Heliyon 10 (2024) e29361

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Baicalin reduces inflammation to inhibit lung cancer via targeting SOCS1/NF-κB/STAT3 axis

Lijuan Guo [1], Ming Yue [1], Chengyuan Ma, Yunjing Wang, Jiejie Hou, Hong Li [*]

School of Pharmacy, Henan University, Kaifeng, 475004, China

ARTICLE INFO

Keywords:
Baicalin
Lung cancer
Inflammation
SOCS1/NF-κB/STAT3 axis

ABSTRACT

Inflammation affects several aspects of lung cancer progression including cell proliferation, metastasis, apoptosis, angiogenesis, and drug resistance. Baicalin, an active component of *Scutellaria baicalensis* Georgi, exhibits anticancer activity in various cancers. However, the effects of baicalin on lung cancer and the underlying molecular mechanisms remain largely unknown. This study is to explore the effect and mechanism of baicalin on lung cancer cell A549 and urethane-induced mouse lung cancer. A cell viability assay, colony formation assay, wound healing assay, acridine orange/ethidium bromide (AO/EB) staining assay, Western blot assay, urethane-induced mouse lung cancer model, hematoxylin and eosin (HE) staining, immunohistochemistry (IHC), and ELISA assay were performed to investigate the effects of baicalin on lung cancer *in vitro* and *in vivo*. Network pharmacology analysis, molecular docking, gene silencing assays, and LPS-induced inflammation model were utilized to explore the molecular mechanisms underlying the effect of baicalin on lung cancer. Baicalin showed significant anti-proliferative, anti-migratory, anti-inflammatory and pro-apoptotic effects *in vitro*; it also inhibited the progression of urethane-induced mouse lung cancer *in vivo*. Mechanistically, suppressor of cytokine signaling 1 (SOCS1) was the key determinant for baicalin-induced inhibition of lung cancer. Baicalin increased SOCS1 expression to inactivate the NF-κB/STAT3 pathway to inhibit lung cancer *in vitro* and *in vivo*. Taken together, baicalin reduces inflammation to inhibit lung cancer via targeting SOCS1/NF-κB/STAT3 axis, providing a prospective compound and novel target for lung cancer treatment.

1. Introduction

Scutellaria baicalensis Georgi, or Huangqin, is a medicinal plant, widely used in China and other Asian countries. Baicalin, a vital monomer of *Scutellaria baicalensis* Georgi, exhibits anticancer potential against various cancer types, including bladder [1], colon [2], breast [3], ovarian [4], bone [5], liver cancers [6]. Further, an increasing number of reports have shown that baicalin is closely associated with lung diseases, especially lung cancer. However, the effects of baicalin on lung cancer and its underlying molecular mechanisms remain largely unknown.

Inflammation is a predisposing factor for tumorigenesis, is strongly associated with cancer, and affects many aspects of malignancy, including cell proliferation, apoptosis, ferroptosis, metastasis, angiogenesis, and drug tolerance. Inflammation plays a central role in lung cancer, and may provide reversible targets for its prevention and treatment [7]. However, inflammation itself is not the cause of

* Corresponding author.
E-mail address: lihong@vip.henu.edu.cn (H. Li).
[1] Lijuan Guo and Ming Yue contributed equally.

https://doi.org/10.1016/j.heliyon.2024.e29361
Received 24 November 2023; Received in revised form 3 April 2024; Accepted 7 April 2024
Available online 9 April 2024

the lung cancer onset. Instead, a sustained atmosphere rich in inflammatory cells and inflammatory factors promotes lung cancer emergence [8].

Suppressor of cytokine signaling 1 (SOCS1), the most potent member of the SOCS family consisting of SOCS1-7 and CIS (cytokine-induced SH2 containing protein), was discovered in 1997 from the secretion system of M1 line of IL-6-induced mouse mononuclear leukemia cells; and it was found to be the primary regulator of several cytokines involved in the inflammation and the immune response [9,10]. Decreased SOCS1 expression has been observed in various cancers including lung cancer [11], HCCs [12], acute myeloid leukemia [13], and lymphoma [14]. Abnormal expression of SOCS1 has been reported in different human cancer cells associated with dysregulation of various signals from JAK-STAT [15], TLR4-NFκB [16], hormone receptors [17], and cytokine receptors [18], resulting in malignancies. In recent years, baicalin has been reported to modulate the expression levels of SOCS1/3 [19], and inhibit/stimulate JAK/STAT [20], TLRs [21], and NF-κB pathways [22]. However, whether baicalin affects lung cancer by regulating the SOCS1/NF-κB/STAT3 axis remains unclear.

In this study, a cell viability assay, colony formation assay, wound healing assay, AO/EB staining assay, Western blot assay, urethane-induced mouse lung cancer model, HE staining, IHC, and ELISA assay were performed to investigate the effects of baicalin on lung cancer *in vitro* and *in vivo*. Network pharmacology analysis, molecular docking, gene silencing assays, and LPS-induced inflammation model were utilized to explore the molecular mechanisms underlying the effect of baicalin on lung cancer. Our study will hopefully provide a prospective compound and novel target for lung cancer treatment.

2. Materials and methods

2.1. Cell culture and treatments

Based on the previous study, A549 was selected as the lung cancer cell line for the later study. The human lung cancer cell line A549 was a gift from Professor Du Gangjun from Henan University. The cells were maintained in RPMI 1640 medium (Gibco, USA), supplemented with 10 % foetal bovine serum (FBS; Gibco, USA), 100 units/ml penicillin (Invitrogen, USA) and 100 units/ml streptomycin (Invitrogen, USA), and cultured in 5 % CO_2 atmosphere at 37 °C. The experiment was carried out after three generations of cell transmission.

2.2. Animal breeding and treatments

Male BALB/c mice, 4–6 weeks of age, 20–24 g, were obtained from Henan Provincial Medical Laboratory Animal Center (No. SCXK 2020-0005). All mice were housed under a 12-h light-dark cycle at 25 °C, and were maintained under pathogen-free conditions within the institutional animal facility. All animal experiments were subjected to ethical review (HUSOM2021-067, Biomedical Research Ethics Committee, Henan University). All mice were randomly divided into different groups: the normal group, the model group, and the treatment groups with different doses of baicalin (n = 25). In the normal group, normal saline was injected intraperitoneally, whereas in the model and treatment groups, 6 % urethane (Sigma-Aldrich) solution was injected intraperitoneally, once a week for ten weeks. In the treatment groups, baicalin was administered intragastrically at doses 50, 100, and 200 mg/kg for 6 months. Subsequently, all mice were maintained normally for 6 months. The animals were then euthanized by cervical dislocation and dissected. Serum and lung tissues were collected to confirm the successful induction of lung cancer and for subsequent experiments. Lung tissues were fixed in 4 % paraformaldehyde (GBCBIO Technologies Inc.) and frozen in −80 °C. All the procedures were approved by the Institutional Animal Care and Use Committee of Henan University (No. HUSOM2021-067).

2.3. Colony formation assay

In 6-well plates, 200 cells were seeded per well and cultured for 14 days. The resulting colonies were counted after staining with 0.3 % crystal violet for 15 min at room temperature.

2.4. Wound healing assay

In 6-well plates, 2×10^5 cells were plated per well. The resulting monolayer was scratched using a 10 μL pipet tip and washed with PBS to remove detached cells. The cells were then cultured in medium supplemented with 1 % FBS. Wound areas were photographed at 0, 12, 24, 36, and 48 h after-wounding. The closure area of the wound was calculated as follows: $R = (A_0 - A_n)/A_0 \times 100$, where R represents migration area (%), A_0 represents the initial wound area, A_n represents the area remaining at the metering point.

