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Original Article

FL118 inhibits proliferation, migration, invasion, while promoting apoptosis in non-small cell lung cancer

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Abstract: Background: FL118, a novel camptothecin analogue, has been extensively studied in human cancers due to its superior antitumor properties. However, significantly less is known about its effect and underlying mechanisms in non-small cell lung cancer (NSCLC). Therefore, this study aimed to investigate the effects of FL118 on NSCLC cell growth, migration, invasion and apoptosis. Methods: CCK-8 assay was used to detect the effect of FL118 on the cell viability of A549 and H520 cells. Western Blot, Hoechst 33258 staining and Annexin V-FITC/PI double staining followed by flow cytometry were used to detect the effect of FL118 on apoptosis of A549 and H520 cells. Transwell assay and wound scratch experiment were used to detect the effect of FL118 on the invasion and migration of A549 and H520 cells. Immunofluorescence and Western Blot were applied to detect the effects of FL118 on the expression levels of EMT marked proteins E-cadherin and N-cadherin. Results: FL118 inhibited cell viability in a dose-dependent manner. Moreover, FL118 treatment led to a significant increase of apoptotic cells, cleaved PAPR, Bax, as well as the decrease of Bcl-2 level. FL118 treatment also dramatically reduced the migratory and invasive capabilities of NSCLC cells. Furthermore, FL118 reversed the epithelial to mesenchymal transition (EMT) process in A549 and H520 cells. Conclusion: FL118 could effectively suppress NSCLC cell growth, migration, invasion, and EMT process, while promoting apoptotic cell death.

Keywords: FL118, NSCLC, apoptosis, invasion, migration, EMT

Introduction

Throughout the world, cardiovascular disease is considered the major cause of mortality and morbidity that is closely followed by cancer [1]. According to an estimation, there will be 18.1 million newly diagnosed cases and 9.6 million cancer deaths worldwide in 2018. Among various human malignant tumors, lung cancer represents the most commonly diagnosed cancer and the leading cause in cancer death. The number of new cases and deaths were approximately 2,093,876 (11.6% of total cases) and 1,761,007 (18.4% of total cancer deaths) in 2018, respectively [2]. Lung cancer is classified into two types according to distinct pathological patterns, including small cell lung cancer and non-small cell lung cancer (NSCLC) [3]. NSCLC is the most frequently diagnosed and accounts for 85% of lung cancer. Surgery, chemotherapy and radiotherapy still represent the conventional treatment regime for patients [4]. Currently, the median survival for NSCLC patients treated with chemotherapy was approximately 8-10 months. Most recently, the survival time and disease-free survival time of NSCLC patients were substantially improved with the advent of targeted therapy and immunotherapy [5, 6]. However, only a subgroup population benefit due to the inherent and/or acquired resistance of molecular-targeted drugs, as well as the individual heterogeneity of immunotherapy [7, 8]. The 5-year overall survival time was still short and unsatisfactory. Accordingly, identifying novel strategies for the treatment of NSCLC is urgently needed.

FL118 is an anti-cancer small molecule that targeted Survivin gene [9]. It shares similar chemical structure features with irinotecan and

topotecan, which are two FDA-approved anticancer agents for colon cancer treatment. Interestingly, accumulating preclinical studies have reported that FL118 exhibits superior anti-cancer potency than irinotecan and topotecan [10]. Moreover, it has been well-established that irinotecan and topotecan are the substrate of the efflux pump proteins P-gp/MDR1 and ABCG2/BCRP, which led to the occurrence of inherent and/or acquired resistance. In contrast, FL118 is not a substrate of P-gp and ABCG2 and seems to overcome multiple efflux pump protein-induced resistance [11]. Accordingly, FL118 has attracted many investigators owing to its anti-tumourigenic function and that it is well-tolerated. Previously, A great deal of research has confirmed that FL118 exerts anti-proliferative and anti-metastatic effects against a variety of human malignant tumors, such as colorectal cancer [12], breast cancer [13], head and neck cancer [9]. Additionally, it also has been found that FL118 could potentiate the antitumor effect of AMR-MeOAc in K-ras mutation pancreatic cancer cells [14]. However, the effect of FL118 on lung cancer, especially in NSCLC, has not been explored. To the best of current knowledge, only one study has reported that FL118 effectively halts the cancer stem cell-like properties of cancer stem cells in lung cancer. Surprisingly, FL118 showed greater antitumor efficacy than cisplatin [15]. Thus, the study of the effects of FL118 on cognition in NSCLC cells remains challenging and underrepresented in the literature.

The purpose of this investigation is to evaluate the anti-tumor properties of FL118 in NSCLC cell lines. Our data showed FL118 effectively inhibited cell growth and metastasis, while promoting apoptosis, suggesting FL118 could be considered as a potential therapeutic candidate for NSCLC patients.

Materials and methods

Reagents

FL118, 11-methylenedioxy-camptothecin (**Figure 1A**), was a gift from the laboratory of American Roswell Park Cancer Institute (RPCI). FL118 was dissolved with dimethyl sulfoxide (DMSO) for indicated concentration. Antibodies against Bax (#32503, 1:1000) Bcl-2 (#1964-95, 1:1000), pro-PARP, and cleaved PARP (#32-064, 1:1000) were purchased from Abcam (Cambridge, UK). Antibodies against glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and goat anti-rabbit immunoglobulin horse radish peroxide (IgG-HRP) or anti-mouse IgG-HRP were from Beyotime Biotechnology Co. Ltd. (Shanghai, China). The anti-E-cadherin (#3195, 1:200 (IF), 1:1000 (wb)) and anti-N-cadherin (#13116, 1:200 (IF), 1:1000 (wb)) were from Cell signaling technology (Danvers, MA, US). Cell counting kit-8 (CCK-8) (#C0038), Annexin V-FITC/PI staining (#C1062L), and Hoechst 33258 staining (#C1018) were purchased from Beyotime Institute of Biotechnology (Haimen, China).

