Article

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Mechanism of Corylin Inhibiting the Development of Osteosarcoma: Regulating *HMGB1*/p38 MAPK Signaling

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Background: High-mobility group box 1 (*HMGB1*) participates in the progression of osteosarcoma (OS) through the p38 mitogen-activated protein kinase (MAPK) signaling pathway. Corylin, one of the active components of *Psoralea corylifolia L.*, has anti-oxidant, anti-inflammatory, and anti-tumor effects. This study investigates the association between corylin and *HMGB1*, and their impact and mechanism of action on OS.

Methods: OS cells and osteoblasts were transfected with/without *HMGB1* overexpression plasmid and si*HMGB1*. Cell viability was examined using the Cell Counting Kit-8 (CCK-8) assay after treatment with corylin (0, 2.5, 5, 10, 30 µM). The effects of corylin on cell malignant behaviors were examined by cell function assays. The mRNA expression level of high-mobility group box 1 (*HMGB1*) was determined by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The protein levels of HMGB1, matrix metalloproteinase-2 (MMP-2), MMP-9, and alpha-smooth muscle actin (α-SMA) were measured by western blotting. The effects of corylin and *HMGB1* on the expression of p38 MAPK signaling pathway-related proteins were also assessed.

Results: Corylin decreased OS cell viability, proliferation, migration, and invasion but increased apoptosis in a concentration-dependent manner. Corylin concentration-dependently suppressed the levels of *HMGB1*, MMP-2, MMP-9, and α-SMA. Overexpression of *HMGB1* was partially reversed, while knockdown of *HMGB1* enhanced the above effects of corylin.

Conclusion: Corylin inhibits OS cell migration and invasion through regulation of the *HMGB1*-mediated p38 MAPK signaling pathway.

Keywords: osteosarcoma; corylin; *HMGB1*; p38 MAPK signaling pathway

Introduction

Osteosarcoma (OS) is a frequently encountered malignant bone tumor clinically [1], featuring early lung metastasis and high recurrence rates [2]. Despite the overall age-standardized incidence rate of OS at 5.2 cases/million in England, invasion and metastasis pose a high risk of death [3]. After neoadjuvant chemotherapy and surgical resection, the 5-year average survival rate of OS patients without local metastasis is 60–70% [4]. However, metastatic lesions in the lungs are often detected at initial diagnosis in most patients, and strict adherence to the standard treatment strategy only results in a 5-year survival rate of 20–30% [5,6]. Furthermore, high-dose chemotherapy drugs, such as methotrexate, adriamycin, cisplatin, and ifosfamide, have serious adverse events [7]. Therefore, it is critical that safe and effective treatments be identified.

Psoralea corylifolia is the dried and mature fruit of the legume *Psoralea corylifolia L.*, and pharmacological research has shown that Psoralea functions as an immunoregulator, and anti-tumor and anti-bacterial agent, as well as in the relief of asthma, osteoporosis, and vitiligo [8]. Accordingly, *Psoralea corylifolia*-related preparations have been widely applied in the treatment of arthritis, osteoporosis, and other conditions [9]. Corylin, a mixture of flavonoids extracted from *Psoralea corylifolia*, has strong anti-oxidant, anti-inflammatory, and anti-tumor activities [10]. For example, corylin attenuates inflammatory responses and protects against lipopolysaccharide (LPS)-induced sepsis [11]. Corylin suppresses the proliferation and migration of vascular cells [12], and hinders the progression of hepatocellular carcinoma through suppression of the epithelial-to-mesenchymal transition (EMT) [13]. Additionally, corylin can induce osteoblastic proliferation and differentiation [14]. Although corylin has been proven to have anti-inflammatory and anti-cancer effects, its effect on OS has not been reported so far. This study thus investigated the effect of corylin on OS and elucidated its possible molecular mechanism.

High-mobility group box 1 (*HMGB1*) protein is a type of non-histone chromosomal binding protein that exists in eukaryotic cells [15]. In the context of necrosis, hypoxia, and lack of nutrients and growth factors, *HMGB1* protein in the nucleus is released in the extracellular environ-

ment where it binds to toll-like receptor (TLR) 2 and TLR4 [16,17]. The activation of the *HMGB1*-membrane receptor axis can lead to a series of changes in intracellular signal transduction (including mitogen-activated protein kinase [MAPK]) and biological behaviors [18]. It has been documented that the p38 MAPK signaling pathway is involved in cell proliferation, differentiation, apoptosis, and invasion [19,20]. Moreover, *HMGB1* plays a role in inflammation, immunity, and apoptosis [16]. *HMGB1* promotes colon epithelial cell proliferation in diabetes [21], and cell proliferation and migration in OS [22]. However, whether corylin can regulate the proliferation, migration, and apoptosis of OS cells by mediating *HMGB1* remains unclear.

The current study investigated the effects of corylin and *HMGB1* on OS by observing cell malignant behaviors in an attempt to elucidate how corylin inhibits the progression of OS.

Materials and Methods

Cell Culture

MG-63 cells (CRL-1427, ATCC, Manassas, VA, USA) were cultivated in Eagle's Minimum Essential Medium (M5650, Sigma-Aldrich, St. Louis, MO, USA) with 10% fetal bovine serum (FBS, 16140071, Gibco, Grand Island, NY, USA) at 37 °C under 5% CO_2. U2OS cells (HTB-96, ATCC, Manassas, VA, USA) were cultured in McCoy's 5a Medium (M9309, Sigma-Aldrich, St. Louis, MO, USA) with 10% FBS at 37 °C. Human osteoblastic cells hFOB 1.19 (CRL-11372, ATCC, Manassas, VA, USA) were incubated in DMEM/F12 Medium (51445C, Sigma-Aldrich, St. Louis, MO, USA), with 2.5 mM L-glutamine (G7513, Sigma-Aldrich, St. Louis, MO, USA), 0.3 mg/mL G418 (A1720, Sigma-Aldrich, St. Louis, MO, USA), and 10% FBS at 34 °C. All cells were identified by short tandem repeats and tested for mycoplasma free.

Experimental Grouping and Drug Treatment

MG-63, U2OS, and hFOB 1.19 cells were divided into several groups, where some groups of cells were treated with corylin (M13462, purity: >99%, Abmole, Shanghai, China) for 72 hours and other groups of cells were not treated. The groups were as follows: control group (normally cultured cells); corylin 2.5 group (2.5 µM corylin-treated cells); corylin 5 group (5 µM corylin-treated cells); corylin 10 group (10 µM corylin-treated cells); corylin 30 group (30 µM corylin-treated cells); vector group (cells transfected with pcDNA3.1 empty vector); si*HMGB1* group (si*HMGB1*-transfected cells); *HMGB1* group (*HMGB1* overexpression vector-transfected cells); corylin 30+vector group (30 µM corylin-treated cells transfected with empty vector); corylin 30+*HMGB1* group (30 µM corylin-treated cells transfected with *HMGB1* overexpression vector); and corylin 30+si*HMGB1* group (30 µM corylin-treated cells transfected with si*HMGB1*).

Transfection

The coding sequence (CDS) of the *HMGB1* gene was inserted into the pcDNA3.1 vector (HG-VPI0017, Honorgene, Changsha, China) to construct the overexpression plasmid. Next, the *HMGB1* overexpression vector, pcDNA3.1 empty vector, and small interfering RNA against *HMGB1* (si*HMGB1*) (siB140818110615-1-5, GATGCAGCTTATACGAAATAA, Ribobio, Guangzhou, China) were separately transfected into MG-63 and U2OS cells using ViaFect transfection reagent (E4981, Promega, Madison, WI, USA). In short, the plasmids (2.5 µg *HMGB1* overexpression plasmid and pcDNA3.1 empty/100 pmol si*HMGB1*) and ViaFect reagent (5 µL) were separately diluted in the cell medium, then mixed and cultured at 25 °C for 20 min. The complex was incubated with MG-63 and U2OS cells for 24 hours.

