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Research article

BET bromodomain inhibitors PFI-1 and CPI-203 suppress the development of follicular lymphoma via regulating Wnt/β-catenin signaling

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ABSTRACT

Objective: Follicular lymphoma (FL) is an indolent B-cell lymphoproliferative disorder, characterized by a lymphoid follicular pattern of growth. PFI-1 or CPI-203 has been known to effectively promote the inhibition of primary effusion lymphoma progression. This study aimed at investigating the anti-tumor properties of PFI-1 and CPI-203 on FL cells and uncover the underlying mechanism of action.

Methods: FL cells were treated with PFI-1 and CPI-203, and the treated cells were evaluated for their cell viability, cell cycle and apoptosis using CCK8, flow cytometry, and Western blot assays. A xenograft mouse model was used for assessing the *in vivo* effects of CPI-203 on tumorigenesis.

Results: PFI-1 or CPI-203 showed potential inhibitory effects on the cell viability of DOHH2 and RL cells in a dose-response-dependent manner. Furthermore, PFI-1 and CPI-203 inhibited cell growth, induced apoptosis of FL cells *in vitro*, and facilitated the translocation of β-catenin into cytoplasm both *in vitro* and *in vivo*. After engrafted with FL cells, CPI-203-treated mice got a longer duration of survival and a smaller tumor size than control mice. Mechanistically, PFI-1 and CPI-203 impede the activity of β-catenin and its downstream molecules by regulating the DVL2/GSK3β axis.

Conclusion: In conclusion, PFI-1 and CPI-203 may serve as potential anti-tumor inhibitors for the therapy of FL.

1. Introduction

Follicular lymphoma (FL) is the second most common type of lymphoma diagnosed in the United States and Western Europe, and the incidence has been slightly increased among relatives of patients with FL [1]. Despite being an indolent disease with a median survival of >15 years, FL is still an incurable malignancy that can be difficult to diagnose due to its various morphological variants and specific clinical subtypes [2]. A significant challenge in clinical management is that approximately 20% of patients relapse within 2

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years after treatment with a poor prognosis [2]. Additionally, the original lymphoma often transforms into a more aggressive subtype, i.e., diffuse large B-cell lymphoma (DLBCL), leading to poor clinical outcomes [3]. The current standard of care for FL includes the use of monoclonal CD-20 antibodies (e.g., rituximab), alone or in combination with chemotherapy, which has been shown to significantly improve overall survival [4]. However, patients treated with monoclonal antibodies and chemotherapy may still experience relapse and resistance [5,6]. Therefore, the exploration and development of novel agents to reduce FL transformation and improve patient outcomes are essential.

The bromodomain and extra-terminal (BET) family of proteins, including BRD2, BRD3, BRD4, and BRDT, contain two domains (BD1 and BD2) that recognize acetylated lysine residues on histones to regulate cell cycle and apoptosis [7]. Small molecule BET inhibitors have been shown to suppress cell viability in various cancer models, primarily by downregulating oncogene expression [8,9]. PFI-1 is a highly selective and potent BET inhibitor that efficiently blocks the interaction of BRDs with acetylated histone tails [10]. PFI-1 facilitates the inhibitory effects of lenalidomide on primary effusion lymphoma by suppressing C-MYC transcriptional activity [11]. CPI-203 is another BET inhibitor that targets the BRD4 protein, which can activate the Wnt pathway by downregulating C-MYC expression [12]. Furthermore, C-MYC has been reported to stimulate the expression of lncRNA FIRRE and accelerate the development of DLBCL [13], suggesting that PFI-1 and CPI-203 may have a therapeutic effect on FL progression by modulating C-MYC and its downstream pathway.

In this study, the ability of PFI-1 and CPI-203 to significantly reduce the proliferation of FL cells, and facilitate its apoptosis, was validated *in vitro*. Furthermore, PFI-1 and CPI-203 promoted the nuclear translocation of β-catenin, increasing Histone 3 expression in the cytoplasm. CPI-203 restrained xenograft tumor growth *in vivo* and prolonged the survival time of mice. PFI-1 and CPI-203 suppressed FL cell growth by blocking Wnt/β-catenin signaling, while CPI-203 repressed tumor growth *in vivo* by the same pathway. Our results indicate that the deactivation of the Wnt/β-catenin signaling pathway is critical in interrupting the development of FL, proving to be a potential anti-tumor agent in the treatment of FL.

2. Materials and methods

2.1. Cell culture

The human FL cell lines, DOHH2 and RL, were obtained from Jennio Biotech (Guangzhou, China). All the cells were maintained in RPMI 1640 medium (Gibco, Grand Island, USA) supplemented with 10% fetal bovine serum (Gibco) and incubated at 37 °C in a 5 % CO_2 incubator.