Cell lines and culture

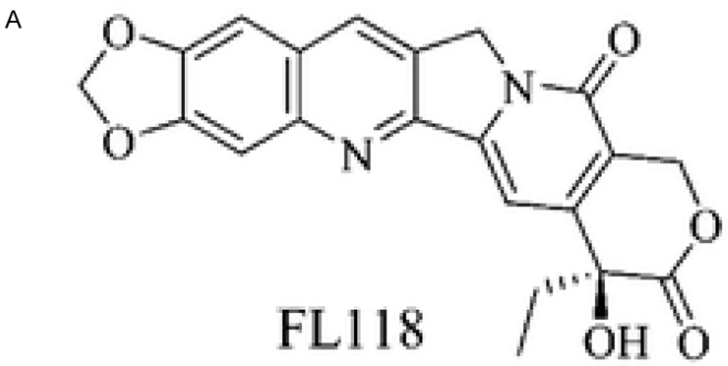
Human NSCLC cell lines, A549 and H520, were purchased from the American Type Culture Collection. Cells were cultured in McCoy's 5a medium (A549) or L-15 medium (H520) containing 10% fetal bovine serum at 37°C under 5% CO_2.

Cell viability assessment

CCK-8 assay was used to detect the effect of FL118 on NSCLC cell proliferation. Briefly, A549 and H520 cells were plated in 96-well plates. Each well had 5×10^3 cells. Cells were treated with different concentrations of FL118 for 24 h and 48 h. Then, the medium was aspirated and cells were washed with 1 × PBS, followed by incubation with 10 μM CCK-8 liquor at an environment of 37°C for 1 h. A spectrophotometer (Tecan Group Ltd., Männedorf, Switzerland) was used to measure the optical density of living cells at 450 nm.

Hoechst 33258 staining

A549 and H520 cells were digested with 0.05% trypsin, dispersed and plated in a 96-well plate at a density of 1×10^5 cells per well. Following exposure to FL118 (0, 10, and 20 nM) for 48 h, cells were washed 3 times with PBS, then fixed in 4% paraformaldehyde at 4°C for 20 min, stained with Hoechst 33258 staining solution (125 μl/well, Beyotime Institute of Biotechnology, Haimen, China) for 5 min, rewashed three times with PBS and covered with anti-fading solution. The nuclear morphology of apoptotic cells were observed under inverted phase contrast fluorescence microscope (magnification, × 400) (Carl Zeiss, Heidenheimer, Germany).



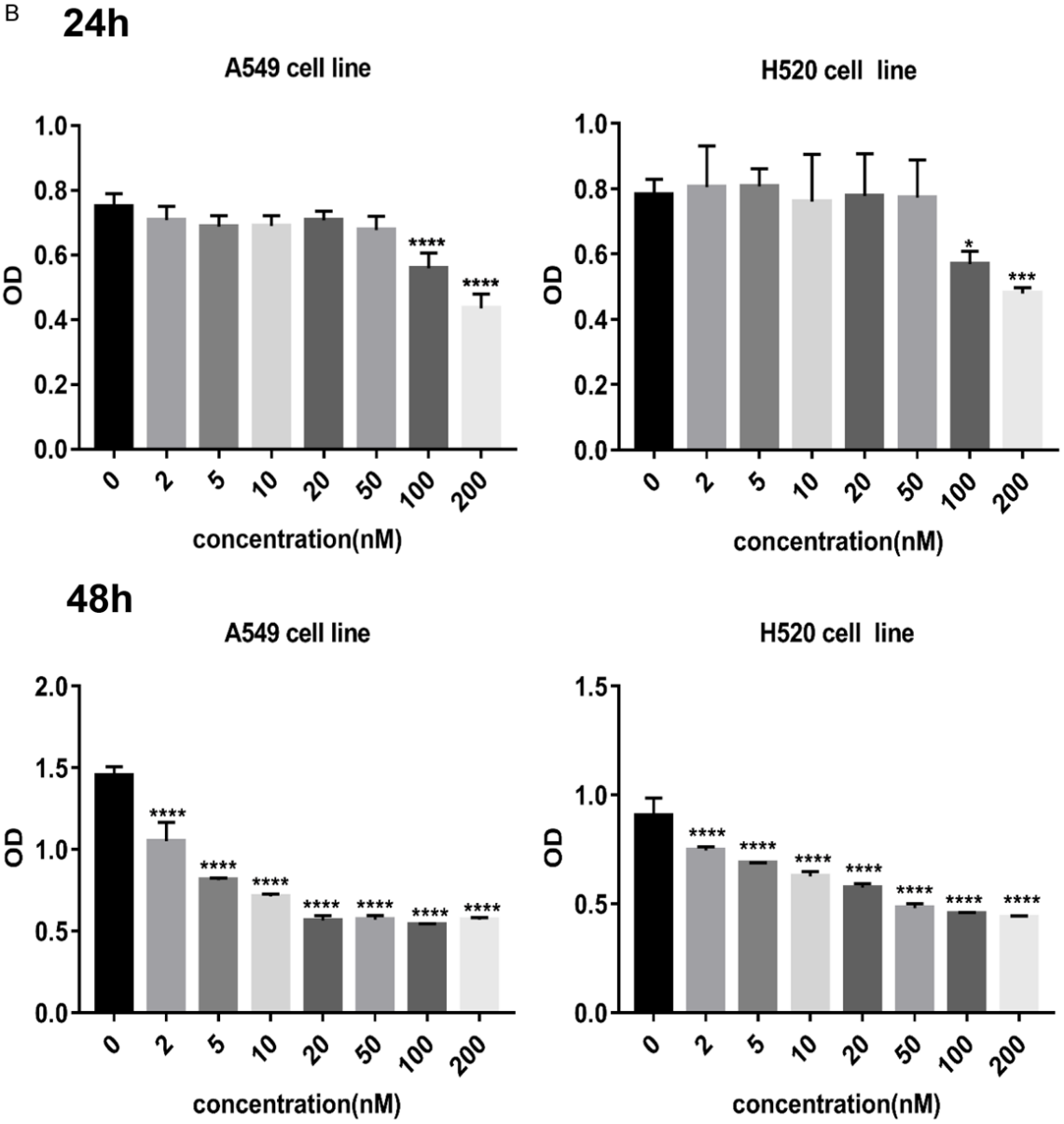


Figure 1. The effect of FL118 on NSCLC cell viability. A. The chemical structure of FL118. B. A549 and H520 cells were cultured with various concentrations FL118 (0-200 nM) for 24 and 48 h. Cells were then subjected for cell viability assessment using CCK-8 assay. Data were presented as mean ± SD (standard deviation). $*P < 0.05$, $***P < 0.001$, $****P < 0.0001$.

