

MicroRNA-1271 inhibits proliferation and promotes apoptosis of multiple myeloma cells through inhibiting smoothened-mediated Hedgehog signaling pathway

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Abstract. MicroRNAs (miRNAs) have emerged as important regulators in multiple myeloma (MM). miR-1271 is a tumor suppressor in many cancer types. However, the biological role of miR-1271 in MM remains unclear. In the present study, we elucidated the biological role of miR-1271 in MM. Results showed that miR-1271 was significantly decreased in primary MM cells from MM patients and MM cell lines. Overexpression of miR-1271 inhibited proliferation and promoted apoptosis of MM cells. Conversely, suppression of miR-1271 showed the opposite effect. Bioinformatics algorithm analysis predicted that smoothened (SMO), the activator of Hedgehog (HH) signaling pathway, was a direct target of miR-1271 that was experimentally verified by a Dual-luciferase reporter assay. Furthermore, overexpression of miR-1271 inhibited SMO expression and HH signaling pathway. Conversely, the restoration of SMO expression markedly abolished the effect of miR-1271 overexpression on cell proliferation, apoptosis and HH signaling pathway in MM cells. Taken together, the present study suggests that miR-1271 functions as a tumor suppressor that inhibits proliferation and promotes apoptosis of MM cells through inhibiting SMO-mediated HH signaling pathway. This finding implies that miR-1271 is a potential therapeutic target for the treatment of MM.

Introduction

Multiple myeloma (MM) is one of the most common hematological cancers characterized by high proliferation of plasma cells expressing the surface marker CD138 in the bone marrow (1,2). Despite the advancement in treatment options over the past decades, MM remains an intractable disease with a poor survival rate and a relatively high incidence rate in recent years (3,4). The underlying mechanism of the pathogenesis of MM remains poorly understood. Therefore, it is of great importance to develop novel and effective therapies for MM.

In recent years, microRNAs (miRNAs) have been taken as promising therapeutic targets for cancer treatments, including MM (5). miRNAs are a type of small, non-coding regulatory RNA molecules (20-25 nucleotides) that post-transcriptionally and negatively modulate target gene expression by targeting the 3'-untranslated region (UTR) of target gene (6,7). Therefore, miRNAs regulate a variety of cellular processes, including cell proliferation, apoptosis, differentiation, migration and invasion (8). Increasing evidence has reported that numerous miRNAs are frequently dysregulated in MM serving as oncogenes or tumor suppressors in regulating MM cell proliferation, apoptosis, cell cycle, drug resistance, migration and invasion (9-15). In addition, the dysregulated miRNAs also represent potential biomarkers for MM diagnosis and prognosis (16-18). However, the precise mechanism of miRNAs in MM remains largely unknown.

Hedgehog (HH) signaling pathway is a highly conserved pathway from *Drosophila* to vertebrates that regulate embryonic development and adult tissue homeostasis (19). Three HH ligands exist including desert hedgehog, Indian hedgehog and sonic hedgehog in mammals (20,21). In the state of inactivation, the HH receptor patched1 (Ptch1) inhibits smoothened (SMO), which is a seven-transmembrane protein essential for HH activation (22). When HH ligands bind to Ptch1, SMO is released and activated, which then initiates the transcriptional program of HH signaling including Ptch1 and glioma-associated oncogene homolog 1 (Gli1) (20,23). Although HH signaling pathway is essential for embryonic development, tissue repair, and homeostasis, the abnormal activation of HH signaling pathway can lead to tumorigenesis (22,24-27). The

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Abbreviations: miRNAs, microRNA; MM, multiple myeloma; SMO, smoothened; UTR, untranslated region; HH, Hedgehog; Gli1, glioma-associated oncogene homolog 1

Key words: multiple myeloma, miR-1271, Hedgehog, SMO, cell proliferation, cell apoptosis

abnormal activation of HH signaling pathway has been found in solid tumors (prostate, pancreatic and lung cancers) (28-30) and hematologic malignancies (MM and B-cell lymphoma) (31-33). HH signaling pathway regulates cell proliferation, apoptosis, survival, drug resistance, colony growth and self-renewal of MM cells (34-36). Therefore, strategies inhibiting HH signaling may be a promising and effective anticancer intervention.

A recent study has suggested that miR-1271 functions as a tumor suppressor gene in various cancer types (37-40). However, whether miR-1271 plays a potential role in MM is unknown. In this study, we investigated the potential role and underlying mechanism of miR-1271 in MM. We found that miR-1271 was significantly decreased in MM samples and MM cell lines. Overexpression of miR-1271 inhibited proliferation and promoted apoptosis of MM cells. Bioinformatics algorithms analysis showed that SMO was a predicted target gene of miR-1271 that was confirmed by a Dual-luciferase reporter assay. The overexpression of miR-1271 inhibited SMO expression as well as HH signaling in MM cells. The restoration of SMO expression significantly abrogated the effects of miR-1271. The present study suggests that miR-1271 functions as a tumor suppressor of MM, indicating a potential molecular candidate for MM treatment.

