ORIGINAL ARTICLE

# Downregulation of lncRNA UCA1 facilitates apoptosis and reduces proliferation in multiple myeloma via regulation of the miR-1271-5p/*HGF* axis

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## Abstract

**Background:** Long noncoding RNAs (lncRNAs) are considered to be a novel prognostic and therapeutic target in many cancers. This study identified dysregulation of lncRNA urothelial carcinoma associated 1 (UCA1) and hepatocyte growth factor (HGF) mRNA via the Gene Expression Omnibus (GEO) database, which was traced to the mutual target miRNA, miR-1271-5p, and their effects were explored in multiple myeloma (MM).

**Methods:** RNA expression profiles of MM were downloaded from the GEO database and analyzed using R packages. The expression of RNAs in MM tissue samples and cells was evaluated through quantificational real-time polymerase chain reaction (qRT-PCR). A luciferase reporter assay was utilized to confirm the binding relationships between UCA1/*HGF* and miR-1271-5p. To assess cell proliferation and apoptosis, CCK-8 assays and flow cytometry were conducted. Additionally, tumor progression was demonstrated in vivo.

**Results:** LncRNA UCA1 and *HGF* expression was higher in the cells and samples of patients with MM than in normal plasma cells. miR-1271-5p was confirmed to be the target of lncRNA UCA1 and *HGF* and to be negatively correlated with them. Moreover, downregulation of lncRNA UCA1 and *HGF* inhibited cell proliferation and facilitated cell apoptosis in RPMI 8226 cells (human MM cell line). However, miR-1271-5p overexpression affected the proliferation decrease and apoptosis increase. Moreover, in vivo experiments indicated that down or upregulation of lncRNA UCA1 repressed or enhanced the tumor growth of MM, respectively, in xenograft models.

**Conclusion:** LncRNA UCA1 promoted proliferation and inhibited apoptosis by regulating miR-1271-5p and *HGF* in the human MM cell line RPMI 8226. Our investigations might contribute to a better understanding of the lncRNA UCA1/miR-1271-5p/*HGF* axis as a potential therapeutic strategy in MM.

**Keywords:** HGF; lncRNA; miR-1271-5p; Multiple myeloma; UCA1

## 1. INTRODUCTION

Multiple myeloma (MM) is a kind of neoplasm of postgerminal center, terminally differentiated B cells. It is the second most common hematologic cancer in the United States. The transformation of long-lived plasma cells (PCs) is considered to be the initial event in MM. PCs can produce antibodies and survive in the bone marrow for years to maintain immunologic memory.[1] Long-lived PCs will first transform to cause monoclonal gammopathy of undetermined significance (MGUS), and MGUS then turns into smoldering MM (SMM). Finally, SMM becomes MM. During transformation, a cell will experience IgH translocations, hyperdiploidy, secondary translocations, gene deletions, and chromosome gains.[2] The immunophenotypes of MM cells will be altered at the same time. For example, they will strongly express CD138, CD56, CD28, and CD38 but will express little CD19, CD27, and CD45.[3] These immunophenotypes allow researchers to separate normal PCs and MM cells by using multiparametric flow cytometry. Several novel molecular target therapies have been approved by the US Food and Drug Administration (FDA) over the past 15 years, such as thalidomide and lenalidomide.[2] Lenalidomide is a stronger derivative of thalidomide; thus, thalidomide is the first-line agent, and lenalidomide is the second-line agent. Both agents function through cereblon (CRBN), an E3 ubiquitin-ligase complex.[4] However, MM treatment has not been completely realized. The development of MM requires a cascade of genetic events, so in addition to thalidomide and lenalidomide, RNA regulation might be a new approach for curing MM.

RNAs, including long noncoding RNAs (lncRNAs), microRNAs (miRNAs), and mRNAs, have been reported to act as suppressors or enhancers of many cancers.[5–7] Among these RNAs, miRNAs and lncRNAs belong to an assemblage of noncoding RNAs (ncRNAs) with or without a limited protein coding capacity. LncRNAs constitute a crucial class of ncRNAs consisting of mRNA-like transcripts transcribed by RNA polymerase II. In addition, they are longer than 200 nucleotides in

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Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

Journal of Chinese Medical Association. (2019) 82: 699-709.

Received November 23, 2018; accepted February 25, 2019.

doi: 10.1097/JCMA.0000000000000145.

length.[8] MiRNAs are shorter RNAs compared with lncRNAs and act as negative regulators of mRNA expression by binding with sequences that are usually located in the 3′-untranslated region (UTR) of mRNAs.[9] Many ncRNAs have been found to exhibit functions in tumor development, but the majority of ncRNAs are yet to be identified in different diseases. Regarding the abilities of ncRNAs, it has recently been revealed that lncRNAs may act as suppressors of miRNAs by sponging them, preventing miRNAs from regulating the translation of mRNAs, or acting as intermediaries between chromosomes and proteins as usual.[10]

A number of lncRNAs have been investigated in MM. For instance, lncRNA PDIA3P has been reported to interact with c-Myc and regulate MM proliferation.[11] LncRNAs MALAT1 and PCAT1 have been reported as poor prognostic biomarkers for MM.[12,13] Furthermore, lncRNA MALAT1 can regulate MM development by modulating different molecules.[14,15] Some scientists have studied the interaction between lncRNAs and miRNAs. For example, the lncRNA OIP5-AS1 is found to target miR-40 and downregulate *KLF10*. Loss of lncRNA OIP5-AS1 results in the accumulation of miR-410, which regulates cell proliferation and apoptosis through activation of the PTEN/PI3K/AKT signaling pathway in MM.[16] Drug resistance and chemoresistance are promoted by lncRNA NEAT1 and linc00515, respectively. LncRNA NEAT1 functions through targeting miR-193a,[17] and linc00515 operates via downregulating miR-140-5p.[18] LncRNA CRNDE targets miR-451 to further impede MM cell growth.[19] However, researches are limited in which the functions of lncRNAs in MM remain vague, and additional investigations are needed to overcome MM.

In particular, human urothelial carcinoma associated 1 (UCA1) is one of the dysregulated lncRNAs in MM, and it was first identified in human bladder carcinoma.[20] UCA1 contains three exons and two introns and is located on the chromosome 19p13.12 positive strand. In addition, the UCA1 sequence contains multiple stop codons and does not contain any conserved long open reading frames (ORFs). The full-length cDNA of UCA1 does not produce protein products in an in vitro translation assay.[20] Although UCA1 has three isoforms, including 1.4, 2.2, and 2.7 kb isoforms, most researchers focus on the 1.4 kb isoform of UCA1 because it is the most abundant isoform of UCA1 in diverse cancers, such as bladder cancer,[21] breast cancer,[22] and hepatocellular carcinoma.[23] Due to its various modulatory mechanisms in a variety of cancers, what role UCA1 plays in MM is a question of interest.

Hepatocyte growth factor (HGF) is a multidomain glycoprotein and is physiologically produced by mesenchymal cells as a single-chain precursor (pro-HGF).[24] HGF is a known ligand of the MET receptor. The combination of HGF and MET could act as a mesenchymal-derived growth factor.[25] High expression of HGF generally causes human cells to become tumorigenic, metastatic, and progressive.[26] This tumorigenic effect has been reported in both blood tumors[27] and solid tumors.[28–30] HGF has also been suggested to be upregulated in the bone marrow blood of MM patients and is indicated as a prognostic factor.[31] Based on the previous studies, HGF has the ability to cause normal cells to become cancerogenic. Additionally, the modulation of HGF in MM is still unknown.