RNA Isolation and Reverse Transcription-Quantitative Polymerase Chain Reaction (RT-qPCR)

Total RNA was extracted from cells using TriReagent (T9424, Sigma-Aldrich, St. Louis, MO, USA), and reversely transcribed into cDNA using HyperScribe T7 High Yield RNA Synthesis Kit (K1401, APExBio, Houston, TX, USA) according to the manufacturer's instructions. Next, RT-qRCR on an ABI 7900 system (Applied Biosystems, Waltham, MA, USA) was implemented using Fast Taq PCR Master Mix (K1116, APExBio, Houston, TX, USA). The primer sequences are as follows (listed 5′ to 3′): *HMGB1* (NM_001363661.2) (F) GGGCAAAGGAGATCCTAAGAAG, (R) GTTGACTGAAGCATCTGGGT; β-actin (F) FCTCCATCCTGGCCTCGCTGT, (R) GCTGTCACCTTCACCGTTCC. For calculating the gene expression levels, the $2^{-\Delta\Delta Ct}$ method was employed [23], and β-actin served as the reference gene.

Cell Counting Kit-8 (CCK-8) Assay

The viability of MG-63, U2OS, and hFOB 1.19 cells was determined using the CCK-8 Kit Assay (C0037, Beyotime, Shanghai, China). In brief, cells (2×10^3/well) in 96-well plates were cultivated with 10 µL of CCK-8 solution for 2 hours in the dark. Next, the absorbance (450 nm) was measured with a microplate reader (Z742711-1EA, Sigma-Aldrich, St. Louis, MO, USA).

Flow Cytometry

A total of 5×10^4 MG-63 and U2OS cells were rinsed in phosphate-buffered saline (PBS, C0221A, Beyotime, Shanghai, China), and incubated in 195 µL of binding buffer, followed by a reaction with 5 µL of Annexin V-FITC and 10 µL of propidium iodide (C1062S, Beyotime, Shanghai, China) under isothermal (20 °C) and dark conditions for 20 min. Subsequently, an Attune NxT Flow Cytometer (Thermo Fisher Scientific, Waltham, MA, USA) was used for the analysis of apoptotic cells.

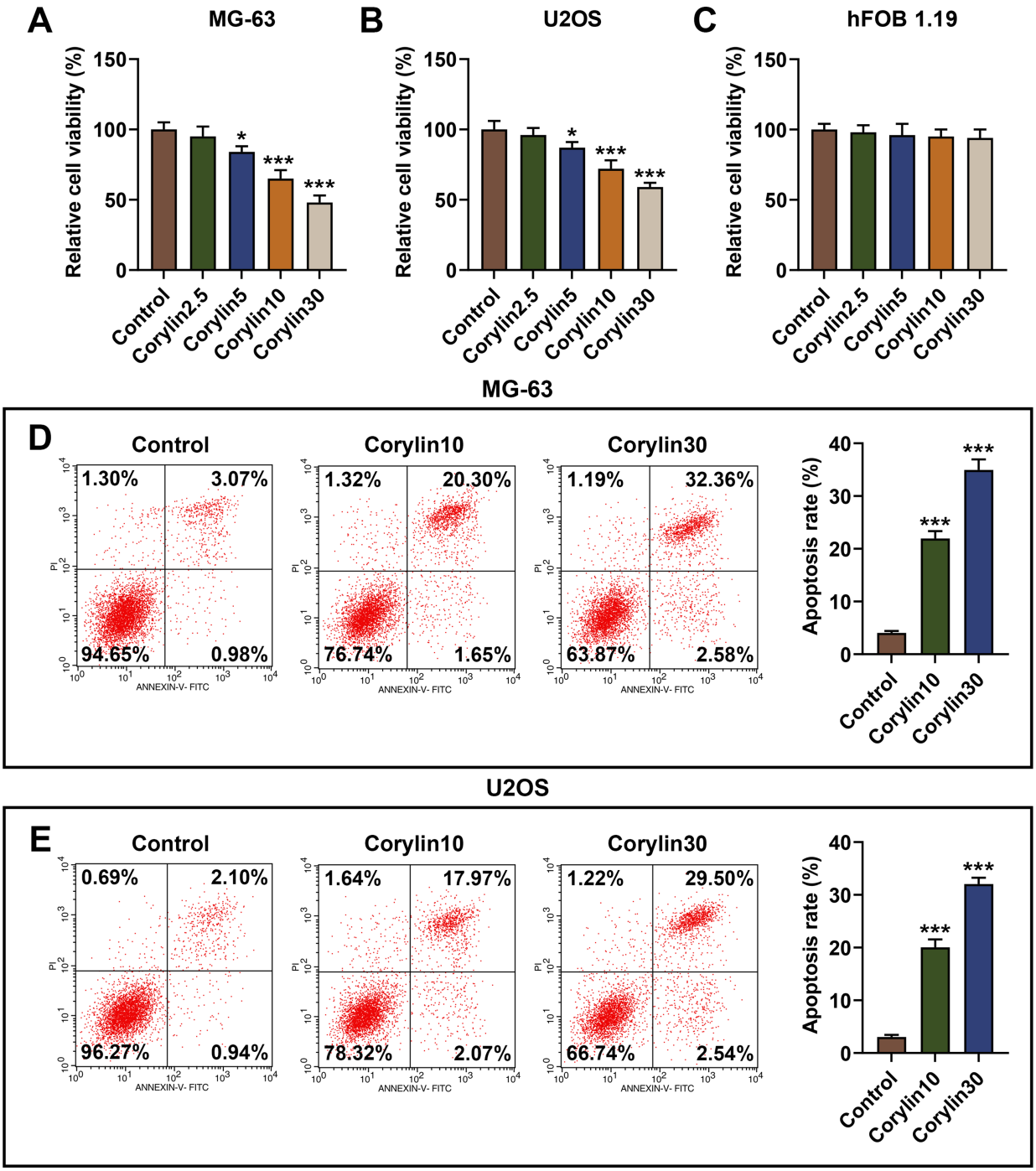

Fig. 1. Effects of corylin on viability and apoptosis of MG-63, U2OS, and hFOB 1.19 cells. (A–C) The viability of MG-63, U2OS, and hFOB 1.19 cells in control and corylin (2.5, 5, 10 or 30) groups was evaluated using the Cell Counting Kit-8 (CCK-8) assay. (D,E) Apoptosis of MG-63 and U2OS cells in control and corylin (10 or 30) groups was analyzed by flow cytometry. All experiments were repeated at least three independent times; *$p < 0.05$; ***$p < 0.001$ vs control.

