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KAN0438757: A NOVEL PFKFB3 INHIBITOR THAT INDUCES PROGRAMMED CELL DEATH AND SUPPRESSES CELL MIGRATION IN NON-SMALL CELL LUNG CARCINOMA CELLS

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Aim. PFKFB3 is glycolytic activator that overexpressed in human lung cancer and plays a crucial role in multiple cellular functions including programmed cell death. Despite the many small molecules described as PFKFB3 inhibitors, some of them have shown disappointing results *in vitro* and *in vivo*. On the other hand KAN0438757, selective and potent, small molecule inhibitor has been developed. However, the effects of KAN0438757, in non-small cell lung carcinoma cells remain unknown. Herein, we sought to decipher the effect of KAN0438757 on proliferation, migration, DNA damage, and programmed cell death in non-small cell lung carcinoma cells

Methods. The effects of KAN0438757 on cell viability, proliferation, DNA damage, migration, apoptosis, and autophagy in non-small cell lung carcinoma cells were tested by WST-1, real-time cell analysis, comet assay, wound-healing migration test, and MMP/JC-1 and AO/ER dual staining assays as well as western blot analysis.

Results. Our results revealed that KAN0438757 significantly suppressed the viability and proliferation of A549 and H1299 cells and inhibited migration of A549 cells. More importantly, KAN0438757 caused DNA damage and triggered apoptosis and this was accompanied by the up-regulation of cleaved PARP in A549 cells. Furthermore, treatment with KAN0438757 resulted in increased LC3 II and Beclin1, which indicated that KAN0438757 stimulated autophagy.

Conclusions. Overall, targeting PFKFB3 with KAN0438757 may be a promising effective treatment approach, requiring further *in vitro* and *in vivo* evaluation of KAN0438757 as a therapy in non-small cell lung carcinoma cells.

Key words: PFKFB3; KAN0438757; Apoptosis; Autophagy; Lung Cancer.

Transformed cells more than non-transformed cells exert heavy demands on glycolysis to support their increased energy consumption owing to their rapid rates of cell division. The glycolysis pathway is the basic enzymatic process in cell metabolism. However, under limited oxygen, normal cells respond physiologically by increasing glycolysis. The dysregulation of glucose metabolism is one of the hallmarks of cancer cells. High glucose consumption by tumors was first reported by Warburg in the 1920s and later suggested that cancer cells exhibit a respiratory disorder [1, 2]. Cancer cells choose the aerobic glycolysis (known as "Warburg" effect), which is an efficient way of rapidly synthesizing nucleotides, amino acids and lipids required to form biomass [3] 6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatases (PFKFBs) are enzyme family, best known for its role in the glycolysis. PFKFBs enzyme family consists of four homologs are encoded by separate genes: PFKFB1, PFKFB2, PFKFB3, and PFKFB4 [4]. Among these members, the

PFKFB3 enzyme exhibit kinase activity higher than phosphatase activity, has the highest Kinase/Biphosphatase activity ratio (740-fold) expressed by PFKFBs [5]. The essential activity of PFKFB3 is to catalyze the synthesis of fructose-2,6-bisphosphate (F2,6BP), which subsequently activates phosphofructokinase-1 (PFK-1) that enables upregulating of the glycolytic flux [6]. Previous findings indicated that PFKFB3 can be regulated by several tumor-related genes, including phosphatase and tensin homolog (PTEN) [7], mitogen activated protein kinase (MAPK) [8], phosphoinositide 3-kinases (PI3K) [9], and hypoxia-inducible factor 1-alpha (HIF-1a) [10], in cancer cell lines. However, in spite of this abundance of potential targets in glycolytic flux, PFKFB3, has received considerable attention. In recent years, potent and selective PFKFB3 inhibitors have been identified and investigated as potential anticancer agents. 3PO (3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one), a chalcone group, is the first small-molecule inhibitor of PFKFB3 in this class to be evaluated *in vitro* and human-derived tumor studies. 3PO has demonstrated impressive single-agent activity via prohibit tumorigenic growth of breast, leukemia, and lung adenocarcinoma cells [11]. Moreover, it exhibits synergistic activity when administered in combination with a multi-kinase inhibitor Sunitinib L-malate in preclinical studies in Human endothelial cells (HUVECs) [12]. On the other hand, PFK15, also a potent and selective PFKFB3 inhibitor, displayed a more (aprx.100-fold) potent effect than 3PO in preclinical studies [13]. PFK15 inhibit cell migration and invasion in HNSCC cell culture and block the metastasis to lung in the xenograft mice models [14]. PFK158, an PFK15-based synthetic inhibitor assessed through active phase I clinical trials, exhibits a high affinity to the ATP binding site and elicits a significant antitumor effect on lung, glioblastoma, melanoma, pancreatic, and colon cancer. In more recent study, Gustafsson et al. revealed that RNAi or pharmacological inhibition of PFKFB3 is involved in DNA damage response and deoxynucleotide incorporation upon DNA repair. In addition, the results showed that PFKFB3 inhibition plays a critical role in homologous recombination and defective nucleotide incorporation. KAN0438757 is a newly developed small molecule inhibitor of PFKFB3 that impairs DNA repair by disrupting deoxynucleotide incorporation and inhibits survival of transformed cells [15]. Previous studies have found that PFKFB3 expression is increased significantly in some cancers [16] including lung cancer; however, whether KAN0438757, a novel and specific small molecule inhibitor of PFKFB3, has anti-neoplastic activity on lung cancer is unknown. Since lung cancer is very resistant to chemotherapy, and PFKFB3 has been shown to crucial effects on its survival, the objective of the study was to reveal the potential therapeutic role of KAN0438757 by targeting PFKFB3.