Materials and methods

Cell cultures. Bone marrow aspirates were collected from 10 MM patients and 10 healthy donors in Xi'an Jiaotong University Medical College Red Cross Hospital. The primary MM cells were isolated from bone marrow aspirates as previously described (41). Briefly, mononuclear cells were separated from bone marrow aspirates by Ficoll-Hypaque density gradient centrifugation (Amersham, Little Chalfont, UK). Then, the cells were suspended in ice-cold phosphate-buffered saline (PBS) and incubated with microbeads labeled with a mouse anti-human CD138 monoclonal antibody (Miltenyi Biotech, Auburn, CA, USA). The CD138⁺ cells were sorted on a BD fluorescence-activated cell sorting FACS Aria flow cytometer (BD Biosciences, San Jose, CA, USA) in accordance with the manufacturer's instruction. The collection and use of clinical samples were approved by the Institutional Human Experiment and Ethics Committee of Xi'an Jiaotong University Medical College Red Cross Hospital with written informed consents from all MM patients and healthy donors. The human MM cell lines including NCI-H929, U266 and MM1.R were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). The KMS-12-BM MM cell line was purchased from Beijing Abace Biology, Co., Ltd. (Beijing, China). The primary MM cells, human MM cell lines, and normal plasma cells (nPCs) were cultured in RPMI-1640 (Gibco, Rockville, MD, USA) in supplement with 10% fetal bovine serum (FBS; Gibco) and 1% penicillin/streptomycin mix (Sigma-Aldrich, St. Louis, MO, USA) in a humidified atmosphere containing 5% CO₂ at 37°C.

Real-time quantitative PCR (RT-qPCR) analysis. Total RNA from tissues or cells was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Complementary DNA was synthesized using M-MLV reverse transcriptase (BioTeke,

Beijing, China) for mRNA expression analysis or miScript reverse transcription kit (Qiagen, Dusseldorf, Germany) for miRNA expression analysis. PCR amplification was performed on an Applied Biosystems AB 7500 Real-Time PCR system (Applied Biosystems, Inc., Carlsbad, CA, USA) using Power SYBR-Green PCR Master Mix (Applied Biosystems). The primers used were as follows: miR-1271 forward, 5'-CA GCACTTGGCACCTAGCA-3' and reverse, 5'-TATGGTTGT TCTCCTCTCTGTCTC-3'; U6 forward, 5'-CGCTTCGGC AGCACATATACTAA-3' and reverse, 5'-TATGGAACGC TTCACGAATTTGC-3'; SMO forward, 5'-TGCTCATCGT GGGAGGCTACTT-3' and reverse, 5'-ATCTTGCTGGCAG CCTTCTCAC-3'; Gli1 forward, 5'-TATGGACCTGGCTTT GGA-3' and reverse, 5'-CCTATGTGAAGCCCTATTT GC-3'; Ptch1, CTCTGGAGCAGATTTCCAAGG; Ptch1 forward, 5'-CTCTGGAGCAGATTTCCAAGG-3' and reverse, 5'-TGCCGCAGTTCTTTTGAATG-3' and GAPDH forward, 5'-CCATGTTTCGTCATGGGTGTG-3' and reverse, 5'-GGTG CTAAGCAGTTGGTGGTG-3'. U6 was used as the internal control of miR-1271 and GAPDH was used as the internal control of mRNA. The relative gene expression was determined by 2^{-ΔΔC_t} method, normalized against GAPDH or U6, and then compared with control.

Cell transfection. The miR-1271 mimics, miR-1271 inhibitor, and their scrambled controls (Scr) were purchased from Shanghai GenePharma (Shanghai, China). Cells were transfected with miR-1271 mimics or miR-1271 inhibitor at a final concentration of 50 nM using Lipofectamine 2000 (Invitrogen). The open reading frame of SMO without 3'-UTR was inserted into pcDNA3.1 (BioVector, Beijing, China). For overexpression of SMO, the pcDNA/SMO constructs were transfected into cells using Lipofectamine 2000 (Invitrogen).

Colony formation assay. Cells were transfected with miR-1271 mimics or miR-1271 inhibitor for 48 h. Then, the cells (200 cells/well) were seeded in 6-well plates and grown in a medium containing 0.3% noble agar for 2 weeks at 37°C. After fixing with 100% methanol for 30 min, the cells were stained with 0.1% crystal violet (Sigma-Aldrich). The number of colonies was observed and counted under a microscope (Olympus Corp., Tokyo, Japan).

3-(4,5-Dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cell proliferation was assessed by MTT assay. Briefly, cells were seeded into a 96-well plate at a density of 5×10³ cells/well and transfected with miR-1271 mimics or miR-1271 inhibitor for 24, 48 and 72 h. After replacement with fresh medium, 20 μl MTT (0.5 mg/ml in PBS; Sigma) was added to each well and incubated for 4 h at 37°C. Then, the supernatant was discarded and 200 μl dimethyl sulfoxide (Sigma-Aldrich) was added to each well. After incubation for 15 min, the absorbance at 490 nm was measured with a microplate reader (Thermo Fisher Scientific, Rockford, IL, USA).

