

Effects of sesamin on the chemosensitivity, invasiveness and immune evasion mechanism of human lung adenocarcinoma

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Abstract. Lung adenocarcinoma (LUAD) is a major cause of cancer-related mortality worldwide. Sesamin is a lignan with potent anticancer properties and promising therapeutic potential. In the present study, it was aimed to investigate the specific mechanisms through which sesamin reduces cell invasiveness and cancer-associated immunosuppression in LUAD cells. The effects of sesamin on LUAD cell invasiveness were investigated using a wound healing assay and anoikis resistance assay. NK-92 MI cells were used to analyze cancer-associated immunosuppression upon sesamin treatment. The therapeutic effect of sesamin in LUAD was measured using a subcutaneous mouse model. Our results indicated that sesamin inhibited the proliferation, survival and migration of LUAD cells (A549 and CL1-5) in a dose-dependent manner. Sesamin also enhanced the proapoptotic effects of chemotherapeutic agents such as docetaxel and paclitaxel through the activation of the caspase-3/poly(ADP-ribose) polymerase pathway. In addition, sesamin reduced cancer cell migration and anoikis resistance by downregulating the expression of N-cadherin and inhibiting the phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR) pathway. It also induced the downregulation of programmed death ligand 1 through hsa-microRNA-34a-5p, resulting in the increased cytotoxicity of natural killer cells. This sequence of events consequently interfered with the immune evasion mechanism of LUAD cells. In conclusion, sesamin has a multifaceted effect on the migration, anoikis resistance and antitumor immunity of LUAD cells, indicating its potential as adjunctive therapy.

Introduction

Lung adenocarcinoma (LUAD), the most prevalent subtype of non-small-cell lung cancer, is a leading cause of cancer-related mortality worldwide and accounts for ~40% of all cases of lung cancer (1). Despite advancements in chemoradiotherapy, the 5-year overall survival rate of LUAD remains 15% in Western countries, with particularly high mortality rates observed in Taiwan (2). Given the challenges associated with the early detection of LUAD and the limitations in the current therapeutic strategies, innovative treatment modalities are urgently required.

Naturally derived compounds, particularly those from dietary sources, have attracted considerable attention because of their potential for augmenting conventional cancer therapies (3). Sesamin, a major lignan extracted from sesame seeds (*Sesamum indicum*), exhibits diverse biological effects, including antioxidative, anti-inflammatory and antineoplastic effects (3,4). Its anticancer effects, particularly its ability to inhibit cell proliferation and metastasis while promoting apoptosis and autophagy, render it a promising treatment for cancer (5). Sesamin is also effective against various cancer cell lines, indicating its broad therapeutic potential (6-8). It influences the activation of caspase-3, a key process in apoptotic pathways, and the subsequent cleavage of poly(ADP-ribose) polymerase (PARP) (9,10). Despite the aforementioned benefits of sesamin, the specific mechanisms through which it induces apoptosis in LUAD cells, particularly when used in combination with chemotherapy, remain unknown.

Epithelial-mesenchymal transition (EMT) is a key process in cancer pathogenesis, conferring cells with increased invasive and metastatic potential through the modulation of markers such as E-cadherin and N-cadherin (11). Although the inhibitory effects of sesamin on cell migration and invasion through EMT pathways have been well documented in prostate cancer, gallbladder cancer and head and neck squamous cell carcinoma (12), the detailed mechanisms underlying these effects remain to be fully elucidated. Regarded as an integral component of cell proliferation and survival, the PI3K/AKT/mTOR signaling pathway has been implicated in the development of resistance to cancer therapies (13,14). In the present study, the signaling pathways underlying the inhibitory effects of

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sesamin on EMT were investigated to evaluate the potential of sesamin as an antitumor agent for LUAD.

The expression of programmed death ligand 1 (PD-L1), a major ligand of programmed death receptor 1, plays a key role in immune checkpoint inhibition and tumor immune evasion (15,16). PD-L1 blockade offers valuable insights into its role in immune-mediated tumor suppression through checkpoint-based immunotherapy (17). In various cancers, including gastric cancer, colorectal cancer, B-cell lymphoma and prostate cancer, numerous microRNAs (miRNAs) act as negative regulators of PD-L1 and immune evasion mechanisms (18,19). Specifically, hsa-miR-34a has been found to play a critical role in regulating antitumor immunity and the tumor immune microenvironment (20). It targets PD-L1 and serves as a potential immunotherapeutic target in acute myeloid leukemia (21). However, it remains unknown whether sesamin regulates PD-L1 and immune evasion through hsa-miR-34a in LUAD.

In the present study, the tumor-suppressing effects of sesamin on LUAD cells were examined. The present results indicated that sesamin enhanced the therapeutic efficacy of chemotherapeutic agents and modulated EMT through the PI3K/AKT/mTOR signaling pathway. The findings also indicated that sesamin had a regulatory effect on the antitumor immune response through the miR-34a/PD-L1 axis, thereby providing valuable insights into the potential of sesamin as an adjunctive therapy for patients with LUAD.

Materials and methods

Cell culture. The human lung cancer cell line A549 (cat. no. CCL-185) and the human NK cell line NK-92MI (cat. no. CRL-2408) were obtained from the American Type Culture Collection. The lung cancer cell line CL1-5 was established by Professor Pan-Chyr Yang (22). A549 and CL1-5 cells were cultured on Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS; both from Gibco; Thermo Fisher Scientific, Inc.). The NK-92MI cells were maintained in alpha minimum essential medium (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 0.1 mM 2-mercaptoethanol, 0.2 mM inositol, 0.02 mM folic acid, 12.5% horse serum, and 12.5% FBS. All cell lines were subcultured until they reached 80% confluence and were incubated in a humidified atmosphere with 5% CO₂ at 37°C.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide cell viability assay. Cell viability was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, A549 and CL1-5 cells were plated at a density of 1x10⁴ cells per well in 96-well culture plates containing DMEM and were allowed to adhere overnight. Subsequently, the cells were treated with various concentrations of sesamin (0, 25, 50 and 100 µM) for 24 h, followed by incubation with 50 µl of MTT dye (0.5 mg/ml; MilliporeSigma) for 4 h at 37°C in a CO₂ incubator. After the supernatant was discarded, formazan crystals were dissolved in 150 µl of dimethyl sulfoxide. Finally, a spectrophotometric plate reader was used to measure the absorbance of the dissolved formazan at 540 nm, with a reference wavelength of 630 nm.

Colony formation assay. LUAD cells were seeded onto 6-well plates at a density of 3x10³ cells per well. A colony was defined as a group of at least 50 cells. After 7 days of incubation for colony formation, the colonies were fixed with 3.7% formaldehyde for 20 min and stained with 0.05% crystal violet (w/v) for 20 min at room temperature. Finally, after the dye was extracted with 10% acetic acid, the colonies were quantified by measuring their absorbance at 590 nm.

Immunofluorescence (IF) staining. LUAD cells were seeded onto a chamber slide (Sigma-Aldrich; Merck KGaA) and treated with or without sesamin (50 µM) for 24 h. IF staining was conducted following a previously described protocol (23). Briefly, LUAD cells were incubated with a primary antibody against Ki-67 (1:50; cat. no. ab15580; Abcam) for 1 h at room temperature. Subsequently, the cells were incubated with a secondary antibody conjugated to Alexa Fluor 647 red fluorescent dye (1:50; cat. no. ab150079; Abcam) for another 1 h. Finally, Ki-67⁺ cells were visualized using a Nikon Ti2 fluorescence microscope (Nikon Corporation).

Transfection of miRNA mimics and inhibitors. A synthetic negative control (NC) mimic (sense: UUGUACUACACA AAAGUACUG; antisense: GUACUUUUGUGUAGUACA ANN), an NC inhibitor (CAGUACUUUUGUGUAGUACAA; full-length nucleotide with 2'-methoxy modification), an hsa-miR-34a-5p mimic (sense: UGGCAGUGUCUUAGC UGGUUGU; antisense: AACCAGCUAAGACACUGC CANN), and an hsa-miR-34a-5p inhibitor (ACAACCAGCUAAGACACUGC; full-length nucleotide with 2'-methoxy modification) were purchased from MedChemExpress. Transfection was performed using a ViaFect Transfection Reagent (Promega Corporation) following the manufacturer's instructions. LUAD cells were seeded onto a 6-well plate at a density of 3x10⁵ cells per well and were transfected with either the NC mimic (25 nM), NC inhibitor, hsa-miR-34a-5p mimic (25 nM), or hsa-miR-34a-5p inhibitor (25 nM) for 24 h in a 37°C incubator. Following transfection, the cells were treated with sesamin for an additional 24 h under the same incubation conditions.

Western blot analysis. The LUAD cells were treated with activators of PI3K (740 Y-P; cat. no. HY-P0175; MedChemExpress), AKT (SC79; cat. no. 305834-79-1; Sigma-Aldrich; Merck KGaA), or mTOR (MHY1485; cat. no. 326914-06-1; Sigma-Aldrich; Merck KGaA) for 30 min, followed by treatment with sesamin. Cells were then harvested and washed with ice-cold phosphate-buffered saline (PBS). Total cell lysates were obtained using a 1X radioimmunoprecipitation (RIPA) lysis buffer (Thermo Fisher Scientific, Inc.). After protein concentrations were quantified using a BCA Protein Assay kit (Thermo Fisher Scientific, Inc.), electrophoresis and transfer were conducted as previously described (24). In brief, a 12% SDS-PAGE gel was prepared using 30% acrylamide. Protein extracts (30 µg) were separated by electrophoresis and subsequently transferred onto immobilized polyvinylidene fluoride (PVDF) membranes. After blocking with 5% skim milk for 1 h, PVDF membranes were incubated with primary antibodies at 4°C for 2 h, followed by incubation with horseradish-peroxidase-conjugated anti-mouse

(1:3,000; cat. no. GTX213111-01; GeneTex, Inc.) and anti-rabbit (1:3,000; cat. no. GTX213110-01; GeneTex, Inc.) secondary antibodies for 1 h. The primary antibodies used were the following: E-cadherin (1:3,000; cat. no. ab231303; Abcam), N-cadherin (1:3,000; cat. no. ab76011; Abcam), vimentin (1:3,000; cat. no. GTX100619; GeneTex, Inc.), phosphorylated (p)-PI3K/PI3K (1:1,000; cat. no. GTX636838/GTXGTX641922; GeneTex, Inc.), p-AKT/AKT (1:1,000; cat. no. GTX640358/GTX26076; GeneTex, Inc.), p-mTOR/mTOR (1:1,000; cat. no. GTX132803/GTX638220; GeneTex, Inc.), PD-L1 (1:3,000; cat. no. GTX635975; GeneTex, Inc.) and glyceraldehyde 3-phosphate dehydrogenase (1:5,000; cat. no. GTX100118; GeneTex, Inc.). Immunoreactive bands were visualized using an ImageQuant LAS 4000 biomolecular imager (GE Healthcare Life Sciences), and protein expression levels were quantified with UN-SCAN-IT gel analysis software (version 6.1).

