

Dysregulated circular RNAs are closely linked to multiple myeloma prognosis, with circ_0026652 predicting bortezomib-based treatment response and survival via the microRNA-608-mediated Wnt/ β -catenin pathway

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Abstract. Circular RNA (circRNA/circ) profiles have been suggested to be involved in the prognosis of several types of solid tumors and hematological malignancies, including multiple myeloma (MM). Therefore, the aim of the present study was to comprehensively explore the involvement of circRNA profiles in MM prognosis. A total of 60 patients with MM that underwent bortezomib-based induction therapy were enrolled. Next, eight patients with complete response (CR) and eight with no response (NR) were randomly selected to detect their circRNA profiles in bone marrow plasma cells (BMPCs) by microarray. Next, 10 candidate circRNAs were verified via reverse transcription-quantitative PCR (RT-qPCR) in the BMPCs of 60 patients with MM. Finally,

the molecular mechanism of circ_0026652 knockdown underlying the regulation of chemosensitivity to bortezomib was assessed. Microarray showed that 79 circRNAs were upregulated and 167 were downregulated in CR compared with NR cases, which were found to be enriched in carcinogenic and chemoresistance-related pathways (Wnt, mTOR and MAPK pathways). RT-qPCR showed that 8/10 circRNAs (circ_0026652, circ_0068708, circ_0088128, circ_0001566, circ_0031113, circ_0083587, circ_0005552 and circ_0007171) were associated with treatment response [CR or objective response rate (ORR)] and 5/10 circRNAs (circ_0026652, circ_0068708, circ_0001566, circ_0031113 and circ_0005552) were associated with progression-free survival (PFS) or overall survival (OS). Of note, circ_0026652 was a key prognostic marker simultaneously associated with CR, ORR, PFS and OS. Cellular experiments showed that circ_0026652 knockdown enhanced chemosensitivity to bortezomib through the microRNA (miR)-608-mediated Wnt/ β -catenin pathway in U266 and RPIM-8226 cells. In conclusion, dysregulated circRNA profiles were closely associated with MM prognosis, with circ_0026652 being linked to bortezomib-based treatment response and survival through the miR-608-mediated Wnt/ β -catenin pathway.

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Abbreviations: MM, Multiple myeloma; PFS, progressive-free survival; OS, overall survival; CircRNAs, Circular RNAs; miRNAs, microRNAs; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; IDA, iron deficiency anemia; CR, complete response; NR, non-response; IMWG, International Myeloma Working Group; ISS, International Staging System; VGPR, very good partial response; SD, stable disease; PD, progressive disease; FC, fold-change; PCA, principal component analysis; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; HP, Human Phenotype; IQR, interquartile range

Key words: multiple myeloma, circular RNA profiles, circular RNA 0026652, prognosis, chemosensitivity to bortezomib

Introduction

Multiple myeloma (MM) is a heterogenous hematological malignancy that arises from the clonal proliferation of plasma cells in the bone marrow, which is frequently accompanied by the secretion of monoclonal immunoglobulin in serum and/or urine (1). Clinically, patients with MM experience hypercalcemia, renal insufficiency, anemia and bony lesions (2). Despite the current lack of a definitive cure for MM, the introduction of immunomodulatory drugs and proteasome inhibitors, as well as the implementation of hematopoietic stem cell transplantation in suitable candidates, have substantially improved both progressive-free survival (PFS) and overall survival (OS) in patients with MM (1,2). However, the vast majority of patients with MM inevitably relapse after one or more treatment

regimens, or become refractory to the treatments due to drug resistance and treatment-related toxicities (3,4). Advancements in next-generation sequencing technologies and bioinformatics tools have facilitated the research of the pathophysiology underlying MM and the identification of novel treatment targets, as well as potential biomarkers for patients with MM.

Circular RNAs (circRNAs/circs), a group of single-stranded non-coding RNAs, are covalently closed ring structures that lack 5' end caps and 3' polyadenylated tails, are highly stable and have cell- and tissue-specific features (5,6). In recent years, numerous studies have uncovered the circRNA expression pattern, regulatory network and potential function [such as the regulation of important cellular events by sponging microRNAs (miRNAs/miRs), proteins or DNA] in hematological malignancies, including MM (7-12). In MM, only two previous studies have explored the circRNA profiles of patients with MM and analyzed the clinical implications of certain dysregulated circRNAs in patients with MM (11,12). One study revealed that 382 circRNAs were dysregulated in patients with MM, compared with healthy participants. Further reverse transcription-quantitative PCR (RT-qPCR) validation showed that 10 circRNAs (including circ-PTK2, circ-RNF217, circ-RERE, circ-NAGPA, circ-KCNQ5, circ-AFF2, circ-WWC3, circ-DNAJC5, circ-KLHL2, circ-IQGAP1 and circ-AL137655) were dysregulated in patients with MM, compared with healthy participants (12). Another study reported that 147 circRNAs were dysregulated in patients with MM compared with patients with iron deficiency anemia (IDA), and circRNA_101237 was confirmed to be increased in patients with MM compared with patients with IDA (11). However, research regarding the comprehensive evaluation of circRNA profiles in the prognosis (such as treatment response to induction treatment and survival benefits) of patients with MM is still lacking.

In the present study, microarray and bioinformatics analyses were initially performed to assess the circRNA profiles in eight complete response (CR) and eight non-response (NR) MM cases. Subsequently, 10 candidate circRNAs (top five upregulated and top five downregulated circRNAs in CR compared with NR MM cases) were screened using microarray to validate their prognostic value via RT-qPCR in 60 patients with MM. Finally, since circ_0026652 was clinically observed to be a key prognostic marker in MM, the molecular mechanism of circ_0026652 knockdown on regulating MM chemosensitivity was further assessed.

Materials and methods

Study patients. A total of 60 newly diagnosed symptomatic patients with MM from Changzheng Hospital, Second Military Medical University (Shanghai, China) between January 2017 and December 2019 were enrolled in the present study. Patients were eligible for inclusion if they had a confirmed diagnosis of symptomatic MM, in accordance with the International Myeloma Working Group (IMWG) criteria (13), were >18 years old and agreed to pre-treatment bone marrow sample collection. Patients were excluded from the study if they had any other hematologic malignancies, bone marrow diseases or cancer, had previously undergone hematopoietic stem cell transplantation, or had a history of radiotherapy

or chemotherapy. The present study was approved by the Institutional Review Board of Changzheng Hospital, Second Military Medical University (approval no. 2017SL002), and all patients provided written informed consent.

Sampling and data collection. Bone marrow samples were collected from the patients prior to the initiation of induction therapy. Plasma cells were separated from the bone marrow samples using CD138⁺ Plasma Cell Isolation Kit (Miltenyi Biotec GmbH) and were stored in liquid nitrogen for further detection. The clinical data of patients, including age, sex, immunoglobulin subtype, disease features, laboratory findings, Durie-Salmon stage (14), International Staging System (ISS) (14) and cytogenetics abnormalities, were recorded following the initial examinations. All patients were treated with 3-4 cycles of bortezomib-based induction treatment (bortezomib/lenalidomide/dexamethasone and bortezomib/cyclophosphamide/dexamethasone; usually four cycles), according to the IMWG Guidelines (15). Treatment response was evaluated using the IMWG uniform response criteria (16,17) and classified as CR, very good partial response (VGPR), partial response (PR), stable disease (SD) and progressive disease (PD). The objective response rate (ORR) was defined as CR + VGPR + PR. NR was defined as SD + PD. Patients who died during induction therapy or whose response to induction therapy was not recorded for any reason were excluded from the final analysis. In addition, all patients were continuously followed up in accordance with the IMWG Guidelines (15). PFS and OS were then evaluated based on the follow-up records (the date of the last follow-up was Jan 31, 2020). The PFS was defined as the time elapsed between the start of the treatment and disease progression or death, whichever came first. OS was defined as the time elapsed between the start of the treatment and death.

Study design. Plasma cell samples from eight CR cases and eight NR cases (age- and sex-matched) were selected for circRNA microarray analysis aimed at identifying the dysregulated circRNAs. Next, the top five upregulated and top five downregulated circRNAs were screened out from the dysregulated circRNAs by ranking the absolute value of Log₂ fold-change (FC). Detailed information of the 10 candidate circRNAs is listed in Table I. Subsequently, the expression of these 10 circRNAs was further determined via RT-qPCR in plasma cell samples from 60 patients with MM to verify its association with treatment response and survival profiles. Following clinical data analysis, circ_0026652 was found to be associated with the treatment response and survival of patients with MM. Next, *in vitro* experiments were carried out to study the role and potential mechanisms of circ_0026652 in regulating chemosensitivity to bortezomib.

Total RNA extraction and purification. Total RNA was extracted from the plasma cell samples using RNeasy Protect Mini Kit (Qiagen GmbH), according to the manufacturer's instructions. The purity and concentration of total RNA were then determined using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Inc.), and the integrity of the RNA was measured using 1.2% denaturing agarose gel electrophoresis. Next, the RNA was processed using RNase-Free

Table I. Top 10 dysregulated (five upregulated and five downregulated) circRNAs in microarray assay.

CircRNAs	Probe ID	Type	Chrom	Strand	Start	End	Log ₂ FC	P-value	P _{adj} value	Trend
Circ_0026652	ASCRP001444	Exonic	chr12	+	53856276	53862616	-3.41	5.33x10 ⁻⁷	1.87x10 ⁻⁴	DOWN
Circ_0068708	ASCRP003866	Exonic	chr3	-	197009549	197009716	-3.08	8.18x10 ⁻⁵	3.32x10 ⁻³	DOWN
Circ_0088128	ASCRP005132	Exonic	chr9	+	116018393	116019465	-3.01	4.26x10 ⁻⁷	1.62x10 ⁻⁴	DOWN
Circ_0001566	ASCRP004311	Exonic	chr5	-	179688683	179707608	-2.90	9.33x10 ⁻⁸	6.28x10 ⁻⁵	DOWN
Circ_0005327	ASCRP001893	Exonic	chr15	-	60674540	60678285	-2.76	1.45x10 ⁻⁸	3.31x10 ⁻⁵	DOWN
Circ_0031113	ASCRP001671	Exonic	chr14	-	20863608	20864108	3.06	3.21x10 ⁻⁵	2.00x10 ⁻³	UP
Circ_0083587	ASCRP004820	Exonic	chr8	+	21958950	21959041	2.72	4.15x10 ⁻³	4.27x10 ⁻²	UP
Circ_0005552	ASCRP003051	Exonic	chr2	+	63206322	63223901	2.55	6.30x10 ⁻⁵	2.82x10 ⁻³	UP
Circ_0007171	ASCRP002789	Exonic	chr19	+	17264776	17267848	2.24	4.73x10 ⁻⁵	2.35x10 ⁻³	UP
Circ_0007521	ASCRP004957	Exonic	chr8	-	1.42E+08	1.42E+08	2.00	4.47x10 ⁻⁶	6.39x10 ⁻⁴	UP

Top five upregulated and five downregulated circRNAs were selected by the rank of absolute value of Log₂FC. circRNA, circular RNA; ID, identification; chrom, chromosome; FC, fold-change; adj, adjust; +, sense; -, antisense.

DNase Set (Qiagen GmbH) for purification, followed by linear RNA digestion using RNase R (Epicentre; Illumina, Inc.).

