Antcin K inhibits chondrosarcoma motility by reducing MMP-7 expression via downregulation of the PI3K, Akt, mTOR and NF-κB signaling pathway

YAT-YIN LAW[1,2], NGUYEN BAO TRAN[3], CHANG-YU SONG[3], YU-YING WU[1,2,4], HSIEN-TE CHEN[5,6], YI-CHIN FONG[5-7], HSIAO-CHI TSAI[8], YUEH-HSIUNG KUO[9,10] and CHIH-HSIN TANG[3,10-12]

[1]School of Medicine, Chung Shan Medical University, Taichung, Taiwan 40201, Taiwan, R.O.C.; [2]Department of Orthopedics, Chung Shan Medical University Hospital, Taichung, Taiwan 402, Taiwan, R.O.C.; [3]Graduate Institute of Biomedical Sciences, China Medical University, Taichung, Taiwan 404, Taiwan, R.O.C.; [4]Department of Orthopedics, Penghu Hospital, Ministry of Health and Welfare, Penghu, Taiwan 880, Taiwan, R.O.C.; [5]Department of Sports Medicine, College of Health Care, China Medical University, Taichung, Taiwan 404, Taiwan, R.O.C.; [6]Department of Orthopedic Surgery, China Medical University Hospital, Taichung, Taiwan 404, Taiwan, R.O.C.; [7]Department of Orthopedic Surgery, China Medical University Beigang Hospital, Taichung, Yunlin, Taiwan 651, Taiwan, R.O.C.; [8]Department of Medicine Research, China Medical University Beigang Hospital, Yunlin, Taiwan 651, Taiwan, R.O.C.; [9]Department of Chinese Pharmaceutical Sciences and Chinese Medicine Resources, China Medical University, Taichung, Taiwan 404, Taiwan, R.O.C.; [10]Department of Pharmacology, School of Medicine, China Medical University, Taichung, Taiwan 404333, Taiwan, R.O.C.; [11]Chinese Medicine Research Center, China Medical University, Taichung, Taiwan 404, Taiwan, R.O.C.; [12]Department of Medical Laboratory Science and Biotechnology, College of Medical and Health Science, Asia University, Taichung, Taiwan 41354, Taiwan, R.O.C.

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Abstract. Chondrosarcoma is the second most common form of primary bone cancer originating from cartilage. Chondrosarcoma cells have a high propensity to spread to other organs during the advanced stage, with the lung being a preferred site. Although surgery is the most effective treatment for chondrosarcoma, it has low efficacy in the metastasis stage. *Antrodia cinnamomea* is the source of the triterpenoid antcin K, which exhibits immunomodulatory and anti-inflammatory properties. However, the therapeutic function of antcin K on chondrosarcoma has not yet been elucidated. The inhibitory effect of antcin K was evaluated using migration and invasion assays while cell toxicity was determined using the MTT assay. Molecular function regulation by antcin K was investigated by RNA sequencing and Ingenuity Pathway Analysis. The present study revealed that antcin K decreases migration and invasion in two chondrosarcoma cell lines. RNA sequencing revealed that MMP-7 serves a key role in antcin K-mediated motility of chondrosarcoma cells. Antcin K diminished MMP-7 expression, and overexpression of MMP-7 antagonized antcin K-induced inhibition of cell migration and invasion. Antcin K abolished the activation of PI3K, Akt, mTOR and NF-κB pathways. The present study demonstrated that antcin K is a novel candidate for chondrosarcoma motility inhibition by decreasing the PI3K, Akt, mTOR and NF-κB signaling cascades, which inhibits MMP-7 production.

Correspondence to: Professor Yueh-Hsiung Kuo, Department of Chinese Pharmaceutical Sciences and Chinese Medicine Resources, China Medical University, Taichung, 91 Xueshi Road, Taiwan 404333, Taiwan, R.O.C.
E-mail: kuoyh@mail.cmu.edu.tw

Professor Chih-Hsin Tang, Department of Pharmacology, School of Medicine, China Medical University, 91 Xueshi Road, Taichung, Taiwan 404333, Taiwan, R.O.C.
E-mail: chtang@mail.cmu.edu.tw

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Introduction

After osteosarcoma, chondrosarcoma is the second most common type of bone cancer (accounting for 20-30% of bone cancer cases) and presents with a range of morphological and clinical characteristics, from low-grade, biologically benign tumors to aggressive high-grade variants (1,2). Chondrosarcoma is the predominant malignancy of cartilage, attributed to somatic mutations in the isocitrate dehydrogenase (IDH) 1 and 2 genes (3). Surgery is typically the first line treatment for chondrosarcoma. However, surgical intervention is ineffective if the tumor has metastasized to other parts of the body or is located in an unresectable position such as the skull or pelvis (4). Between 50 and 70% of advanced-stage chondrosarcoma cases involve metastasis to other organs, such as the lung (5), markedly affecting patient prognosis with median overall survival of 12.7 months in

the prospective database of the French Sarcoma Group (6,7). Furthermore, numerous types of chemotherapy have limited efficacy against chondrosarcoma (8). These challenges highlight the need for alternative therapeutic strategies to address chondrosarcoma metastasis.

The leading cause of mortality in patients with cancer is metastases (9); the involvement of MMPs is key to the metastatic process (10). MMP-7 is distributed in endothelial and vascular smooth muscle cells (11). Additionally, it may be released by epithelial cells or retained within the cytoplasm of breast cancer cells (12). Furthermore, a direct association between MMP-7 expression and the onset of cancer has been demonstrated (13). Specifically, chondrosarcomas exhibit high expression levels of MMP-7, an enzyme that controls colony formation and cell invasion in response to fluid shear stress (14). MicroRNA-520f-3p regulates the activity of MMP-7, which is key for the progression and metastasis of chondrosarcoma (15). Therefore, MMP-7 may be a viable option for treating chondrosarcoma metastases.

A previous study estimated that 60% of anticancer drugs were wholly or partially derived from natural sources (16). Researchers are screening natural medications for active anticancer constituents and studying the processes underlying their antitumor action, which is a growing trend in the development of antitumor therapy (17-19). Natural ingredients serve as a unique therapeutic alternative for chondrosarcoma research (20). *Antrodia cinnamomea*, a rare medicinal mushroom native to Taiwan exhibits anti-inflammatory, hepatoprotective, anticancer, immunomodulatory and antioxidative properties (21-23). Antcin K, a triterpenoid derived from *A. cinnamomea*, has been demonstrated to have anti-angiogenesis and anti-inflammatory function in both *in vitro* and *in vivo* studies (24-26). Antcin K has considerable antiproliferative effects on liver cancer cells and induces cell death by promoting the generation of reactive oxygen species and ATP depletion, leading to endoplasmic reticulum stress and changes in mitochondrial membrane permeability (27), and also inhibits hepatoma cancer cell integrin-mediated adhesion, migration and invasion (28). Antcin K exerts anticancer activities by regulating levels of MMP-2 and MMP-9 (28). The present study aimed to investigate the effects of antcin K on chondrosarcoma progression and metastasis. These findings may offer novel insight and approaches for chondrosarcoma treatment in future.