2.1.1. Cell counting kit-8 (CCK-8) assay

Cells were treated with different concentrations of PFI-1 or CPI-203 for 24h. Cells were cultured for 4 h at 37 °C after adding 10 μl of CCK-8 solution (Beyotime, Shanghai, China) to each well. The absorbance was detected using a microplate reader (Bio-Rad Laboratories, Hercules, USA) at 450 nm.

2.1.2. Cell cycle assay

Cells treated with PFI-1, CPI-203, PFI-1+CPI-203, PFI-1+ lithium chloride (LiCl), CPI-203+ LiCl, or PFI-1+CPI-203+LiCl were collected, washed with PBS, and fixed overnight with 70% ethanol at 4 °C. Fixed cells were incubated using 2 μg/ml RNase I (Sigma-Aldrich, MO, USA) at 37 °C for 30 min, followed by incubation with 10 μg/ml PI (Sigma-Aldrich) at 4 °C for 30 min. The stained cells were analyzed using flow cytometry (Becton Dickinson, Franklin Lakes, USA).

2.1.3. Cell apoptosis assay

After treatment with PFI-1, CPI-203, PFI-1+CPI-203, PFI-1+ LiCl, CPI-203+ LiCl, or PFI-1+CPI-203+LiCl for 24h, cells were washed and resuspended in the binding buffer. In 100 μL of cell suspension at the density of 1.0×10^6 cells/ml, 5 μl of Annexin V-FITC (Sigma-Aldrich) and 5 μl of PI (Sigma-Aldrich) were then added. The cell suspension was detected and analyzed within 30 min using flow cytometry (Becton Dickinson, Franklin Lakes, USA).

2.1.4. Quantitative real-time PCR (qRT-PCR)

TRIzol reagents (Invitrogen, Carlsbad, USA) were used for total RNA extraction of cells or tissues, followed by reversing the RNA to cDNA using moloney murine leukemia virus (MMLV) reverse transcriptase (Promega, Madison, USA). qRT-PCR was carried out with an SYBR™ Select Master Mix kit (Applied Biosystems, Foster City, USA) on an ABI 7900HT PCR System (Applied Biosystems). The relative expression was quantified using the $2^{-\Delta\Delta CT}$ method, with β-actin as the standard internal control. The PCR primer sequences used in this study were as follows:

β-catenin: forward 5′- GCCTGACACACTAACCAAGCT-3′, reverse 5′-TCCATTCTTGTGCATTCTTCA-3′; U6: forward 5′-CTCGCTTCGGCAGCACA-3′, reverse 5′- AACGCTTCACGAATTTGCGT-3′;

β-actin: forward 5′- CTCCATCCTGGCCTCGCTGT-3′, reverse 5′- ACTAAGTCATAGTCCGCCTAGA-3′; DVL2: forward 5′-GCCTATCCAGGTTCCTCCTC-3′, reverse 5′-AGAGCCAGTCAACCACACATCC-3′; GSK-3β: forward 5′-ACGCTCCCTGTGATTTAT-3′, reverse 5′-CTCTGATTTGCTCCCTTG-3′; C-MYC: forward 5′- CCCTCCACTCGGAAGGACTAT-3′, reverse 5′-CCTCTTGACATTCTCCTCGGT; TCF4: forward 5′-AGCAGAAGGCAGAGCGTGAG-3′, reverse 5′-GGTCTGGGGCTTGTCACTCTT-3′.