Cell apoptosis assay. Cell apoptosis was determined by the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) and caspase-3 activity assay. For TUNEL assay, briefly, cells were fixed with 4% paraformaldehyde and permeabilized with 0.2% Triton X-100 followed by incuba-

tion with TUNEL reaction mixtures (Roche Diagnostics, Indianapolis, IN, USA) for 1 h at 37°C. The apoptotic cells were observed under a microscope (Olympus), and five fields per slide were randomly chosen to quantitatively calculate the apoptotic cells. For caspase-3 activity assay, cells were lysed and the protein concentration was measured. A total of 100 µg of protein with 50 µl of reaction buffer was treated with 5 µl DEVD-pNA substrate (4 mM; Roche Diagnostics) for 2 h at 37°C. The absorbance at 405 nm was measured with a microplate reader (Thermo Fisher Scientific).

Dual-luciferase reporter assay. The 3'-UTR of SMO containing the target sequence of miR-1271 was inserted into pmirGLO vector (Promega, Madison, WI, USA) to obtain pmirGLO-SMO 3'-UTR, and the 3'-UTR of SMO containing mutant miR-1271 target sites was inserted into pmirGLO vector (Promega) to obtain pmirGLO-mutant SMO 3'-UTR. NCI-H929 cells were co-transfected with pmirGLO-SMO 3'-UTR or pmirGLO-mutant SMO 3'-UTR and miR-1271 mimics, miR-1271 inhibitor or Scr controls. At 4 h after incubation, the luciferase activity was detected by a Dual-luciferase reporter assay system (Promega).

Western blot analysis. Total proteins were extracted, separated and transferred onto a nitrocellulose membrane (Bio-Rad Laboratories, Hercules, CA, USA). The membrane was blocked by 5% non-fat milk and incubated with primary antibodies at 4°C overnight. After three washes with Tris-buffered saline containing 0.1% Tween-20, the membrane was incubated with horseradish peroxidase conjugated secondary antibodies (1:2,000; Bosis, Beijing, China) for 1 h at 37°C. Then, the protein was detected using enhanced chemiluminescence (Millipore, Boston, MA, USA). The primary antibodies (anti-SMO and anti-GAPDH) used in this study were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The intensities of protein bands were quantified by Image-Pro Plus 6.0 software (Media Cybernetics, Inc., Rockville, MD, USA). The relative protein expression was normalized against GAPDH and then compared with the control.

Data analysis. All the data were reported as means ± standard deviation. Statistical analyses were analyzed by the Student's t-test for two group comparison or one-way analysis of variance followed by Bonferroni post hoc for multiple group comparison (>2) using SPSS version 11.5 (SPSS, Inc., Chicago, IL, USA). At $P < 0.05$, the difference was considered statistically significant.

Results

Expression of miR-1271 is downregulated in MM. To investigate whether miR-1271 plays a role in MM, we firstly examined the miR-1271 expression in primary MM cells isolated from MM patient bone marrow samples. The results showed that the expression of miR-1271 was significantly lower in MM primary cells than in plasma cells from healthy donors (Fig. 1A). We then evaluated miR-1271 expression in MM cell lines including NCI-H929, KMS-12-BM, U266 and MM1.R. As compared with normal plasma cells (nPCs), miR-1271 was significantly decreased in MM cell lines

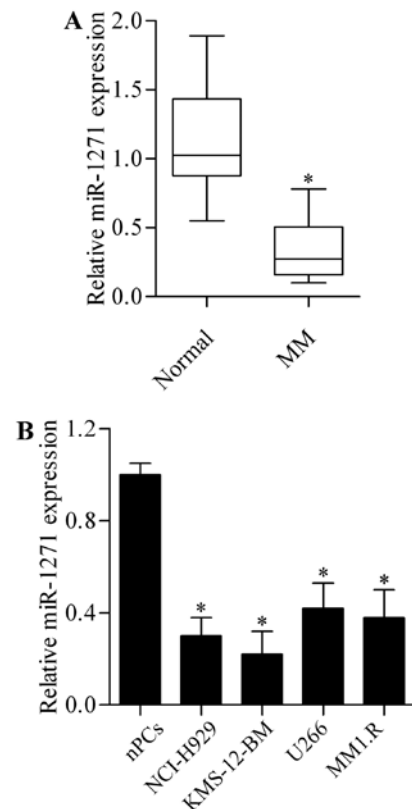


Figure 1. Detection of miR-1271 expression in MM by RT-qPCR. (A) Expression of miR-1271 in primary MM cells derived from MM patients. Plasma cells from healthy donors (normal) were used as control. * $P < 0.05$ vs. normal. (B) The expression of miR-1271 in MM cell lines including NCI-H929, KMS-12-BM, U266 and MM1.R compared with normal plasma cells (nPCs). * $P < 0.05$ vs. nPCs.

