

Long noncoding RNA UCA1 regulates proliferation and apoptosis in multiple myeloma by targeting miR-331-3p/IL6R axis for the activation of JAK2/STAT3 pathway

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Abstract. – **OBJECTIVE:** We attempted to clarify the regulatory mechanism of UCA1/miR-331-3p/IL6R on cell progression in multiple myeloma (MM).

PATIENTS AND METHODS: The expression of UCA1, miR-331-3p, and IL6R in tumor tissues and cells was measured by qRT-PCR. Cell Counting Kit-8 (CCK-8) was conducted to detect cell proliferation, and flow cytometry assay was applied to examine cell apoptosis. Protein expression of L6R, p-JAK2, p-STAT3, c-Myc, CyclinD1, Bcl-2, and Bax was detected by Western blot assay. The interaction among miR-331-3p, UCA1, and IL6R was determined by Luciferase reporter system. Murine xenograft assay was performed to confirm the biological function of UCA1 *in vivo*.

RESULTS: The expression of UCA1 and IL6R was up-regulated, while miR-331-3p was down-regulated in MM tumors and cell lines compared with normal tissues and cells. By calculation, miR-331-3p was correlated with UCA1 or IL6R inversely. In addition, UCA1 knockdown suppressed cell proliferation and promoted apoptosis *in vitro* and *in vivo*. Luciferase reporter system confirmed the interaction between miR-331-3p and UCA1 or IL6R. More importantly, UCA1 restored miR-331-3p mediated inhibition of proliferation and promotion on apoptosis of MM cells. Consistently, IL6R rescued UCA1 knockdown caused repression on MM cell growth and elevation on apoptosis. Besides, UCA1 facilitated the activation of the JAK2/STAT3 signaling pathway by enhancing IL6R expression via targeting miR-331-3p.

CONCLUSIONS: UCA1 accelerates proliferation and suppresses apoptosis in MM by targeting miR-331-3p/IL6R axis to activate JAK2/STAT3 pathway, providing potential targets for the diagnosis and therapy of MM.

Key Words:

UCA1, miR-331-3p, IL6R, JAK2/STAT3 signaling pathway, MM.

Introduction

Multiple myeloma (MM), ranking as the second common hematologic malignancy, is characterized by the accumulation of monoclonal immunoglobulins secreted by clonal growth of plasma cells in the bone marrow¹. Clinical symptoms of MM are complex and include anemia, hypercalcemia, bone pain, and renal failure². Currently, the traditional diagnosis of MM is the assessment of abnormal paraprotein levels induced by plasma cells in the peripheral blood and urinary examination³. Although the intervention of proteasome-inhibitors such as bortezomib and immunomodulatory drugs has improved the therapeutic outcome, 5-year survival rate is still poor⁴. However, the pathogenesis of MM is still elusive.

Long noncoding RNAs (lncRNAs) refer to a class of highly conserved transcripts with longer than 200 endogenous nucleotides⁵. Despite with limited protein-coding capacity, lncRNAs are involved in a variety of physiological and pathological processes including chromatin remodeling, cell cycle, migration, pluripotency, embryonic development, and apoptosis by modulating RNA transcription and protein translation⁶. Human urothelial carcinoma associated 1 (UCA1), which is comprised of 1442 nucleotides, was initially discovered in bladder cancer and identified as biomarker in multiple cancer types⁷. For instance, UCA1 was clarified to activate Wnt/ β -catenin signaling pathway and enhance viability, migration, and invasion of laryngeal squamous cell carcinoma cells⁸. On the contrary, UCA1 knockdown significantly hindered cell cycle, metabolism, and development in hemangioma by up-regulating miR-200c⁹. However, the exact role of UCA1 in MM progression requires further exploration.

MicroRNAs (miRNAs) are small non-coding RNAs (18-24 nucleotides) that play fundamental roles in the modulation of cell behavior by base-pairing 3'UTR region of the messenger RNA, causing RNA degradation and protein translation suppression¹⁰. Aberrant expression of miRNAs contributes to tumorigenesis, cell cycle, infiltration, inflammation, and metastasis¹¹. Serum miR-331-3p has been validated as diagnostic, prognostic marker, and personalized therapy target in multiple cancers, like hepatic carcinoma, non-small cell lung cancer, and esophageal adenocarcinoma¹². For example, up-regulation of miR-331-3p enhanced hepatitis B virus-associated hepatocellular carcinoma cell proliferation by targeting ING5¹³. Similarly, miR-331-3p was reported to be overexpressed in pancreatic cancer, and the abundance of miR-331-3p facilitated cell growth by regulating ST7L¹⁴. However, the pathological function of miR-331-3p in MM remains elusive.

In this present study, we attempted to clarify the regulatory mechanism of UCA1/miR-331-3p/IL6R on cell progression in MM. Up-regulation of UCA1 and IL6R and down-regulation of miR-331-3p was certified by qRT-PCR. In addition, miR-331-3p was correlated with UCA1 or IL6R negatively. More importantly, UCA1 was found to promote proliferation and suppress apoptosis by the activation of JAK2/STAT3 signaling pathway induced by IL6R overexpression by targeting miR-331-3p.

Patients and Methods

Tissue Samples

A total of 35 MM patients and 20 healthy volunteers were recruited from the Second Hospital of Shanxi Medical University. All the participants signed informed consent, and the protocols were approved by the Ethics Committee of Second Hospital of Shanxi Medical University. Tumor tissues and normal tissues were harvested by surgery and immediately transferred to -80°C refrigerator until use.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Total RNA extraction was conducted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The cDNA for UCA1, miR-331-3p, and IL6R was synthesized by All-in-One™ First-Strand cDNA Synthesis Kit (FulenGen, Guangzhou, China). Then, qRT-PCR was performed using SYBR green (Applied Biosystems, Fos-

ter City, CA, USA) under the standard procedure. The primers for UCA1, miR-331-3p, and IL6R were listed as follows: UCA1, (Forward, 5'-CTCTCCATTGGGTTACCATTC-3'; Reverse, 5'-CTCTCCATTGGGTTACCATTC-3'); miR-331-3p, (Forward, 5'-GCGCCCCTGGG-CCTATC-3'; Reverse, 5'-CGATGACCTATGAATTGACA-3'); IL6R (Forward, 5'-CCCCTCAGCAATGTTGTTTGT-3'; Reverse, 5'-CTCCGGGACTGCTAACTGG-3'); U6, (Forward, 5'-ACCCTGAGAAATACCCTCACAT-3'; Reverse, 5'-GACGACTGAGCCCCTGATG-3').

