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LncRNA OIP5-AS1 Promotes the Autophagy-Related Imatinib Resistance in Chronic Myeloid Leukemia Cells by Regulating miR-30e-5p/ATG12 Axis

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Abstract

Background: Resistance to tyrosine kinase inhibitors (TKIs) in patients with chronic myeloid leukemia (CML) remains a problem in clinical treatment, and the mechanism has not been fully clarified. Autophagy can protect cancer cells under chemotherapeutic stimulation. Long noncoding RNAs (lncRNAs) are critical in drug resistance of CML. The role of lncRNAs in autophagy and drug resistance of CML needs to be further explored. **Methods:** Western blot and immunofluorescence were used to evaluate the autophagy activity in the drug-resistant CML cell line K562/G01 and its parental cell line K562. Then the sensitivity of K562/G01 cells to the first generation TKI imatinib (IM) after autophagy inhibition was determined by CCK-8 assays. The lncRNA OIP5-AS1 related to the drug resistance of CML cells was determined by Gene Expression Omnibus database analysis. Western blot and drug-sensitivity assays were used to detect changes in autophagy and sensitivity to the IM in resistant CML cells after OIP5-AS1 knockdown. The interactions of OIP5-AS1, miR-30e-5p, and ATG12 were explored by RNA immunoprecipitation and dual-luciferase reporter assays. **Results:** In this study, we found that autophagy was associated with drug resistance in CML cells. Moreover, the upregulation of OIP5-AS1 in K562/G01 cells was related to the enhancement of autophagy. Knockdown of OIP5-AS1 suppressed autophagy and enhanced the sensitivity of K562/G01 cells to IM. Furthermore, OIP5-AS1 regulated ATG12 by competitively binding miR-30e-5p, thereby affecting autophagy-related drug resistance. **Conclusion:** Our study reveals that OIP5-AS1 promotes the autophagy-related IM resistance in CML cells by regulating miR-30e-5p/ATG12 axis, providing new insights into the drug resistance mechanism of CML.

Keywords

LncRNA OIP5-AS1, autophagy, ATG12, chronic myeloid leukemia, chemoresistance

Abbreviations

ATG12, autophagy related 12; BMMNCs, bone marrow mononuclear cells; CML, chronic myeloid leukemia; ceRNA, competitive endogenous RNA; CQ, chloroquine; GEO, Gene Expression Omnibus; IM, imatinib; IC50, 50% inhibition of growth; LncRNA, long noncoding RNA; LSC, leukemia stem cell; MMR, major molecular response; OD, optical density; RIP, RNA immunoprecipitation; shRNA, short hairpin RNA; TKI, tyrosine kinase inhibitor

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Introduction

Chronic myeloid leukemia (CML) is a clonal myeloproliferative disease originating from hematopoietic stem cells. It is characterized by translocation of chromosomes 9q34 and 22q11, which generates a fusion gene, bcr-abl, that encodes a protein with constitutive tyrosine kinase activity.[1,2] Tyrosine kinase inhibitors

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(TKIs) targeting BCR-ABL are used as first-line treatments in CML patients, and imatinib (IM) is a first-generation TKI. Most CML patients can achieve a major molecular response (MMR), but 26% of CML patients experience TKIs treatment failure.[3] However, the mechanism is far from fully understood. Therefore, understanding novel resistance mechanisms and identifying new therapeutic targets are critical for new drug development for CML.

In recent studies, autophagy-related molecules have been shown to be promising new therapeutic targets for the treatment of various malignant tumors. Autophagy is the process of degrading macromolecular substances and damaged organelles in autophagosomes through lysosomes. Under chemotherapeutic stress conditions, it can maintain the energy requirements of cells and reduce cell damage.[4,5] Inhibition of autophagy sensitizes cancer cells to chemotherapy, and long noncoding RNAs (lncRNAs) play a crucial role in the process of autophagy and drug resistance in cancer.[6–8]

LncRNAs, which are defined as RNA molecules with a length of more than 200 nucleotides, are involved in cancer cell proliferation, differentiation, apoptosis, and chemoresistance through transcriptional, post-transcriptional, and epigenetic mechanisms.[9–13] The most classical regulatory role of lncRNAs is their regulation of mRNA expression as competitive endogenous RNAs (ceRNAs). LncRNAs can reduce the inhibitory effect of microRNAs (miRNAs) on mRNAs by competitively binding miRNAs.[14] Recent studies have demonstrated that the lncRNA OIP5-AS1 can promote tumor progression by regulating the proliferation, migration, invasion and drug resistance of tumor cells.[15–18] However, the role of OIP5-AS1 in CML has not been reported in the literature.

In this study, we found that OIP5-AS1 was highly expressed in drug-resistant CML cells. Knockdown of OIP5-AS1 sensitized resistant CML cells to chemotherapy by decreasing chemotherapy-induced autophagy. Moreover, OIP5-AS1 sponged miR-30e-5p to facilitate ATG12-mediated autophagy via a ceRNA network, thereby promoting the resistance of CML cells to TKI.

Materials and Methods

Bioinformatic Analysis

We analyzed single-cell sequencing data of CML BCR-ABL$^+$ leukemia stem cells (LSCs) from the Gene Expression Omnibus (GEO) database with the accession number GSE76312. Patients were stratified as good (n = 11) or poor responders (n = 5) according to whether their response to TKI achieved MMR.

Clinical samples

Bone marrow (BM) of normal individuals (2 cases) and CML patients (6 cases) were obtained from the second affiliated hospital of Chongqing Medical University, Chongqing, China. Mononuclear cells were isolated using human bone marrow mononuclear cells (BMMNCs) isolation kit (Tbd science). The basic information of CML patients were shown in Supplemental Table S1. Patients signed the written informed consent, because the BMMNCs we obtained are left over from the patient's necessary medical testing, it will not harm the patients' interests.

Cell Culture

The human CML cell line K562 and the BCR-ABL-non-expressing cell line 293T were purchased from the Cell Bank of Shanghai Institute of Cell Biology. K562/G01 is drug-resistant cell line without BCR-ABL mutations obtained by K562 cells through IM screening. Cells were maintained in RPMI-1640 or DMEM (Gibco) supplemented with 10% fetal bovine serum (Gibco). Cells were cultured at 37 °C in an atmosphere containing 5% CO_2.