(Fig. 1B). These results suggest a tumor suppressor role of miR-1271 in MM.

miR-1271 inhibits MM cell proliferation. To explore the biological role of miR-1271 in MM, we performed gain-of- and loss-of-function experiments in KMS-12-BM and NCI-H929 cells by transfection of miR-1271 mimics or miR-1271 inhibitor. The results showed that transfection of miR-1271 significantly increased the expression of miR-1271 in KMS-12-BM (Fig. 2A) and NCI-H929 (Fig. 2B) cells, whereas miR-1271 inhibitor markedly decreased miR-1271 expression. We then detected the effect of miR-1271 on cell proliferation by MTT assay. The results showed that overexpression of miR-1271 significantly suppressed MM cell proliferation and miR-1271 inhibition markedly promoted cell proliferation (Fig. 2C and D). Furthermore, the colony growth of KMS-12-BM (Fig. 2E) and NCI-H929 (Fig. 2F) cells was also significantly decreased or increased by miR-1271 overexpression or miR-1271 inhibition, respectively. These data indicate that miR-1271 inhibit MM cell proliferation.

miR-1271 induces MM cell apoptosis. To further investigate the function of miR-1271 in MM, we then evaluated its effect on cell apoptosis. TUNEL assay showed that miR-1271 overexpression markedly induced cell apoptosis of MM cells, whereas miR-1271 inhibition markedly suppressed MM cell

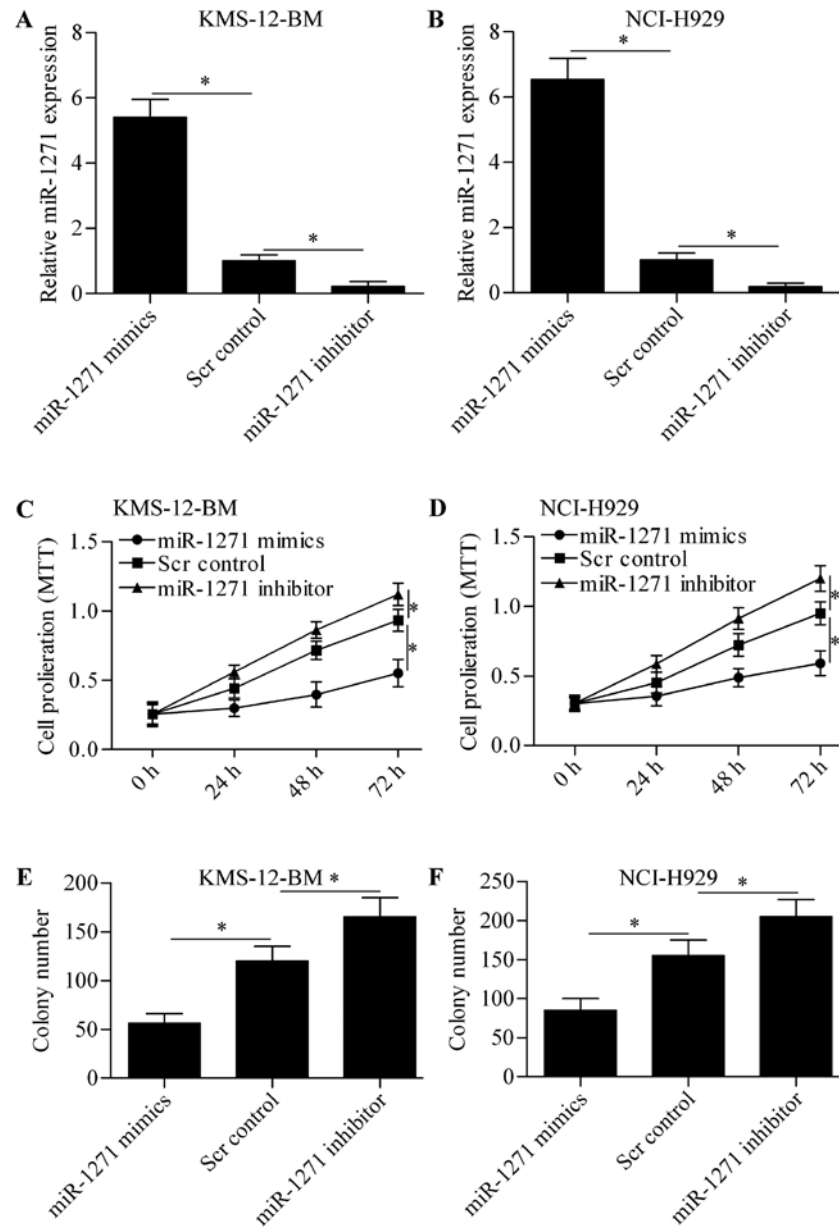


Figure 2. miR-1271 inhibits MM cell proliferation. RT-qPCR analysis of miR-1271 expression in KMS-12-BM (A) and NCI-H929 (B) cells transfected with miR-1271 mimics or miR-1271 inhibitor. Cells transfected with scrambled oligonucleotides (Scr) were used as control. The effect of miR-1271 on cell proliferation of KMS-12-BM (C) and NCI-H929 (D) cells was detected by MTT assay. The effect of miR-1271 on colony forming ability of KMS-12-BM (E) and NCI-H929 (F) cells was detected by colony formation assay. *P<0.05.