Gap closure assay. A wound healing assay was conducted using a 3-well silicone insert (Ibidi GmbH), and uniform scratches were created across the cell monolayer. A549 and CL1-5 cells (3×10^4) were treated with various concentrations of sesamin (0, 25, 50 and 100 μ M) for 24 h. Following treatment, non-adherent cells were gently washed away with PBS, and the remaining adherent cells were cultured on a serum-free medium for an additional 24 h. Migratory cells in the wound area were imaged using a ZEISS microscope (Carl Zeiss AG). Finally, the gap width was measured at multiple defined points along each scratch by using ImageJ software v.1.44p (National Institutes of Health).

Anoikis resistance assay. Briefly, 24-well plates were coated with poly(2-hydroxyethyl methacrylate) (20 mg/ml in ethanol) and allowed to dry for 24 h at room temperature. A549 cells (3×10^5) were seeded onto these low-attachment plates containing DMEM. After 24 h of seeding, the cells were treated with sesamin at concentrations of 0, 25 and 50 μ M. At 2, 4 and 6 days after treatment, cell aggregates were collected and disaggregated into single-cell suspensions through gentle pipetting. Finally, the cells were stained using a 0.4% trypan blue solution to determine their viability and were counted using a hemocytometer.

Luciferase reporter assay. A DNA fragment containing the 3'-untranslated regions (3'UTRs) of either the wild-type (WT) PD-L1 sequence (5'...TCAGACTGCCA...3') or the mutant PD-L1 sequence (5'...TCAGACGGACT...3'), was cloned into the pmirGLO vector (Promega Corporation) with the technical assistance of MDBio, Inc. BC cells were seeded into 12-well plates and transfected with either the empty vector or the 3'UTR reporter constructs (1 μ g/ μ l) using ViaFect transfection reagent (Promega Corporation) for 24 h. After transfection, cells were treated with either a control or miR-34a inhibitor for an additional 24 h. The activities of firefly and *Renilla* luciferase were subsequently measured using the Dual-Luciferase Reporter Assay System (Promega Corporation) according to the manufacturer's protocol. Relative activity was expressed as firefly/*Renilla* luciferase ratio.

Apoptosis analysis by flow cytometry. Following anoikis resistance analysis (as aforementioned), apoptotic cells

were detected using the fluorescein isothiocyanate (FITC) Annexin V Apoptosis Detection Kit (BD Biosciences) in accordance with the manufacturer's instructions. Early apoptotic (Annexin V⁺/PI⁻) and late apoptotic (Annexin V⁺/PI⁺) cells were analyzed using an Accuri C5 flow cytometer (BD Biosciences), and the data were processed with CellQuest Pro software (version 5.1; BD Biosciences).

Cytotoxicity of NK cells. A549 and CL1-5 cells (1×10^4) were seeded onto a 48-well plate and treated with 100 μ M sesamin for 24 h. After treatment, the cells were stained with calcein acetoxymethyl (calcein AM, green fluorescent dye, 1 μ g/ μ l) for 1 h at 37°C in a CO₂ incubator. Following staining, the cells were washed three times with 500 μ l of PBS to remove excess dye. Subsequently, NK-92MI cells were added to the wells at a ratio of 1:5 (cancer cells to NK cells) and incubated for 2-4 h. The green fluorescence of calcein AM, indicative of live cancer cells, was captured using an Eclipse Ti2 microscope (Nikon Corporation), with appropriate filters installed for green fluorescence. Finally, the cells were lysed in 0.1% Triton X-100, and the fluorescence of was measured using a Varioskan LUX multimode microplate reader (Thermo Fisher Scientific, Inc.).

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from BC cells using TRIzol[®] manufacturer's protocol (Thermo Fisher Scientific, Inc.). RNA concentration was measured using Nanodrop (Thermo Fisher Scientific, Inc.). After total RNA was isolated from A549 and CL1-5 cells, a Mir-X[™] miRNA First-Strand Synthesis (Takara Bio, Inc.) was used to reverse-transcribe this total RNA to complementary DNA (100 ng). The forward primer for hsa-miR-34a-5p is 5'-TGGCAGTGTCTTAGCTGGTTGTT-3', while the universal reverse primer for hsa-miR-34a-5p was included in the Mir-X[™] miRNA First-Strand Synthesis Kit. The forward and reverse primers for U6 (RNU6-1) snRNA, used as an internal control, were also provided in the kit. RT-qPCR was conducted by using a TaqMan[®] one-step PCR Master Mix (Applied Biosystems; Thermo Fisher Scientific, Inc.), following a previously described methodology (25). The cycling conditions consisted of polymerase activation at 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 60 sec. Quantification of hsa-miR-34a-5p transcript levels was performed using the 2^{- $\Delta\Delta$ C_q} method, with U6 used for normalization (26).

Animal model. All experimental procedures involving animals were approved (approval no. 112SKH015) by the Institutional Animal Care and Use Committee of Shin Kong Wu Ho-Su Memorial Hospital (Taipei, Taiwan) and were conducted following the institution's Guidelines for Animal Experimentation. A total of eight male BALB/c nude mice (6 weeks old; weight, ~25 g) were obtained from BioLASCO (Taipei, Taiwan) and housed in a specific pathogen-free facility under controlled conditions (temperature: ~27°C; humidity: ~50%) with a 12/12-h light/dark cycle. The mice had free access to sterilized drinking water and standard food pellets. A total of ~ 1×10^6 A549 cells suspended in 50 μ l of PBS mixed with 50 μ l of Matrigel were subcutaneously inoculated into the mice. Tumor formation was considered successful when

the subcutaneous tumor volume reached over 100 mm³ and became palpable one week after cell inoculation. The mice were then divided into two groups (a treatment group and a control group; each comprising five animals) to receive different treatment regimens. The control group received injections of PBS, and the treatment group received sesamin at a dose of 20 mg/kg, delivered twice weekly for a total of three weeks. Tumor progression was monitored twice per week, in accordance with the treatment schedule. At the end of the treatment period, the mice were euthanized via CO₂ asphyxiation. CO₂ was introduced at a flow rate of 40% chamber air displacement per min (40% V/min; 2.4 l/min). The gas was administered for at least 4 min to ensure complete euthanasia, and death was confirmed by the cessation of heartbeat and respiratory movement. Tumor tissues were subsequently excised, and tumor growth was assessed by measuring tumor weight.

Immunohistochemistry (IHC) staining. Paraffin-embedded tissue sections (5 µm) obtained from the aforementioned animal model were stained with anti-Ki-67 (1:400; cat. no. ab15580; Abcam), PD-L1 (1:200; cat. no. GTX635975, GeneTex, Inc.) and N-cadherin (1:200; cat. no. ab76011; Abcam) at room temperature for 2 h, following a previously described methodology (27). The IHC staining results were evaluated using ImageJ software, considering both the percentage of tumor tissue displaying specified protein staining (ranging from 0 to 100%) and the intensity of staining (negative: 0, weak: 1, moderate: 2, and strong: 3). These assessments were independently performed by three pathologists, yielding a final score ranging from 0 to 300.

Statistical analysis. All experiments were performed at least three times, with each experiment conducted in triplicate. Data are presented as the mean ± standard deviation (SD). Statistical significance between two groups was determined using an unpaired Student's t-test. For experiments involving three or more groups, one-way analysis of variance (ANOVA) was performed, followed by Tukey's multiple comparisons test for post hoc analysis. P<0.05 was considered to indicate a statistically significant difference. All statistical analyses were carried out using GraphPad Prism software (version 9; Dotmatics).

Results

Sesamin inhibits the viability of human LUAD cells in a dose-dependent manner. First of all, it was investigated whether sesamin exerts similar anticancer mechanisms across different types of LUAD cells. To this end, experiments were conducted using two LUAD cell lines, A549 and CL1-5. A549 exhibits typical lung epithelial cell characteristics, whereas CL1-5, a highly invasive subline of the CL1 series, possesses strong metastatic potential. Using MTT assays, cell viability was evaluated for A549 and CL1-5 cells treated with various concentrations of sesamin (0, 25, 50, 100, 200 and 400 µM) for 24 h. Moreover, the 50% inhibitory concentration (IC₅₀) values of sesamin for A549 and CL1-5 cells were determined to be 317.3 and 240.7 mM, respectively. The results indicated that sesamin significantly reduced the viability of A549 and CL1-5 cells in a dose-dependent manner (Fig. 1A and B).

Colony formation assays further confirmed the reduction in cell survival (Fig. 1C and D). IF staining was performed on A549 and CL1-5 cells to evaluate the expression of Ki-67, a well-known marker of cell proliferation (28). As shown in Fig. 1E and F, IF analysis revealed the reduced expression of Ki-67 in cells treated with 100 µM sesamin. Overall, these findings indicate that sesamin exerts an antitumor effect on LUAD cells by inhibiting their proliferation.

Sesamin promotes the chemosensitivity of LUAD cells. Administering chemotherapeutic agents such as docetaxel and paclitaxel is a standard clinical practice for managing LUAD (29). In the present study, the potential of sesamin for enhancing the efficacy of these chemotherapeutic agents against LUAD cells was investigated. Our results indicated that co-treatment with sesamin (100 µM) and either docetaxel (12.5 µM) or paclitaxel (5 µM) significantly reduced the viability of A549 and CL1-5 cells (Fig. 2A and B). Colony formation assays further confirmed a reduction in cell survival (Fig. 2C-F). Moreover, the combination treatment of sesamin with docetaxel or paclitaxel had a synergistic effect (CI<1) (Fig. S1). The activation of caspase-3 and cleavage of PARP are well-established indicators of apoptosis (9,30). In the present study, it was investigated whether sesamin contributes to the induction of apoptosis in LUAD cells. Our results indicated that the co-administration of sesamin with either docetaxel or paclitaxel significantly upregulated the expression of cleaved caspase-3 and PARP (Fig. 2G and H). These results suggest that sesamin potentiates the apoptotic effects of chemotherapeutic agents in LUAD cells by increasing the cleavage of caspase-3 and PARP.