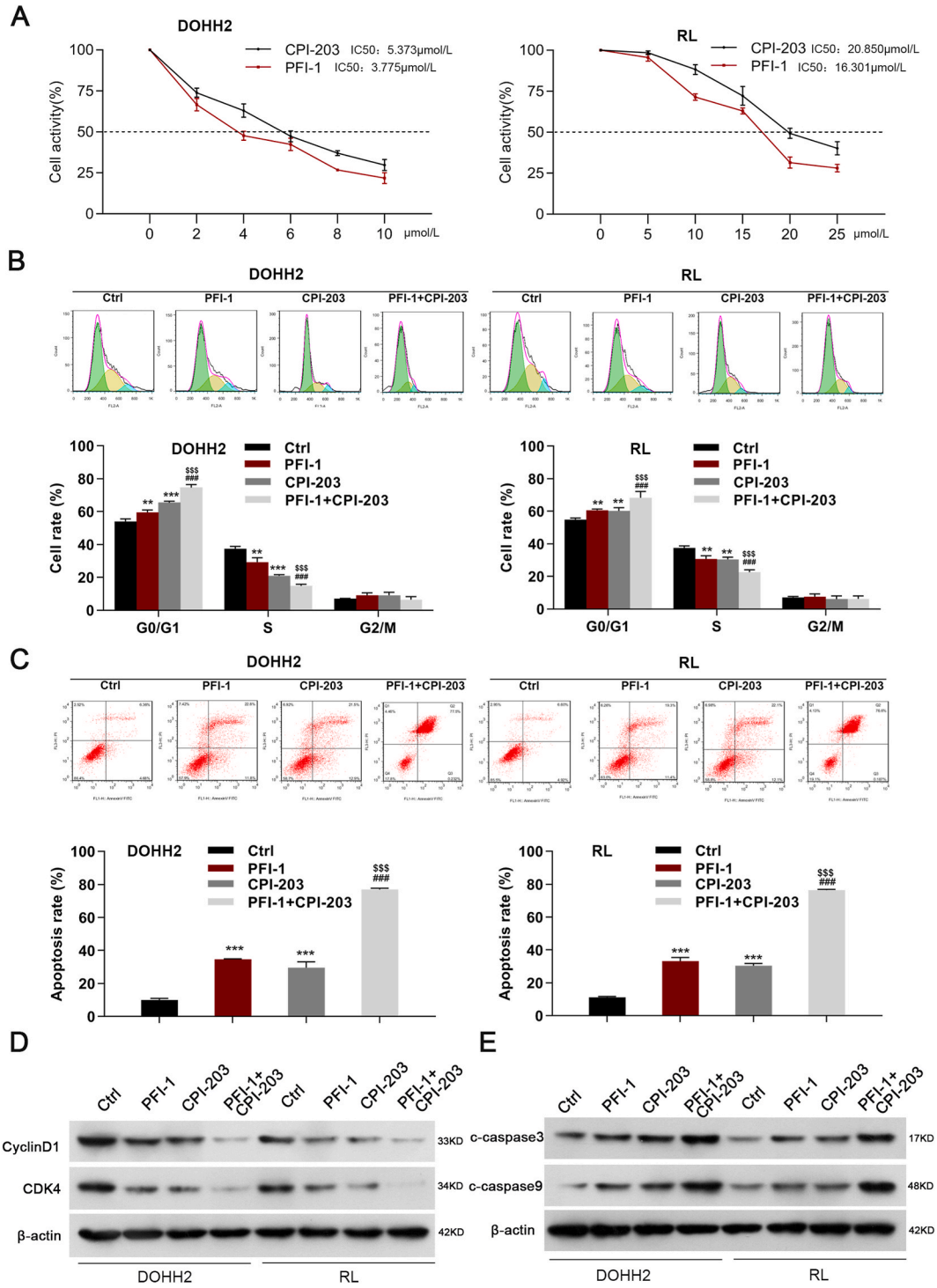

FIG. (1). PFI-1 and CPI-203 ameliorate cell proliferation and induce apoptosis in FL cells. (a) Cell viability evaluated using CCK-8 assay. (b) The cell cycle distribution of the DOHH2 and RL cells treated with PFI-1, CPI-203 or PFI-1+CPI-203, detected using flow cytometry analysis. (c) Cell apoptosis assessed by flow cytometry through Annexin-V/FITC staining. (d, e) The expression of Cyclin D1, CDK4, c-caspase 3, and c-caspase 9 examined by Western blot assay. The data are presented as the mean ± SD of triplicate experiments, $^{**}P < 0.01$ versus the control group, $^{***}P < 0.001$ versus the control group, $^{\#\#\#}P < 0.001$ versus the PFI-1 group, $^{\$\$\$}P < 0.001$ versus the PFI-1 group.

2.2. Isolation of nuclear and cytoplasmic RNA

Nuclear and cytoplasmic RNA isolation of the DOHH2 cells was carried out using a PARIS Kit (Invitrogen, Carlsbad, USA) as per the manufacturer's instructions. The subcellular fractions were then obtained and analyzed by qRT-PCR.

2.3. Protein extraction and Western blot

Cells or tissues were collected and lysed with RIPA buffer (Beyotime), and the total protein was detected using a BCA protein assay kit (Beyotime). The equivalent volume of protein was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS PAGE) and transferred onto the polyvinylidene fluoride membrane. After blocking, the membrane was incubated overnight at 4 °C with primary antibodies, including CyclinD1 (1:1000, Cell Signaling Technology, Danvers, MA, USA), CDK4 (1:1000, Abcam, Cambridge, MA, USA), active caspase 3 (c-caspase 3; 1:1000, Abcam), active caspase 9 (c-caspase 9; 1:1000, Abcam), β-catenin (1:1000, Cell Signaling Technology), and Histone H3 (1:1000, Cell Signaling Technology), DVL2 (1:1000, Abcam), GSK3β (1:1000, Cell Signaling Technology), TCF 4 (1:1000, Abcam), C-MYC (1:1000, Cell Signaling Technology). Anti-β-actin antibody (1:2000, Abcam) acted as an internal control. The membrane was then hybridized with an HRP-conjugated secondary antibody (1:5000, Cell signaling Technology) and the signals were measured using chemiluminescence analysis.