CircRNA microarray analysis. For microarray analysis, purified RNA samples from eight CR and eight NR cases were subjected to amplification and transcribed into fluorescent cRNA using Arraystar Super RNA Labeling Kit (Arraystar, Inc.), and the labeled cRNAs were purified using RNeasy Mini Kit (Qiagen GmbH). Subsequently, the samples were hybridized using Arraystar Human CircRNA Array v1 (AS-S-CR-H-V2.0; Arraystar) and Gene Expression Microarray Hybridization Kit (Agilent Technologies, Inc.), according to the manufacturer's instructions in an Agilent Hybridization Oven (Agilent Technologies, Inc.). Following hybridization, the hybridized arrays were scanned using an Agilent Microarray Scanner (Agilent Technologies, Inc.) and resulting images were analyzed in Agilent Feature Extraction software (Version 11.0.1.1; Agilent Technologies, Inc.) to obtain raw data.

Bioinformatics analysis. Quantile normalization and low-intensity filtering were performed using R software package (R Version 3.1.2) (18). Next, the circRNAs detected in >50% samples were subjected to further analysis. The principal component analysis (PCA) plots of circRNA profiles were constructed using Factoextra package (Version 3.3.3) (19), and a heatmap of circRNA profiles was plotted using Pheatmap package (Version 1.0.8) (20). The FC of circRNA expression was calculated, and the circRNA expression difference between CR and NR cases was determined using an unpaired t-test. The P-value was corrected using the Benjamini-Hochberg method, which was marked as P_{adj}-value. The dysregulated circRNAs were defined as the circRNAs with a FC ≥2.0 (or FC≤0.5) and P_{adj}<0.05, as shown by volcano plots with R (Version 3.1.2). Gene Ontology (GO) annotation of dysregulated circRNAs based on located genes was obtained from the GO database (<http://www.geneontology.org/>), and pathway annotation of dysregulated circRNAs based on located genes was obtained from Kyoto Encyclopedia of Genes and Genomes (KEGG) database (<https://www.kegg.jp/>). The target miRNAs of dysregulated circRNAs were predicted using miRNA target prediction software miRanda (Version 1.0b) (<http://www.microrna.org/>). Annotation of the target miRNAs was performed using GO resource, KEGG, Human Phenotype Ontology (HP; <https://hpo.jax.org/>) and Disease Ontology Identification (DOID; <http://www.disease-ontology.org/>) databases. Enrichment analysis was performed using Fisher's exact test. In addition, a circRNA-miRNA network of the top five upregulated and top five downregulated circRNAs was established through miRanda database.

Cell culture. Human U266 and RPMI-8226 MM cell lines were purchased from the American Type Culture Collection and cultured in RPMI-1640 medium (Thermo Fisher Scientific, Inc.) containing 10% FBS (Thermo Fisher Scientific, Inc.), 100 U/ml penicillin and 100 µg/ml streptomycin (Thermo Fisher Scientific, Inc.) at 37°C with 5% CO₂.

Transfection. Negative control (NC) small interfering RNA (siRNA; Guangzhou RiboBio Co., Ltd.) (50 pM) and circ_0026652 siRNA (Guangzhou RiboBio Co., Ltd.) (50 pM)

were transfected into 2×10^5 U266 and RPMI-8226 cells using Lipofectamine® 2000 transfection reagent (Invitrogen; Thermo Fisher Scientific, Inc.) at 37°C for 6 h. Following transfection, the cells were divided into si-NC and si-circ cells. The U266 and RPMI-8226 cells without transfection were used as the control cells. A total of 24 h after transfection, the expression of circ_0026652 in control, NC and circ(-) cells were evaluated using RT-qPCR. The sequences were as follows: circ_0026652 siRNA sense, 5'-ACUCACCAUCCAAACGAUUU-3' and antisense, 5'-UUUGAGUGGUAAGGUUUGCUA-3'; and NC siRNA sense, 5'-GAAUUAUUAAGAUGGCCCGUUGUACU-3' and antisense, 5'-UCAUCGAAGUUAUAGGGAUACAUUACGUGAUC-3'.

Chemosensitivity detection. Following transfection, cells were cultured with different concentrations of bortezomib (Selleck Chemicals) for 48 h (the concentration range of U266 and RPMI-8226 cells were 0-16 and 0-4 nM, respectively) at 37°C. Next, a Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Inc.) assay was used to evaluate cell viability, according to the manufacturer's instructions. Relative cell viability was calculated as previously described (21). Furthermore, 4 nM bortezomib was used to treat U266 cells for 24, 48 and 72 h, and 1 nM bortezomib was used to treat RPMI-8226 cells for 24, 48 and 72 h at 37°C, followed by relative cell viability detection.

Cell apoptosis assessment. Following transfection, cells were incubated with bortezomib (Selleck Chemicals; 4 nM for U266 cells, 1 nM for RPMI-8226 cells) for 48 h. The bortezomib concentration for cell incubation was selected using the IC₅₀, as shown in the 'Chemosensitivity detection' subsection. Following incubation, Annexin V-FITC Apoptosis Detection Kit (MilliporeSigma) was used to determine cell apoptosis, according to the manufacturer's instructions. The data were collected by FACSCalibur flow cytometer (Becton, Dickinson and Company) and analyzed by Flowjo 7.6 (Becton, Dickinson and Company).

Target miRNA assessment. Using miRanda, miR-608, miR-762 and miR-939 were identified as the potential targets of circ_0026652. RT-qPCR was performed to assess the expression of miR-608, miR-762 and miR-939 at 24 h after transfection.

Rescue experiment. The control, si-NC and si-circ cells were constructed as described in the 'Transfection' subsection. Next, 50 pM NC inhibitor (Guangzhou RiboBio Co., Ltd.) and 50 pM miR-608 inhibitor (Guangzhou RiboBio Co., Ltd.) were transfected into si-NC or si-circ cells using Lipofectamine 2000 for 6 h at 37°C. The cells were termed as si-NC + NC-inhibitor, si-NC + miR-inhibitor, si-circ + NC-inhibitor and si-circ + miR-inhibitor cells. At 24 h after transfection, the expression of circ_0026652 and miR-608 in the control, si-NC + NC-inhibitor, si-NC + miR-inhibitor, si-circ + NC-inhibitor and si-circ + miR-inhibitor cells was determined via RT-qPCR. Following transfection (24 h), chemosensitivity was detected as described in the 'Chemosensitivity detection' subsection, and cell apoptosis was evaluated with the method described

in 'Cell apoptosis assessment' subsection. Furthermore, to detect the transfection efficiency, 50 pM NC inhibitor alone and 50 pM miR-608 inhibitor alone were transfected into 2×10^5 U266 and RPMI-8226 cells using Lipofectamine 2000 transfection reagent for 6 h at 37°C, followed by detection of miR-608 expression via RT-qPCR. The sequences were as follows: miR-608 inhibitor, 5'-ACGGAGCUGUCCCAACAC CACCCCU-3'; and NC inhibitor, 5'-CAGUACUUUUGU GUAGUACAA-3'.

Pathway assessment. In previous studies, miR-608 was reported to regulate the Wnt/ β -catenin pathway in carcinomas (22). In other studies, the Wnt/ β -catenin pathway was reported to play a major role in the regulation of the chemosensitivity to bortezomib (23-25). Hence, the expression of Wnt family member 3A (WNT3A) and β -catenin in control, si-NC + NC-inhibitor, si-NC + miR-inhibitor, si-circ + NC-inhibitor and si-circ + miR-inhibitor cells was assessed via western blot analysis at 24 h after transfection.

RT-qPCR. Briefly, total RNA in cells was extracted using RNeasy Protect Mini Kit (Qiagen GmbH). The purity (A260/A280) range was 1.90-2.10 and the RNA integrity number was 7.5-9.0. For the detection of circRNAs, the linear RNA in isolated RNA was digested using RNase R (Illumina, Inc.) prior to cDNA synthesis. For the detection of miRNAs, GAPDH and U6, linear RNA digestion was not performed. Next, cDNA synthesis was carried out using PrimeScript™ RT reagent kit according to the manufacturer's protocol (Takara Bio, Inc.), followed by cDNA amplification using TB Green™ Fast qPCR Mix (Takara Bio, Inc.). The thermocycling conditions were as follows: Initial denaturation at 95°C for 30 seconds (sec); 40 cycles of 95°C for 5 sec and 61°C for 10 sec. Finally, the relative expression of circRNAs and miRNAs was calculated using the $2^{-\Delta\Delta C_q}$ method (26); GAPDH, which is a well-known and commonly used internal reference for circRNA detection, served as an internal reference for circRNAs, and U6 served as an internal reference for miRNAs (27-29). The primers used are listed in Table SI.

Western blot analysis. Protein in cells was extracted using RIPA Lysis and Extraction Buffer (Thermo Fisher Scientific, Inc.), and protein quantification was performed using Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, Inc.). Next, the 20 μ g protein was separated on 4-20% TruPAGE™ Precast Gels (MilliporeSigma) for electrophoresis and then transferred to a PVDF membrane (Pall Life Sciences). The membrane was blocked by 5% BSA (MilliporeSigma) at 37°C for 1 h, incubated with primary antibodies at 4°C overnight and then a secondary antibody for 90 min at room temperature. Finally, the protein was detected by chemiluminescence and visualized using Immobilon ECL Ultra Western HRP Substrate (MilliporeSigma). The antibodies used in western blot analysis are listed in Table SII.

Statistical analysis. Data are presented as the mean \pm SD, median interquartile range or number with percentage [n, (%)]. For the analysis of clinical data, Student's unpaired t-test or Wilcoxon rank-sum test was used to determine the difference in clinical features or the difference in circRNA expression

between two groups. Kaplan-Meier curve was used to evaluate PFS and OS, and log-rank test was used to determine the difference in PFS and OS between two groups. For the analysis of the experimental data, one-way ANOVA followed by Tukey's multiple comparisons was used for multiple comparisons between groups. Clinical data analysis was carried out using SPSS 21.0 statistical software (IBM Corp.) and experimental data analysis was performed using GraphPad Prism 7.02 (GraphPad Software Inc.). $P < 0.05$ was considered to indicate a statistically significant difference. The experiments were carried out in triplicate.

Results

Clinical characteristics of patients with MM. Of the 60 patients with MM, 26 (43.3%) were women and 34 (56.7%) were men, with a mean age of 54.1 ± 8.3 years (Table II). Among these patients with MM, 32 (53.3%), 14 (23.3%) and 14 (23.3%) cases secreted IgG, IgA or other IgG types, respectively. Furthermore, 33 (55.0%) and 28 (46.7%) cases exhibited bone lesion and renal impairment, respectively. In addition, seven (11.7%) and 53 (88.3%) cases presented with Durie-Salmon stage II and III, respectively. Finally, 14 (23.3%), 14 (23.3%) and 32 (53.3%) cases had ISS stage I, II and III, respectively. Detailed information on laboratory findings and cytogenetic abnormalities is displayed in Table II.

circRNA profiles in eight CR and eight NR cases. A total of eight CR cases were randomly selected from 16 patients who achieved CR using the random number method. A total of eight NR cases were randomly selected from 17 patients with NR using the random number method. These eight CR and eight NR cases were then subjected to circRNA microarray and bioinformatics analysis. A clear distinction in circRNA profiles between eight CR and eight NR cases was revealed by PCA plots, which suggested that CR cases could be distinguished from NR cases based on their circRNA profiles (Fig. 1A). Heatmap analysis of circRNA profiles revealed a relatively good consistency of circRNA profiles in CR and NR cases (Fig. 1B). Furthermore, volcano plots showed that 79 circRNAs were upregulated and 167 circRNAs were downregulated in patients with CR, compared with NR cases [$FC \geq 2.0$ (or $FC \leq 0.5$) and $P_{adj} < 0.05$; Fig. 1C].

