells, we treated A549 and H460 cells with DMSO, BAY-598, DOX, and a combination of BAY-598 and DOX at optimal concentrations for 48 h. Apoptosis and cell cycle progression were then analyzed using flow cytometry. As shown in Fig. 3A and B, the combined treatment with BAY-598 and DOX significantly promoted apoptosis in NSCLC cells. Additionally, Western blot analysis revealed that the combined treatment significantly increased the expression of apoptosis-related proteins in NSCLC cells (Fig. 3C and D). Furthermore, cell cycle analysis indicated that the combined treatment significantly inhibited cell cycle progression in NSCLC cells (Fig. 3E and F). Western blotting further corroborated these cell cycle findings (Fig. 3G and H). In summary, the combination of BAY-598 and DOX enhances apoptosis induction and cell cycle inhibition in NSCLC cells.

3.4. Concurrent treatment with BAY-598 and DOX impedes non-small cell lung cancer cell growth via JAK-STAT signaling pathways

Our earlier findings indicated that the combination of BAY-598 and DOX markedly suppresses NSCLC cell growth, migration, invasion, apoptosis, and cell cycle progression. However, the exact pathways involved remained unclear. To address this, we examined potential mechanisms, focusing on classical pathways such as MAPK and JAK-STAT in NSCLC cells treated with BAY-598 and DOX, both separately and together. Interestingly, the JAK-STAT pathway was notably inhibited in A549 and H460 cells subjected to the combined treatment compared to those receiving either agent alone. This observation suggests that the JAK-STAT pathway could play a crucial role in the synergistic suppression of NSCLC cell proliferation by BAY-598 and DOX (Fig. 4A–D).

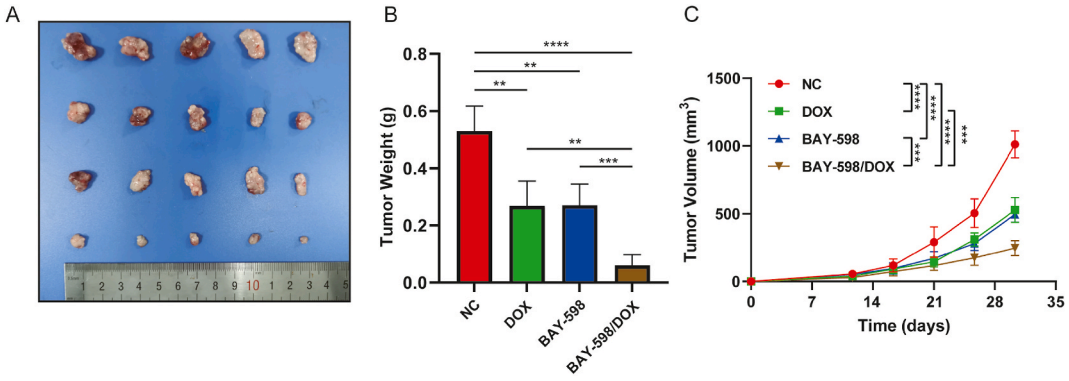

Fig. 5. BAY-598 demonstrated a synergistic inhibition of subcutaneous tumor growth with DOX. (A). A549 cells were subcutaneously injected into mice, and on the third day, the following treatments were administered via intraperitoneal injection: the control group received PBS, DOX, BAY-598, and DOX in combination with BAY-598. After two weeks of treatment, the morphology and size of the xenografts excised from nude mice are depicted in figure. (B). Starting from the fifth day, tumor volume changes for the four treatment groups were recorded every two days. (C). Final tumor weight changes for the four treatment groups. (**P < 0.01, ***P < 0.001, ****P < 0.0001).

3.5. Joint administration of BAY-598 and DOX elicits synergistic effects in vivo to suppress non-small cell lung cancer xenograft tumor growth

To better validate the inhibitory effects of BAY-598 and DOX on the proliferation of lung cancer cells, we evaluated the anti-tumor efficacy of BAY-598 alone, DOX alone, and their combination on lung cancer xenograft models in immunocompromised mice. The findings demonstrated that both BAY-598 and DOX, when administered individually, significantly suppressed the growth and mass of lung cancer tumors in NOD-SCID mice. Notably, the concomitant administration of BAY-598 and DOX exhibited a more potent inhibition of tumor growth compared to either agent alone (see Fig. 5A–C). This indicates that the combination of BAY-598 and DOX can effectively impede the proliferation of lung cancer cells in vivo.