Transwell Assay

The migration and invasion capacities of OS cells were evaluated using Transwell inserts (353097, Corning Incorporated, Corning, NY, USA). Matrigel (354234, Corning Incorporated, Corning, NY, USA) was applied to the upper surface of the membrane of Transwell inserts in the invasion assay but not the migration assay. Next, 3×10^4 cells suspended in the serum-free medium were added to the upper chamber, whereas medium supplemented with 10% FBS was added to the lower chamber. Following a 24-hour incubation period, cells that had passed through

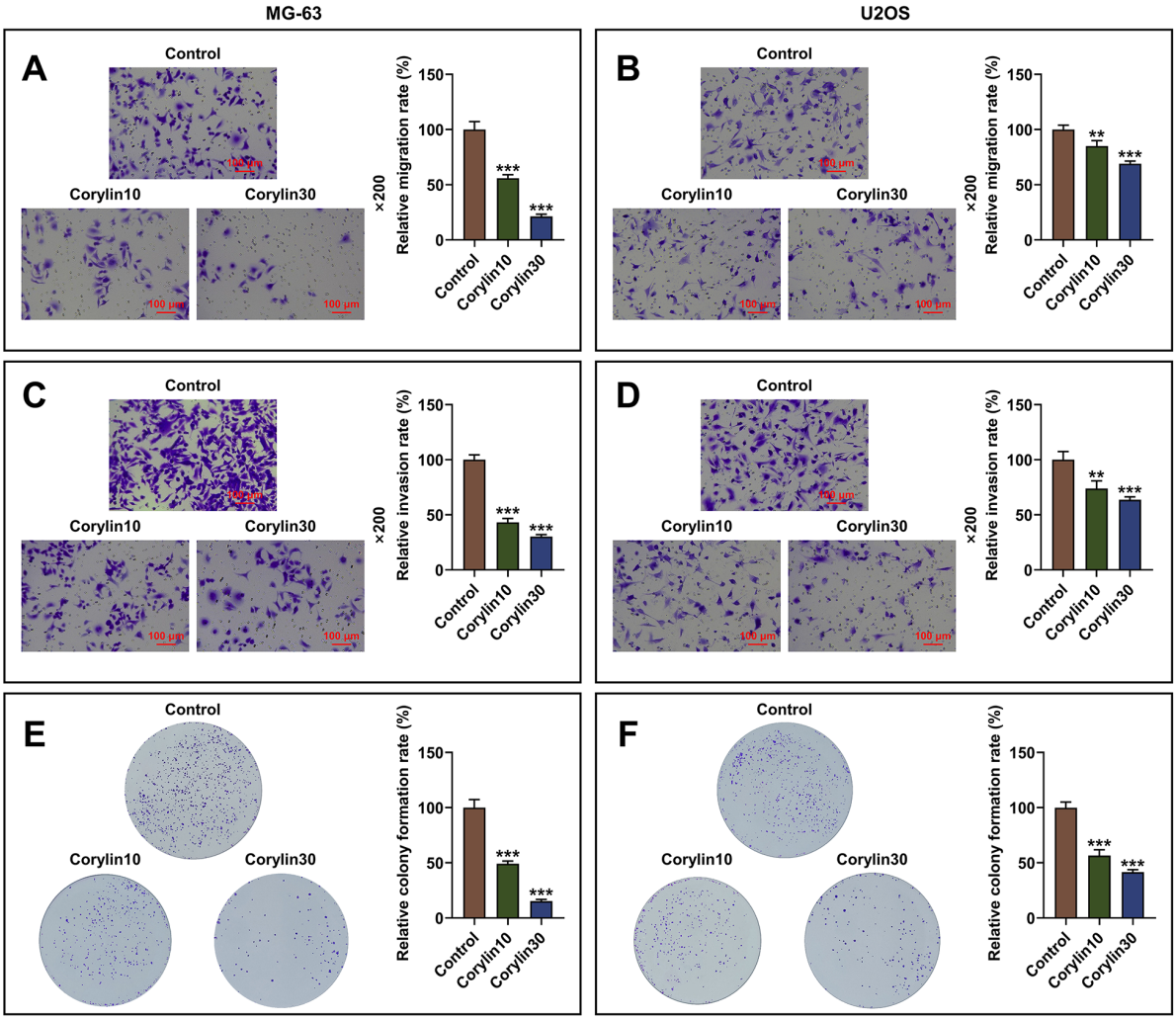

Fig. 2. Effects of corylin on osteosarcoma (OS) cell proliferation, migration, and invasion. (A–D) Transwell assay was used to examine the migration and invasion of MG-63 and U2OS cells in control and corylin (10 and 30) groups. (E,F) Proliferation of OS cells in control and corylin (10 and 30) groups was determined using colony formation assay. All experiments were repeated at least three independent times; $^{**}p < 0.01$; $^{***}p < 0.001$ vs control.

the Matrigel layer and entered the lower chamber were fixed with 4% paraformaldehyde (ABD-20010, Biomol, Hamburg, Germany) and dyed with crystal violet (CDX-C0252, Biomol, Hamburg, Germany). Cells were viewed under a microscope (magnification: ×200; EVOS M5000, Thermo Fisher Scientific, Waltham, MA, USA). The migration and invasion rates were calculated as follows: (migrated/invaded cells at 24 hour-migrated/invaded cells at 0 h)/total cell number × 100%.

Colony Formation Assay

OS cells seeded in 6-well plates (1000 cells/well) were cultured for 2 weeks, with the medium replaced every 2 days. After the removal of the medium, cells were fixed for 10 min with 4% paraformaldehyde at 25 °C and then stained for 15 min with crystal violet. Cells were viewed under a microscope.

Western Blot

RIPA lysis buffer (R301899, Aladdin, Shanghai, China) was used to extract total protein from MG-63 cells and U2OS cells. The separated lysates underwent 5-min high-speed centrifugation at 2000 rpm at 4 °C, after which the supernatant was collected for measurement of protein concentration with the bicinchoninic acid (BCA) Assay Kit (23225, Thermo Fisher Scientific, Waltham, MA, USA). Next, equivalent amounts of protein underwent electrophoresis by SDS-PAGE (M00659, Jinsilui Biotechnology Co., Ltd., Haikou, China) and separated proteins were electrophoretically transferred onto polyvinylidene fluoride membranes (88518, Thermo Fisher Scientific, Waltham, MA, USA). Membranes were blocked for 10 min with blocking buffer (YT067, BIORAB, Beijing, China), exposed to primary antibodies at 4 °C overnight, and incubated with secondary antibodies, goat anti-rabbit im-