Reverse Transcription-Quantitative Polymerase Chain Reaction

RNA was extracted using TRIzol reagent (Accurate Biology) following the manufacturer's protocol and reversed transcribed into cDNA using the Evo M-MLV RT Kit (Accurate Biology). Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was performed using the SYBR® Green Pro Taq HS premixed qPCR kit (Accurate Biology). The primers involved were OIP5-AS1 forward (AGGAACTAACCGAACATTCT), OIP5-AS1 reverse (GCCTGTTTGGTGGTCTC). ATG12 forward (TTTGCTAAAGGCTGTGGG), ATG12 reverse (AAGGAGCAAAGGACTGAT), hsa-miR-30e-5p reverse transcription primer (GTCGTATCCAGTGCAGGGTCCGAGG TATTCGCACTGGATACGACCTTCCA), hsa-miR-30e-5p forward (GCGCGTGTAAACATCCTTGAC), hsa-miR-30e-5p reverse (AGTGCAGGGTCCGAGGTATT), and U6 forward (CTCGCTTCGGCAGCACA), U6 reverse (AACGCTTCACG AATTTGCGT). ACTB was used as an endogenous control for normalization, and the $2^{-\Delta\Delta Ct}$ method was used to evaluate the comparative quantification.

Western Blot

The cells were collected and lysed on ice with **Radio-Immunoprecipitation Assay (RIPA) lysis buffer** lysis buffer supplemented with protease and phosphatase inhibitors for 30 min. After centrifugation at 13 000*g* for 30 min at 4 °C, the supernatant was collected. The protein concentration was measured with the BCA protein concentration determination kit (Biosharp). Then 40 μg extract was separated by 10% to 12% SDS-PAGE and transferred to the PVDF membrane. The membrane was blocked with 5% milk in TBST and then incubated with the target antibody at 4 °C overnight. Next, the membranes were washed and incubated with HRP-conjugated secondary antibodies (1:5000, GAR007 and GAM007, MultiSciences) for 1 to 1.5 h. General ECL Luminescent Liquid LumiQ ECL

(Share-bio) was used for detection. The antibodies for the Western blot targeted LC3B (1:1000, A5202, Bimake), ATG12 (1:1000, A5219, Bimake), p62 (1:1000, A5180, Bimake), and β-actin (1:1000, TA-09, ZSGB-BIO).

OIP5-AS1 Knockdown

The lentiviral vector (hU6-MCS-Ubiquitin-EGFP-IRES-puromycin) encoding OIP5-AS1 shRNA and its negative control (sh-NC) were synthesized by GeneChem. The target sequence was 5′-CAAACAGGCTTTGTGTTCCTTATCA-3′ (sh-OIP5-AS1). OIP5-AS1 shRNA was transfected into K562/G01 cells, and stable shRNA-OIP5-AS1-transfected cells were selected with 2 μg/ml puromycin (Solarbio).

Cell Counting Kit-8 (CCK-8)

In the drug-sensitivity assay, K562 and K562/G01 cells with or without chloroquine (CQ; an autophagy inhibitor) (Target Mol), and K562/G01 cells transfected with sh-NC, or sh-OIP5-AS1 were added to a 96-well plate (2000 cells/well), and medium containing different concentrations of IM. Each concentration had 3 replicates. After incubation for 72 h, 10 μL of CCK-8 solution (Target Mol) was added to each well. Two hours later, the absorbance was measured with a microplate reader (Bio Teck). Next, the corresponding cell viability was determined.

Flow Cytometry

K562/G01 cells transfected with sh-NC or sh-OIP5-AS1 were treated with or without IM (5 μM) for 48 h. Cells were stained using an Annexin V-PE/7-AAD Apoptosis Detection Kit (Yeasen Biotech). Apoptosis was detected using the flow cytometer (CytoFLEX).

Colony Formation Assay

K562/G01 cells transfected with sh-NC or sh-OIP5-AS1 were seeded in a 96-well plate (100 cells/well) with the medium with or without IM (2 μM). Colonies were observed and counted after 7 to 15 days of incubation.

Immunofluorescence

K562/G01 cells with or without knockdown of OIP5-AS1 were fixed in 4% paraformaldehyde for 30 min, permeabilized for 15 min in 1% Triton-100, and then blocked for 1 h in goat serum (BOSTER) at 4 °C. The cells were incubated with a target antibody against LC3 (1: 500, PM036, MBL) at 4 °C overnight. Next, the cells were incubated with Cy3-conjugated goat-anti-rabbit secondary antibody (1:1000, A10520, Invitrogen) at 37 °C for 1 h in the dark, and then stained with DAPI (1:1000, C1002, Beyotime) for 15 min under the same conditions. Then, images were observed and photographed by fluorescence microscope (Nikon 80i).

Cytoplasmic and Nuclear RNA Isolation

To determine the cellular localization of OIP5-AS1, the PARIS™ Kit (Thermo Fisher) was used to separate and extract cytoplasmic and nuclear RNA from CML cells. The expression of OIP5-AS1, ACTB, and U6 in the cytoplasm and nucleus was detected by RT-qPCR.

RNA Immunoprecipitation

RNA immunoprecipitation (RIP) was performed with an RIP kit (BersinBio). A total of 2×10^7 cells were collected and lysed with RIP lysis buffer. The lysate was divided into IP, IgG, and input groups. An anti-Ago2 antibody (Abcam, ab32381) and an IgG antibody were added to the corresponding sample groups and mixed at 4 °C for 16 h. Then, protein A/G beads were added to precipitate antibody complexes. The complexes were subjected to RNA extraction and used for RT-qPCR.

Dual-Luciferase Reporter Assay

Fragments of OIP5-AS1 and ATG12 containing the possible binding site for miR-30e-5p and corresponding mutants (Wt-OIP5-AS1, Mut-OIP5-AS1, and Wt-ATG12, Mut-ATG12) were synthesized and inserted into the pmiR-GLO dual luciferase miRNA target expression vector (Genecreate). Next, 293T cells were seeded into a 96-well plate. The constructed plasmids and miR-30e-5p mimics or NC mimics (Biomics) were co-transfected into cells using Lipoplus 2000 transfection reagent (GenTech). After 48 h, the cells were harvested and analyzed for luciferase activity according to the instructions of the Dual-Luciferase Reporter Assay System (Promega).