2.4. Immunohistochemistry staining

Tumor masses were dissected, fixed, dehydrated and embedded in paraffin. The tissue sections (4 μm) were then dewaxed, rehydrated, and incubated with Ki67 antibody (1:200, Abcam), followed by a secondary antibody (1:3000, Abcam). Chromogenic detection was carried out using the 3,3′-diaminobenzidine (DAB) Kit (Beyotime Biotechnology) and hematoxylin (Sigma Aldrich).

2.4.1. Xenograft model of human diffuse large B cell lymphoma

Study protocols involving the use of mouse models were approved by the Cancer Hospital Affiliated with Shanxi Medical University (No. 2022038) and all experiments were performed as per the relevant ethical regulations. Six weeks of male Nod-SCID mice were obtained from the Animal Center of Southern Medical University (Guangzhou, China) and maintained under specified pathogen-free conditions (temperature 22 °C, humidity 50%) with a 12h light and dark cycle. In the xenograft model, 1×10^6 DOHH2 cells expressing luciferase were subcutaneously injected into Nod-SCID mice. *In vivo* imaging system (IVIS, PerkinElmer, Waltham, USA) was used for assessing tumor engraftment and progression. Tumor size was measured weekly using a vernier caliper and the tumor volume was calculated according to the following formula: tumor size = length × width^2 × 0.5. When tumors reached volumes of approximately 100 mm^3, mice were randomly divided into two groups (n = 6 per group): (1) The control group in which mice were intraperitoneally injected with the same amount of solvent once daily and (2) The CPI-203 group in which mice were intraperitoneally injected with 50 mg/kg CPI-203 once daily. After 4 weeks, mice were observed until death or sacrificed. The tumors were then excised, weighed and frozen in liquid nitrogen until further analyses.

A total of 20 SCID mice were intravenously injected with DOHH-2 cells at a concentration of 1×10^7 cells/mouse on day 0 via the tail vein. On day 3, the mice were randomized into two groups (n = 10 per group): (1) the vehicle group, in which the mice were intraperitoneally injected with the same amount of solvent once daily and (2) the CPI-203 group in which the mice were intraperitoneally injected with 50 mg/kg CPI-203 once daily. Mice were observed daily and followed for survival.

2.5. Statistical analysis

Statistical analysis was carried out using GraphPad Prism 7 software. The difference between the two groups was analyzed by Student's *t*-test, and the differences between multiple groups were evaluated using one-way ANOVA with Tukey's multiple comparisons tests. All data are presented as mean ± standard deviation (SD). Statistically significant differences were indicated as $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$.

3. Results

3.1. PFI-1 and CPI-203 ameliorate cell proliferation and induce apoptosis in FL cells

Two follicular lymphoma cell lines, DOHH2 and RL, were used to investigate the effect of PFI-1 or CPI-203 on FL cell viability. CPI-203 or PFI-1 exhibited significant concentration-dependent growth inhibition in both DOHH2 and RL. After 24 h treatment, the IC50 value for PFI-1 or CPI-203 was 5.373 μM, 3.775 μM, 20.850 μM, 16.301 μM, respectively (Fig. 1A). Flow cytometry analysis revealed that there was an increase in the accumulation of cells treated with PFI-1 or CPI-203 in the G0/G1 phase, whereas the subpopulations of the cells in the S phase decreased (Fig. 1B). Co-treatment with PFI-1 and CPI-203 further inhibited the progression of the cell cycle (Fig. 1B). Moreover, a growing percentage of apoptotic cells was observed in PFI-1 or CPI-203 group than in the control group, and the combination of PFI-1 and CPI-203 further enhanced this effect (Fig. 1C). Similarly, PFI-1 or CPI-203 downregulated the expression of tumor proliferative markers, such as CyclinD1 and CDK4, and remarkably upregulated the protein levels of apoptins, including c-caspase 9 and c-caspase 3. The combination of PFI-1 and CPI-203 further reduced CyclinD1 and CDK4 expression and increased the expression of c-caspase 9 and c-caspase 3 (Fig. 1D and E, Supplementary Fig. 2A). These data suggest that the inhibitory effects of PFI-1

and CPI-203 are synergistic in the progression of FL *in vitro*.