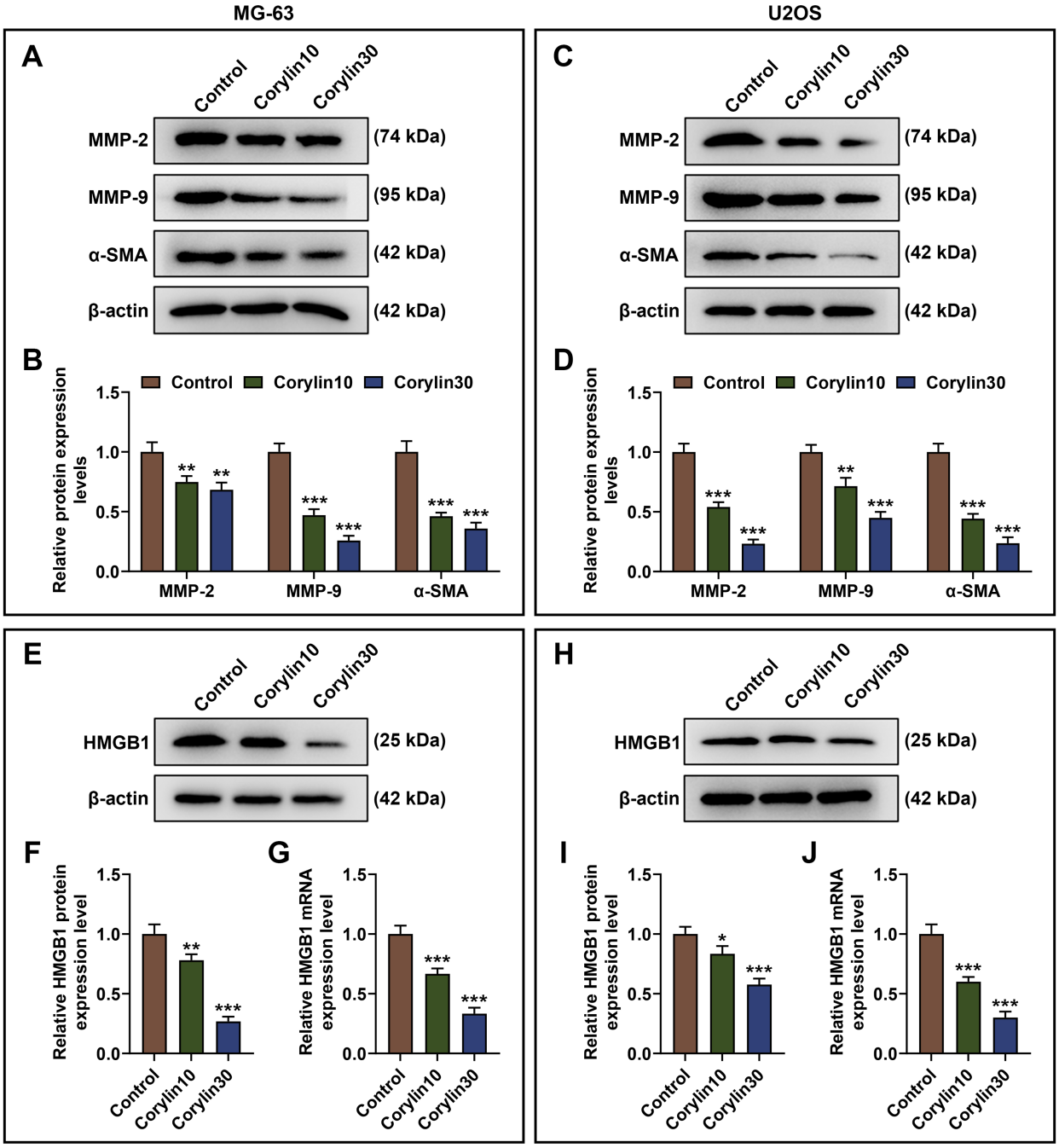

Fig. 3. Effects of corylin on the protein levels of MMP-2, MMP-9, α-SMA, and HMGB1. (A–D) Levels of MMP-2, MMP-9, and α-SMA in MG-63 and U2OS cells from control and corylin (10 and 30) groups were detected by western blotting. (E,F) HMGB1 protein expression in MG-63 cells was determined by western blotting. (G) *HMGB1* mRNA expression in MG-63 cells was quantified by RT-qRCR. (H–J) HMGB1 protein and mRNA levels in U2OS cells were examined by western blotting and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). All experiments were repeated at least three independent times; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ vs control. Abbreviations: MMP-2, matrix metalloproteinase-2; α-SMA, alpha-smooth muscle actin; *HMGB1*, high-mobility group box 1.

munoglobulin G (IgG) (ab6721, 1:2000, Abcam, London, UK) or rabbit anti-mouse IgG (ab6728, 1:2000, Abcam, London, UK) for 1 hour. Next, the target proteins were visualized using the enhanced chemiluminescence (ECL) Plus Kit (WBKlS0100, Millipore, Boston, MA, USA) and exposed in a Kodak *in vivo* Imaging System FX Pro (Kodak, Rochester, NY, USA). ImageJ software (version 1.52s, National Institutes of Health, Bethesda, MA, USA) was used