Statistical Analysis

All data are presented as the mean ± SD from 3 independent experiments. Statistical analysis was performed using GraphPad Prism 5. The data between 2 groups were compared using Student's *t*-test, and one-way analysis of variance was used to compare the variance of multiple groups. $P < .05$ was considered statistically significant.

Results

Autophagy was Related to the Resistance in CML Cells

Autophagy can be induced by chemotherapy. This process relieves cancer cells from chemotherapy-related cell damage, thereby promoting chemoresistance.[7] Thus, we hypothesized that drug-resistant CML cells might exhibit increased autophagy. The LC3 markers are widely used in the detection of autophagy. During the progression of autophagy, LC3-| is converted into LC3-||. Therefore, the ratio of LC3-|| to LC3-| can be used to evaluate the level of autophagy.[19] We treated K562 cells with IM and found that the ratio of LC3-|| to LC3-| increased (Figure 1A and B), but in K562/G01 cells, it did not change

significantly (Figure 1C and D). Moreover, K562/G01 cells had a higher LC3-‖ to LC3-| ratio than K562 cells (Figure 1E and F). Immunofluorescence was used to evaluate the number of LC3 bright spots as a measure of autophagy. LC3-| is dispersed in the cytoplasm, and LC3-‖ aggregates on the autophagosome membrane, appearing as bright spots. Therefore, autophagy increased when the number of LC3 bright spots increased. There were more LC3 bright spots in K562/G01 cells than in K562 cells (Figure 1G). These results demonstrated that autophagy was induced under chemotherapeutic stress, and IM-resistant CML cells exhibited increased autophagy. The CQ can inhibit autophagy by preventing the fusion of autophagosomes with lysosomes, increasing LC3-‖ due to hindered degradation. We treated K562/G01 cells with CQ and found that LC3 bright spots increased (Figure 1H). In addition, p62 is substrate of autophagy, autophagy decreases when p62 increases. The protein levels of LC3-‖ and p62 increased in K562/G01 cells with CQ (10 μM) treatment (Figure 1I and J), indicating that CQ did inhibit the autophagy of K562/G01 cells. We found that treatment with CQ (10 μM) reduced the IC50 value of IM in K562/G01 cells (Figure 1K). The result indicates that CQ can sensitize K562/G01 cells to IM by inhibiting autophagy.

OIP5-AS1 was Highly Expressed in LSCs From CML Patients With Poor Response to TKI and K562/G01 Cells

To further identify differentially expressed lncRNAs in CML, we analyzed single-cell sequencing data from the GEO database with the accession number GSE76312. We found that the expression level of OIP5-AS1 in LSCs from CML patients with poor response to TKI was notably higher than that in LSCs from CML patients with good response (Figure 2A). Patients were stratified as good or poor responders according to whether their response to TKI achieved MMR.[20] RT-qPCR showed that the expression of OIP5-AS1 was higher in the IM-resistant CML cell line K562/G01 than in IM-sensitive K562 cells (Figure 2B). We speculate that OIP5-AS1 correlates with drug resistance in CML. We also found that the expression of OIP5-AS1 in BMMNCs of CML patients was higher than that in normal control (Figure 2C). The basic information of CML patients were shown in Supplemental Table S1.

OIP5-AS1 Knockdown Increased the Sensitivity of K562/G01 Cells to IM and Inhibited Autophagy in CML Cells

OIP5-AS1 was upregulated in K562/G01 cells. To determine whether OIP5-AS1 is related to drug resistance, we silenced OIP5-AS1 with shRNA. CML cells stably transfected with shRNA-OIP5-AS1 were obtained after puromycin screening, and green fluorescent protein (GFP) fluorescence was detected by flow cytometry. We found that the transfection efficiency of OIP5-AS1 shRNA in CML cells was close to 100% (Figure 3A). The RT-qPCR results showed that the knockdown efficiency of OIP5-AS1 shRNA was more than 70% (Figure 3B). Flow cytometry assays indicated that under the IM (5 μM) treatment, the apoptosis rates of K562/G01 cells were enhanced by OIP5-AS1 knockdown (Figure 3C). In addition, the drug-sensitivity assays showed that OIP5-AS1 knockdown significantly enhanced the sensitivity of K562/G01 cells to IM as illustrated by decreased IC50 values (Figure 3D and E). Moreover, the colony formation assay indicated that under the IM (2 μM) treatment, OIP5-AS1 silencing prominently inhibited the growth of K562/G01 cells (Figure 3F and G). Our results showed that OIP5-AS1 knockdown increased the sensitivity of K562/G01 cells to IM. We next explored whether OIP5-AS1 affects the drug resistance of K562/G01 cells by regulating autophagy. Western blot showed that OIP5-AS1 knockdown decreased the ratio of LC3-‖ to LC3-| (Figure 3H and I). We found that the LC3 bright spots in K562/G01 cells were reduced after OIP5-AS1 knockdown (Figure 3J). These data suggest that knockdown of OIP5-AS1 increases the sensitivity of K562/G01 cells to IM by inhibiting autophagy.

ATG12 was Identified as a Downstream Molecule of OIP5-AS1 Regulating Autophagy

To determine how OIP5-AS1 regulates autophagy, we first determined the location of OIP5-AS1 in CML cells. Cytosolic/nuclear RNA isolation experiments suggested that OIP5-AS1 was mainly located in the cytoplasm of CML cells (Figure 4A). By using the starBase V3.0 (http://starbase.sysu.edu.cn/), we found that OIP5-AS1 might act as a ceRNA to regulate the autophagy-related molecules ATG12, ATG5, ATG2B, ULK2, and so on. They were presented in Supplemental Table S2. We chose ATG12 because it has a high correlation with OIP5-AS1 and has not been studied in CML. By utilizing the GEPIA database (http://gepia.cancer-pku.cn/), we found that the expression of OIP5-AS1 was positively correlated with ATG12 (Figure 4B). Moreover, the RT-qPCR and western blot results illustrated that the expression of ATG12 in K562/G01 cells was higher than that in K562 cells (Figure 4C to E), and the suppression of OIP5-AS1 downregulated the mRNA and protein levels of ATG12 (Figure 4F to H). These data reveal that OIP5-AS1 regulates ATG12.