Sesamin suppresses the motility and anoikis resistance of LUAD cells by downregulating N-cadherin. In cancer cells, cadherin switching, a phenomenon characterized by the upregulation of N-cadherin and the concomitant downregulation of E-cadherin, is associated with increased migratory and invasive capabilities, which are a hallmark of EMT (31). Next, it was examined whether sesamin suppresses EMT activity. Treatment of LUAD cells with sesamin resulted in changes in cell morphology from a mesenchymal-like to an epithelial-like phenotype (Fig. S2A). Sesamin also downregulated the expression of N-cadherin in both A549 and CL1-5 cells in a concentration-dependent manner (Fig. 3A and B). In Fig. 3A, a statistically significant change in vimentin expression was observed at 50 µM compared with the control group, and in Fig. 3B at 100 µM compared with 0 µM. Regarding E-cadherin, a significant increase was noted at 25 µM in Fig. 3B. However, no clear dose-dependent changes were observed in the expression levels of vimentin or E-cadherin.

Cell migration and anoikis resistance are the hallmarks of oncogenic EMT in cancers (11,32). To further investigate the role of sesamin-inhibited EMT activity in alleviating the migratory and metastatic potential of LUAD cells, wound healing and anoikis resistance assays were conducted. The wound healing assay revealed that sesamin inhibited the migration of both A549 and CL1-5 cells in a dose-dependent manner (Fig. 3C and D). As shown in Fig. 3E, the anoikis resistance of A549 cells decreased with treatment at various sesamin concentrations for 2, 4 and 6 days. Importantly, a significant

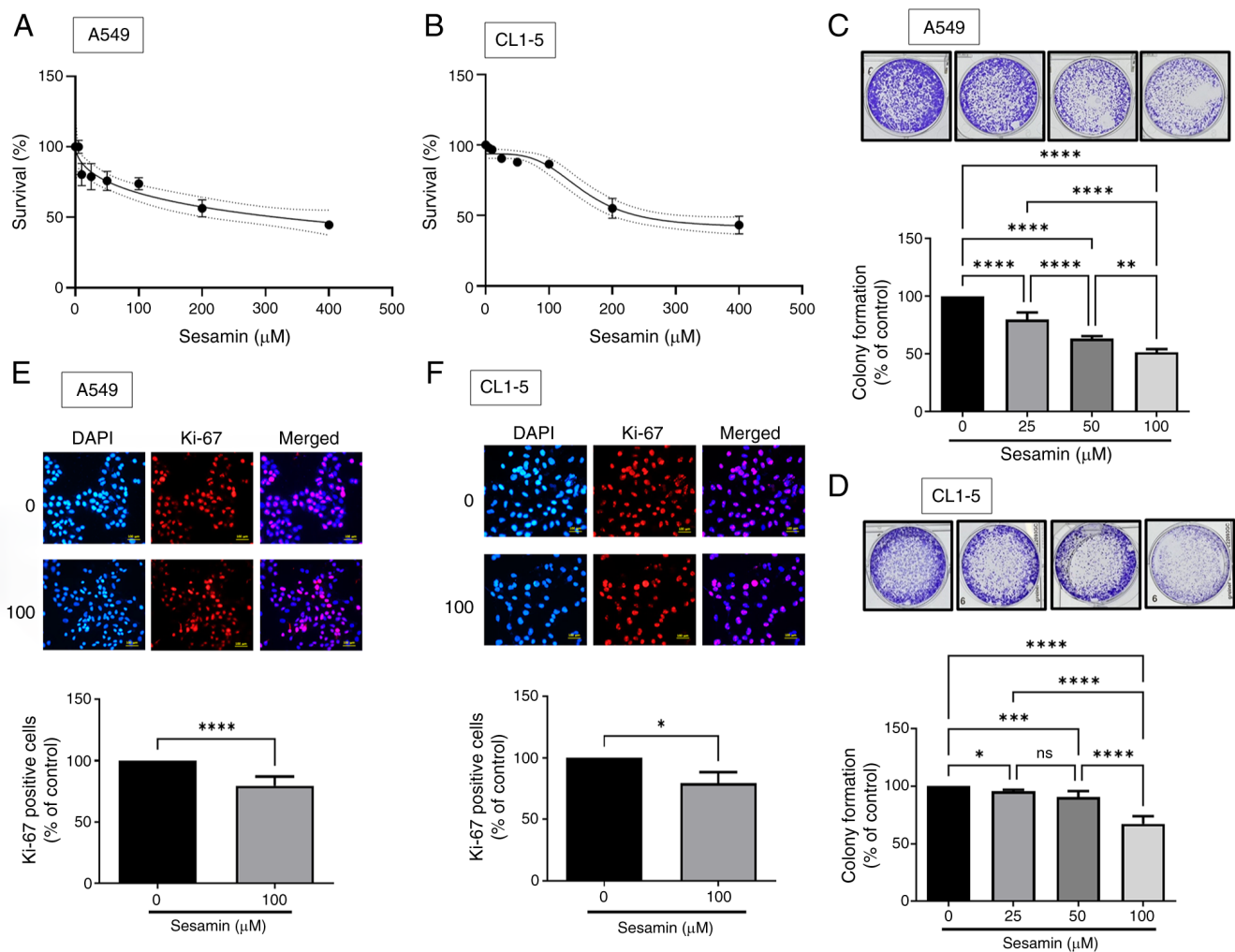


Figure 1. Sesamin reduces the viability of human LUAD cells in a dose-dependent manner. (A-D) The effect of various concentrations of sesamin (0-100 μ M) on the viability and survival of LUAD cells was evaluated using MTT and colony formation assays, respectively. (E and F) LUAD cells were treated with sesamin at a concentration of 100 μ M, and cell proliferation was evaluated using Ki-67 staining. Scale bars, 100 μ m. Data are expressed as mean $\pm$ SD for triplicate samples. *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001 relative to the control group. LUAD, lung adenocarcinoma; ns, not significant.

reduction in anoikis resistance was particularly notable after 6 days of sesamin treatment. Additionally, it was confirmed during the anoikis resistance analysis that sesamin induced LUAD cells to undergo apoptotic cell death (Fig. S2B). Overall, these results indicated that the inhibitory effects of sesamin on the motility and anoikis resistance of LUAD cells may be attributed to its downregulation of N-cadherin expression.

Sesamin suppresses the motility and anoikis resistance of LUAD cells by downregulating PI3K/AKT/mTOR. To elucidate the molecular mechanism underlying the inhibitory effects of sesamin on cell motility and anoikis resistance, the PI3K/AKT/mTOR signaling pathway was examined in LUAD cells. It was discovered that sesamin inhibited the expression of p-PI3K, AKT and mTOR in a time-dependent manner (Fig. 4A and B; Fig. S3A-C). The co-administration of sesamin and a PI3K activator interrupted sesamin-inhibited AKT phosphorylation (Fig. 4C and D; Fig. S3D). Moreover, the PI3K and AKT activator also interrupted sesamin-inhibited mTOR activation, demonstrating that sesamin could downregulate the PI3K/AKT/mTOR signaling pathway in LUAD cells (Fig. 4E and F; Fig. S3E).

To confirm whether the PI3K/AKT/mTOR signaling pathway is involved in the sesamin-mediated suppression of N-cadherin, cell motility and anoikis resistance, A549 and CL1-5 cells were pretreated with the specific activators of PI3K, AKT and mTOR for 30 min before the addition of sesamin. Co-administration of sesamin with specific activators of PI3K, AKT, or mTOR significantly attenuated the sesamin-mediated suppression of N-cadherin protein expression (Fig. 4G-L; Fig. S3F-H). Furthermore, treatment with these activators significantly alleviated the sesamin-induced reductions in cell motility, anoikis resistance and chemosensitivity (Fig. 4M-O; Fig. S4A and B). Overall, these findings indicate that the inhibitory effect of sesamin on cell migration and its anoikis-conductive effect may be attributable to its downregulation of the PI3K/AKT/mTOR signaling pathway; this mechanism may contribute to the antitumor effects of sesamin.

Sesamin attenuates the expression of PD-L1 and mitigates cancer-associated immunosuppression. PD-L1 is an essential biomarker of immune evasion that functions as an inhibitory checkpoint (16). In the present study, the effect of sesamin on cancer-associated immunosuppression was examined by