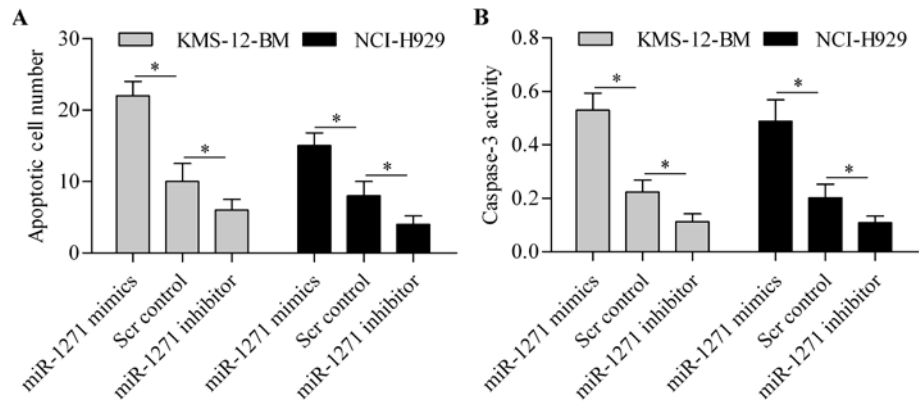


Figure 3. miR-1271 induces MM cell apoptosis. KMS-12-BM or NCI-H929 cells were transfected with miR-1271 mimics or miR-1271 inhibitor and incubated for 48 h. Cell apoptosis was determined by TUNEL assay (A) or (B) caspase-3 activity assay. *P<0.05.

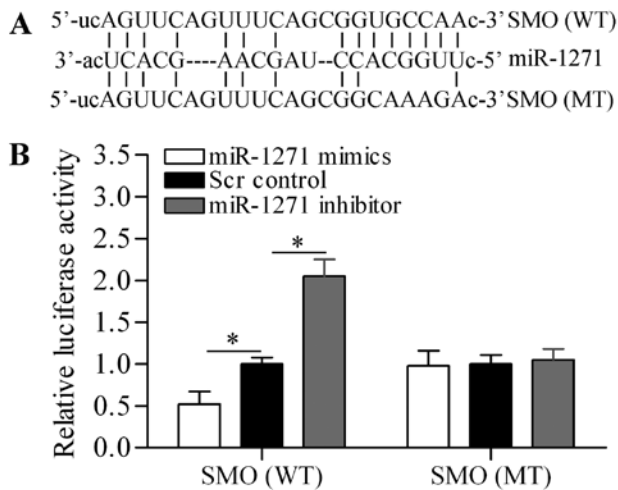


Figure 4. miR-1271 targets the 3'-UTR of SMO. (A) The targeting sites between miR-1271 and the 3'-UTR of SMO. Mutant sequences in the 3'-UTR of SMO are indicated. (B) Relative luciferase activity of NCI-H929 cells co-transfected with WT or MT SMO 3'-UTR luciferase reporter constructs and miR-1271 mimics, miR-1271 inhibitor, or Scr control. *P<0.05.

apoptosis (Fig. 3A). Moreover, caspase-3 activity assay showed that the activity of caspase-3 was significantly upregulated by miR-1271 overexpression but decreased by miR-1271 inhibition (Fig. 3B). These results suggest that miR-1271 induces apoptosis of MM cells.

miR-1271 targets the 3'-UTR of SMO. To understand the underlying mechanism by which miR-1271 inhibits proliferation and induces apoptosis of MM cells, we used bioinformatics analysis to seek the potential targets of miR-1271. Notably, we found that SMO, an important oncogene (42), was predicted as a potential target gene of miR-1271 (Fig. 4A). To verify the targeting relationship, luciferase reporter vectors containing wild-type (WT) or mutant (MT) SMO 3'-UTR were constructed. The Dual-luciferase reporter assay showed that miR-1271 mimics significantly decreased the luciferase activity of SMO 3'-UTR (WT), whereas miR-1271 inhibitor significantly increased the luciferase activity of SMO 3'-UTR (WT) (Fig. 4B). However, neither miR-1271 mimics nor miR-1271 inhibitor showed significant effect on the luciferase activity of SMO 3'-UTR (MT). Subsequently, we performed RT-qPCR and western blot analysis to detect the direct effect of miR-1271 on SMO expression. The results showed that expression of both mRNA (Fig. 5A and B) and protein (Fig. 5C and D) was significantly decreased by miR-1271 mimics or increased by miR-1271 inhibitor in MM cells. Taken together, these results indicate that miR-1271 inhibits SMO expression by directly targeting the 3'-UTR of SMO.

miR-1271 inhibits the HH signaling pathway. Because SMO is the critical regulator of HH signaling pathway, we speculated that miR-1271 might affect the HH signaling pathway. To test this hypothesis, we detected the effect of miR-1271 on Gli1 and Ptch1 expression. The results showed that the mRNA expression of Gli1 (Fig. 6A) and Ptch1 (Fig. 6B) was significantly downregulated by miR-1271 overexpression. By contrast, suppression of miR-1271 markedly increased the expression of