Cell apoptosis detection

A549 and H520 cells in the logarithmic growth phase were collected at the density of 1 × 10^6 cells/mL and incubated with indicated concentrations of FL118 for 48 h. Subsequently, cells were stained with Annexin V-FITC/Propidium Iodide (KeyGen Biotech Co., Ltd., Nanjing,

Jiangsu, China) according to the protocols. Then apoptotic cells were determined using a flow cytometer (Thermo Fisher Scientific, Shanghai).

Western blot analysis

Following FL118 treatment, RIPA lysate buffer (Beyotime Biotechnology Co., Ltd., Shanghai, China) was used to extract total protein from A549 and H520 cells. Quantitative analysis of total protein was performed using Bradford Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). Then, 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to separate 40 μg protein and the protein was transferred onto polyvinylidene fluoride (PVDF) membranes. Then 5% skim milk was used to block the membranes. After that, a shaking table was used to shake the membranes at an environment of 37°C for 1 h. Then they were co-incubated with Bcl-2, Bax, pro-PARP, cleaved PARP, E-cadherin, N-cadherin and GAPDH rabbit anti-mouse monoclonal antibody at a condition of 4°C overnight. The membranes were washed by TBST for three times and each time lasted 5 minutes. Membranes and horseradish peroxidase (HRP)-conjugated goat anti-rabbit antibody (1:3000, Cell Signaling Technology, USA) were co-incubated for 2 hours. Lastly, TBST was used to wash the membranes for three times (5 min each). Luminol reagent and peroxide solution (Millipore, Billerica, MA, USA) were mixed at 1:1. Then the mixed solution was used to detect proteins.

Wound scratch assay

A549 and H520 cells were resuspended with FBS free medium. Then 5×10^5 cells in 2 mL medium were seed into 6-well plate and cultured overnight. When the confluency of the cells reached 90%, a line was scraped on the cell monolayer with the 20 μl pipette tip. Then cells were rinsed gently with phosphate buffer saline (PBS) for 3 times. The cell migration was observed under a microscope (magnification, × 100). Scratch pre-experiment was used to judge whether the cells had healing ability according to the migration area.

Transwell assay

Transwell assay was carried out to evaluate the effect of FL118 on cell invasion ability. Briefly, A549 and H520 cells were digested with 0.05% trypsin and then resuspended with 100 μl serum-free medium (with 1% FBS). The cells with the density of 5×10^5 cells/ml were added in upper chamber. 500 μl 10% FBS medium were added into the lower chamber. Subsequently, cells were incubated at a condition of 37°C for 24 h. The microscope was used to observe cells that were on the lower side. Before observation, the cells were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet for 0.5 h.

Immunofluorescence

Cells were cultured on coverslips, fixed in 4% paraformaldehyde, permeabilized in 0.5% Triton X-100, incubated with the primary anti-E-cadherin and N-cadherin antibodies (Sigma Aldrich) for 1 h, and AlexaFluor 594-conjugated secondary antibody (Invitrogen, Grand Island, NY, USA). After counterstaining with DAPI (Molecular Probes, Invitrogen), cells were examined and photographed using a confocal microscope (Zeiss Axiovert 200 M).

Statistical analysis

All data was expressed as mean ± SD (standard derivation). Each experiment was repeated at least 3 times. Statistical analysis was performed with SPSS 20.0 (IBM, Armonk, NY, USA) and Graph Prism 7.0 (La Jolla, CA, USA). One-way ANOVA was used to assess the difference between two groups. *P* values less than 0.05 was considered as statistically significant.

Results

FL118 reduces the viability of NSCLC cells in a time and dose-dependent manner

FL118 (**Figure 1A**), a novel camptothecin analogue, has been extensively studied in head-and-neck and colon cancer owing to its superior antitumor potency [9, 10]. However, the influence of FL118 on NSCLC has remained unclear. In the current study, we first investigated the cytotoxicity effect of FL118 on two well-established NCSCL cell lines, A549 and H520. Cells were exposed to various concentrations (0-200 nM) of FL118 for 24 h and 48 h. As shown in **Figure 1B**, only 100 and 200 nM could effectively reduce the cell viability of A549 and H520 cells at 24 h, while 0-50 nM FL118 did not

cause obvious cytotoxicity effect. In contrast, both tested concentrations of FL118 led to a significant reduction of cell viability at 48 h. Moreover, the anti-proliferative effect of FL118 was more obvious in the high dose group, indicating FL118 suppresses cell viability in a dose-dependent manner. Collectively, these findings suggested that FL118 could effectively reduce NSCLC cell viability in a time and dose-dependent fashion.