Materials and methods

Materials. Antcin K (Fig. 1A) was synthesized as previously described (27). The phosphorylated (p)-p85 (1:2,000; cat. no. 4228S), p-Akt (1:2,000; cat. no. 4060S), p-mTOR (1:2,000; cat. no. 5536S), mTOR (1:2,000; cat. no. 2983S) and p-p65 (1:2,000; cat. no. 3033) antibodies were obtained from Cell Signaling Technology, Inc. Antibodies for MMP-7 (1:500; cat. no. sc-515703), Akt (1:500; cat. no. sc-5298) and p85 (1:500; cat. no. sc-1637) were purchased from Santa Cruz Biotechnology, Inc. p65 (1:2,000; cat. no. GTX102090) and β-actin (1:5,000; cat. no. GT5512) antibodies were purchased from GeneTex International Corporation. MTT was obtained from Sigma-Aldrich (Merck KGaA) and Lipofectamine® 2000 was supplied by Invitrogen (Thermo Fisher Scientific, Inc.).

Cell culture. Dr Sean P. Scully (Miller School of Medicine, University of Miami; Miami; USA) gifted the chondrosarcoma JJ012 cell line. The chondrosarcoma SW1353 cell line was supplied by American Type Culture Collection. The JJ012 and SW1353 cell culture conditions were as previously described (29,30).

MTT assay. Chondrosarcoma cells ($5x10^3$ cells/well) were seeded in 96-well culture plates and incubated at 37˚C with or without antcin K (0.3, 1.0, 3.0 or 10 μM) for 24 h. Dimethylsulfoxide was used to dissolve the purple formazan crystals formed by MTT solution, which was added at a concentration of 0.5 mg/ml for 2 h. Absorbance was measured at 570 nm using a BioTek microplate reader (Agilent Technologies, Inc.), as previously described (26,31).

Cell migration assay. For the migration assay, 48-well Micro Chemotaxis Chambers (Neuro Probe Inc.) were used (32,33). A total of 50 μl serum-free DMEM (Invitrogen; Thermo Fisher Scientific, Inc.) was used to seed ~$2.5x10^3$ cells into the upper chamber. In the lower chamber, 30 μl DMEM (Invitrogen; Thermo Fisher Scientific, Inc.) containing 10% FBS (Corning, Inc.) with or without antcin K (0.3, 1.0, 3 or 10 μM) was added. Following a 24 h incubation, cells were fixed at room temperature for 15 min using 3.7% formaldehyde and stained for 15 min at room temperature using 0.1% crystal violet. PBS was used to wash the GVS 8 μm membrane (Data Support Company). The migrated cell was visualised by an Olympus CKX53 microscope. ImageJ (version 1.53; National Institutes of Health) was used to analyze the number of migrated cells in one field of view.

Cell invasion assay. Invasion experiments were conducted using an 8-μm pore-size Corning Costar Transwell chamber (Corning, Inc.). A total of ~$1x10^4$ cells were inserted into the upper chamber, which was pre-covered with a thin layer of matrix gel for 30 min at 37˚C. In the lower chamber, 330 μl 10% FBS-containing medium with or without antcin K (0.3, 1, 3 or 10 μM) were added for 24 h at 37˚C. All invasive cells adhering to the lower surface were fixed with 3.7% formaldehyde for 30 min, stained with 0.1% crystal violet for 20 min at room temperature, and then washed with PBS at room temperature. ImageJ (version 1.53; National Institutes of Health) was used to evaluated the number of cell invasions.

RNA sequencing (RNA-seq). Total RNA from the JJ012 cells treated for 30 min at 37˚C with or without antcin K (10 μM) was isolated by TRIzol® (cat. no. 12183555, Invitrogen; Thermo Fisher Scientific, Inc.) for RNA-seq. Transcriptome sequencing experiments included RNA extraction and quantity control, library construction, purification, quality control and quantitation, sequencing cluster generation and high through-put sequencing, were performed as previously described (34). Differentially expressed genes (DEGs) from RNA-seq analysis were uploaded to Ingenuity Pathway Analysis (IPA; digitalinsights.qiagen.com/) and Kyoto Encyclopedia Genes and Genomes (KEGG; genome.jp/kegg/pathway.html) database to investigate potential pathways and biological function analyses.

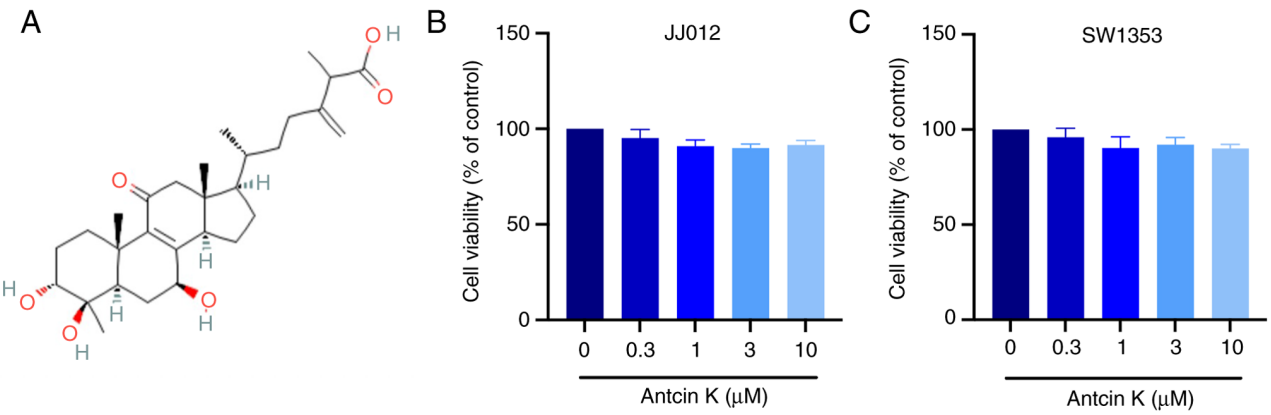

Figure 1. Non-cytotoxic effect of antcin K. (A) Chemical structure of antcin K. (B) JJ012 and (C) SW1353 cells were incubated with antcin K and cell viability was examined using the MTT assay.