In this study, we analyzed off-the-peg MM data in the GEO database and selected lncRNA UCA1 and *HGF* mRNA for subsequent analysis due to their dysregulation in MM. Our investigations indicated that lncRNA UCA1 and *HGF* targeted both miR-1271-5p, and their influences on the proliferation and apoptosis of MM cells were revealed in vitro. LncRNA UCA1 showed similar utility in MM in vivo.

## 2. METHODS

### 2.1. Bioinformatic analysis

LncRNA and mRNA expression data from MM were downloaded from the Gene Expression Omnibus database (GEO database: https://www.ncbi.nlm.nih.gov/geo/). The lncRNA data came from GSE109116, and the mRNA data came from GSE47552. The "limma" package was utilized to identify dysregulated RNAs ($\log_2$|Fold Change|>1 and $p$. adjust<0.05). The "pheatmap" R-package was applied to generate a heatmap of the top 20 differentially expressed RNAs via the pheatmap function. LncRNA UCA1 and *HGF* mRNA were then screened out. Potential common miRNA targets of lncRNA UCA1 and HGF were predicted and filtered by using the TargetScan 7.2 (http://www.targetscan.org/vert_72/), miRanda (http://34.236.212.39/microrna/home.do), and HMDD 3.0 (http://www.cuilab.cn/hmdd) databases.

### 2.2. Clinical samples and cell culture

Clinical samples from 15 patients diagnosed with MM and 15 healthy donors were collected from the Department of Hematology, Second Affiliated Hospital of Chongqing Medical University, from January 2017 to January 2018. All enrolled people were provided with informed consent, and this study was supported by the Ethics Committee of the Second Affiliated Hospital of Chongqing Medical University. Normal plasma cells and MM samples from bone marrow aspirates were purified using $CD138^-$ and $CD138^+$ magnetic beads (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany), respectively, with a Mini-MACS separator (Miltenyi Biotec GmbH, Germany). The human myeloma cell line (HMCL) RPMI 8226 was obtained from the BeNa Culture Collection (Beijing, China; http://www.bnbio.com/). Cells were cultured in RPMI 1640 medium (BasalMedia, Shanghai, China) with 10% FBS, 2 mmol/L L-glutamine, and penicillin/streptomycin at 37°C in a humidified atmosphere with 5% $CO_2$.

### 2.3. Quantificational real-time polymerase chain reaction (qRT-PCR)

Total RNA extraction was performed using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) based on the manufacturer's guidance. cDNA was synthesized by using the PrimeScript RT reagent kit with gDNA Eraser (Takara, Tokyo, Japan). Transcript levels were determined by qRT-PCR using SYBR Green PCR Master Mix (Takara, Japan). U6 and β-actin were used as internal controls, and RNA expression was quantified via the $2^{-\Delta\Delta Ct}$ method. All primer sequences for qRT-PCR are listed in Table.

### 2.4. Plasmid construction and transfection

After the extracted total RNA was reverse transcribed into cDNA, the cDNA fragment of lncRNA UCA1 was amplified by RT-PCR. LncRNA UCA1 was cloned into the expression vector pCDH-CMV-MCS-EF1-copGFP (System Biosciences, CA, USA) between the BamHI and EcoRI restriction sites using a Cold Fusion kit (System Biosciences, USA). The construction of a shRNA targeting lncRNA UCA1 (sh-UCA1) was conducted by Ribobio (Guangzhou, China). An siRNA targeting *HGF* (si-HGF), miR-1271-5p mimics, and a negative control were purchased from Genepharma (Shanghai, China). All vectors were transfected into RPMI 8226 cells with Lipofectamine 2000 (Invitrogen, USA), according to the manufacturer's instructions.

### 2.5. Luciferase reporter assay

On the basis of complementary sequence analysis between miR-1271-5p and lncRNA UCA1, the full-length cDNA of lncRNA UCA1 was subcloned into the pMIR-REPORT Luciferase vector (UCA1-wt). pMIR-REPORT Luciferase-UCA1-mut (UCA1-mut)

**Table**

**Primer sequences used for qRT-PCR in this study**

| Gene names | Primer sequences |
|---|---|
| *LncRNA UCA1* | F: TGCACCCTAGACCCGAAACT |
| | R: CAAGTGTGACCAGGGACTGC |
| *HGF* | F: TCCACGGAAGAGGAGATGAGA |
| | R: GGCCATATACCAGCTGGGAAA |
| *β-actin* | F: AAGGCCAACCGCGAG |
| | R: TAATGTCACGCACGATTCCCG |
| *miR-1271-5p* | F: CAGCACTTGGCACCTAGCA |
| | R: TATGGTTGTTCTCCTCTCTGTCTC |
| *U6* | F: CGCAAGGATGACACGCAAATTC |
| | R: TAGCAGCACGTAAATATTGGCG |

HGF = hepatocyte growth factor; lncRNA = long noncoding RNAs; MM = multiple myeloma; UCA1 = urothelial carcinoma associated 1.

was synthesized by BioSune (Shanghai, China). The potential binding site in the *HGF* 3′-UTR and a corresponding mutant sequence were cloned into the pMIR-REPORT Luciferase vector and named HGF-wt or HGF-mut. 293T cells were transfected with miR-1271-5p mimics or the control vector and reporter plasmids using Lipofectamine 2000 (Invitrogen, USA), and a Dual Luciferase Reporter Assay Kit (Promega, Madison, WI, USA) was employed to conduct this assay according to the manufacturer's instructions.

### 2.6. Western blot analysis

Pellets of RPMI 8226 cells were lysed in radio immunoprecipitation assay (RIPA; Beyotime, Shanghai, China) buffer with phenylmethanesulfonyl fluoride (PMSF; Beyotime, China) on ice for 30 minutes. Equal amounts of protein were separated by 8% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The membranes were blocked with 5% nonfat milk in phosphate buffer solution with Tween-20 (PBST) at room temperature for 1 hour, followed by incubation with a rabbit polyclonal primary antibody against *HGF* (ab83760, 1:1000; Abcam, Cambridge, MA, USA) and the loading control α-tubulin antibody (ab7291, 1:5000; Abcam, USA) overnight at 4°C. The membranes were then washed twice with PBST and incubated with horseradish peroxidase-labeled secondary anti-rabbit IgG antibodies (ab7090, 1:5000; Abcam, USA). Chemiluminescence was detected using an ECL kit (Beyotime, China).

### 2.7. CCK-8 assay

Cell proliferation was evaluated using a Cell Counting Kit-8 (CCK-8; Beyotime, China) in accordance with the manufacturer's instructions. Cells were seeded into 96-well plates for 24, 48, or 72 hours, after which WST-8 solution was added, followed by incubation for 3 hours. The optical density (OD) was measured with an EnSpire Reader (PerkinElmer, Waltham, MA, USA) at 450 nm.