OIP5-AS1 was Found to Regulate ATG12 by Competitively Binding miR-30e-5p

We further determined whether OIP5-AS1 regulated the expression of ATG12 in CML cells in a miRNA-dependent manner. RIP experiments proved that the fold enrichment of OIP5-AS1 and ATG12 in the Ago2 group was significantly higher than that in the negative control group (Figure 5A). Since Ago2 is an essential component of the RNA-induced silencing complex, this experiment showed that OIP5-AS1 and ATG12 can bind miRNA. Next, we tried to look for miRNAs with which OIP5-AS1 competitively binds. By searching the starBase, LncBase (http://carolina.imis.athena-innovation.gr/diana_tools/

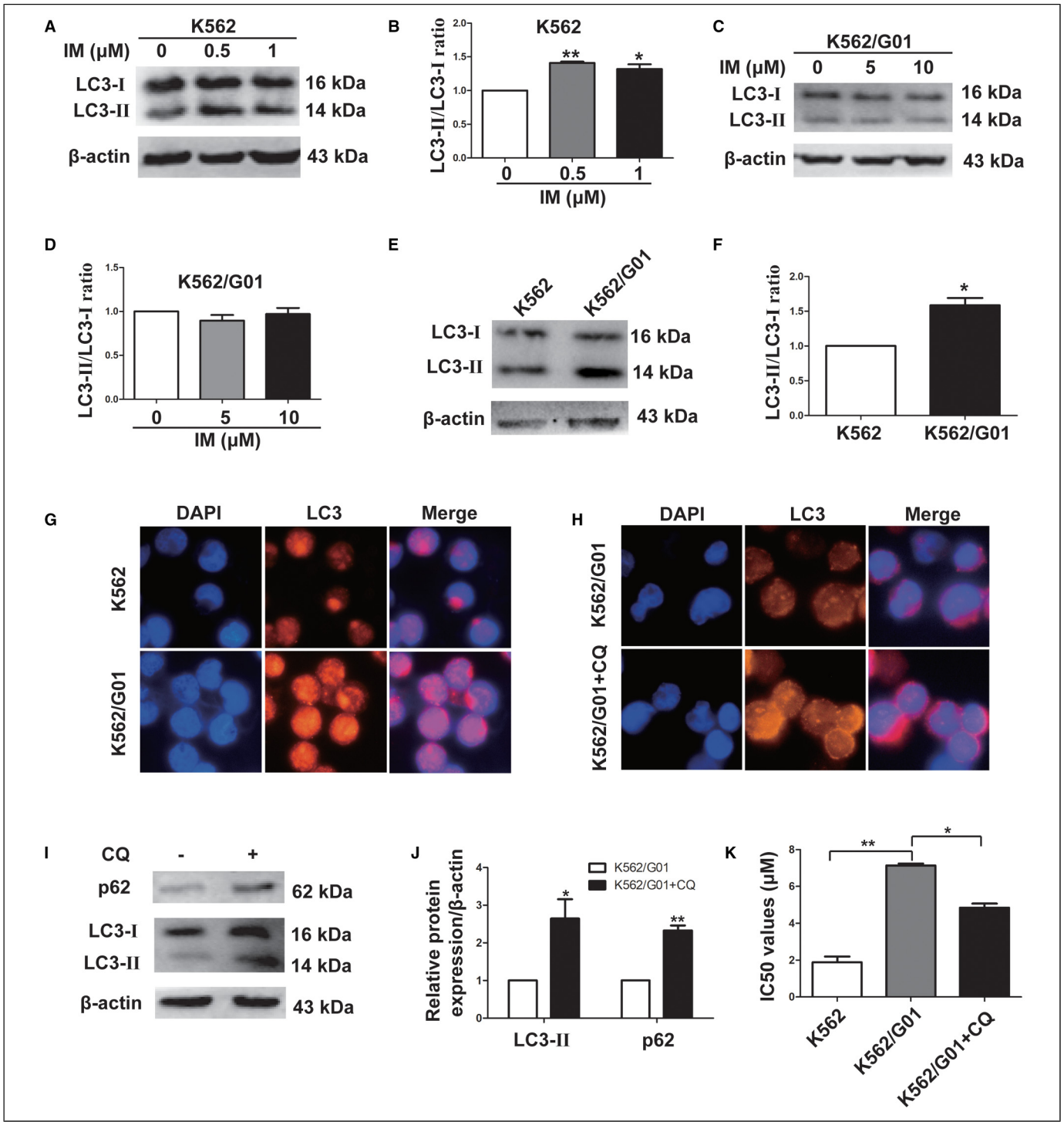

Figure 1. Autophagy is related to resistance in CML cells. (A, B) Western blot analysis of the expression of the autophagy-related protein LC3 in K562 cells treated with different concentrations of IM. (C, D) Western blot analysis of the expression of the LC3 in K562/G01 cells treated with different concentrations of IM. (E, F) Western blot analysis of the expression of LC3 in K562 and K562/G01 cells. (G) K562 and K562/G01 cells were stained with an anti-LC3B antibody, which was detected by fluorescence microscopy. Magnification 1000×. (H) K562/G01 cells with or without CQ (10μM) treatment were stained with an anti-LC3B antibody, which was detected by fluorescence microscopy. Magnification 1000×. (I, J) Autophagy in K562/G01 cells treated with CQ (10 μM) was determined by Western blot assay using LC3 and p62 antibodies. (K) CCK-8 were used to measure the relative cell viability of K562 and K562/G01 cells treated with or without CQ (10 μM) plus IM at different concentrations for 72 h; the corresponding IC50 values were calculated. *$P < .05$, **$P < .01$.

Abbreviations: CML, chronic myeloid leukemia; IM, imatinib; CQ, chloroquine.