4. Discussion

In this research, we investigated the synergistic effects and underlying mechanisms of the SMYD2 inhibitor BAY-598 combined with the chemotherapeutic agent DOX for the treatment of NSCLC. NSCLC is a highly fatal malignancy with significant therapeutic challenges [19]. Despite some advancements, drug resistance continues to be a major hurdle impeding treatment efficacy [20–22]. Consequently, developing new therapeutic strategies to enhance treatment sensitivity and mitigate drug resistance is crucial for NSCLC patients. This study aimed to elucidate the synergistic mechanisms of action of BAY-598 and DOX in NSCLC treatment and to assess their impact on cell proliferation, migration, invasion, apoptosis, and cell cycle progression.

BAY-598 is a highly potent and selective inhibitor of the SMYD2. It inhibits the methyltransferase activity of SMYD2 by competitively binding to the substrate recognition site [23]. Kinetic analysis of SMYD2 inhibition revealed that BAY-598 acts as an uncompetitive inhibitor against the SAM cofactor and a competitive inhibitor against the p53 peptide substrate, suggesting it preferably binds to the SMYD2-SAM complex [23]. BAY-598 suppresses the methylation of important SMYD2 substrates such as p53 and AHNAK. Both in vitro and in vivo studies demonstrated that BAY-598 decreases the monomethylation levels of p53 and AHNAK. Mouse xenograft models also validated that BAY-598 decreases AHNAK methylation levels in tumor cells in vivo [23]. BAY-598 sensitizes cells to apoptosis induced by chemotherapeutic agents like doxorubicin, consistent with the biological functions of SMYD2. Co-treatment of BAY-598 and doxorubicin in mice showed enhanced antitumor efficacy. In summary, BAY-598 selectively inhibits the binding of peptide substrates to the binding site of SMYD2, leading to reduced methylation of SMYD2 substrates and inhibition of SMYD2 biological functions, which contributes to its antitumor activity.

DOX is one of the first-line chemotherapeutic agents for NSCLC treatment. It inhibits tumor growth by interacts with DNA, causing DNA damage and triggering cell apoptosis, induces oxidative stress, generating oxygen-free radicals that damage cellular molecules, alters the structure of DNA, disrupting normal replication and transcription and induces apoptosis in lung cancer cells through multiple pathways, such as interfering with apoptosis signaling pathways. inserting DNA double strands and inducing DNA damage [24–28]. Certainly, DOX treatment, while effective in many cases, frequently encounters challenges due to its high drug toxicity and the development of acquired resistance. These issues have spurred ongoing research and innovation in the field of oncology. Researchers are actively exploring novel drug delivery systems to minimize the adverse effects of DOX and improve its safety profile. Additionally, the development of combination therapies that incorporate DOX alongside other agents holds promise in enhancing its efficacy and reducing the likelihood of resistance. The quest to address these hurdles not only drives progress in cancer treatment but also underscores the importance of personalized medicine approaches tailored to individual patient needs [29–32]. Given the distinct mechanisms of action of BAY-598 and DOX, their combined use may produce synergistic effects and enhance chemotherapy sensitivity. Relevant research has also shown that combined use of SMYD2 inhibitors and apatinib can synergistically kill colorectal cancer [11]. This provides a theoretical basis for the combined use of BAY-598 and DOX for NSCLC treatment. But its specific synergistic mechanisms and modes of action need to be systematically elucidated through more preclinical studies.

The key discovery of this study is that the combined treatment with BAY-598 and doxorubicin exhibits synergistic effects in NSCLC cells. This synergism is evident in the suppression of cell proliferation, migration, and invasion, along with the induction of apoptosis and cell cycle arrest. These findings suggest that BAY-598 and doxorubicin may enhance their anti-cancer efficacy through multiple pathways. Further investigations into the molecular mechanisms reveal that the combination of BAY-598 and doxorubicin may synergistically exert anti-cancer effects by inhibiting the JAK-STAT signaling pathway. The JAK-STAT pathway is crucial for cancer cell growth, metastasis, and drug resistance [33–35]. Combined use of BAY-598 and doxorubicin can significantly reduce JAK-STAT pathway activity, which may be an important mechanism for its inhibition of NSCLC cell growth and migration. In addition, this study also validated the synergistic anti-tumor effects of combined application in vivo models. Through mouse xenograft models, we observed that the combination could significantly inhibit tumor growth, further confirming the potential of this combination therapy strategy. The results of this study firstly confirmed the synergistic inhibitory effect of BAY-598 on the growth of NSCLC cell lines in combination with DOX. We further found that this synergistic effect is related to the induction of cell cycle arrest and apoptosis, which is consistent with previous reports that BAY-598 alone can downregulate anti-apoptotic factors and upregulate apoptosis-related genes. We also found that the two drugs in combination synergistically inhibit the migration and metastasis of NSCLC cells. This may be related to the synergistic inhibition of epithelial-mesenchymal transition (EMT). Finally, we confirmed that the combination of the two drugs can synergistically inhibit NSCLC cell growth through inhibition of the JAK-STAT pathway. Preclinical studies have confirmed the synergistic antitumor efficacy of this combination strategy in significantly inhibiting tumor xenograft growth.