FL118 triggers apoptosis in NSCLC cells

Next, we also evaluated the effect of FL118 on the apoptosis of A549 and H520. Hoechst 33258 staining results indicated the fragmented nuclei and condensed chromatin pattern was significantly increased in FL118-treated cells (**Figure 2A**). Similarly, Flow cytometry results also confirmed that both 10 and 20 nM FL118 could notably elevate the number of apoptotic cells in comparison with control cells. In A549 cells, FL118 (0, 10, and 20 nM)-induced apoptosis rates were about 3.3%, 22.9% and 35.8%, respectively. Moreover, the apoptosis rates in H520 cells were about 3.8%, 7.9%, and 9.3%, respectively (**Figure 2B**). Interestingly, A549 cells were more sensitive to FL118 than H520 cells in same dose and time. We also examined the expression level of several apoptosis-related proteins, including Bax, Bcl-2, pro-PARP, and cleaved PARP. Consistent with above results, western blot analysis demonstrated FL118 (5-20 nM) treatment led to a marked increase of Bax and cleaved PARP, as well as the decrease of Bcl-2 and pro-PARP (**Figure 2C**). Taken together, these data suggested that FL118 induced apoptosis of NSCLC cell in a dose-dependent manner.

FL118 suppresses migration and invasion of NSCLC cells

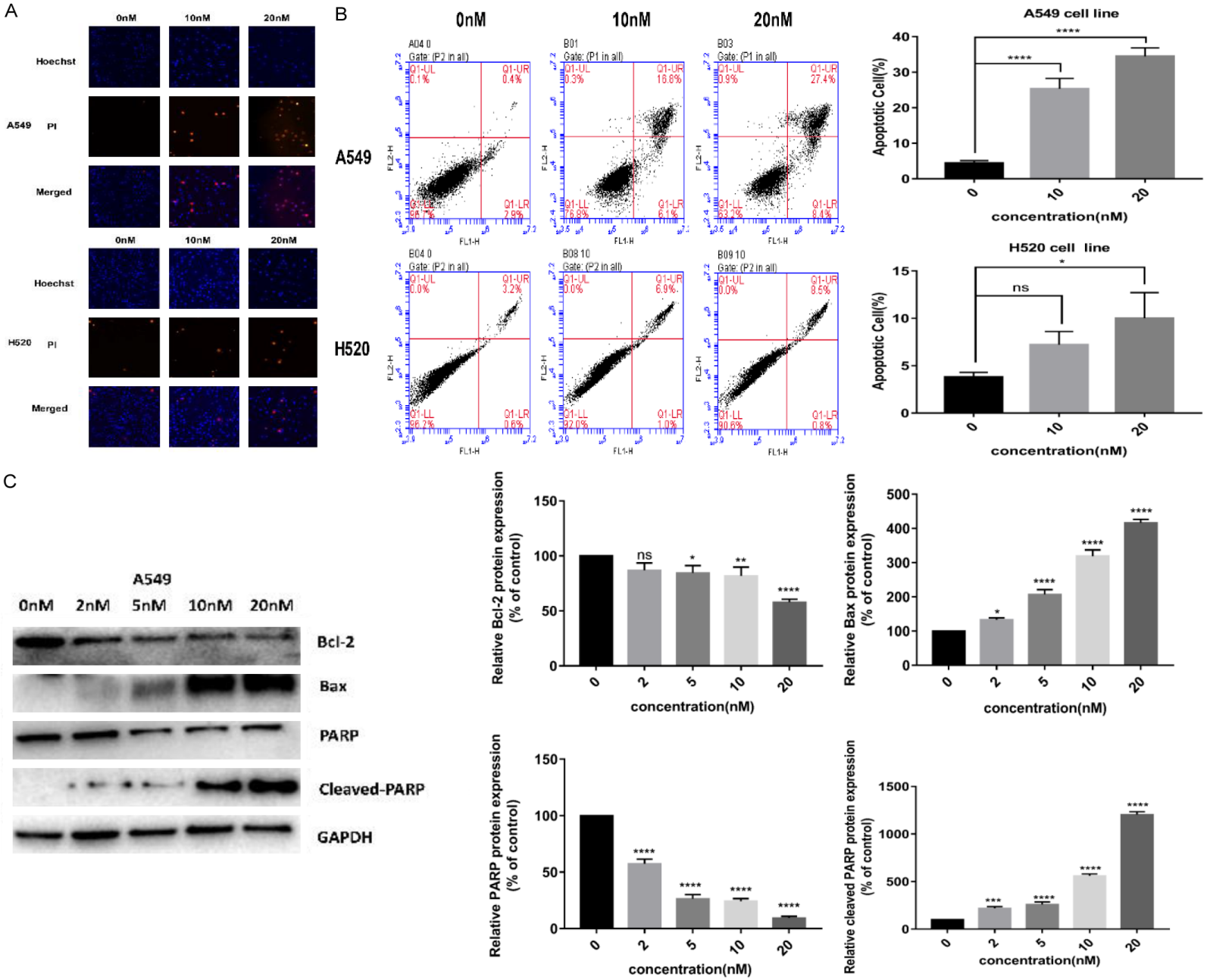
The anti-metastatic effect of FL118 has been reported in some tumors. Thus, we also examined migratory and invasive activities of NSCLC cells. **Figure 3A** showed that both 20 nM and 50 nM FL118 could effectively suppress the migration of A549 and H520 cells. Wound scratch assay results showed that FL118 significantly reduced the migration of cells in a dose-dependent manner, the percent of migrated wound area were about 45% (0 nM), 30% (20 nM), and 12% (50 nM) in A549 cells, while about 48% (0 nM), 32% (20 nM), and 28% (50 nM) for H520 cells. Similarly, Transwell assay results revealed that the invasive capability was significantly reduced in FL118-treated cells. Collectively, these results concluded that FL118 suppressed the migration and invasion of NSCLC cells.

