

Original Article

MiR-144-3p inhibits cell proliferation and induces apoptosis in multiple myeloma by targeting c-Met

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Abstract: MicroRNA-144-3p (miR-144-3p) has been implicated in the development of many types of cancer. However, its role in multiple myeloma (MM) remains largely unknown. In this study, we found that miR-144-3p was downregulated in both MM cell lines and plasma from patients with MM. *In vitro* studies further showed that transfection of an miR-144-3p mimic into MM cells inhibited their proliferation and colony formation, and promoted cell cycle arrest at the G0/G1 phase and apoptosis. In addition, we found that miR-144-3p could directly target the 3'-untranslated region of cellular-mesenchymal to epithelial transition factor (*c-MET*) and suppress *c-MET* expression and its downstream signaling pathway (PI3K/AKT). Rescue experiments revealed that overexpression of *c-MET* partially reversed the inhibition effect of miR-144-3p in MM cells. *In vivo* studies confirmed that restoration of miR-144-3p suppressed tumor growth in xenograft nude mice by repressing *c-MET*. Overall, these findings demonstrate that miR-144-3p functions as a tumor suppressor in MM by targeting *c-MET*, suggesting that miR-144-3p might serve as a potential therapeutic target in MM.

Keywords: MiR-144-3p, multiple myeloma, c-MET, proliferation, apoptosis

Introduction

Multiple myeloma (MM) is a genetically complex hematologic malignancy characterized by the aberrant expansion of plasma cells within the bone marrow [1]. Despite increased understanding of the pathobiology of MM and remarkable progress in the management of the disease, its resistance to current therapies means that it is still incurable [2, 3]. Therefore, a better understanding of the molecular mechanisms underlying MM development and progression is urgently needed.

MicroRNAs (miRNAs) are small (18-25 nt), single stranded, noncoding RNAs, which can regulate gene expression by binding to the 3'-untranslated region (3'-UTR) of messenger RNAs (mRNAs), leading to mRNA degradation and/or suppression of mRNA translation [4, 5]. Increasing evidence suggests that miRNAs are involved in multiple biological processes such as cell proliferation, cell migration and invasion, glucose and lipid metabolism, signal transduction [6, 7]. In addition, miRNAs have

recently been linked to tumorigenicity and tumor progression by acting as oncogenes or tumor suppressors in various cancers [8, 9]. A growing body of evidence has demonstrated that aberrant miRNA expression is associated with MM development and progression, which may lead to new insights into the mechanisms of human MM [10, 11].

MicroRNA-144-3p (miR-144-3p) has been reported to be involved in both tumorigenesis and suppression of many types of cancers depending on the organ and the tissues [12-17]. However, the expression status, specific functions, and underlying mechanisms of miR-144-3p in MM remain unclear. In the present study, we found that miR-144-3p was significantly downregulated in MM cell lines and plasma from patients with MM. Importantly, ectopic expression of miR-144-3p in MM cells decreased their proliferation and colony formation, and induced cell cycle arrest at the G0/G1 phase and apoptosis *in vitro*. Cellular-mesenchymal to epithelial transition factor (*c-MET*) was identified as a potential target of

miR-144-3p. We also showed that miR-144-3p retarded tumor growth *in vivo* by repressing the expression of c-MET. These results suggest that miR-144-3p could be a potential target for MM therapy.

Materials and methods

Plasma samples and cell lines

This study was approved by the research ethics committee of China-Japan Union Hospital of Jilin University (Changchun, China). Prior to recruitment, all participants signed an informed consent for the use of their samples, according to committee's regulations. Plasma samples were obtained from 38 patients with MM and 16 normal individuals at the China-Japan Union Hospital of Jilin University (Changchun, China).

The four MM cell lines, MM1S, RPMI-8226, NCI-H929, and U266 (all from the American Type Culture Collection, Manassas, VA, USA) were cultured in complete RPMI-1640 medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; HyClone, Logan, UT, USA), 100 units/mL penicillin, 100 µg/mL streptomycin, and 2 mM L-glutamine in a humidified chamber supplemented with 5% CO₂ at 37°C.

Cell transfection

MiR-144-3p mimic (UCAUGUAGUAGAUUAUGAC-AU), the corresponding negative control (miR-NC, UUCUCGAACGUGUCACGUUUU), siRNA against c-MET (si-c-MET), and the corresponding scramble control (si-NC) were purchased from GenePharma (Shanghai, China). The c-MET overexpression vector was constructed by introducing the c-MET gene into the pCDNA3.1 vector (GenePharma), and was designated as pCDNA3.1-c-MET. These constructs were transfected into MM1S cells, grown to 80-90% confluence, using Oligofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

RNA extraction and quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from cultured cells and plasma by using TRIzol (Invitrogen), per the manufacturer's instructions. cDNA was synthesized from total RNA by using the PrimeScript

RT Reagent Kit (TaKaRa, Dalian, China). Expression levels of miR-144-3p were determined using TaqMan MicroRNA Assays (Applied Biosystems, Shanghai, China). Quantitative real-time PCR was performed using them iScript SYBR Green PCR kit (TaKaRa) and a 7900HT Fast Real-Time PCR System (Applied Biosystems) following the manufacturers' instructions. The primers for c-MET and GAPDH used in this study were described previously [18]. The expression of miR-144-3p and c-MET was normalized to that of U6 snRNA and GAPDH, respectively, using the 2^{-ΔΔCt} method.

Cell proliferation

To examine cell proliferation, transfected cells were seeded in a 96-well plate at a density of 1 × 10⁴ cells/well and grown at 37°C in a 5% CO₂ incubator. Cell viability was assessed at 24, 48, and 72 h by using the Cell Counting Kit-8 (CCK-8; Dojindo, Kumamoto, Japan), in accordance with the manufacturer's instructions. The absorbance of each well was detected at 450 nm using a Quant Universal Microplate Spectrophotometer (BioTek Instruments, Inc., Winooski, VT, USA).