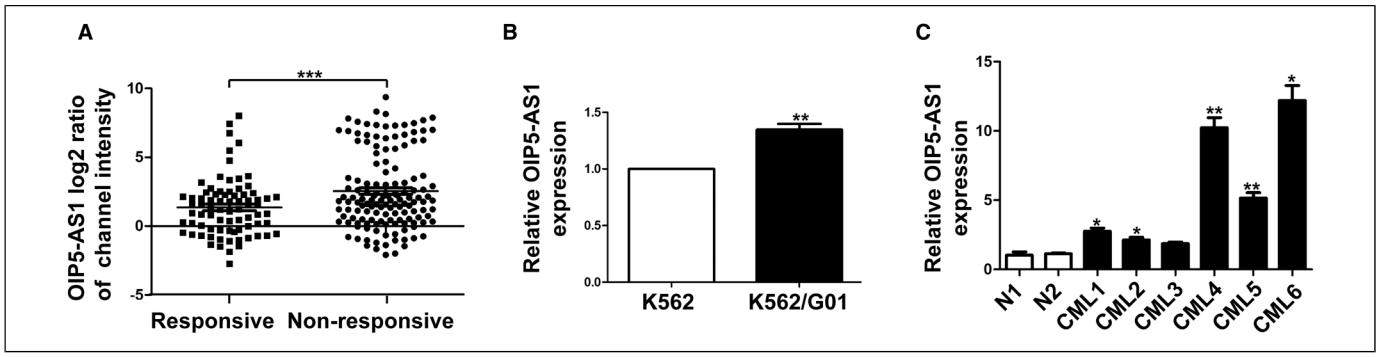

Figure 2. OIP5-AS1 is upregulated in LSCs from CML patients with poor response to TKI and drug-resistant cells. (A) The expression of OIP5-AS1 in BCR-ABL$^+$ LSCs from CML patients with good response to TKI and CML patients with poor response to TKI was analyzed using the GEO dataset GSE76312. (B) RT-qPCR was used to determine the expression of OIP5-AS1 in the drug-resistant cell line K562/G01 and its parental cells. (C) RT-qPCR was used to detect the expression of OIP5-AS1 in BMMNCs of CML patients and normal control. $*P < .05$, $**P < .01$, $***P < .001$.

Abbreviations: LSC, leukemia stem cells; CML, chronic myeloid leukemia; TKI, tyrosine kinase inhibitor; GEO, Gene Expression Omnibus; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; BMMNCs, bone marrow mononuclear cells.

web/index.php?r=lncbasev2/index-predicted), and TargetScan (http://www.targetscan.org/vert_72/) databases, we found 48 candidate miRNAs that interact with OIP5-AS1 and ATG12 (Figure 5B). Among them, only hsa-miR-30e-5p was reported to be related to CML in the literature.[21] In addition, the expression of miR-30e-5p in K562/G01 cells was lower than that in K562 cells (Figure 5C). The expression of ATG12 and OIP5-AS1 were downregulated in K562/G01 cells transfected with miR-30e-5p mimics. On the contrary, the expression of ATG12 and OIP5-AS1 were upregulated with miR-30e-5p inhibitors treatment (Figure 5D and E). The protein level of ATG12 was also negatively regulated by miR-30e-5p (Figure 5F and G). Moreover, the IC50 values of K562/G01 cells to IM decreased when miR-30e 5p was overexpressed, and increased when miR-30e 5p was suppressed (Figure 5H and I). Next, we predicted the miR-30e-5p binding sites of OIP5-AS1 and ATG12 via the LncBase database (Figure 5J) and constructed OIP5-AS1 and ATG12 constructs containing the wild-type sequence of the binding site and the corresponding mutant sequences of the binding site into dual-luciferase reporter gene plasmids. The results of the dual-luciferase reporter gene assays demonstrated that the luciferase activity of cells co-transfected with the miR-30e-5p mimics and the wild-type OIP5-AS1 and ATG12 sequences was decreased (Figure 5K and L), while the luciferase activity of the NC groups and the mutant groups remained unchanged, indicating that OIP5-AS1 and ATG12 can bind to miR-30e-5p. These data reveal that OIP5-AS1 regulates the expression of ATG12 by competitively binding miR-30e-5p.

Discussion

TKIs have greatly improved the prognosis for CML patients, but about 25% of CML patients experience TKIs treatment failure. Resistance of CML to TKI involves BCR-ABL1-dependent and independent mechanisms. The BCR-ABL1 independent drug resistance mechanism includes the reactivation of BCR-ABL1 downstream signals, transcription and epigenetic changes, changes in microenvironmental factors, changes in autophagy activity, and so on.[22–24] Targeting autophagy provides new strategy for the treatment of CML.

Autophagy has various roles in cancer. The role of autophagy in cancer may depends on stage of cancers and factors related to the microenvironment.[5] Some tumors undergo autophagy cell death after being induced by certain anticancer drugs, which is a cytotoxic process for cancer cells.[25] However, autophagy can prolong cells survival during stress in more tumors.[26,27] In this case, suppressing autophagy sensitizes cancer cells to chemotherapy.[28,29] Furthermore, a growing volume of literature illustrates that suppression of autophagy can increase the sensitivity of CML cells to chemotherapy drugs.[4,30,31] Our study revealed that autophagy was related to chemoresistance in CML cells. The autophagy level in K562/G01 cells was higher than that in K562 cells, and the sensitivity of K562/G01 cells to IM was increased by inhibiting autophagy. Therefore, autophagy played a cytoprotective role in K562/G01 cells. We found that treatment with IM increased the autophagy in K562 cells (Figure 1A and B). This result can be attributed to the fact that autophagy provides energy for cells by degrading macromolecular substances in autophagosomes and reduces the damage to CML cells with drug stimulation. But the treatment with IM could not induce autophagy in K562/G01 cells (Figure 1C and D), we speculate that this is related to the higher basal autophagy level of K562/G01 cells.