Enrichment analyses. GO enrichment analysis of the located genes revealed that dysregulated circRNAs were enriched in various biological processes (such as 'positive regulation of cell proliferation', 'positive regulation of GTPase activity' and 'positive regulation of transcription, DNA-templated'), cellular components (such as 'cytosol', 'membrane' and 'perinuclear region of cytoplasm') and molecular functions (such as 'protein binding', ' β -catenin binding' and 'protein kinase binding'; Fig. 2A). KEGG enrichment analysis of the located genes showed that dysregulated circRNAs were mainly enriched in the 'Wnt signaling pathway', 'mTOR signaling pathway' and 'MAPK signaling pathway' (Fig. 2B). Furthermore, GO enrichment analysis of target miRNAs showed that dysregulated circRNAs were enriched in multiple biological processes (such as 'positive regulation of T cell-mediated cytotoxicity', 'translational termination'

Table II. Clinical features of patients with MM (n=60).

Clinical characteristics	Patients
Age, years, mean \pm SD	54.1 \pm 8.3
Sex, n (%)	
Female	26 (43.3)
Male	34 (56.7)
Immunoglobulin subtype, n (%)	
IgG	32 (53.4)
IgA	14 (23.3)
Other types	14 (23.3)
Disease features, n (%)	
Bone lesion	33 (55.0)
Renal impairment	28 (46.7)
Laboratory findings	
Hb, g/l, mean \pm SD	99.8 \pm 23.4
Calcium, mg/dl, mean \pm SD	9.7 \pm 2.2
Scr, mg/dl, median (IQR)	1.9 (1.6-2.2)
Albumin, g/l, median (IQR)	34.0 (29.0-37.0)
β 2-MG, mg/l, median (IQR)	6.1 (3.3-10.1)
LDH, U/l, median (IQR)	214.8 (175.4-244.3)
Durie Salmon stage, n (%)	
Stage II	7 (11.7)
Stage III	53 (88.3)
ISS stage, n (%)	
Stage I	14 (23.3)
Stage II	14 (23.3)
Stage III	32 (53.4)
Cytogenetics abnormalities, n (%)	
t (4; 14)	7 (11.7)
t (14; 16)	4 (6.7)
Del (17p)	9 (15.0)

MM, multiple myeloma; SD, standard deviation; IgG, immunoglobulin G; IgA, immunoglobulin A; Hb, hemoglobin; Scr, serum creatinine; IQR, interquartile range; β 2-MG, β 2-microglobulin; LDH, lactate dehydrogenase; ISS, International Staging System.

and 'translational initiation'), cellular components (such as 'cytosolic large ribosomal subunit', 'B cell receptor complex' and 'T cell receptor complex') and molecular function (such as 'protein binding involved in protein folding', 'methyltransferase activity' and 'satellite DNA binding'; Fig. 2C). KEGG/HP/DOID enrichment analysis of the target miRNAs revealed that the dysregulated circRNAs in acute myeloid leukemia were enriched in disease ontology (such as 'congenital nonspherocytic hemolytic anemia', 'alcoholic pancreatitis' and 'pilocytic astrocytoma of cerebellum'), HP (such as 'impaired neutrophil bactericidal activity', 'abnormality of neutrophil physiology' and 'increased prevalence of valvular diseases') and cellular signaling pathways (such as 'ribosome', 'RNA polymerase' and 'DNA replication'; Fig. 2D). So as to the detailed information of enrichment analyses, they are exhibited in Table SIII-SVI.

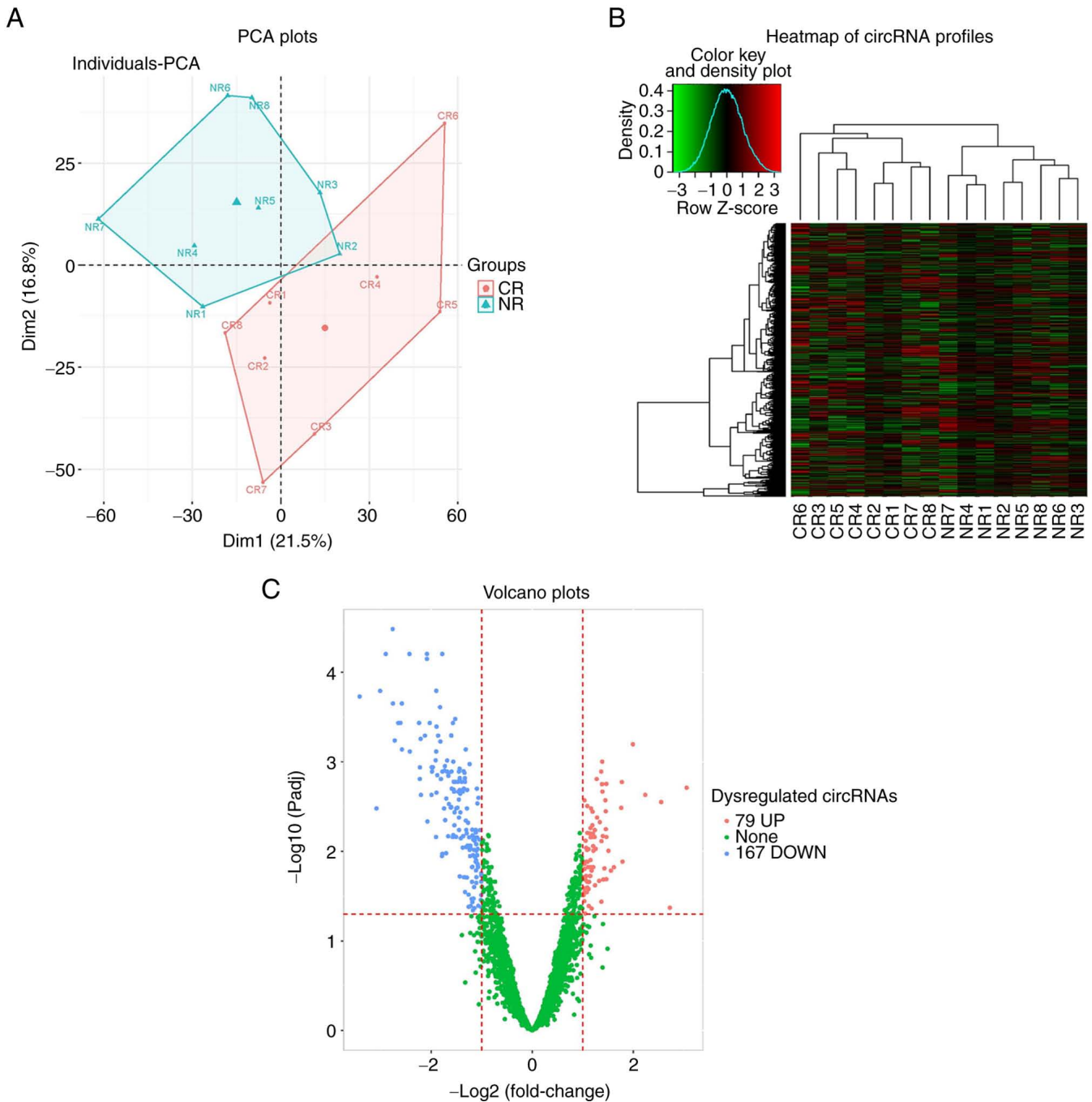


Figure 1. PCA plots, heatmap analysis and volcano plots. (A) PCA plots and (B) heatmap analysis of circRNA profiles between eight CR patients and eight NR patients. (C) Volcano plots of dysregulated circRNAs between eight CR patients and eight NR patients. PCA, principal component analysis; circRNA, circular RNA; CR, complete response; NR, no response.

CircRNA-miRNA network of top five upregulated and top five downregulated circRNAs. Among the dysregulated circRNAs, 10 candidate circRNAs (top five upregulated and top five downregulated) were further screened out by ranking the absolute value of Log_2FC . Then, circRNA-miRNA network of top five upregulated and top five downregulated circRNAs was constructed using the miRanda database. As shown in the circRNA-miRNA network, half of the dysregulated circRNAs (including circ_0026652, circ_0007171, circ_0007521, circ_0005552 and circ_0083587) had a large number of target miRNAs, while circ_0001566 and circ_0088128 had four target miRNAs; Circ_0031113 had three target miRNAs,

circ_0068708 had two target miRNAs and circ_0005327 did not have any (Fig. 3).

Validation of association between 10 candidate circRNAs and treatment response via RT-qPCR. To validate the association between 10 candidate circRNAs (screened out using microarray and bioinformatics analysis) and treatment response in 60 patients with MM, their expression was further assessed via RT-qPCR. A total of 16 cases (26.7%) achieved CR, 43 cases (71.7%) achieving ORR and 17 (28.3%) had NR (data not shown).

It was found that the expression levels of circ_0026652 ($P < 0.001$; Fig. 4A), circ_0068708 ($P = 0.017$; Fig. 4B),