### 2.8. Flow cytometry analysis for apoptosis detection

Apoptosis was detected through flow cytometric (FACS) analysis with Annexin V-FITC/PI (Yeasen, Shanghai, China) and analyzed with FlowJo Software (Tree Star, Ashland, OR, USA), following the manufacturer's instructions. Annexin V-FITC$^+$/PI$^-$ staining was representative of early apoptotic cells, whereas Annexin V-FITC$^+$/PI$^-$ staining was representative of late apoptotic cells. The apoptosis rate is defined as the early apoptosis rate and late apoptosis rate combined in this study.

### 2.9. Animal experiment

A total of twelve, 6- to 8-week-old female CB-17 severe combined immunodeficient (SCID) mice (Vital River, Beijing, China) were used to establish the xenograft model of human MM. PRMI 8226 cells were divided into four groups. Then, the cells received the following treatments: lncRNA UCA1 upregulation (UCA1 group), lncRNA UCA1 downregulation (sh-UCA1), empty vector (NC group), and no treatment (blank group). Mice were subcutaneously injected with $5 \times 10^6$ treated PRMI 8226 cells in their flanks. The tumor volume was observed every 7 days from the day of injection. On the 28th day, the tumors were isolated after mice were anesthetized. The tumor volume was calculated with the formula: tumor volume = length × width2/2. The isolated tumors were weighed before being stored at –80°C for further investigations. All experimental procedures were approved by the Animal Care Committee of the Second Affiliated Hospital of Chongqing Medical University.

### 2.10. Statistical analysis

Data are presented as the mean ± SD, and $p < 0.05$ was regarded as statistically significant. All experiments were performed with at least three independent replications. GraphPad Prism 7.0 (GraphPad Software, San Diego, CA, USA) was applied to perform statistical analyses including the two-tailed Student's *t* test, Spearman correlation analysis, and analysis of variance.

## 3. RESULTS

### 3.1. LncRNA UCA1 and *HGF* were overexpressed in MM tissue and MM cell lines

Based on GEO data, lncRNA expression data were downloaded from GSE109116, which included 30 MM samples and four normal controls, and mRNA expression data were downloaded from GSE47552, which included 41 MM samples and five normal controls. The heatmaps displayed the top 20 differentially expressed lncRNAs and mRNAs. In particular, lncRNA UCA1 and *HGF* mRNA were upregulated in MM patient samples (Fig. 1A, B). The overexpression of lncRNA UCA1 and *HGF* was verified in 15 MM samples and in the RPMI 8226 cell line (Fig. 1C, D).

### 3.2. miR-1271-5p targeted both lncRNA UCA1 and *HGF*

The target analysis was carried out in silico, as shown in the Venn diagram. The three components of the data consisted of predicted miRNAs targeting lncRNA UCA1, *HGF*, and the miRNAs reported in the HMDD v3.0 database. The intersection of the three components included five miRNAs, miR-18a-5p, miR-96-5p, miR-182-5p, miR-202-5p, and miR-1271-5p (Fig. 2A). The expression levels of these five miRNAs were determined in MM samples and normal controls. The results indicated that miR-202-5p was upregulated and that miR-1271-5p was downregulated in MM compared with normal controls. Additionally, the expression of other miRNAs was not significantly different (Fig. 2B). Because of endogenous competitive inhibition, the upregulation of lncRNA UCA1 usually means that its target miRNAs should be downregulated. Therefore, miR-1271-5p was selected for further investigation. Moreover, Spearman correlation analysis revealed a negative correlation between miR-1271-5p and lncRNA UCA1 or *HGF* (Fig. 2C, D). The predicted binding sites between miR-1271-5p and lncRNA UCA1 are shown in Fig. 2E. The luciferase reporter assays confirmed that miR-1271-5p was a target of lncRNA UCA1 (Fig. 2F). Similarly, Fig. 2G shows the potential binding site of miR-1271-5p and *HGF*. A luciferase reporter assay verified that miR-1271-5p was a target of *HGF* (Fig. 2H).