Cell Transfection

Human MM cell lines U266, NCI-H929, and RPMI-8226 were purchased from Cell Bank of Chinese Academy of Science (Shanghai, China) and cell line LP-1 was purchased from American Type Culture Collection (ATCC; Manassas, VA, USA). All the cell lines and normal plasma cells (nPCs) were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (Gibco, Rockville, MD, USA) supplemented with 10% fetal bovine serum (FBS) and 0.05% penicillin/streptomycin. Small hairpin RNA (shRNA) targeting UCA1 (sh-UCA1-1, sh-UCA1-2), shRNA targeting IL6R (sh-IL6R), shRNA negative control (sh-NC), pcDNA, pcDNA-UCA1 overexpression vector (UCA1), and pcDNA-IL6R overexpression vector (IL6R) were synthesized by Genepharma (Shanghai, China). The miRNA mimic (miR-331-3p), miR-331-3p inhibitor (anti-miR-124-3p), miRNA negative control (miR-NC), and miRNA negative control inhibitor (anti-miR-NC) were purchased from RiboBio (Guangzhou, China). Those plasmids were transfected in NCI-H929 and RPMI-8226 cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

Cell Viability and Apoptosis Assays

Cell viability was analyzed by Cell Counting Kit-8 (CCK-8) assay. Transfected NCI-H929 and RPMI-8226 cells (5000 cells/well) were seeded onto 96-well plates for 24 h, 48 h, and 72 h. Then, 10 μL CCK-8 reagent (Beyotime, Shanghai, China) was added to each well for 2 h, and the optical density (OD) value at 450 nm was measured by a spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Cell apoptosis was evaluated by flow cytometry assay. Briefly, cells were harvested at 48 h post-transfection, co-strained using Annexin V-FITC/PI Apoptosis Detection Kit (Vazyme, Nanjing, China) and counted by BD FACS Canto II flow cytometry (BD Biosciences, Franklin Lakes, NJ, USA).

Luciferase Reporter Assay

Wild type (WT-UCA1, IL6R 3'UTR-WT) and mutant type luciferase vectors (MUT-UCA1, IL6R 3'UTR-MUT) were constructed and co-transfected with miR-331-3p or miR-NC into NCI-H929 and RPMI-8226 cells using Lipofectamine 2000 transfection reagent. Luciferase activities were detected by Dual-Luciferase reporter system.

Murine Xenograft Assay

5-week-old Male Balb/c nude mice were purchased from Vital River Laboratory Animal Technology (Beijing, China). All animal experiments were conducted following the guidelines of the National Animal Care and Ethics Institution. Murine xenograft models were established by subcutaneously injecting with RPMI-8226 cells stably transfected with sh-UCA1-1 or sh-NC. Tumor volume was measured every week till 5 weeks, and tumor weight was measured after 5 weeks.

Statistical Analysis

All the experiments were conducted at least in triplicate, and data were presented as means \pm standard deviation (SD). Statistical analysis was carried out using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 7 (GraphPad Inc., San Diego, CA, USA). The correlation between miR-331-3p and UCA1 or IL6R was analyzed by Pearson's correlation coefficient analysis. p -value less than 0.05 ($p < 0.05$) was considered statistically significant.

Results

Up-Regulation of UCA1 and Down-Regulation of MiR-331-3p in MM

The expression of UCA1 and miR-331-3p in MM tumors and the corresponding normal tissues were evaluated using qRT-PCR. As illustrated in Figure 1A-B, the expression of UCA1 was up-regulated, whereas miR-331-3p was down-regulated in MM tumors compared with normal tissues. Meanwhile, up-regulation of UCA1 expression and down-regulation of miR-331-3p expression were observed in MM cell lines (U266, LP-1, NCI-H929, and RPMI-8226) compared with normal plasma cells (NPCs) (Figure 1C-D). Through the Pearson correlation coefficient analysis, we discovered that UCA1 was correlated with miR-331-3p inversely (Figure 1E). In addition, the association between prognosis of MM patients and

UCA1 or miR-331-3p expression was detected by Kaplan-Meier analysis and log-rank test. The data indicated that low level of UCA1 contributed to relatively high survival rate while high level of UCA1 resulted in low survival rate (Figure 1F). Oppositely, patients with low level of miR-331-3p had relatively poor overall survival rate in comparison with that with high expression (Figure 1G). Collectively, we concluded that UCA1 functions as oncogene while miR-331-3p functions as tumor suppressor in MM.

UCA1 Depletion Inhibits Cell Proliferation and Induces Apoptosis in MM

The regulatory effects of UCA1 on MM cell proliferation and apoptosis were further explored by CCK-8, flow cytometry, and Western blot assay, respectively. Extremely high transfection efficiency was detected in NCI-H929, and RPMI-8226 cells were stably transfected with sh-UCA1-1, sh-UCA1-2, and sh-NC (Figure 2A). The subsequent CCK-8 assay revealed that silencing of UCA1 significantly suppressed the proliferation ability of NCI-H929 and RPMI-8226 cells compared with sh-NC group (Figure 2B-C). Furthermore, the apoptotic rate was enhanced in cells stably transfected with sh-UCA1-1 and sh-UCA1-2 (Figure 2D). In addition, the expression of proliferation-related protein c-Myc, CyclinD1, and anti-apoptosis protein Bcl-2 was decreased dramatically after UCA1 silencing (Figure 2E). Conversely, the expression of apoptosis-related protein Bax was increased in cells transfected with sh-UCA1. These findings implicated that deficiency of UCA1 inhibits cell proliferation and induces apoptosis in MM.

Restoration of UCA1 Promotes Cell Proliferation and Inhibits Apoptosis in MM

Previous studies have demonstrated that lncRNA participates in cell regulation by sponging the target miRNA. Bioinformatics tool StarBase2.0 displayed that miR-331-3p includes the binding sites of UCA1 (Figure 3A). Then, wild (WT-UCA1) or mutant type UCA1 (MUT-UCA1) vector and miR-331-3p or miR-NC were co-transfected in NCI-H929 and RPMI-8226 cells to confirm the prediction. Luciferase activity decreased in both NCI-H929 and RPMI-8226 cells transfected with WT-UCA1 and miR-331-3p compared with that of MUT-UCA1 group (Figure 3B-C). In addition, the sufficiency of UCA1 reduced miR-331-3p expression while deficiency of UCA1 enhanced miR-331-3p expression in NCI-H929 and

RPMI-8226 cells (Figure 3D). Moreover, UCA1 attenuated miR-331-3p mediated inhibition on MM cell proliferation (Figure 3E-F). Besides, the cell apoptosis rate was increased by miR-331-3p and decreased UCA1 (Figure 3G). Meanwhile, UCA1 reversed the suppressive effect induced by miR-331-3p on the expression of proliferation-related protein c-Myc, CyclinD1, and anti-apoptosis protein Bcl-2. However, the expression of apoptosis-related protein Bax showed the opposite results (Figure 3 H-I). All the data indicated that restoration of UCA1 promotes cell proliferation and inhibits apoptosis in MM.