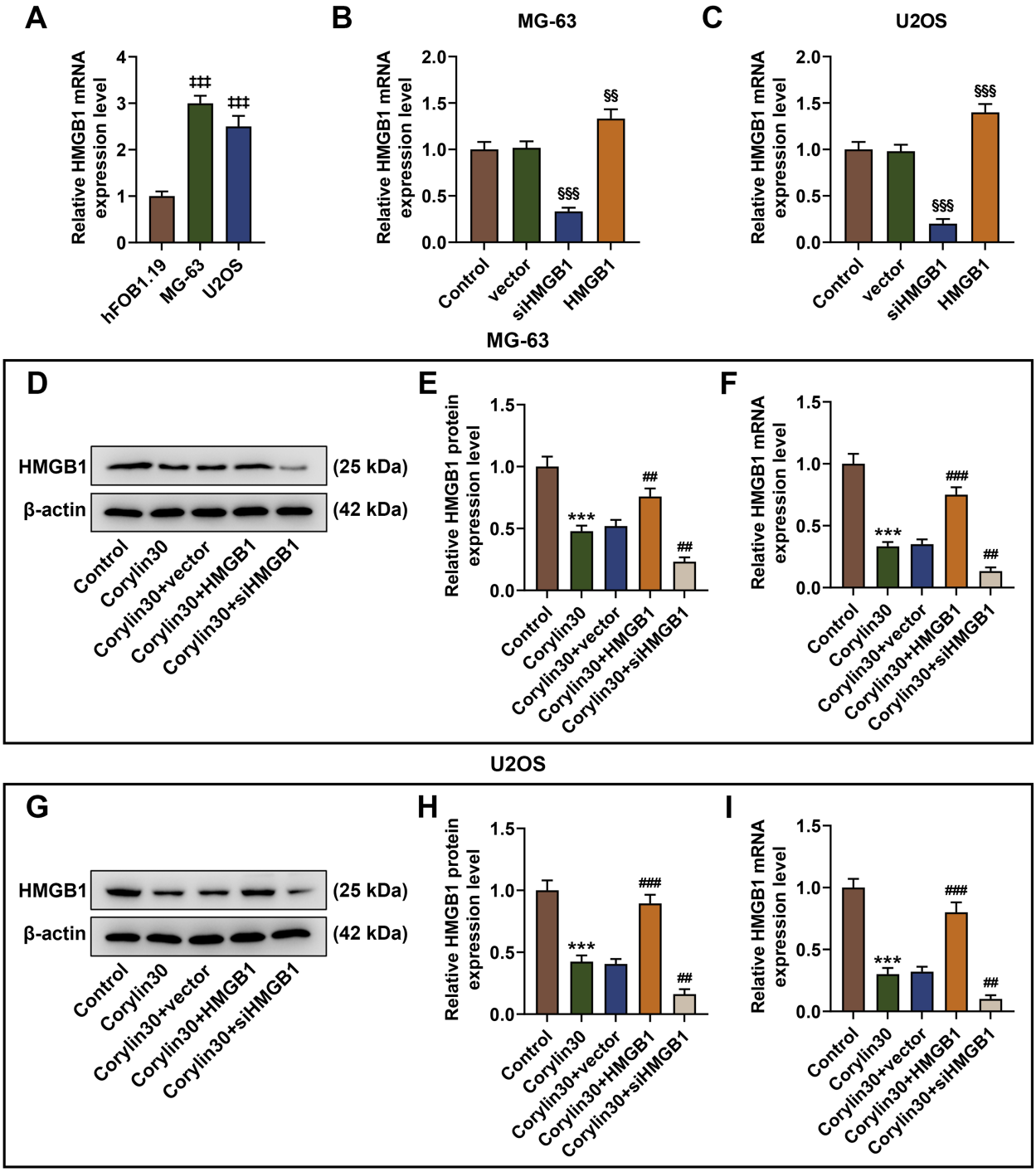

Fig. 4. Expression of *HMGB1* in OS cells and the effects of corylin, *HMGB1* overexpression, and *HMGB1* knockdown on the expression of *HMGB1*. (A) *HMGB1* mRNA expression level in MG-63, U2OS, and hFOB 1.19 cells. (B,C) *HMGB1* mRNA expression level in MG-63 and U2OS cells in control, vector, si*HMGB1*, and *HMGB1* groups. (D–I) HMGB1 mRNA and protein levels in OS cells in control, corylin 30, corylin 30+vector, corylin 30+*HMGB1*, and corylin 30+si*HMGB1* groups. All experiments were repeated at least three independent times; ***$p < 0.001$ vs control; $^{\ddagger\ddagger\ddagger}p < 0.001$ vs hFOB1.19; $^{\S\S}p < 0.01$; $^{\S\S\S}p < 0.001$ vs vector; $^{\#\#}p < 0.01$; $^{\#\#\#}p <$ 0.001 vs Corylin 30+vector. Abbreviation: *HMGB1*, high-mobility group box 1.

for the quantification of target proteins, with β-actin serving as the internal reference. Primary antibodies were purchased from Abcam (London, UK) and used as follows: matrix metalloproteinase-2 (MMP-2) (1:1000; ab215986, 74 kDa), MMP-9 (1:1000; ab73734, 95 kD), alpha-smooth muscle actin (α-SMA) (1:1000; ab5694, 42 kDa), β-actin

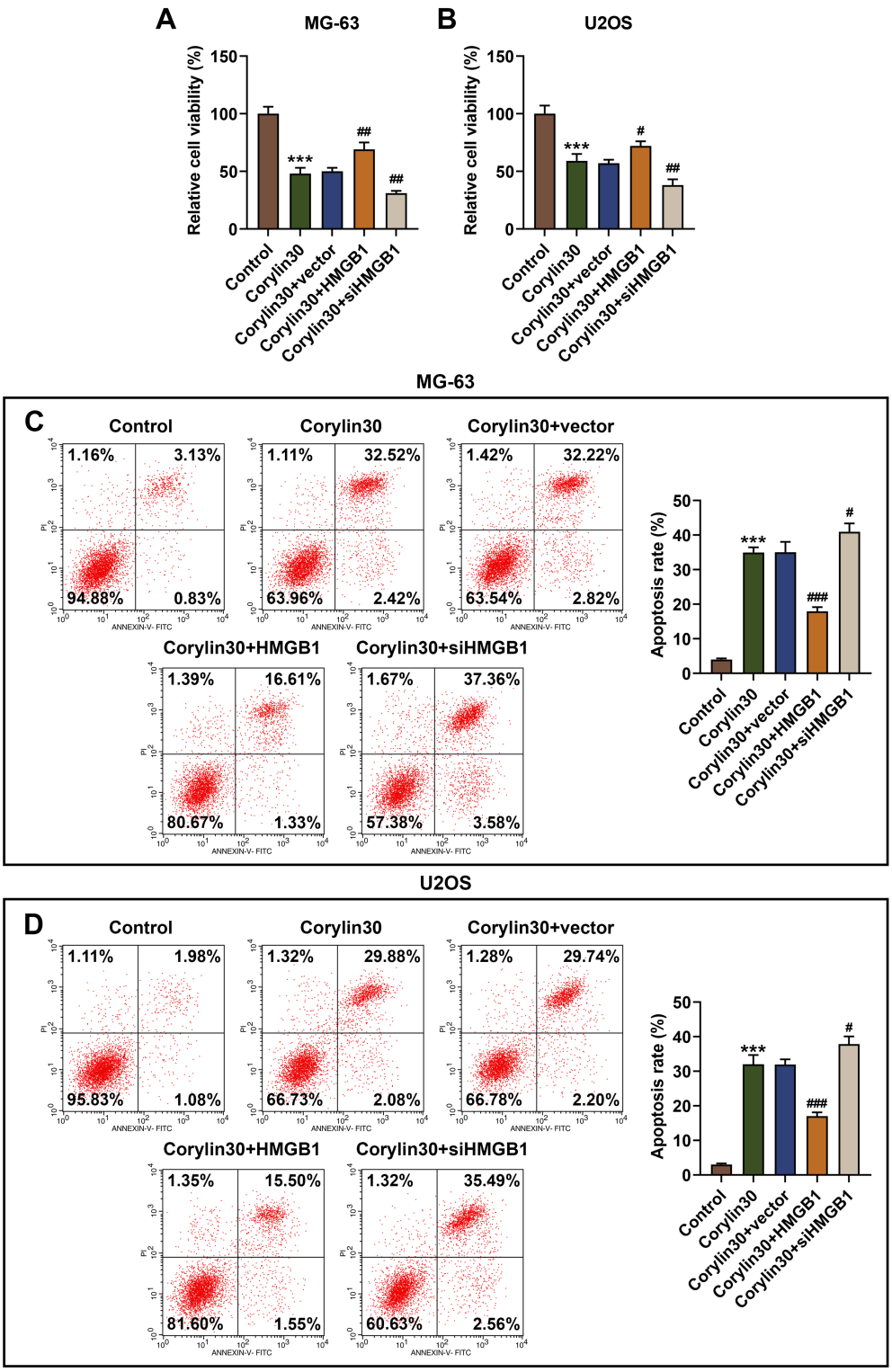

Fig. 5. Effects of *HMGB1* overexpression and *HMGB1* knockdown on OS cell survival and apoptosis. (A,B) OS cell viability in control, corylin 30, corylin 30+vector, corylin 30+*HMGB1*, and corylin 30+si*HMGB1* groups. (C,D) OS cell apoptosis in control, corylin 30, corylin 30+vector, corylin 30+*HMGB1*, and corylin 30+si*HMGB1* groups. All experiments were repeated at least three independent times; ***$p < 0.001$ vs control; $^{\#}p < 0.05$; $^{\#\#}p < 0.01$; $^{\#\#\#}p < 0.001$ vs Corylin 30+vector. Abbreviation: *HMGB1*, high-mobility group box 1.