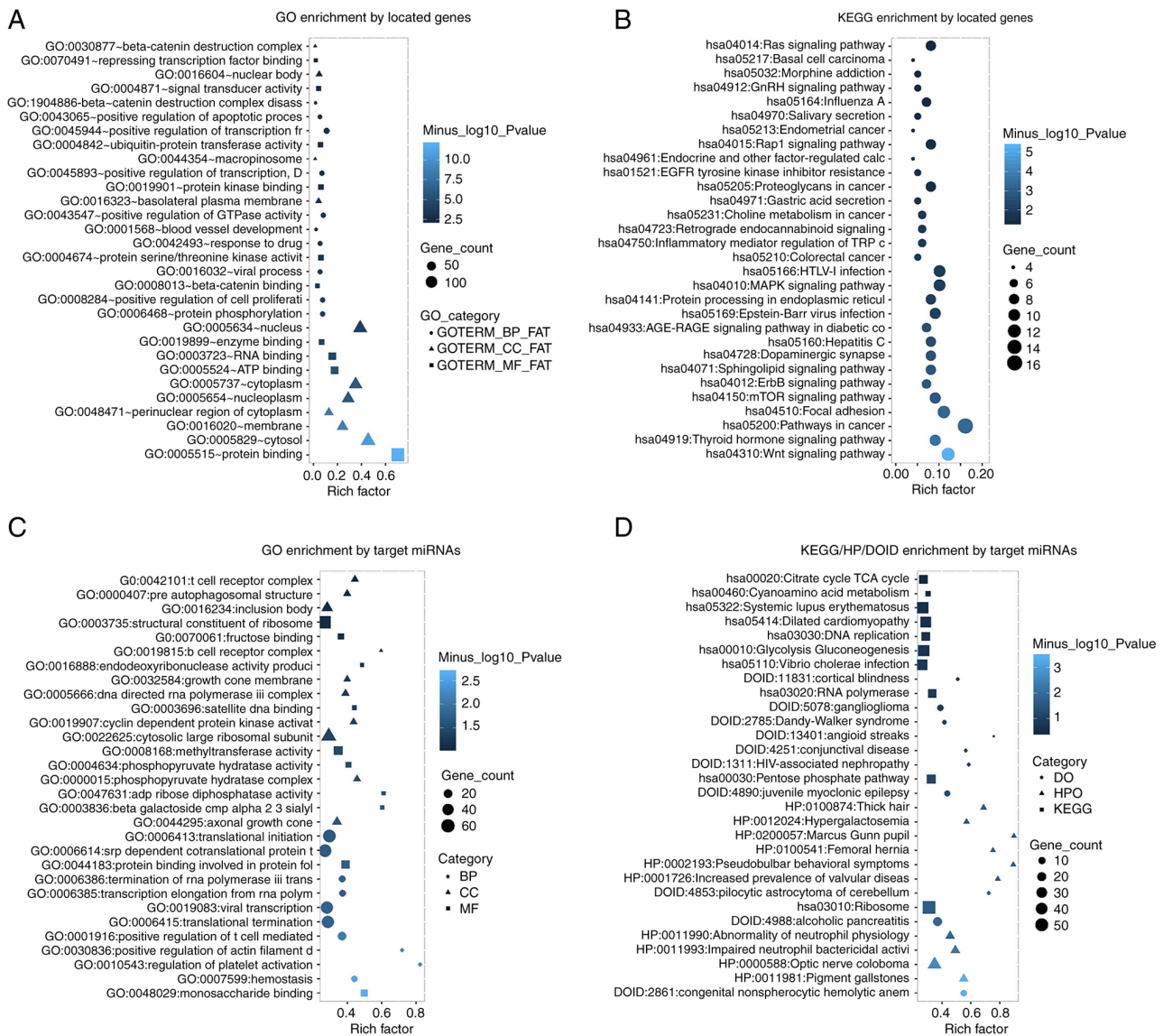


Figure 2. Enrichment analyses for dysregulated circRNAs. (A) GO and (B) KEGG enrichment analyses based on located genes of dysregulated circRNAs in patients with MM. (C) GO and (D) KEGG/HP/DOID enrichment analyses based on target miRNAs of dysregulated circRNAs in patients with MM. CircRNA, circular RNA; GO, Gene Ontology; KEGG, Kyoko Encyclopedia of Genes and Genomes; HP, Human Phenotype Ontology; DOID, Disease Ontology Identification; miRNAs, microRNAs; MM, multiple myeloma.

circ_0088128 ($P=0.009$; Fig. 4C) and circ_0001566 ($P=0.034$; Fig. 4D) were decreased, that of circ_0031113 ($P=0.001$; Fig. 4F), circ_0083587 ($P=0.011$; Fig. 4G), circ_0005552 ($P=0.028$; Fig. 4H) and circ_0007171 ($P=0.019$; Fig. 4I) were increased, and that of circ_0005327 ($P=0.068$; Fig. 4E) and circ_0007521 ($P=0.127$; Fig. 4J) showed no change in patients with CR compared with NR cases.

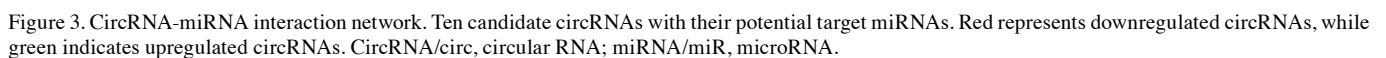
The expression levels of circ_0026652 ($P=0.002$; Fig. 5A), circ_0068708 ($P=0.045$; Fig. 5B) and circ_0088128 ($P=0.001$; Fig. 5C) were decreased, that of circ_0031113 ($P=0.007$; Fig. 5F), circ_0083587 ($P=0.041$; Fig. 5G), circ_0005552 ($P=0.010$; Fig. 5H) and circ_0007171 ($P=0.009$; Fig. 5I) were increased, and that of circ_0001566 ($P=0.159$; Fig. 5D), circ_0005327 ($P=0.093$; Fig. 5E) and circ_0007521 ($P=0.240$; Fig. 5J) showed no change in patients with CR compared with non-CR cases.

In addition, the expression levels of circ_0026652 ($P<0.001$; Fig. 6A) and circ_0001566 ($P=0.027$; Fig. 6D) were decreased, that of circ_0031113 ($P=0.015$; Fig. 6F) and

circ_0083587 ($P=0.012$; Fig. 6G) were increased, and that of circ_0068708 ($P=0.063$; Fig. 6B), circ_0088128 ($P=0.326$; Fig. 6C), circ_0005327 ($P=0.175$; Fig. 6E), circ_0005552 ($P=0.262$; Fig. 6H), circ_0007171 ($P=0.216$; Fig. 6I) and circ_0007521 ($P=0.171$; Fig. 6J) showed no change in patients with ORR compared with non-ORR cases.

Furthermore, it was also noted that these ten candidate circRNAs were of no difference between BLD regimen treated patients and BCD regimen treated patients (Fig. S1A-J). Besides, the treatment outcomes, such as response, PFS and OS, were of no difference between BLD regimen treated patients and BCD regimen treated patients (data not shown).

Validation of the association between 10 candidate circRNAs and survival via RT-qPCR. circ_0026652 high ($P=0.017$; Fig. 7A) and circ_0068708 high ($P=0.040$; Fig. 7B) expression were associated with a shorter PFS, circ_0031113 high ($P=0.019$; Fig. 7F) and circ_0005552 high ($P=0.014$; Fig. 7H)



circ_0026652 high (P=0.031; Fig. 7K) and circ_0001566 high (P=0.008; Fig. 7N) expression were associated with a short OS, while no association of circ_0068708 high (P=0.288; Fig. 7L), circ_0088128 high (P=0.102; Fig. 7M), circ_0005327 high (P=0.253; Fig. 7O), circ_0031113 high (P=0.051; Fig. 7P), circ_0083587 high (P=0.118; Fig. 7Q), circ_0005552 high (P=0.248; Fig. 7R), circ_0007171 high (P=0.355; Fig. 7S) and circ_0007521 high (P=0.897; Fig. 7T) expression with OS was observed.

In RPMI-8226 cells, circ_0026652 expression was lower in si-circ than in si-NC cells ($P<0.001$; Fig. 8E). Relative cell viability following treatment with 0.25, 0.5, 1, 2 and 4 nm

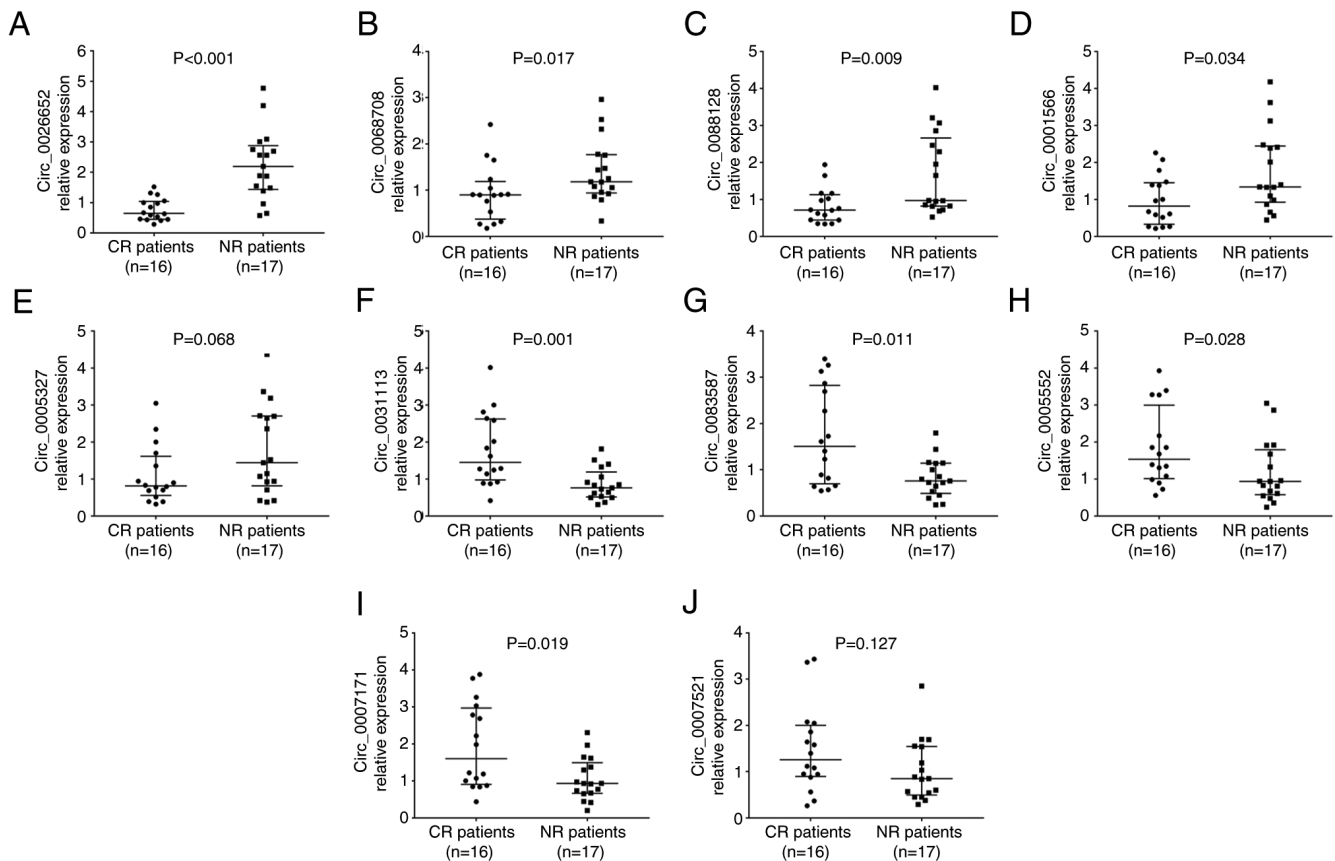


Figure 4. Ten candidate circRNAs between CR patients and NR patients. Comparisons of (A) circ_0026652, (B) circ_0068708, (C) circ_0088128, (D) circ_0001566, (E) circ_0005327, (F) circ_0031113, (G) circ_0083587, (H) circ_0005552, (I) circ_0007171 and (J) circ_0007521 expression levels between CR patients (n=16) and NR patients (n=17). CircRNA/circ, circular RNA; CR, complete response; NR, no response.

bortezomib was decreased in si-circ compared with si-NC cells (all $P<0.05$; Fig. 8F). In addition, following treatment with 1 nM bortezomib, relative cell viability was decreased in si-circ compared with si-NC cells at 24, 48 and 72 h (all $P<0.05$; Fig. S2B). Next, cell apoptosis was detected following treatment with 1 nM (IC_{50} value) bortezomib, and the results showed that the cell apoptosis rate was elevated in si-circ compared with si-NC cells ($P<0.01$; Fig. 8G and H).

Collectively, these data indicated that circ_0026652 knockdown promoted chemosensitivity to bortezomib in MM.

Circ_0026652 knockdown facilitates MM chemosensitivity to bortezomib by regulating the miR-608-mediated Wnt/ β -catenin pathway. In both U266 (Fig. 9A-C) and RPMI-8226 cells (Fig. 9D-F), miR-608 and miR-939 expression levels were increased (all $P<0.05$) and miR-762 expression showed no change (both $P>0.05$) in si-circ cells compared with si-NC cells. miR-608 has been reported to regulate the Wnt/ β -catenin pathway, which plays an essential role in the regulation of chemosensitivity to bortezomib (22-25), hence, further rescue experiments were performed to explore how the interaction between circ_0026652 and miR-608 regulated MM chemosensitivity to bortezomib. miR-608 inhibitor transfection efficiency is shown in Fig. S3A and B.

