



# Potential of microRNA expression profile in predicting renal impairment risk in multiple myeloma patients

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**Background:** This study aimed to investigate the correlation of microRNA (miRNA) expression profile with renal impairment (RI) risk in multiple myeloma (MM) patients.

**Methods:** Plasma cell samples were isolated from bone marrows of 20 RI-MM patients and 20 non-RI-MM patients, and then proposed to microarray assay. Then 5 candidate miRNAs were selected and further validated by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) in plasma cell samples from bone marrows of 60 RI-MM patients and 60 non-RI-MM patients.

**Results:** Principal component analysis and heatmap analysis revealed that miRNA expression profile could clearly distinguish RI-MM patients from non-RI-MM patients, further Volcano plots identified 28 upregulated and 13 downregulated miRNAs in RI-MM patients compared to non-RI-MM patients, and enrichment analysis observed that these dysregulated miRNAs were enriched in renal/inflammatory/apoptosis pathways and renal/inflammatory diseases. Subsequent RT-qPCR validation discovered that miR-103a-3p, miR-449c-5p and let-7a-5p were greatly increased, while miR-877-5p and miR-455-3p were dramatically decreased in RI-MM patients compared to non-RI-MM patients, and all of them could predict RI risk in MM patients by receiver operating characteristic (ROC) curve analysis. Most importantly, the combination of these five miRNAs presented with a great predictive value for RI risk in MM patients with an area under the curve (AUC) of 0.934, 95% CI: 0.895–0.974.

**Conclusions:** MiRNA expression profile is closely implicated in the RI development, and miR-103a-3p, miR-449c-5p, miR-877-5p, miR-455-3p and let-7a-5p may serve as novel biomarkers for RI risk in MM patients.

**Keywords:** MicroRNA expression profile; renal impairment; multiple myeloma (MM); microarray; reverse transcription-quantitative polymerase chain reaction

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## Introduction

Multiple myeloma (MM), as a hematological malignancy featured by clonal proliferation of malignant plasma cells which accounts for around 1% of neoplastic diseases and

13% of hematologic malignancies, commonly accompanied with severe symptoms such as renal impairment (RI), extensive skeletal destruction, infections, anemia, hypercalcemia and so on (1). Among these complications, RI occurs in 20–50% MM patients worldwide, while in

China, RI is reported in 24.0%, 19.7% and 30.8% of total MM patients in China mainland, Hong Kong and Taiwan, respectively (2,3). Although bortezomib and dexamethasone triplet combinations are proposed to be the current standard of therapy for MM patients with RI (RI-MM patients), some patients refractory to these indications or with low-income are hard to benefit from the treatment, besides, the RI still greatly weakens the prognosis of MM patients (4,5). Thus, exploration of novel biomarkers for RI risk in MM patients is essential for the early prevention, treatment and prognosis improvement.

MicroRNA (miRNA), as a group of non-coding RNAs, has disclosed its wide application as biomarkers for many diseases including renal diseases (6). For instance, a previous study reports that the miRNA expression pattern is correlated with the vascular remodeling in patients with type 2 diabetes mellitus (7); another study uncovers that abnormal miRNA expression profile is suggestive for renal fibrosis in patients with Fabry disease (8); besides, a meta-analysis reveals the potential of miRNA expression profile as biomarker for renal fibrosis (6). Apart from these evidences, miRNA expression profile is also discovered to be deeply involved in the development and progression of MM (9,10). Considering these above-mentioned data, we hypothesized that miRNA expression profile might have the potential to be biomarker for RI risk in MM patients, while no-related study has ever been reported.

Thus, this present study aimed to investigate the potential of miRNA expression profile in predicting RI risk in MM patients.

## Methods

### Patients

Between January 2016 and February 2019, 60 RI-MM patients and 60 MM patients without RI (non-RI-MM patients) treated in our hospital were recruited in this study. The inclusion criteria for RI-MM patients were as follows: (I) diagnosed as primary MM in accordance with the International Myeloma Working Group (IMWG) updated criteria for the diagnosis of MM (11); (II) presented with RI, which was defined as serum creatinine  $>1.73 \mu\text{mol/L}$  (or  $>2 \text{ mg/dL}$ ) or estimated creatinine clearance  $<40 \text{ mL/min}$  and confirmed by the renal biopsy (11); (III) age  $\geq 18$  years. And the inclusion criteria for non-RI-MM patients were: (I) diagnosed as primary MM according to the IMWG criteria (11); (II) absence of RI confirmed by clinical and

laboratory examinations; (III) age  $\geq 18$  years. Both the RI-MM patients and the non-RI-MM patients were excluded from the study if they had any of following conditions: (I) secondary or relapsed MM; (II) concomitant with other malignancies; (III) pregnant or lactating women. The present study was approved by the Institutional Review Board of our hospital with ethical approval number 2015-018, and all patients provided the written informed consents.

### Data and sample collection

After confirmation of patients' eligibility, clinical data and biochemical indexes were recorded prior to initial treatment, which mainly covered age, gender, body weight and the level of haemoglobin (Hb), calcium, serum creatinine (Scr), creatinine clearance rate (Ccr), albumin (ALB),  $\beta_2$  microglobulin ( $\beta_2$ -MG) and lactate dehydrogenase (LDH). Before initial therapy, bone marrow samples were extracted from all patients, then the plasma cells were isolated from the samples using CD138<sup>+</sup> Plasma Cell Isolation Kit (Miltenyi, Bergisch Gladbach, Germany) and stored at  $-80^\circ\text{C}$  for further determination.