2.5. Cell apoptosis assay

In 12-well plates, 5×10^5 cells/well were plated, and cultured for 48 h in a medium supplemented with baicalin. The apoptosis rate was measured after staining with AO and EB solutions for 5 min.

2.6. Western blot analysis

RIPA lysis buffer (Solarbio, China) was utilized to lyse the cells. SDS–PAGE (10 %) was utilized to separate the total proteins before

transferring them to a polyvinylidene fluoride (PVDF) membrane (Millipore, Germany). The membrane was blocked with BSA (2.5 %) for 1 h at room temperature, followed by overnight incubation with the primary antibody at 4 °C. The antibody-related information was presented in Table 1. On the next day, the membrane was incubated with a secondary antibody at room temperature for 45 min before visualisation using ECL (Solarbio, Beijing).

2.7. Molecular docking

The chemical structure of Baicalin (Molecule ID MOL002935) was obtained from the TCMSP (http://tcmspnw.com/). The SOCS1 (PDB code 6C7Y) and JKA2 (PDB code 7F7W) protein structures were obtained from RCSB PDB (http://www.rcsb.org/pdb/). Autodock tools 1.5.6 software was used to explore the interaction between baicalin and SOCS1. The molecular docking results were visualized using PyMOL 2.3.2 software.

2.8. SOCS1 knockdown in lung cancer cells

In 6-well plates, 2×10^5 cells/well were seeded and cultured as monolayers until ~50 % confluence. SOCS1 knockdown was performed using the genOFFTM k-it and the transfection kit ribo FECT™ CP (RiboBio, China). Cells were then transfected with 5 nmol of siRNA (5′-TGCACCTCCTACCTCTTCATGTTTCAAGAGAACATGAAGAGGTAGGAGGTGCTTTTTTC-3′and5′-TCGAGAAAAAAGCACC TCCTACCTCTTCATGTTCTCTTGAAACATGAAGAGGTAGGAGGTGCA-3′) (RiboBio, China).

2.9. HE staining

The tissues were fixed in 4 % paraformaldehyde for 48 h at room temperature. After deparaffinization and rehydration, sections (8 μm) were stained with hematoxylin solution for 3–6 min followed by 5–8 dips in the color separation solution (1 % HCl in 70 % ethanol); they were then rinsed in cold water. Subsequently, the sections were stained with eosin solution for 2–5 min followed by dehydration and clearing. Images were taken utilizing Olympus BX53 fluorescence microscope (Olympus, Tokyo, Japan).

2.10. Immunohistochemical staining

Tissue sections (3 μm) were kept at 60 °C for 90 min in the oven and subsequently de-paraffinised and hydrated. After incubating with 3 % H_2O_2 for 30 min at room temperature, the sections were incubated overnight at 4 °C with primary antibody (1:50). The following day, sections were incubated with the corresponding secondary antibody for 45 min at room temperature followed by staining with DAB and hematoxylin staining. Images were captured using an Olympus BX53 fluorescence microscope (Olympus, Tokyo, Japan) and analysed by Image-Pro Plus 6.0.

2.11. Statistical analysis

All experiments were repeated at least 3 times unless otherwise stated in the figure legends. Statistical analysis was performed using GraphPad Prism 7.0 and Origin 8.0. The data are presented as means ± SD except where stated otherwise. $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ were considered statistically significant.

Table 1
Antibody information.

antibody	company
anti-IL6 antibody	Proteintech, CN
anti-iNOS antibody	Proteintech, CN
anti-β-actin antibody	Proteintech, CN
anti-BAX antibody	Proteintech, CN
anti-Bcl-2 antibody	Proteintech, CN
anti-Caspase 3 antibody	Proteintech, CN
anti-Cleaved Caspase 3 antibody	Proteintech, CN
anti-TLR4 antibody	Proteintech, CN
anti-PCNA antibody	Proteintech, CN
anti-MMP2 antibody	Proteintech, CN
anti-SOCS1 antibody	Proteintech, CN
anti–NF–κB antibody	Proteintech, CN
anti-JAK2 antibody	Proteintech, CN
anti-STAT3 antibody	Proteintech, CN
HRP goat anti-mouse antibody	Proteintech, CN
HRP goat anti-rabbit antibody	Proteintech, CN

3. Results

3.1. Baicalin inhibited cell inflammation, proliferation, and migration and induced cell apoptosis in lung cancer cells

Gene Ontology (GO) analysis was performed to determine the effect of baicalin on lung cancer cells. These results suggest that baicalin affects lung cancer cells apoptosis, inflammation, proliferation, and migration (Fig. 1A). Baicalin concentrations of 30, 60, and 120 μM and a process-time of 48 h were chosen for further confirmatory tests (Fig. S1). Cell apoptosis was assessed using AO/EB staining. Baicalin treatment was found to induce a high percentage of cell death and apoptosis in A549 cells ($P < 0.01$) (Fig. 1B and C). Subsequently, the protein expression of apoptosis markers was analysed by Western blotting. The results showed that the levels of pro-apoptosis markers BAX ($P < 0.001$), Caspase 3 ($P < 0.01$), and Cleaved Caspase 3 ($P < 0.01$) were increased, and that the anti-apoptosis