FL118 inhibits EMT process of NSCLC cells

A plethora of literature has confirmed the role of epithelial to mesenchymal transition (EMT) in tumor metastasis. During an EMT, epithelial cells lose their polarized organization and acquire migratory and invasive capabilities [16]. Accordingly, we also evaluated the effect of FL118 through detecting the relative abundance of EMT-markers. As shown in **Figure 4A**, FL118-treated A549 and H520 cells showed a stronger red fluorescence in cell membranes stained with E-cadherin, while a weaker red fluorescence when stained with N-cadherin antibody compared with the control cells. Moreover, FL118 regulated the relative abundance of E-cadherin and N-cadherin in a dose-dependent manner. Consistent with above results, western blot analysis also confirmed that FL118 led to a significant increase of E-cadherin and a decrease of N-cadherin (**Figure 4B**). Based on these results, we concluded that FL118 might suppress metastasis by inhibiting EMT process in NSCLC cells.

Discussion

NSCLC represents the most commonly diagnosed lung cancer and with shorter survival. Based on the pathological types, NSCLC is clarified into squamous cell cancer, adenocarcinoma, as well as large cell carcinoma. Despite considerable developments in the treatment of NSCLC, including molecular targeted therapy and immunotherapy, the current situation for NSCLC patients still remains poor. The long-term survival (5-year survival rate is ~60%) and mortality remained unchanged. Therefore, novel therapeutic strategies are urgently required that can effectively act via various anticancer mechanisms. FL118 is firstly identified as the inhibitor of Survivin through high-throughput screen [9]. Recently, accumulating evidence has reported that FL118 exerts anti-tumor effect against various human malignancies through multiple distinct mechanisms [17]. As we all known, p53 status (wild type, mutant or null) in tumor cells is strongly associated the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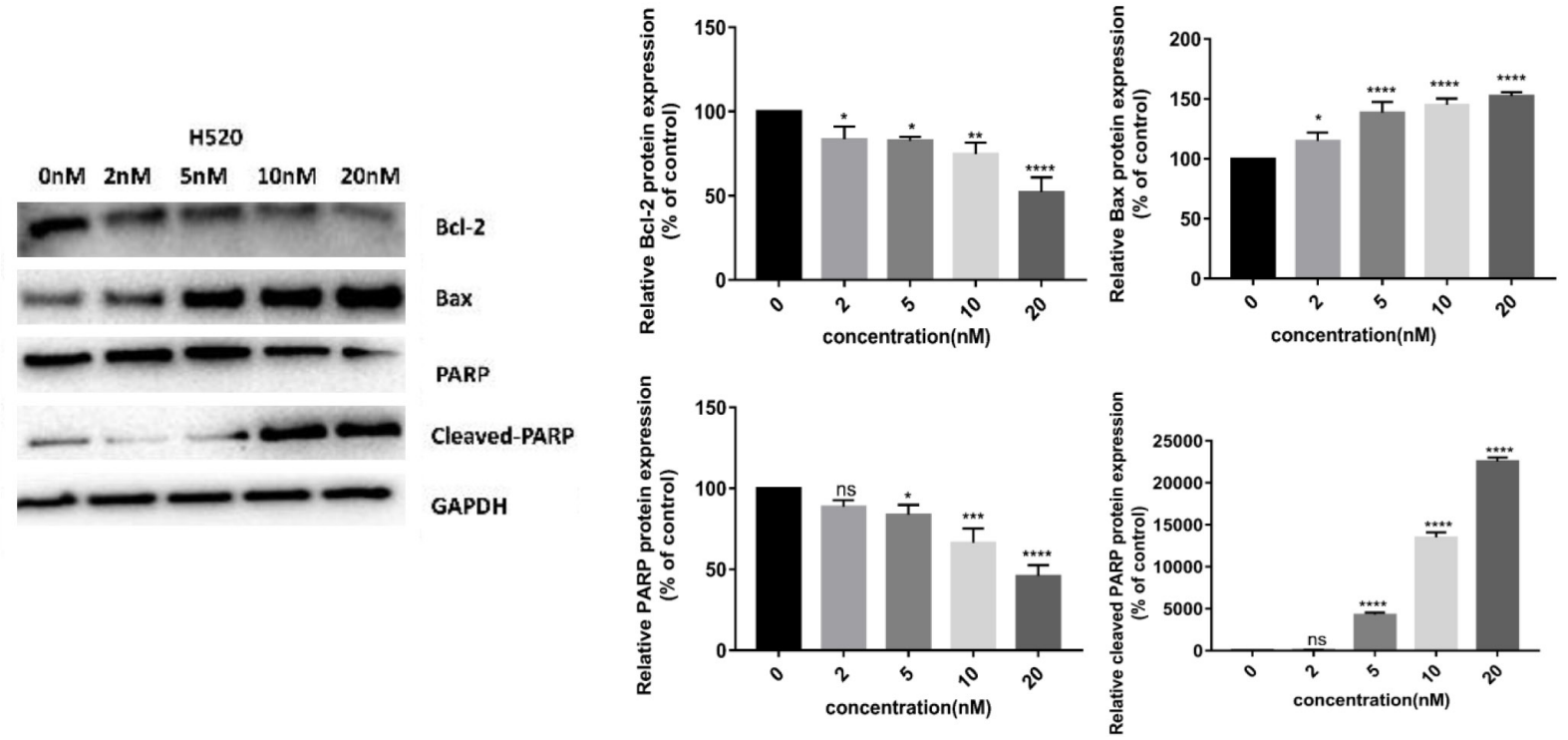

Figure 2. Effect of FL118 on apoptosis and apoptosis-related proteins expression in NSCLC cells. A549 and H520 cells were treated with FL118 (0, 10, and 20 nM) for 48 h. A. Apoptosis analysis of FL118-treated cells through microscopy using Hoechst 33258 staining (magnification, × 200). Hoechst 33258 staining enrichment represents the apoptotic cells. B. Following Annexin V-FITC/PI staining, apoptotic cell rate in every group was quantified by using flow cytometry. C. Western blot analyzes the expression level of apoptosis-related proteins (Bax, Bcl-2, pro-PARP, cleaved PARP) in indicated cells. GAPDH was chosen as the internal control. Data were presented as mean ± SD (standard deraition). ns: no significant, $*P < 0.05$, $**P < 0.01$, $****P < 0.0001$.