Western blotting. Total protein from JJ012 and SW1353 cells was extracted using RIPA lysis buffer (cat. no. P0013; Beyotime Institute of Biotechnology). A total of 25 μg protein calculated using the BCA kit (Invitrogen; Thermo Fisher Scientific, Inc.) was loaded into each lane. Proteins were separated using 8% or 10% of resolving gels. Proteins were transferred to Immobilon® PVDF membranes following separation via SDS-PAGE. The membrane was blocked for 1 h at room temperature with a 5% non-fat milk solution. The membranes were incubated with the primary antibodies (MMP-7 (1:500), p-p85 (1:2,000), p85 (1:500), p-Akt (1:2,000), Akt (1:500), p-mTOR (1:2,000), mTOR (1:2,000), p-p65 (1:2,000), p65 (1:2,000) and β-actin (1:5,000)) overnight at 4˚C, followed by a horseradish peroxidase-conjugated secondary antibodies (goat anti-rabbit IgG, cat. no. sc-2357; 1:3,000; goat anti-mouse IgG, cat. no. sc-516102; 1:3,000; Santa Cruz Biotechnology, Inc.) for 1 h at room temperature. An ECL kit (MilliporeSigma) was used to detect immunoreactive bands by the chemiluminescent imaging system (Invitrogen iBright CL1500 Imaging Systems). Following normalization to β-actin, the optical density of the blot was quantified using ImageJ v1.53 software (National Institutes of Health).

Reverse transcription-quantitative (RT-q)PCR. JJ012 and SW1353 cells (~1x10^4) were seeded onto 6-well dishes and incubated with antcin K (0, 0.3, 1, 3 or 10 μM) for 24 h at 37˚C. A total of 1 μg RNA was extracted using TRIzol® (cat. no. 12183555, Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. The RNA was transformed into cDNA using an oligo-DT primer. The KAPA SYBR® FAST qPCR kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) was used to mix 100 ng cDNA sample with primers specific, with GAPDH used as the internal control. PCR primer (5'-3') sequences were as follows: MMP-7 forward, GAGTGAGCTACAGTGGGAAC and reverse, CTA TGACGCGGGAGTTTA ACAT; programmed cell death ligand 1 (PD-L1) forward, TGCCGACTACAAGCGAATTAC TG and reverse, CTGCTTGTCCAGATGACTTCGG; IL-6 forward, AGACAGCCACTCACCTCTTCAG and reverse, TTCTGCCAGTGCCTCTTTGCTG; IL-1β forward, ATG ATGGCTTATTACAGTGGCAA and reverse, GTCGGAGAT TCGTAGCTGGA; TNF-α forward, CCTCTCTCTA ATCAG CCCTCTG and reverse, GAGGACCTGGGAGTAGATGAG and GAPDH forward, ACCACAGTCCATGCCATCAC and reverse, TCCACCACCCTGTTGCTGTA. Thermocycling conditions were as follows: Initial denaturation at 95˚C for 10 min, followed by 40 cycles of 95˚C for 15 sec and 60˚C for 1 min. A StepOnePlus sequence detection system (Applied Biosystems; Thermo Fisher Scientific, Inc.) was used to perform the qPCR tests in triplicate. MMP-7 mRNA expression was determined using the ΔCq comparative methods (35-37).

Establishment of stable cell lines. MMP-7-overexpressing chondrosarcoma cell lines were established by transfecting JJ012 and SW1353 cells with 1 μg pcDNA3.1(+)/MMP7 vector (MDBio Inc.) with Lipofectamine® 2000 at 37˚C. After 24 h, transfectants were selected by 200 μg/ml of G418 (Geneticin) (Life Technologies).

Luciferase activity assay. Chondrosarcoma cells were transfected with 1 μg of NF-κB luciferase plasmid (Stratagene; Agilent Technologies, Inc.) using Lipofectamine 2000® for 24 h at 37˚C and treated with antcin K (10 μM) for an additional 24 h at 37˚C. The Dual-Luciferase® Reporter Assay System (Promega) was used to detect the luciferase activity following the company protocol. Firefly luciferase activity was normalized to *Renilla* luciferase activity.

Statistical analysis. Data were analyzed using GraphPad Prism 10 (Dotmatics). Statistical significance was assessed using an unpaired Student's t-test for comparisons between two groups. Comparisons involving a control group and multiple drug concentrations were conducted using one-way ANOVA followed by Dunnett's test. Comparisons involving >2 groups were analyzed using one-way ANOVA followed by Tukey's post hoc test. Results are expressed as the mean ± standard deviation of at least 3 independent experiments. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Antcin K inhibits chondrosarcoma cell migration and invasion. JJ012 (derived from a grade 2 chondrosarcoma