In conclusion, our preliminary study provides first evidence that SMYD2 inhibitor BAY-598 can synergistically enhance the anticancer efficacy of the chemotherapeutic agent DOX against NSCLC, possibly through coordinated inhibition of the JAK-STAT pathway. Our findings may provide a rationale for this combination strategy for the treatment of NSCLC and shed light on the

development of synergistic therapy for other cancer types. We believe that with the in-depth translational research, this strategy will ultimately bring clinical benefits to NSCLC patients.

Ethics and consent to participate

Ethics committee belonging to Third Affiliated Hospital of Nanchang University ratified the current study (No. 2021101201, Nanchang, Jiangxi, China), and the study was reported according to the ARRIVE guidelines.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

Funding

This study was supported by Science and Technology Research Project of Education Department of Jiangxi Province (GJJ190021).

CRediT authorship contribution statement

Jiaqi Meng: Writing – original draft, Visualization, Methodology, Formal analysis. **Weichang Yang:** Writing – review & editing, Validation, Data curation. **Can Li:** Writing – review & editing, Supervision, Project administration. **Fengyuan Li:** Writing – review & editing, Validation, Supervision, Resources, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e32015.

References

[1] V.M.L. de Sousa, L. Carvalho, Heterogeneity in lung cancer, Pathobiology 85 (2018) 96–107, https://doi.org/10.1159/000487440, 2018.
[2] C. Gridelli, A. Rossi, D.P. Carbone, et al., Non-small-cell lung cancer, Nat. Rev. Dis. Prim. 1 (1) (2015) 1–16, https://doi.org/10.1038/nrdp.2015.9, 2015.
[3] H. Mithoowani, M. Febbraro, Non-small-cell lung cancer in 2022: A review for general Practitioners in oncology, Curr. Oncol. 29 (2022) 1828–1839, https://doi.org/10.3390/curroncol29030150, 2022.
[4] J. Wu, Z. Lin, Non-small cell lung cancer targeted therapy: drugs and mechanisms of drug resistance, Int. J. Mol. Sci. 23 (23) (2022) 15056, https://doi.org/10.3390/ijms232315056, 2022.
[5] M. Wang, R.S. Herbst, C. Boshoff, Toward personalized treatment approaches for non-small-cell lung cancer, Nat. Med. 27 (2021) 1345–1356, https://doi.org/10.1038/s41591-021-01450-2.
[6] Y.Q. Yu, A. Herrmann, V. Thonn, et al., SMYD2 inhibition downregulates TMPRSS2 and decreases SARS-CoV-2 infection in human intestinal and airway epithelial cells, Cells 11 (8) (2022) 1262, https://doi.org/10.3390/cells11081262.
[7] D. Boehm, M. Jeng, G. Camus, A. Gramatica, R. Schwarzer, J.R. Johnson, et al., SMYD2-mediated histone methylation contributes to HIV-1 latency, Cell Host &amp, Microbe 21 (2017), https://doi.org/10.1016/j.chom.2017.04.011.
[8] L. Wu, F. Kou, Z. Ji, B. Li, B. Zhang, Y. Guo, et al., SMYD2 promotes tumorigenesis and metastasis of lung adenocarcinoma through RPS7, Cell Death &amp, Diseases 12 (2021), https://doi.org/10.1038/s41419-021-03720-w.
[9] F. Weijin, S. Liying, L. Zuojun, M. Peipei, Z. Shanru, L. Shikun, Effect of miRNA-200b on the proliferation of liver cancer cells via targeting SMYD2/p53 signaling pathway, J. Cent. S. Univ. Med. Sci. 47 (2022) 1303–1314, https://doi.org/10.11817/j.issn.1672-7347.2022.210521.
[10] N. Reynoird, P.K. Mazur, T. Stellfeld, N.M. Flores, S.M. Lofgren, S.M. Carlson, et al., Coordination of stress signals by the lysine methyltransferase SMYD2 promotes pancreatic cancer, Genes &amp, Development 30 (2016) 772–785, https://doi.org/10.1101/gad.275529.115.
[11] Y. Zhang, L. Zhou, Y. Xu, J. Zhou, T. Jiang, J. Wang, et al., Targeting SMYD2 inhibits angiogenesis and increases the efficiency of apatinib by suppressing egfl7 in colorectal cancer, Angiogenesis 26 (2022) 1–18, https://doi.org/10.1007/s10456-022-09839-4.
[12] S. Sulthana, T. Banerjee, J. Kallu, S.R. Vuppala, B. Heckert, S. Naz, et al., Combination therapy of NSCLC using hsp90 inhibitor and doxorubicin carrying functional nanoceria, Mol. Pharm. 14 (2017) 875–884, https://doi.org/10.1021/acs.molpharmaceut.6b01076.
[13] M. Dragoj, Z. Milosevic, J. Bankovic, N. Tanic, M. Pesic, T. Stankovic, Targeting CXCR4 and FAK reverses doxorubicin resistance and suppresses invasion in non-small cell lung carcinoma, Cell. Oncol. 40 (2016) 47–62, https://doi.org/10.1007/s13402-016-0304-6.
[14] Y. Wu, Z. Wang, L. Han, Z. Guo, B. Yan, L. Guo, et al., PRMT5 regulates RNA M6A demethylation for doxorubicin sensitivity in breast cancer, Mol. Ther. 30 (2022) 2603–2617, https://doi.org/10.1016/j.ymthe.2022.03.003.
[15] T. Wang, J. Tang, H. Yang, R. Yin, J. Zhang, Q. Zhou, et al., Effect of apatinib plus pegylated liposomal doxorubicin vs pegylated liposomal doxorubicin alone on platinum-resistant recurrent ovarian cancer, JAMA Oncol. 8 (2022) 1169, https://doi.org/10.1001/jamaoncol.2022.2253.