### Microarray

Twenty plasma cell samples from RI-MM patients and 20 plasma cell samples from non-RI-MM patients were subjected to microarray assay. In brief, total RNA was extracted from each sample by Trizol reagent (Invitrogen, Carlsbad, USA) followed by RNA integrity assessment by Agilent 2100 Bioanalyzer (Agilent, City of Santa Clara, USA) and quantification by NanoDrop ND-1000 spectrophotometer (Thermo, Wilmington, USA). Subsequently, miRNA expression profile of each sample was detected using Affymetrix Multispecies miRNA-4 Array (Agilent, City of Santa Clara, USA) by Genergy Bio (Shanghai, China) as the methods described in previous studies (12,13).

### Bioinformatics

As for data processing, quantile normalization and low-intensity filtering were performed by R software package (R version 3.1.2), and the miRNAs detected in above 50 percent samples were proposed to further analysis. Bioinformatics analysis was performed using R software package (R version 3.1.2) as well. In brief, principal component analysis (PCA) of miRNA expression profile

**Table 1** Primers used in the study

Gene	Forward (5'→3')	Reverse (5'→3')
hsa-miR-103a-3p	ACACTCCAGCTGGGAGCAGCATTGTACAGG	TGTCGTGGAGTCGGCAATTC
hsa-miR-449c-5p	ACACTCCAGCTGGGTAGGCAGTGTATTGCT	TGTCGTGGAGTCGGCAATTC
hsa-miR-877-5p	ACACTCCAGCTGGGGTAGAGGAGATGGCGC	TGTCGTGGAGTCGGCAATTC
hsa-miR-455-3p	ACACTCCAGCTGGGGCAGTCCATGGGCATA	TGTCGTGGAGTCGGCAATTC
hsa-let-7a-5p	ACACTCCAGCTGGGTGAGGTAGTAGTTGT	TGTCGTGGAGTCGGCAATTC
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT

miRNA, microRNA.

was performed by Factoextra package; heatmap analysis of miRNA expression profile was performed by pheatmap package; differentially expressed miRNAs were analyzed by Limma package, and miRNAs with a fold change (FC)  $\geq 1.5$  and an adjusted P value (FDR, False discovery rate)  $< 0.1$  were identified as differentially expressed miRNAs and exhibited by Volcano Plots according to the definition of previous studies (14,15); heatmap plot of differentially expressed miRNAs was performed by pheatmap package; enrichment analysis of dysregulated miRNAs was performed by Fisher exact test based on annotations from Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), human-phenotype-ontology (HP), Disease Ontology (DOID).

#### **Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) validation**

Using the screening criteria: absolute value of FC  $> 2$  and the adjusted P ( $P_{adj}$ ) value  $< 0.01$ , a total of 5 candidate miRNAs were selected from differentially expressed miRNAs in microarray, then those 5 candidate miRNAs were further verified in the total 60 RI-MM patients and 60 non-RI-MM patients by RT-qPCR. In brief, total RNA was extracted from plasma cells using TRIzol™ Reagent (Thermo Fisher Scientific, Waltham, USA), and then cDNA was reversely transcribed by QuantiTect Rev. Transcription Kit (Qiagen, Frankfurt, Germany). Subsequently, polymerase chain reaction was performed using Direct SYBR® Premix (Clontech, Mountain View, USA). All the procedures were in line with the instructions of the manufacturers. The primers used in RT-qPCR were listed in the *Table 1*. The miRNA expression was then calculated by  $2^{-11Ct}$  with the U6 as the internal reference.

#### **Statistical analysis**

Kolmogorov-Smirnov test was performed to determine the normality of quantitative data. Normally distributed data were expressed as mean and standard deviation (SD), while the non-normally distributed data were displayed as median and inter-quartile range (IQR). And the qualitative data were presented as number (percentage). The student's t test and Wilcoxon rank sum test were used to determine the comparison of quantitative data between two groups as appropriate, and the Chi-square test was applied to determine the comparison of qualitative data between two groups. Receiver operating characteristic (ROC) curve and the derived area under the curve (AUC) were used to assess the predicting ability of miRNAs for RI risk in MM patients. SPSS 21.0 statistical software (IBM, New York, USA) was used for statistical data processing, and the GraphPad Prism 7.02 (GraphPad Software Inc., New York, USA) was applied for plotting graphs. All tests were two-sided, and P value  $< 0.05$  was considered as significant.

## **Results**

#### **Patients' characteristics in microarray assay**

A total of 20 RI-MM patients and 20 non-RI-MM patients were included in the microarray assay as the detailed characteristics shown in *Table 2*. In brief, no difference of age ( $P=0.806$ ), gender ( $P=0.736$ ), body weight ( $P=0.643$ ), calcium ( $P=0.314$ ) was observed between RI-MM patients and non-RI-MM patients, while Hb ( $P=0.004$ ), ALB ( $P=0.005$ ) and Ccr ( $P<0.001$ ) were lower, Scr ( $P<0.001$ ),  $\beta_2$ -MG ( $P<0.001$ ) and LDH ( $P=0.004$ ) were higher in RI-MM patients compared to non-RI-MM patients.