In this study, we found that the pharmacological inhibition of PFKFB3 It also arrested the migration and induced DNA damage and apoptosis *in vitro*. Furthermore, KAN0438757 disrupted Mitochondrial Membrane Potential (MMP) and induced Autophagy biomarkers; LC3 and Beclin1. Overall, KAN0438757 treatment may be a novel approach to suppressing Lung cancer.

Materials and Methods

Main reagents, Antibodies, and KAN0438757

Protease-phosphatase inhibitor cocktail, trypan blue, Tris HCl, trypsin-EDTA, dimethyl sulfoxide (DMSO), TEMED, Sodium azide (NaN3), luminol, Skim milk powder, P-coumaric acid, bovine serum albumin (BSA) and others was obtained from Sigma Aldrich company. Dulbecco's modified Eagle's medium (DMEM) fetal bovine serum (FBS), penicillin/streptomycin, and phosphate buffered saline (PBS) were purchased from Gibco. WST-1 assay was obtained from Boster (USA). Mouse anti-GAPDH, p62, Beclin 1, LC3, PARP antibodies used in Western blot studies were obtained from SantaCruz Biotechnology (USA), and Anti-mouse antibody horseradish peroxidase conjugated was obtained from Invitrogen (USA).

PFKFB3 inhibitor KAN0438757 (Cat No: S0400) was purchased from Selleck Chemicals GmbH (Houston, TX 77014 USA). It was dissolved in sterile dimethyl sulfoxide (DMSO, sterile-filtered, suitable for cell culture) at a final concentration of 1 mM, and then stored in aliquots at –80 °C and working concentrations were diluted in culture medium.

Cell Lines and Culture Conditions

All cell lines used (A549, H1299, and COS7) were provided by ATCC (American Type Culture Collection, Manassas, USA). A549 and COS7 were maintained in DMEM (Dulbecco's modified Eagle's medium containing with 10%

fetal bovine serum (FBS) and 1% penicillin-streptomycin (Gibco, Grand Island, NY, USA). H1299 cells were cultured in RPMI (Roswell Park Memorial Institute) 1640 medium added with 10% FBS and 1% penicillin-streptomycin. Cell lines used in this study were grown by culturing at 37 ˚C, 95% humidity and 5–6% CO_2.

Cell Viability

The viability of A549, H1299, and COS7 cells was assessed with the WST-1 assay kit (Boster, USA). Briefly, the cells were treated with KAN0438757 at concentrations of 1, 5, 10, 25, 50, and 100 µM) and time point (24, 48, 72h). Then, 10µl WST-1 solution was added into the wells. The plates were kept out of light and incubated in a 37 °C CO_2 (5%) incubator for 3h. After that, it was measured spectrophotometrically at 420–480 nm using an ELISA microplate reader (Spectra Max 384 Plus).

Real-Time Cell Analysis (RCTA)

A549 and H1299 cells (1.5×10^4 cells/well) and A549 cells (1×10^4 cells/well) were plated into the E-Plate16 and then incubated in xCELLigence RTCA instrument (ACEA Bioscience, CA, USA) at 37 °C and %5 CO_2. E-16 plates have microelectrodes to measure the cellular impedance which was recorded every 15 min for 96 h by xCELLigence RTCA. After attaching them to the plate, the cells were given different doses of KAN0438757 and then incubated for 48 hours. Recorded data were analyzed by RTCA Software (v1.2, ACEA Biosciences Inc.).

Single Cell Gel Electrophoresis (Comet Assay)

A549 cells were seeded in 6-well plates (2×10^5) and treated with incubated with indicated doses of KAN0438757 for 48 h. At the end of the incubation, the wells were washed with PBS and the cells were dissociated using trypsin. The cells were then centrifuged and re-suspended with PBS and mixed with 0.5% low melting point agarose (LMA). After taking 20 µl of this mixture, it was spread on a slide covered with normal melting point agarose (NMA) and the agarose was frozen at 4 °C by covering the coverslip for 45 minutes. Coverslips were removed and placed in cold lysis solution (2.5M NaCl, 100mM EDTA, 10 mM Tris, 10% DMSO, 1% Triton X-100, (pH 10)) at 4 °C for 60 min. Slides were rinsed with PBS and placed in a buffer-filled gel electrophoresis. Electrophoresis was performed at 28V, 300 mA for 25 minutes and slides were neutralized with solution (pH 7.5) and rinsed with PBS. Slides were stained with Ethidium bromide (10 µg/mL) for 15 minutes and viewed under a fluorescent microscope. Opencomet program was used for analysis.