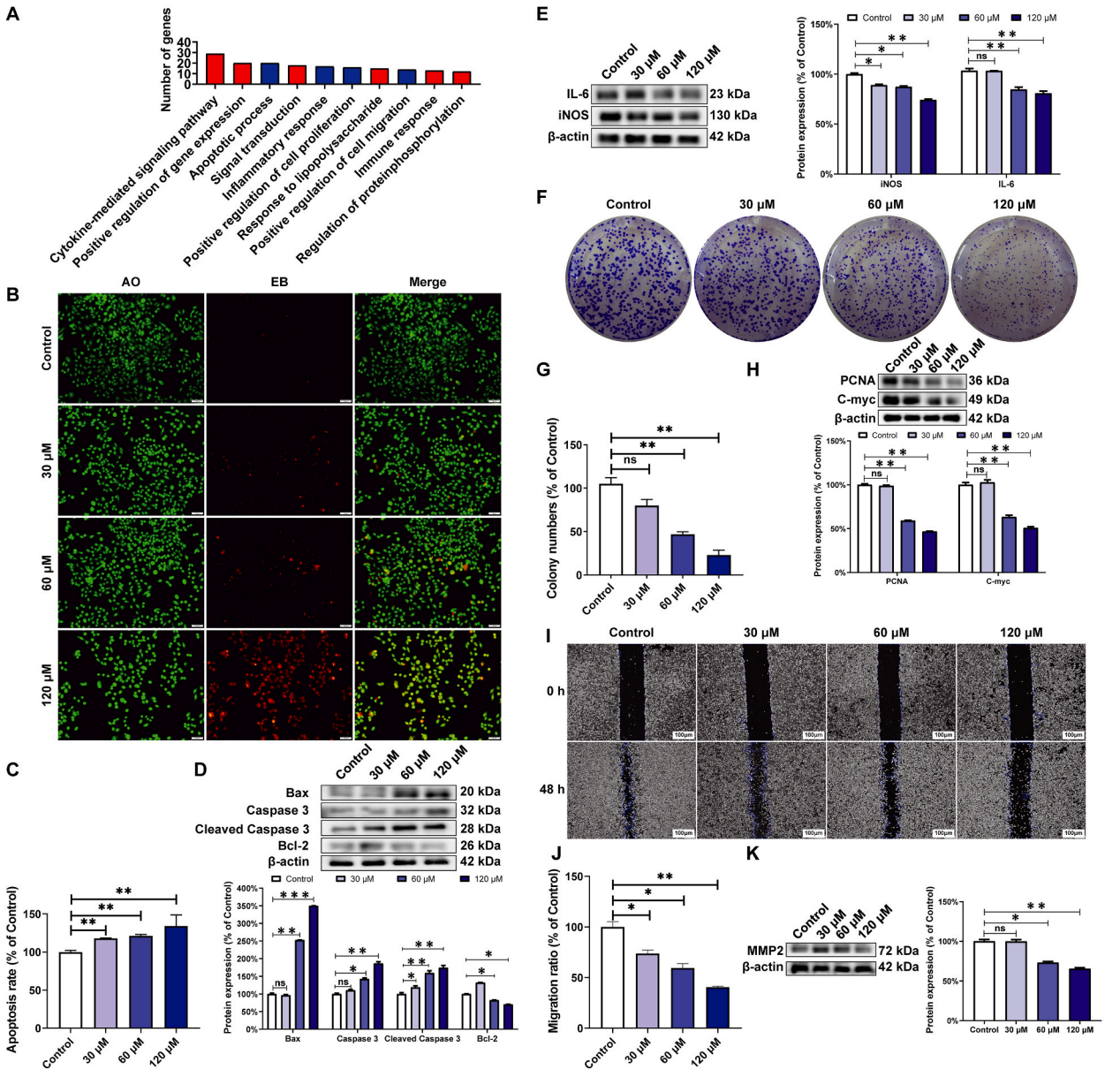

Fig. 1. Effect of baicalin on A549 cells. A, GO analysis of the tentative inference regarding the effect of baicalin on lung cancer cells. B, AO/EB staining for A549 cell apoptosis. C, Quantification results of AO/EB staining shown in histograms. D, E, H, and K, Western blot and quantification results for histograms of protein levels. D, Markers of cell apoptosis. E, Markers of cell inflammation. H, Cell proliferation markers. K, Migration marker. F, Colony formation assay for cell proliferation. G, Quantification results of colony formation shown as histograms. I, Wound healing assay for cell migration. J, Quantification results of migration shown in histograms. All experiments were repeated at least 3 times. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ were considered statistically significant. The uncropped versions of D, E, H, K have been provided as *Supplement file*.

marker Bcl-2 ($P < 0.05$) was decreased (Fig. 1D). Further, the protein expression of cell inflammation markers, IL-6 ($P < 0.01$) and iNOS ($P < 0.01$), were decreased (Fig. 1E). A colony formation assay was performed to examine cell proliferation. The results showed that baicalin inhibited A549 cells proliferation ($P < 0.01$) (Fig. 1F and G). Further, the cell proliferation markers PCNA ($P < 0.01$) and C-myc ($P < 0.01$) were decreased (Fig. 1H). The results of the wound-healing assay demonstrated that baicalin inhibited A549 cells migration ($P < 0.01$) (Fig. 1I and J). Western blotting data suggested that the expression of the migration marker MMP2 was significantly decreased ($P < 0.01$) (Fig. 1K). All results were dose-dependent. Overall, baicalin was found to inhibit lung cancer in a dose-dependent manner *in vitro*.

3.2. Baicalin reduced inflammation in lung cancer cells

LPS, a specific agonist of the TLR4-mediated pathway, promotes the expression of inflammation-related proteins and triggers an inflammatory response. LPS (2 μg/mL) was thus utilized to induce cellular inflammation. Cotreatment with baicalin significantly reversed the LPS-induced increased in the expression of inflammatory proteins, including IL-6 ($P < 0.05$) and iNOS ($P < 0.001$) (Fig. 2A). LPS-induced cell proliferation ($P < 0.001$), migration ($P < 0.01$), and anti-apoptosis markers ($P < 0.001$) were inhibited by baicalin (Fig. 2B–D). Further, LPS-induced high expression of the cell proliferation marker PCNA ($P < 0.01$), migration marker MMP2

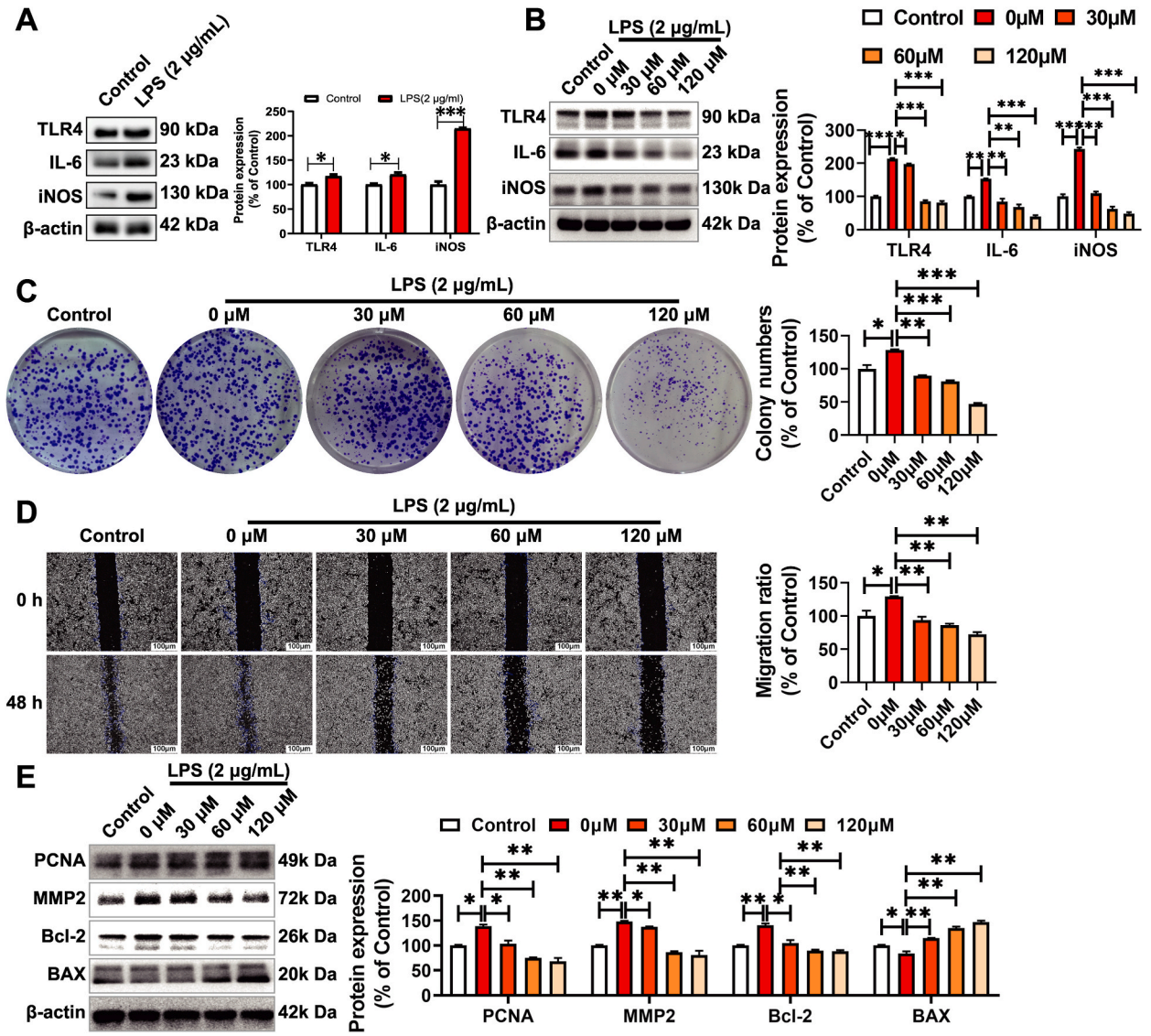

Fig. 2. Effect of baicalin on inflammation in A549 cells. A, B, and E, Western blot analysis and quantification results as histograms of protein levels. A and B, Markers of cell inflammation. E, Markers of cell proliferation, migration, and apoptosis. C, Colony formation assay for cell proliferation. D, Wound healing assay for cell migration. All experiments were repeated at least 3 times. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ were considered statistically significant. The uncropped versions of A, B, E have been provided as *Supplement file*.