In the si-NC + miR-inhibitor and si-circ + miR-inhibitor U266 cells (Fig. 10A-E) and si-NC + miR-inhibitor and si-circ + miR-inhibitor RPMI-8226 cells (Fig. 10F-J),

miR-608 expression was decreased (all $P<0.01$) and did not affect circ_0026652 expression (all $P>0.05$). Furthermore, in the si-NC + miR-inhibitor and si-circ + miR-inhibitor groups, relative cell viability was increased (all $P<0.05$) and cell apoptosis was decreased (all $P<0.05$) following bortezomib treatment, thus indicating that the miR-608 inhibitor enhanced chemosensitivity to bortezomib.

In addition, the effect of circ_0026652 and miR-608 knockdown on the Wnt/ β -catenin pathway was assessed; in both U266 and RPMI-8226 cells, WNT3A and β -catenin protein expression was decreased in si-circ + NC-inhibitor compared with si-NC + NC-inhibitor cells and partially increased in si-NC + miR-inhibitor cells compared with si-NC + NC-inhibitor cells. Further rescue experiments showed that transfection with the miR-608 inhibitor compensated the suppressive effect of circ_0026652 knockdown on the protein expression of WNT3A and β -catenin to some extent (Fig. 11A and B).

Collectively, the results showed that circ_0026652 knockdown enhanced chemosensitivity to bortezomib by regulating the miR-608-mediated Wnt/ β -catenin pathway in MM.

Discussion

Due to their high stability, and cell- and tissue-specific expression patterns, the study of circRNA is a research hotspot, and previous studies using next-generation sequencing technologies

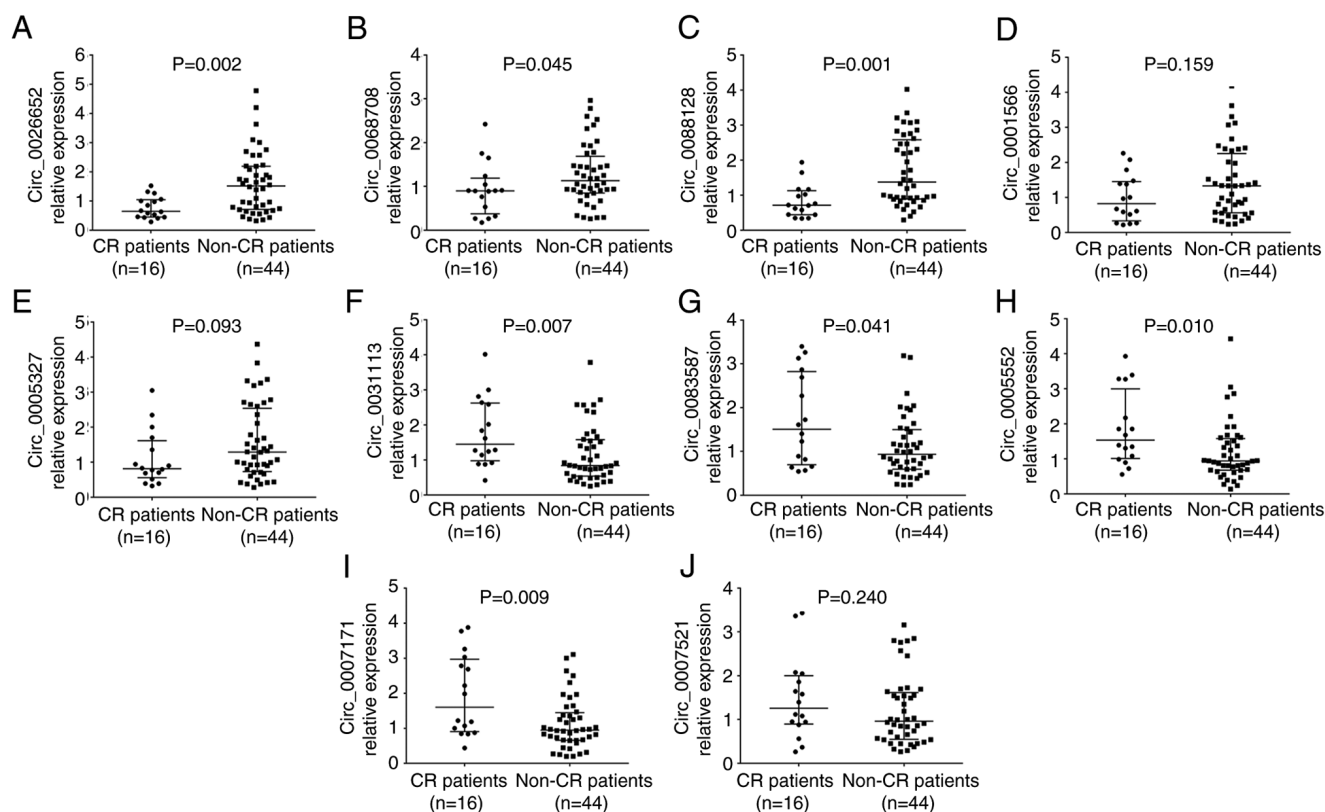


Figure 5. Ten candidate circRNAs between CR patients and non-CR patients. Comparisons of (A) circ_0026652, (B) circ_0068708, (C) circ_0088128, (D) circ_0001566, (E) circ_0005327, (F) circ_0031113, (G) circ_0083587, (H) circ_0005552, (I) circ_0007171 and (J) circ_0007521 expression levels between CR patients (n=16) and non-CR (n=44) patients. CircRNA/circ, circular RNA; CR, complete response.

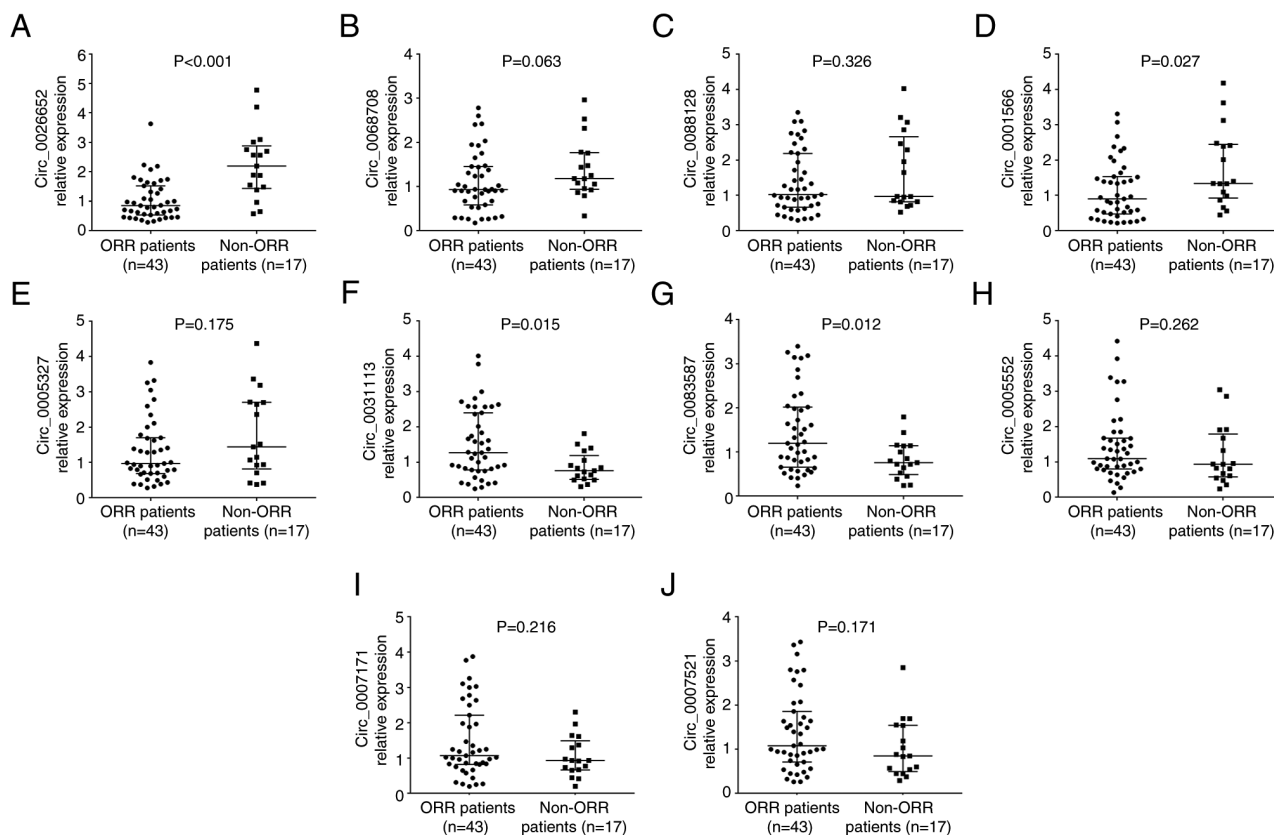


Figure 6. Ten candidate circRNAs between ORR patients and non-ORR patients. Comparisons of (A) circ_0026652, (B) circ_0068708, (C) circ_0088128, (D) circ_0001566, (E) circ_0005327, (F) circ_0031113, (G) circ_0083587, (H) circ_0005552, (I) circ_0007171 and (J) circ_0007521 expression levels between ORR patients (n=43) and non-ORR (n=17) patients. CircRNA/circ, circular RNA; ORR, objective response rate.