Colony formation assay

Transfected cells were cultured in 6-well plates at a density of 1 × 10³ cells/well for 2 wk. The resulting colonies were fixed in 4% paraformaldehyde for 5 min before being stained with 1.0% crystal violet for 1 min. The number of surviving colonies was determined with an × 71 inverted microscope (Olympus, Tokyo, Japan). The colony formation rate was calculated as follows: (number of colonies/number of seeded cells) × 100%.

Cell cycle analysis

Cell cycle analysis was performed using a Cell Cycle Detection Kit (Beckman Coulter, Brea, CA, USA) in accordance with the manufacturer's instructions. Briefly, transfected cells were digested with EDTA-free trypsin and harvested by centrifugation at 1000 × g for 5 min. After washing twice in PBS, the cells were permeabilized in cold 70% ethanol and incubated overnight at 4°C in the dark. After washing with PBS, the cells were incubated in 500 µL staining solution (10 µg/mL propidium iodide and 5 U/mL RNaseA) for 30 min at 37°C. Cell cycle

stage was determined using a flow cytometer (FACSsort; Becton Dickinson, Franklin Lakes, NJ, USA) with an excitation wavelength of 488 nm and an emission wavelength of 525 nm. The cell cycle stage was analyzed by ModFit 3.2 (Beckman Coulter).

Cell apoptosis assay

The Annexin V-FITC Apoptosis Detection Kit I (Abcam, Cambridge, MA, USA) was employed to determine and quantify apoptosis by flow cytometry (FACSsort).

Luciferase reporter assays

The 3'-UTR of the human *c-MET* gene was amplified by PCR from human liver cDNA, and cloned into the *XhoI* and *NotI* sites downstream of the luciferase reporter gene in the pGL3-control vector (Ambion, Austin, TX, USA). The resulting plasmid was named WT-*c-MET*-3'UTR. The mutant construct was generated by replacing the 3'-UTR with custom-made synthetic whole 3'-UTR DNAs with mismatched seed region mutations and inserted into the pGL3-control vector (Ambion). The resulting plasmid was named Mut-*c-MET*-3'UTR. All constructs were verified by sequencing. For luciferase activity assay, MM1S cells (5×10^5) were seeded in triplicate in 6-well plates and allowed to settle for 24 h. The cells were then co-transfected with miR-144-3p mimic or miR-NC and *c-MET* reporter plasmid (wild-type or mutant) by using Lipofectamine 2000 (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's recommendation. The relative Renilla and firefly luciferase activities were measured 48 h post-transfection using the Dual Luciferase Reporter Assay Kit (Promega, Madison, WI, USA), following the manufacturer's instructions.

Western blot analysis

Total protein was extracted from cultured cells by using modified radioimmunoprecipitation assay buffer (50 mM Tris-HCl, pH 7.4; 120 mM NaCl; 1% Nonidet P-40; 0.25% deoxycholate; 0.1% sodium dodecyl sulfate) supplemented with protease inhibitor cocktail (Beyotime, Shanghai, China). Protein concentration was measured using a BCA protein assay kit (Pierce, Rockford, IL, USA). Equal amounts of protein were separated by electrophoresis on an 8-12%

SDS polyacrylamide gel and transferred to a nitrocellulose membrane (Amersham Biosciences, Piscataway, NJ, USA). After blocking with 5% nonfat milk in PBS, the membranes were probed with antibodies against *c-MET* (1:1000, cat. no. sc-8057; Santa Cruz Biotechnologies, Dallas, TX, USA), PI3K (1:1000, cat. no. sc-136298; Santa Cruz Biotechnologies), p-PI3K (1:500, cat. no. 4228; Cell Signaling Technology, Danvers, MA, USA), AKT (1:1000, cat. no. sc-81434; Santa Cruz Biotechnologies), p-AKT (1:1000, cat. no. sc-7985-R; Santa Cruz Biotechnologies), or GAPDH (1:5000, cat. no. sc-32233; Santa Cruz Biotechnologies), followed by horseradish peroxidase-conjugated secondary antibodies (1:6000, cat. no. sc-2004 or sc-2005, Santa Cruz Biotechnologies) for 1 h at room temperature. GAPDH was used as an internal loading control. The signals were detected using an enhanced chemiluminescence detection reagent (ECL; Thermo Scientific, Rockford, IL, USA) according to the manufacturer's instructions.

Xenograft tumor model

BALB/c-nu mice (5-6 week of age and weighing 20-25 g) were purchased from the Experimental Animal Center of Jilin University, and cared in accordance with the institutional guidelines. The mice were randomly divided into two groups ($n = 10$ mice per group). One group of mice was inoculated subcutaneously with 2×10^6 MM1S cells expressing miR-NC (MM1S/miR-NC cells) in the left dorsal flank, and the other with 2×10^6 MM1S cells expressing miR-144-3p (MM1S/miR-144-3p cells) in the right dorsal flank. Tumor volumes were measured weekly from the first injection until mice were sacrificed, using the following formula: $V = (L \times W^2)/2$ (V , volume of tumor; L , length; W , width). Mice were sacrificed 42 d after inoculation, tumors were excised, and the wet weight of each tumor was determined. Tumor tissues were stored at -80°C for further analysis.