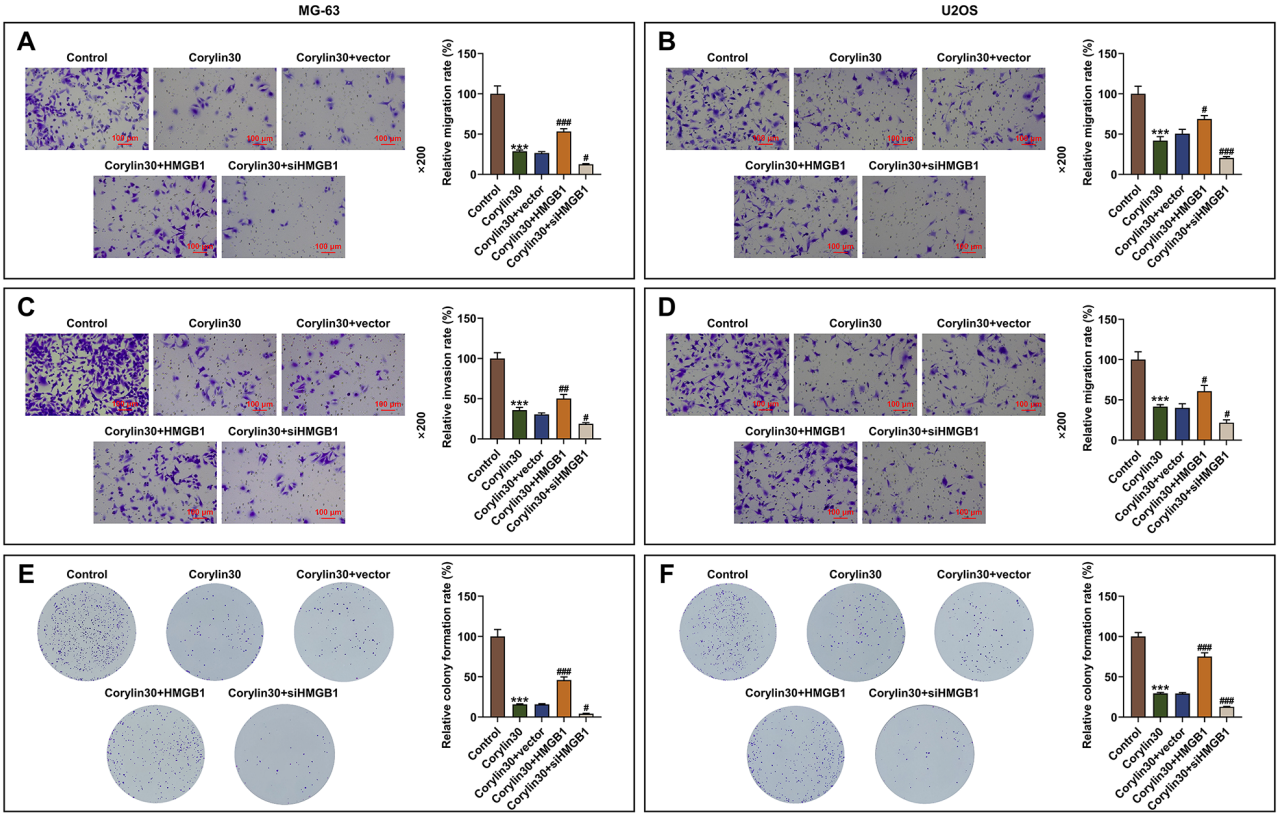

Fig. 6. Effects of *HMGB1* overexpression and *HMGB1* knockdown on OS cell proliferation, migration, and invasion. (A,B) Migration of OS cells in control, corylin 30, corylin 30+vector, corylin 30+*HMGB1*, and corylin 30+si*HMGB1* groups. (C,D) Cell invasion of OS cells in control, corylin 30, corylin 30+vector, corylin 30+*HMGB1*, and corylin 30+si*HMGB1* groups. (E,F) OS cell colony formation in control, corylin 30, corylin 30+vector, corylin 30+*HMGB1*, and corylin 30+si*HMGB1* groups. All experiments were repeated at least three independent times; ***$p < 0.001$ vs control; $^{\#}p < 0.05$; $^{\#\#}p < 0.01$; $^{\#\#\#}p < 0.001$ vs Corylin 30+vector. Abbreviation: *HMGB1*, high-mobility group box 1.

(1:5000; ab8226, 42 kDa), HMGB1 (1:10000; ab79823, 25 kDa), p-ERK (1:400; ab223500, 42–44 kDa), ERK (1:1000; ab17942, 42–44 kDa), p-p38 (1:1000; ab47363, 41 kDa), p38 (1:2000; ab170099, 42 kDa), p-JNK (1:1000; ab124956, 46–54 kDa), and JNK (1:1000; ab179461, 46–54 kDa). The normalized relative protein expression level was calculated as follows: x' (normalized data) = (x (original data) – mean data (x)) / std data (x, internal control).

Statistical Analyses

The measurement data were denoted as mean ± standard deviation, and multi-group comparisons were performed with one-way ANOVA. All statistical analyses were conducted by GraphPad Prism 8.0 (GraphPad Software, Inc., San Diego, CA, USA), and $p < 0.05$ was considered to be statistically significant.

Results

Corylin Reduces Viability and Promotes Apoptosis of MG-63 and U2OS Cells

To investigate the effect of corylin on OS cells, cells were divided into control and corylin (2.5, 5, 10, and 30) groups. As shown in Fig. 1A–C, there was no significant difference in the viability of MG-63 and U2OS cells between the control group and the corylin 2.5 group, while the viability of cells in the corylin (5, 10 and 30) groups decreased concentration-dependently compared to the control group. However, corylin did not impact the viability of hFOB 1.19 cells. As shown in Fig. 1D,E, apoptosis was promoted by 10 and 30 μM corylin compared to the control group ($p < 0.001$, Fig. 1D,E). Therefore, corylin can concentration-dependently decrease viability and increase apoptosis of OS cells but exert no effect on normal human osteoblasts.