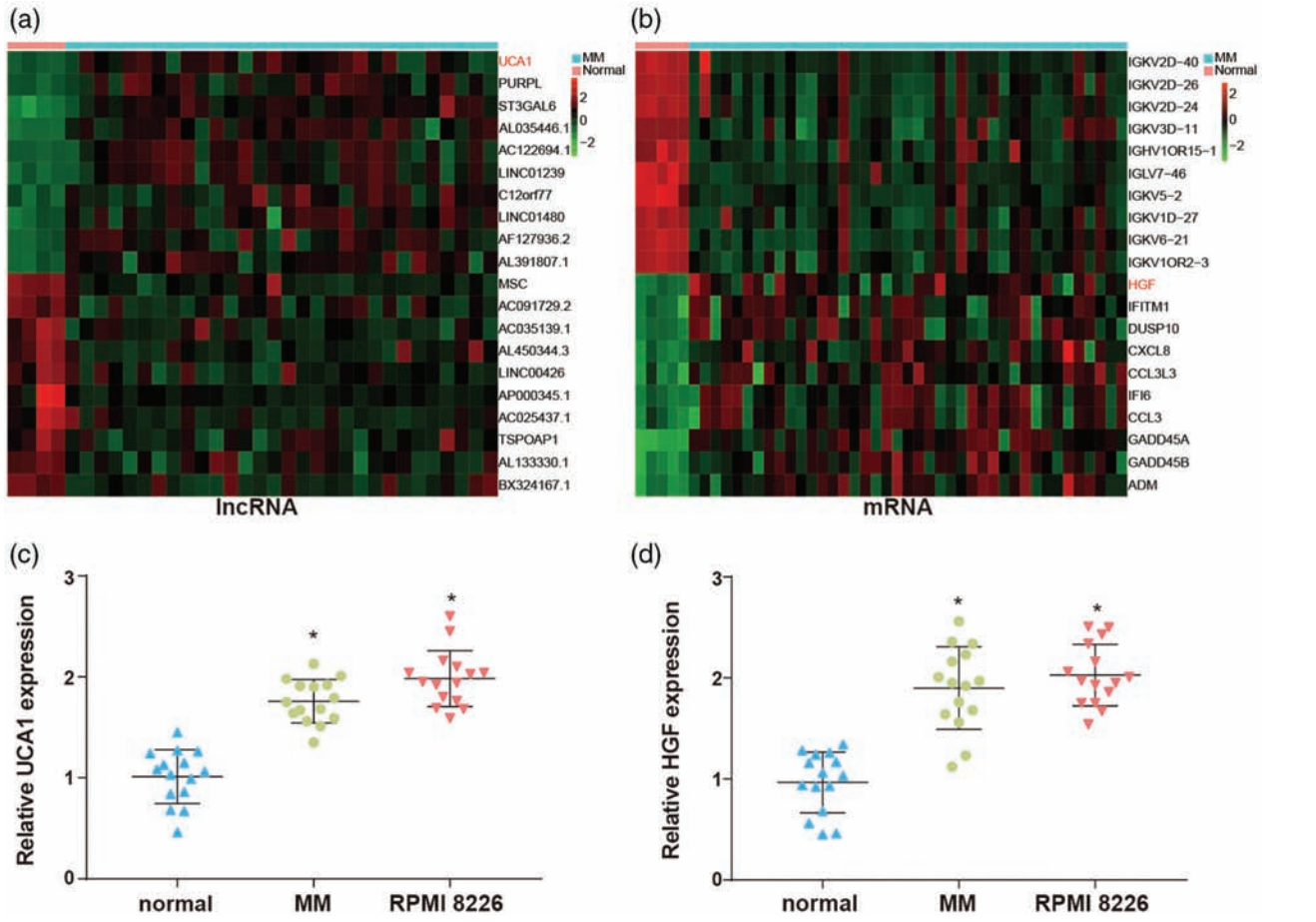


**Fig. 1** LncRNA UCA1 and *HGF* were upregulated in MM. (A) The lncRNA expression data were downloaded from GSE109116. Through differential expression analysis, lncRNA UCA1 was found to be upregulated in MM. (B) The mRNA expression data were downloaded from GSE47552. Through differential expression analysis, *HGF* was found to be upregulated in MM. (C, D) Fifteen pairs of clinical samples were obtained from healthy donors and MM patients. The expression measurements of lncRNA UCA1 and HGF in the MM cell line RPMI 8226 were repeated 15 times using qRT-PCR. LncRNA UCA1 and HGF were both upregulated in MM patient samples and cells. $^{*}p < 0.05$, compared with the normal group. HGF, hepatocyte growth factor; lncRNA, long noncoding RNAs; MM, multiple myeloma; UCA1, urothelial carcinoma associated 1.

### 3.3. Downregulation of lncRNA UCA1 decreased proliferation and facilitated apoptosis in MM cells

The utility of sh-UCA1 was confirmed in qRT-PCR assays (Fig. 3A). Under the downregulation of lncRNA UCA1, miR-1271-5p was significantly increased (Fig. 3B). Furthermore, the expression of *HGF* mRNA and HGF protein was suppressed when lncRNA UCA1 was downregulated (Fig. 3C–E). Cell proliferation and apoptosis detection assays were also conducted. The CCK-8 assay indicated that knocking down lncRNA UCA1 reduced proliferation and induced apoptosis in RPMI 8226 cells (Fig. 3F, G).

### 3.4. Upregulation of miR-1271-5p inhibited proliferation and facilitated apoptosis in MM cells

miR-1271-5p mimics were transfected into RPMI 8226 cells, and miR-1271-5p upregulation was confirmed using an qRT-PCR assay (Fig. 4A). The miR-1271-5p mimics had no influence on the expression of lncRNA UCA1 (Fig. 4B). However, they could inhibit the expression of HGF at both the mRNA and protein levels compared with the NC group (Fig. 4C–E). LncRNA UCA1 upregulation significantly reversed the changes induced by miR-1271-5p mimics (Fig. 4A–E). The reverse effect appeared in proliferation and apoptosis analyses as well. The miR-1271-5p mimics obviously suppressed cell proliferation and facilitated cell apoptosis in RPMI 8226 cells, and these alterations were restored by lncRNA UCA1 upregulation (Fig. 4F, G).

### 3.5. Downregulation of *HGF* repressed proliferation and facilitated apoptosis in MM cells

The effects of *HGF* on RPMI 8226 cell function were evaluated via *HGF* downregulation or lncRNA UCA1 overexpression. The expression of lncRNA UCA1 and miR-1271-5p was not affected by the downregulation of *HGF* (Fig. 5A, B). The expression levels of *HGF* mRNA and HGF protein were successfully decreased in the si-HGF group (Fig. 5C–E). However, HGF expression was not significantly different from that in the NC group when RPMI 8226 cells were cotreated with lncRNA UCA1 upregulation and HGF downregulation (Fig. 5C–E). In the CCK-8 assay, the results showed that proliferation in the si-HGF group was inhibited, and proliferation in the HGF downregulation and lncRNA UCA1 upregulation cotreatment group was not different from that in the NC group (Fig. 5F). Furthermore, apoptosis was increased in the si-HGF group, but the increase in apoptosis was then suppressed by lncRNA UCA1 overexpression (Fig. 5G, H).