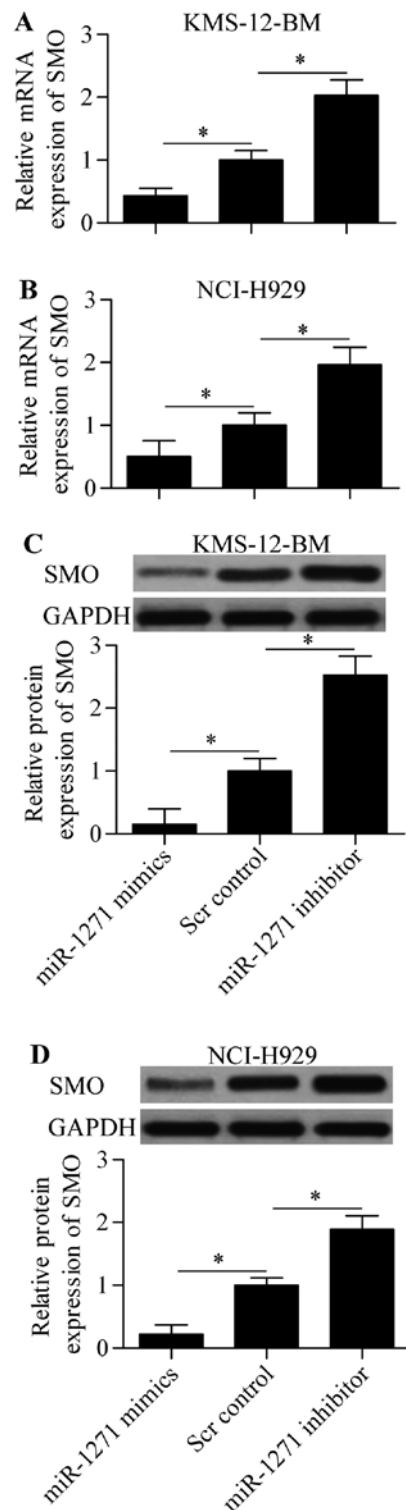


Figure 5. miR-1271 regulates SMO expression. KMS-12-BM or NCI-H929 cells were transfected with miR-1271 mimics and miR-1271 inhibitor for 48 h and then subjected to analysis. The mRNA expression of SMO in KMS-12-BM (A) and NCI-H929 (B) cells was detected by RT-qPCR analysis. The protein expression of SMO in KMS-12-BM (C) and NCI-H929 (D) cells was detected by western blot analysis. *P<0.05.

Gli1 (Fig. 6A) and Ptch1 (Fig. 6B) in MM cells. These results suggest that miR-1271 regulates the HH signaling pathway.

Overexpression of SMO reverses the effect of miR-1271. To validate whether miR-1271 functions through SMO,

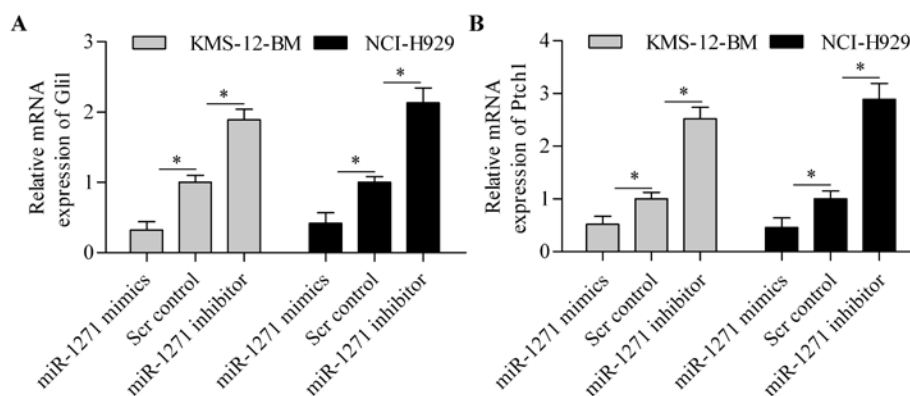


Figure 6. miR-1271 inhibits the HH signaling pathway. RT-qPCR analysis of Gli1 (A) and Ptch1 (B) expression in KMS-12-BM and NCI-H929 cells. KMS-12-BM and NCI-H929 cells were transfected with miR-1271 mimics or miR-1271 inhibitor for 48 h and then subjected to analysis. * $P < 0.05$.