[16] J. Xu, D. Liu, H. Niu, G. Zhu, Y. Xu, D. Ye, et al., Resveratrol reverses doxorubicin resistance by inhibiting epithelial-mesenchymal transition (EMT) through modulating PTEN/Akt signaling pathway in gastric cancer, Journal of Experimental &amp, Clin. Cancer Res. 36 (2017), https://doi.org/10.1186/s13046-016-0487-8.
[17] T.I. Wi, J.E. Won, C.M. Lee, J.-W. Lee, T.H. Kang, B.C. Shin, et al., Efficacy of combination therapy with linalool and doxorubicin encapsulated by liposomes as a two-in-one hybrid carrier system for epithelial ovarian carcinoma, Int. J. Nanomed. 15 (2020) 8427–8436, https://doi.org/10.2147/IJN.S272319.
[18] H. Yi, W. Lu, F. Liu, G. Zhang, F. Xie, W. Liu, et al., Ros-responsive liposomes with NIR light-triggered doxorubicin release for combinatorial therapy of breast cancer, J. Nanobiotechnol. 19 (2021), https://doi.org/10.1186/s12951-021-00877-6.
[19] M. Alexander, S.Y. Kim, H. Cheng, Update 2020: management of non-small cell lung cancer, Lung 198 (2020) 897–907, https://doi.org/10.1007/s00408-020-00407-5.
[20] Z.-F. Lim, P.C. Ma, Emerging insights of tumor heterogeneity and drug resistance mechanisms in lung cancer targeted therapy, Journal of Hematology &amp, Oncology 12 (2019), https://doi.org/10.1186/s13045-019-0818-2.
[21] K. Fu, F. Xie, F. Wang, L. Fu, Therapeutic strategies for EGFR-mutated non-small cell lung cancer patients with osimertinib resistance, Journal of Hematology &amp, Oncology 15 (2022), https://doi.org/10.1186/s13045-022-01391-4.
[22] J. He, Z. Huang, L. Han, Y. Gong, C. Xie, Mechanisms and management of 3rd-generation EGFR-TKI resistance in advanced non-small cell lung cancer, Int. J. Oncol. 59 (2021), https://doi.org/10.3892/ijo.2021.5270 (review).
[23] E. Eggert, R.C. Hillig, S. Koehr, D. Stöckigt, J. Weiske, N. Barak, et al., Discovery and characterization of a highly potent and selective aminopyrazoline-based in vivo probe (Bay-598) for the protein lysine methyltransferase SMYD2, J. Med. Chem. 59 (2016) 4578–4600, https://doi.org/10.1021/acs.jmedchem.5b01890.
[24] X. Zheng, Y. Zhao, Y. Jia, D. Shao, F. Zhang, M. Sun, et al., Biomimetic co-assembled nanodrug of doxorubicin and berberine suppresses chemotherapy-exacerbated breast cancer metastasis, Biomaterials 271 (2021) 120716, https://doi.org/10.1016/j.biomaterials.2021.120716.
[25] X. Tai, X.-B. Cai, Z. Zhang, R. Wei, In vitro and in vivo inhibition of tumor cell viability by combined dihydroartemisinin and doxorubicin treatment, and the underlying mechanism, Oncol. Lett. 12 (2016) 3701–3706, https://doi.org/10.3892/ol.2016.5187.