**Table 2** Clinical characteristics of MM patients

Items	Microarray assay			RT-qPCR validation		
	RI-MM patients (N=20)	Non-RI-MM patients (N=20)	P value	RI-MM patients (N=60)	Non-RI-MM patients (N=60)	P value
Age (years), mean $\pm$ SD	56.6 $\pm$ 7.4	57.3 $\pm$ 9.1	0.806	57.7 $\pm$ 7.4	55.8 $\pm$ 8.9	0.222
Gender, No. (%)			0.736			0.336
Female	7 (35.0)	6 (30.0)		23 (38.3)	18 (30.0)	
Male	13 (65.0)	14 (70.0)		37 (61.7)	42 (70.0)	
Body weight (kg), mean $\pm$ SD	60.9 $\pm$ 9.5	62.4 $\pm$ 11.6	0.643	59.0 $\pm$ 10.5	60.3 $\pm$ 10.4	0.486
Hb (g/dL), mean $\pm$ SD	8.8 $\pm$ 2.3	10.8 $\pm$ 1.8	0.004	9.8 $\pm$ 2.4	10.4 $\pm$ 2.5	0.168
Calcium (mg/dL), mean $\pm$ SD	9.9 $\pm$ 1.6	10.4 $\pm$ 1.3	0.314	10.1 $\pm$ 1.7	10.1 $\pm$ 1.9	0.962
Scr (mg/dL), median (IQR)	2.8 (2.4–3.0)	1.4 (1.0–1.6)	<0.001	3.0 (2.5–3.6)	1.4 (1.2–1.7)	<0.001
Ccr (mL/min), median (IQR)	25.2 (22.1–30.8)	49.0 (41.1–68.8)	<0.001	23.2 (17.8–26.7)	45.6 (41.6–54.2)	<0.001
ALB (mg/dL), mean $\pm$ SD	3.2 $\pm$ 0.7	3.8 $\pm$ 0.7	0.005	3.2 $\pm$ 0.8	4.0 $\pm$ 0.7	<0.001
$\beta$ 2-MG (mg/L), median (IQR)	5.6 (4.4–9.1)	2.7 (1.1–3.8)	<0.001	6.3 (4.1–9.8)	2.7 (1.2–4.4)	<0.001
LDH (U/L), median (IQR)	227.2 (190.0–285.9)	182.9 (125.3–218.3)	0.004	224.9 (194.3–258.7)	176.5 (140.4–207.6)	<0.001

Normality was determined by Kolmogorov-Smirnov test. Comparison was determined by Student's t test, Chi-square test or Wilcoxon rank sum test. RT-qPCR, reverse transcription-quantitative polymerase chain reaction; MM, multiple myeloma; RI, renal impairment; SD, standard deviation; Hb, hemoglobin; IQR, interquartile range; Scr, serum creatinine; Ccr, creatinine clearance; ALB, albumin;  $\beta$ 2-MG, beta-2-microglobulin; LDH, lactate dehydrogenase.

### PCA and heatmap analysis of miRNA expression profile

PCA plots exhibited that miRNA expression profile could clearly distinguish RI-MM patients from non-RI-MM patients (Figure 1A), and heatmap analysis also showed that miRNA expression profile could obviously differentiate RI-MM patients from non-RI-MM patients (Figure 1B).

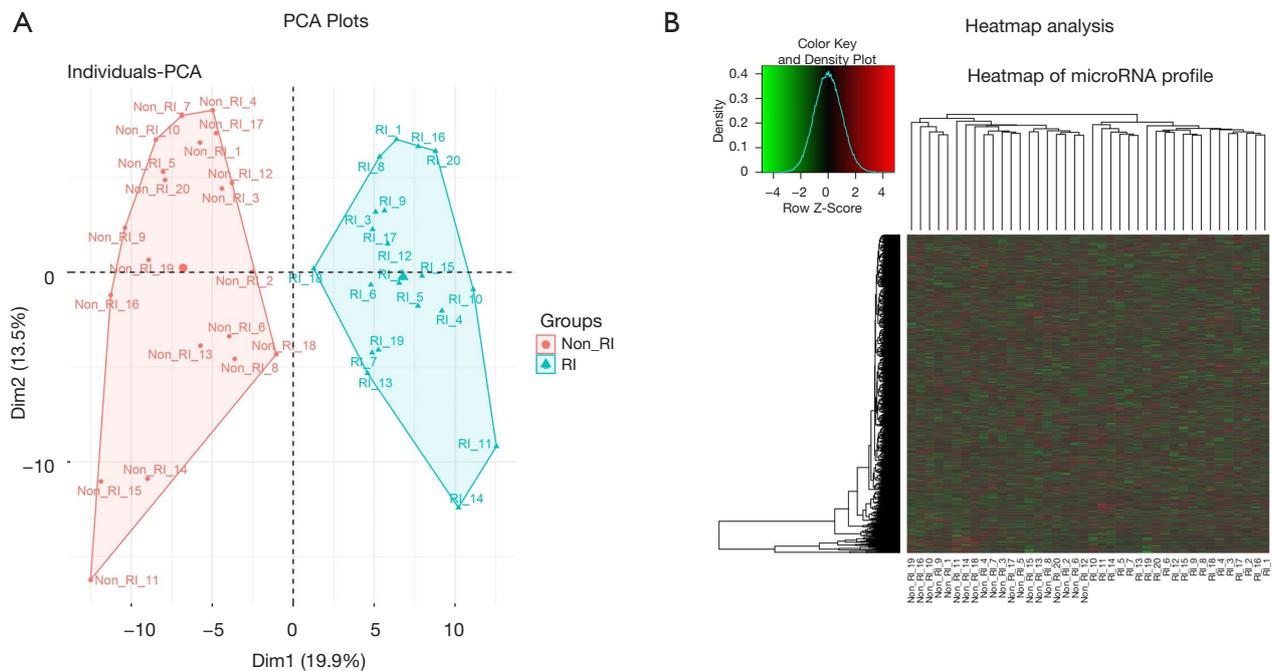
### Volcano plots and heatmap analysis of differentially expressed miRNAs