Wound-Healing Assay

Cells were seeded in 6-well cell culture dishes at 25×10^4 in each well. After the cells adhered to the surface, the cells were scraped to form a straight line with the help of a 20 µl pipette tip. Each well was washed twice with PBS so that the removed cells would not re-adhere to the surface. Wound areas were observed with an inverted microscope and photographed at 0, 24, and 48 hour time periods [17].

Acridine Orange And Ethidium Bromide double staining

Acridine orange and Ethidium bromide (EtBr/AO) staining was performed according to the protocol described previously [18]. A549 cells were treated with the indicated doses of KAN0438757 and then EtBr/AO(1:1) and added to the wells for 15 minutes at room temperature. Stained cells were observed with a fluorescence microscope (Olympus, Japan).

Mitochondrial membrane potential ($\Delta\Psi m$) Assay

Monolayers cells in the logarithmic growth stage were inoculated into glass-bottom culture plates at 2×10^5 cells per well. Then, cells were treated with KAN0438757 for 48 h. Slides were then washed and stained with the cationic dye JC-1 (5 mg/mL) for 15 min at 37 ℃. The stained cells were analyzed by fluorescence microscope (Olympus, Japan). After staining, seven areas per well were randomly captured by the microscope. Each experiment was repeated at least three times.

Cell lysate preparation and Western Blot Analysis

Cells were collected with a plastic scraper by washing three times with cold PBS after 48 hours of treatment and transferred to numbered microcentrifuge tubes. After centrifugation at 6600 rpm for 10 minutes, it was dissolved in lysis (RIPA) buffer and then incubated on ice for 60 minutes. After the incubation period, it was centrifuged for 10 minutes at 14,000 rpm at 4 °C. Protein content in the supernatants was measured by their absorbance at 595 nm in the spectrophotometer using BSA as a

standard. (Biomethod-GBC (Bradford)). With the help of electrophoresis system (Bio-RadTrans-Blot cell, BioRad, USA), protein samples and marker were fractionated on SDS-PAGE (10–12%) gel. Then, the proteins separated in the gel were transferred to Polyvinylidenedifluoride (PVDF) membrane. Blockage was made with BSA. The membrane was incubated overnight with primary antibodies first and then with secondary antibodies. Subsequently, washing was done with TBS-T. The results were determined by chemiluminescence method so that the membranes were visualized by X-Ray device.

Statistical Analysis

A statistical analysis were performed using the GraphPad Prism 8.0.2 software (USA). Data were obtained from three separate experiments. The multiple comparison Post-Hoc Tests in "One-way ANOVA" method or independent two-sample Student's t-test were performed. $P < 0.05$ was accepted as significant in the analyzes.

Results and Discussion

The PFKFB3 inhibitor KAN0438757 effectively blocked the proliferation of non-small cell lung carcinoma cells

Colorimetric assay (WST-1 based) and Real-Time Cell Analysis (RCTA) was carried out to investigate the effect of KAN0438757 on cell viability and the proliferation of cell lines. The Non-small cell lung cancer cell lines (NSCLC H1299 and A549) or COS7 were treated with increasing concentrations of KAN0438757 (0, 5, 10, 25, 50 and 100 μM). The WST-1 results showed that KAN0438757 inhibited cell viability in a dose-time dependent manner (supplementary data, 24/48/72 h) when compared to untreated control and the cell viability of the A549 and H1299 cell lines with KAN0438757 for 48 h were shown in Fig. 1, *A* and *B*. The IC50s of A549 for KAN0438757 was pronouncedly lower (35.2 μM) than that of H1299 (52.4 μM) for 48h. RCTA was performed to further confirm the effect of KAN0438757 on the inhibition of lung cancer cells proliferation for 48 h. The RCTA results of A549 cells (Fig. 1, *D*) further proved that