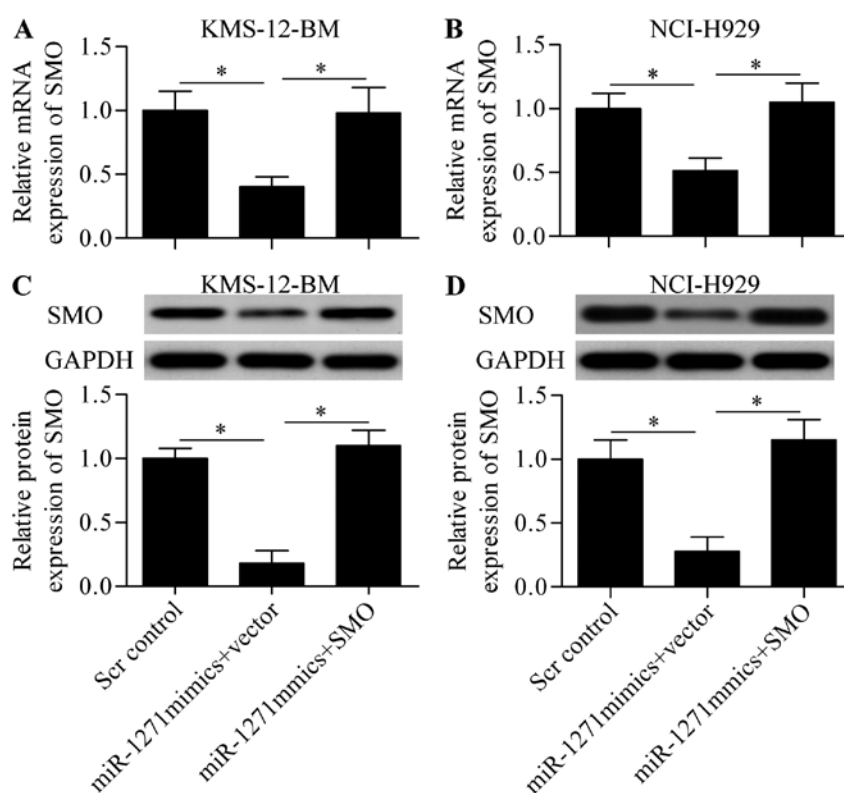


Figure 7. Restoration of SMO expression. Cells were transfected with Scr control, miR-1271 mimics plus null vector, or miR-1271 mimics plus SMO-overexpressing vectors and incubated for 48 h. The mRNA of SMO in KMS-12-BM (A) and NCI-H929 (B) cells was detected by RT-qPCR. The protein expression of SMO in KMS-12-BM (C) and NCI-H929 (D) was detected by western blot analysis. * $P < 0.05$.

we constructed a SMO-overexpressing vector harboring no 3'-UTR of SMO and performed a rescue experiment. The cells were co-transfected with miR-1271 mimics and SMO-overexpressing vector. The results showed that the decreased mRNA (Fig. 7A and B) and protein (Fig. 7C and D) expression induced by miR-1271 was significantly restored by SMO-overexpressing vector transfection. Notably, overexpression of SMO expression significantly restored MM cell proliferation, which was suppressed by miR-1271 (Fig. 8A). Furthermore, the promotion effect of miR-1271 on cell apoptosis was markedly reversed by SMO overexpression (Fig. 8B). In addition, the inhibitory effect of miR-1271 on Gli1 (Fig. 8C) and Ptch1 (Fig. 8D) expression was restored by SMO overex-

pression, thereby implying that SMO overexpression reversed the inhibitory effect of miR-1271 on HH signaling pathway.

Discussion

In the present study, we established a tumor suppressor role of miR-1271 in MM. The expression of miR-1271 was significantly downregulated in MM cells. Importantly, overexpression of miR-1271 inhibited proliferation and promoted apoptosis of MM cells. Further data demonstrated that SMO was a direct target gene of miR-1271 that overexpression of miR-1271 inhibited SMO expression. Suppression of SMO by miR-1271 overexpression also significantly inhibited the HH