3.1.1. PFI-1 and CPI-203 blocked Wnt/β-catenin signaling in FL cells

The Wnt/β-catenin signaling plays an important role in tumor development [14–16]. This study investigated the effect of PFI-1 and CPI-203 on the Wnt/β-catenin signaling pathway. qRT-PCR and Western blot assays were used to assess the mRNA level and protein expression of Wnt/β-catenin signaling-related mediators. PFI or CPI-203 treatment downregulated DVL2, but upregulated the expression of *p*-GSK3β (Supplementary Figs. 1A, B, E, F, G). Further, a reduced mRNA and protein levels of β-catenin was observed in the nucleus of cells, while an increase β-catenin expression was shown in the cytoplasm (Fig. 2A–D, Supplementary Figs. 2B and C). PFI and CPI-203 also suppressed the protein expression of downstream molecules, TCF4 and C-MYC (Supplementary Figs. 1C, D, E, H, I). Notably, combination treatment was more potent than separate administration. To further evaluate the regulatory role of PFI-1 and CPI-203 in the Wnt/β-catenin signaling pathway, an activator LiCl, was used. As shown in Fig. 2E and F, Supplementary Fig. 1J-M, Supplementary Fig. 2D, the inhibition of nuclear translocation of β-catenin, TCF4 and C-MYC induced by PFI-1, CPI-203, or combination treatment was largely reversed by LiCl. These findings collectively suggest that PFI-1 and CPI-203 can disrupt the Wnt/β-catenin pathway by suppressing the translocation of β-catenin into the nucleus.

3.1.2. PFI-1 and CPI-203 inhibited the growth of FL cells by deactivation of the Wnt/β-catenin pathway

To investigate whether the suppressive effects of PFI-1 or CPI-203 on FL cell growth were mediated via the Wnt/β-catenin pathway, DOHH2 cells were treated with PFI-1, CPI-203, PFI-1+CPI-203, PFI-1+LiCl, CPI-203+LiCl or PFI-1+CPI-203+LiCl for further investigations. As expected, the cell viability, which was repressed by PFI-1, CPI-203 or combination treatment, was partly restored by treatment with LiCl (Fig. 3G). Cell cycle arrest and apoptosis induced by PFI-1 or CPI-203 were significantly improved in Licl-treated groups (Fig. 3A, B, D, E). Consistently, the downregulated expression of tumor proliferative protein and upregulated expression of apoptins in the PFI-1, CPI-203, or combination treatment group were significantly reversed after LiCl treatment (Fig. 3C–F, Supplementary Fig. 2E). Overall, these results suggest that PFI-1 and CPI-203 can interrupt the Wnt/β-catenin pathway to affect FL cell viability, proliferation, and apoptosis.