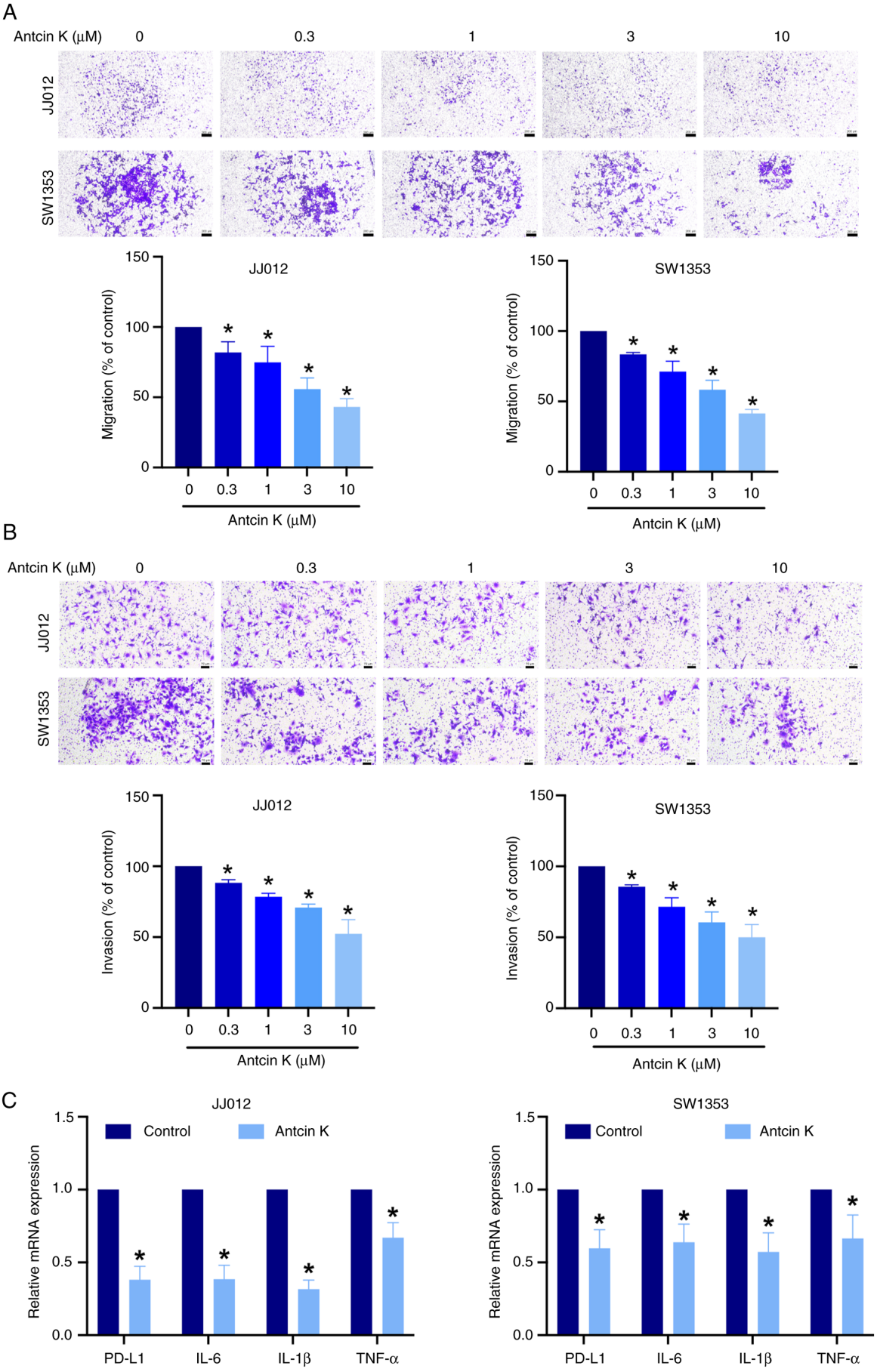

Figure 2. Inhibitory effect of antcin K on chondrosarcoma cell migration and invasion. Following 24 h incubation with antcin K, cell (A) cell (scale bar; 200 μm) and (B) invasion (scale bar; 70 μm) were determined. (C) Reverse transcription-quantitative PCR analysis detected mRNA expression of immunomodulatory and inflammatory-associated genes. *P<0.05 vs. control. PD-L1, programmed cell death ligand 1.

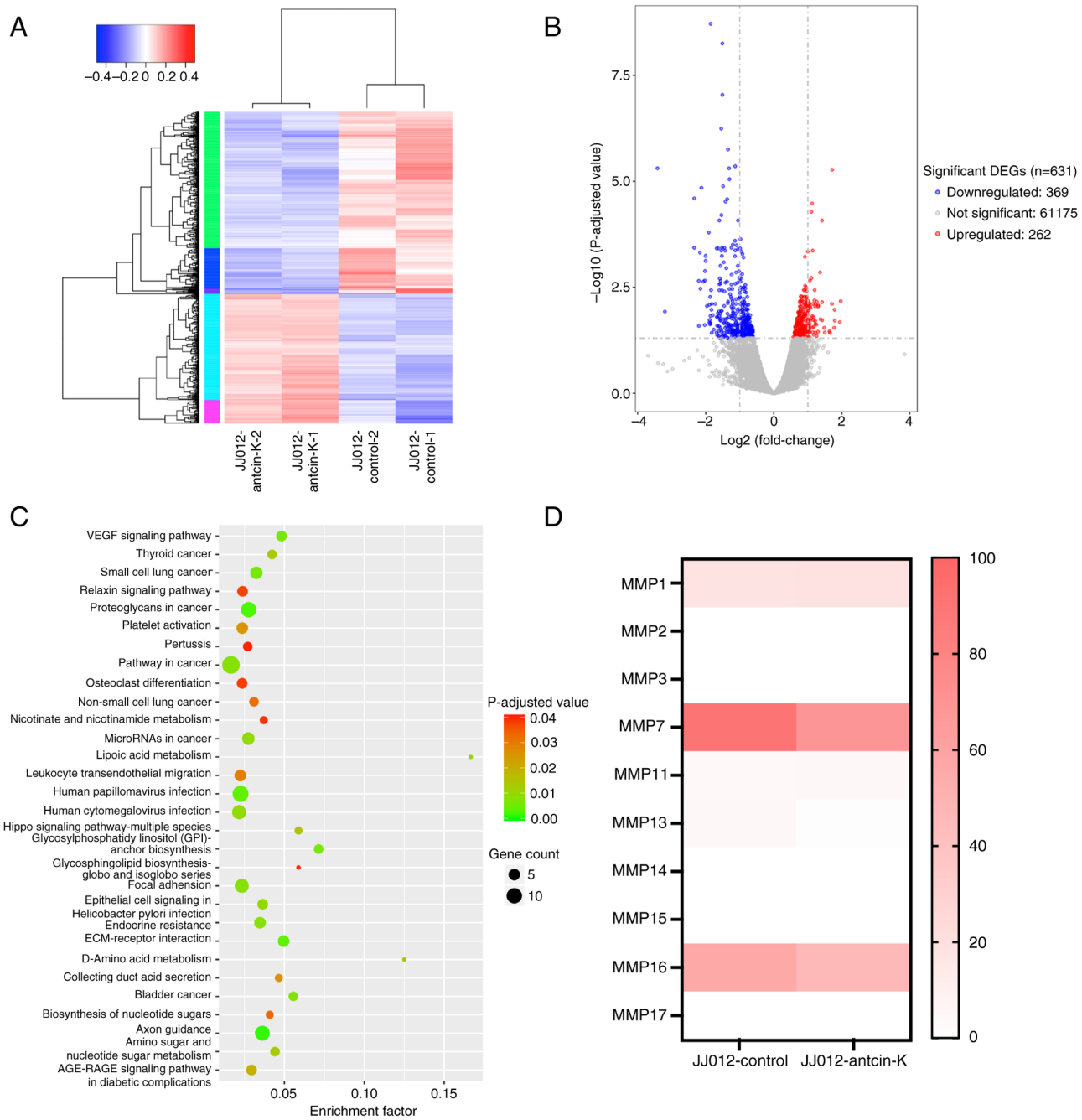

Figure 3. RNA-sequencing revealed that MMP-7 serves a key role in antcin K-mediated motility of chondrosarcoma cells. (A) Gene expression in control compared with JJ012 cells treated with antcin K. (B) Fold-change in gene expression following antcin K treatment. (C) Significantly enriched signaling pathways determined by Kyoto Encyclopedia of Genes and Genomes analysis. (D) MMP expression levels in control compared with JJ012 cells treated with Antcin K were analyzed by RNA-sequencing. DEG, differentially expressed gene.

tumor; IDH1 mutation) and SW1353 (derived from a grade 2 chondrosarcoma tumor; IDH2 mutation) cell lines (38) were used to evaluate the effect of antcin K. Antcin K markedly influences the induction of apoptosis in human hepatoma cells (27). MTT assay was used to assess the cytotoxic effects of antcin K on JJ012 and SW1353 cell lines. Antcin K had no effect on the viability of chondrosarcoma cells, including at the maximum dosage of 10 μM (Fig. 1B and C). Migration assay was performed using the same concentration range to assess the effects of antcin K on chondrosarcoma motility. Antcin K suppressed migration in both chondrosarcoma cell lines (Fig. 2A). The invasion of chondrosarcoma cells was also inhibited by antcin K (Fig. 2B). Antcin K exhibits anti-inflammatory and immunomodulatory properties (39). Antcin K exerted a notable inhibitory effect on the mRNA expression of genes associated with immunomodulation (PD-L1) and inflammation (IL-6, IL-1β and TNF-α; Fig. 2C). Collectively, these data suggested that antcin K significantly reduces chondrosarcoma cell motility.