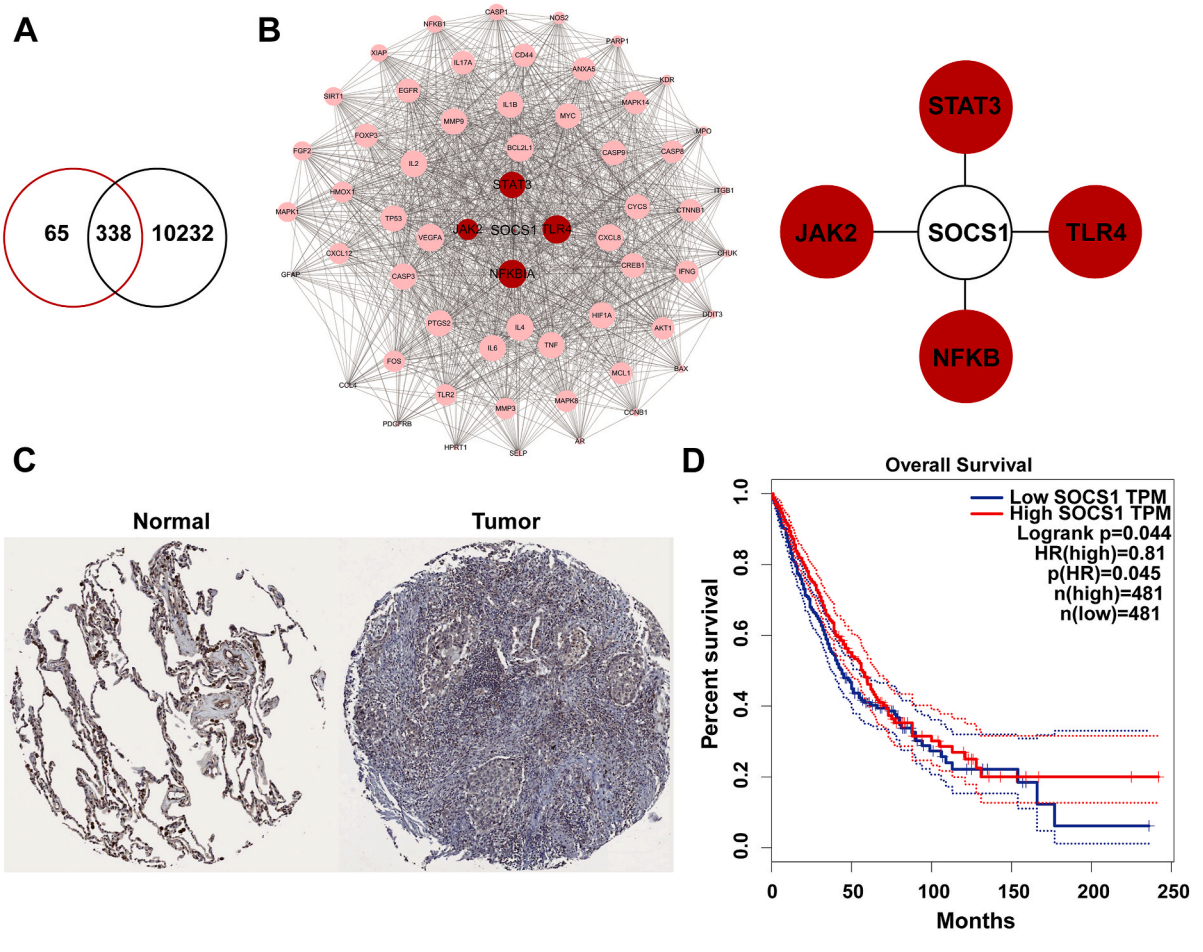

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Fig. 3. Target of baicalin-induced inhibition inflammation in A549 cells. A-B, Tentative inference regarding the target of baicalin in lung cancer using network pharmacology analysis. C, Protein expression of SOCS1 in patient lung cancer tissues and normal lung tissues obtained from The Human Protein Altas database. D, Survival analysis of SOCS1 in patients with lung cancer. E-H, Validation of SOCS1 as the baicalin target. Left panel, Western blot of SOCS1 protein expression. Right panel, Quantification results in histograms. All experiments were repeated at least 3 times. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ were considered statistically significant. The uncropped versions of E-H have been provided as *Supplement file*.

($P < 0.01$), and anti-apoptosis marker Bcl-2 ($P < 0.01$) were also decreased by baicalin. Oppositely, expression of the pro-apoptosis marker BAX ($P < 0.01$) was increased (Fig. 2E). All results were dose-dependent.

3.3. SOCS1 was a key determinant for baicalin-induced anti-inflammatory action in lung cancer

Network pharmacology analysis and Gene Expression Profiling Interactive Analysis (GEPIA2, http://gepia2.cancer-pku.cn; The Human Protein Atlas, https://www.proteinatlas.org/) were performed to identify the targets of baicalin in lung cancer. The potential target of baicalin in lung cancer is SOCS1 (Fig. 3A and B). SOCS1 expression was decreased in lung cancer tissues than in normal lung tissues (Fig. 3C) and gradually decreased with lung cancer progression (stages I-IV). Low SOCS1 expression was unfavourable for the overall survival of patients with lung cancer (Fig. 3D). Following LPS-induced cell inflammatory conditions, the protein expression of SOCS1 decreased significantly ($P < 0.01$) (Fig. 3E). On the contrary, cotreatment with baicalin significantly reversed the LPS-induced decrease in SOCS1 protein expression ($P < 0.01$) (Fig. 3F). To further identify the key role of SOCS1, SOCS1-siRNA was transfected into baicalin-treated A549 cells (Fig. 3G). Baicalin did not significantly reverse LPS-induced decrease in SOCS1 protein expression ($P > 0.05$) (Fig. 3H).

To identify the key residues in SOCS1 that interact with baicalin, molecular docking of SOCS1 & baicalin and protein structure analysis of SOCS1 & JAK2 were performed. Molecular docking showed that SOCS1 protein could bond well with baicalin (Molecule ID MOL002935) with a docking score of −8.45 and that the key amino acids were R56, T57, R59, D63, N110, and F130 (Fig. 4A). Furthermore, amino acids R56, T57, R59, N110, R127, and D131 played important roles in SOCS1 (PDB code 6C7Y) binding with JAK2 (PDB code 7F7W) (Fig. 4B). The structures of SOCS1&JAK2 and SOCS1&baicalin were aligned, demonstrating a spatial overlap between them and the key amino acids R56, T57, R59 and N110 overlap (Fig. 4C). Sequences of Human-SOCS1, Scrofa-SOCS1, Oryctolagus-SOCS1, Musculus-SOCS1, and Gallus-SOCS1 were aligned. Residues R56, T57, R59 and N110 were found to be highly conserved (Fig. 4E), and were the four putative kinase inhibitory region, inferred from the sequence homology (Fig. 4D).