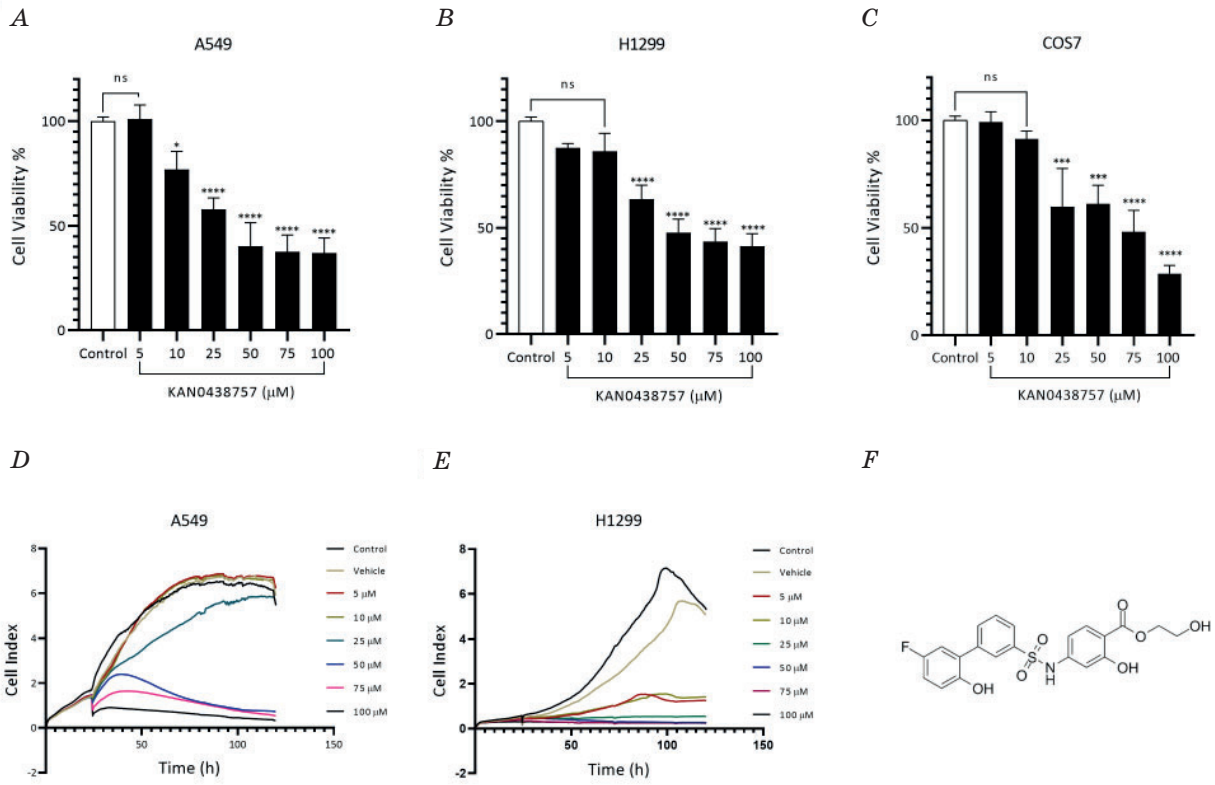

Fig. 1. **KAN0438757 inhibits the proliferation and viability of human non-small cell lung carcinoma cell:**
A, *B* and *C* — A549, H1299 and COS7 cells were treated with KAN0438757 (0, 5, 10, 25, 50, and 100 μM) for 48 h, and cell viability was examined using WST-1 assays;
D and *E* — A549 and H1299 were seeded in the xCELLigence system plate for 24 h and were then exposed to increasing concentrations of KAN0438757 for 120 h; *F* —the chemical structure of KAN0438757

KAN0438757 had a stronger antiproliferative activity. Similar results also observed in H1299 cells (Fig. 1, *E*).

KAN0438757 stimulate DNA damage

In order to investigate the effects of KAN0438757 on DNA damage, the Comet Assay was performed in A549 cell line using alkaline single-cell gel electrophoresis (Fig. 2). Almost all of comets in the control and 5, 10 μM -treated cells showed no fluorescent tails, which indicates that the nuclear DNA was intact. In contrast, the exposure of the cells to 25 and 50 μM concentrations of KAN0438757 for increased the number of typical comets with tails of different fluorescence intensities, which is an evident indicator of DNA strand breakage (Fig. 2, *A*). The values of the% tail DNA, the tail length, were significantly increased in the KAN0438757 treated groups compared with the control group (Fig. 2, *B*, *C*). These results suggest that KAN0438757 can induce DNA damage in A549 cells.

KAN0438757 inhibits migration of A549 lung cancer cells

Furthermore, having established that a low dose of KAN0438757 has no effect on cell viability, we used a wound-healing assay in order to investigate whether these concentrations affect cell migration in the A549 cell line. The data showed that a low concentration of KAN0438757 (less than 25 μM) effectively inhibited the cell migration (Fig. 3, *A*). The distance was found to be wide in A549 cells treated with KAN0438757 compared with the control (Fig. 3, *A*), indicating that KAN0438757 effectively inhibits cell migration ability in A549 cells (Fig. 3, *B*).

AO/EB double-staining in KAN0438757 treated-lung adenocarcinoma cells

After treatment with KAN0438757 inhibitors for 48 h, apoptotic cell death was determined by using Double-Staining with Acridine Orange-Ethidium Bromide on A549 lung cancer cells. As shown Fig.4, *A*, in the untreated control group, the green color labeled cells were visible. On the other hand, KAN0438757 treated groups, the intensity of the orange color also increased, illustrating a higher rate of apoptosis, which had the highest number of apoptotic cells in the 25 μM group.