Statistical analysis

All data were expressed as mean \pm standard deviation from at least three independent experiments. Statistical analysis was performed using the SPSS 19 software package (SPSS Inc., Chicago, IL, USA). Independent *t*-tests was used to compare differences between two groups. One-way ANOVA with

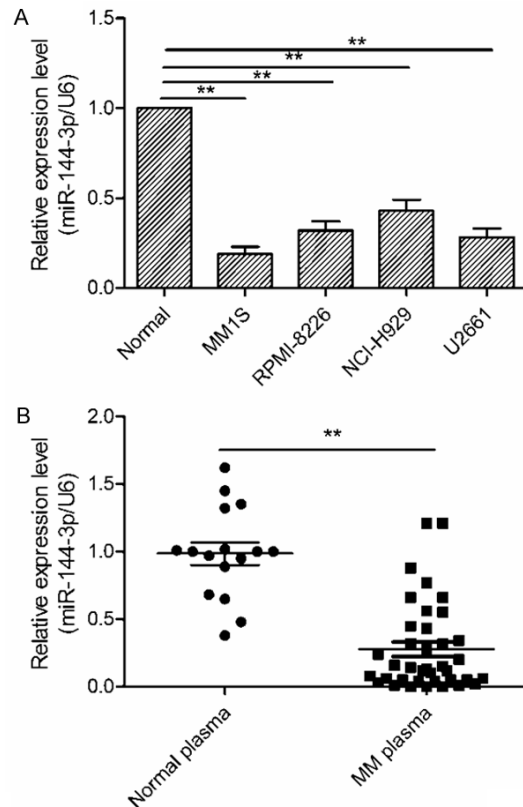


Figure 1. Expression of miR-144-3p was downregulated in MM cells and plasma from MM patients. A. qRT-PCR analysis of miR-144-3p expression in four MM cell lines (MM1S, RPMI-8226, NCI-H929, and U266) and plasma from normal individuals. B. qRT-PCR analysis of miR-144-3p expression in 38 plasma samples from patients with MM and 16 plasma samples from normal individuals. ** $P < 0.01$.

Bonferroni post-hoc tests was performed to compare differences between three or more groups. A P value < 0.05 was considered as statistically significant.

Results

miR-144-3p was downregulated in MM cell lines and patient plasma

To determine the expression status of miR-144-3p in MM, we first assessed miR-144-3p expression in four MM cell lines (MM1S, RPMI-8226, NCI-H929, and U266) and plasma derived from normal individuals. As shown in **Figure 1A**, miR-144-3p expression was significantly lower in the four MM cell lines than in the plasma samples from normal individuals. MM1S cells exhibited the lowest expression among the four MM cell lines (**Figure 1A**), and

thus, were selected for subsequent analysis. We next examined miR-144-3p expression in plasma samples obtained from normal subjects and from patients with MM. We found that miR-144-3p expression was significantly lower in the plasma from patients with MM than in plasma from normal individuals (**Figure 1B**). These results indicate that miR-144-3p expression was decreased in MM cell lines and in plasma from patients with MM.

miR-144-3p inhibits cell growth in MM cells

To explore the biological function of miR-144-3p in MM, MM1S cells transfected with 100 nM miR-144-3p or miR-NC by using Lipofectamine 2000, according to the manufacturer's instructions. The efficacy of transfection was assessed by qRT-PCR 48 h after transfection. It was found that miR-144-3p expression was significantly increased in MM1S cells transfected with miR-144-3p mimic compared with cells transfected with miR-NC (**Figure 2A**). After confirming the efficacy of transfection, the effects of miR-144-3p on proliferation, colony formation, cell cycle, and apoptosis were determined. The CCK-8 assay revealed that the proliferation of MM1S cells transfected with miR-144-3p mimic was significantly reduced compared with cells transfected with miR-NC (**Figure 2B**). Similarly, transfection with miR-144-3p mimic significantly reduced the number of colonies formed (**Figure 2C**). FACS analysis showed that compared to transfection with miR-NC, transfection with the miR-144-3p mimic significantly increased the proportion of cells in the G0/G1 phase and reduced the proportion of cells in the S phase (**Figure 2D**). We also showed that transfection with miR-144-3p mimic significantly increased apoptosis compared to transfection with miR-NC (**Figure 2E**). These results suggest that miR-144-3p overexpression inhibits MM cell proliferation and induces apoptosis.

c-MET is a direct target of miR-144-3p in MM cells

Using three miRNA databases (TargetScan, PicTar, and miRanda), we found a putative miR-144-3p-binding site located in the 3'-UTR of *c-MET* mRNA (**Figure 3A**). To confirm whether miR-144-3p directly binds to *c-MET*, we cloned wild-type or mutant *c-MET* 3'-UTR, inserted each clone into a luciferase reporter vector, and determined the luciferase activity. We