3.4. Baicalin inhibited inflammation by regulating SOCS1/NF-κB/STAT3 axis in lung cancer

Network pharmacology analysis was performed to identify the downstream pathways and SOCS1 genes. KEGG pathway enrichment analysis showed that baicalin may inhibit lung cancer through the TLR4/NF-κB and JAK2/STAT3 pathways (Fig. 5A). With the LPS-induced cell inflammation condition, SOCS1 protein expression decreased significantly ($P < 0.01$) (Fig. 3E), and key proteins of the NF-κB/STAT3 axis increased significantly, including TLR4 ($P < 0.001$) (Fig. 2A), NF-κB ($P < 0.01$), JAK2 ($P < 0.001$), and STAT3 ($P < 0.001$) (Fig. 5B). On the contrary, cotreatment with baicalin significantly reversed the expression of SOCS1 ($P < 0.001$) (Fig. 3F), TLR4 ($P < 0.001$) (Fig. 2B), NF-κB ($P < 0.001$), JAK2 ($P < 0.001$), and STAT3 ($P < 0.001$) (Fig. 5C).

To further identify the role of the SOCS1/NF-κB/STAT3 axis in lung cancer. SOCS1-siRNA was transfected into A549 cells treated with baicalin. The data suggested that baicalin promoted the protein expression of SOCS1, and inhibited the protein expression of key components in the NF-κB/STAT3 axis (NF-κB, JAK2, and STAT3) ($P < 0.01$) (Fig. 5B) as well as inflammatory markers ($P < 0.001$) (IL-6 and iNOS) (Fig. 2B). Upon silencing SOCS1, baicalin did not affect the protein expression of NF-κB, JAK2, STAT3, IL-6, or iNOS ($P > 0.05$) (Fig. 5D).

3.5. Baicalin inhibited inflammation by regulating SOCS1/NF-κB/STAT3 axis in vivo

To investigate the therapeutic potential of baicalin *in vivo*, we used a urethane-induced lung cancer mouse model (Fig. 6A). Pre-experiments were performed to determine the *in vivo* experimental conditions. BALB/c mice were randomly divided into five experimental groups (twenty-five mice per group): normal (normal saline), model, and baicalin treatment (50, 100, and 200 mg/kg) groups. Mouse body weight (Fig. 6B), independent activity (Fig. 6C), and overall survival (Fig. 6D) were found to be improved by baicalin treatment compared to that in the model group ($P < 0.01$). A significant decrease in pulmonary nodules was also detected in the baicalin-treated groups compared to that in the model group ($P < 0.001$) (Fig. 6E). The effect of baicalin on inflammation in mice with lung cancer was investigated using HE staining and ELISA. The results suggested that baicalin could inhibit inflammatory cells infiltration in mouse lung cancer tissues (Fig. 6F) and reduce the level of inflammatory factors, including IL-6 ($P < 0.001$), TNF-α ($P < 0.01$), and TGF-β ($P < 0.01$), in mouse serum (Fig. 6G). Finally, PCA analysis was obtained based on the data of body weight, independent activity, overall survival, pulmonary nodules, severity of pulmonary lesions, and levels of inflammatory factors. The model group deviated significantly from the other groups, whereas the treatment groups were clustered and were closer to the normal group (Fig. 6H). This suggests that baicalin exerted therapeutic effects in mice with lung cancer.

Immunohistochemical staining indicated that SOCS1 expression increased in the baicalin-treated group compared to that in the model group ($P < 0.001$) (Fig. 7A). Meanwhile, key proteins of the SOCS1/NF-κB/STAT3 axis, including TLR4 ($P < 0.01$), NF-κB ($P <$

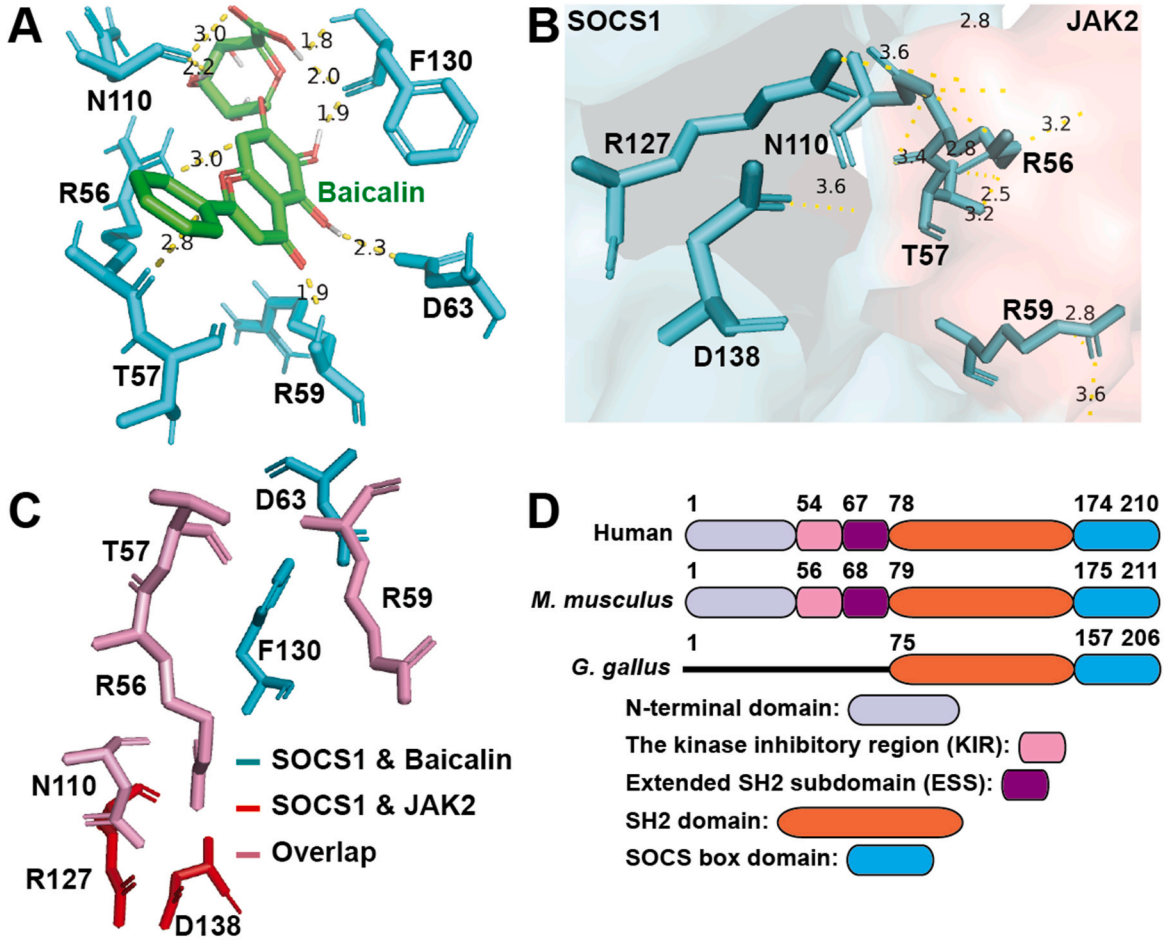

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Fig. 4. Key residues in SOCS1 that interact with baicalin. A-E, Identification of key residues in SOCS1 combining with baicalin. A, Overall structure of SOCS1 docking with baicalin. SOCS1 and baicalin are colored in cyan and green, respectively. The side chains of the key residues are shown as sticks. B, The overall structure of SOCS1 in complex with JAK2. SOCS1 and JAK2 are colored in cyan and pink, respectively. The side chains of the key residues are shown as sticks. C, Alignment of the combined domains of SOCS1&JAK2 and SOCS1&baicalin. The overall structure shown in the ribbon rendition in two orthogonal views. The side chains of the key residues are shown as sticks. Overlapping residues are colored in purple. D, Schematic representation of the SOCS1 domain architecture. It consists of the N-terminal, KIR, ESS, SH2, and SOCS box domains. E, Sequences of Human-SOCS1, Scrofa-SOCS1, Oryctolagus-SOCS1, Musculus-SOCS1, and Gallus-SOCS1 proteins. Conserved residues are highlighted in red. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