Volcano plots identified 28 upregulated and 13 downregulated miRNAs in RI-MM patients compared to non-RI-MM patients (Figure 2A), and heatmap illuminated that these differentially expressed miRNAs clearly differentiate RI-MM patients from non-RI-MM patients (Figure 2B). Besides, the detailed information of these differentially expressed miRNAs were shown in Table 3.

### Enrichment analysis

GO enrichment analysis illuminated that the differentially expressed miRNAs were enriched in molecular function

(such as cyclin binding, endopeptidase inhibitor activity, cyclin dependent protein kinase inhibitor activity, etc.), cellular component (such as cyclin dependent protein kinase holoenzyme complex, eukaryotic translation elongation factor 1 complex, cytoplasmic vesicle membrane, etc.) and biological process (such as negative regulation of cyclin dependent protein kinase activity, osteoblast development, negative regulation of apoptotic process, etc.) (Figure 3A). KEGG enrichment analysis illustrated that the differentially expressed miRNAs were enriched in renal/inflammatory/apoptosis pathways such as ErbB signaling pathway, RIG I like receptor signaling pathway, B cell receptor signaling pathway, p53 signaling pathway, NOD like receptor signaling pathway, etc. (Figure 3B). HP enrichment analysis disclosed that the differentially expressed miRNAs were enriched in renal-dysfunction/mitochondrial related phenotypes such as renal tubular dysfunction, decreased activity of mitochondrial complex I, renal dysplasia, etc. (Figure 3B). Finally, DOID enrichment analysis revealed that the differentially expressed miRNAs were enriched in renal or inflammatory diseases such as metastatic renal cell carcinoma, systemic inflammatory response syndrome, autoimmune hemolytic anemia, immune thrombocytopenic



**Figure 1** PCA plots analysis and heatmap analysis. PCA plots analysis of miRNA expression profile between RI-MM patients and non-RI-MM patients (A); heatmap analysis of miRNA expression profile between RI-MM patients and non-RI-MM patients (B). PCA, principal component analysis; miRNA, microRNA; RI-MM, multiple myeloma patients with renal impairment; non-RI-MM, multiple myeloma patients without renal impairment.

purpura, etc. (Figure 3B).

**Patients’ characteristics in RT-qPCR validation**

A total of 60 RI-MM patients and 60 non-RI-MM patients were included in the RT-qPCR validation as the detailed characteristics shown in Table 2. In brief, no difference of age (P=0.222), gender (P=0.336), body weight (P=0.486), Hb (P=0.168), calcium (P=0.962) was observed between RI-MM patients and non-RI-MM patients, while ALB (P<0.001) and Ccr (P<0.001) were lower, Scr (P<0.001), β2-MG (P<0.001) and LDH (P<0.001) were higher in RI-MM patients compared to non-RI-MM patients.

**Expressions of candidate miRNAs**

Using the screening criteria: absolute value of FC >2 and the P<sub>adj</sub> value <0.01, a total of 5 candidate miRNAs were selected from differentially expressed miRNAs in microarray, then those 5 candidate miRNAs were further verified in the total 60 RI-MM patients and 60 non-RI-MM patients by RT-qPCR. As shown in Figure 4, miR-103a-3p,