IL6R is a Target of MiR-331-3p

Based on bioinformatics analysis searched by StarBase2.0, miR-331-3p has the potential to bind to 3'UTR of UCA1 (Figure 4A). As illustrated in Figure 4B-C, an evident reduction of luciferase

ase activity was noticed in NCI-H929 and RPMI-8226 cells co-transfected with IL6R 3'UTR-WT and miR-331-3p. However, the luciferase activity remained unchanged in IL6R 3'UTR-MUT co-transfection group. Furthermore, the expression of IL6R protein was repressed by miR-331-3p and boosted by miR-331-3p inhibitor (Figure 4D-E). In addition, the expression of IL6R at mRNA and protein levels was up-regulated in MM tumor tissues in comparison with the corresponding normal tissues (Figure 4F-G). Person correlation coefficient analysis indicated that miR-331-3p was negatively correlated with IL6R (Figure 4H). More importantly, high level of IL6R caused low survival rate whereas low level of IL6R led to high survival rate of MM patients (Figure 4I). Similarly, the expression of IL6R protein was up-regulated in MM cell lines (U266, LP-1, NCI-H929, and RPMI-8226) compared with nPCs (Figure 4J).

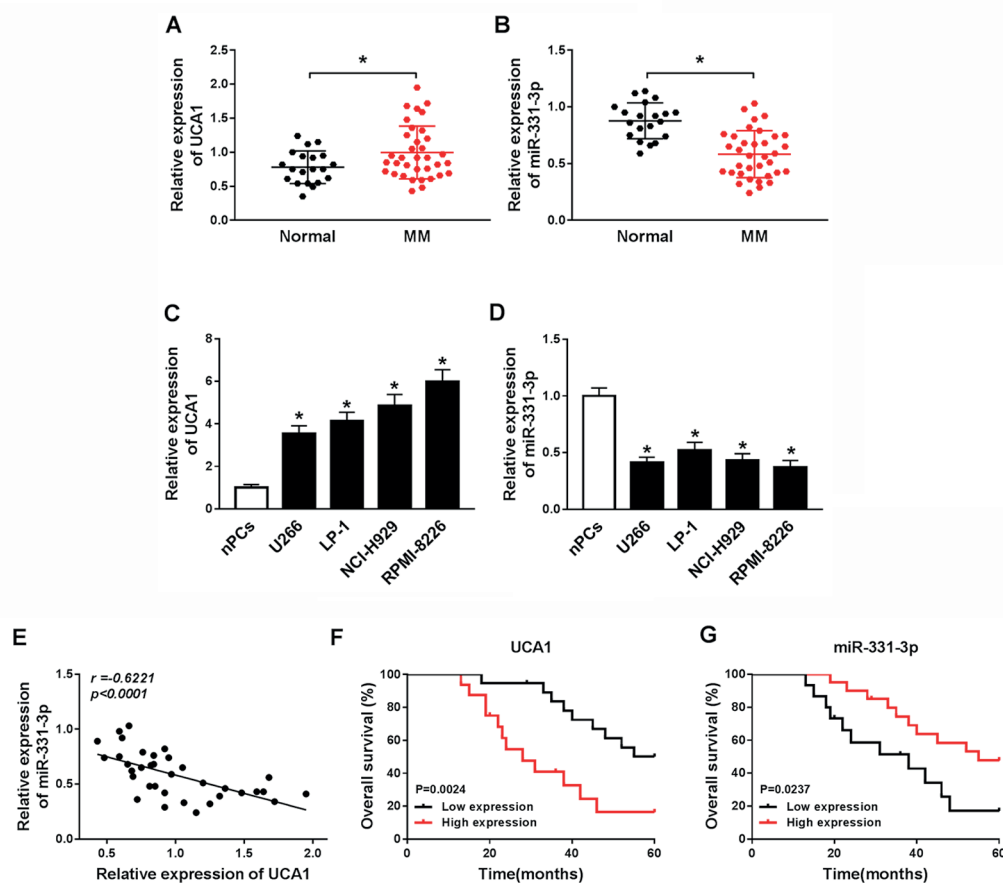


Figure 1. The expression of UCA1 and miR-331-3p in MM tumors and cell lines. **A-B**, Expression of UCA1 (**A**) and miR-331-3p (**B**) in MM tumor tissues compared with the corresponding normal tissues measured by qRT-PCR. **C-D**, Expression of UCA1 (**C**) and miR-331-3p (**D**) in MM cell lines (U266, LP-1, NCI-H929, and RPMI-8226) compared with normal plasma cells (nPCs). **E**, Correlation between UCA1 and miR-331-3p ($R = -0.6221$, $p < 0.0001$). **F-G**, Overall survival rate of patients with different level of UCA1 (**F**) and miR-331-3p (**G**). * $p < 0.05$.