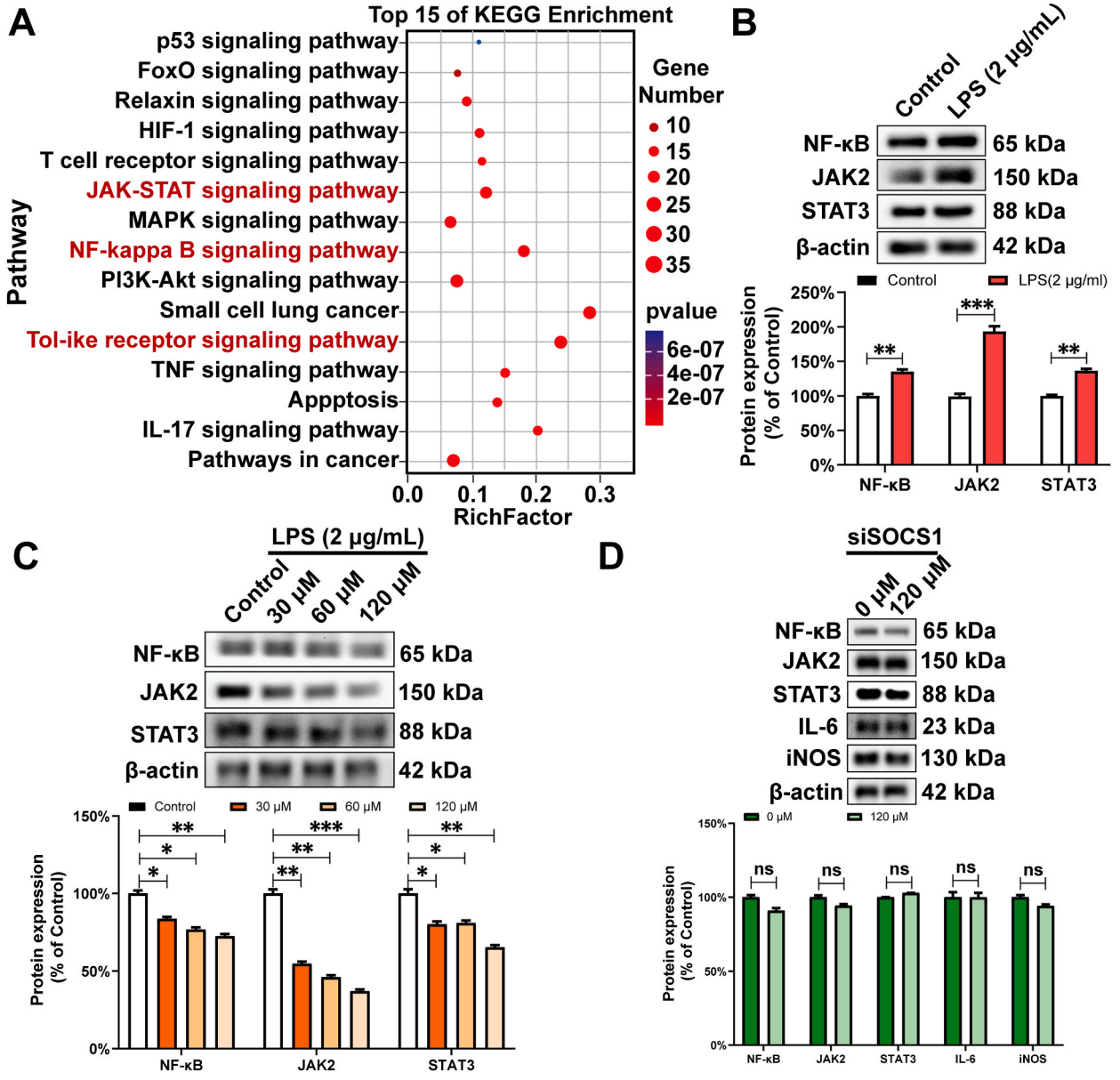

Fig. 5. Target signal axis of baicalin-induced inhibition of inflammation in A549 cells. A, Network pharmacology analysis. B-D, Western blot and quantification results as histograms of protein levels. B, LPS-induced inflammation in A549 cells. C, Baicalin inhibited LPS-induced inflammation in A549 cells. D, Silencing of SOCS1 in A549 cells. All experiments were repeated at least 3 times. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ were considered statistically significant. The uncropped versions of B-D have been provided as *Supplement file*.