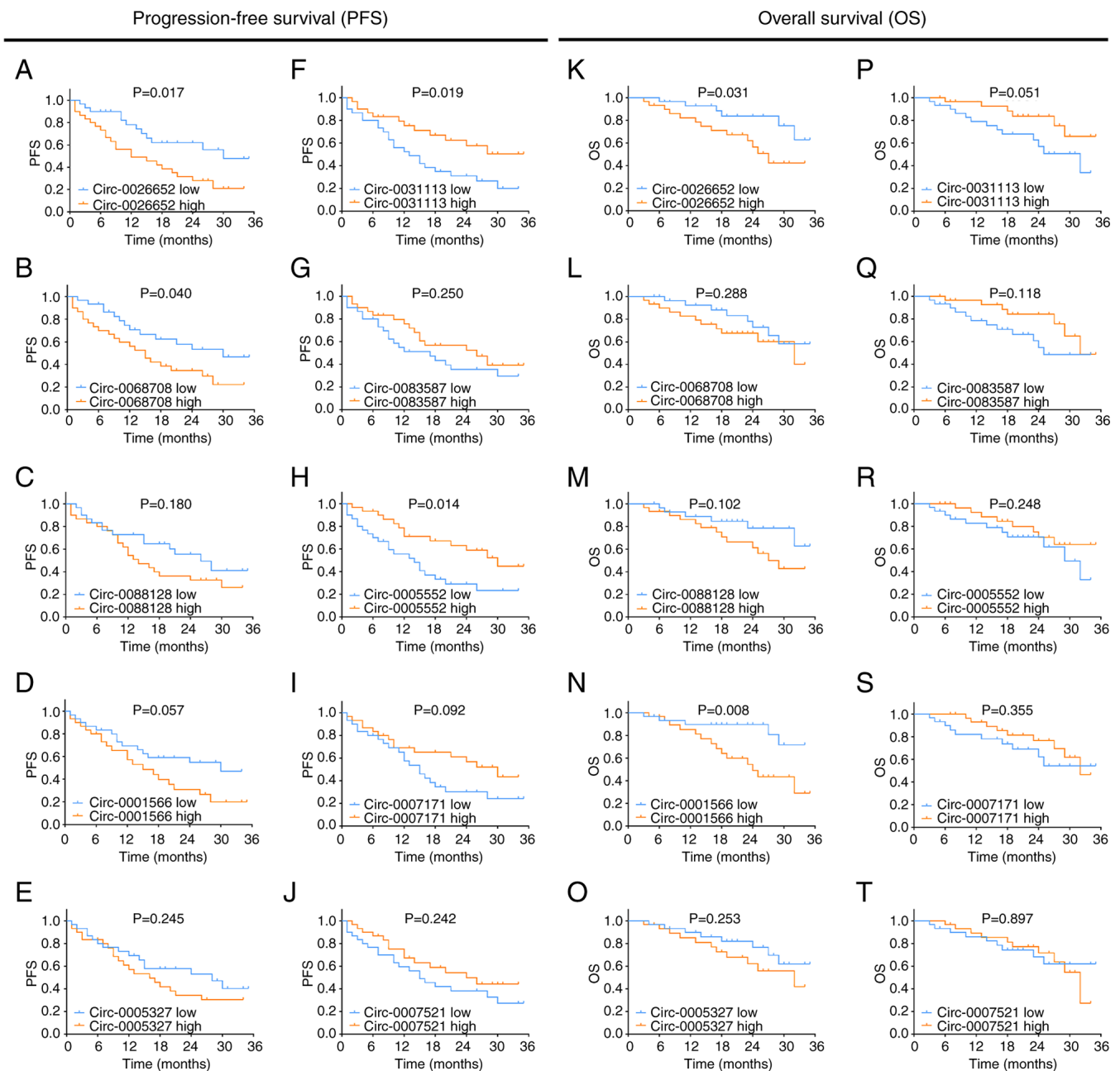


Figure 7. Associations between candidate circRNAs and PFS and OS. Associations between circ_0026652, circ_0068708, circ_0088128, circ_0001566, circ_0005327, circ_0031113, circ_0083587, circ_0005552, circ_0007171 and circ_0007521 expression levels and (A-J) PFS and (K-T) OS. CircRNA/circ, circular RNA; PFS, progressive-free survival; OS, overall survival.

and bioinformatics tools have reported that dysregulated circRNAs are essential for the development and progression of hematological malignancies (7-12), including MM (11,12). One study reported 122 upregulated and 260 downregulated circRNAs in patients with MM compared with healthy participants. Among these dysregulated circRNAs, circ-PTK2, circ-RNF217, circ-RERE, circ-NAGPA, circ-KCNQ5, circ-AFF2, circ-WWC3, circ-DNAJC5, circ-KLHL2, circ-IQGAP1 and circ-AL137655, were confirmed by RT-qPCR to be dysregulated in MM compared with the controls (12). Another study showed that circRNA microarray analysis revealed 40 upregulated and 10 downregulated circRNAs in patients with MM compared with patients with IDA (11). Subsequent RT-qPCR validation studies showed that

circ_101237 is upregulated in patients with MM compared with patients with IDA, and its upregulation is associated with 13q14 deletion, 1q21 amplification, P53 deletion, t(4,14) and t(14,16) chromosomal abnormalities (11). The role of circRNA expression profiles in the prognosis of MM has not been comprehensively explored.

In the present study, microarray assays and bioinformatics analyses were initially performed in eight CR and eight NR MM cases to comprehensively characterize the circRNA profiles in the prognosis of MM. A total of 246 circRNAs were found to be dysregulated (79 upregulated and 167 downregulated) in CR MM cases compared with NR MM cases. Subsequent enrichment analyses revealed that the dysregulated circRNAs were mainly enriched in MM-related

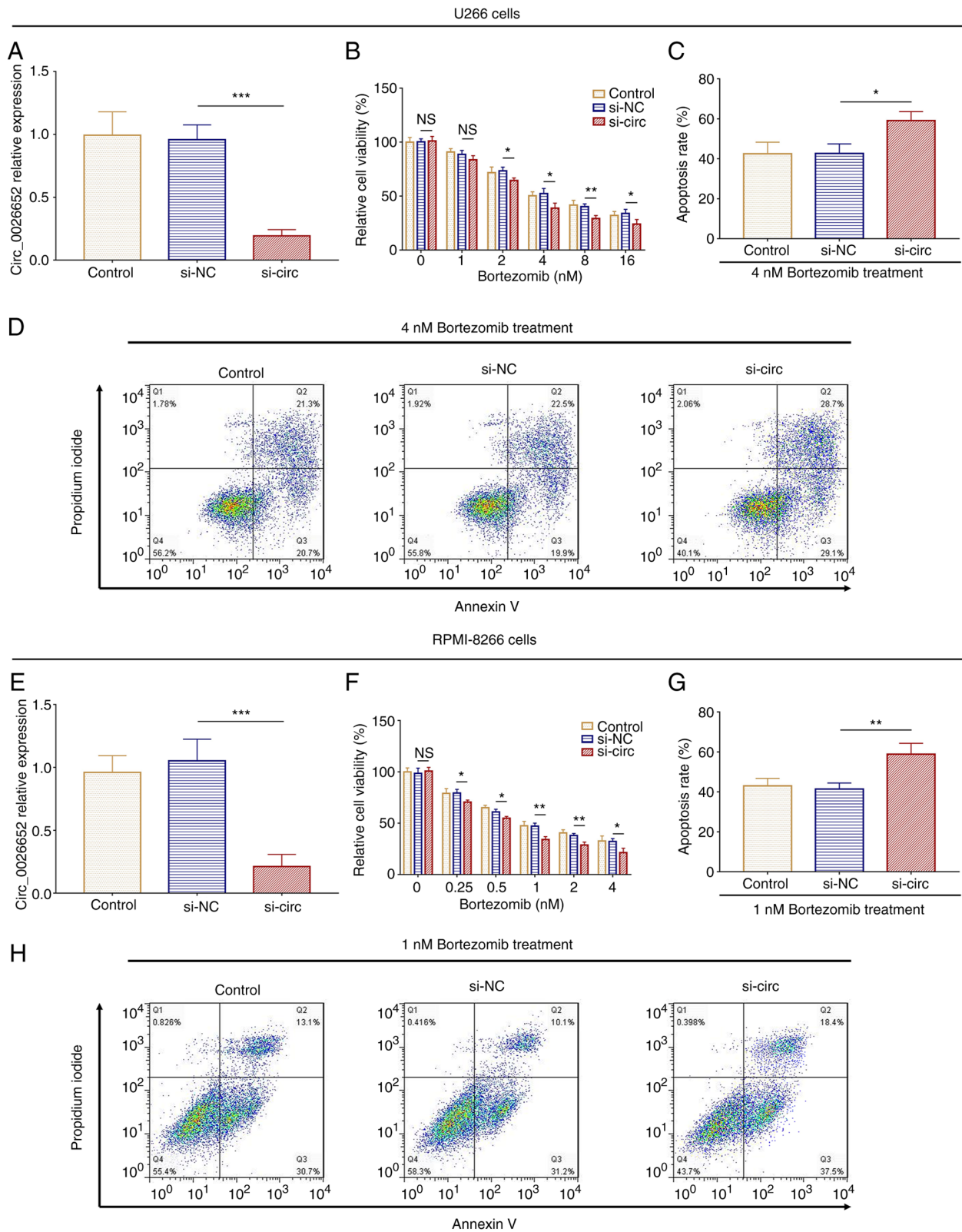


Figure 8. Effect of circ_0026652 knockdown on multiple myeloma chemosensitivity to bortezomib. (A) circ_0026652 expression, (B) relative cell viability under different bortezomib treatment (0, 1, 2, 4, 8 and 16 nM) and (C and D) cell apoptosis under 4 nM bortezomib treatment in U266 cells transfected with si-circ and si-NC. (E) circ_0026652 expression, (F) relative cell viability under different bortezomib concentrations (0, 0.25, 0.5, 1, 2 and 4 nM) and (G and H) cell apoptosis under 1 nM bortezomib treatment in RPMI-8226 cells transfected with si-circ and si-NC. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. circ, circular RNA; si, small interfering RNA; NC, negative control.

signaling pathways, such as the ‘Wnt signaling pathway’, ‘mTOR signaling pathway’ and ‘MAPK signaling pathway’. Wnt signaling functions as an important regulator of cell

proliferation, cell fate, migration and cell polarity (30). In MM, aberrant Wnt signaling pathway activation induces MM cell proliferation, migration and invasion by modulating the

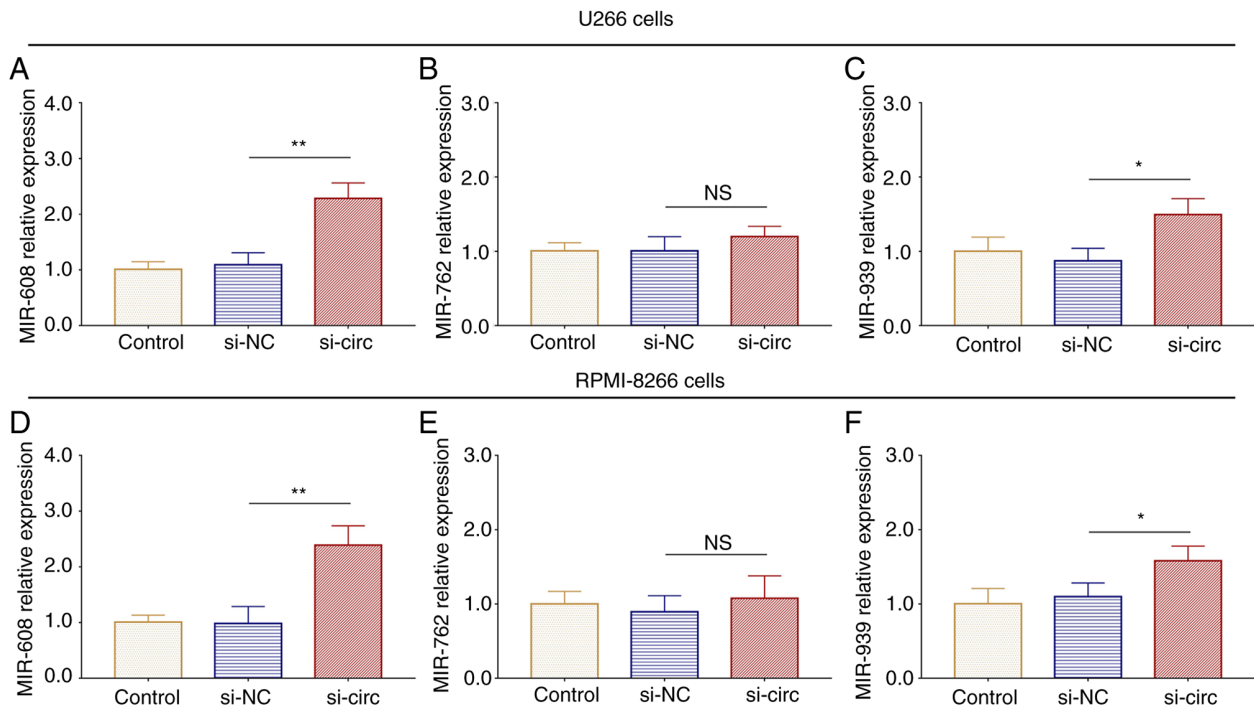


Figure 9. miR-608, miR-762 and miR-939 expression levels. Expression levels of (A) miR-608, (B) miR-762 and (C) miR-939 in U266 cells transfected with si-circ and si-NC. Expression levels of (D) miR-608, (E) miR-762 and (F) miR-939 in RPMI-8226 cells transfected with si-circ and si-NC. * $P < 0.05$, ** $P < 0.01$. miR, microRNA; si, small interfering RNA; NC, negative control; circ, circular RNA.

expression of cell cycle genes, Rho-associated protein kinase and protein kinase C family members, which cause drug resistance and malignancy progression (31). mTOR (a highly conserved PI3K-related serine/threonine kinase) is essential in regulating cell proliferation, survival, invasion, migration and chemoresistance, which is involved in the pathogenesis of MM (32,33). The MAPK (a serine-threonine protein kinase) signaling pathway plays a vital role in modulating MM cell proliferation, survival and drug resistance (34). In combination, dysregulated circRNAs appear to play a key role in drug resistance in MM.