Antcin K suppresses the motility of chondrosarcoma cells by inhibiting the production of MMP-7. To investigate the molecules responsible for the anti-motility effects of antcin K, RNA-seq analysis was performed in JJ012 cells treated

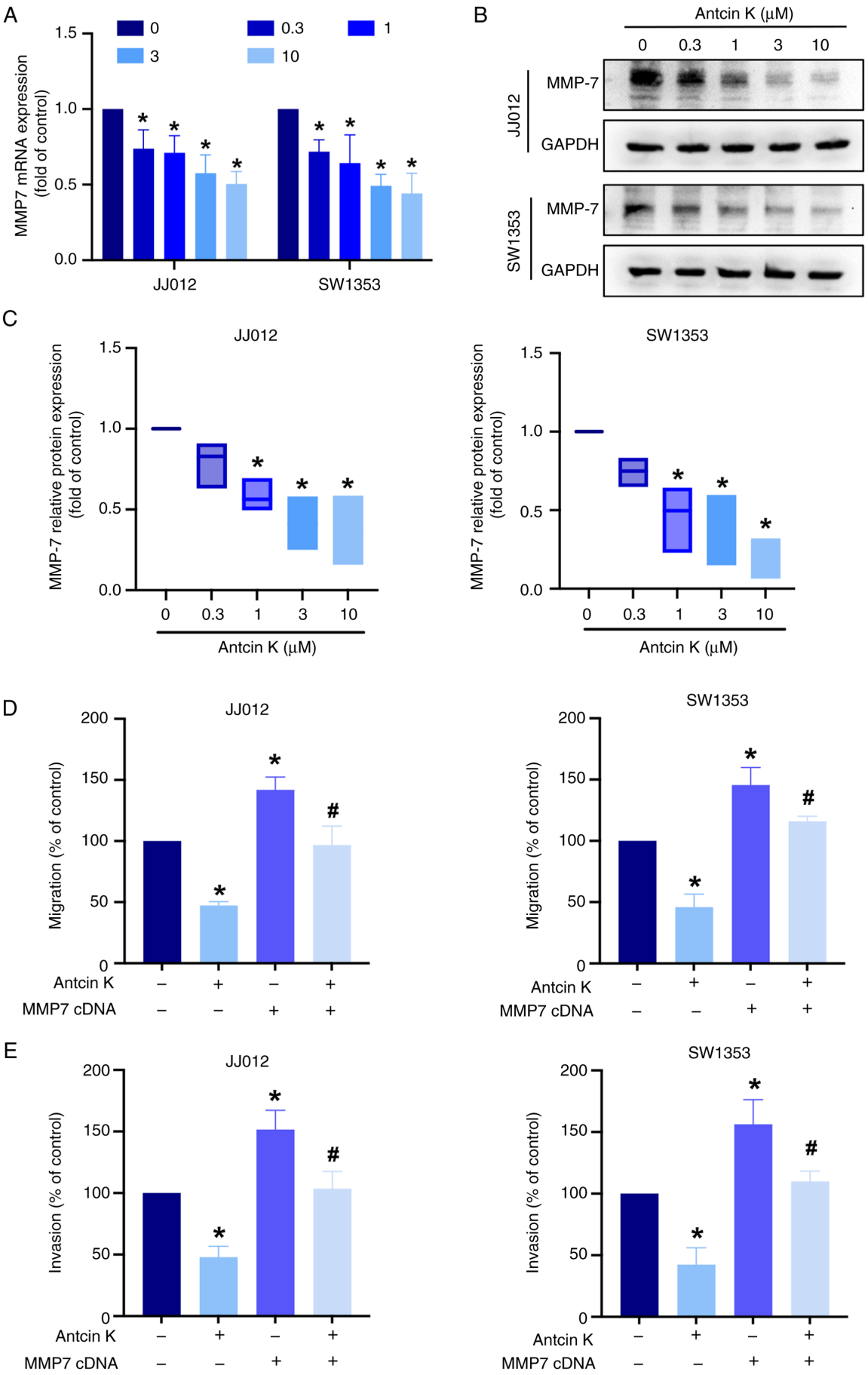

Figure 4. Antcin K inhibits MMP-7-dependent chondrosarcoma motility. Cells were stimulated with antcin K for 24 h. MMP-7 production was assessed by (A) quantitative PCR and (B) western blotting. (C) Protein expression. Chondrosarcoma cells were stably transfected with MMP-7 cDNA and treated with Antcin K for 24 h. (D) Migration and (E) invasion were assessed. *P<0.05 vs. control; #P<0.05 vs. antcin K.