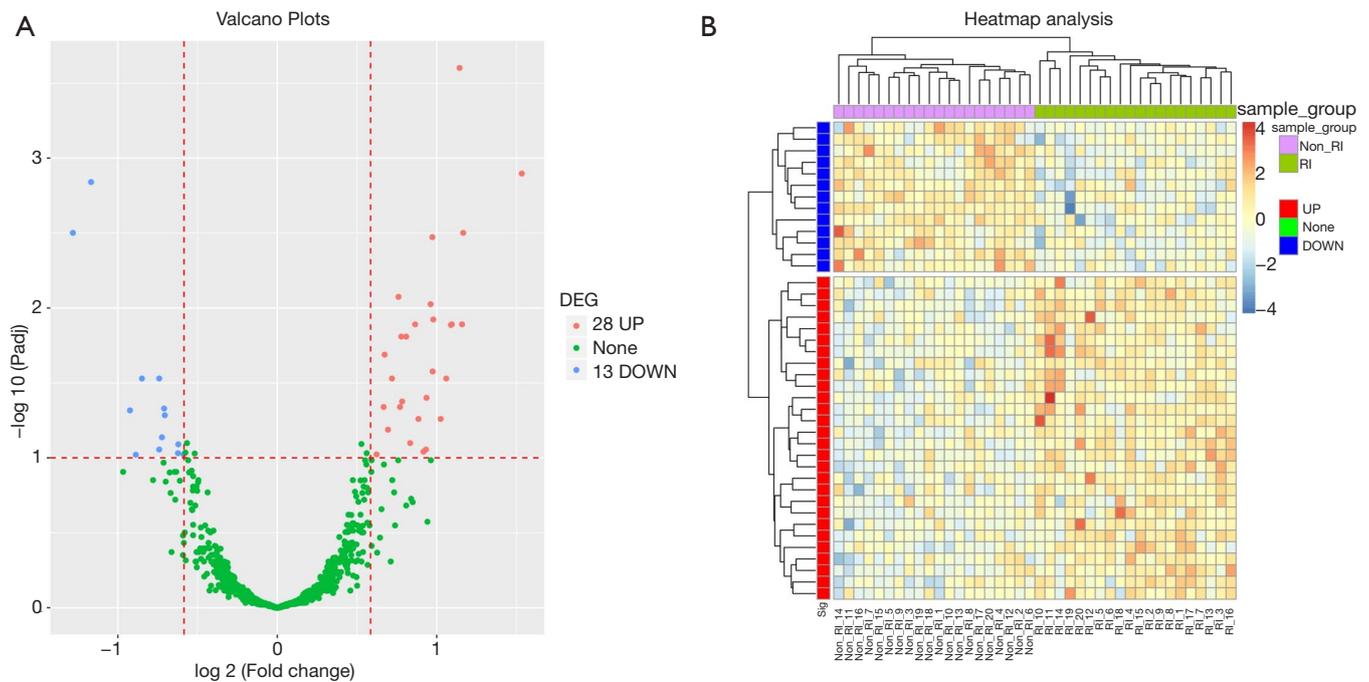
miR-449c-5p and let-7a-5p were greatly increased, while miR-877-5p and miR-455-3p were dramatically decreased in RI-MM patients compared to non-RI-MM patients (all P<0.001, P<sub>adj</sub><0.001).

**Predictive value of candidate miRNAs for RI risk in MM patients**

ROC curve analysis showed that miR-103a-3p, miR-449c-5p, miR-877-5p, miR-455-3p and let-7a-5p could all predict RI risk in MM patients, most importantly, the combination of these five miRNAs presented with a great predictive value for RI risk in MM patients with an AUC of 0.934, 95% CI: 0.895–0.974 (Figure 5).

**Discussion**

In this study, we discovered that: (I) the expression profile of miRNA notably differentiated RI-MM patients from non-RI MM patients; (II) 28 upregulated and 13 downregulated miRNAs in RI-MM patients compared to non-RI-MM patients were identified, which were mainly enriched



**Figure 2** Valcano plots analysis and heatmap analysis. Valcano plots analysis of differentially expressed miRNAs between RI-MM patients and non-RI-MM patients (A); heatmap analysis of differentially expressed miRNAs between RI-MM patients and non-RI-MM patients (B). miRNA, microRNA; RI-MM, multiple myeloma patients with renal impairment; non-RI-MM, multiple myeloma patients without renal impairment.

**Table 3** Differentially expressed miRNAs in microarray

miRNA name	Probe ID	Log <sub>2</sub> FC	P value	Adjusted P value	Trend
hsa-miR-103a-3p	MIMAT0000101	1.14348	4.84E-07	0.00025	UP
hsa-miR-449c-5p	MIMAT0010251	1.533664	2.94E-06	0.001263	UP
hsa-miR-877-5p	MIMAT0004949	-1.16843	3.91E-06	0.00144	DOWN
hsa-miR-455-3p	MIMAT0004784	-1.28229	1.10E-05	0.003157	DOWN
hsa-let-7a-5p	MIMAT0000062	1.165801	1.10E-05	0.003157	UP
hsa-miR-151a-3p	MIMAT0000757	0.972224	1.30E-05	0.003364	UP
hsa-miR-20b-5p	MIMAT0001413	0.760544	3.59E-05	0.008425	UP
hsa-miR-320a	MIMAT0000510	0.962337	4.39E-05	0.009424	UP
hsa-miR-620	MIMAT0003289	0.979332	6.01E-05	0.01191	UP
hsa-miR-488-5p	MIMAT0002804	0.865665	7.62E-05	0.012839	UP
hsa-miR-17-5p	MIMAT0000070	1.09423	7.23E-05	0.012839	UP
hsa-miR-663a	MIMAT0003326	1.158985	7.97E-05	0.012839	UP
hsa-let-7g-5p	MIMAT0000414	1.089788	8.54E-05	0.012955	UP
hsa-miR-559	MIMAT0003223	0.77763	0.000114	0.015509	UP

**Table 3** (continued)

Table 3 (continued)