PFKFB3 inhibitor KAN0438757 triggers mitochondrial membran instability in A549 cells

The mitochondrial membrane potential (ΔΨm) is crucial for mitochondrial homeostasis which is a fundamentally important determinant of cell predestination.

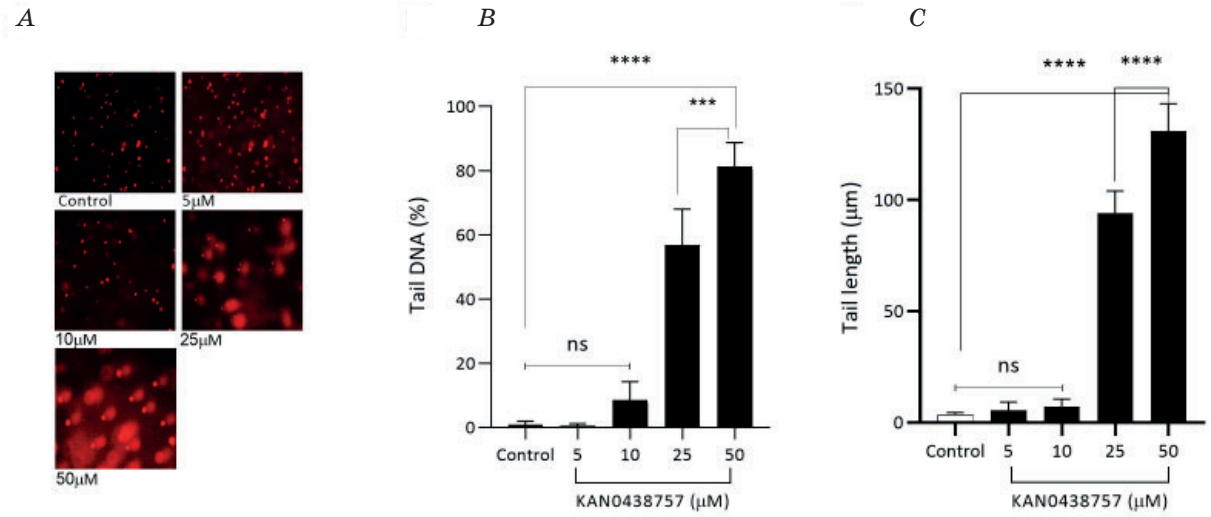

Fig. 2. **Single-cell gel electrophoresis for effects of KAN0438757 on DNA damage in A549 cells**
DNA damage was detected by the alkaline single-cell gel electrophoresis in A549 cells exposed to KAN0438757 at 5, 10, 25, 50 μM for 48h. DNA fragments were stained with Ethidium bromide (20 μg/mL) for 10 minutes and images (*A*) obtained under a fluorescent microscope. Opencomet program was used for analysis; *B* — Tail DNA; *C* — Tail length

To investigate whether KAN0438757 induces A549 cell mitochondrial membrane instability, we used JC-1 staining which is the cationic dye; red-colored cells indicate healthy mitochondria while green-colored indicate loss of mitochondrial membrane potential. As depicted in Fig. 4, *B*, with increasing the KAN0438757 concentration, the intensity of green color also increased, indicating a higher rate of loss of mitochondrial membrane potential in A549 cells. Furthermore, the results showed a significant positive correlation between the loss of mitochondrial membrane potential and the KAN0438757 concentration indicating that KAN0438757 triggers mitochondrial membrane instability.

KAN0438757 induces autophagy in lung adenocarcinoma cell

To further assess whether KAN0438757 affects autophagy, which are key events that maintains cellular homeostasis. To investigate the underlying mechanism of autophagy induced by KAN0438757, the expression of p62/SQSTM1, LC3 I-II, and Beclin1 was measured by western blotting. Results showed that KAN0438757 in A549 cells did not change the protein expression level of p62/SQSTM1. As shown in Fig. 4, *D*, the protein expression of LC3 I was down-regulated and LC3 II was up-regulated in A549 cell line. Besides, KAN0438757 treatment increased the Beclin1 protein expression level. Collectively, these findings indicated that KAN0438757 treatment could induce autophagy in A549 cells.