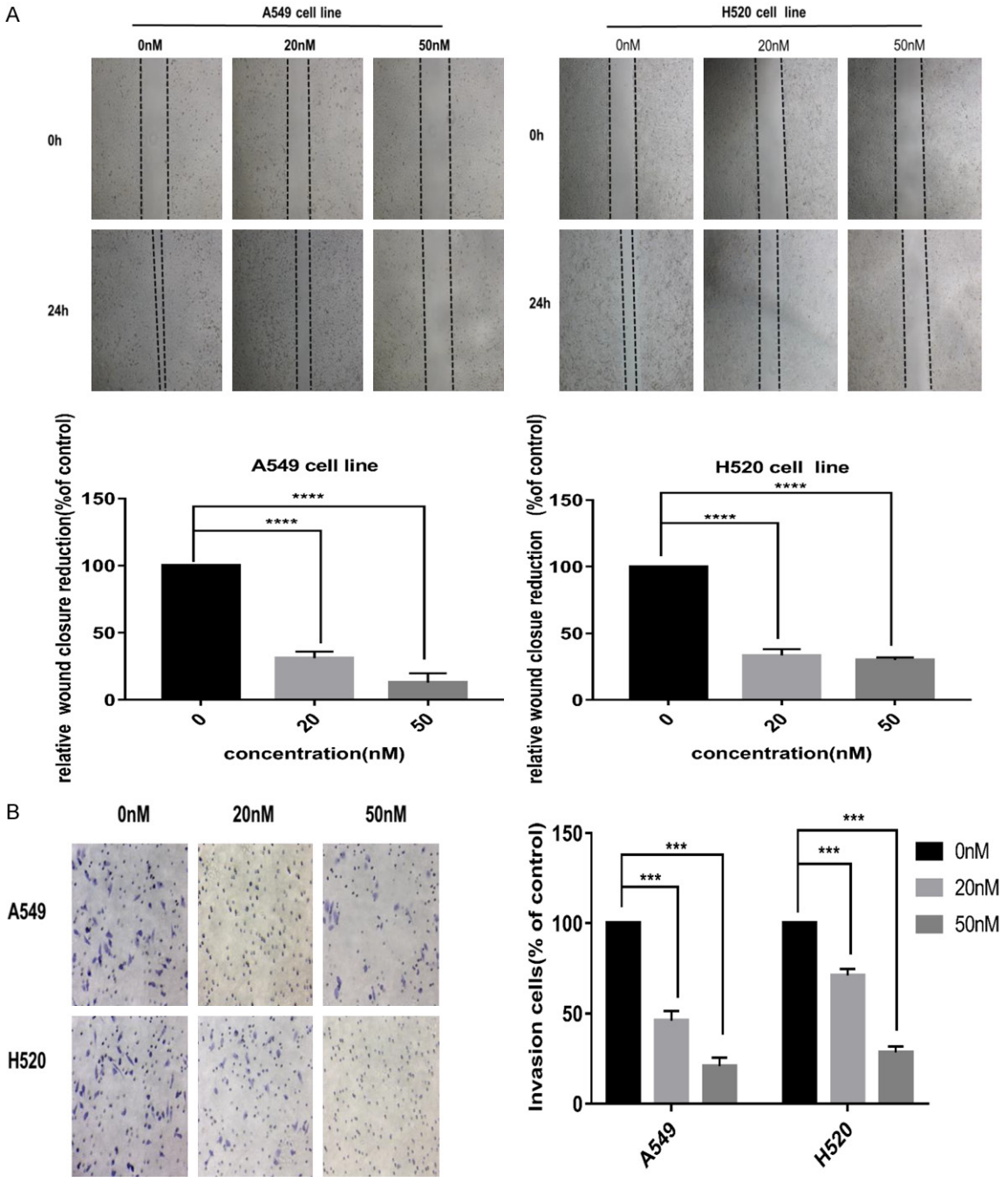

Figure 3. Effect of FL118 on the migratory and invasive capabilities of NSCLC cells. A549 and H520 cells in logarithmic growth phase were incubated with FL118 (0, 20, and 50 nM) for 24 h. A. The cells were subjected to evaluate the migratory properties by using Wound scratch assay. Data were presented as mean ± SD (standard deviation). B. The invasion of A549 and H520 cells were examined by Transwell assay. Data were presented as mean ± SD (standard deviation). $*P < 0.05$, $**P < 0.01$, $****P < 0.0001$.

efficacy of chemotherapeutic drugs in clinical setting. Surprisingly, preclinical research has reported that FL118 could effectively suppress neoplasm growth and metastasis in regardless of the status of p53. Additionally, it has been well-established that the inherent and/or acquired resistance of chemotherapeutic drugs represents the major challenge for improving overall survival time. Available evidence also reveals that FL118 could overcome the resis-