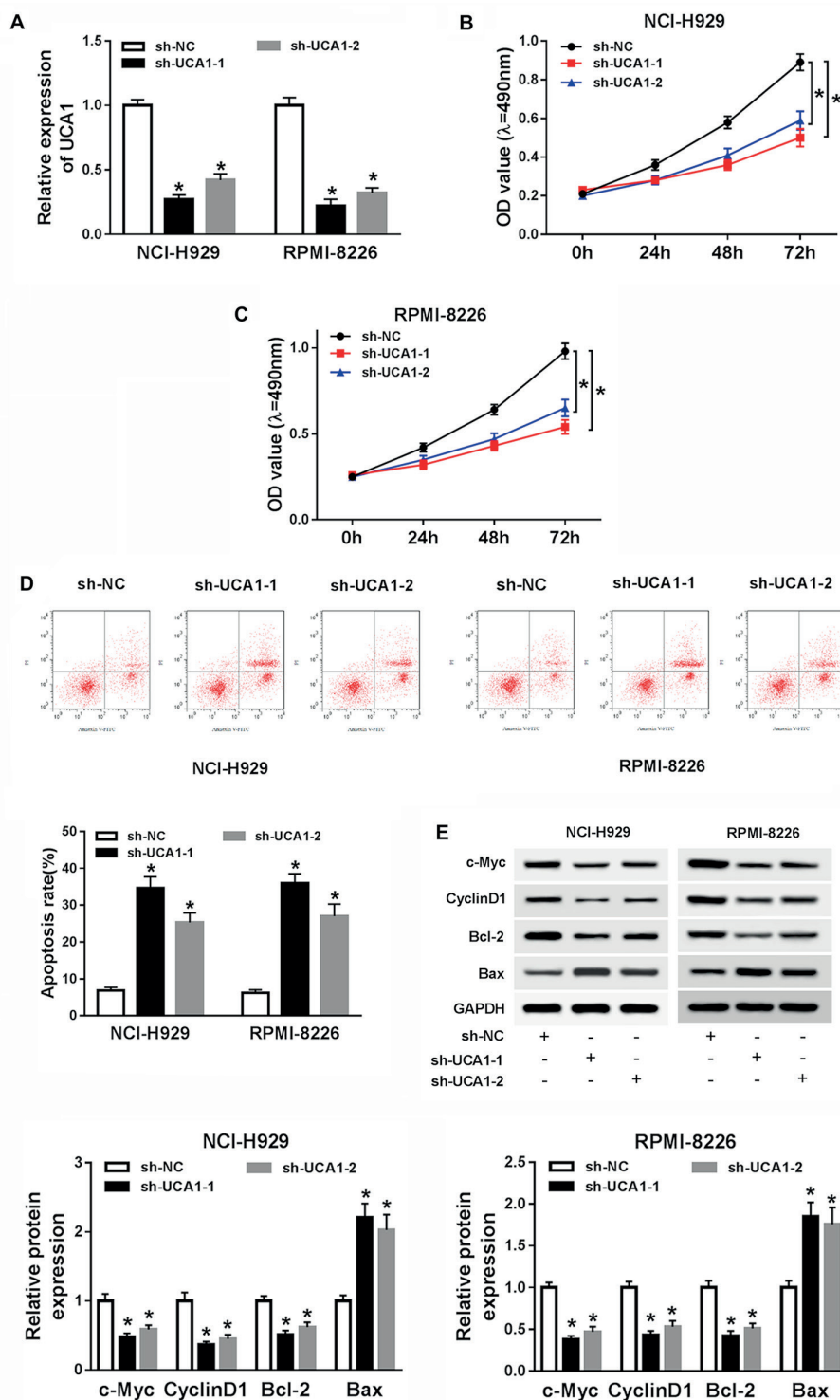


Figure 2. UCA1 knockdown suppressed proliferation and induced apoptosis in MM. NCI-H929 and RPMI-8226 cells were stably transfected with UCA1 (sh-UCA1-1, sh-UCA1-2) or sh-NC. **A**, Expression of UCA1 in transfected cells. **B-C**, Cell viability of NCI-H929 (**B**) and RPMI-8226 cells (**C**) after transfection for 24 h, 48 h and 72 h detected by CCK-8 assay. **D**, Apoptotic rate of NCI-H929 and RPMI-8226 cells at 48 h post-transfection assessed by flow cytometry. **E**, Protein expression of c-Myc, CyclinD1, Bcl-2, and Bax in transfected cells examined by Western blot assay. GAPDH was used as internal reference. * $p < 0.05$.