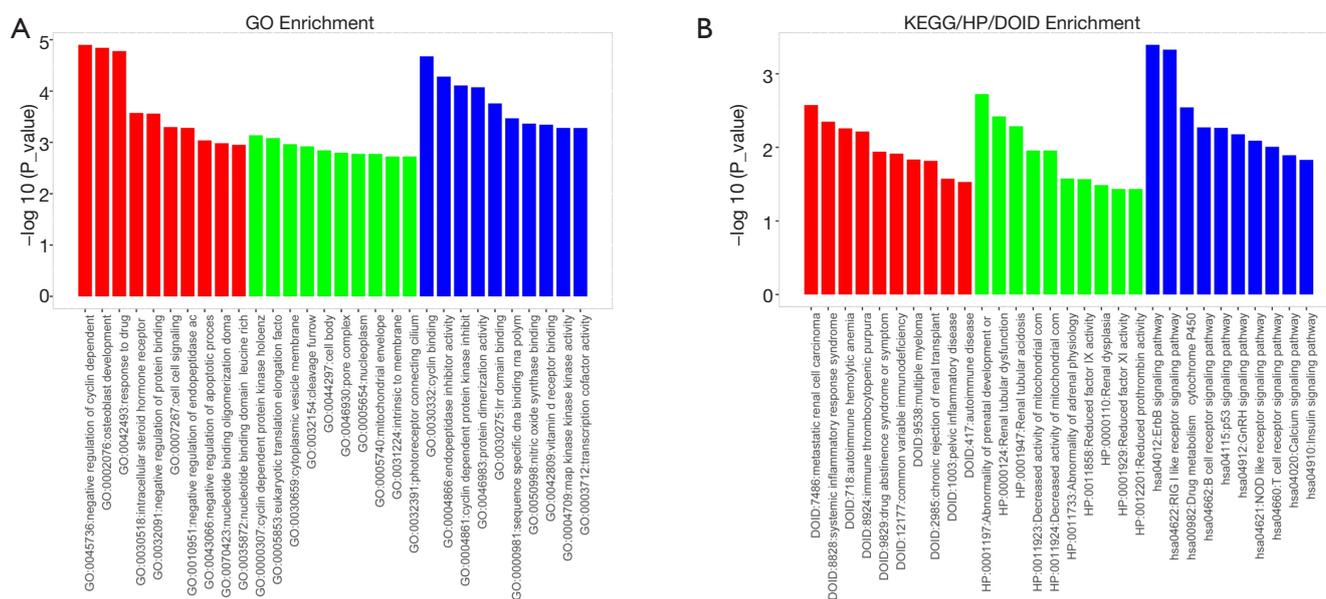
miRNA name	Probe ID	Log <sub>2</sub> FC	P value	Adjusted P value	Trend
hsa-miR-127-3p	MIMAT0000446	0.809156	0.000114	0.015509	UP
hsa-miR-146a-5p	MIMAT0000449	0.672637	0.000159	0.020467	UP
hsa-miR-520a-3p	MIMAT0002834	0.974852	0.000216	0.026478	UP
hsa-miR-155-5p	MIMAT0000646	-0.84853	0.000276	0.029466	DOWN
hsa-miR-4463	MIMAT0018987	-0.73943	0.000286	0.029466	DOWN
hsa-miR-4640-5p	MIMAT0019699	0.720609	0.000273	0.029466	UP
hsa-miR-3128	MIMAT0014991	1.059139	0.000267	0.029466	UP
hsa-miR-181a-2-3p	MIMAT0004558	0.9362	0.000401	0.03978	UP
hsa-miR-361-3p	MIMAT0004682	0.783039	0.000442	0.042082	UP
hsa-miR-190a-3p	MIMAT0026482	0.667474	0.000532	0.045674	UP
hsa-miR-520b	MIMAT0002843	0.769241	0.000514	0.045674	UP
hsa-miR-6865-5p	MIMAT0027630	-0.71012	0.000562	0.046769	DOWN
hsa-miR-642a-3p	MIMAT0020924	-0.92327	0.000597	0.048087	DOWN
hsa-miR-6511a-5p	MIMAT0025478	-0.70514	0.000665	0.051972	DOWN
hsa-miR-4423-3p	MIMAT0018936	0.885864	0.000745	0.054903	UP
hsa-miR-6799-3p	MIMAT0027499	1.024948	0.000744	0.054903	UP
hsa-miR-326	MIMAT0000756	0.694285	0.000903	0.064696	UP
hsa-miR-7154-5p	MIMAT0028218	-0.72389	0.001046	0.072906	DOWN
hsa-miR-92a-3p	MIMAT0000092	0.833052	0.001207	0.079787	UP
hsa-miR-6511a-3p	MIMAT0025479	-0.62136	0.001289	0.081048	DOWN
hsa-miR-1268a	MIMAT0005922	-0.74005	0.00145	0.087705	DOWN
hsa-miR-181c-5p	MIMAT0000258	0.933365	0.001463	0.087705	UP
hsa-miR-1281	MIMAT0005939	0.917223	0.00155	0.090821	UP
hsa-miR-4775	MIMAT0019931	-0.62303	0.00166	0.092911	DOWN
hsa-miR-508-5p	MIMAT0004778	-0.58759	0.001799	0.094644	DOWN
hsa-miR-96-5p	MIMAT0000095	0.623519	0.001837	0.094705	UP
hsa-miR-6511b-5p	MIMAT0025847	-0.88698	0.001882	0.095124	DOWN

miRNA, microRNA; ID, identity; FC, fold change.