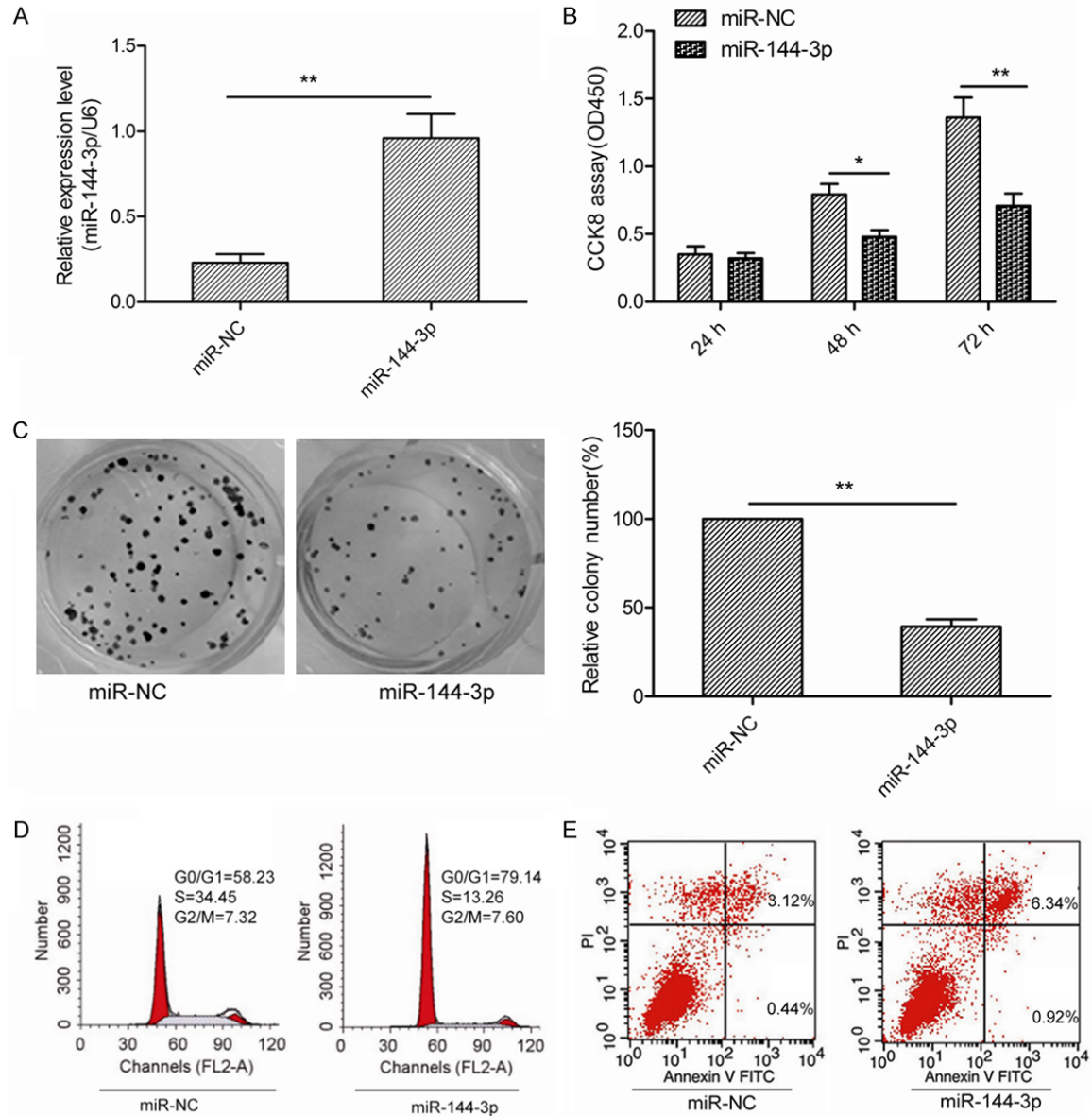


Figure 2. MiR-144-3p inhibits cell proliferation and induces apoptosis in MM cells. A. qRT-PCR analysis of miR-144-3p expression in MM1S cells transfected with miR-144-3p mimic and miR-NC. B-E. Cell proliferation, colony formation, cell cycle stage and apoptosis were assessed in MM1S cells transfected with miR-144-3p mimic or miR-NC. *P < 0.05, **P < 0.01.

found that miR-144-3p overexpression remarkably reduced the luciferase activity of the wild-type c-MET-3'UTR, but not that of the mutant c-MET-3'UTR (Figure 3B). To further confirm the effect of miR-144-3p on c-MET, we determined c-MET expression at the mRNA and protein level in MM1S cells transfected with miR-144-3p mimic or miR-NC. The results showed that c-MET expression at both the mRNA and protein level was lower in MM1S cells transfected with miR-144-3p than in cells transfected with

miR-NC (Figure 3C and 3D). We also found that miR-144-3p overexpression significantly inhibited the expression of PI3K/AKT, which are effector molecules downstream of c-MET, in MM1S cells (Figure 3D). In addition, we found that c-MET expression was upregulated both at the mRNA and protein level in the four MM cell lines compared to that in plasma from normal individuals (Figure 3E and 3F). These results suggest that c-MET is a direct target of miR-144-3p in MM1S cells.