LncRNAs can regulate the function and activity of autophagy-related DNA, RNA or protein, or affect autophagy-related stress factors and energy receptors, thereby participating in the regulation of autophagy. Considering that the autophagy pathway is involved in the occurrence and development of a variety of tumors, lncRNA related to autophagy is more likely to have potentially important value in the treatment and prognosis of tumors. Based on the analysis of single-cell sequencing data from CML patients in the GEO database, we found that the expression level of OIP5-AS1 in LSCs from CML patients with poor response to TKI was notably higher

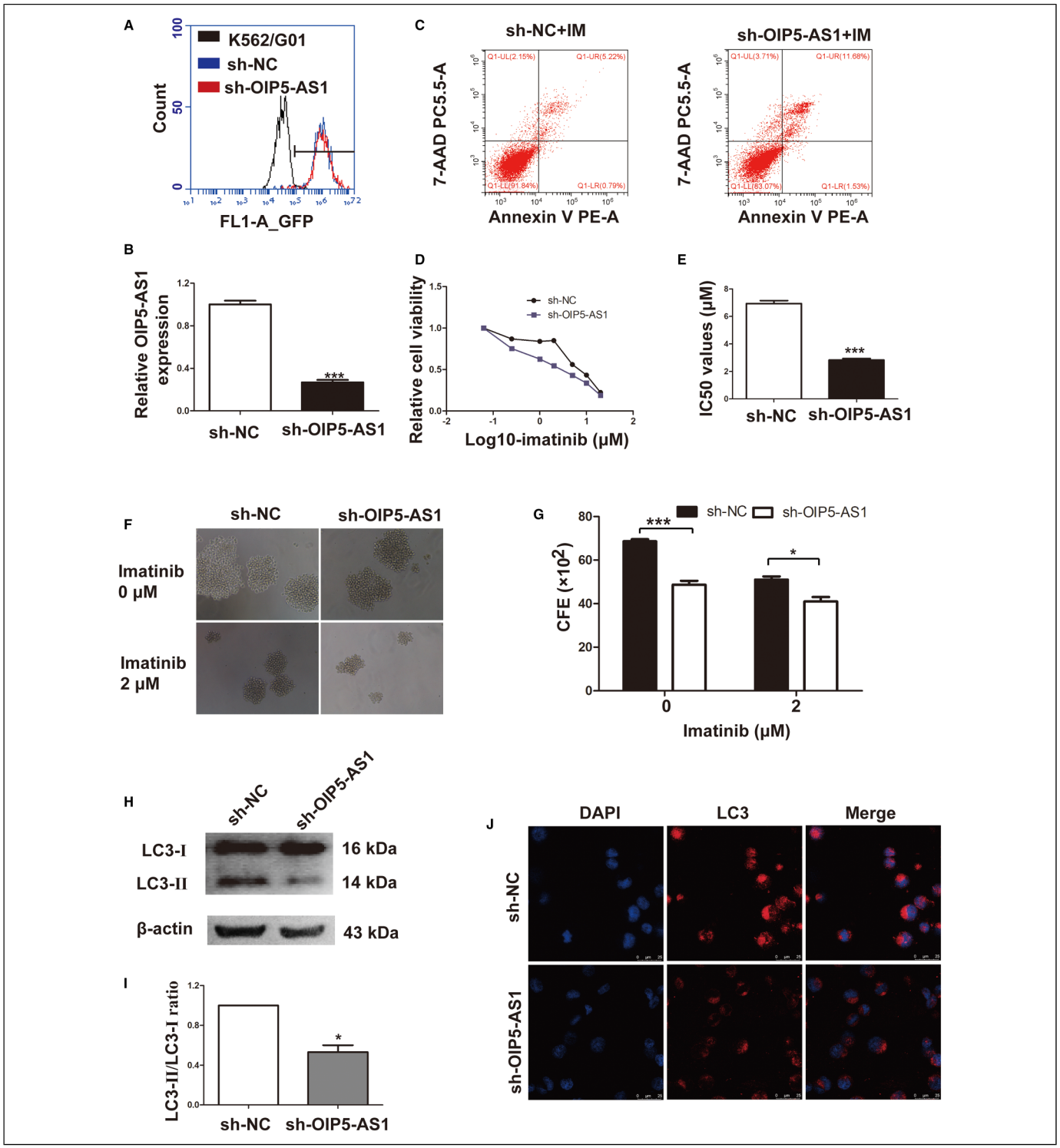

Figure 3. OIP5-AS1 silencing suppresses the autophagy of CML cells and enhances the sensitivity of resistant CML cells to IM. (A) Flow cytometry to detect the GFP fluorescence of K562/G01 cells transfected with sh-NC or sh-OIP5-AS1. (B) Knockdown efficiency of OIP5-AS1 shRNA in K562/G01 cells. (C) The apoptosis rates of K562/G01 cells in the sh-NC and sh-OIP5-AS1 groups treated with IM (5 μM) for 48 h was detected by flow cytometry. (D, E) CCK-8 assays were used to measure the relative cell viability of K562/G01 cells in the sh-NC and sh-OIP5-AS1 groups treated with IM at different concentrations for 72 h; the corresponding IC50 values were calculated. (F, G) Under IM (2 μM) treatment, the colony morphology and colony formation efficiency (CFE) of K562/G01 cells transfected with sh-NC or sh-OIP5-AS1 were detected by colony formation assays. Magnification 200×. (H, I) Western blot assays were used to identify changes in the autophagy-related proteins LC3 in K562/G01 cells after OIP5-AS1 knockdown. (J) The same cells were stained with an anti-LC3B antibody and analyzed by confocal microscopy. Scale bar: 25 μm. $^{*}P < .05$, $^{***}P < .001$.

Abbreviations: CML, chronic myeloid leukemia; IM, imatinib.