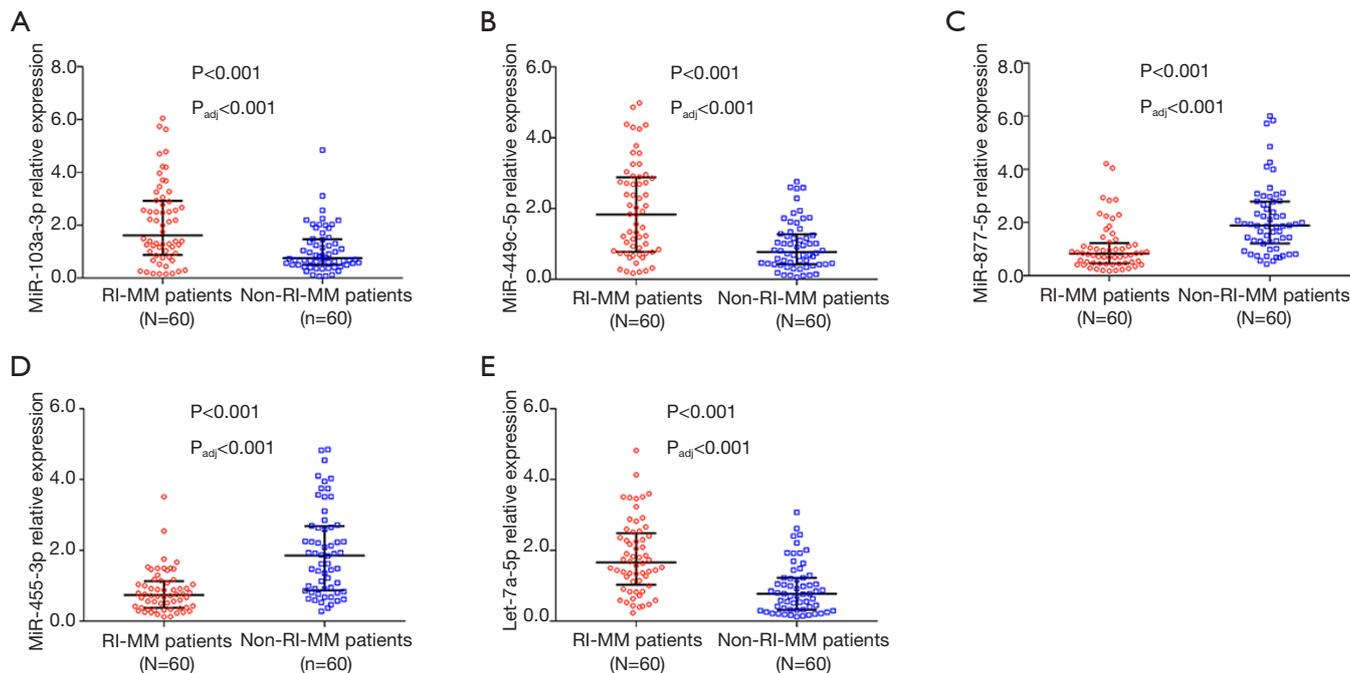
in renal/inflammatory/apoptosis pathways and renal/inflammatory diseases; (III) furthermore, 5 differentially expressed miRNAs in microarray were selected as candidate miRNAs for RT-qPCR validation in a large sample size population, which displayed that there were 3 upregulated miRNAs (miR-103a-3p, miR-449c-5p, let-7a-5p) and 2 downregulated miRNAs (miR-877-5p and miR-455-3p) in RI-MM patients compared with non-RI MM patients;

then the ROC curve analysis disclosed that combining the 5 candidate miRNAs had great value for predicting the RI risk in MM patients.

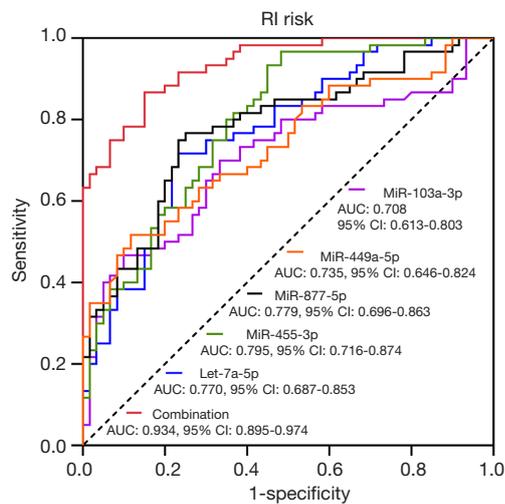
RI is a frequent comorbidity and complication in MM as a result of the insufficient renal function derived from the accumulation of immunoglobulin light chains in the kidney, which often results in the cast nephropathy (5,16). It has been reported that RI attacks approximately 20–50% MM



**Figure 3** Enrichment analysis of differentially expressed miRNAs. GO enrichment analysis of differentially expressed miRNAs between RI-MM patients and non-RI-MM patients (A); KEGG/HP/DOID enrichment analysis of differentially expressed miRNAs between RI-MM patients and non-RI-MM patients (B). miRNA, microRNA; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; HP, phenotype-ontology; DOID, Disease Ontology; RI-MM, multiple myeloma patients with renal impairment; non-RI-MM, multiple myeloma patients without renal impairment.