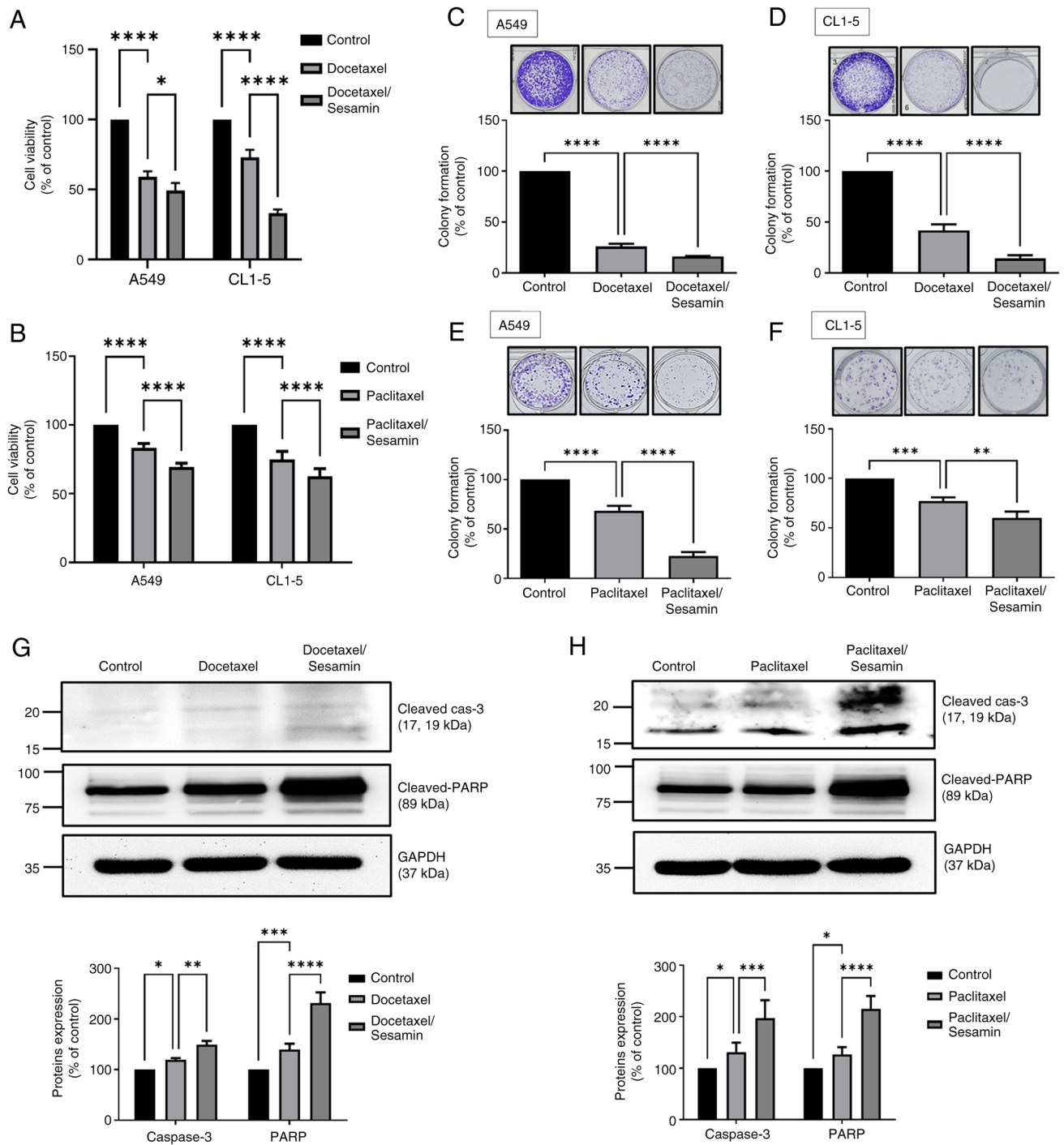


Figure 2. Sesamin increases the chemosensitivity of LUAD cells. (A-F) Viability and survival of LUAD cells treated with sesamin in combination with a chemotherapeutic agent (12.5 μ M docetaxel or 5 μ M paclitaxel), evaluated using both an MTT assay after incubation for 24 h and a colony formation assay after incubation for 1 week. (G and H) The protein expression levels of cleaved caspase-3 and PARP in LUAD cells were determined using a western blot assay after treatment with sesamin in combination with a chemotherapeutic agent (docetaxel or paclitaxel). Data are expressed as the mean $\pm$ SD for triplicate samples. * P <0.05, ** P <0.01, *** P <0.001 and **** P <0.0001 relative to the control group. LUAD, lung adenocarcinoma; PARP, poly(ADP-ribose) polymerase.

measuring the expression of PD-L1 and analyzing the cytotoxic activity of NK cells. The current results revealed that sesamin downregulated the expression of PD-L1 in both A549 and CL1-5 cells in a dose-dependent manner (Fig. 5A and B). Calcein AM staining of A549 and CL1-5 cells also revealed increased NK-cell-mediated cytotoxicity following the administration of sesamin (Fig. 5C and D). This downregulation of the expression of PD-L1 suggests that sesamin plays a role in mitigating cancer-associated immunosuppression.

Hsa-miR-34a mediates the sesamin-induced downregulation of PD-L1 and enhances the cytotoxicity of NK cells. In various types of cancer cells, PD-L1 is regarded as a target gene of hsa-miR-34a-5p (33-35). Experiments were conducted to determine the effect of sesamin on the levels of hsa-miR-34a-5p in LUAD cells. According to the RT-qPCR results, sesamin upregulated the levels of hsa-miR-34a-5p in A549 and CL1-5 cells in a dose-dependent manner (Fig. 6A and B). To determine the regulatory role of hsa-miR-34a-5p in the expression

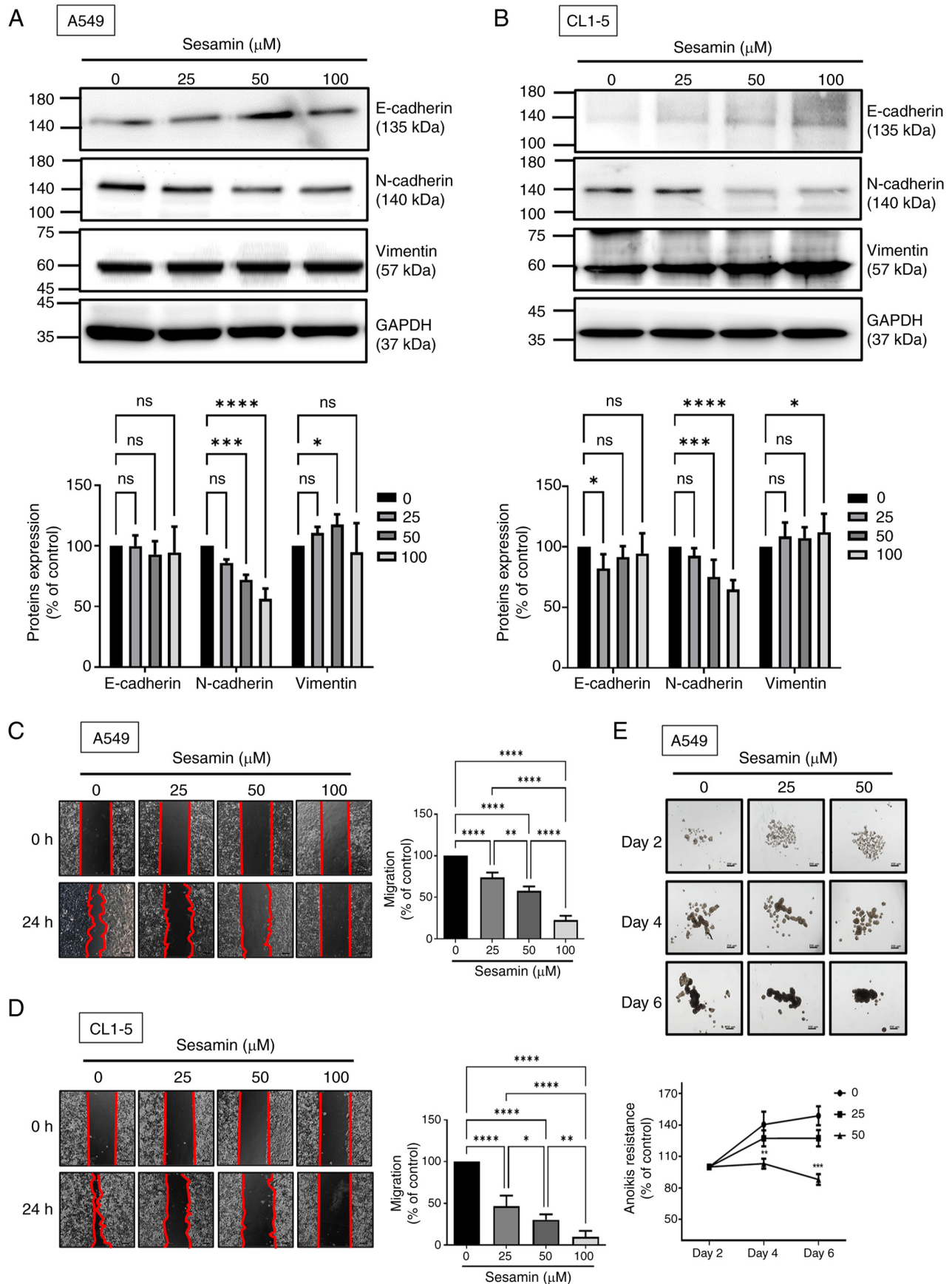


Figure 3. Sesamin reduces the motility and anoikis resistance of LUAD cells by downregulating N-cadherin. (A and B) The expression levels of epithelial to mesenchymal transition-related markers, including E-cadherin, vimentin and N-cadherin, were determined using a western blot assay in lung adenocarcinoma cells treated with various concentrations of sesamin (0-100 μM). (C and D) Cell motility determined using a wound healing assay after 24 h of treatment with sesamin. Scale bars, 100 μm . (E) A549 cells treated with sesamin (0-50 μM) for 2, 4 and 6 days, with an anoikis resistance assay by measuring cell numbers. Scale bars, 250 μm . Data are expressed as the mean $\pm$ SD for triplicate samples. * P <0.05, ** P <0.01, *** P <0.001 and **** P <0.0001 relative to the control group. ns, not significant.