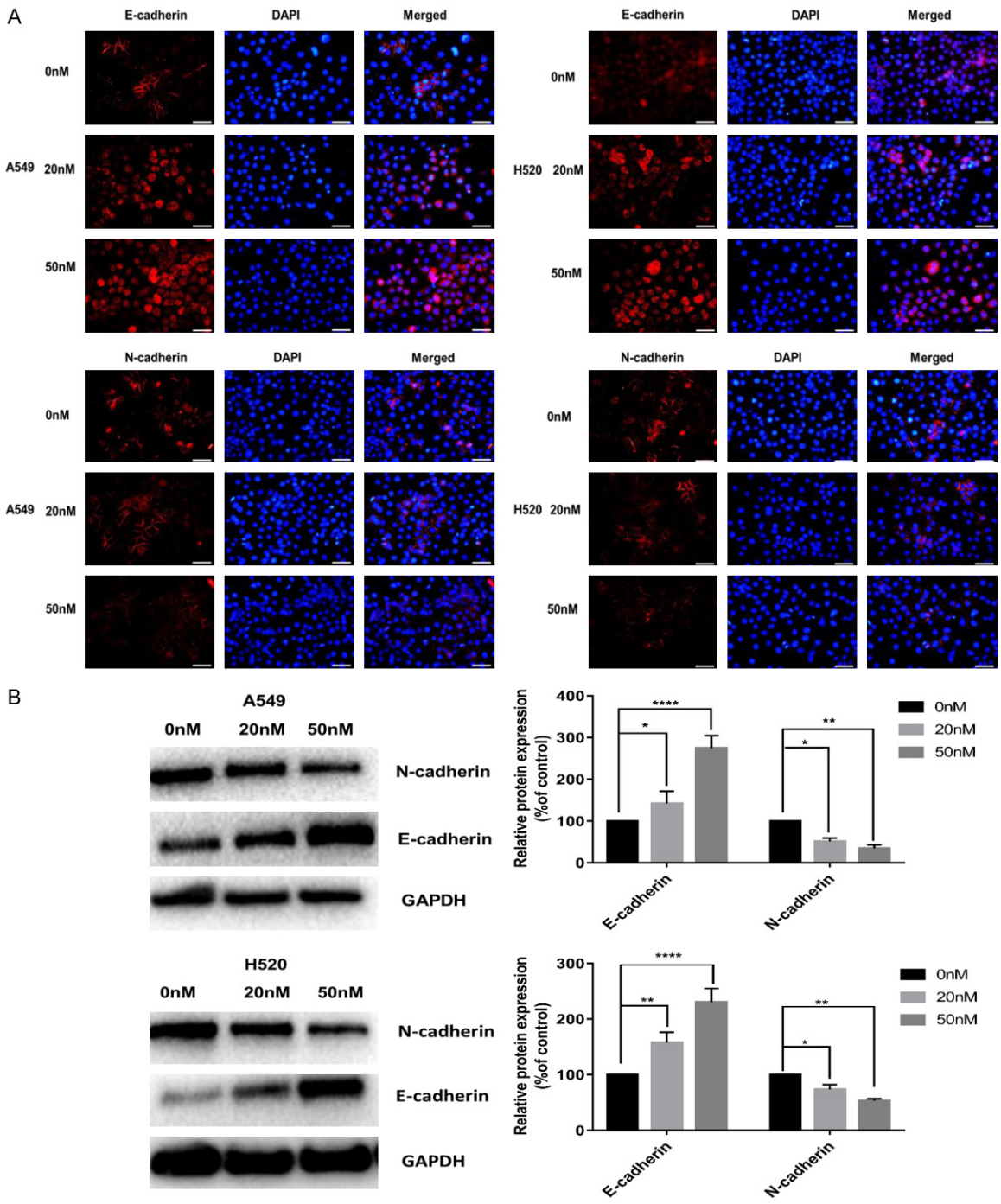

Figure 4. Effect of FL118 on E-cadherin and N-cadherin expression in NSCLC cells. A549 and H520 cells in logarithmic growth phase were incubated with FL118 (0, 20, and 50 nM) for 24 h. A. Localization and quantification of EMT marker proteins (E-cadherin and N-cadherin) in indicated cells were examined by using immunofluorescence staining (magnification, × 400). Representative pictures from immunofluorescence staining. Red: EMT markers, blue: DAPI. B. Western blot analyzes the expression level of EMT marker proteins (E-cadherin and N-cadherin) in A549 and H520 cells. GAPDH was chosen as the internal control. Data were presented as mean ± SD (standard deviation). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.0001$.

tance mediated by efflux pump system in tumor cells. Collectively, FL118 represents an attractive therapeutic option for cancer treatment. In the current study, we preliminary evaluated the anticancer activity of FL118 in NSCLC cell lines and found FL118 markedly inhibited prolifera-

tion, migration, and invasion, while promoted apoptosis in A549 and H520 cells. Sustaining proliferative signaling and resisting cell death are two key hallmarks of tumor cells, which could make them have abilities of oncogenicity and malignant transformation [18]. Thereby, blocking cell proliferation and inducing apoptotic cell death represent an effective strategy to halt tumorigenesis and disease progression [19]. The anti-proliferation and pro-apoptosis ability of FL118 have been confirmed in a variety of human malignancies. For instance, previous study found that FL118 treatment led to significant growth inhibition and cell cycle arrest in breast cancer MDA-MB-231 cells [13]. Similarly, FL118 also exerts obvious anti-proliferative effect in colon cancer cells [20]. Consistent with published results, our current findings demonstrated the growth and clonogenic capacity of NSCLC cells were dramatically halted by FL118. Additionally, recent studies have shown that FL118 triggered apoptotic cell death through regulating multiple apoptosis-related proteins, including Bax and Bcl-2. Consistently, our data demonstrated that FL118 exposure resulted in an increase of apoptotic cells and the level of Bax, while it decreased the expression level of anti-apoptotic protein Bcl-2. Conjointly, these results showed that FL118 would be a potential therapeutic candidate drug for NSCLC through inducing growth inhibition and apoptosis.

To a great deal, tumors from epithelial tissues progressing to higher pathological grades of malignancy are caused by activating invasion and metastasis [21, 22]. During an EMT process, epithelial cells lose their polarized organization and acquire migratory and invasive capabilities [23]. E-cadherin, a key cell to cell adhesion molecule, assists to enable the epithelial cell sheets to fit together and keep the cells quiescence within these sheets [24]. The phenomenon that E-cadherin expresses higher is confirmed to antagonize invasion and metastasis. On the contrary, the reduction of its expression is identified to enhance these phenotypes. In general, during organogenesis, N-cadherin is expressed in migrating neurons and mesenchymal cells. In addition, in many invasive tumor cells, N-cadherin is up-regulated [25]. Consequently, the abundance of E-cadherin and N-cadherin in tumor cells reflects the invasion and metastasis capabilities. Wound scratch experiment and Transwell assay demonstrated that FL118 effectively alleviated the migration and invasion of NSCLC cells. Furthermore, the abundance of E-cadherin was significantly elevated in FL118-treated cells, while N-cadherin level was reduced. Consistent with our results, previous study found that FL118 inhibits migration, invasion and EMT process in breast cancer cell via inactivating Wnt/β-catenin signaling pathway [13]. However, the underlying mechanism that contributes the anti-metastatic effect of FL118 in NSCLC is largely unknown.