PFKFB3 is a member of a the bifunctional enzyme family of 4 such proteins of which are known a rate-limiting enzyme and essential control point in the glycolytic pathway. Beyond its glycolytic activities, PFKFB3 is involved in numerous biological processes such as promoting cell cycle progression [13], inhibiting apoptosis [19], stimulating migration/invasion [29] and having a critical factor for homologous recombination [15]. Previous studies have revealed that PFKFB3 expression is increased significantly both non-small cell lung cancer (NSCLC) [16] and small-cell lung cancer (SCLC) [20]. It identified that PFKFB3 has a crucial role in homologous recombination and developed a selective PFKFB3 small molecule inhibitor-KAN0438757 [15]. However, to the best of our knowledge, there is currently no study available that describes the effects of KAN0438757 on lung cancer. Moreover, the properties of KAN0438757 regarding anti-neoplastic and induction of programmed cell death and the underlying mechanism(s) in lung cancer cells are unclear. Therefore, we assessed the effect of KAN0438757 in human lung cancer and normal cells.

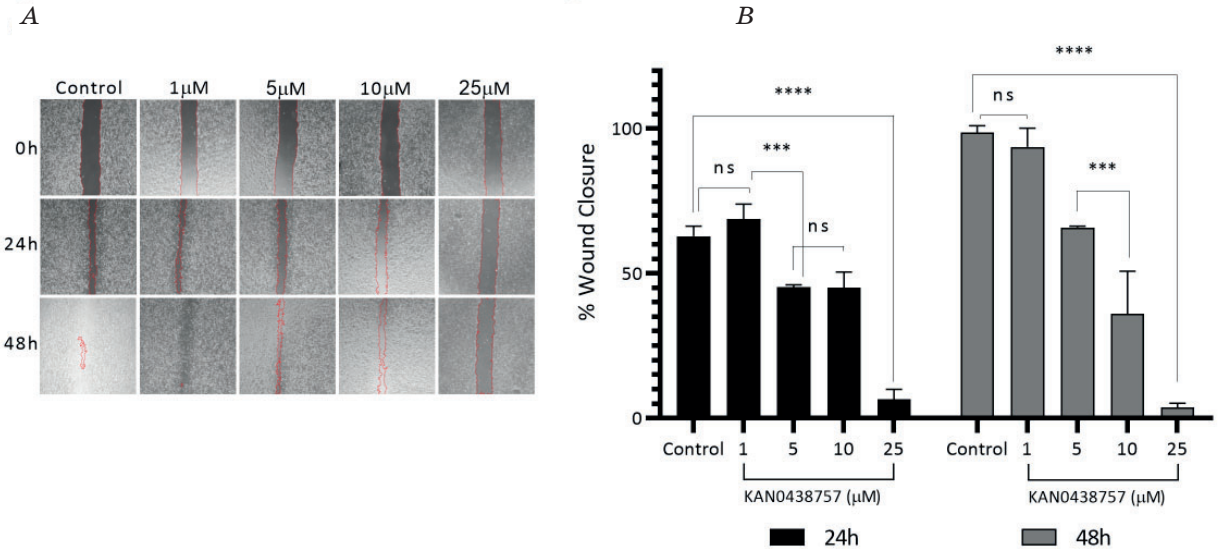

Fig. 3. **KAN0438757 inhibits cell migration**
The A549 cells were treated with KAN0438757 (1–25 μM) for 48 h, and the migration of cells was determined by the wound-healing assay:
A — Microscopic images of A549 cells after different treatments of KAN0438757; *B* — Wound closure. All results are representative of three separate experiments, and representative results are shown

In this study, we investigated the potential anti-neoplastic effect of KAN0438757 in lung cancer cell lines, and the possible mechanisms for it by determining cell viability, cell proliferation, DNA damage, and cell migration, the morphological analysis of apoptosis, apoptosis, and autophagy protein expression.

Firstly, the anti-proliferative effects of KAN0438757 were analyzed on A549 and H1299 cells. Similar to previous research [21], our results demonstrated that KAN0438757 effectively reduced cell viability and inhibited the growth of A549 and H1299 lung cancer cells.