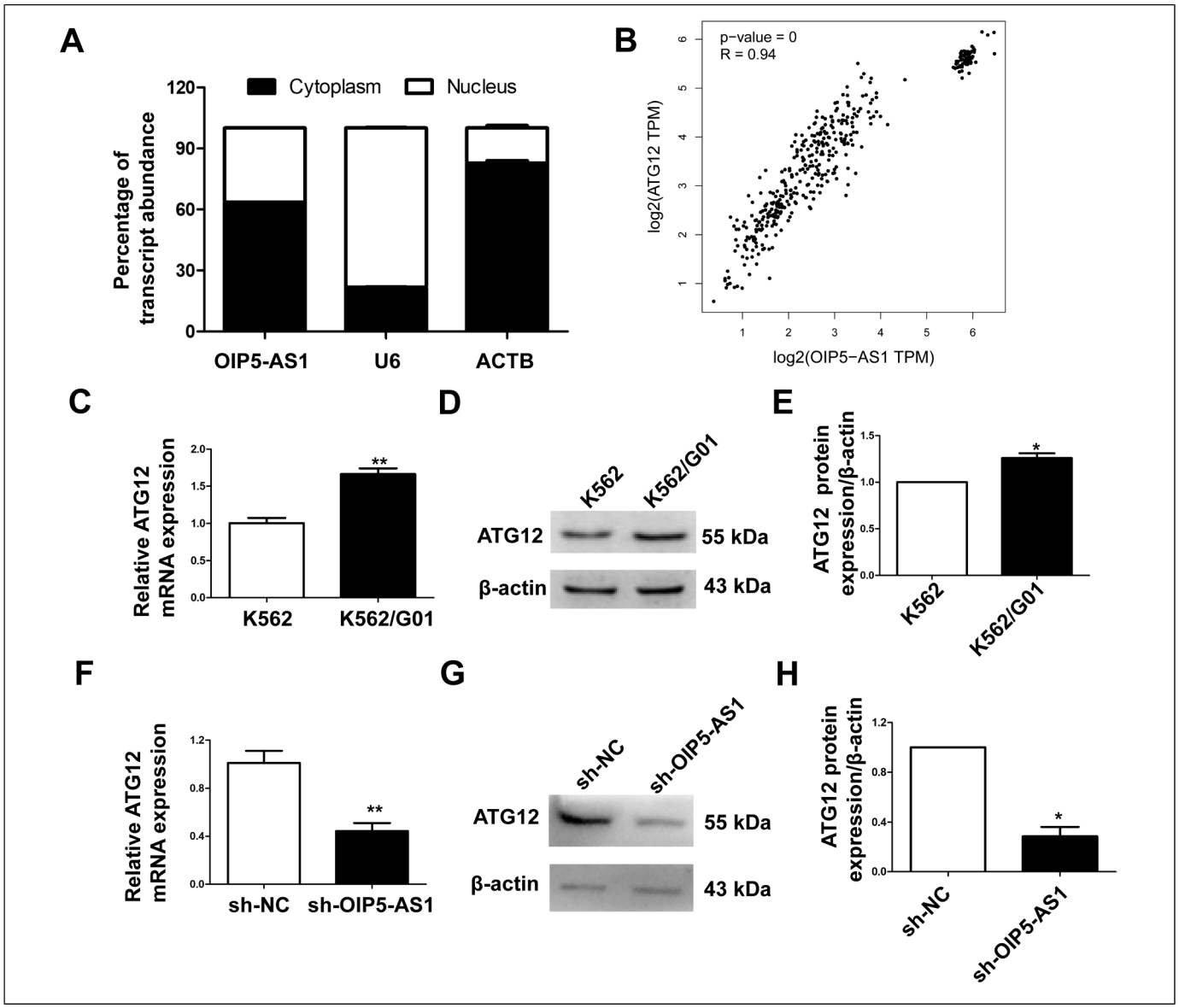

Figure 4. OIP5-AS1 regulates ATG12. (A) Cytosolic/nuclear RNA was isolated to detect the localization of OIP5-AS1 in CML cells. (B) Co-expression analysis of OIP5-AS1 and ATG12 by GEPIA. (C-E) RT-qPCR and western blot were used to detect the expression of ATG12 in K562 and K562/G01 cells. (F-H) RT-qPCR and Western blot was used to detect the expression of ATG12 in K562/G01 cells with or without OIP5-AS1 knockdown. *$P < .05$, **$P < .01$.
Abbreviations: CML, chronic myeloid leukemia; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

than that in LSCs from CML patients with good response to TKI. We also found that the expression of OIP5-AS1 was higher in K562/G01 cells than in K562 cells. Furthermore, OIP5-AS1 was highly expressed in BMMNCs of CML patients. Knockdown of OIP5-AS1 inhibited autophagy and increased the sensitivity of CML cells to IM. These results indicate that OIP5-AS1 can promote autophagy-related IM resistance in CML cells.

We continue to explore the mechanism by which OIP5-AS1 regulates autophagy-related drug resistance in CML cells. We found that OIP5-AS1 could regulate the autophagy-related molecule ATG12 by competitively binding miR-30e-5p. ATG12 is one of the key molecules in the process of autophagy. The process of autophagy mainly includes autophagy initiation, vesicle nucleation and elongation, fusion of autophagosomes and lysosomes, and degradation and recovery of autophagosome contents. The autophagy process is completed by various autophagy-related proteins such as mTOR, ULK1, Beclin-1, LC3, ATG5, ATG12, and so on. The ATG5-ATG12 complex conjugates with ATG16 to expand the auto-phagosome membrane, eventually forming an auto-phagosome.[32] Several studies have verified that ATG12 is a promoter of chemotherapy and radiotherapy resistance.[33,34] For example, the lncRNA MALAT1 acts as a ceRNA to promote the expression of ATG12 by sponging miR-23b-3p and thus increases chemoresistance related to autophagy in