[26] W. Sheng, H. Mao, C. Wang, N. Yang, Z. Zhang, J. Han, Dehydrocostus lactone enhances chemotherapeutic potential of doxorubicin in lung cancer by inducing cell death and limiting metastasis, Med. Sci. Mon. Int. Med. J. Exp. Clin. Res. 24 (2018) 7850–7861, https://doi.org/10.12659/MSM.911410.
[27] P. Koczurkiewicz-Adamczyk, B. Gąsiorkiewicz, K. Piska, A. Gunia-Krzyżak, M. Jamrozik, A. Bucki, et al., Cinnamamide derivatives with 4-hydroxypiperidine moiety enhance effect of doxorubicin to cancer cells and protect cardiomyocytes against drug-induced toxicity through CBR1 inhibition mechanism, Life Sci. 305 (2022) 120777, https://doi.org/10.1016/j.lfs.2022.120777.
[28] L. Li, S. He, L. Yu, E.H. Elshazly, H. Wang, K. Chen, et al., Codelivery of Dox and Sirna by folate-biotin-quaternized starch nanoparticles for promoting synergistic suppression of human lung cancer cells, Drug Deliv. 26 (2019) 499–508, https://doi.org/10.1080/10717544.2019.1606363.
[29] Y. Zhou, G. Quan, Q. Wu, X. Zhang, B. Niu, B. Wu, et al., Mesoporous silica nanoparticles for drug and Gene Delivery, Acta Pharm. Sin. B 8 (2018) 165–177, https://doi.org/10.1016/j.apsb.2018.01.007.
[30] N. Koleini, B.E. Nickel, A.L. Edel, R.R. Fandrich, A. Ravandi, E. Kardami, Oxidized phospholipids in doxorubicin-induced cardiotoxicity, Chem. Biol. Interact. 303 (2019) 35–39, https://doi.org/10.1016/j.cbi.2019.01.032.
[31] M. Mohajeri, A. Sahebkar, Protective effects of curcumin against doxorubicin-induced toxicity and resistance: a Review, Crit. Rev. Oncol. Hematol. 122 (2018) 30–51, https://doi.org/10.1016/j.critrevonc.2017.12.005.
[32] A. Shafei, W. El-Bakly, A. Sobhy, O. Wagdy, A. Reda, O. Aboelenin, et al., A review on the efficacy and toxicity of different doxorubicin nanoparticles for targeted therapy in metastatic breast cancer, Biomedicine &, Pharmacotherapy 95 (2017) 1209–1218, https://doi.org/10.1016/j.biopha.2017.09.059.
[33] P. Xin, X. Xu, C. Deng, S. Liu, Y. Wang, X. Zhou, et al., The role of jak/STAT signaling pathway and its inhibitors in diseases, Int. Immunopharm. 80 (2020) 106210, https://doi.org/10.1016/j.intimp.2020.106210.
[34] Z. Yan, S.A. Gibson, J.A. Buckley, H. Qin, E.N. Benveniste, Role of the JAK/STAT signaling pathway in regulation of innate immunity in neuroinflammatory diseases, Clin. Immunol. 189 (2018) 4–13, https://doi.org/10.1016/j.clim.2016.09.014.
[35] J. Liu, F. Wang, F. Luo, The role of JAK/STAT pathway in fibrotic diseases: molecular and cellular mechanisms, Biomolecules 13 (1) (2023) 119, https://doi.org/10.3390/biom13010119.