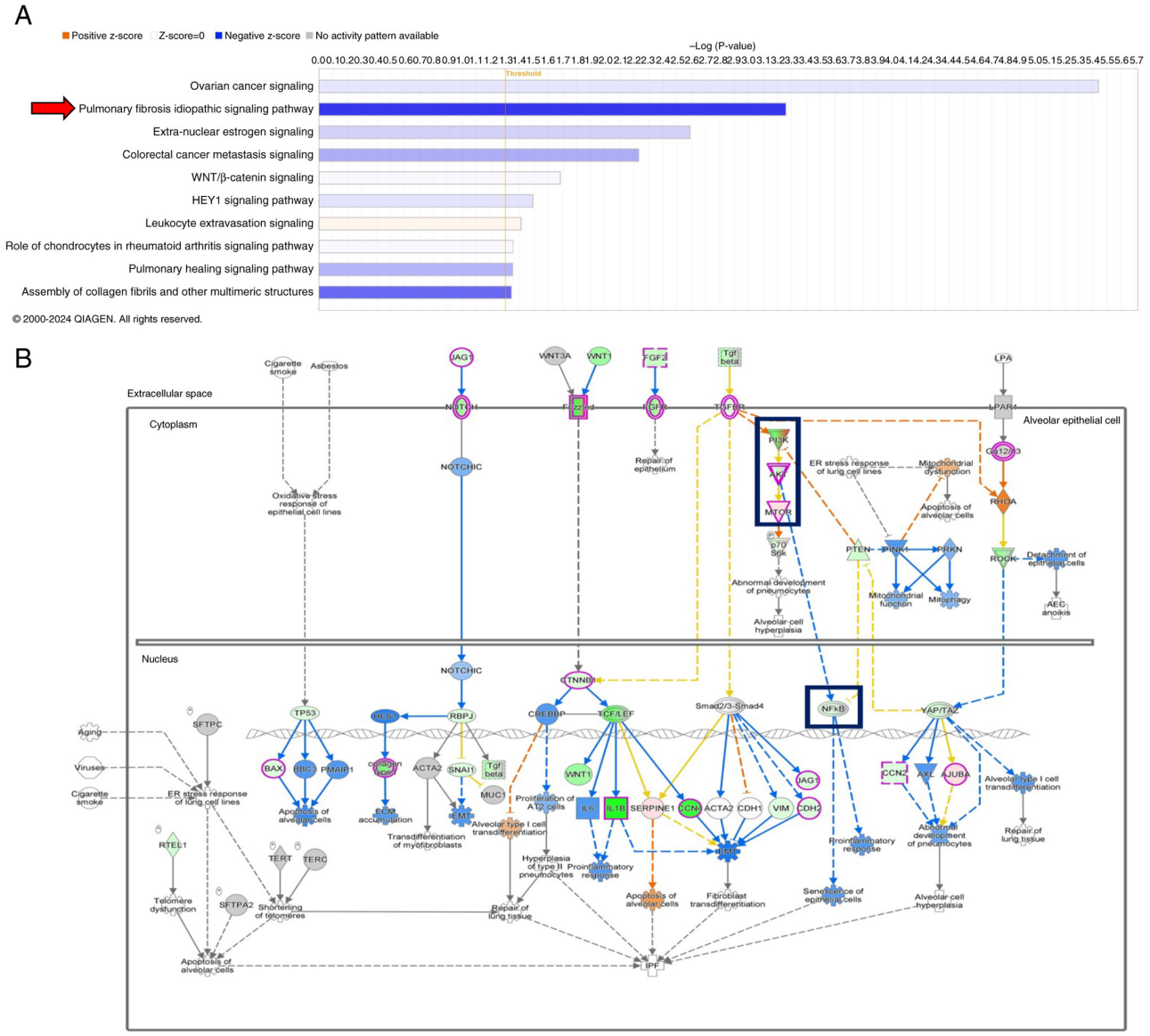

Figure 5. Biological processes regulated by antcin K. IPA pathway enrichment of RNA sequencing data comparing control with JJ012 cells treated with antcin K. (A) Histogram of 10 canonical pathways. (B) Map of 'pulmonary fibrosis idiopathic signaling pathway' enriched by IPA. IPA, Ingenuity Pathway Analysis; AEC, alveolar epithelial cell; ACTA2, alpha smooth muscle actin 2; CCL2, C-C motif chemokine ligand 2; CDKN1A (p21), cyclin-dependent kinase inhibitor 1A; COL1A1, collagen type I alpha 1 chain; connective tissue growth factor; EDN1, endothelin 1; ER, endoplasmic reticulum; FGF2, fibroblast growth factor 2; FN1, fibronectin 1; FOXO3, forkhead box O3; HIF1A, hypoxia inducible factor 1 subunit alpha; ITGA5, integrin subunit alpha 5; JAG1, jagged canonical Notch ligand 1; MAPK1, mitogen-activated protein kinase 1; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homolog; RARA, retinoic acid receptor alpha; RBPJ, recombination signal binding protein for immunoglobulin kappa J region; RIDA, regulator of inflammatory and damage-associated responses; SERPINE1 (PAI-1), serpin family E member 1; SNAI1, snail family transcriptional repressor 1; SOX2, SRY-box transcription factor 2; TERT, telomerase reverse transcriptase; TGFB1, transforming growth factor beta 1; TNF, tumor necrosis factor; TP53, tumor protein p53; VEGFA, vascular endothelial growth factor A; VIM, vimentin.

with or without antcin K. Following treatment with antcin K, 369 genes were upregulated and 262 genes were downregulated (Fig. 3A and B). Kyoto Encyclopedia of Genes and Genomes analysis indicated that 'ECM-receptor interaction' was enriched (Fig. 3C), which was associated with metastasis clear cell renal cell carcinoma (40). Among the MMPs, MMP-7 expression was the most decreased following antcin K treatment (Fig. 3D). Antcin K inhibited MMP-7 mRNA and protein production in a concentration-dependent manner (Fig. 4A-C). Moreover, the overexpression of MMP-7 antagonized the inhibitory effects of antcin K on cell migration and invasion (Fig. 4D and E), indicating that antcin K blocked chondrosarcoma motility by inhibiting MMP-7 production.

Antcin K downregulates PI3K, Akt, mTOR and NF-κB signaling pathways in chondrosarcoma. Ingenuity Pathway Analysis (IPA) was used to identify the enriched canonical pathways involved in chondrosarcoma regulation to assess the molecular mechanism underlying the regulatory effects of antcin K on chondrosarcoma cell motility. 'Pulmonary fibrosis idiopathic signaling pathway' demonstrated the strongest downregulation (Fig. 5A), and was associated with the PI3K, Akt, mTOR and NF-κB pathways (Fig. 5B). Antcin