To validate the association between the 10 candidate circRNAs and MM prognosis, their expression was further determined via RT-qPCR in a larger sample of patients with MM. It was revealed that 8/10 circRNAs (including circ_0026652, circ_0068708, circ_0088128, circ_0001566, circ_0031113, circ_0083587, circ_0005552 and circ_0007171) were dysregulated in CR compared with NR cases, 7/10 circRNAs (including circ_0026652, circ_0068708, circ_0088128, circ_0031113, circ_0083587, circ_0005552 and circ_0007171) were dysregulated in CR compared with non-CR cases, 4/10 circRNAs (including circ_0026652, circ_0001566, circ_0031113 and circ_0083587) were dysregulated in ORR compared with non-ORR cases, 4/10 circRNAs (including circ_0026652, circ_0068708, circ_0031113 and circ_0005552) were associated with PFS and 2/10 circRNAs (including circ_0026652 and circ_0001566) were associated with OS. In combination, these findings indicated that dysregulated circRNAs were closely linked with prognosis in patients with MM. The possible explanations were as follows: i) The dysregulated circRNAs may regulate the transcription of their parental genes either positively or negatively. For instance,

according to a previous study, circ_0031113 likely enhances the transcription of its parental gene telomerase protein component 1 (TEP1), and TEP1 acts as a tumor suppressor gene that may inhibit MM cell proliferation, while promoting apoptosis; therefore, circ_0031113 could ameliorate disease progression and improve prognosis in patients with MM (35). ii) The dysregulated circRNAs may regulate the pathogenesis of MM by sponging their target miRNAs. For instance, through the use of the miRanda database, it has been found that circ_0083587 may serve as a sponge for miR-6875-5p to repress the function of miR-6875-5p in maintaining cell survival and enhancing cell proliferation, resulting in alleviated disease progression and is associated with favorable prognosis in patients with MM (36).

Of note, a key prognostic marker, circ_0026652, was identified in the present study, which was not only associated with CR and ORR, but also with PFS and OS in patients with MM. The possible explanations were as follows: i) Subsequent experiments revealed that circ_0026652 knockdown enhanced chemosensitivity to bortezomib treatment, and high circ_0026652 expression is likely associated with poor treatment response and survival in patients with MM; ii) subsequent experiments revealed that circ_0026652 knockdown suppressed the Wnt/ β -catenin pathway; meanwhile, the Wnt/ β -catenin pathway was demonstrated to induce MM cell proliferation, migration and invasion, and thus high circ_0026652 expression may promote the survival and aggressiveness of MM cells via the Wnt/ β -catenin pathway, leading to a poor prognosis in patients with MM (31).

To further explore the potential mechanisms of circ_0026652 in the regulation of chemosensitivity to

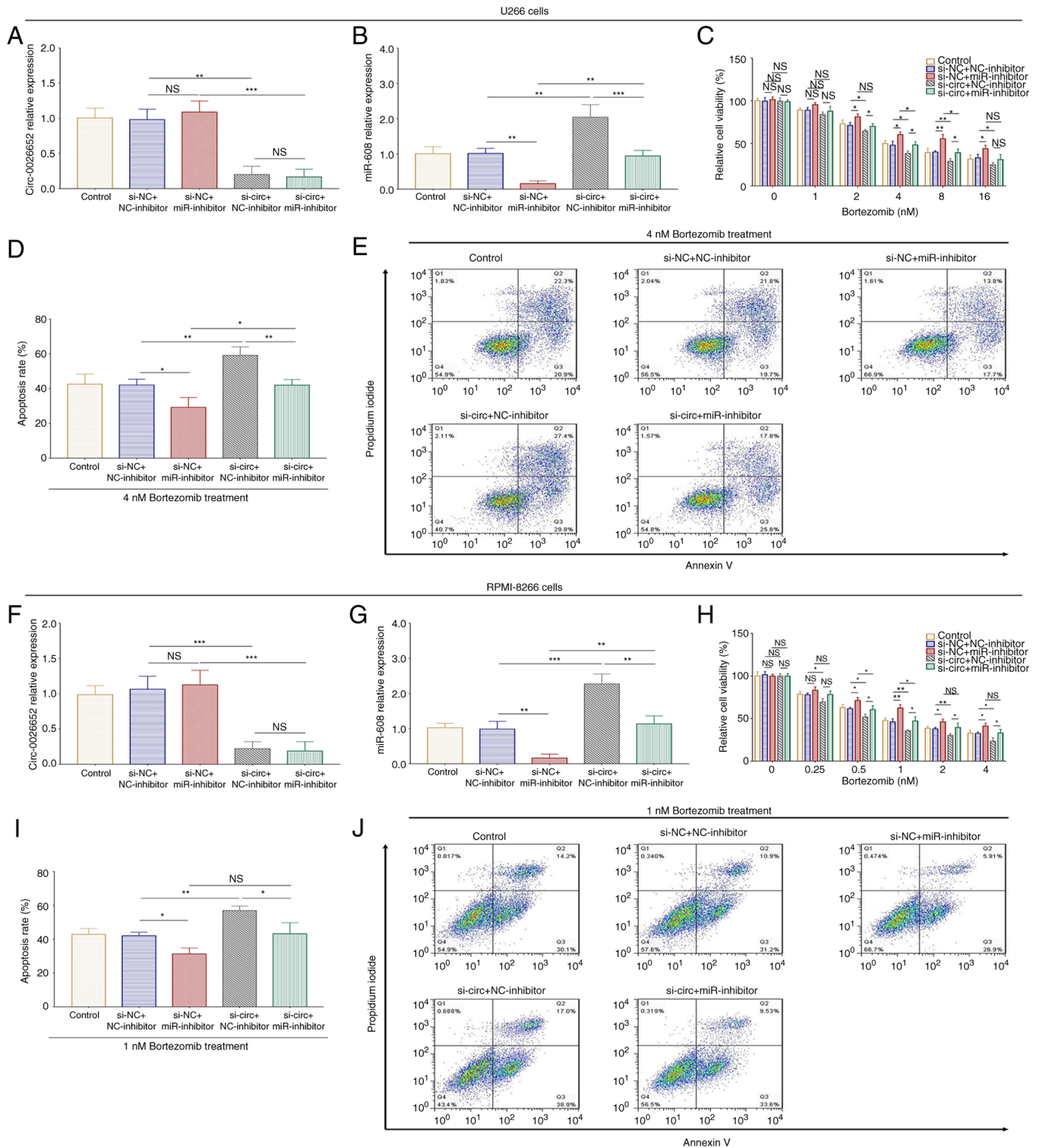


Figure 10. miR-608 knockdown reverses the effects of circ_0026652 knockdown on multiple myeloma chemosensitivity to bortezomib. (A) circ_0026652 expression, (B) miR-608 expression, (C) relative cell viability under different bortezomib treatment (0, 1, 2, 4, 8 and 16 nM) and (D and E) cell apoptosis under 4 nM bortezomib treatment in U266 cells in the control, si-NC + NC-inhibitor, si-NC + miR-inhibitor, si-circ + NC-inhibitor and si-circ + miR-inhibitor groups. (F) circ_0026652 expression, (G) miR-608 expression, (H) relative cell viability under different bortezomib treatment (0, 0.25, 0.5, 1, 2 and 4 nM) and (I and J) cell apoptosis under 1 nM bortezomib treatment in RPMI-8226 cells in the control, si-NC + NC-inhibitor, si-NC + miR-inhibitor, si-circ + NC-inhibitor and si-circ + miR-inhibitor groups. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. miR, microRNA; circ, circular RNA; si, small interfering RNA; NC, negative control.

bortezomib in MM, cellular experiments were performed. The results showed that circ_0026652 knockdown promoted MM chemosensitivity to bortezomib treatment by regulating the miR-608-mediated Wnt/ β -catenin pathway. These results

can be explained as follows: i) circ_0026652 may act as a miRNA sponge to directly suppress the activity of miR-608; meanwhile, miR-608 has been reported to directly inhibit the expression of frizzled family receptor 4 that induces the

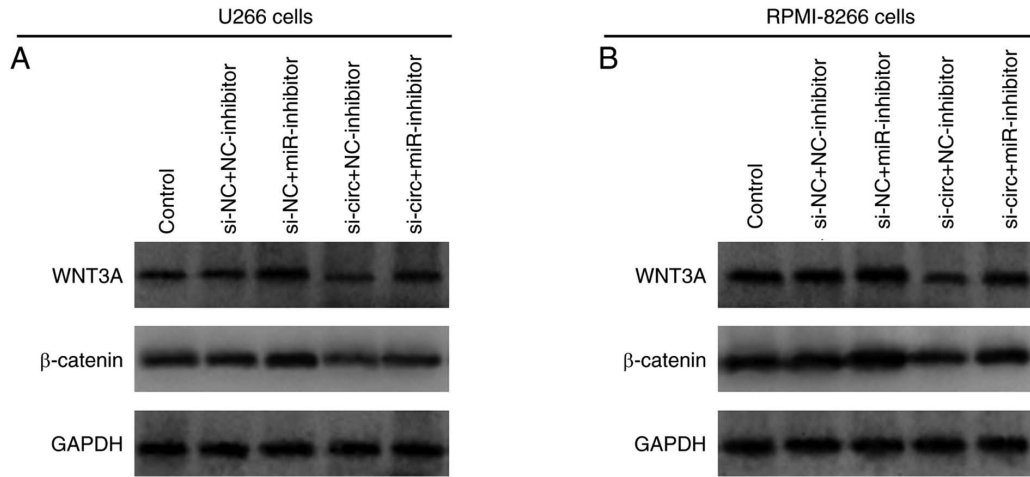


Figure 11. miR-608 knockdown reverses the effects of circ_0026652 knockdown on the Wnt/ β -catenin pathway. (A) Protein expression levels of WNT3A and β -catenin in U266 cells in the control, si-NC + NC-inhibitor, si-NC + miR-inhibitor, si-circ + NC-inhibitor and si-circ + miR-inhibitor groups. (B) Protein expression levels of WNT3A and β -catenin in RPMI-8226 cells in the control, si-NC + NC-inhibitor, si-NC + miR-inhibitor, si-circ + NC-inhibitor and si-circ + miR-inhibitor groups. miR, microRNA; circ, circular RNA; si, small interfering RNA; NC, negative control; WNT3A, Wnt family member 3A.

activation of the oncogenic Wnt/ β -catenin pathway (22,37); in addition, the Wnt/ β -catenin pathway is known to mediate proliferation, migration and drug resistance of MM cells by modulating the expression of cell cycle genes (such as cyclin D1, MYC proto-oncogene, BHLH transcription factor and aurora kinase A) (31). Thereby, circ_0026652 knockdown likely promotes chemosensitivity to bortezomib through the miR-608-mediated Wnt/ β -catenin pathway in MM (22,31,37).