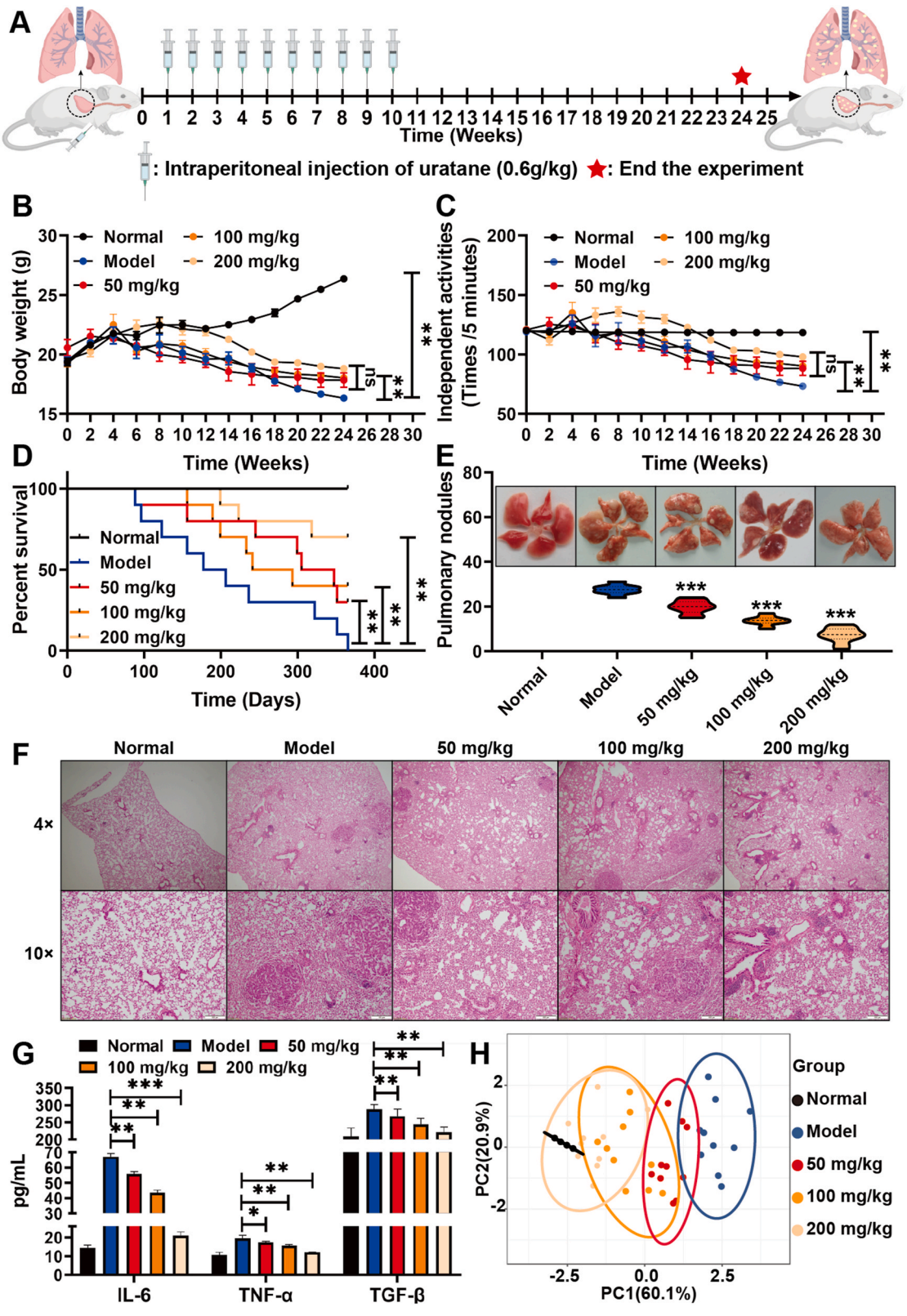

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Fig. 6. Effect of baicalin on mouse lung cancer. A, Schematic diagram of the urethane-induced mouse lung cancer model. B, Mouse body weight. C, Mouse independent activity. D, Mouse survival. E and F, Comparison of the therapeutic effects of baicalin on urethane-induced mouse lung cancer in different dose groups. E, Effect of baicalin on mouse pulmonary nodules. F, Effect of baicalin on histopathological in mouse. Lung tissues were stained with HE. G, ELISA for inflammatory factors in mouse serum. H, PCA analysis of the effect of baicalin on mouse body weight, independent activity, overall survival, pulmonary nodules, severity of pulmonary lesions, and levels of inflammatory factors. All experiments were repeated at least 3 times. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ were considered statistically significant.

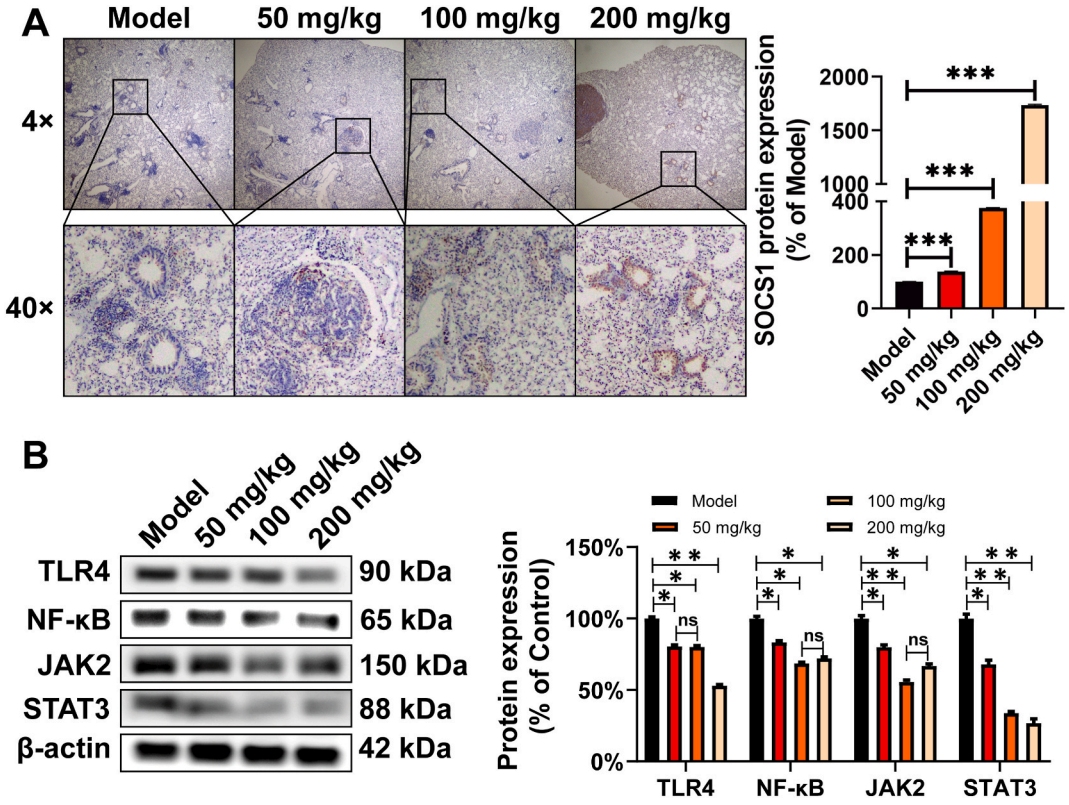

Fig. 7. Effect of baicalin on SOCS1/NF-κB/STAT3 axis *in vivo*. A, Immunohistochemistry (IHC) for SOCS1 expression in mouse lung cancer tissues. Right panel, quantification results of IHC shown as histograms (n = 15). B, Western blot for key proteins of the SOCS1/NF-κB/STAT3 axis in mouse lung tissues. Right panel, quantification of Western blot results shown as histograms. All experiments were repeated at least 3 times. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ were considered statistically significant. The uncropped versions of B have been provided as *Supplement file*.

0.05), JAK2 ($P < 0.05$), and STAT3 ($P < 0.01$), were decreased (Fig. 7B). Taken together, our results demonstrated that baicalin inhibited inflammation by regulating the SOCS1/NF-κB/STAT3 axis *in vivo*.

4. Discussion and conclusions

In recent years, it has been widely explored natural bioactive products for tumor inhibition. Baicalin is found to exert cytotoxicity and anti-proliferation in lung cancer cells A549 by inhibiting activity and regulating abnormal expression of Aurora Kinase B [23]. Inhibitor of differentiation 1 (Id1) is overexpressed in lung cancer and involved in promoting its progression and metastasis. Baicalin is found to trigger Id1 inhibition to inhibit lung cancer proliferation, EMT and angiogenesis [24]. Baicalin is also a useful radioprotective agent, inhibiting the radiation-induced epithelial-mesenchymal transformation of primary type II alveolar epithelial cells by TGF-β and ERK/GSK3β pathways and preventing lung injury that may be induced by tumor radiation therapy [25]. Further, baicalin inhibits mTOR phosphorylation and MMP expression by activating SIRT1/AMPK signal transduction, and induces apoptosis in non-small cell lung cancer cells [26]. In addition, baicalin is found to reduces cisplatin resistance in lung cancer therapy by down-regulating the expression of MAPK2 and P-AKT [27]. Also, baicalin has been reported to inhibit lung cancer both *in vivo* and *in vitro* by directly combining with PBK/TOPK [28]. Baicalin inhibits the proliferation and invasion of lung cancer cells A549 and H1299 by regulating the miR-340-5p/NET1 axis [29]. Although there are many basic studies on baicalin in lung cancer treatment, there are few clinical application studies on it. The clinical application of baicalin in lung cancer treatment is still limited, mainly because its mechanism has not been completely cleared. Therefore, it is crucial to study the inhibitory effect of baicalin on lung cancer from a new perspective, and propose a new mechanism.

In 1863, Virchow hypothesised that tumours originated at sites of chronic inflammation; inflammation has thus been associated with tumour ever since [8,30]. The tumour microenvironment, an indispensable participant in cancer cells proliferation and migration, is now clarified to be largely orchestrated by inflammatory cells [31,32]. Baicalin has demonstrated remarkable anti-inflammatory activities in inflammatory diseases [33], lung injury [34], autoimmune hepatitis, and rheumatoid arthritis [35]. Baicalin has been reported to suppress lung oxidative injury by down-regulating NLRP3 inflammasomes and TLRs/NF-κB signaling but by up-regulating Nrf2-HO-1 signaling [34,36,37]. In this study, we found that baicalin could rescue inflammation and inhibit lung cancer both *in vitro* and *in vivo*.