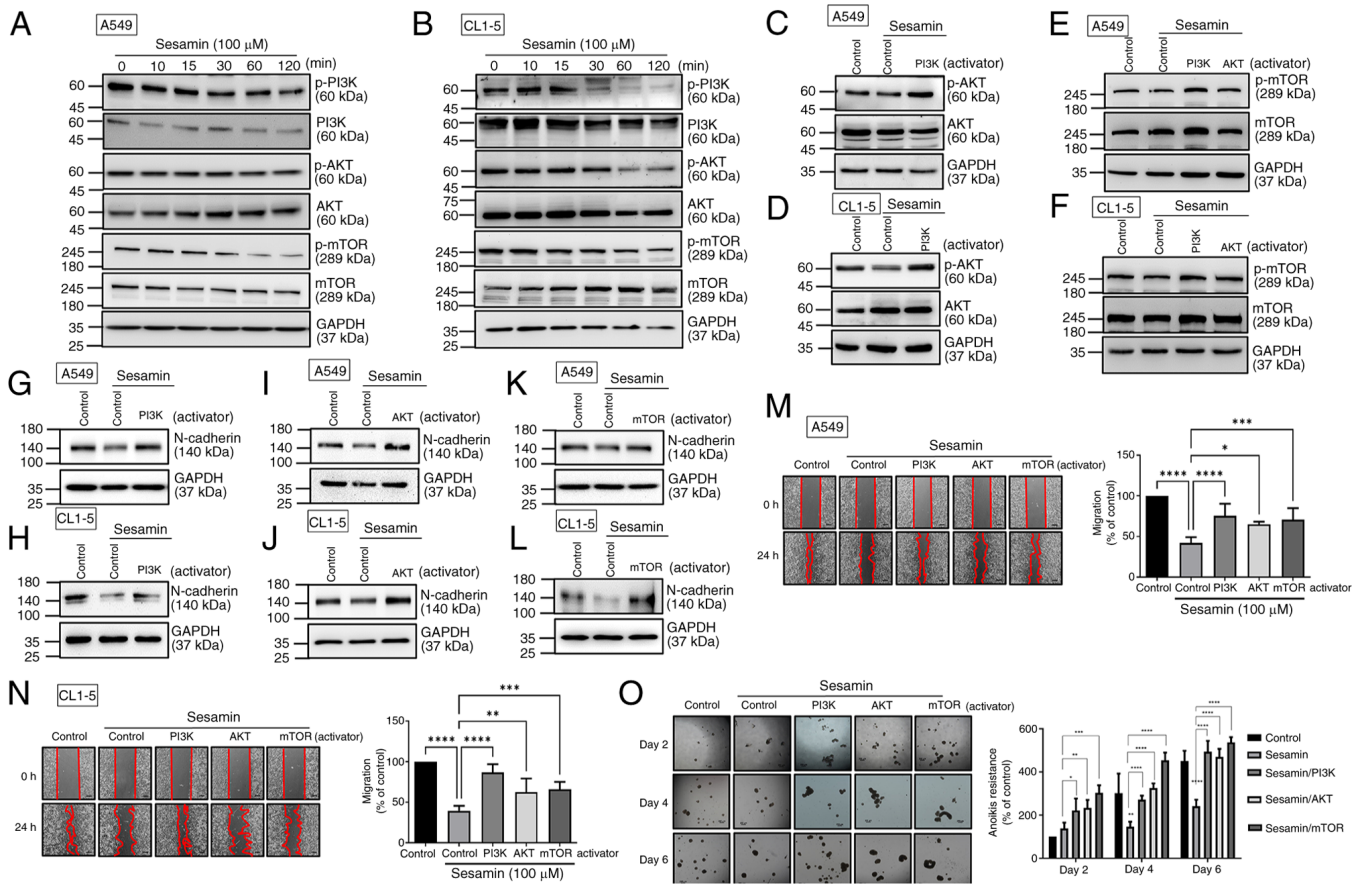


Figure 4. Sesamin reduces the motility and anoikis resistance of LUAD cells by downregulating PI3K/AKT/mTOR. (A and B) The protein expression levels of p-PI3K, p-AKT, and p-mTOR in LUAD cells were determined using a western blot assay after treatment with sesamin (100 μ M) at various time points. (C-F) Pretreatment of LUAD cells with activators of PI3K (740 Y-P, 1 mM), AKT (SC79, 0.5 μ g/ml) and mTOR (MHY1485, 1 mM) for 30 min was followed by treatment with sesamin (100 μ M) for 2 h. The protein levels of AKT and mTOR phosphorylation were detected using a western blot assay. (G-L) Pretreatment of LUAD cells with activators of PI3K (740 Y-P, 1 mM), AKT (SC79, 0.5 μ g/ml), and mTOR (MHY1485, 1 mM) for 30 min, followed by treatment with sesamin (100 μ M) for 24 h. The protein levels of N-cadherin were determined by western blot assay. (M-O) Cell migration and anoikis resistance were determined using wound healing and anoikis resistance assays, respectively. Scale bars, 100 μ m (wound healing) and 250 μ m (anoikis resistance). Data are expressed as the mean $\pm$ SD for triplicate samples. * P <0.05, ** P <0.01, *** P <0.001 and **** P <0.0001 relative to the control group. LUAD, lung adenocarcinoma; p-, phosphorylated.

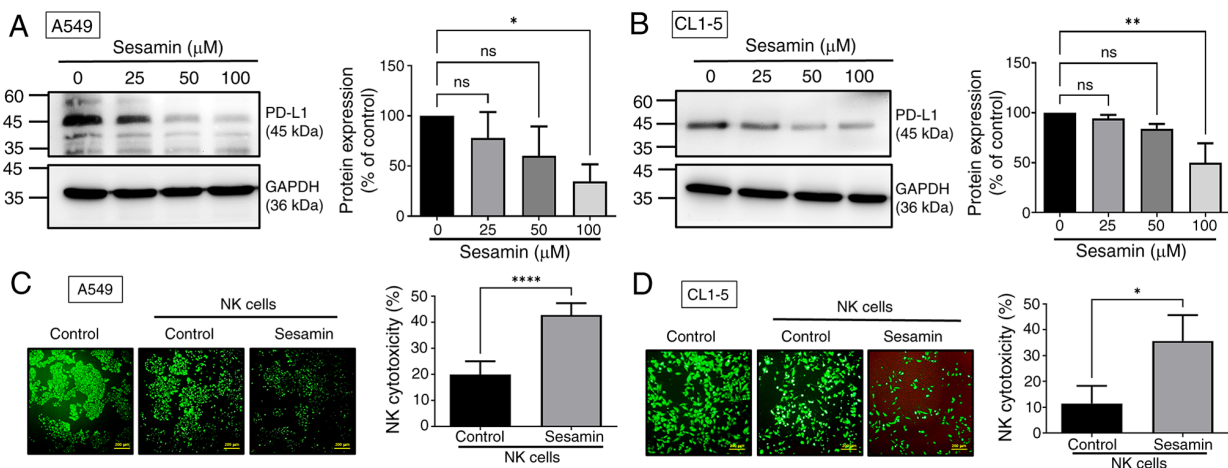


Figure 5. Sesamin downregulates the expression of PD-L1 and mitigates cancer-associated immunosuppression. (A and B) The protein expression of PD-L1 in LUAD cells treated with various concentrations of sesamin (0-100 μ M) was determined using a western blot assay. (C and D) LUAD cells were stained with calcein AM (a green fluorescent marker) after a 24-h sesamin treatment (100 μ M). Following the introduction of NK cells, cytotoxicity was quantitatively evaluated by measuring the fluorescence values. Scale bars, 200 μ m. Data are expressed as the mean $\pm$ SD for triplicate samples. * P <0.05, ** P <0.01 and **** P <0.0001 relative to the control group. PD-L1, programmed death ligand 1; LUAD, lung adenocarcinoma; NK cells, natural killer cells; ns, not significant.

of PD-L1, a hsa-miR-34a-5p inhibitor was used. First, it was confirmed that the inhibitor successfully suppressed

hsa-miR-34a-5p expression in A549 and CL1-5 cells (Fig. S5A and B). Next, our results indicated that transfection

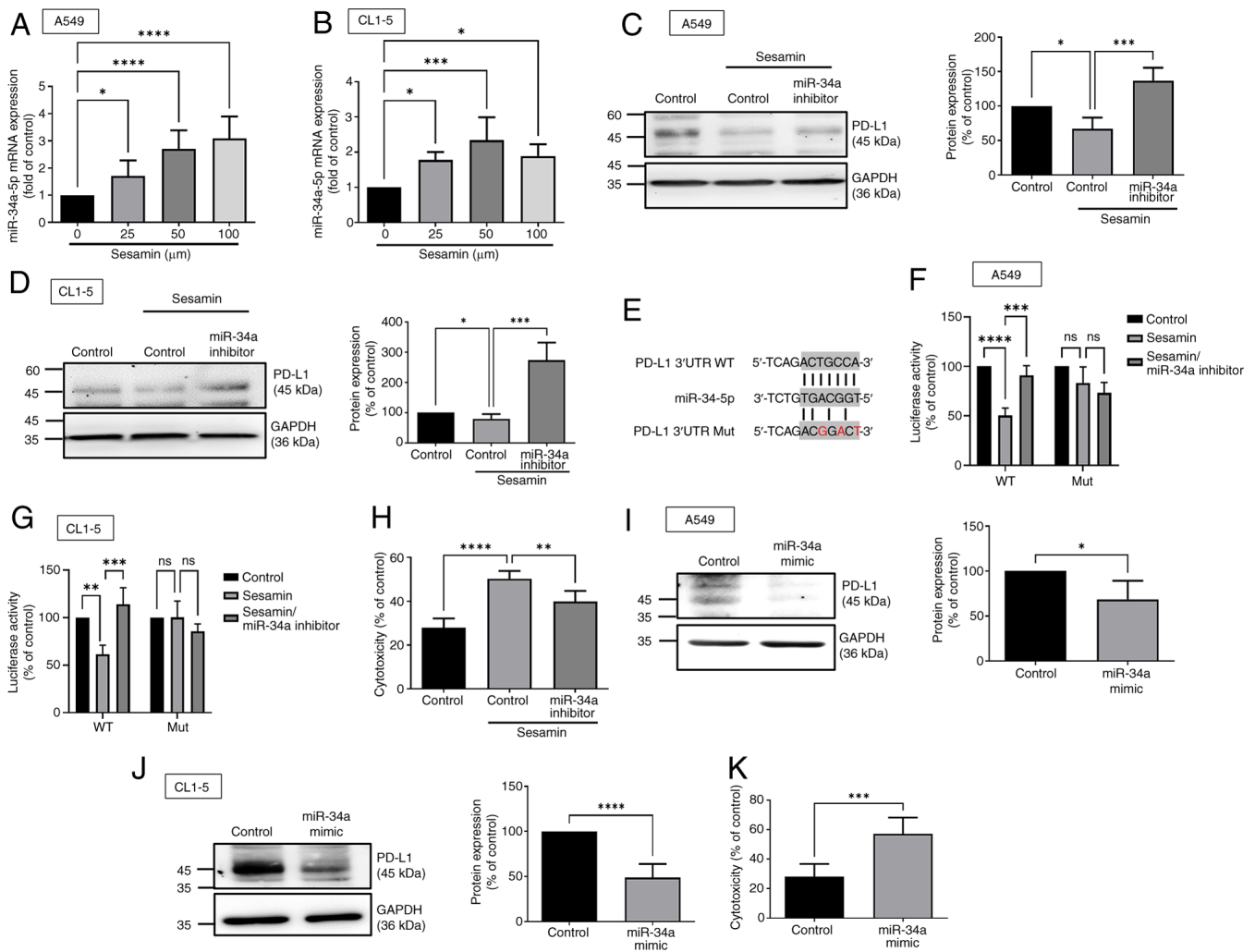


Figure 6. Hsa-miR-34a mediates the sesamin-induced downregulation of PD-L1 and enhances the cytotoxicity of NK cells. (A and B) The expression of hsa-miR-34a-5p in LUAD cells, treated with various concentrations of sesamin (0-100 μ M), was determined using a reverse transcription-quantitative PCR assay. (C and D) LUAD cells transfected with either a control inhibitor or hsa-miR-34a-5p inhibitor (25 nM) for 24 h, followed by treatment with sesamin (100 μ M) for another 24 h. The protein levels of PD-L1 were detected using a western blot assay. (E) Schematic representation of the 3'-UTR of the WT PD-L1 sequence and the mutant PD-L1 sequence containing the miR-34a binding site. (F and G) LUAD cells were co-transfected with a PD-L1 plasmid (1 μ g/ μ l) and a miR-34a inhibitor (25 nM) for 24 h, followed by stimulation with sesamin for an additional 24 h. Relative luciferase and Renilla activities were then measured. (H) NK cytotoxicity assay revealing the immune evasion of CL1-5 cells transfected with a hsa-miR-34a-5p inhibitor (25 nM) for 24 h, followed by treatment with sesamin (100 μ M). (I and J) LUAD cells were transfected with either a control mimic or hsa-miR-34a-5p mimic (25 nM) for 24 h, and PD-L1 protein expression was measured using a western blot assay. (K) Immune evasion was assessed through a NK cytotoxicity assay after transfecting CL1-5 cells with a hsa-miR-34a-5p mimic (25 nM) for 24 h. Data are expressed as the mean $\pm$ SD for triplicate samples. *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001 relative to the control group. PD-L1, programmed death ligand 1; NK cells; natural killer cells; miR, microRNA; LUAD, lung adenocarcinoma; UTR, untranslated region; WT, wild-type; ns, not significant.