A small molecule inhibitor were reported inhibition of PFKFB3 could lead to DNA

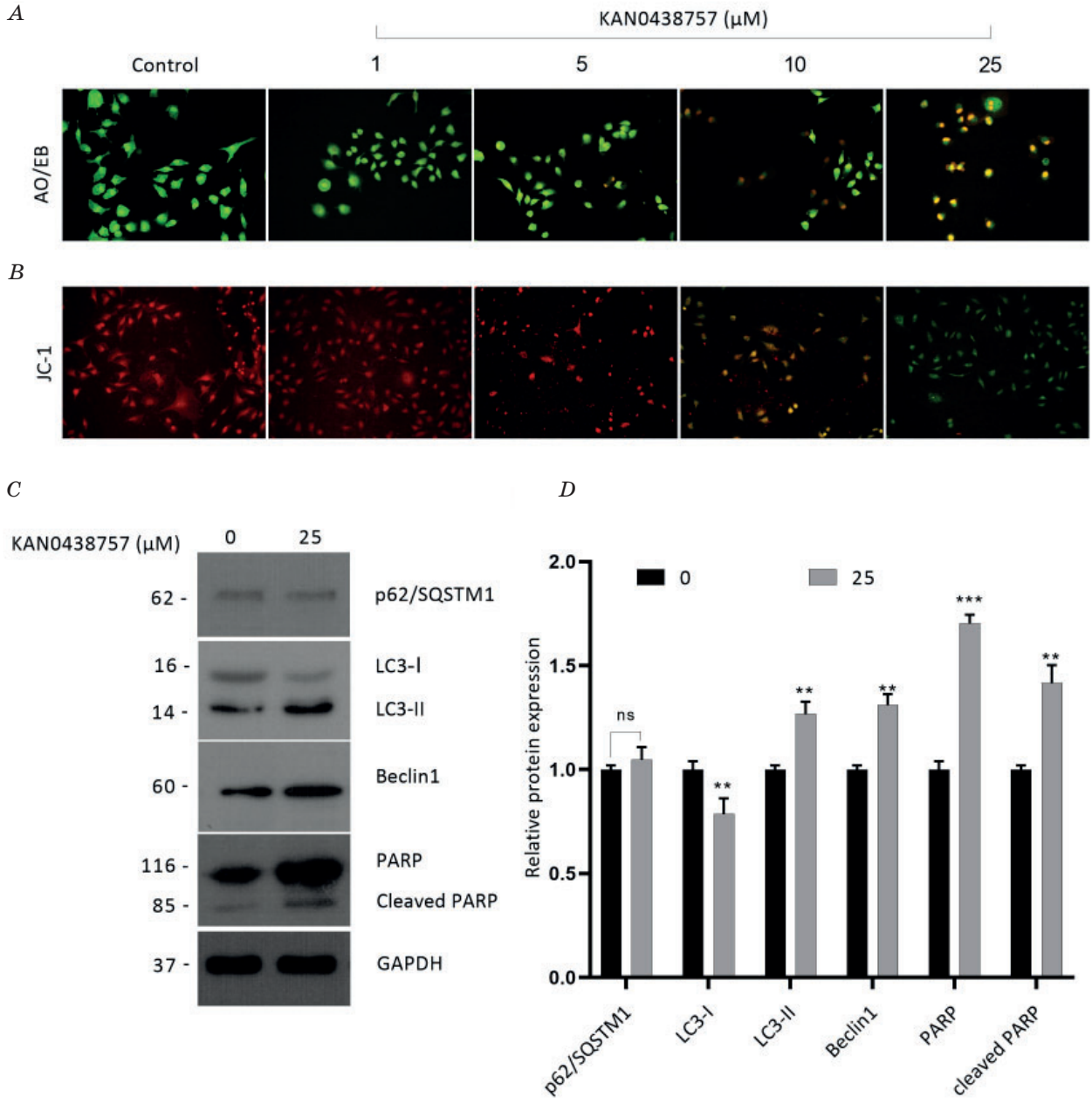

Fig. 4. **KAN0438757 induce apoptosis and causes the activation of autophagy-related markers in A549 lung cancer cells**

Representative fluorescence inverted microscopic images of AO/EB (*A*) and Δψm labeled with JC-1 (*B*). Expression of p62/SQSTM1, LC3 I-II, Beclin1, PARP, and GAPDH were examined by Western Blotting analysis (C–D). The experiment was repeated at least 3 times.
$n = 3$, $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with that of control

damage and cell death. PFK15, a PFKFB3 inhibitor, cause DNA damage impairing DNA repair through AKT in HCC cells [22]. In this study, a comet assay quantitatively detected KAN0438757-induced DNA damage in A549 cells. In line with previous reports, our results showed that the Tail DNA and Tail length of the KAN0438757 treatment groups (25 and 50 μM) were higher than those of the other groups, indicating more severe DNA damage. These results indicated that KAN0438757 triggered DNA damage at the single-cell level, which may lead to apoptosis in lung cancer cell line.

The disruption of mitochondrial membrane potential (Δψm) can lead to the activation of the caspase cascade including caspase-9 and caspase-3 is activated, which are crucial effectors of apoptosis [25]. Once the caspase-3 is activated, the downstream effector proteins are cleaved such as PARP, which plays essential roles in DNA repair and the programmed cell death process [26]. In the current study, it was observed that treatment with KAN0438757 induced cleavage of PARP. In addition, KAN0438757 treatment of A549 cells significantly increased the red fluorescence intensity, indicating loss of Δψm, which is an early event in the apoptotic process. Furthermore, our AO/EB dual staining data confirmed that KAN0438757 treatment increased the apoptosis at 10 and 25 μM dose of KAN0438757. These results are consistence with the former results that either siRNA silencing [19] or the pharmacological inhibition of PFKFB3 [27] induces cell apoptosis. All the results discussed above directly suggest that KAN0438757 induces A549 cell apoptosis via mitochondria-mediated pathway.