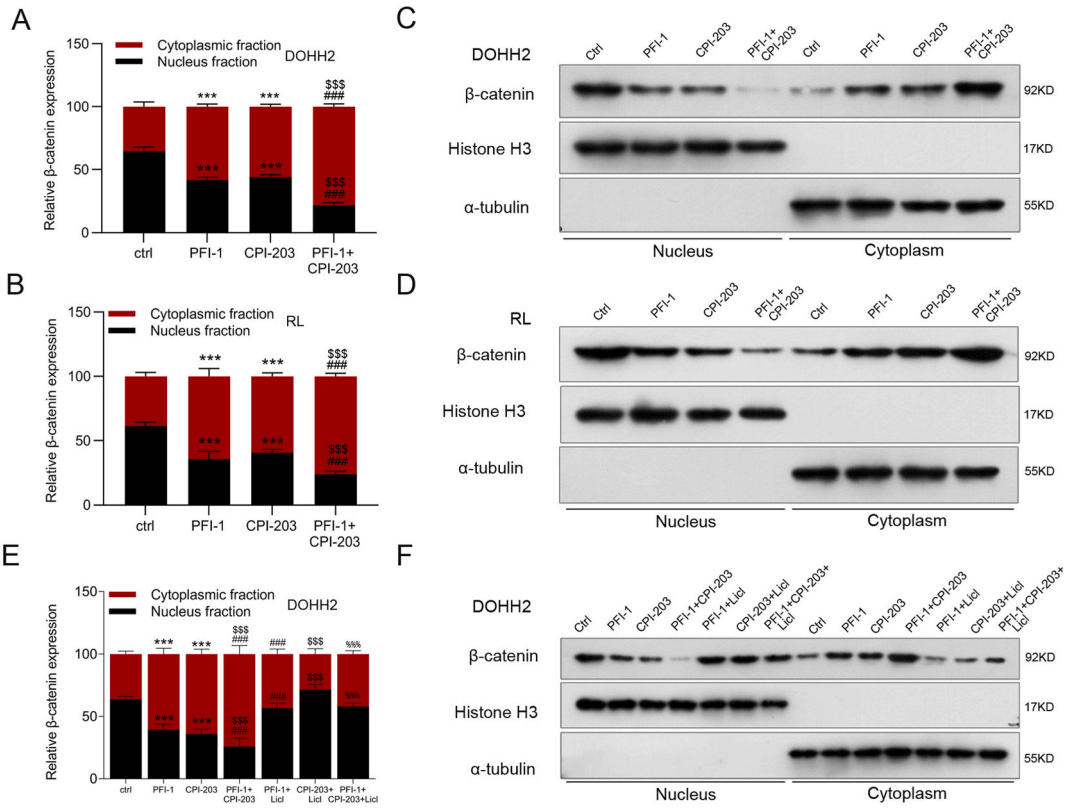

Fig. 2. PFI-1 and CPI-203 block the Wnt/β-catenin signaling in FL cells. (a–d) The cytoplasm translocation of β-catenin in DOHH2 and RL cells treated with PFI-1, CPI-203, or PFI-1+CPI-203, determined by qRT-PCR and Western blot analysis. (e–f) The intracellular distribution of β-catenin in DOHH2 cells treated with PFI-1, CPI-203, PFI-1+CPI-203, PFI-1+lithium chloride (LiCl), CPI-203+ LiCl, or PFI-1+CPI-203+ LiCl. The data are presented as the mean ± SD of triplicate experiments, ***P < 0.001 versus the control group, $^{###}$P < 0.001 versus the PFI-1 group,$^{\$\$\$}$P < 0.001 versus the PFI-1 group, $^{\%\%\%}$P < 0.001 versus the PFI-1+CPI-203 group.

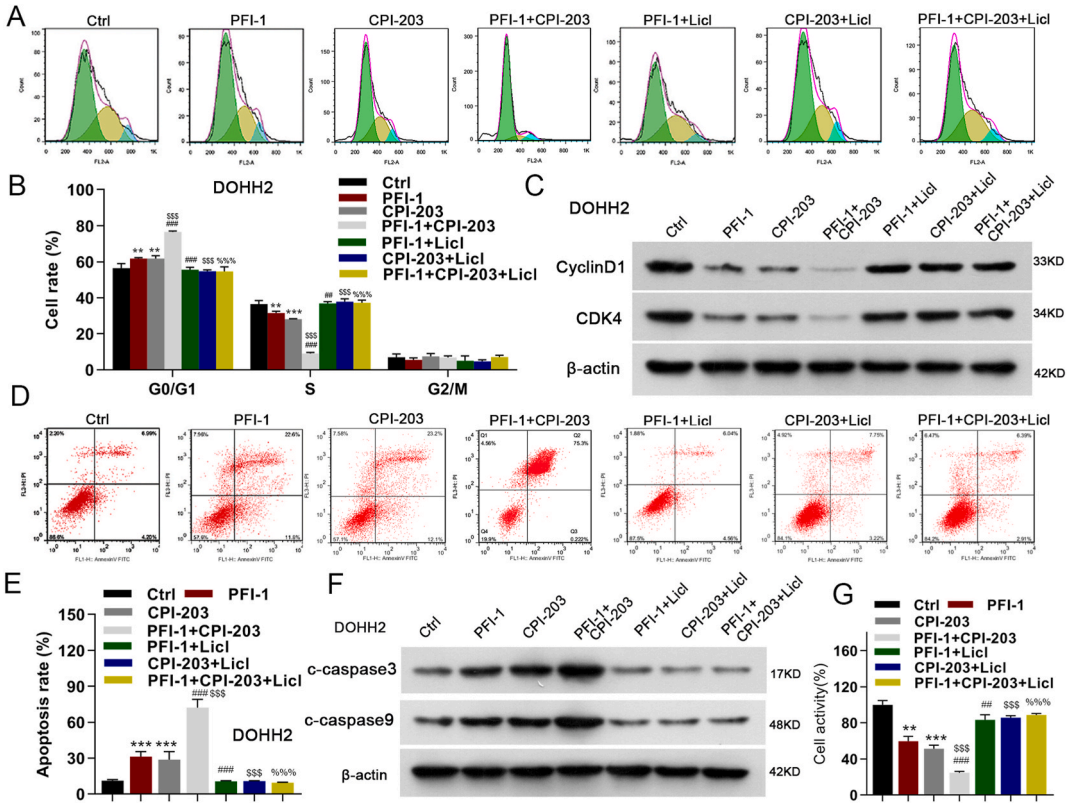

Fig. 3. PFI-1 and CPI-203 inhibit the growth of FL cells by deactivating the Wnt/β-catenin pathway. (a, b, d, e) Cell growth and apoptosis determined by flow cytometry assays. (c, f) The protein expression of Cyclin D1, CDK4, caspase 3, and caspase 9 detected by Western blot analysis. (g) CCK-8 assay is used to detected Cell viability. The data are presented as the mean ± SD of triplicate experiments, $^{**}P < 0.01$ versus the control group, $^{***}P < 0.001$ versus the control group, $^{\#\#}P < 0.01$ versus the PFI-1 group, $^{\#\#\#}P < 0.001$ versus the PFI-1 group, $^{\$\$\$}P < 0.001$ versus the PFI-1 group, $^{\%\%\%}P < 0.001$ versus the PFI-1+CPI-203 group.