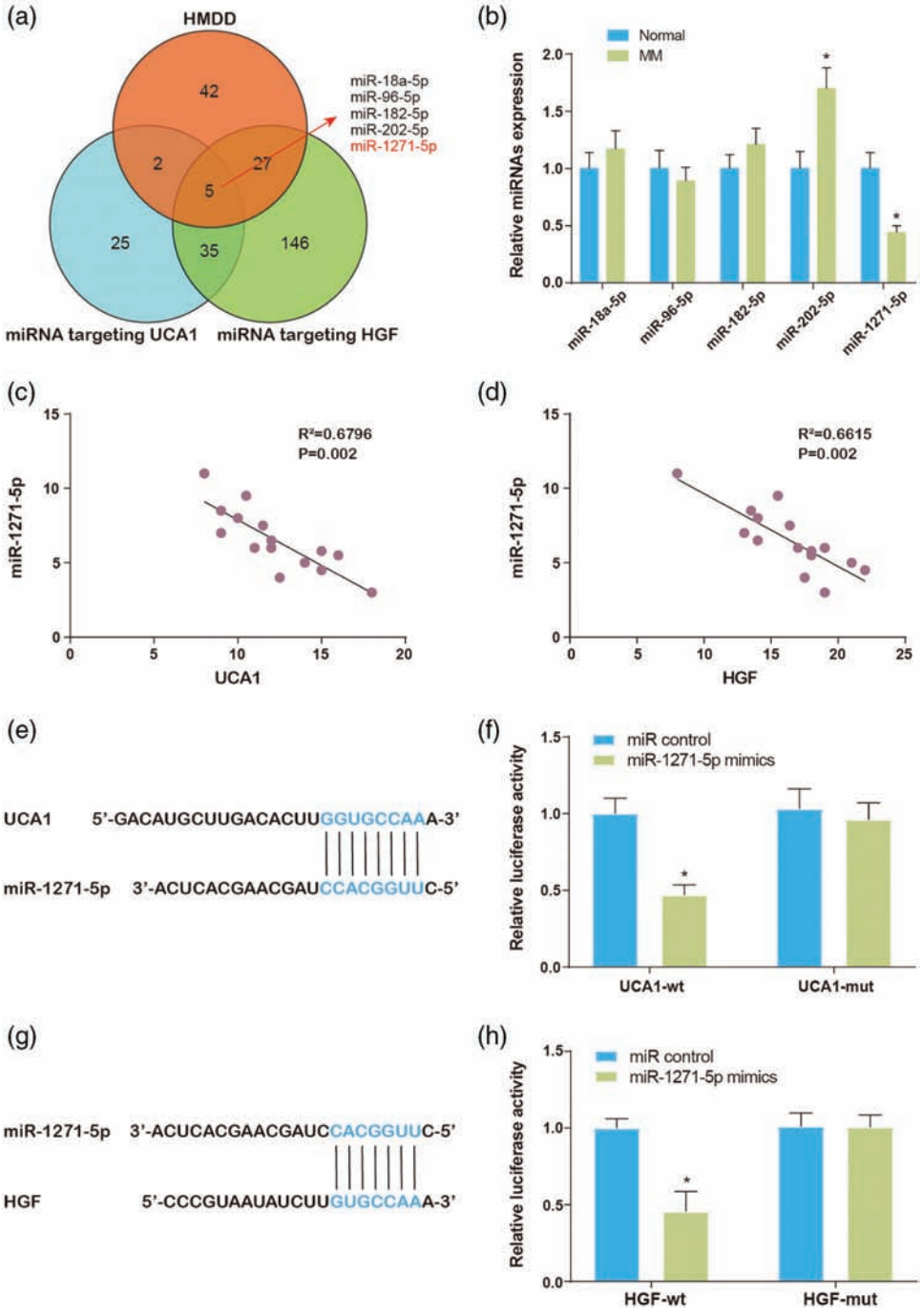


**Fig. 2** miR-1271-5p targeted both lncRNA UCA1 and *HGF*. (A) Venn diagram revealed overlapping miRNAs that were predicted targets of lncRNA UCA1 and *HGF* and were simultaneously reported in the HMDD v3.0 database. (B) The overlap in miRNA expression was evaluated by qRT-PCR, and only miR-1271-5p was significantly downregulated in MM patient samples. *$p < 0.05$, compared with the normal group. (C, D) The negative correlations between lncRNA UCA1 or *HGF* and miR-1271-5p were discovered by Spearman correlation analysis. (E) The predicted binding sites of lncRNA UCA1 and miR-1271-5p are shown. (F) Luciferase reporter assays confirmed that lncRNA UCA1 could target miR-1271-5p. *$p < 0.05$. (G) The predicted binding sites of miR-1271-5p and *HGF* are shown. (H) Luciferase reporter assays verified that miR-1271-5p was targeted by *HGF*. *$p < 0.05$. HGF, hepatocyte growth factor; lncRNA, long noncoding RNAs; MM, multiple myeloma; UCA1, urothelial carcinoma associated 1.

### 3.6. LncRNA UCA1 promoted tumor growth of MM via regulation of miR-1271-5p and HGF in vivo

Based on the results of in vitro experiments, an animal experiment was conducted to verify the previous findings. RPMI 8226 cells with lncRNA UCA1 promotion (UCA1 group) or inhibition (sh-UCA1 group) were subcutaneously injected into mice. The volume and weight of tumors were then estimated each week, and tumors were isolated on the 28th day. Tumor growth was significantly inhibited in the sh-UCA1 group and was markedly enhanced in the UCA1 group compared with the blank group (Fig. 6A–C), which indicated that lncRNA UCA1 could promote MM tumor generation in vivo. Additionally, the expression levels of lncRNA UCA1, miR-1271-5p, and *HGF* were estimated. LncRNA UCA1 was suppressed in the sh-UCA1 group and induced in the UCA1

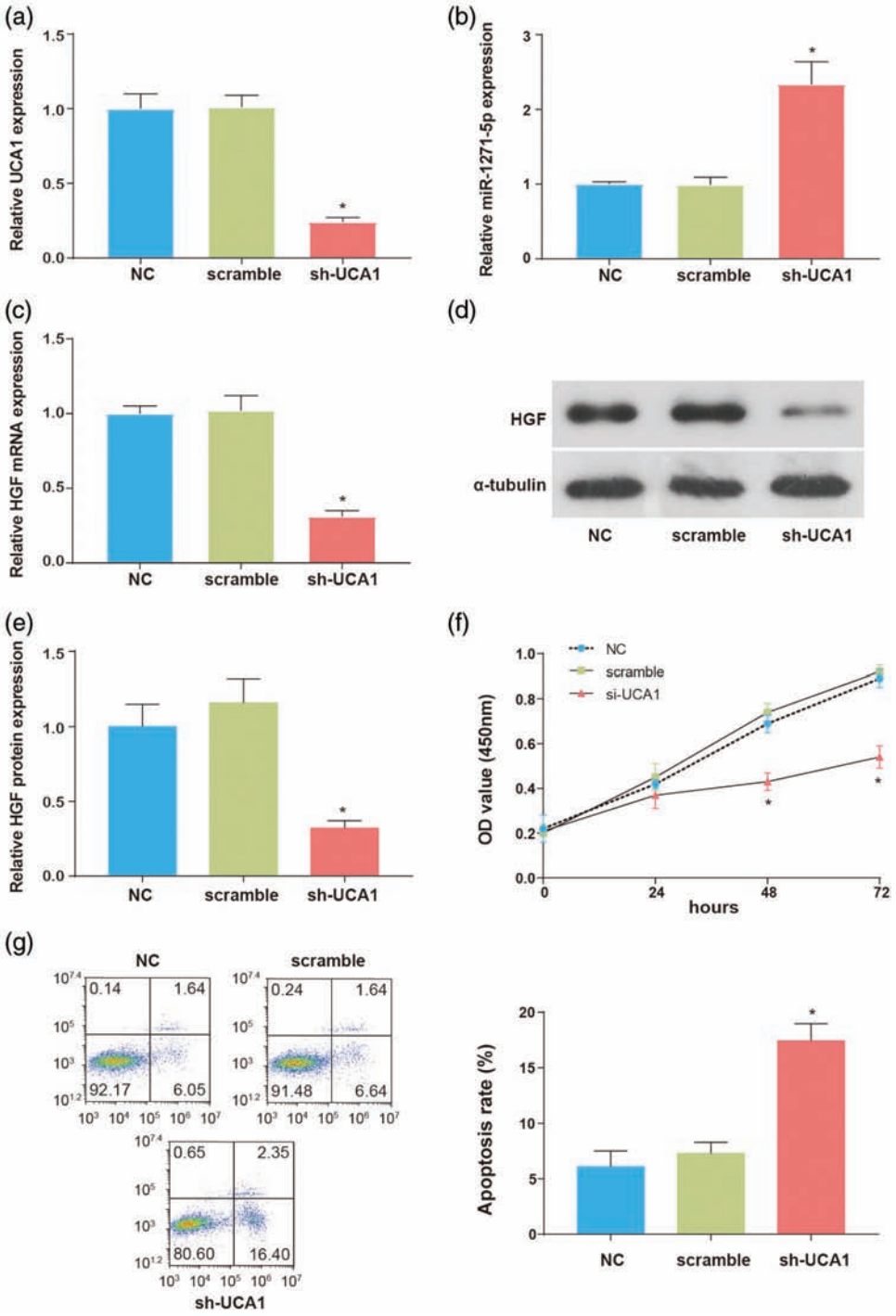


**Fig. 3** The downregulation of lncRNA UCA1 reduced proliferation and induced apoptosis in MM. (A) The expression of lncRNA UCA1 detected by qRT-PCR was downregulated in the sh-UCA1 group as expected. (B) miR-1271-5p detected by qRT-PCR was significantly upregulated due to the suppression of lncRNA UCA1. (C–E) *HGF* mRNA and HGF protein expression detected by qRT-PCR and western blotting, respectively, was significantly inhibited in the sh-UCA1 group. (F) CCK-8 assays revealed that the proliferation of RPMI 8226 cells was significantly decreased when lncRNA UCA1 was inhibited. (G) Flow cytometry assays demonstrated that cell apoptosis was significantly increased in the sh-UCA1 group. *$p < 0.05$, compared with the NC group. CCK-8, cell counting kit-8; HGF, hepatocyte growth factor; lncRNA, long noncoding RNAs; MM, multiple myeloma; UCA1, urothelial carcinoma associated 1.

group (Fig. 6D). miR-1271-5p was upregulated, and HGF was downregulated in the sh-UCA1 group. In addition, the expression of miR-1271-5p and *HGF* in the UCA1 group was opposite to that in the sh-UCA1 group (Fig. 6E–H). These findings indicated that lncRNA UCA1 could enhance the tumor growth of MM via regulation of miR-1271-5p and *HGF* in vivo.