In conclusion, our current study demonstrated that FL118 could effectively inhibit cell proliferation, migration, invasion, and EMT process, while promote cell apoptosis in NSCLC cells. These data suggest FL118 may be a potential therapeutic candidate drug for NSCLC. However, the mechanisms underlying the anti-cancer activity of FL118 in NSCLC are largely an enigma. Future studies on the current topic are therefore recommended.

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Disclosure of conflict of interest

None.

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References

[1] Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Pineros M, Znaor A and Bray F. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer 2019; 144: 1941-1953.

[2] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68: 394-424.

[3] Molina JR, Yang P, Cassivi SD, Schild SE and Adjei AA. Non-small cell lung cancer: epidemi-

ology, risk factors, treatment, and survivorship. Mayo Clin Proc 2008; 83: 584-594.
[4] Herbst RS, Morgensztern D and Boshoff C. The biology and management of non-small cell lung cancer. Nature 2018; 553: 446-454.
[5] Vansteenkiste J, Wauters E, Reymen B, Ackermann CJ, Peters S and De Ruysscher D. Current status of immune checkpoint inhibition in early stage NSCLC. Ann Oncol 2019; 30: 1244-1253.
[6] Heigener DF and Reck M. Lung cancer in 2017: giant steps and stumbling blocks. Nat Rev Clin Oncol 2018; 15: 71-72.
[7] Peters S, Reck M, Smit EF, Mok T and Hellmann MD. How to make the best use of immunotherapy as first-line treatment for advanced/metastatic non-small-cell lung cancer. Ann Oncol 2019; [Epub ahead of print].
[8] Rotow J and Bivona TG. Understanding and targeting resistance mechanisms in NSCLC. Nat Rev Cancer 2017; 17: 637-658.
[9] Ling X, Cao S, Cheng Q, Keefe JT, Rustum YM and Li F. A novel small molecule FL118 that selectively inhibits survivin, Mcl-1, XIAP and cIAP2 in a p53-independent manner, shows superior antitumor activity. PLoS One 2012; 7: e45571.
[10] Ling X and Li F. An intravenous (i.v.) route-compatible formulation of FL118, a survivin, Mcl-1, XIAP, and cIAP2 selective inhibitor, improves FL118 antitumor efficacy and therapeutic index (TI). Am J Transl Res 2013; 5: 139-154.
[11] Ling X, Liu X, Zhong K, Smith N, Prey J and Li F. FL118, a novel camptothecin analogue, overcomes irinotecan and topotecan resistance in human tumor xenograft models. Am J Transl Res 2015; 7: 1765-1781.
[12] Li F, Ling X, Harris DL, Liao J, Wang Y, Westover D, Jiang G, Xu B, Boland PM and Jin C. Topoisomerase I (Top1): a major target of FL118 for its antitumor efficacy or mainly involved in its side effects of hematopoietic toxicity? Am J Cancer Res 2017; 7: 370-382.
[13] Yang Z, Ji L, Jiang G, Liu R, Liu Z, Yang Y, Ma Q and Zhao H. FL118, a novel camptothecin analogue, suppressed migration and invasion of human breast cancer cells by inhibiting epithelial-mesenchymal transition via the Wnt/beta-catenin signaling pathway. Biosci Trends 2018; 12: 40-46.
[14] Rabi T and Li F. Multiple mechanisms involved in a low concentration of FL118 enhancement of AMR-MeOAc to induce pancreatic cancer cell apoptosis and growth inhibition. Am J Cancer Res 2018; 8: 2267-2283.
[15] Wang J, Liu Z, Zhang D, Liu R, Lin Q, Liu J, Yang Z, Ma Q, Sun D, Zhou X and Jiang G. FL118, a novel survivin inhibitor, wins the battle against drug-resistant and metastatic lung cancers through inhibition of cancer stem cell-like properties. Am J Transl Res 2017; 9: 3676-3686.
[16] Diepenbruck M and Christofori G. Epithelial-mesenchymal transition (EMT) and metastasis: yes, no, maybe? Curr Opin Cell Biol 2016; 43: 7-13.
[17] Li F. Anticancer drug FL118 is more than a survivin inhibitor: where is the Achilles' heel of cancer? Am J Cancer Res 2014; 4: 304-311.
[18] Hanahan D and Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144: 646-674.
[19] Normile D. Cell proliferation. Common control for cancer, stem cells. Science 2002; 298: 1869.
[20] Westover D, Ling X, Lam H, Welch J, Jin C, Gongora C, Del Rio M, Wani M and Li F. FL118, a novel camptothecin derivative, is insensitive to ABCG2 expression and shows improved efficacy in comparison with irinotecan in colon and lung cancer models with ABCG2-induced resistance. Mol Cancer 2015; 14: 92.
[21] Hanahan D and Weinberg RA. The hallmarks of cancer. Cell 2000; 100: 57-70.
[22] Coghlin C and Murray GI. Current and emerging concepts in tumour metastasis. J Pathol 2010; 222: 1-15.
[23] Bruner HC and Derksen PWB. Loss of E-cadherin-dependent cell-cell adhesion and the development and progression of cancer. Cold Spring Harb Perspect Biol 2018; 10.
[24] Lecuit T and Yap AS. E-cadherin junctions as active mechanical integrators in tissue dynamics. Nat Cell Biol 2015; 17: 533-539.
[25] Giannelli G, Koudelkova P, Dituri F and Mikulits W. Role of epithelial to mesenchymal transition in hepatocellular carcinoma. J Hepatol 2016; 65: 798-808.