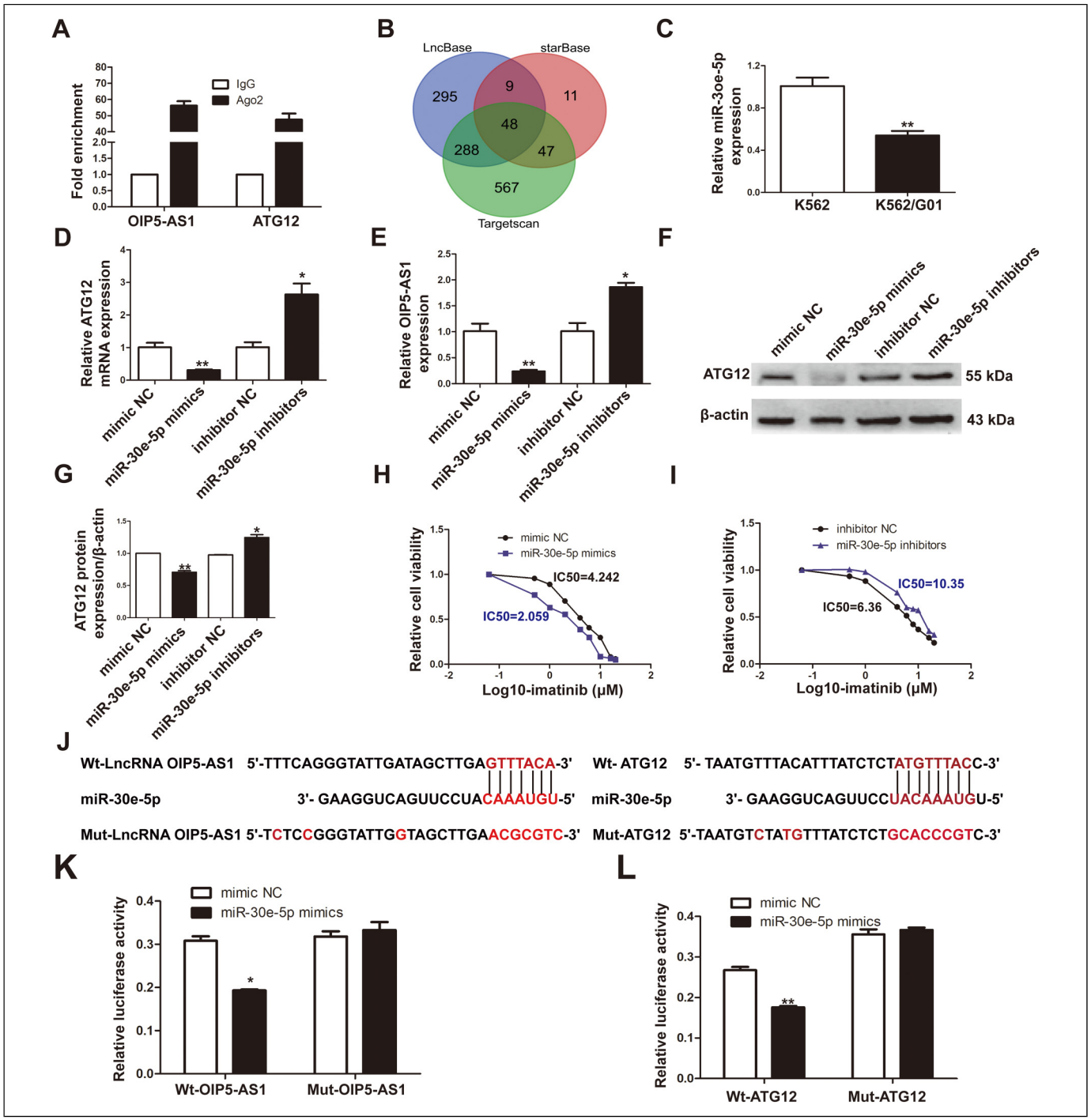

Figure 5. OIP5-AS1 regulates ATG12 by competitively binding miR-30e-5p. (A) The ability of OIP5-AS1 and ATG12 to bind with Ago2 in CML cells (IgG was used as a control) was measured by RIP assays. (B) miRNAs predicted to bind with OIP5-AS1 and ATG12 by 3 databases (starBase, LncBase, and TargetScan). (C) The expression level of miR-30e-5p in in K562 and K562/G01 cells. (D, E) RT-qPCR was used to detect the expression of ATG12 and OIP5-AS1 in K562/G01 cells treated with miR-30e-5p mimics and miR-30e-5p inhibitors. (F, G) Western blot was used to detect the expression of ATG12 in K562/G01 cells treated with miR-30e-5p mimics and miR-30e-5p inhibitors. (H, I) CCK-8 assays were used to measure the relative cell viability of K562/G01 cells transfected with miR-30e-5p mimics and miR-30e-5p inhibitors treated with IM at different concentrations for 72 h; the corresponding IC50 values were calculated. (J) LncBase predicted the miR-30e-5p binding sites of OIP5-AS1 and ATG12. (K, L) The dual-luciferase reporter assays were used to detect the binding of miR-30e-5p to OIP5-AS1 and ATG12. $*P < .05$, $**P < .01$.

Abbreviations: CML, chronic myeloid leukemia; RIP, RNA immunoprecipitation.

gastric cancer cells. It also shows that changes in ATG12 will affect changes in autophagy levels.[6] We also found the level of ATG12 and drug-resistance of CML cells to IM were negatively regulated by miR-30e-5p. Therefore, ATG12 mediated promotion of drug resistance in CML cells is related to the activation of autophagy.

The regulation of autophagy by OIP5-AS1 is not only reflected in changes in drug resistance. A study showed that OIP5-AS1 promotes the autophagy of PLK2-α-synuclein by targeting the miR-126 axis with pathogenic factors, thus reducing the aggregation toxicity of α-synuclein. This may be a potential method to improve the pathogenesis of Parkinson's disease.[35] In addition, OIP5-AS1 can also affect resistance in cancer through other pathways. A study demonstrated that OIP5-AS1 increased the resistance of osteosarcoma cells to doxorubicin through the OIP5-AS1/miR-200b-3p/FN1 axis.[15] The study by Liu et al. showed that OIP5-AS1 promotes the resistance of osteosarcoma cells to cisplatin by regulating the miR-377-3p/FOSL2 axis.[36] Therefore, exploration of how OIP5-AS1 regulates the resistance of CML cells in other ways, not just via autophagy, is warranted.

In summary, our study indicated that upregulation of OIP5-AS1 in drug-resistant CML cells was related to the enhancement of autophagy. The OIP5-AS1/miR-30e-5p/ATG12 pathway promotes autophagy-related resistance in CML cells. Knockdown of OIP5-AS1 can enhance the sensitivity of CML cells to IM, providing a new therapeutic strategy for patients with drug-resistant CML.

Authors' Note

HDD and HZ conceived and designed the experiments. Material preparation, data collection, and analysis were performed by JMW, ZLH, XW, and QL. The draft of the manuscript was written by HDD. JMW, ZLH, and WLF supervised the experimental work and revised critically the manuscript. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

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Availability of Data and Material

The data and materials in the current study are available from the corresponding author on reasonable request.

Ethical Statement

The research conformed to the standard stipulated by Declaration of Helsinki and was performed with the approval of the ethical committee of Chongqing Medical University. All patients are informed of the experimental method and purpose, and the patients signed informed consent.

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Supplemental Material

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