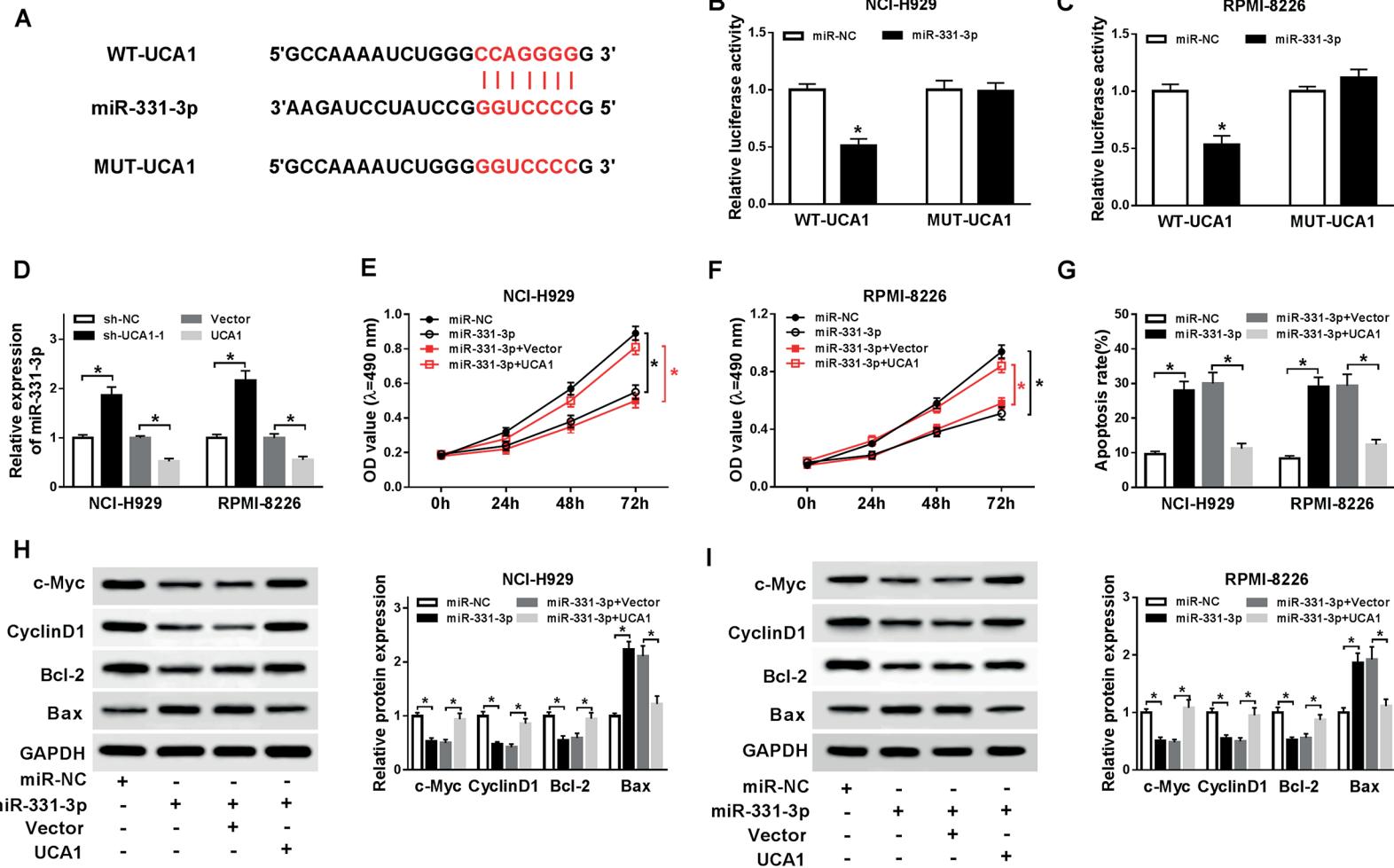


Figure 3. UCA1 attenuated miR-331-3p mediated inhibition on cell proliferation. **A**, The putative binding sites between UCA1 and miR-331-3p. **(B-C)** Luciferase activity of NCI-H929 **(B)** and RPMI-8226 cells **(C)** co-transfected with WT-UCA1 or MUT-UCA1 and miR-331-3p or miR-NC. **D**, Expression of miR-331-3p in NCI-H929 and RPMI-8226 cells stably transfected with UCA1, sh-UCA1-1, Vector and sh-NC. **E-I**, NCI-H929 and RPMI-8226 cells were transfected with miR-NC, miR-331-3p, miR-331-3p+Vector or miR-331-3p+UCA1. **(E-F)** Cell viability of NCI-H929 **(E)** and RPMI-8226 cells **(F)** after transfection for 24 h, 48 h and 72 h. **G**, The apoptotic rate of NCI-H929 and RPMI-8226 cells at 48 h post-transfection. **H-I**, Protein expression of c-Myc, CyclinD1, Bcl-2 and Bax in transfected NCI-H929 **(H)** and RPMI-8226 cells **(I)**. GAPDH was used as internal reference. * $p < 0.05$.

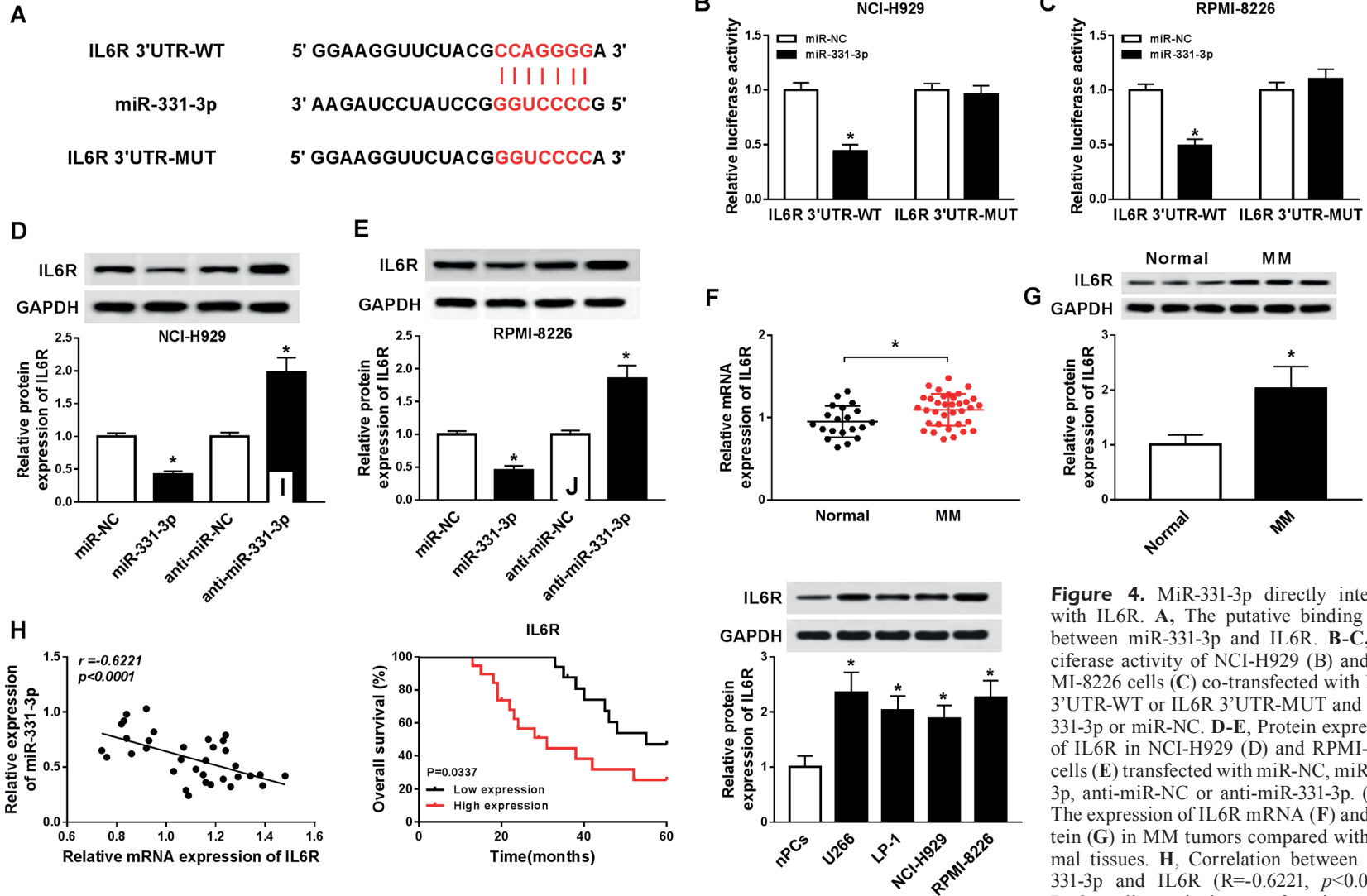


Figure 4. MiR-331-3p directly interacts with IL6R. **A**, The putative binding sites between miR-331-3p and IL6R. **B-C**, Luciferase activity of NCI-H929 (**B**) and RPMI-8226 cells (**C**) co-transfected with IL6R 3'UTR-WT or IL6R 3'UTR-MUT and miR-331-3p or miR-NC. **D-E**, Protein expression of IL6R in NCI-H929 (**D**) and RPMI-8226 cells (**E**) transfected with miR-NC, miR-331-3p, anti-miR-NC or anti-miR-331-3p. (**F-G**) The expression of IL6R mRNA (**F**) and protein (**G**) in MM tumors compared with normal tissues. **H**, Correlation between miR-331-3p and IL6R ($R = -0.6221$, $p < 0.0001$). **I**, Overall survival rate of patients with different level of IL6R. **J**, Expression of IL6R protein in MM cell lines (U266, LP-1, NCI-H929, and RPMI-8226) compared with nPCs. * $p < 0.05$.