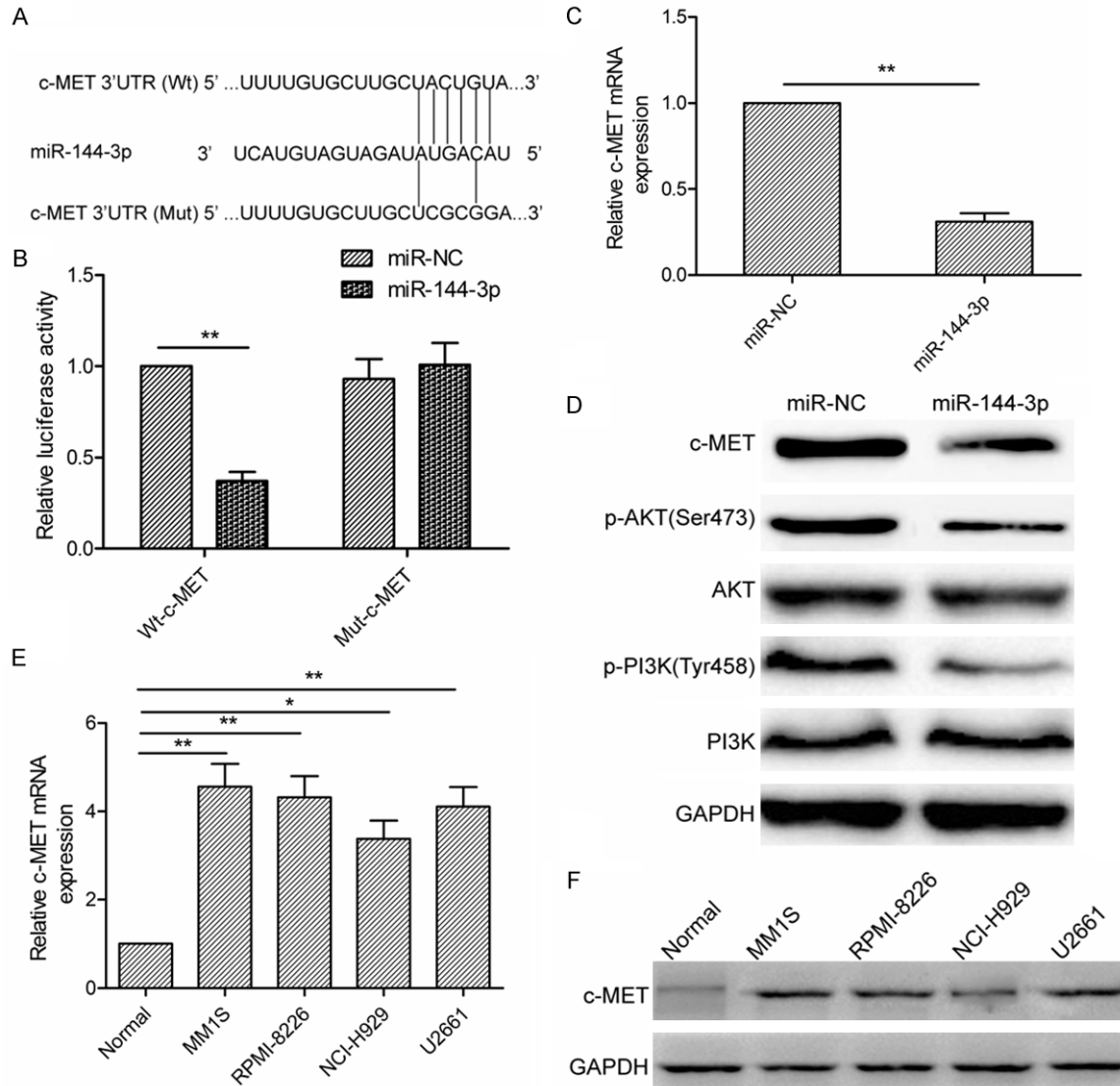


Figure 3. c-MET is a direct target of miR-144-3p in MM cells. **A.** Schematic of the construction of wild-type or mutant c-MET-3'UTR vectors. Wt: Wild-type; Mut: Mutant. **B.** Relative luciferase activities were analyzed in MM1S cells co-transfected with c-MET 3'UTR (wild-type or mutant) reporter plasmid, miR-144-3p mimic, or miR-NC. **C.** qRT-PCR analysis of c-MET mRNA expression in MM1S cells transfected with miR-144-3p mimic and miR-NC. GAPDH served as the loading control. **D.** Western blot analysis of c-MET, PI3K, p-PI3K, AKT, and p-AKT expression in MM1S cells transfected with miR-144-3p mimic or miR-NC. GAPDH served as the loading control. **E, F.** c-MET expression at the mRNA and protein level was determined by qRT-PCR and western blot analysis of four MM cell lines and plasma from normal individuals. GAPDH served as the loading control. *P < 0.05, **P < 0.01.

c-MET silencing inhibits cell proliferation and induces apoptosis in MM cells

To evaluate the effects of c-MET downregulation, we silenced its expression in MM1S cells by transfection with si-c-MET or si-NC. We found that MM1S cells transfected with si-c-MET had significantly reduced c-MET expression at both the mRNA and protein level (**Figure 4A** and **4B**). In addition, c-MET silencing significantly inhibited cell proliferation and colony for-

mation, and induced cell cycle arrest at the G0/G1 phase and apoptosis in MM1S cells (**Figure 4C-F**), which resembled the suppressive effects of miR-144-3p overexpression in MM1S cells.

Overexpression of c-MET reverses the effect of miR-144-3p in MM cells

To determine whether the role of miR-144-3p in MM is mediated by c-MET, we restored c-MET expression by transfection with the c-MET over-

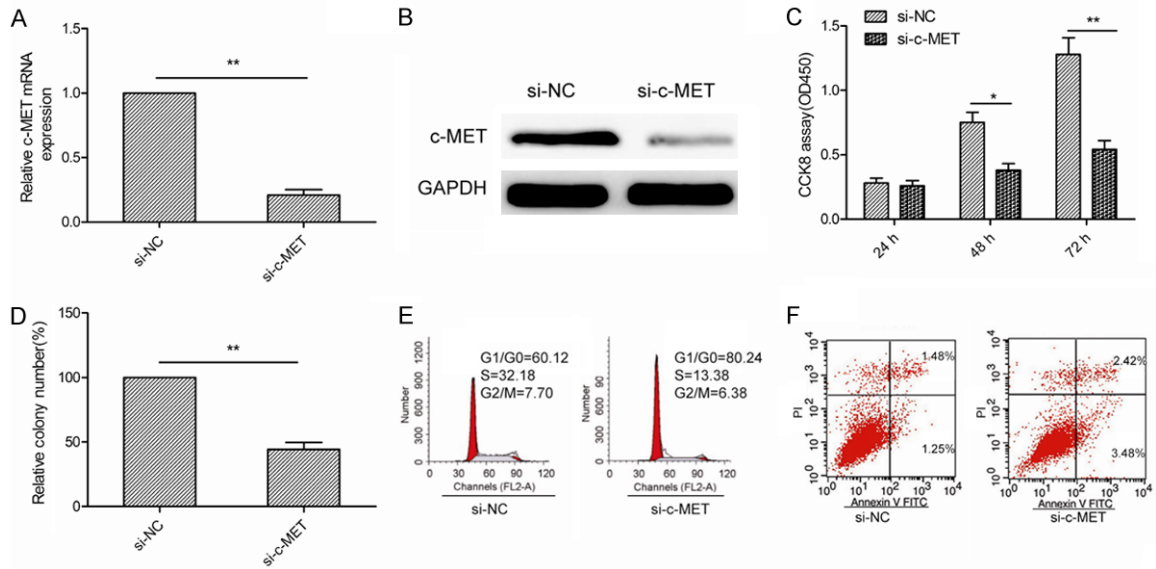


Figure 4. *c-MET* silencing inhibits cell proliferation and induces apoptosis in MM cells. A, B. *c-MET* expression at the mRNA and protein level was determined by qRT-PCR and western blot analysis in MM1S cells transfected with si-*c-MET* or si-NC. GAPDH served as the loading control. C-F. Cell proliferation, colony formation, cell cycle stage, and apoptosis were assessed in MM1S cells transfected with si-*c-MET* or si-NC. * $P < 0.05$, ** $P < 0.01$.