PFKFB3 also plays a role in angiogenesis and metastasis, which can indirectly promote tumor growth [28]. Previous *in vivo* and *in vitro* studies have indicated that PFKFB3 is involved in the migration in nasopharyngeal carcinoma [29], breast cancer [28], non-small-cell lung cancer [16], gastric cancer[30], and osteosarcoma Saos-2 cells [31]. In the present study, KAN0438757 was applied as an PFKFB3 inhibitor in lung cancer cell line. The results indicated that KAN0438757 significantly blocked migration of A549 cells. This result was in accordance with previous reports, where KAN0438757 was observed to reduced the migration capabilities of HCT-116 and HT-29 cell line, as well as HUVECs cells [21].

Autophagy may play different roles not only in the degradation of macromolecules and damaged organelles, but also in different steps of tumor development. It may play a role in inhibiting proliferation and metastasis of cancer cells during tumorigenesis by autophagic activity. On the other hand, as a pro-survival mechanism, cancer cells initiate autophagy by breaking down intracellular organelles and proteins, thus providing the most important energy support of cancer cells [32, 33]. Previous *in vitro* experiments showed that either knockdown of PFKFB3 or pharmacological inhibition, including PFK158, and 3-PO, participated in autophagic flux and markers of autophagy induction [34, 35]. Knockdown of PFKFB3 resulted in the degradation of p62/SQSTM1. Meanwhile, PFKFB3 siRNA transfection, led to a significant increase LC3-II which involved in the assembly of autophagosomes in the inner and outer membrane [35]. Indeed, we found that the inhibiting of PFKFB3 after KAN0438757 treatment is positively connected with autophagy in A549 cells. Western blotting results revealed that the protein expression LC3 II and Beclin1 were significantly increased in the KAN0438757 treatment, while the expression of p62/SQSTM1 was not significantly increased in A549, suggesting that the KAN0438757 treatment promotes autophagy. Accumulating evidence has shown that autophagy can promote cell survival and maintenance of cellular homeostasis by degrading damaged organelles and macromolecules in cells, but it should not be overlooked that autophagy can also promote cell death due to its tight association with apoptosis [36]. In conjunction with analysis of AO/EB, Δψm, and western blotting demonstrated that KAN0438757 treatment (25 μM, 48 h) induced cell death.

Conclusions

KAN0438757 may hold great promise as a novel small molecules agent for lung cancer treatment. Further investigation is needed to validate the contribution of KAN0438757 to lung cancer therapy *in vitro* and *in vivo*.

Declaration of competing interest

Authors have no conflict of interest.

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KAN0438757 — ИНГИБИТОР PFKFB3, ЯКИЙ ІНДУКУЄ ЗАПРОГРАМОВАНУ КЛІТИННУ СМЕРТЬ І ПРИГНІЧУЄ КЛІТИННУ МІГРАЦІЮ В КЛІТИНАХ НЕДРІБНОКЛІТИННОЇ КАРЦИНОМИ ЛЕГЕНІВ

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Мета. PFKFB3 є гліколітичним активатором, який надмірно експресується при раку легенів людини і відіграє вирішальну роль у багатьох клітинних функціях, включаючи запрограмовану клітинну смерть. Незважаючи на велику кількість малих молекул, описаних як інгібітори PFKFB3, деякі з них продемонстрували невтішні результати *in vitro* та *in vivo*. З іншого боку, булол розроблено KAN0438757, селективний і потужний маломолекулярний інгібітор. Однак вплив KAN0438757 на клітини недрібноклітинної карциноми легень залишається невідомим. Ми намагалися розшифрувати вплив KAN0438757 на проліферацію, міграцію, пошкодження ДНК і запрограмовану клітинну смерть у клітинах недрібноклітинної карциноми легенів.

Методи. Вплив KAN0438757 на життєздатність клітин, проліферацію, пошкодження ДНК, міграцію, апоптоз і аутофагію в клітинах недрібноклітинної карциноми легені було перевірено за допомогою WST-1, клітинного аналізу в реальному часі, кометного аналізу, тесту міграції загоєння ран, аналізів подвійного фарбування MMP/JC-1 і AO/ER, а також вестерн-блоту.

Результати. Показано, що KAN0438757 значно пригнічував життєздатність і проліферацію клітин A549 і H1299 і пригнічував міграцію клітин A549. Що ще важливіше, KAN0438757 викликав пошкодження ДНК і апоптоз, і це супроводжувалося посиленням розщепленого PARP у клітинах A549. Крім того, лікування KAN0438757 призвело до збільшення LC3 II і Beclin1, що вказувало на те, що KAN0438757 стимулював аутофагію.

Висновки. Загалом, націлювання на PFKFB3 за допомогою KAN0438757 може бути багатообіцяючим ефективним підходом до лікування, що потребує подальшої *in vitro* та *in vivo* оцінки KAN0438757 як терапії клітин недрібноклітинної карциноми легенів.

Ключові слова: PFKFB3; KAN0438757; апоптоз; аутофагія; рак легенів.