## 4. DISCUSSION

Our investigations suggested that lncRNA UCA1 and *HGF* could enhance cell proliferation and reduce cell apoptosis in MM both in vitro and in vivo, while miR-1271-5p produced the opposite results. The effects of lncRNA UCA1, miR-1271-5p, and *HGF* on MM cells and the targeted combination of lncRNA

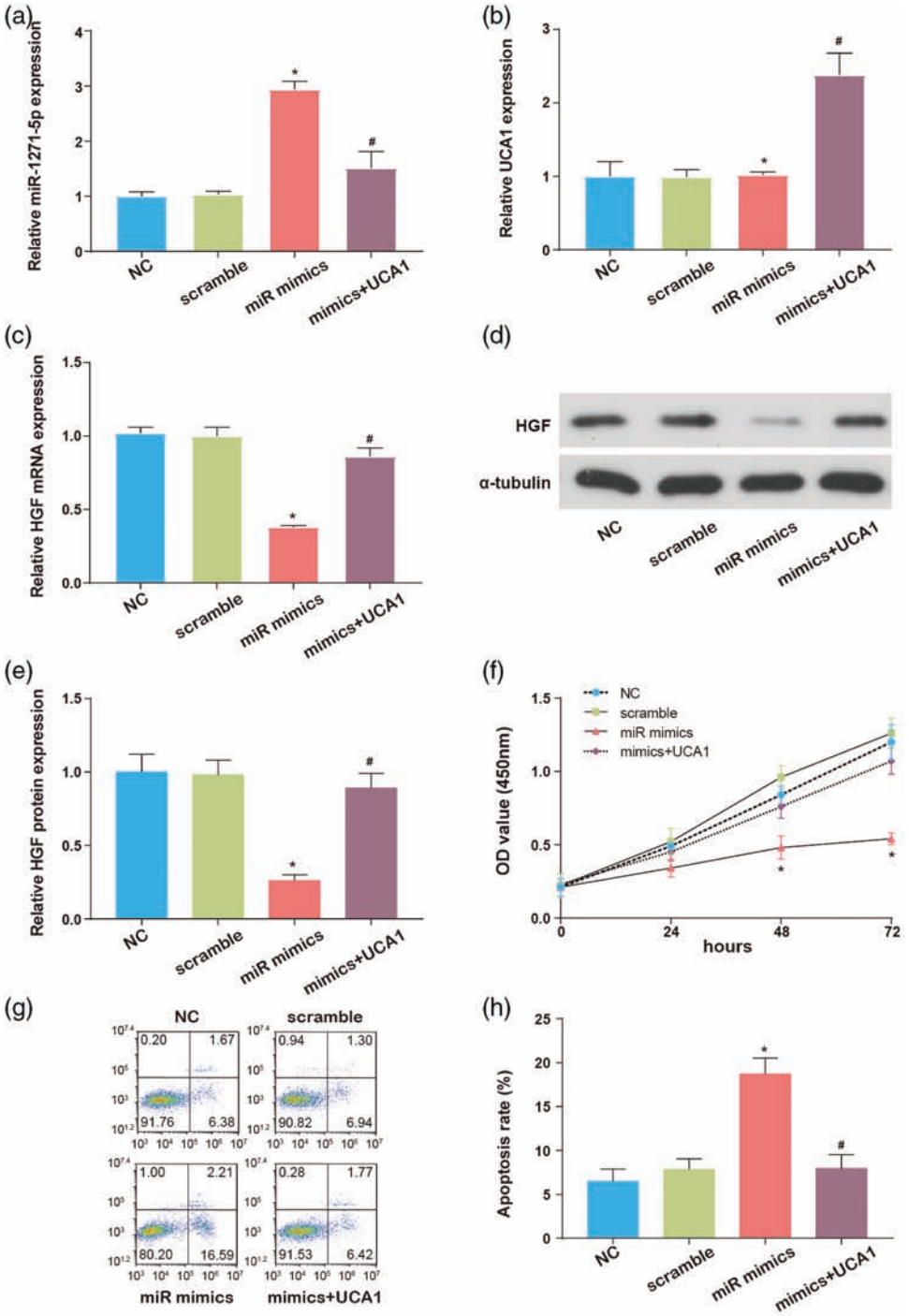


**Fig. 4** Upregulation of miR-1271-5p inhibited proliferation and promoted apoptosis, and lncRNA UCA1 reversed the alterations caused by miR-1271-5p in MM cells. (A) miR-1271-5p expression detected by qRT-PCR was increased by miR-1271-5p mimics as expected, but the increase was repressed by lncRNA UCA1 upregulation. (B) LncRNA UCA1 expression was detected in treated RPMI 8226 cells using qRT-PCR. (C–E) *HGF* mRNA and HGF protein expression detected by qRT-PCR and western blotting, respectively, was significantly suppressed in the miR-1271-5p mimic group, whereas the suppression was reversed when lncRNA UCA1 was upregulated. (F) CCK-8 assays revealed that the proliferation of RPMI 8226 cells was suppressed in the miR-1271-5p mimic group and that suppression was restored when lncRNA UCA1 was upregulated. (G) Flow cytometry assays demonstrated that cell apoptosis was significantly increased in the miR-1271-5p mimic group, and the increase was restored by lncRNA UCA1 upregulation. $^{*}p < 0.05$, compared with the NC group. $^{\#}p < 0.05$, compared with the miR mimic group. HGF, hepatocyte growth factor; lncRNA, long noncoding RNAs; MM, multiple myeloma; UCA1, urothelial carcinoma associated 1.

UCA1/*HGF* and miR-1271-5p were confirmed. Under simultaneous lncRNA UCA1 downregulation and miR-1271-5p upregulation, we found that the influence of miR-1271-5p was abolished. These results indicated that the UCA1/miR-1271-5p/*HGF* axis might regulate MM development.