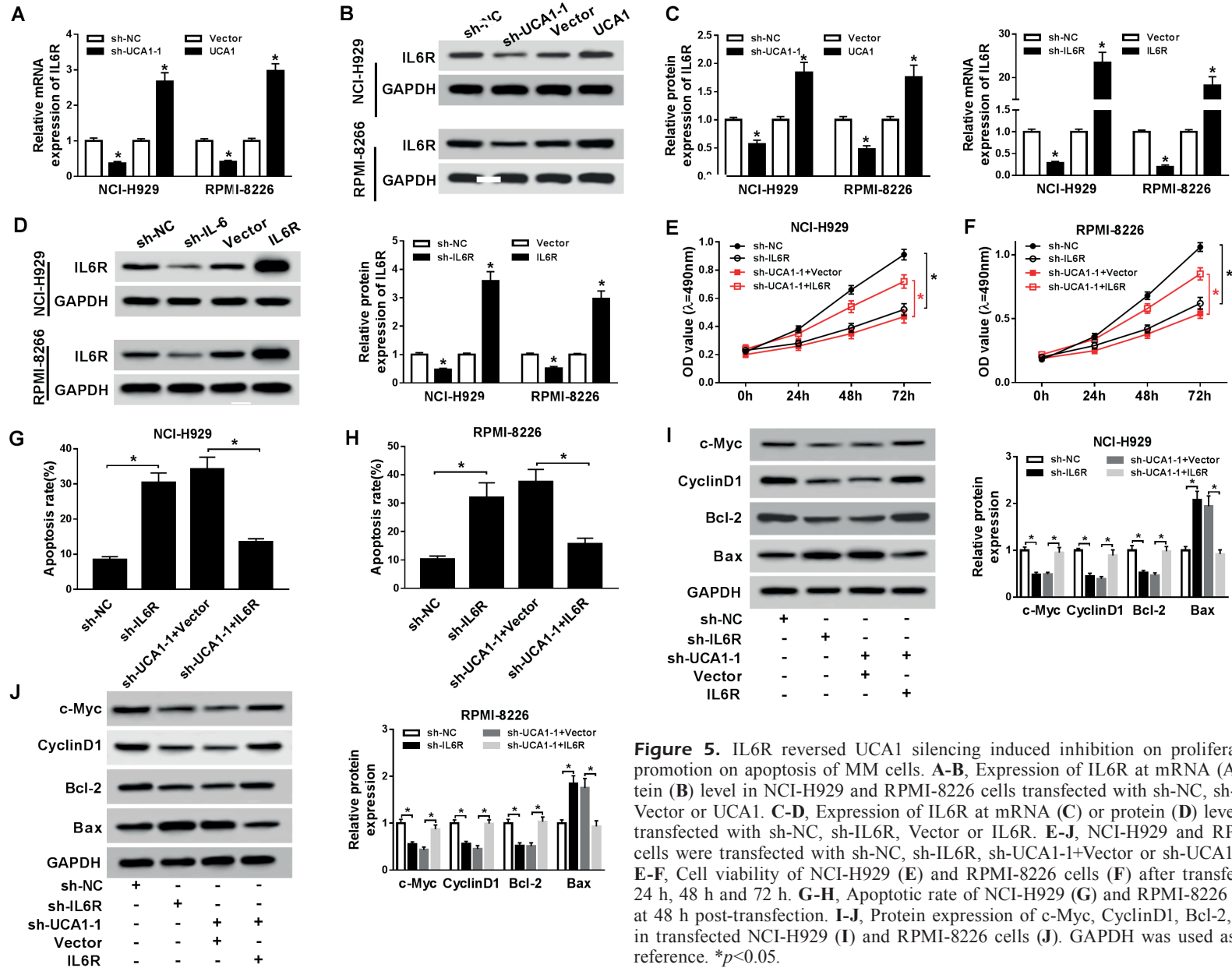


Figure 5. IL6R reversed UCA1 silencing induced inhibition on proliferation and promotion on apoptosis of MM cells. **A-B**, Expression of IL6R at mRNA (**A**) or protein (**B**) level in NCI-H929 and RPMI-8226 cells transfected with sh-NC, sh-UCA1-1, Vector or UCA1. **C-D**, Expression of IL6R at mRNA (**C**) or protein (**D**) level in cells transfected with sh-NC, sh-IL6R, Vector or IL6R. **E-J**, NCI-H929 and RPMI-8226 cells were transfected with sh-NC, sh-IL6R, sh-UCA1-1+Vector or sh-UCA1-1+IL6R. **E-F**, Cell viability of NCI-H929 (**E**) and RPMI-8226 cells (**F**) after transfection for 24 h, 48 h and 72 h. **G-H**, Apoptotic rate of NCI-H929 (**G**) and RPMI-8226 cells (**H**) at 48 h post-transfection. **I-J**, Protein expression of c-Myc, CyclinD1, Bcl-2, and Bax in transfected NCI-H929 (**I**) and RPMI-8226 cells (**J**). GAPDH was used as internal reference. * $p < 0.05$.

Altogether, we concluded that miR-331-3p directly interacts with IL6R.

UCA1 Promotes Proliferation and Suppresses Apoptosis in MM Cells by Regulating IL6R

Subsequently, NCI-H929 and RPMI-8226 cells were transfected with UCA1, IL6R, sh-UCA1-1, sh-IL6R, sh-UCA1-1+Vector, sh-UCA1-1+IL6R, sh-NC, and Vector to explore the regulatory effect of UCA1/IL6R axis. As displayed in Figure 5A-B, UCA1 increased the mRNA and protein expression of IL6R markedly, while UCA1 silencing inhibited the expression of IL6R. Con-

sistently, the expression of IL6R at mRNA and protein levels was reduced distinctly after IL6R silencing, while elevated after IL6R introduction (Figure 5C-D). CCK-8 results clarified that IL6R abrogated the inhibition in cell viability of NCI-H929 and RPMI-8226 cells induced by UCA1 silencing (Figure 5E-F). Flow cytometry assay indicated that silencing of IL6R enhanced apoptosis and abundance of IL6R attenuated reduced apoptosis induced by sh-IL6R in MM cells (Figure 5G-H). Moreover, the expression of proliferation-related protein c-Myc, CyclinD1, and anti-apoptosis protein Bcl-2 was decreased after IL6R silencing, and the decline was weakened