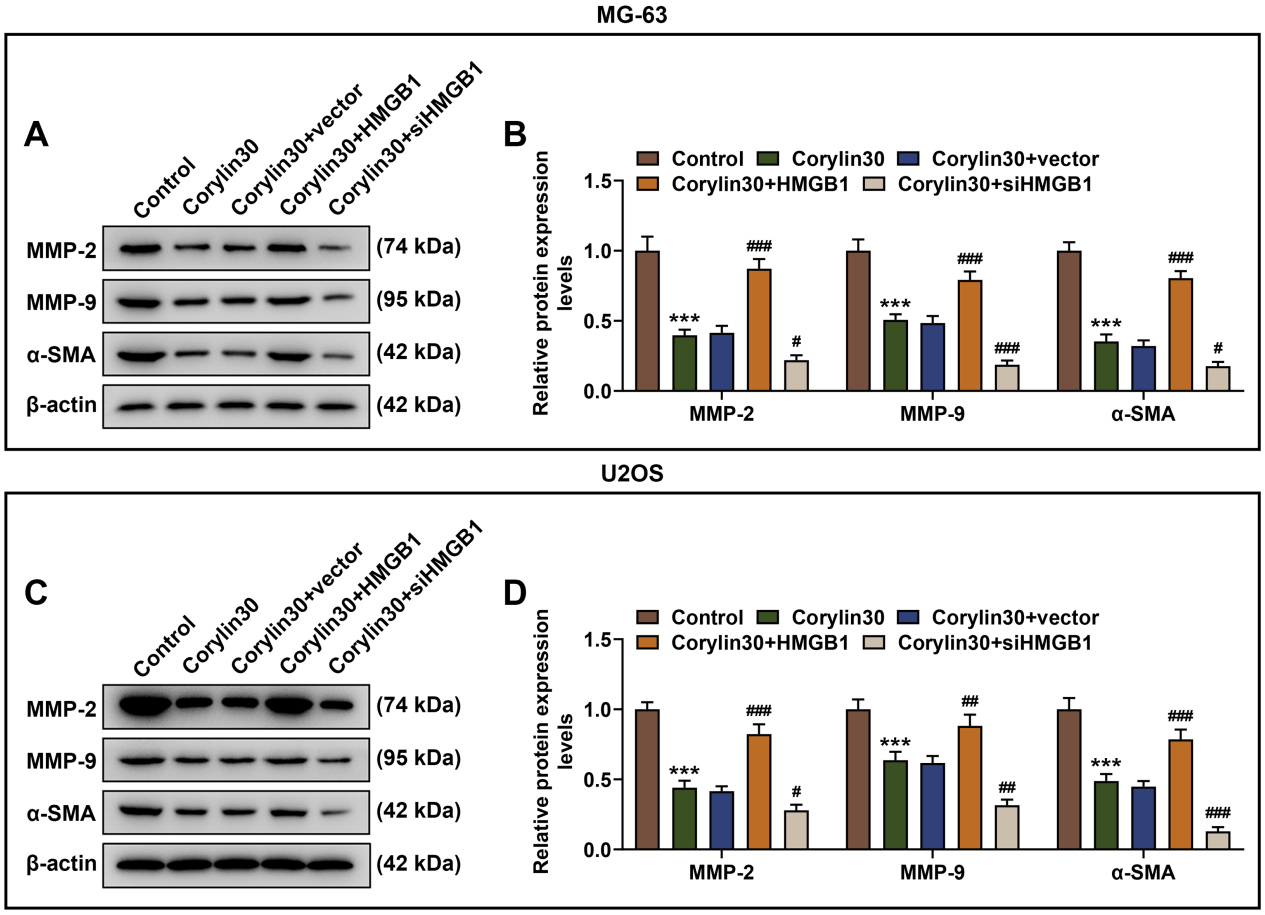

Fig. 7. Effects of *HMGB1* overexpression and *HMGB1* knockdown on MMP-2, MMP-9, and α-SMA expression. (A,B) Protein expression levels of MMP-2, MMP-9, and α-SMA in MG-63 cells. (C,D) Protein expression levels of MMP-2, MMP-9, and α-SMA in U2OS cells in control, corylin 30, corylin 30+vector, corylin 30+*HMGB1*, and corylin 30+si*HMGB1* groups. All experiments were repeated at least three independent times; ***$p < 0.001$ vs control; $^{\#}p < 0.05$; $^{\#\#}p < 0.01$; $^{\#\#\#}p < 0.001$ vs Corylin 30+vector. Abbreviations: MMP-2, matrix metalloproteinase-2; α-SMA, alpha-smooth muscle actin; *HMGB1*, high-mobility group box 1.

Corylin Suppresses Migration, Invasion, and Colony Formation in MG-63 and U2OS Cells

As shown in Fig. 2A,B, corylin (10 and 30 µM) decreased MG-63 and U2OS cell migration compared to the control group ($p < 0.01$, Fig. 2A,B). Additionally, corylin 10 and 30 groups showed lower rates of cell invasion and colony formation compared to the control group ($p < 0.01$, Fig. 2C–F). These data indicate that corylin suppresses OS cell migration, invasion, and proliferation in a concentration-dependent manner.

Corylin Suppresses the Protein Levels of MMP-2, MMP-9, α-SMA, and HMGB1

As shown in Fig. 3A–D, the protein levels of MMP-2, MMP-9, and α-SMA significantly decreased in the corylin 10 and 30 groups compared to the control group ($p < 0.001$). Corylin (10 and 30 µM) also downregulated the *HMGB1* level ($p < 0.001$, Fig. 3E–J).

HMGB1 Expression Increases but is Reduced by Corylin in OS Cells

As shown in Fig. 4A, the mRNA level of *HMGB1* in MG-63 and U2OS cells was significantly higher than that in hFOB 1.19 cells ($p < 0.001$). Additionally, the *HMGB1* expression level in the si*HMGB1* group was lower than that in the vector group. *HMGB1* was upregulated in MG-63 and U2OS cells transfected with *HMGB1* overexpression vector ($p < 0.001$, Fig. 4B,C). Similarly, compared to the corylin 30+vector group, the HMGB1 protein expression levels in MG-63 and U2OS cells were increased in the corylin 30+*HMGB1* group and decreased in the corylin 30+si*HMGB1* group ($p < 0.01$, Fig. 4D–I). Our results show that *HMGB1* expression was upregulated in OS cells. Furthermore, corylin 30-induced suppression of *HMGB1* level was partially reversed by overexpression of *HMGB1*.

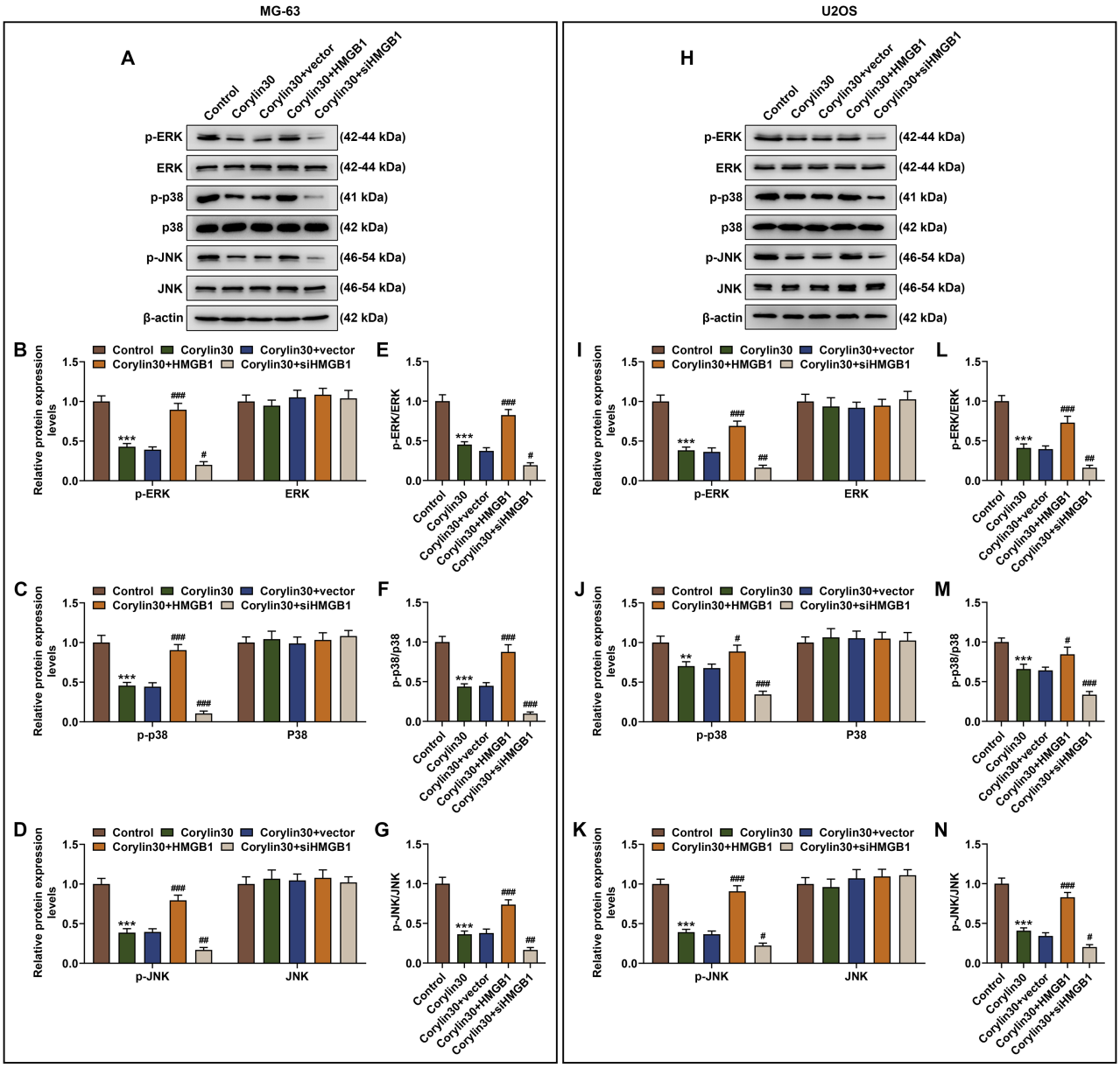

Fig. 8. Effects of *HMGB1* overexpression and *HMGB1* knockdown on p38 MAPK signaling pathway-related proteins. (A–G) Expression levels of p38/MAPK pathway-related proteins in MG-63 cells. (H–N) Levels of p38/MAPK pathway-related proteins in U2OS cells in control, corylin 30, corylin 30+vector, corylin 30+*HMGB1*, and corylin 30+si*HMGB1* groups. All experiments were repeated at least three independent times; $^{**}p < 0.01$, $^{***}p < 0.001$ vs control; $^{\#}p < 0.05$; $^{\#\#}p < 0.01$; $^{\#\#\#}p < 0.001$ vs Corylin 30+vector. Abbreviation: *HMGB1*, high-mobility group box 1; MAPK, mitogen-activated protein kinase.