with this inhibitor reversed the sesamin-induced reduction of PD-L1 protein levels (Fig. 6C and D). To investigate the potential interaction between hsa-miR-34a-5p and PD-L1 in LUAD cells, the 3'UTRs of both the WT PD-L1 sequence and the mutant PD-L1 sequence were constructed (Fig. 6E). Luciferase activity was increased by myostatin in the WT PD-L1 3'-UTR plasmid; no such change was observed with the mut PD-L1 3'-UTR plasmid, while the hsa-miR-34a-5p inhibitor reduced sesamin-suppressed WT PD-L1 3'-UTR activity (Fig. 6F and G). Most importantly, hsa-miR-34a-5p inhibitor reduced sesamin-enhanced NK cell cytotoxicity against LUAD cells (Fig. 6H). By contrast, successful transfection with a hsa-miR-34a-5p mimic was confirmed (Fig. S5C and D), and overexpression of hsa-miR-34a in LUAD cells resulted in diminished PD-L1 protein levels (Fig. 6I and J) and increased

NK cell cytotoxicity (Fig. 6K). Overall, these results suggested that sesamin potentiates the cytotoxicity of NK cells through the miR-34a/PD-L1 axis, thereby disrupting the immune evasion strategies of LUAD cells.

Sesamin inhibits lung tumor growth in vivo. To further investigate the inhibitory effects of sesamin on LUAD cells *in vivo*, a subcutaneous animal model was established using BALB/c nude mice. Briefly, A549 cells (1×10^6) were subcutaneously injected into the mice. One week after injection, to ensure sufficient tumor growth, the mice were divided into two groups: a control group (receiving PBS) and a treatment group (receiving sesamin at a dosage of 20 mg/kg twice a week). Each group initially included five mice; however, one mouse in each group failed to develop a measurable tumor (Fig. 7A). Consequently, analysis of the

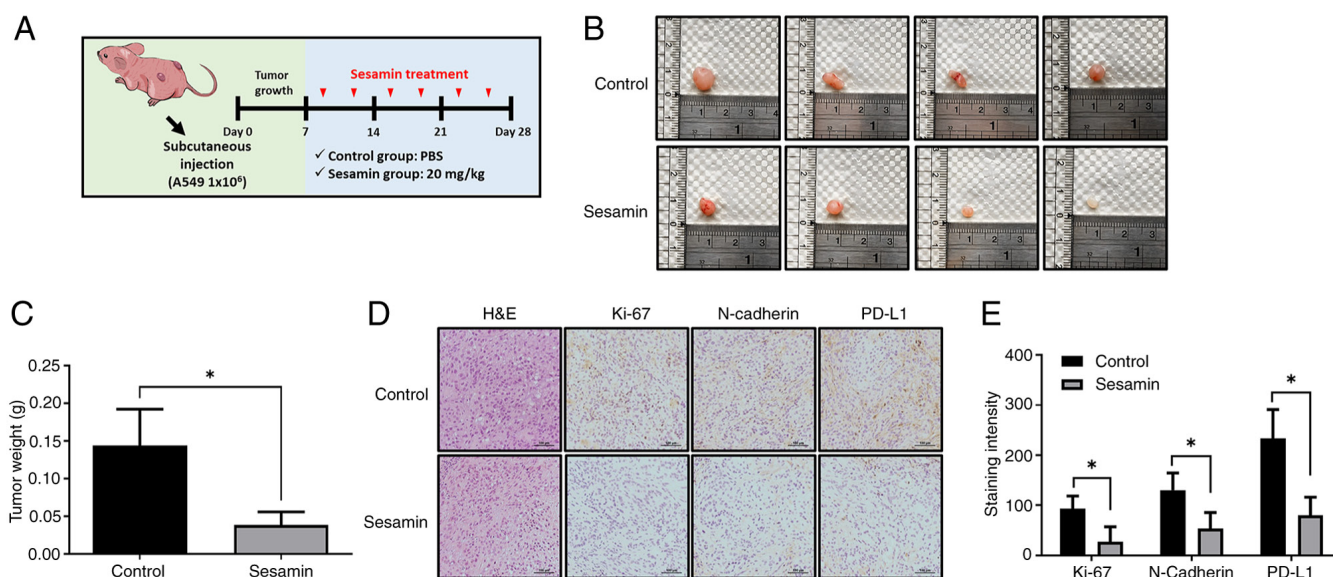


Figure 7. Sesamin inhibits lung tumor growth *in vivo*. (A) Flowchart demonstrating the protocol for sesamin treatment with a subcutaneous animal model. (B) Representative images of tumors excised from each group were obtained after 4 weeks of sesamin treatment. (C) Tumor weights of the treatment and control groups. (D) Expression of N-cadherin and PD-L1 proteins in tumor tissues, determined using an IHC analysis. (E) Quantitative IHC analysis of N-cadherin and PD-L1 expression. Data are expressed as the mean $\pm$ SD for triplicate samples. * $P < 0.05$ relative to the control group. PD-L1, programmed death ligand 1; IHC, immunohistochemical.

remaining four tumors revealed that, compared with the control group, the treatment group exhibited significantly lower tumor weights, indicating that sesamin effectively suppressed tumor growth (Fig. 7B and C). IHC analysis was conducted on mouse tumor tissues to examine the expression of N-cadherin and PD-L1. The results revealed a significant reduction in the expression of N-cadherin and PD-L1 in tumors treated with sesamin, which was consistent with the results of cell-based experiments (Fig. 7D and E). These findings confirmed the substantial anti-tumor efficacy of sesamin against lung tumor growth.

Discussion

Paclitaxel and docetaxel, as chemotherapeutic agents, induce anticancer effects by inhibiting the disassembly of microtubules, leading to cell cycle arrest and apoptosis (36,37). Some cases of severe toxicity associated with these drugs have been reported, as well as the development of resistance to these drugs in certain cancer cells (38,39). Addressing the challenge of reducing the side effects and drug resistance caused by chemotherapy is crucial. Many natural compounds have been identified as safe and potent adjunctive therapies against cancer (40). For instance, lignans, a diverse group of phenolic compounds from dietary fibers, have been confirmed to have anticancer effects, particularly against lung cancer (41,42). Sesamin is a prominent lignan found in sesame seeds. In cervical cancer cells, sesamin induces apoptosis by activating the p53 pathway, which is facilitated by the upregulation of phosphatase and tensin homolog expression (43). Sesamin triggers cell cycle arrest by upregulating p53 and Chk2, and induces apoptosis through the activation of the Bax and caspase-3 pathways in breast cancer (44). Caspase-3 is a key indicator of apoptosis, a physiological process elicited by various stimuli, such as stress, cytotoxic drugs and variations in reactive oxygen species levels in LUAD (7). Additionally,

sesamin suppresses TNF-induced expression of cell-proliferative gene products such as cyclin D1 and cell-survival gene products such as Bcl-2 and survivin. Sesamin exhibits its chemo-preventive effects by inhibiting NF- κ B-mediated cell survival and proliferation in LUAD (45). The results of the present study support the aforementioned findings, indicating that sesamin, when used in combination with paclitaxel and docetaxel, reduces cell viability and survival in human LUAD cells through caspase-3 and PARP activation. This highlights the potential of sesamin as a supplementary agent to optimize chemotherapy in clinical applications.

EMT molecular markers, including E-cadherin and N-cadherin, are key indicators of the phenotypic shift from the epithelial to the mesenchymal state, which is associated with an increase in cancer aggressiveness and metastatic potential (46). N-cadherin has been reported to be aberrantly expressed in various human malignancies, promoting cancer cells invasion, adhesion, apoptosis, angiogenesis and metastasis (47). In addition, N-cadherin is associated with worse recurrence-free survival and is considered a prognostic biomarker in patients with non-muscle-invasive bladder cancer (48). Downregulation of N-cadherin promotes tumor suppression in pancreatic intraepithelial neoplasia and inhibits metastatic spread in neuroblastoma (49,50). These findings provide evidence that N-cadherin is a promising target for the development of innovative onco-therapeutic agents. The present results suggested that sesamin comprehensively inhibits N-cadherin expression both *in vitro* and *in vivo*, resulting in the disruption of cell migration and anoikis resistance in LUAD. In the future, it is worth investigating whether sesamin could serve as a natural onco-therapeutic agent in other cancers characterized by aberrant N-cadherin expression.

Elevated PD-L1 expression significantly reduces the activity of immune cells, facilitating cancer cells in evading immunosurveillance (17). Consequently, PD-L1 expression

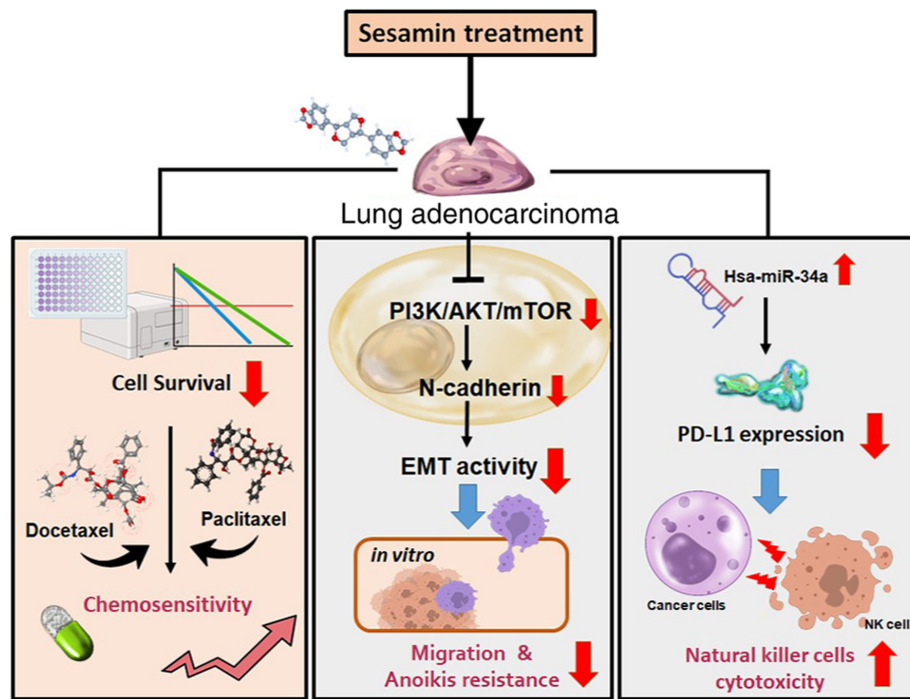


Figure 8. Graphical representation of the mechanisms underlying the effect of sesamin on LUAD cells. Sesamin inhibits cancer cell motility and anoikis resistance primarily through the suppression of EMT. Sesamin also enhances antitumor immunity by downregulating the expression of PD-L1, thereby confirming the tumor-suppressive effects of sesamin in human LUAD. LUAD, lung adenocarcinoma; EMT, epithelial-mesenchymal transition; PD-L1, programmed death ligand 1.