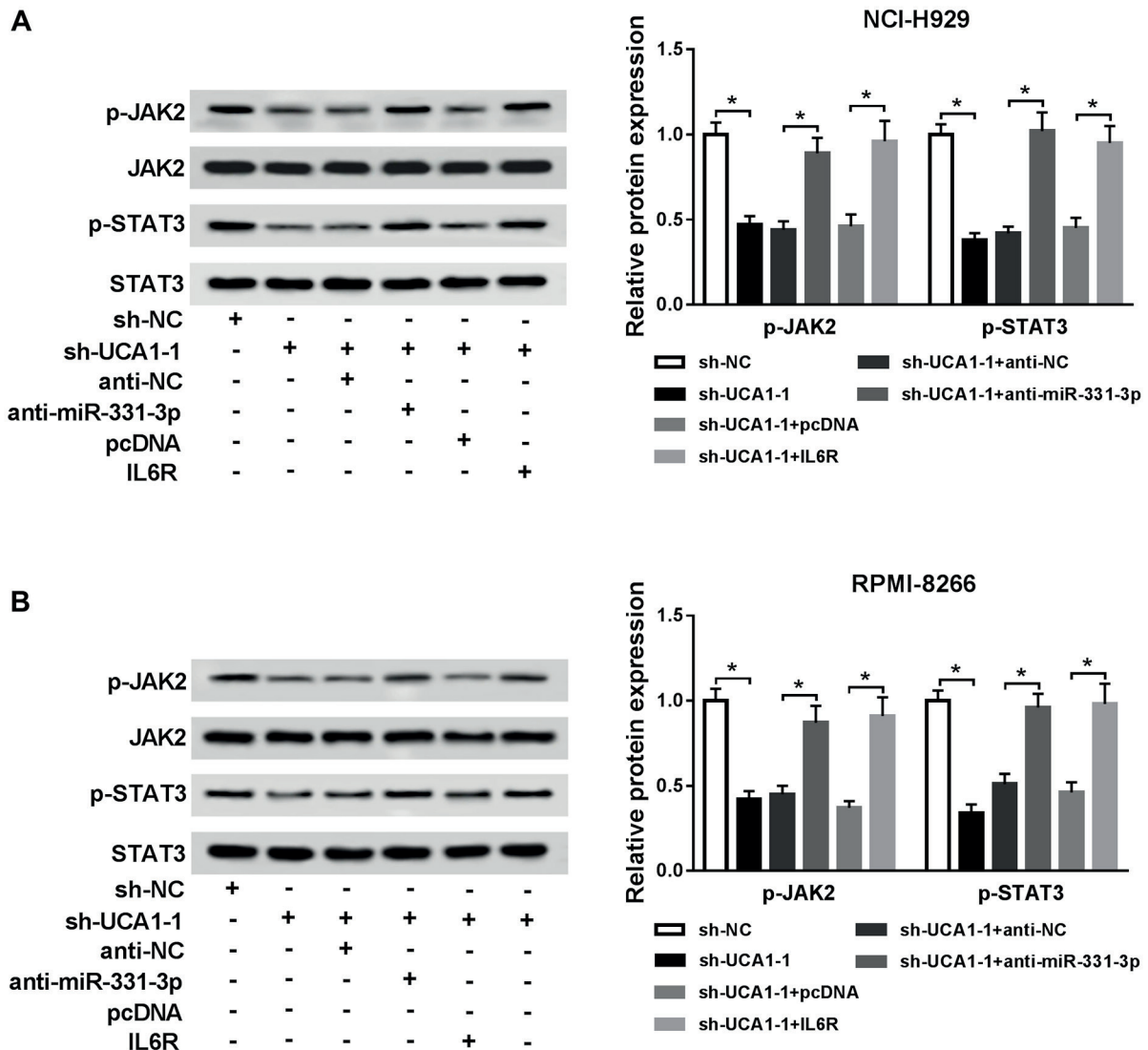


Figure 6. UCA1/miR-331-3p/IL6R axis regulated JAK2/STAT3 signaling pathway. NCI-H929 and RPMI-8226 cells were transfected with sh-NC, sh-UCA1-1, sh-UCA1-1+anti-NC, sh-UCA1-1+anti-miR-331-3p, sh-UCA1-1+pcDNA or sh-UCA1-1+IL6R. **A-B**, Protein expression of p-JAK2, JAK2, p-STAT3, and STAT3 in transfected NCI-H929 (A) and RPMI-8226 cells (B). * $p < 0.05$.

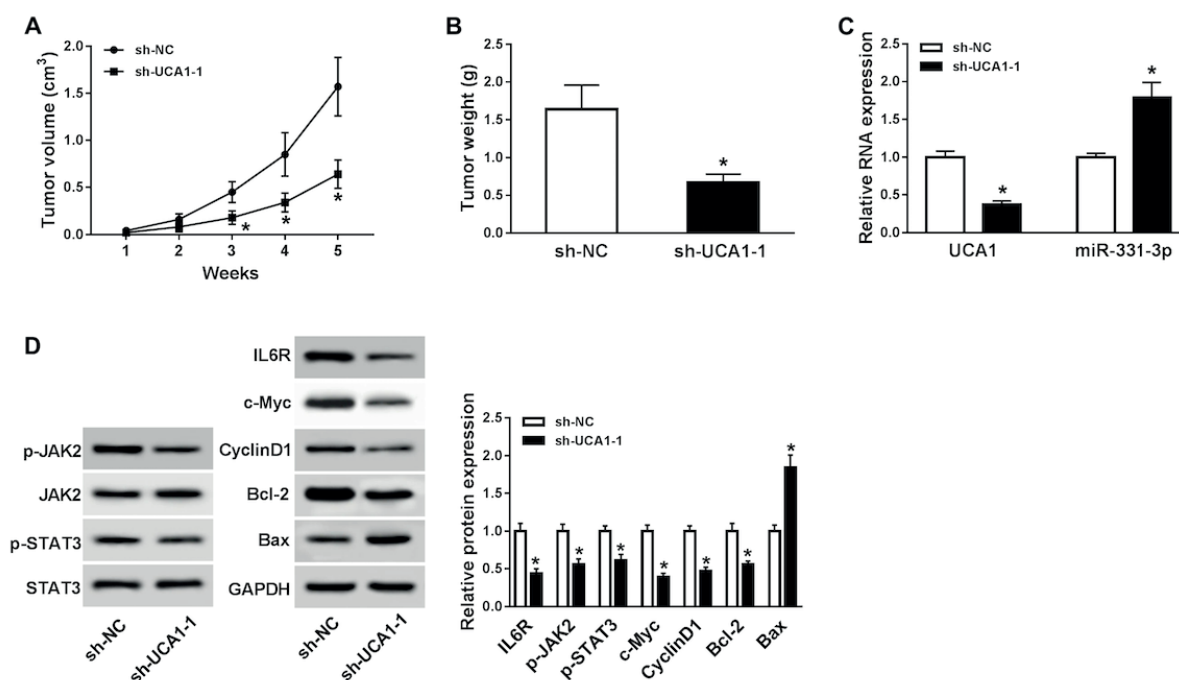


Figure 7. UCA1 silencing suppressed tumor growth *in vivo*. **A**, Tumor volume of RPMI-8266 xenograft mice was measured every week. **B**, Tumor weight was measured when RPMI-8266 xenograft mice were sacrificed after 5 weeks. **C**, Expression of UCA1 and miR-331-3p in tumor tissues. **D**, Protein expression of IL6R, p-JAK2, p-STAT3, c-Myc, CyclinD1, Bcl-2, and Bax in tumor tissues. * $p < 0.05$.