HMGB1 Overexpression Partially Reverses the Effect of Corylin while Enhancing MG-63 and U2OS Cell Survival and Inhibiting Apoptosis

OS cell viability was enhanced in the corylin 30+*HMGB1* group and reduced in the corylin 30+si*HMGB1* group compared to the corylin 30+vector group ($p < 0.05$, Fig. 5A,B). Moreover, the rates of MG-63 and U2OS cell apoptosis were lower in the corylin 30+*HMGB1* group but higher in the corylin 30+si*HMGB1* group compared to the corylin 30+vector group ($p < 0.05$, Fig. 5C,D). These results show that the upregulation of *HMGB1* partially counteracts the effect of corylin while potentiating OS cell viability and attenuating cell apoptosis.

HMGB1 Overexpression Offsets the Effect of Corylin and Enhances OS Cell Migration, Invasion, and Colony Formation

Overexpression of *HMGB1* increased cell migration and invasion, while knockdown of *HMGB1* showed the re-

verse effect ($p < 0.05$, Fig. 6A–D) compared to cells treated with corylin (30 μM) and transfected with a control vector. *HMGB1* overexpression had a similar effect on cell colony formation, as evidenced by increased colony formation in the corylin 30+*HMGB1* group and decreased colony formation in the corylin 30+si*HMGB1* group compared to the corylin 30+vector group ($p < 0.05$, Fig. 6E,F). Overall, *HMGB1* overexpression partially reverses the effect of corylin and promotes OS cell migration, invasion, and proliferation.

HMGB1 Overexpression Counteracts the Effect of Corylin on the Expression of MMP-2, MMP-9, and α-SMA

As shown in Fig. 7A–D, MMP-2, MMP-9, and α-SMA expression levels were elevated by *HMGB1* overexpression, but reduced by si*HMGB1* compared to cells treated with corylin (30 μM) and transfected with vector ($p < 0.05$). Thus, *HMGB1* overexpression partially attenuates the effect of corylin and increases the expression of MMP-2, MMP-9, and α-SMA.

HMGB1 Overexpression Neutralizes the Effect of Corylin and Activates the p38 MAPK Signaling Pathway

The levels of p38 MAPK signaling pathway-related proteins are depicted in Fig. 8. The levels of p-ERK, p-p38, and p-JNK and the ratios of p-ERK/ERK, p-p38/p38, and p-JNK/JNK in the corylin 30+vector group were increased by *HMGB1* overexpression but decreased by corylin (30 μM) and si*HMGB1* ($p < 0.05$, Fig. 8A–N), indicating that si*HMGB1* blocks while *HMGB1* overexpression activates the p38 MAPK signaling pathway in corylin (30 μM)-treated cells.

Discussion

Corylin, the main flavonoid extracted from *Psoralea corylifolia L.*, has been demonstrated to induce osteoblastic differentiation by regulating estrogen and Wnt/β-catenin signaling pathways [14], as well as to exert anti-tumor and anti-inflammatory effects [11,13]. Nevertheless, the impact of corylin on OS has not been explored. This study found that corylin suppressed OS cell proliferation, invasion, and migration, and promoted apoptosis. Moreover, the effect of corylin was enhanced as the concentration increased, which indicates that corylin dose-dependently inhibits the growth of OS cells. However, additional experiments are needed to determine the optimal concentration of corylin to be used.

Metastasis is the primary cause of OS-induced death; therefore, its targeting is a major strategy in OS treatment [24]. A-SMA is an isoform of actin and a marker of EMT [25]. EMT is a process where tumor cells develop the ability to metastasize. Tumor cell progression is followed by cell composition and extracellular matrix component alterations [26]. Increased expression of α-SMA can herald the progress of ovarian neoplasms [27]. MMP-2 and MMP-9 are endogenous Zn^{2+}-dependent proteolytic enzymes that degrade the extracellular matrix and play a vital role in the invasion, migration, and metastasis of various tumors [28]. The inhibition of MMP-2 and MMP-9 expression can attenuate cell migration and invasion during esophageal squamous cell carcinoma progression [29], decrease cell migration in retinoblastoma, and suppress HCCLM3 cell invasion and migration [30,31]. The current study demonstrates that corylin decreases the expression levels of MMP-2, MMP-9, and α-SMA, which further validated that OS cell migration and invasion were suppressed by corylin possibly via downregulation of MMP-2, MMP-9 and α-SMA.

HMGB1 has been confirmed to promote osteoblastic proliferation and differentiation [14]. According to a recent study, *HMGB1* enhances cell proliferation and invasion and suppresses cell apoptosis in OS [32]. OS cell necrosis induces the production and secretion of *HMGB1* [33], whereas MALAT1 promotes the development of OS by regulating *HMGB1* [34]. In our study, the highly expressed *HMGB1* in OS cells was found to be significantly suppressed by corylin. We also verified that the upregulation of *HMGB1* increased OS cell viability, enhanced cell proliferation, migration, and invasion, and inhibited apoptosis, whereas si*HMGB1* generated opposite effects. Additionally, the above effects of *HMGB1* on the OS cell malignant phenotype were reversed by corylin, which proves that corylin exerts an inhibitory effect on OS cell growth by suppressing *HMGB1* expression.

Multiple complex signaling pathways mediate the function of OS cells. The previous study has reported that Paris saponin VII suppresses OS cell migration and invasion through the p38 MAPK signaling pathway [24]. Moreover, *HMGB1* targets the p38/p-JNK signaling pathway [35]. Therefore, we subsequently investigated the effect of corylin on the activation of the *HMGB1*/p38 MAPK signaling pathway in OS cells. The results showed that corylin and si*HMGB1* block, whereas *HMGB1* overexpression activates the p38 MAPK signaling pathway, suggesting that treatment with corylin can hinder OS progression *in vitro* by blocking the activation of the *HMGB1*/p38 MAPK signaling pathway.

Conclusion

Corylin blocks OS cell migration and invasion by suppressing the *HMGB1*/p38 MAPK signaling pathway, which can be used as a promising therapeutic agent. Moreover, OS alleviation is more apparent in dose-escalating exposures to corylin, that is, the greater the dosage, the more obvious the effect. However, there are knowledge gaps to be filled, including what the capping dose of corylin is and whether other mechanisms are involved.

Availability of Data and Materials

The analyzed data sets generated during the study are available from the corresponding author upon reasonable request.

Author Contributions

RYY designed the research study; HW, ZYC, and ZYZ performed the research and collected and analyzed the data. All the authors have been involved in drafting the manuscript and have been involved in revising it critically for important intellectual content. All the authors have given the final approval of the version to be published. All the authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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