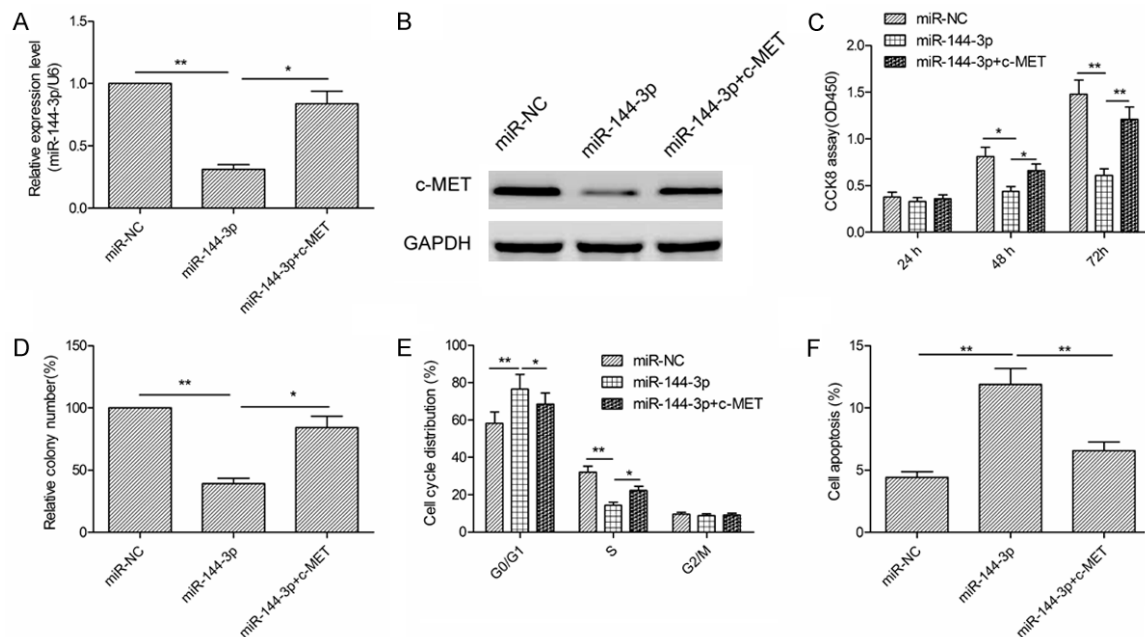


Figure 5. Overexpression of *c-MET* reverses the effect of miR-144-3p in MM cells. A, B. *c-MET* expression at the mRNA and protein level was determined in MM1S cells transfected with miR-144-3p mimic or miR-NC, and with or without pCDNA3.1-*c-MET* vector. GAPDH served as the loading control. C-F. Cell proliferation, colony formation, cell cycle stage, and apoptosis were assessed in MM1S cells transfected with miR-144-3p mimic or miR-NC, and with or without plasmid pCDNA3.1-*c-MET* vector.

expression plasmid pCDNA3.1-*c-MET* in miR-144-3p mimic-transfected MM1S cells (**Figure 5A** and **5B**). In addition, we found that overex-

pression of *c-MET* abolished the effects of cell proliferation, colony formation, cycle arrest, and apoptosis induced by miR-144-3p in MM1S

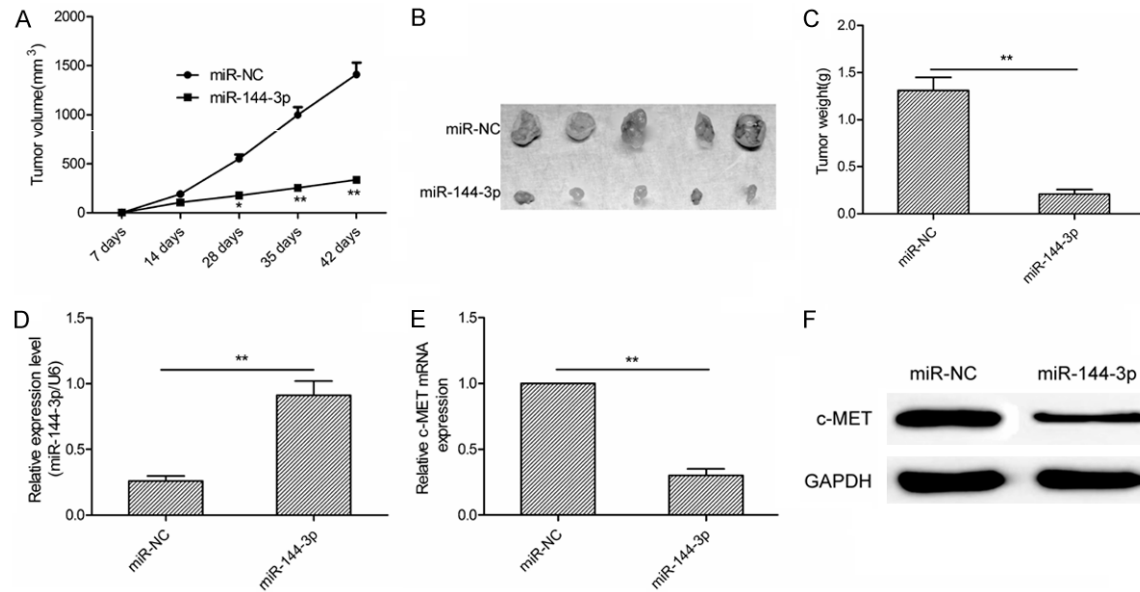


Figure 6. miR-144-3p inhibits tumor growth *in vivo* by targeting c-MET. A. Growth curves for tumor volumes in xenografts of nude mice in the MM1S/miR-144-3p and MM1S/miR-NC group. B. Photographs of tumor tissues from mice in the MM1S/miR-144-3p and MM1S/miR-NC group. C. Tumor tissue weights of mice in the MM1S/miR-144-3p and MM1S/miR-NC group. D. qRT-PCR analysis of miR-144-3p expression in tumor tissues obtained from mice in the MM1S/miR-144-3p and MM1S/miR-NC group. E, F. c-MET expression at the mRNA and protein level was determined in tumor tissues obtained from mice in the MM1S/miR-144-3p and MM1S/miR-NC group. GAPDH served as the loading control. *P < 0.05, **P < 0.01.