Despite the notable findings, the present study had certain limitations. First, due to the limited number of patients with MM in our hospital, only 60 patients with MM were enrolled, which may have reduced the statistic power of the analyses. Secondly, only patients with MM who underwent bortezomib-based induction treatment were enrolled, and thus the findings may not be applicable to patients with MM who have not been treated with bortezomib. Finally, although cellular experiments were conducted to explore the potential mechanism of circ_0026652 in regulating chemosensitivity to bortezomib in MM, further *in vivo* studies are needed for validation.

In conclusion, the present study provided a comprehensive overview of the role of dysregulated circRNAs in MM prognosis, and confirmed that circ_0026652, circ_0068708, circ_0088128, circ_0001566, circ_0031113, circ_0083587, circ_0005552 and circ_0007171 may serve as potential prognostic biomarkers for treatment response or survival in patients with MM. Among these circRNAs, circ_0026652 is a key and promising prognostic marker of MM. In addition, circ_0026652 knockdown promoted chemosensitivity to bortezomib by regulating the miR-608-mediated Wnt/ β -catenin pathway in MM. These findings may lay the foundations for developing potential drug targets, as well as optimizing the treatment and improving the prognosis of MM.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available due to institutional policy prohibits data sharing, but are available from the corresponding author on reasonable request.

Authors' contributions

WF conceived and designed the present study. WF and LL confirm the authenticity of all the raw data. LL, JLi and JD performed the experiments and collected the data. HJ, HH and JLu analyzed the data. WQ and NH mainly designed the experiments and wrote the manuscript. PG and YZ are responsible for analysis and interpretation of data and revising the article. approved the final manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by Institutional Review Board of Changzheng Hospital, Second Military Medical University (Shanghai, China), and all patients provided written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Röllig C, Knop S and Bornhäuser M: Multiple myeloma. *Lancet* 385: 2197-2208, 2015.
2. Kunacheewa C and Orlowski RZ: New drugs in multiple myeloma. *Annu Rev Med* 70: 521-547, 2019.
3. Pinto V, Bergantim R, Caires HR, Seca H, Guimãraes JE and Vasconcelos MH: Multiple myeloma: Available therapies and causes of drug resistance. *Cancers (Basel)* 12: 407, 2020.

4. Pawlyn C and Davies FE: Toward personalized treatment in multiple myeloma based on molecular characteristics. *Blood* 133: 660-675, 2019.
5. Jamal M, Song T, Chen B, Faisal M, Hong Z, Xie T, Wu Y, Pan S, Yin Q, Shao L and Zhang Q: Recent progress on circular RNA research in acute myeloid leukemia. *Front Oncol* 9: 1108, 2019.
6. Kristensen LS, Andersen MS, Stagsted LV, Ebbesen KK, Hansen TB and Kjems J: The biogenesis, biology and characterization of circular RNAs. *Nat Rev Genet* 20: 675-691, 2019.
7. Wu Z, Sun H, Li J and Jin H: Circular RNAs in leukemia. *Aging (Albany NY)* 11: 4757-4771, 2019.
8. Li W, Zhong C, Jiao J, Li P, Cui B, Ji C and Ma D: Characterization of hsa_circ_0004277 as a new biomarker for acute myeloid leukemia via circular RNA profile and bioinformatics analysis. *Int J Mol Sci* 18: 597, 2017.
9. Lv C, Sun L, Guo Z, Li H, Kong D, Xu B, Lin L, Liu T, Guo D, Zhou J and Li Y: Circular RNA regulatory network reveals cell-cell crosstalk in acute myeloid leukemia extramedullary infiltration. *J Transl Med* 16: 361, 2018.
10. Ding Y, Dong Y, Lu H, Luo X, Fu J, Xiu B, Liang A and Zhang W: Circular RNA profile of acute myeloid leukaemia indicates circular RNA annexin A2 as a potential biomarker and therapeutic target for acute myeloid leukaemia. *Am J Transl Res* 12: 1683-1699, 2020.
11. Liu X, Tang H, Liu J and Wang X: hsa_circRNA_101237: A novel diagnostic and prognostic biomarker and potential therapeutic target for multiple myeloma. *Cancer Manag Res* 12: 2109-2118, 2020.
12. Zhou F, Wang D, Wei W, Chen H, Shi H, Zhou N, Wu L and Peng R: Comprehensive profiling of circular RNA expressions reveals potential diagnostic and prognostic biomarkers in multiple myeloma. *BMC Cancer* 20: 40, 2020.
13. Rajkumar SV, Dimopoulos MA, Palumbo A, Blade J, Merlini G, Mateos MV, Kumar S, Hillengass J, Kastritis E, Richardson P, *et al*: International myeloma working group updated criteria for the diagnosis of multiple myeloma. *Lancet Oncol* 15: e538-e548, 2014.
14. Chinese Hematology Association and Chinese Society of Hematology: *Zhonghua Nei Ke Za Zhi* 61: 480-487, 2022 (Article in Chinese).
15. Ludwig H, Miguel JS, Dimopoulos MA, Palumbo A, Garcia Sanz R, Powles R, Lentzsch S, Ming Chen W, Hou J, Jurczyszyn A, *et al*: International myeloma working group recommendations for global myeloma care. *Leukemia* 28: 981-992, 2014.
16. Rajkumar SV, Harousseau JL, Durie B, Anderson KC, Dimopoulos M, Kyle R, Blade J, Richardson P, Orlowski R, Siegel D, *et al*: Consensus recommendations for the uniform reporting of clinical trials: Report of the international myeloma workshop consensus panel 1. *Blood* 117: 4691-4695, 2011.
17. Durie BG, Harousseau JL, Miguel JS, Bladé J, Barlogie B, Anderson K, Gertz M, Dimopoulos M, Westin J, Sonneveld P, *et al*: International uniform response criteria for multiple myeloma. *Leukemia* 20: 1467-1473, 2006.
18. Skuja E, Butane D, Nakazawa-Miklasevica M, Daneberga Z, Purkalne G and Miklasevics E: Deletions in metastatic colorectal cancer with chromothripsis. *Exp Oncol* 41: 323-327, 2019.
19. Wang L, Liu X, Yue M, Liu Z, Zhang Y, Ma Y, Luo J, Li W, Bai J, Yao H, *et al*: Identification of hub genes in bladder cancer based on weighted gene co-expression network analysis from TCGA database. *Cancer Rep (Hoboken)*: e1557, 2021 (Online ahead of print).
20. Cheng Q and Wang L: LncRNA XIST serves as a ceRNA to regulate the expression of ASF1A, BRWD1M, and PFKFB2 in kidney transplant acute kidney injury via sponging hsa-miR-212-3p and hsa-miR-122-5p. *Cell Cycle* 19: 290-299, 2020.
21. Zhou Y, Liu X, Xue J, Liu L, Liang T, Li W, Yang X, Hou X and Fang H: Discovery of peptide boronate derivatives as histone deacetylase and proteasome dual inhibitors for overcoming bortezomib resistance of multiple myeloma. *J Med Chem* 63: 4701-4715, 2020.
22. Wang Y, Zhang W, Wang Y and Wang S: HOXD-AS1 promotes cell proliferation, migration and invasion through miR-608/FZD4 axis in ovarian cancer. *Am J Cancer Res* 8: 170-182, 2018.
23. Jin Y, Xu L, Wu X, Feng J, Shu M, Gu H, Gao G, Zhang J, Dong B and Chen X: Synergistic efficacy of the demethylation agent decitabine in combination with the protease inhibitor bortezomib for treating multiple myeloma through the Wnt/ β -catenin pathway. *Oncol Res* 27: 729-737, 2019.
24. Yao R, Sun X, Xie Y, Liu L, Han D, Yao Y, Li H, Li Z and Xu K: Lithium chloride inhibits cell survival, overcomes drug resistance, and triggers apoptosis in multiple myeloma via activation of the Wnt/ β -catenin pathway. *Am J Transl Res* 10: 2610-2618, 2018.
25. Savvidou I, Khong T, Cuddihy A, McLean C, Horrigan S and Spencer A: β -catenin inhibitor BC2059 is efficacious as monotherapy or in combination with proteasome inhibitor bortezomib in multiple myeloma. *Mol Cancer Ther* 16: 1765-1778, 2017.
26. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25: 402-408, 2001.
27. Chen X, Mao R, Su W, Yang X, Geng Q, Guo C, Wang Z, Wang J, Kresty LA, Beer DG, *et al*: Circular RNA circHIPK3 modulates autophagy via MIR124-3p-STAT3-PRKAA/AMPK α signaling in STK11 mutant lung cancer. *Autophagy* 16: 659-671, 2020.
28. Fang Y, Wang X, Li W, Han J, Jin J, Su F, Zhang J, Huang W, Xiao F, Pan Q and Zou L: Screening of circular RNAs and validation of circANKRD36 associated with inflammation in patients with type 2 diabetes mellitus. *Int J Mol Med* 42: 1865-1874, 2018.
29. Zhang M, Wen F and Zhao K: Circular RNA_0001946 is insufficiently expressed in tumor tissues, while its higher expression correlates with less lymph node metastasis, lower TNM stage, and improved prognosis in NSCLC patients. *J Clin Lab Anal* 35: e23625, 2021.
30. Gajos-Michniewicz A and Czyz M: WNT signaling in melanoma. *Int J Mol Sci* 21: 4852, 2020.
31. van Anel H, Kocemba KA, Spaargaren M and Pals ST: Aberrant Wnt signaling in multiple myeloma: Molecular mechanisms and targeting options. *Leukemia* 33: 1063-1075, 2019.
32. Peterson TR, Laplante M, Thoreen CC, Sancak Y, Kang SA, Kuehl WM, Gray NS and Sabatini DM: DEPTOR is an mTOR inhibitor frequently overexpressed in multiple myeloma cells and required for their survival. *Cell* 137: 873-886, 2009.
33. Li J, Zhu J, Cao B and Mao X: The mTOR signaling pathway is an emerging therapeutic target in multiple myeloma. *Curr Pharm Des* 20: 125-135, 2014.
34. Podar K, Chauhan D and Anderson KC: Bone marrow microenvironment and the identification of new targets for myeloma therapy. *Leukemia* 23: 10-24, 2009.
35. Hu TH, Huang CC, Lin PR, Chang HW, Ger LP, Lin YW, Changchien CS, Lee CM and Tai MH: Expression and prognostic role of tumor suppressor gene PTEN/MMAC1/TEP1 in hepatocellular carcinoma. *Cancer* 97: 1929-1940, 2003.
36. Xie Y, Du J, Liu Z, Zhang D, Yao X and Yang Y: MiR-6875-3p promotes the proliferation, invasion and metastasis of hepatocellular carcinoma via BTG2/FAK/Akt pathway. *J Exp Clin Cancer Res* 38: 7, 2019.
37. Gupta S, Iljin K, Sara H, Mpindi JP, Mirtti T, Vainio P, Rantala J, Alanen K, Nees M and Kallioniemi O: FZD4 as a mediator of ERG oncogene-induced WNT signaling and epithelial-to-mesenchymal transition in human prostate cancer cells. *Cancer Res* 70: 6735-6745, 2010.



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