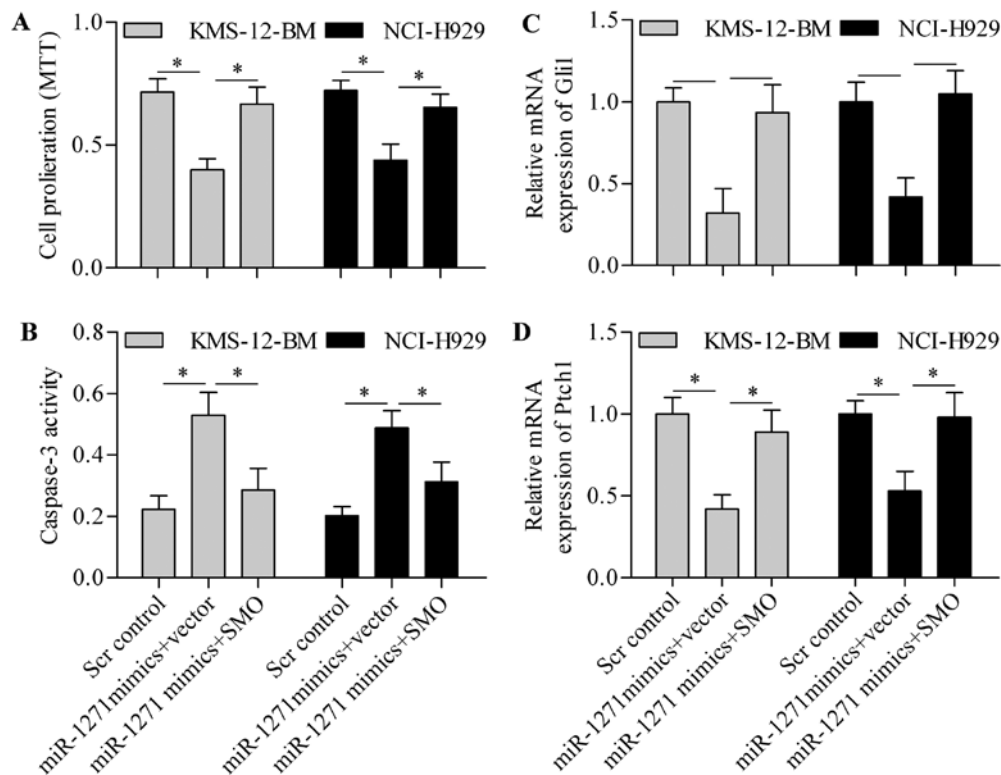


Figure 8. SMO overexpression reverses the effect of miR-1271. Cells were transfected with Scr control, miR-1271 mimics plus null vector, or miR-1271 mimics plus SMO-overexpressing vectors and incubated for 48 h. Cell proliferation (A) and apoptosis (B) were detected by MTT assay and caspase-3 activity, respectively. The expression of Gli1 (C) and Ptch1 (D) was detected by RT-qPCR analysis. *P<0.05.

signaling pathway. However, these effects of miR-1271 overexpression were markedly reversed by SMO overexpression. This study for the first time report the expression and function of miR-1271 in MM.

miRNAs play an important role in tumorigenesis acting as either oncogenes or tumor suppressors, representing an attractive therapeutic target (43). In recent years, miR-1271 has been reported as tumor-associated genes that are involved in various cancer types and functions through different target genes. A functional screening identifies that miR-1271 is a tumor suppressor in hepatocellular carcinoma that is decreased in tumor samples and inhibits tumor cell growth through targeting glypican-3 (44). In gastric cancer, miR-1271 is downregulated and inversely correlated with tumor size, tumor stage and lymph node metastasis (40). Yang *et al* (45) reported that miR-1271 inhibits gastric cancer cell proliferation and promotes the sensitization to cisplatin-induced apoptosis through targeting insulin-like growth factor 1 receptor, insulin receptor substrate 1, serine/threonine-protein kinase mTOR, and anti-apoptotic protein Bcl-2. miR-1271 is decreased in oral squamous cell carcinoma tissues and cell lines and overexpression of miR-1271 inhibits cell proliferation, colony formation, migration, and invasion of oral squamous cell carcinoma cells through targeting anaplastic lymphoma kinase (46). More recently, Liu *et al* (37) reported that miR-1271 functioned as tumor suppressor in pancreatic cancer through targeting ZEB1 and TWIST1. Tumor suppressor role of miR-1271 is also found in ovarian (39) and lung cancer (38) through targeting cyclin G1 or mTOR, respectively. Interestingly, an oncogenic role of miR-1271 is also reported that miR-1271 promotes cell

proliferation and invasion of non-small cell lung cancer cells through inhibiting homeobox A5 (47). However, in this study, we have demonstrated that miR-1271 is downregulated in MM cells and overexpression of miR-1271 inhibits proliferation and induces apoptosis of MM cells, supporting a tumor suppressor role of miR-1271.

SMO is an activator of the HH signaling pathway that functions as oncogene in various cancers (42). Overexpression of SMO is associated with aberrant activation of HH signaling pathway (42). Peacock *et al* (33) reported that HH signaling pathway maintains a tumor stem cell trait of MM cells. In MM cells, inhibition of SMO induces a decrease in cell viability and inhibits the HH signaling pathway (48). Inhibiting HH signaling pathway inhibits proliferation of MM cells (34,36). A new drug named vismodegib involves the inhibition of HH pathway and shows promising results in the treatment of medulloblastoma and basal-cell carcinoma (49). Therefore, strategies inhibiting HH signaling represent promising and effective anticancer intervention for MM.

miRNAs have emerged as promising tools for cancer treatment because of the inhibitory effect of miRNAs on gene expression. Indeed, increasing evidence has accumulated on the potential miRNAs that can directly target and inhibit SMO expression and the HH signaling pathway. miR-388-3p has been reported to target SMO to inhibit liver cancer cell invasion (50) and colorectal cancer cell growth and invasion (51,52). In chronic myeloid leukemia cells, inhibition of SMO by miR-326 inhibits cell proliferation as well as oncogenic HH pathway (53). Similarly, Du *et al* (54) reported that miR-326 inhibits glioma biological behavior and stemness

through targeting and inhibiting SMO-mediated HH signaling pathway. These findings support the notion that targeting SMO-mediated HH signaling by specific miRNAs is a promising strategy for MM. In this study, we identified miR-1271 as a novel miRNA that can target SMO and inhibit the HH signaling pathway in MM suggesting a novel and promising molecular target for MM treatment.

In conclusion, the present study for the first time demonstrated a tumor suppressive role of miR-1271 in MM. We have elucidated that miR-1271 inhibits proliferation and induces apoptosis of MM cells through targeting and inhibition of SMO, leading to the inhibition of HH signaling pathway. Our findings not only improve the understanding of MM pathogenesis, but also provide a potential and promising molecular target for MM therapy development.

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