serves not only as a pivotal biomarker of tumor immune evasion but also as a therapeutic target in checkpoint-based immunotherapy (16,18). There is an increasing number of scholars developing PD-L1 inhibitors, such as anti-PD-L1 antibodies and peptides, which block the activity of PD-L1 and have positive effects on endogenous antitumor immune responses, thus representing a feasible therapeutic strategy (51,52). Our results revealed that sesamin inhibits the expression of PD-L1 in a dose-dependent manner in LUAD cells, resulting in the augmented cytotoxicity of NK cells. In a previous study, it was reported that at a specific concentration, sesamin downregulated the mRNA and protein expression of PD-L1 in breast cancer cells, which is consistent with our findings (16). Another prominent lignan found in sesame seeds is sesamol, which, similar to sesamin, exhibits significant biological activity (53). Notably, sesamol enhances the expression of natural killer group 2D (NKG2D) ligands, key molecules that regulate NK cell-mediated anticancer responses, without reaching toxic concentrations. This upregulation was also observed in other key molecules, including UL-16 binding protein 1 (ULBP1), ULBP2 and MHC class I chain-related protein A (MICA) and B (MICB). Additionally, sesamol increased the phosphorylation of the ERK pathway, which is linked to the regulation of NKG2D ligand expression in Burkitt's lymphoma cells (54). These findings indicate the potential of sesamin as an adjunctive immunotherapeutic agent that can enhance immune-mediated tumor suppression.

Generally, miRNAs are small, non-coding RNA molecules that regulate gene expression after transcription, typically by binding to the 3'-UTR of their target mRNAs (55). The tumor-suppressive miRNA, hsa-miR-34a-5p, induces G0/G1 cell cycle arrest by inhibiting the expression of cyclin D1 and cyclin E2 proteins (56). Additionally, it suppresses cell

motility by downregulating EMT activity in bladder cancer cells (56). In triple-negative breast cancer cells, upregulation of hsa-miR-34a-5p suppresses cellular activity and migration, promotes apoptotic cell death, and enhances cytotoxic antitumor responses (34). *In vivo* studies have shown that hsa-miR-34a-5p agomir inhibits breast tumor growth and downregulates PD-L1 expression (34). An inverse correlation between PD-L1 and hsa-miR-34a levels was also observed in acute myeloid leukemia (AML), where hsa-miR-34a overexpression in AML cells reduced PD-L1-specific T cell apoptosis, suggesting that hsa-miR-34a could serve as a potential immunotherapeutic target (21). In addition to regulating PD-L1, hsa-miR-34a has been identified as a key upstream regulator of C-C motif chemokine ligand 22 and diacylglycerol kinase ζ (DGK ζ) expression, playing a crucial role in modulating the tumor immune microenvironment by enhancing T cell activation and inhibiting Treg cell recruitment (57,58). Consistent with these findings, the present results demonstrated that sesamin upregulates the expression of hsa-miR-34a-5p in LUAD cells, leading to the downregulation of PD-L1 expression. This sesamin-induced upregulation of hsa-miR-34a-5p contributed to the enhanced cytotoxicity of NK cells, thereby disrupting the immune evasion mechanisms of LUAD cells. Although the present study demonstrated these promising *in vitro* results, *in vivo* experiments to validate the role of sesamin in reducing LUAD immune evasion were not conducted. Therefore, future research should focus on *in vivo* studies to improve understanding of the efficacy and potential side effects of sesamin in a more physiologically relevant setting.

In conclusion, sesamin increased the proapoptotic effects of chemotherapeutic agents in LUAD cells. Sesamin also attenuated cancer cell migration and anoikis resistance by downregulating the expression of N-cadherin through the suppression

of the PI3K/AKT/mTOR signaling pathway. In addition, sesamin increased the cytotoxicity of NK cells by upregulating the expression of hsa-miR-34a, resulting in the downregulation of PD-L1 expression and the disruption of the immune evasion mechanisms of LUAD cells (Fig. 8). In summary, sesamin inhibits the aggressiveness of LUAD while reducing immune escape, demonstrating its multifaceted anticancer mechanisms. Sesamin combined with chemotherapy is expected to enhance treatment effectiveness and reduce the occurrence of side effects in LUAD.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

ACC conceptualized and supervised the study, developed methodology, and wrote, reviewed and edited the manuscript. YYL curated data. PWP performed formal analysis, software analysis and wrote the original draft. CCC conducted investigation, project administration and managed resources. CCC and ACC confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

All experimental procedures involving animals were approved (approval no. 112SKH015) by the Institutional Animal Care and Use Committee of Shin Kong Wu Ho-Su Memorial Hospital (Taipei, Taiwan) and were conducted following the institution's Guidelines for Animal Experimentation.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Myers DJ and Wallen JM: Lung adenocarcinoma: In: StatPearls [Internet]. StatPearls Publishing, Treasure Island, FL, 2023.
- Lu TP, Tsai MH, Lee JM, Hsu CP, Chen PC, Lin CW, Shih JY, Yang PC, Hsiao CK, Lai LC and Chuang EY: Identification of a novel biomarker, sema5a, for non-small cell lung carcinoma in nonsmoking women. *Cancer Epidemiol Biomarkers Prev* 19: 2590-2597, 2010.
- Tsai SC, Liu YC, Li CP, Huang TS and Lee CC: Sesamin inhibits vascular endothelial cell growth and angiogenic activity of lung adenocarcinoma cells. *J Cancer Mol* 2: 199-205, 2006.
- Kong X, Ma MZ, Zhang Y, Weng MZ, Gong W, Guo LQ, Zhang JX, Wang GD, Su Q, Quan ZW and Yang JR: Differentiation therapy: Sesamin as an effective agent in targeting cancer stem-like side population cells of human gallbladder carcinoma. *BMC Complement Alternat Med* 14: 1-12, 2014.
- Majdalawieh AF, Massri M and Nasrallah GK: A comprehensive review on the anti-cancer properties and mechanisms of action of sesamin, a lignan in sesame seeds (*Sesamum indicum*). *Eur J Pharmacol* 815: 512-521, 2017.
- Ye H, Sun L, Li J, Wang Y, Bai J, Wu L, Han Q, Yang Z and Li L: Sesamin attenuates carrageenan-induced lung inflammation through upregulation of A20 and TAX1BP1 in rats. *Int Immunopharmacol* 88: 107009, 2020.
- Yang S, Li X, Dou H, Hu Y, Che C and Xu D: Sesamin induces A549 cell mitophagy and mitochondrial apoptosis via a reactive oxygen species-mediated reduction in mitochondrial membrane potential. *Korean J Physiol Pharmacol* 24: 223-232, 2020.
- Dou H, Yang S, Hu Y, Xu D, Liu L and Li X: Sesamin induces ER stress-mediated apoptosis and activates autophagy in cervical cancer cells. *Life Sci* 200: 87-93, 2018.
- Siriwarin B and Weerapreeyakul N: Sesamol induced apoptotic effect in lung adenocarcinoma cells through both intrinsic and extrinsic pathways. *Chem Biol Interact* 254: 109-116, 2016.
- Acar V, Fernandez FL, Buscariolo FF, Novais AA, Pereira RA and de Campos Zuccari DAP: Immunohistochemical evaluation of PARP and caspase-3 as prognostic markers in prostate carcinomas. *Clin Med Res* 19: 183-191, 2021.
- Son H and Moon A: Epithelial-mesenchymal transition and cell invasion. *Toxicol Res* 26: 245-252, 2010.
- Mottaghi S and Abbaszadeh H: A comprehensive mechanistic insight into the dietary and estrogenic lignans, arctigenin and sesamin as potential anticarcinogenic and anticancer agents. Current status, challenges, and future perspectives. *Crit Rev Food Sci Nutr* 62: 7301-7318, 2022.
- Ediriweera MK, Tennekoon KH and Samarakoon SR: Role of the PI3K/AKT/mTOR signaling pathway in ovarian cancer: Biological and therapeutic significance. *Semin Cancer Biol* 59: 147-160, 2019.
- Fang Q, Zhu Y, Wang Q, Song M, Gao G and Zhou Z: Suppression of cyclooxygenase 2 increases chemosensitivity to sesamin through the Akt-PI3K signaling pathway in lung cancer cells. *Int J Mol Med* 43: 507-516, 2019.
- Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, Roche PC, Lu J, Zhu G, Tamada K, *et al*: Tumor-associated B7-H1 promotes T-cell apoptosis: A potential mechanism of immune evasion. *Nat Med* 8: 793-800, 2002.
- Kongtawelert P, Wudtiwai B, Shwe TH, Pothacharoen P and Phitak T: Inhibition of programmed death ligand 1 (PD-L1) expression in breast cancer cells by sesamin. *Int Immunopharmacol* 86: 106759, 2020.
- Cha JH, Chan LC, Li CW, Hsu JL and Hung MC: Mechanisms controlling PD-L1 expression in cancer. *Mol Cell* 76: 359-370, 2019.
- Cortez MA, Ivan C, Valdecanas D, Wang X, Peltier HJ, Ye Y, Araujo L, Carbone DP, Shilo K, Giri DK, *et al*: PDL1 regulation by p53 via miR-34. *J Natl Cancer Inst* 108: djv303, 2016.
- Danbaran GR, Aslani S, Sharafkandi N, Hemmatzadeh M, Hosseinzadeh R, Azizi G, Jadidi-Niaragh F, Babaie F and Mohammadi H: How microRNAs affect the PD-L1 and its synthetic pathway in cancer. *Int Immunopharmacol* 84: 106594, 2020.
- Yin M, Zhang Z and Wang Y: Anti-tumor effects of miR-34a by regulating immune cells in the tumor microenvironment. *Cancer Med* 12: 11602-11610, 2023.
- Wang X, Li J, Dong K, Lin F, Long M, Ouyang Y, Wei J, Chen X, Weng Y, He T and Zhang H: Tumor suppressor miR-34a targets PD-L1 and functions as a potential immunotherapeutic target in acute myeloid leukemia. *Cell Signal* 27: 443-452, 2015.
- Chu YW, Yang PC, Yang SC, Shyu YC, Hendrix MJ, Wu R and Wu CW: Selection of invasive and metastatic subpopulations from a human lung adenocarcinoma cell line. *Am J Respir Cell Mol Biol* 17: 353-360, 1997.
- Tsai HC, Chang AC, Tsai CH, Huang YL, Gan L, Chen CK, Liu SC, Huang TY, Fong YC and Tang CH: CCN2 promotes drug resistance in osteosarcoma by enhancing ABCG2 expression. *J Cell Physiol* 234: 9297-9307, 2019.