LncRNAs can function as sponges of miRNAs to modulate translation indirectly. In addition, dysregulation of lncRNAs frequently occurs in various cancers, and this dysregulation has been proven to influence many aspects of cancer development. LncRNA UCA1 was selected as the research focus among

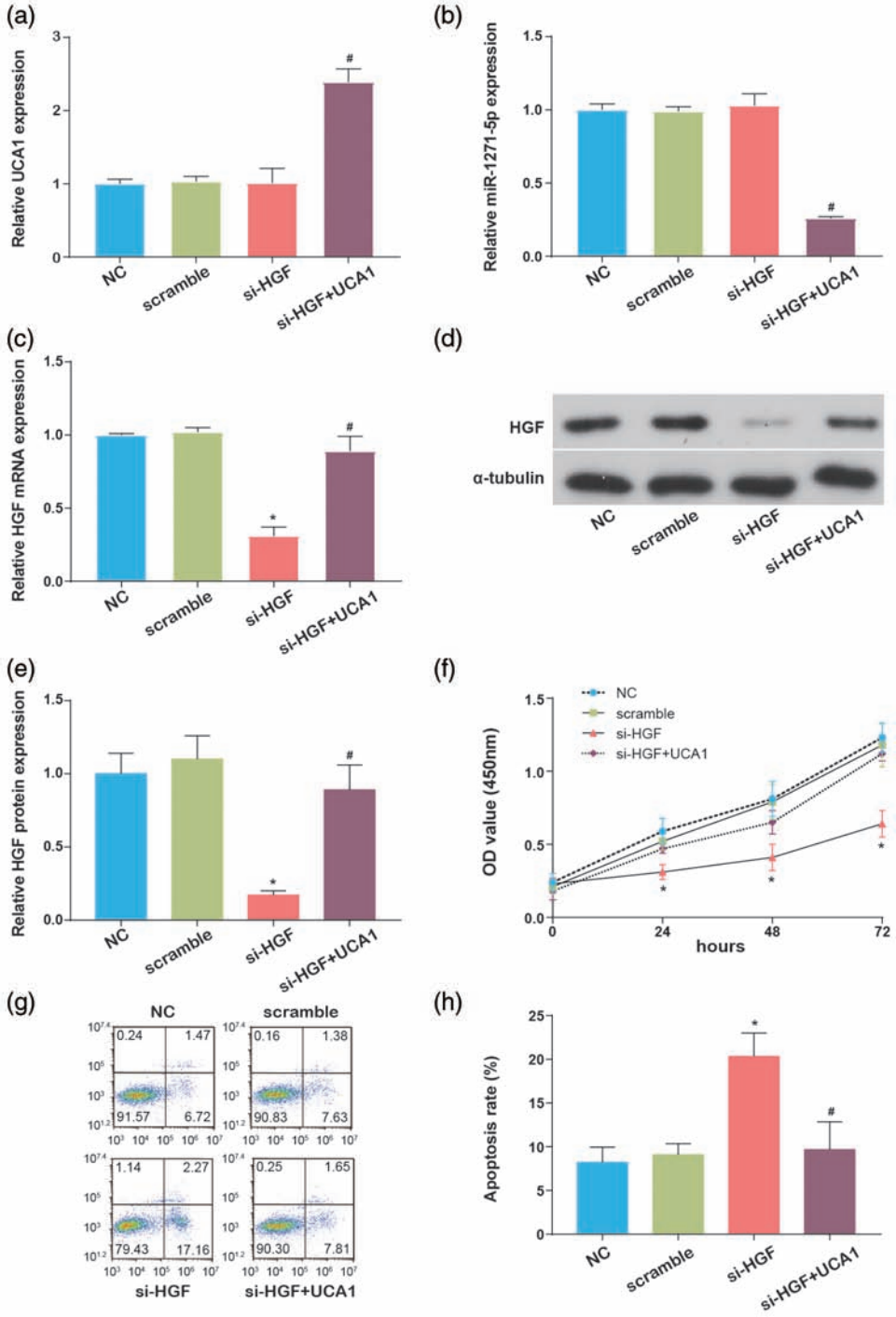


**Fig. 5** Downregulation of *HGF* repressed proliferation and enhanced apoptosis in MM cells. (A) Expression of lncRNA UCA1 was detected by qRT-PCR. LncRNA UCA1 expression was not affected by si-HGF. (B) The expression of miR-1271-5p detected by qRT-PCR was not affected by si-HGF, but its expression was decreased in the si-HGF plus UCA1 group. (C–E) *HGF* mRNA and HGF protein detected by qRT-PCR and western blotting, respectively, was notably inhibited in the si-HGF group, and the inhibition was reversed by lncRNA UCA1 upregulation. (F) CCK-8 assays showed that si-HGF markedly repressed proliferation and that the repression was alleviated by lncRNA UCA1. (G, H) Flow cytometry assays indicated that cell apoptosis was increased in the si-HGF group, and the increase was eased by lncRNA UCA1 upregulation. $^{*}p < 0.05$, compared with the NC group. $^{\#}p < 0.05$, compared with the si-HGF group. HGF, hepatocyte growth factor; lncRNA, long noncoding RNAs; MM, multiple myeloma; UCA1, urothelial carcinoma associated 1.

dysregulated lncRNAs in the current study and has been previously reported in other cancers. In bladder cancer, lncRNA UCA1 increases invasion and migration through regulating the miR-145/ZEB1/2/FSCN1 pathway,[32] promotes glycolysis through the mTOR-STAT3/miR-143-HK2 cascade,[33] and enhances cisplatin/gemcitabine resistance via activating the transcription factor *CREB*, which promotes miR-196a-5p expression through binding with its promoter.[34] The enhancement of drug resistance caused by lncRNA UCA1 and its target miRNAs occurs not only in bladder cancer but also in oral squamous cell carcinoma