cells (Figure 5C-F). These data suggest that miR-144-3p may exert its biological role in MM cells by targeting c-MET.

miR-144-3p inhibits tumor growth *in vivo* by targeting c-MET

To determine whether miR-144-3p inhibits tumor growth *in vivo*, MM1S/miR-144-3p or MM1S/miR-NC cells were subcutaneously injected into nude mice and tumor growth was measured. It was found that tumor growth was slower in the MM1S/miR-144-3p group than in the MM1S/miR-NC group (Figure 6A). Mice were killed 42 days after injection and tumor tissue was isolated and weighed. Tumor size and weight in the MM1S/miR-144-3p group were significantly decreased than in the MM1S/miR-NC group (Figure 6B and 6C). We also investigated the expression of miR-144-3p and c-MET in tumor tissues, and found that miR-144-3p expression was upregulated (Figure 6D), whereas c-MET expression was downregulated at both the mRNA and protein level, in the MM1S/miR-144-3p group (Figure 6E and 6F). These results suggest that miR-144-3p suppresses tumor growth *in vivo* by targeting c-MET.

Discussion

Emerging evidence has indicated that miRNAs play crucial roles in the development and progression of MM by regulating the expression of genes involved in proliferation, cell cycle control, apoptosis, cell migration, and metastasis [10, 11], suggesting that investigating the biological role of miRNAs in MM might help identify novel diagnosis markers and therapeutic agents for MM. Here, a series of experiments were conducted to investigate the role of miRNA-144-3p in MM. Our study showed for the first time that miRNA-144-3p is downregulated in MM cell lines and plasma from patients with MM, and that miR-144-3p inhibits MM growth *in vitro* and *in vivo* by targeting c-MET. These findings suggest that miR-144-3p might serve as a novel therapeutic target for MM.

MiR-144-3p has been reported to be downregulated and function as a tumor suppressor in many types of cancers [12-17, 19, 20]. However, a recent report revealed that miR-144-3p expression is upregulated and that it functions as an oncogene in nasopharyngeal carcinoma [21]. Thus, the biological role of miR-144-3p in carcinogenesis seems to be complex and highly

tissue specific. Here, we found that the expression of miR-144-3p was downregulated in human MM cell lines and plasma from MM patients. Moreover, introduction of miR-144-3p reduced MM cell proliferation and colony formation, induced cell arrest at the G0/G1 phase and apoptosis, and suppressed tumor growth *in vivo*. These results suggest that miR-144-3p might act as a tumor suppressor in MM.

It is well known that miRNAs control biological functions by inhibiting the expression of their target genes [5]; therefore, elucidation of these targets is crucial in studying the role of miRNAs in cancer. To predict the target genes of miR-144-3p, three bioinformatic databases (Target-Scan, miRanda, and PicTar) were employed. *c-MET* (also known as *MET*) was selected as a target gene owing to its known role in promoting tumorigenesis and cancer progression [22, 23]. *c-MET*, a receptor for hepatocyte growth factor (HGF) [24], has been found to be involved in various biological processes such as cellular proliferation, migration, and invasion [25, 26]. It has been reported that *c-MET* expression is elevated in lung, ovarian, rectum, and prostate cancer, and that it functions as an oncogene by enhancing carcinogenesis, cancer invasion, proliferation, and metastasis [22-26]. *c-MET* has been implicated in the development and progression of multiple cancers by regulating several downstream signaling pathways such as STAT3, ERK1/2, and PI3K/AKT [27, 28]. Moreover, previous studies have reported that overexpression of *c-MET* mRNA in plasma from patients with MM is associated with poor response to therapy and poor clinical outcome [29], and that overexpression of *c-MET* could promote MM progression and development [23]. These findings suggest that *c-MET* functions as an oncogene in MM. *c-MET* has been shown to be a target of miR-144-3p in glioblastoma [13], gastric cancer [20], and uveal melanoma [30], but the interaction between miR-144-3p and *c-MET* has not been experimentally validated in MM. Using a luciferase reporter assay, qRT-PCR, and western blotting, the present study has revealed that *c-MET* is a direct target of miR-144-3p. Overexpression of miR-144-3p significantly inhibited *c-MET* expression and its downstream pathway (PI3K/AKT). Furthermore, our data show that knockdown of *c-MET* has an effect similar to that of miR-144-3p overexpression in MM cells, whereas over-

expression of *c-MET* reverses miR-144-3p-mediated suppression of MM cells growth. *In vivo* experiments demonstrated that miR-144-3p overexpression suppresses tumor growth by regulating *c-MET*. These studies suggest that miR-144-3p exerts a biological role in MM by regulating *c-MET*.

In conclusion, the present study has demonstrated that miR-144-3p is downregulated in MM cell lines and patient plasma. Further investigation revealed that ectopic expression of miR-144-3p inhibits cell proliferation and colony formation, induces cell cycle arrest at the G0/G1 phase and apoptosis, as well as suppresses tumor growth *in vivo* by repressing *c-MET*. These results indicate that miR-144-3p may be a potential therapeutic target for MM.

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Disclosure of conflict of interest

None.