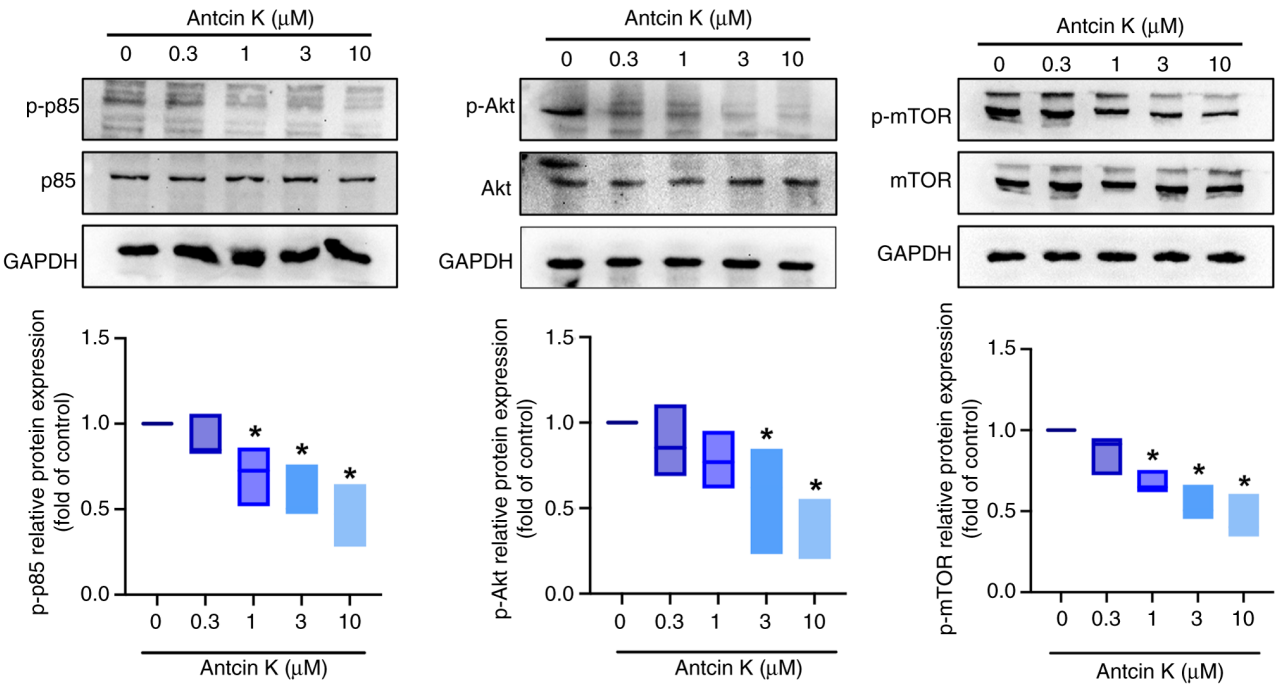

Figure 6. Antcin K suppresses PI3K, Akt and mTOR signaling. Western blotting analysis of levels of p-p85, Akt and mTOR after 24 h incubation with antcin K. *P<0.05 vs. control. p-, phosphorylated.

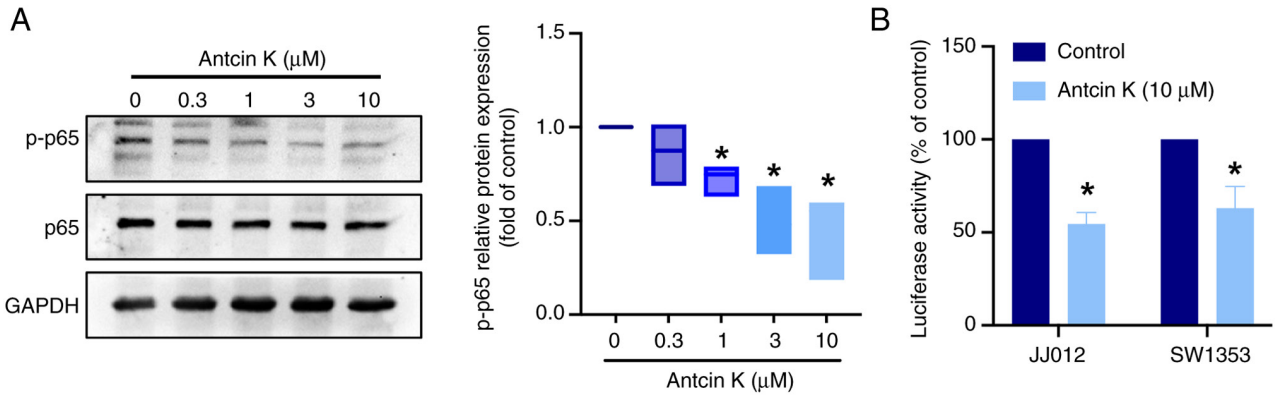

Figure 7. Antcin K suppresses NF-κB signaling. (A) Western blot analysis of the levels of p-p65 after 24 h incubation with antcin K. (B) NF-κB reporter assay detected the NF-κB luciferase activity of JJ012 and SW1353 cells. *P<0.05 vs. control. p-, phosphorylated.

K decreased the levels of p-p85, p-Akt and p-mTOR (Fig. 6). Furthermore, the phosphorylation of p65 decreased following antcin K stimulation (Fig. 7A). NF-κB luciferase reporter assay was performed to evaluate the effects of antcin K on NF-κB activity. Antcin K decreased NF-κB luciferase activity (Fig. 7B). These data support the hypothesis that antcin K abolishes the PI3K, Akt, mTOR and NF-κB pathways in chondrosarcoma.

Discussion

The discovery of natural compounds and their structural analogs has been beneficial to pharmacotherapy, particularly in the treatment of cancer (41). Agents such as vinblastine, taxol and camptothecin are useful in treating numerous types of cancer, including ovarian, lung and breast cancer (42). The fungus *A. cinnamomea* has been used in Taiwanese traditional medicine for centuries to treat hypertension, cancer, liver disease and inflammatory conditions (43). Antcin K, a functional molecule derived from the fruiting bodies of *A. cinnamomea*, has anti-inflammatory abilities that markedly decrease IL-6, IL-1β and TNF-α production (44) and mediates anti-inflammatory effects in arthritic illness (45). Antcin K inhibits proliferation and motility in hepatoma cancer (27,28), however, to the best of our knowledge, the present study is the first to investigate the effects of antcin K on chondrosarcoma motility. Antcin K inhibited chondrosarcoma MMP-7 generation, cell migration and invasion. Antcin K also inhibited the PI3K, Akt, mTOR and NF-κB signaling cascades. Han *et al* (44) reported that antcin K decreases central neuroinflammation, thereby alleviating depression in mice at doses of 5 and 15 mg/kg. Antcin K reduced cartilage degradation in collagen-induced arthritic mice (25).

As triterpenoids progress from laboratory research to clinical application, highlighted by the US Food and Drug