by gain of IL6R (Figure 5 I-J). Taking together, UCA1 promotes proliferation and suppresses apoptosis by regulating IL6R.

UCA1 Modulates JAK2/STAT3 Signaling Pathway by Up-Regulating IL6R Via Sponging MiR-331-3p

We suggested that UCA1/miR-331-3p/IL6R axis affects cell progression by altering the downstream signaling pathway. To clarify the specific molecular mechanism, NCI-H929 and RPMI-8226 cells were transfected with sh-NC, sh-UCA1-1, sh-UCA1-1+anti-NC, sh-UCA1-1+anti-miR-331-3p, sh-UCA1-1+pcDNA or sh-UCA1-1+IL6R. We observed that IL6R and miR-331-3p inhibitor restored UCA1 depletion induced suppression on the expression of p-JAK2 and p-STAT3 protein (Figure 6A-B). The results indicate that UCA1 promotes the activation of JAK2/STAT3 signaling pathway by enhancing IL6R expression via targeting miR-331-3p.

Interference of UCA1 Suppresses RPMI-8266 Xenograft Tumor Growth

To investigate the regulatory effect of UCA1 on tumor growth *in vivo*, RPMI-8266 cells stably transfected with sh-UCA1-1 or sh-NC were

subcutaneously injected in mice to construct RPMI-8266 xenograft model. As illustrated in Figure 7A, tumor growth was inhibited in xenograft model stably transfected with sh-UCA1-1 compared with sh-NC group. Similarly, tumor weight of sh-UCA1-1 group was relatively lower than that of sh-NC group (Figure 7B). Next, we detected the expression of UCA1 and miR-331-3p in tumor tissues by qRT-PCR and found that UCA1 expression was down-regulated while miR-331-3p expression was up-regulated in sh-UCA1-1 group compared with sh-NC group (Figure 7C). Also, UCA1 knockdown suppressed the JAK2/STAT3 signaling pathway and the expression of proliferation-related protein c-Myc, CyclinD1, and anti-apoptosis protein Bcl-2 (Figure 7D). Collectively, UCA1 knockdown could suppress tumor growth *in vivo*.

Discussion

It is well acknowledged that abnormal expression of the relative lncRNA is diagnosed in different diseases such as cardiovascular, neurodegenerative diseases, osteoarthritis, and cancer¹⁵. UCA1, mapped to chromosome region of

19p13.12, was closely associated with tumorigenesis and development in many cancers, like prostate adenocarcinoma, glioma, breast, and ovarian cancer¹⁶. For example, UCA1 acts as oncogene to accelerate cell progression in prostate cancer by sponging miR-204 to enhance CXCR4 expression¹⁷. Consistently, UCA1 promoted invasion and metastasis of bile duct carcinoma by targeting miR-122/CLIC1 axis to active ERK/MAPK signaling pathway¹⁸. Xu et al¹⁹ reported that UCA1 promoted CREB1-mediated epithelial mesenchymal transition (EMT) and metastasis by activating the PI3K/AKT/mTOR pathway in osteosarcoma. In addition, UCA1 was demonstrated to target miR-582-5p to improve drug resistance against bladder cancer through the inhibition of ATG7-mediated autophagy²⁰. Therefore, we thought that UCA1 is a vital regulator in the initiation and development of MM.

Evidence showed that lncRNAs exert their function by acting as competing endogenous RNA (ceRNA) to sponge the target miRNA. According to bioinformatics analysis by Star-Base2.0, miR-331-3p contains the potential binding sites of UCA1. Generally, miR-331-3p played a crucial role as oncogene or tumor suppressor to regulate cell progression²¹. For example, miR-331-3p functioned as oncogene to promote tumorigenesis, proliferation, and metastasis of hepatocellular carcinoma and prostate cancer by targeting PHLPP and aurora kinase inhibitor II, respectively²². Conversely, miR-331-3p functioned as tumor suppressor to inhibit cell proliferation and induce apoptosis in cervical cancer by targeting NRP2 to down-regulate E6/E7 expression, as well as in colorectal cancer, by interacting with HER2 to alter the PI3K/Akt and ERK1/2 pathways²³. However, the regulatory mechanism of UCA1/miR-331-3p axis is still unclear.

In our study, we speculated that UCA1 accelerates cell progression by sponging miR-331-3p to up-regulate IL6R expression. We found the expression of UCA1 and IL6R was up-regulated while miR-331-3p was down-regulated in MM tumors and cell lines compared with normal tissues and cells. Also, miR-331-3p was inversely correlated with UCA1 and IL6R. The subsequent luciferase reporter assay confirmed the interaction between miR-331-3p and UCA1 or IL6R²⁴⁻³⁹. Moreover, the rescue experiments revealed that UCA1 restored miR-331-3p mediated inhibition on proliferation of MM cells. Likewise, IL6R attenuated UCA1 elimination mediated suppressive effects on MM cell proliferation. In addition,

we discovered that UCA1 could activate IL6R induced the JAK2/STAT3 signaling pathway by targeting miR-331-3p. The *in vivo* experiments further validated UCA1 could promote proliferation and suppress apoptosis by activation of JAK2/STAT3 signaling pathway. Meanwhile, UCA1 knockdown suppressed the expression of proliferation-related protein c-Myc, CyclinD1, anti-apoptosis protein Bcl-2, and enhanced the expression of apoptosis-related protein Bax.

Conclusions

We explored the molecular mechanism of UCA1/miR-331-3p/IL6R axis on proliferation and apoptosis in MM. The results revealed that UCA1 promotes proliferation and inhibits apoptosis by enhancing IL6R expression and further activating the JAK2/STAT3 signaling pathway by sponging miR-331-3p. Our findings provide promising biomarkers for targeted therapy of MM.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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