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References

- [1] Barille-Nion S, Barlogie B, Bataille R, Bergsagel PL, Epstein J, Fenton RG, Jacobson J, Kuehl WM, Shaughnessy J and Tricot G. Advances in biology and therapy of multiple myeloma. *Hematology Am Soc Hematol Educ Program* 2003; 248-278.
- [2] Hideshima T, Richardson PG and Anderson KC. Mechanism of action of proteasome inhibitors and deacetylase inhibitors and the biological basis of synergy in multiple myeloma. *Mol Cancer Ther* 2011; 10: 2034-2042.
- [3] Tricot G, Barlogie B and Van Rhee F. Treatment advances in multiple myeloma. *Br J Haematol* 2004; 125: 24-30.
- [4] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004; 116: 281-297.
- [5] Malan-Muller S, Hemmings SM and Seedat S. Big effects of small RNAs: a review of micro-

- NAs in anxiety. *Mol Neurobiol* 2013; 47: 726-739.
- [6] McManus MT. MicroRNAs and cancer. *Semin Cancer Biol* 2003; 13: 253-258.
- [7] Farazi TA, Spitzer JI, Morozov P and Tuschl T. miRNAs in human cancer. *J Pathol* 2011; 223: 102-115.
- [8] Garzon R and Marcucci G. Potential of microRNAs for cancer diagnostics, prognostication and therapy. *Curr Opin Oncol* 2012; 24: 655-659.
- [9] Tie J, Fan D. Big roles of microRNAs in tumorigenesis and tumor development. *Histol Histopathol* 2011; 26: 1353-1361.
- [10] Abdi J, Jian H and Chang H. Role of micro-RNAs in drug resistance of multiple myeloma. *Oncotarget* 2016; 7: 60723-60735.
- [11] Abdi J, Qiu L and Chang H. Micro-RNAs, New performers in multiple myeloma bone marrow microenvironment. *Biomark Res* 2014; 2: 10.
- [12] Liu F, Chen N, Xiao R, Wang W and Pan Z. miR-144-3p serves as a tumor suppressor for renal cell carcinoma and inhibits its invasion and metastasis by targeting MAP3K8. *Biochem Biophys Res Commun* 2016; 480: 87-93.
- [13] Zhang SY, Lu ZM, Lin YF, Chen LS, Luo XN, Song XH, Chen SH and Wu YL. miR-144-3p, a tumor suppressive microRNA targeting ETS-1 in laryngeal squamous cell carcinoma. *Oncotarget* 2016; 7: 11637-11650.
- [14] Lan F, Yu H, Hu M, Xia T and Yue X. miR-144-3p exerts anti-tumor effects in glioblastoma by targeting c-Met. *J Neurochem* 2015; 135: 274-286.
- [15] Huo F, Zhang C, He H and Wang Y. MicroRNA-144-3p inhibits proliferation and induces apoptosis of human salivary adenoid carcinoma cells via targeting of mTOR. *Biotechnol Lett* 2016; 38: 409-416.
- [16] Fan JY, Yang Y, Xie JY, Lu YL, Shi K and Huang YQ. MicroRNA-144 mediates metabolic shift in ovarian cancer cells by directly targeting Glut1. *Tumour Biol* 2016; 37: 6855-6860.
- [17] Yu L, Yang Y, Hou J, Zhai C, Song Y, Zhang Z, Qiu L and Jia X. MicroRNA-144 affects radiotherapy sensitivity by promoting proliferation, migration and invasion of breast cancer cells. *Oncol Rep* 2015; 34: 1845-1852.
- [18] Li B, Yang XX, Wang D and Ji HK. MicroRNA-138 inhibits proliferation of cervical cancer cells by targeting c-Met. *Eur Rev Med Pharmacol Sci* 2016; 20: 1109-1114.
- [19] Huang J, Shi Y, Li H, Yang M and Liu G. MicroRNA-144 acts as a tumor suppressor by targeting Rho-associated coiled-coil containing protein kinase 1 in osteosarcoma cells. *Mol Med Rep* 2015; 12: 4554-4559.
- [20] Liu J, Xue H, Zhang J, Suo T, Xiang Y, Zhang W, Ma J, Cai D and Gu X. MicroRNA-144 inhibits the metastasis of gastric cancer by targeting MET expression. *J Exp Clin Cancer Res* 2015; 34: 35.
- [21] Zhang LY, Ho-Fun Lee V, Wong AM, Kwong DL, Zhu YH, Dong SS, Kong KL, Chen J, Tsao SW, Guan XY and Fu L. MicroRNA-144 promotes cell proliferation, migration and invasion in nasopharyngeal carcinoma through repression of PTEN. *Carcinogenesis* 2013; 34: 454-463.
- [22] Zhang J and Babic A. Regulation of the MET oncogene: molecular mechanisms. *Carcinogenesis* 2016; 37: 345-355.
- [23] Gambella M, Palumbo A and Rocci A. MET/HGF pathway in multiple myeloma: from diagnosis to targeted therapy? *Expert Rev Mol Diagn* 2015; 15: 881-893.
- [24] Skead G and Govender D. Gene of the month: MET. *J Clin Pathol* 2015; 68: 405-409.
- [25] Marano L, Chiari R, Fabozzi A, De Vita F, Boccardi V, Roviello G, Petrioli R, Marrelli D, Roviello F and Patriti A. c-Met targeting in advanced gastric cancer: an open challenge. *Cancer Lett* 2015; 365: 30-36.
- [26] Ho-Yen CM, Jones JL and Kermorgant S. The clinical and functional significance of c-Met in breast cancer: a review. *Breast Cancer Res* 2015; 17: 52.
- [27] Boccaccio C, Luraghi P and Comoglio PM. MET-mediated resistance to EGFR inhibitors: an old liaison rooted in colorectal cancer stem cells. *Cancer Res* 2014; 74: 3647-3651.
- [28] Mazzone M and Comoglio PM. The Met pathway: master switch and drug target in cancer progression. *FASEB J* 2006; 20: 1611-1621.
- [29] Rocci A, Gambella M, Aschero S, Baldi I, Trusolino L, Cavallo F, Gay F, Larocca A, Magarotto V, Omede P, Isaia G, Bertotti A, Liberati AM, Catalano L, De Rosa L, Musto P, Vallone R, Falcone A, Drandi D, Ladetto M, Comoglio PM, Boccadoro M and Palumbo A. MET dysregulation is a hallmark of aggressive disease in multiple myeloma patients. *Br J Haematol* 2014; 164: 841-850.
- [30] Sun L, Bian G, Meng Z, Dang G, Shi D and Mi S. MiR-144 inhibits uveal melanoma cell proliferation and invasion by regulating c-met expression. *PLoS One* 2015; 10: e0124428.