3.1.3. Interference of CPI-203 restrained the xenograft tumor growth in vivo

Based on pharmacokinetics studies in mice, the effective concentration of PFI-1 in an animal experiment cannot be attained [17]. Therefore, only the anti-tumor efficacy of CPI-203 was assessed *in vivo* using a lymphoma xenograft SCID model. As shown in Fig. 4A, the luciferase signals in CPI-203-treated mice were significantly reduced than those in controls. Furthermore, CPI-203-treated mice had a smaller tumor volume than controls (Fig. 4B–C) and the administration of CPI-203 significantly prolonged the survival time of mice (Fig. 4D). Consistent with *in vitro* results, immunohistochemistry staining showed that the percentage of cells with Ki-67 signal in the CPI-203 group was less than those in untreated mice (Fig. 4E). Cyclin D1 and CDK4 expression in the CPI-203 administration group were also significantly downregulated, while there was an upregulation of c-caspase 3 and c-caspase 9 (Fig. 4F, Supplementary Fig. 2F). Furthermore, a Western blot was performed to confirm the involvement of the Wnt/β-catenin pathway *in vivo*. As shown in Fig. 4 G, H, Supplementary Fig. 1N, O, Supplementary Fig. 2G, nuclear translocation of β-catenin and DVL2 expression were inhibited, while the level of *p*-GSK3β was increased by CPI-203, accompanied by the downregulation of TCF4 and C-MYC. These results indicate that the administration of CPI-203 exhibits antitumor activity by the inactivation of the Wnt/β-catenin pathway.

4. Discussion

In this study, the potential therapeutic effects of PFI-1 and CPI-203 on FL progression were investigated. Results revealed that these compounds exhibited suppressive effects on FL progression. Interference of PFI-1 and CPI-203 represses cell proliferation and induces cell apoptosis *in vitro*. PFI-1 and CPI-203 can block the Wnt/β-catenin pathway via inhibition of DVL2, which cause the phosphorylation of GSK3β and suppress the nuclear translocation of β-catenin, thereby repressing the growth of FL cells. *In vivo* studies also showed that CPI-203 could restrain tumor growth through the Wnt/β-catenin signaling pathway. These findings suggest that PFI-1 and CPI-203 may act as potential therapeutic agents for FL.

PFI-1 is a selective BET inhibitor that targets the bromodomains of BRD2/BRD4, repressing the interaction between BRDs and proteins and regulating the expression of lineage-specific genes that are associated with cell cycle progression [18,19]. PFI-1 suppresses cell proliferation and induces apoptosis in leukemic blasts, human prostate cancer cells, and breast cells by downregulating C-MYC expression [10,20,21]. PFI-1 suppresses AR/AR-V7 signaling and proliferation of prostate cell lines by downregulating PSA