SOCS1 is known to be associated with lung cancer, and downregulating SOCS1 promotes lung cancer [11,38]. We also found that SOCS1 expression was decreased in lung cancer tissues. Baicalin is known to modulate SOCS1 expression in viruses [19]. We further demonstrated that SOCS1 was the key target through which baicalin inhibited lung cancer; notably, increased the expression of SOCS1 in both lung cancer cells A549 and mouse lung cancer tissues.

SOCS1 in 1999 was first identified as a JAK-binding inhibitor of cytokine signaling [39]. SOCS1 is significantly enriched in JAK/STAT signaling [40], RNA degradation [41], and NF-κB signaling related to cancer [42], and plays an essential role in the effect of cinnamaldehyde against non-small cell lung cancer [43]. Meanwhile, miR-146a-5p and miR-155-5p promote the activation of CAFs through JAK2-STAT3/NF-κB signaling by targeting SOCS1 [44]. The molecular mechanisms of baicalin working as an antiviral agent involve regulation of the JAK/STAT [45], TLRs [46], and NF-κB pathways [19]. To date, baicalin has been reported to suppresses lung cancer via the miR-340-5p/NET1 axis [29], PDK1/AKT signaling [47], Akt/mTOR signaling [48], SIRT1/AMPK signaling [49], AKT/HIF-1α/p27-associated pathway [50], TGF-β and ERK/GSK3β signaling [51], and PI3K/Akt signaling [52]. In our study, baicalin was found to increase the expression of SOCS1 to inactivate the NF-κB/STAT3 pathway and inhibit lung cancer both *in vivo* and *in vitro*.

Scutellaria baicalensis Georgi is a common Chinese medicinal herb that has been utilized for more than 2000 years. Recently, *Scutellaria baicalensis* Georgi and its constituent components have been shown to exhibit significant activities against lung cancer by regulating the drug-resistance, apoptosis, cell-cycle arrest, proliferation, invasion and metastasis of lung cancer cells [53]. Its constituent components [54], which possessing anti-lung cancer effects, include baicalein, a baicalein derivative, wogonin, wogonoside, baicalin, skullcap flavone I, and oroxylin A. In this study, we suggest the traditional Chinese medicine prescription Huang-qin decoction, exerts significant anti-lung cancer effects *in vivo*, and that baicalin is the vital component of Huang-qin decoction involved in lung cancer inhibition.

There are also some limitations and future directions in our study. First, baicalin targeting SOCS1 reduces inflammation to inhibit lung cancer was found in our study, but how does it target SOCS1 in lung cancer should to be further explored. Second, samples of clinic tissues are needed to validate our findings.

In summary, we demonstrated that baicalin exerts anti-lung cancer activity by inducing apoptosis and inhibiting cells proliferation

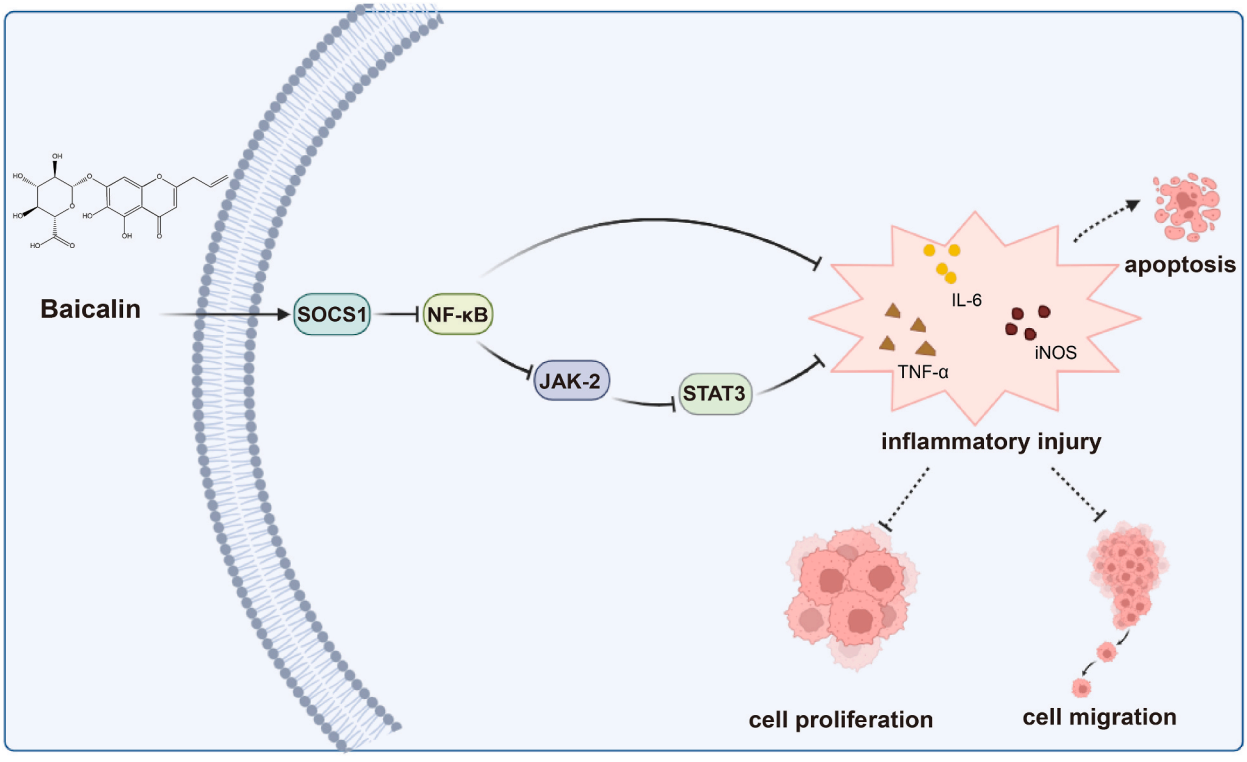

Fig. 8. Molecular mechanism of baicalin-induced lung cancer inhibition.

and migration in a dose-dependent manner. Subsequently, we demonstrated for the first time that baicalin targets SOCS1 to rescue cancer-associated inflammation in lung cancer, both *in vitro* and *in vivo*. Mechanistically, we identified that baicalin increased SOCS1 expression to inactivate the NF-κB/STAT3 pathway and inhibit lung cancer. Interestingly, baicalin was found to be the vital component in Huang-qin decoction for inhibiting lung cancer. In summary, our data suggest that baicalin exerts anti-lung cancer effects by regulating the SOCS1/NF-κB/STAT3 axis, thus providing a prospective compound for lung cancer treatment (Fig. 8).

Ethics approval

The study was approved by the Institutional Animal Care and Use Committee of Henan University (No. HUSOM2021-067).

Funding

This study was supported by Henan Provincial Science and Technology Research Project (No. 202102310168; No. 242102310517).

Data availability statement

All data generated during this study are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Lijuan Guo: Visualization, Validation, Software, Investigation, Data curation. **Ming Yue:** Visualization, Validation, Software, Investigation, Data curation. **Chengyuan Ma:** Software, Data curation. **Yunjing Wang:** Software, Data curation. **Jiejie Hou:** Software, Data curation. **Hong Li:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Investigation, Data curation, Conceptualization.

Declaration of competing interest

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

Acknowledgments

The authors gratefully acknowledge the efforts of the TCGA, GO, PDB, The Human Protein Atlas, and TCMSP datasets for providing high-quality open resources for researchers.

The authors also gratefully acknowledge Elsevier Language Editing Services for providing professional and prompt editing of scientific language for our manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e29361.

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