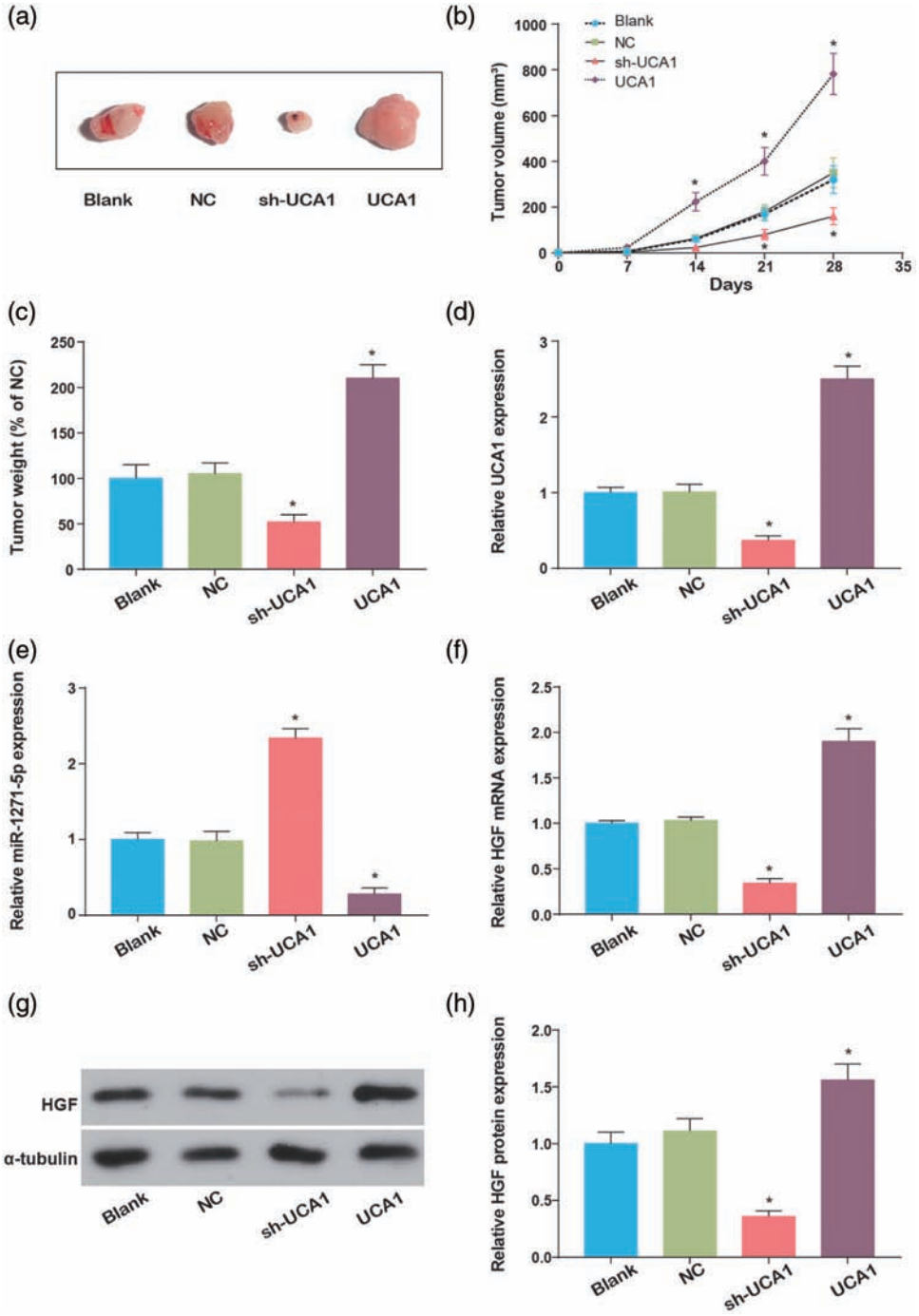


**Fig. 6** LncRNA UCA1 promoted tumor growth of MM via regulation of miR-1271-5p and HGF in vivo. (A, B) Images of in vivo xenograft tumors and their growth curves show that lncRNA UCA1 enhanced tumor growth. (C) The isolated tumors were weighed. (D) Expression levels of lncRNA UCA1 were detected by qRT-PCR. (E) miR-1271-5p detected by qRT-PCR was suppressed in the lncRNA UCA1 group and promoted in the sh-UCA1 group. (F) *HGF* mRNA detected by qRT-PCR was enhanced in the lncRNA UCA1 group and inhibited in the sh-UCA1 group. (G, H) HGF protein detected by western blotting was upregulated in the lncRNA UCA1 group and downregulated in the sh-UCA1 group. $^{*}p < 0.05$, compared with the blank group. HGF, hepatocyte growth factor; lncRNA, long noncoding RNAs; MM, multiple myeloma; UCA1, urothelial carcinoma associated 1.

(OSCC),[35] gastric cancer (GC),[36] and colorectal cancer (CRC).[37] LncRNA UCA1 can increase cell proliferation and promote cancer progression in several cancers as well. For instance, lncRNA UCA1 functions by inhibiting miR-204-5p in CRC[37] and by inhibiting miR-216b and activating the FGFR1/ERK signaling pathway in hepatocellular carcinoma (HCC).[38] Thus, lncRNA UCA1 acts as an oncogene in a variety of cancers and could drive cancer development by sponging different miRNAs and activating different signaling pathways even within the same type of cancer. Zhang et al indicated that lncRNA UCA1 was upregulated in 60 MM patient samples and in 4 MM cell lines.[39] Our research led to similar conclusions to the study of Zhang et al and showed that lncRNA UCA1 sponged miR-1271-5p and enhanced the development of MM in vitro and in vivo.

miR-1271-5p has been found to function as a suppressor in most cancers examined to date, such as GC,[40] CRC,[41,42] PCa,[43,44] HCC,[45] pancreatic adenocarcinoma,[46] and OSCC.[47] Different downstream molecules of miR-1271-5p function differently. miR-1271-5p suppresses the expression of *Capn4*[41] or the metadherin/Wnt signal pathway[42] to inhibit the proliferation and invasion in CRC. By inhibiting *DIXDC1*[48] or *ERG*[43] in prostate cancer, miR-1271-5p is able to suppress proliferation and invasion. However, in nonsmall-cell lung cancer, miR-1271-5p acts as a promoter. The increase in proliferation and invasion is facilitated via miR-1271-5p inhibition of *HOXA5*.[49] A similar situation is discovered in breast cancer. Yu et al found that miR-1271-5p can enhance letrozole resistance in breast cancer by suppressing *Erα*.[50] Regarding the role of miR-1271-5p in MM, our results showed that it could inhibit *HGF* to further decrease the proliferation of MM cells. In the investigation of Xu et al, miR-1271-5p reduces the proliferation of MM cells by suppressing the smoothened-mediated hedgehog signaling pathway.[51] In brief, miR-1271-5p is similar to lncRNA UCA1 in the multiple targets or signaling pathways through which it affects the progression of cancers. Unlike lncRNA UCA1, miR-1271-5p exhibits different functions in different cancers. miR-1271-5p presents distinct effects in some identical cancers, and these effects differ in different studies. As mentioned previously, miR-1271-5p was shown to act as an accelerant in breast cancer,[50] but according to the study of Liang et al, miR-1271-5p is suppressed by circ-ABCBIO, and the effect of miR-1271-5p on the proliferation and progression of breast cancer is negative.[52] In our study, miR-1271-5p was shown to act as a repressor of the proliferation and a promoter of apoptosis in MM.

Hepatocyte growth factor was first discovered as a mitogenic protein in rat hepatocytes in 1984.[53] Soon thereafter, researchers found that *HGF* plays a key role in regulating liver growth and regeneration. Recent studies have indicated that *HGF* is likely to enhance proliferation and migration. HGF not only affects normal cells but also functions in cancer cells. In NSCLC, *HGF* can increase gefitinib resistance,[54] and the proliferation of renal carcinoma can be promoted through the *HGF*/c-Met pathway.[55] The study of NSCLC indicated that *HGF* could induce epithelial-mesenchymal transition (EMT) and angiogenesis, but the effect was inhibited by miR-206.[56] *HGF* induces migration in GC as well; however, this effect can be suppressed by miR-16,[57] and the growth of GC mediated by *HGF* is hampered by miR-26a/b.[58] These reports suggested that *HGF* mostly increases cell proliferation and might induce EMT, which causes cancer to become invasive and mediates angiogenesis. In our investigation, *HGF* enhanced the proliferation of MM cells and reduced apoptosis, which might be in accordance with the finding of Kosai et al in 1998 that *HGF* suppressed the apoptotic death of hepatocytes.[59] Combined with previous reports that the effect of *HGF* can be inhibited by its target miRNAs,[54,56,58,60] our investigation indicated that the 3′-UTR of *HGF* is targeted by miR-1271-5p to function in MM.

In conclusion, our study revealed that lncRNA UCA1 and *HGF* could enhance the proliferation and reduce the apoptosis of MM cells. In contrast, miR-1271-5p had adverse impacts on MM cells. The effect of miR-1271-5p was directly regulated by lncRNA UCA1 due to the target relationship, and there was a negative correlation between lncRNA UCA1 and miR-1271-5p expression. LncRNA UCA1 promoted tumor growth of MM via regulation of miR-1271-5p and HGF in vivo. The lncRNA UCA1/miR-1271-5p/*HGF* axis could be a novel potential therapeutic target for MM patients.

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