24. Yu HS, Wang SW, Chang AC, Tai HC, Yeh HI, Lin YM and Tang CH: Bradykinin promotes vascular endothelial growth factor expression and increases angiogenesis in human prostate cancer cells. *Biochem Pharmacol* 87: 243-253, 2014.
25. Wu MH, Lo JF, Kuo CH, Lin JA, Lin YM, Chen LM, Tsai FJ, Tsai CH, Huang CY and Tang CH: Endothelin-1 promotes MMP-13 production and migration in human chondrosarcoma cells through FAK/PI3K/Akt/mTOR pathways. *J Cell Physiol* 227: 3016-3026, 2012.
26. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25: 402-408, 2001.
27. Chang AC, Chen PC, Lin YF, Su CM, Liu JF, Lin TH, Chuang SM and Tang CH: Osteoblast-secreted WISP-1 promotes adherence of prostate cancer cells to bone via the VCAM-1/integrin $\alpha 4 \beta 1$ system. *Cancer Lett* 426: 47-56, 2018.
28. Sun X and Kaufman PD: Ki-67: More than a proliferation marker. *Chromosoma* 127: 175-186, 2018.
29. Ashrafzadeh M, Ahmadi Z, Mohamadi N, Zarrabi A, Abasi S, Dehghannoudeh G, Tamaddondoust RN, Khanbabaei H, Mohammadinejad R and Thakur VK: Chitosan-based advanced materials for docetaxel and paclitaxel delivery: Recent advances and future directions in cancer theranostics. *Int J Biol Macromol* 145: 282-300, 2020.
30. Hou RC, Huang HM, Tzen JT and Jeng KC: Protective effects of sesamin and sesamol on hypoxic neuronal and PC12 cells. *J Neurosci Res* 74: 123-133, 2003.
31. Loh CY, Chai JY, Tang TF, Wong WF, Sethi G, Shanmugam MK, Chong PP and Looi CY: The E-cadherin and N-cadherin switch in epithelial-to-mesenchymal transition: Signaling, therapeutic implications, and challenges. *Cells* 8: 1118, 2019.
32. Zhou J, Yang S, Zhu D, Li H, Miao X, Gu M, Xu W, Zhang Y, Tang W, Shen R, *et al*: The crosstalk between anoikis and epithelial-mesenchymal transition and their synergistic roles in predicting prognosis in colon adenocarcinoma. *Front Oncol* 13: 1184215, 2023.
33. Zuo Y, Zheng W, Liu J, Tang Q, Wang SS and Yang XS: MiR-34a-5p/PD-L1 axis regulates cisplatin chemoresistance of ovarian cancer cells. *Neoplasma* 67: 93-101, 2020.
34. Deng S, Wang M, Wang C, Zeng Y, Qin X, Tan Y, Liang B and Cao Y: p53 downregulates PD-L1 expression via miR-34a to inhibit the growth of triple-negative breast cancer cells: A potential clinical immunotherapeutic target. *Mol Biol Rep* 50: 577-587, 2023.
35. Wang Y and Wang L: miR-34a attenuates glioma cells progression and chemoresistance via targeting PD-L1. *Biotechnol Lett* 39: 1485-1492, 2017.
36. Verweij J, Clavel M and Chevalier B: Paclitaxel (TaxolTM) and docetaxel (TaxotereTM): Not simply two of a kind. *Ann Oncol* 5: 495-505, 1994.
37. McGrogan BT, Gilmartin B, Carney DN and McCann A: Taxanes, microtubules and chemoresistant breast cancer. *Biochim Biophys Acta* 1785: 96-132, 2008.
38. Cenik M, Abas BI, Kocabiyyik B, Demirbolat GM and Cevik O: Development of a new drug delivery system from hela-derived exosomes and the effect of docetaxel-loaded exosomes on mitochondrial apoptosis. *J Pharm Innov* 17: 931-939, 2022.
39. Ashrafzadeh M, Mirzaei S, Hashemi F, Zarrabi A, Zabolian A, Saleki H, Sharifzadeh SO, Soleymani L, Daneshi S, Hushmandi K, *et al*: New insight towards development of paclitaxel and docetaxel resistance in cancer cells: EMT as a novel molecular mechanism and therapeutic possibilities. *Biomed Pharmacother* 141: 111824, 2021.
40. Chen Y, Li H, Zhang W, Qi W, Lu C, Huang H, Yang Z, Liu B and Zhang L: Sesamin suppresses NSCLC cell proliferation and induces apoptosis via Akt/p53 pathway. *Toxicol Appl Pharmacol* 387: 114848, 2020.
41. Wu M, Jiang Z, Duan H, Sun L, Zhang S, Chen M, Wang Y, Gao Q, Song Y, Zhu X and Zhang L: Deoxydopodophyllotoxin triggers necroptosis in human non-small cell lung cancer NCI-H460 cells. *Biomed Pharmacother* 67: 701-706, 2013.
42. Choi JY, Hong WG, Cho JH, Kim EM, Kim J, Jung CH, Hwang SG, Um HD and Park JK: Podophyllotoxin acetate triggers anticancer effects against non-small cell lung cancer cells by promoting cell death via cell cycle arrest, ER stress and autophagy. *Int J Oncol* 47: 1257-1265, 2015.
43. Kuo TN, Lin CS, Li GD, Kuo CY and Kao SH: Sesamin inhibits cervical cancer cell proliferation by promoting p53/PTEN-mediated apoptosis. *Int J Med Sci* 17: 2292-2298, 2020.
44. Siao AC, Hou CW, Kao YH and Jeng KC: Effect of sesamin on apoptosis and cell cycle arrest in human breast cancer mcf-7 cells. *Asian Pac J Cancer Prev* 16: 3779-3783, 2015.
45. Harikumar KB, Sung B, Tharakan ST, Pandey MK, Joy B, Guha S, Krishnan S and Aggarwal BB: Sesamin manifests chemopreventive effects through the suppression of NF-kappa B-regulated cell survival, proliferation, invasion, and angiogenic gene products. *Mol Cancer Res* 8: 751-761, 2010.
46. Huang RY, Wong MK, Tan TZ, Kuay KT, Ng AH, Chung VY, Chu YS, Matsumura N, Lai HC, Lee YF, *et al*: An EMT spectrum defines an anoikis-resistant and spheroidogenic intermediate mesenchymal state that is sensitive to e-cadherin restoration by a src-kinase inhibitor, saracatinib (AZD0530). *Cell Death Dis* 4: e915, 2013.
47. Cao ZQ, Wang Z and Leng P: Aberrant N-cadherin expression in cancer. *Biomed Pharmacother* 118: 109320, 2019.
48. Abufaraj M, Shariat SF, Haitel A, Moschini M, Foerster B, Chłosta P, Gust K, Babjuk M, Briganti A, Karakiewicz PI and Albrecht W: Prognostic role of N-cadherin expression in patients with non-muscle-invasive bladder cancer. *Urol Oncol* 35: 264-271, 2017.
49. Su Y, Li J, Shi C, Hruban RH and Radice GL: N-cadherin functions as a growth suppressor in a model of K-ras-induced PanIN. *Oncogene* 35: 3335-3341, 2016.
50. Lammens T, Swerts K, Derycke L, De Craemer A, De Brouwer S, De Preter K, Van Roy N, Vandesompele J, Speleman F, Philippé J, *et al*: N-cadherin in neuroblastoma disease: Expression and clinical significance. *PLoS One* 7: e31206, 2012.
51. He J, Hu Y, Hu M and Li B: Development of PD-1/PD-L1 pathway in tumor immune microenvironment and treatment for non-small cell lung cancer. *Sci Rep* 5: 13110, 2015.
52. Liu H, Zhao Z, Zhang L, Li Y, Jain A, Barve A, Jin W, Liu Y, Fetsé J and Cheng K: Discovery of low-molecular weight anti-PD-L1 peptides for cancer immunotherapy. *J Immunother Cancer* 7: 270, 2019.
53. Wu MS, Aquino LBB, Barbaza MYU, Hsieh CL, Castro-Cruz KA, Yang LL and Tsai PW: Anti-Inflammatory and anticancer properties of bioactive compounds from sesamum indicum L.-A review. *Molecules* 24: 4426, 2019.
54. Kim JH and Lee JK: Sesamol enhances NK cell lysis activity by increasing the expression of NKG2D ligands on Burkitt's lymphoma cells. *Int Immunopharmacol* 28: 977-984, 2015.
55. Wang Q, Lin W, Tang X, Li S, Guo L, Lin Y and Kwok HF: The roles of microRNAs in regulating the expression of PD-1/PD-L1 immune checkpoint. *Int J Mol Sci* 18: 2540, 2017.
56. Hwang TI, Cui YC, Chen YC, Chen PC, Tsai TF, Chou KY, Ho CY, Chen HE, Chang PH and Chang AC: Tumor suppressive functions of hsa-miR-34a on cell cycle, migration and protective autophagy in bladder cancer. *Int J Oncol* 62: 66, 2023.
57. Yang P, Li QJ, Feng Y, Zhang Y, Markowitz GJ, Ning S, Deng Y, Zhao J, Jiang S, Yuan Y, *et al*: TGF- β -miR-34a-CCL22 signaling-induced Treg cell recruitment promotes venous metastases of HBV-positive hepatocellular carcinoma. *Cancer Cell* 22: 291-303, 2012.
58. Shin J, Xie D and Zhong XP: MicroRNA-34a enhances T cell activation by targeting diacylglycerol kinase ζ . *PLoS One* 8: e77983, 2013.