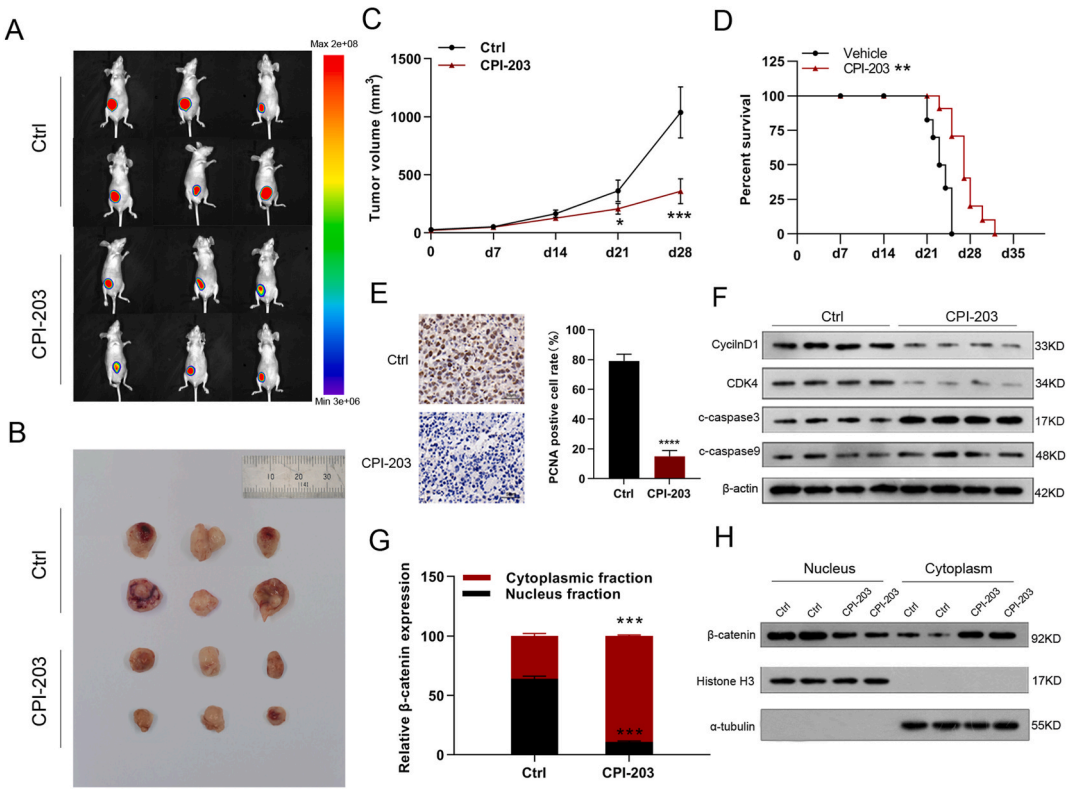

Fig. 4. Interference of CPI-203 restrains the xenograft tumor growth *in vivo*. (a) Living imaging of the subcutaneous DOHH2 tumor-bearing mice in response to CPI-203. (b) Representative images of dissected tumors at 4 weeks after CPI-203 treatment. (c) Tumor volume measured using a clipper once weekly. (d) The Kaplan-Meier survival curves for SCID mice bearing tumor xenografts treated with CPI-203. (e) The expression of Ki67 detected by immunohistochemical staining. (f) The protein expression of Cyclin D1, CDK4, c-caspase-3, and c-caspase-9 in tumor tissues. (g, h) The mRNA and protein levels of β-catenin in the cytoplasm and nucleus of tumor tissues detected by qRT-PCR and Western blot, respectively. The data are presented as the mean ± SD of triplicate experiments, *P < 0.05 versus the control group, **P < 0.01 versus the control group, ***P < 0.001 versus the control group.

expression [20]. PFI-1 has been shown to enhance the inhibitory function of lenalidomide on primary effusion lymphoma, suggesting its inhibitory effects on lymphoma development [11]. Similarly, CPI-203 has also been shown to be an efficient inhibitor in the development of several cancers [22–24]. In acute myeloid leukemia, CPI-203 acts as an enhancer of H4-induced differentiation, which facilitates overcoming therapy resistance [25]. CPI-203 also has potent inhibitory effects on lymphoma cell lines by regulating the PI3K pathway and inducing GSK3β S9 inhibitory phosphorylation [23]. Moreover, CPI-203 has been found to be a synergistic drug combination with PARP inhibitors in ovarian cancer [24]. Here, our findings revealed that PFI-1 and CPI-203 could upregulate the expression of Histone 3 in the cytoplasm and downregulate C-MYC expression by perturbing the interaction between BRDs and Histone H3 leading to G1/G0 cell-cycle arrest and apoptosis in FL cells.

The Wnt/β-catenin signaling pathway is a conserved signaling axis involved in diverse physiological processes and has also been closely associated with cancer [14,26]. Increasing evidence demonstrates that the aberrant activation of the Wnt/β-catenin pathway promotes tumorigenesis, and blocking β-catenin signaling is an effective approach for the treatment of cancer [15,16]. DVL2 and GSK3β are key mediators involved in the Wnt/β-catenin signaling axis, which regulate the activation and inhibition of Wnt signaling [27]. DVL2 inhibits the phosphorylation of GSK3β and promotes the nuclear translocation of β-catenin for activation of Wnt signaling, which in turn transcription of downstream molecules. The interaction between β-catenin and TCF4 plays an essential role in tumorigenesis and cancer development, which is believed to be a promising target in the development of anti-cancer drugs [28]. The other important downstream molecule of β-catenin is C-MYC that regulates the growth and metastasis of many cancer cells [29–31]. FNC has been shown to be an effective chemotherapeutic agent that can suppress the invasion and metastasis of aggressive non-Hodgkin lymphoma through blocking the Wnt/β-catenin pathway [32]. In this study, both PFI-1 and CPI-203 remarkably inhibited FL development by impeding the Wnt/β-catenin signaling pathway.

In conclusion, our data suggest that PFI-1 and CPI-203 are potential anti-tumor inhibitors that suppress FL proliferation by regulating the Wnt/β-catenin pathway. The interruption of this signal pathway can also contribute to the restraint of tumor growth *in vivo*. Of note, the combination treatment used in this study is an effective therapeutic strategy for FL.

Data availability statement

The datasets of this study are available from the corresponding author upon request.

Ethics approval and consent to participate

The experimental protocols using animal models were reviewed and approved by the Ethics Committee of the Cancer Hospital affiliated with Shanxi Medical University (No. 2022038).

CRediT authorship contribution statement

Min Bai: Writing – review & editing, Writing – original draft, Data curation. **Yunpeng Huang:** Project administration, Methodology. **Xinrui Suo:** Writing – review & editing, Data curation. **Lieyang Wang:** Writing – review & editing, Visualization, Data curation. **Weie Han:** Writing – review & editing, Data curation. **Weihua Zhang:** Project administration, Conceptualization.

Declaration of competing interest

No competing financial interests exist.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27149.

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