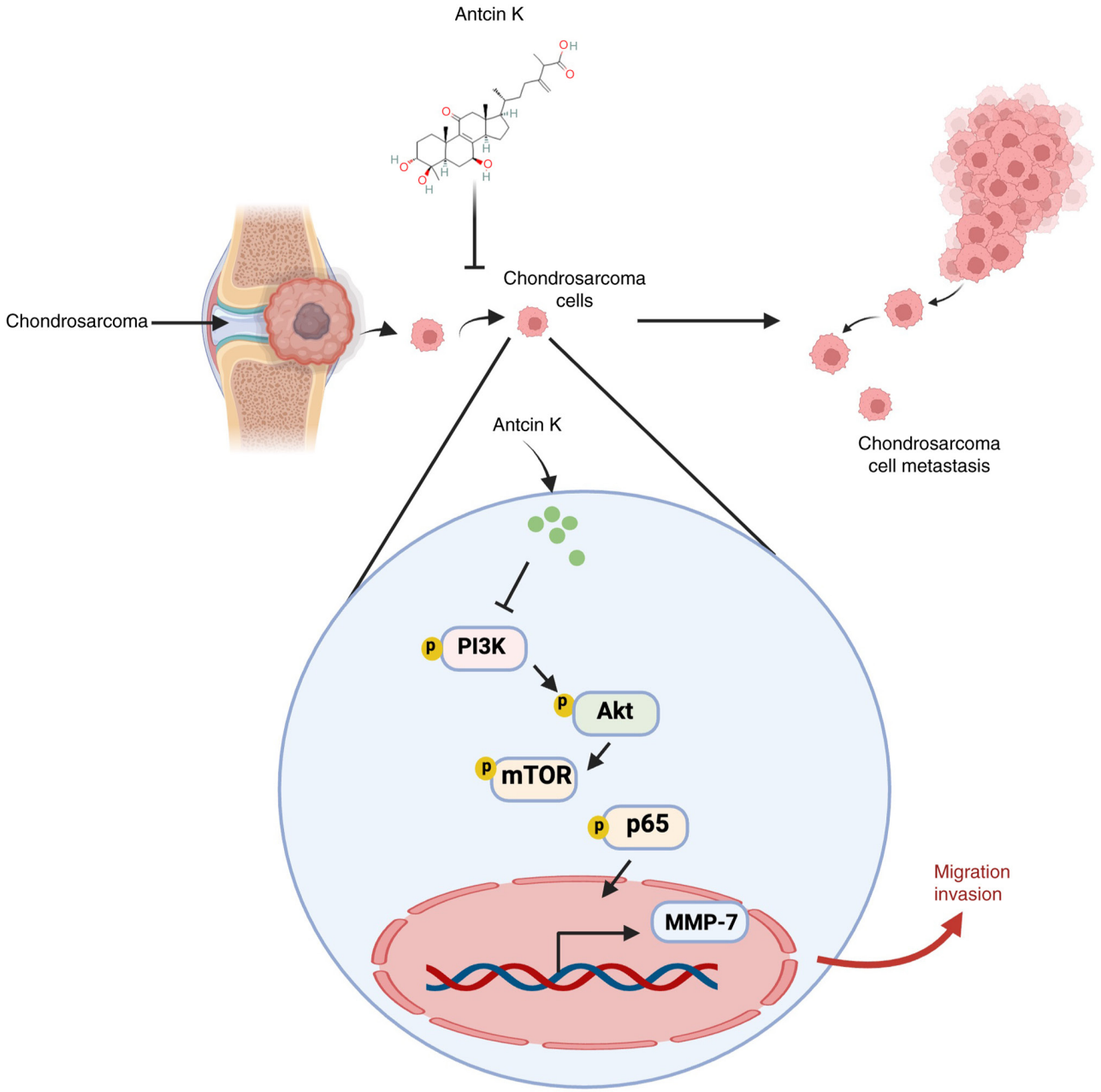

Figure 8. Inhibitory effect of antcin K on chondrosarcoma cell motility. Antcin K inhibits chondrosarcoma cell migration and invasion by decreasing MMP-7 expression by downregulating PI3K, Akt, mTOR and NF-κB signaling cascades. Image created using BioRender.com. p-, phosphorylated.

Administration (FDA) approval of omaveloxolone (46), and numerous clinical trials of triterpenoids in different types of disease (47,48), obtaining an understanding of their biological mechanisms in the context of cancer is key. The present study and previous research (28,49) implicate antcin K, a triterpenoid, in carcinogenesis inhibition and suggest that it may be a potential candidate for clinical trials.

Cancer cells undergo metastasis to colonize additional locations (50). MMPs serve a key role in a number of cancer metastatic processes. Overexpressed and active MMPs facilitate the establishment of a conducive microenvironment in tumor tissue (51). The identification of efficient MMP inhibitors for therapeutic use has yielded encouraging results (52,53). However, due to their poor solubility, lack of potency and adverse clinical trial outcomes, the FDA has not yet licensed any MMP inhibitors for use in cancer treatment (54,55). The smallest enzyme in the MMP family, MMP-7 serves a key role in promoting the progression of numerous types of tumor such as gastric, pancreatic and colorectal cancer (56). By contrast with normal cartilage, MMP-7 is substantially expressed in the tissue of patients with chondrosarcoma (11). The aforementioned study further highlighted that MMP-7 regulates the migration and invasion of chondrosarcoma cells (14). In the present study, RNA-seq analysis revealed that antcin K treatment had a more pronounced effect on downregulating MMP-7, compared with other MMPs. Results from *in vitro* cell migration and invasion assays demonstrated that antcin K markedly diminished chondrosarcoma motility. Transfection with MMP-7 cDNA antagonized antcin K-induced inhibition of cell

motility, indicating that antcin K inhibits MMP-7-dependent chondrosarcoma motility.

Several signaling pathways promote the motility, angiogenesis and proliferation of chondrosarcoma cells (57). IPA demonstrated that the 'pulmonary fibrosis idiopathic signaling pathway', which includes PI3K, Akt, mTOR and NF-κB, is a prime candidate signaling pathway. Antcin K stimulation diminished p85, Akt and mTOR phosphorylation. Accumulating evidence indicates that the PI3K, Akt and mTOR pathways serve key functions in chondrosarcoma metastasis (57-60). First, endothelin-1 facilitates MMP-13 generation and chondrosarcoma metastasis via the PI3K, Akt and mTOR pathway (58). Secondly, the adipokine adiponectin facilitates chondrosarcoma-associated angiogenesis via the PI3K, Akt and mTOR pathway (59). Furthermore, nerve growth factor controls PI3K, Akt and mTOR cascades, upregulating chondrosarcoma motility (60). NF-κB mediates a key role in chondrosarcoma metastasis (61,62) and antcin K activation decreases expression of p-p65. NF-κB is a key transcription factor regulating antcin K-regulated chondrosarcoma motility, as evidenced by the fact that antcin K eliminated NF-κB luciferase activity.

The present study had limitations, including lack of experiments examining the inhibitory effects of antcin K on chondrosarcoma metastasis in mice. The challenges of the synthesis resulted in insufficient quantities of antcin K, limiting the ability to conduct *in vivo* studies to explore its potential effects on chondrosarcoma cell metastasis in animal models and necessitating further research.

In conclusion, antcin K significantly suppressed MMP-7 production and cell motility in chondrosarcoma by inhibiting the PI3K, Akt, mTOR and NF-κB signaling pathways (Fig. 8). These findings demonstrated the key effect of antcin K in metastatic chondrosarcoma.

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Availability of data and materials

The data generated in the present study may be found in the Gene Expression Omnibus database under accession number GSE287361 or at the following URL: ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE287361.

Authors' contributions

YYL performed experiments, analyzed data and wrote the manuscript. NBT and CYS analyzed data. YYW, HTC, YHK, CHT, YCF and HCT designed the experiments. YHK performed experiments. CHT conceived the study and revised the manuscript. YYL, NBT and CHT confirm the authenticity of all raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

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