**Figure 4** Comparison of candidate miRNAs expressions. Comparison of miR-103a-3p (A), miR-449c-5p (B), miR-877-5p (C), miR-455-3p (D) and let-7a-5p (E) expressions between RI-MM patients and non-RI-MM patients. miRNA, microRNA; RI-MM, multiple myeloma patients with renal impairment; non-RI-MM, multiple myeloma patients without renal impairment.



**Figure 5** ROC curve analysis. ROC, receiver operating characteristic; AUC, area under the curve.

patients at diagnosis and is correlated with unsatisfactory clinical outcome and survival profiles, especially for the patients who are already complicated with some chronic disease, which could lead to a chronic damage in the renal function (2,3,17-19). Furthermore, treatment of RI in MM is associated with better survival in patients, for instance, a recent clinical trial reveals that pomalidomide accompanied with low-dose dexamethasone achieves good efficacy in regard to treatment response and overall survival (20). Thus, an early identification of RI in MM patients is necessary in enhancing clinical outcome and prolonging survival of MM patients.

To better improve diagnosis and treatment in RI-MM patients, increasing studies investigating the potential biomarkers for predicting RI in MM patients, however, the predictive value of miRNA for RI in MM patients is still unclear. Nonetheless, there are several studies which report the miRNA expression profile in RI previously. For instance, a recent study identifies 9 miRNAs that are dysregulated among diabetic nephropathy (DN) patients, diabetic patients with membranous nephropathy and patients with normal histology, and 2 of these miRNAs are validated to be involved in the regulation of kidney fibrosis by interacting with ubiquitin-conjugating E2 enzyme variant (UBE2v1) (21). Another study that investigates the miRNA expression profile in the rat models with acute kidney injury identifies 22 downregulated miRNAs and 19 upregulated miRNAs in kidney (22). A study using TaqMan low-density array (TLDA) shows that there are

11 upregulated miRNAs and 11 downregulated miRNAs in plasma of patients with sepsis-induced kidney injury compared with healthy controls (23). In the present study, we investigated the miRNA expression profile in RI-MM patients and found the miRNA expression profile markedly differentiated RI-MM patients from non-RI MM patients, and the dysregulated miRNAs are enriched in renal/inflammatory/apoptosis pathways as well as renal/inflammatory diseases. These results indicate that miRNAs expression profile is markedly dysregulated in RI-MM patients, and may be critical regulators in the pathogenesis of RI in MM patients.

In order to further explore the value of differentially expressed miRNAs in RI-MM patients, 5 differentially expressed miRNAs in microarray were selected for RT-qPCR validation, which disclosed 3 upregulated miRNAs (miR-103a-3p, miR-449c-5p, let-7a-5p) and 2 downregulated miRNAs (miR-877-5p and miR-455-3p) in RI-MM patients compared with non-RI MM patients. Furthermore, the subsequent analyses elucidated that all 5 candidate miRNAs could differentiate RI-MM patients from non-RI MM patients, and their combination exhibited an excellent value for predicting the RI risk in MM patients. And here are several possible explanations to these results: (I) miR-103a-3p: miR-103a has been previously illuminated as a factor promoting inflammation and fibrosis in kidney. For instance, a prior experiment shows that circulating miR-103a expression could be upregulated by angiotensin II, which subsequently results in increased SNRK level in glomerular endothelial cells, leading to renal inflammation and fibrosis in mouse models (24); (II) miR-449c-5p: it has been reported that somatostatin receptor subtype 5 (SSTR5) could inhibit the CRH receptor subtype 1 (CRHR1) expression and function by inducing miR-449c, which subsequently advocates the secondary adrenal dysfunction (25); (III) let-7a-5p: let-7a has been found to be a regulator promoting renal dysfunction. For example, an experiment demonstrates that naringenin reduces kidney injury by regulating the let-7a/ transforming growth factor- $\beta$ 1 receptor 1 (TGFBR1) signaling pathway in diabetic nephropathy rats (26). And another experiment reveals that let-7a enhances hyperplasia and inflammation by increasing cell proliferation and NF- $\kappa$ B activation in systemic lupus erythematosus cell models (27); (IV) miR-877-3p: previous studies report that miR-877 may play an essential role in preventing the progression of RI. For instance, miR-877-3p downregulation results in the over production of interleukin (IL)-1 $\beta$  in human mesangial cells (obtained from IgA

nephropathy patients) that are activated by secretory IgA (28). And another experiment reveals that miR-877 inhibits renal cell cancer cell proliferation and migration via mediating the eukaryotic elongation factor-2 kinase (eEF2K)/eEF2 signaling pathway (29); (V) miR-445-3p: miR-455-3p is reported to inhibit renal fibrosis via suppressing the Rho-associated coiled coil-containing protein kinase 2 (ROCK2) expression in diabetic nephropathy rats (30). Additionally, miR-445-3p and miR-445-5p are illuminated to suppress cancer cell proliferation and invasive abilities through targeting SKA1 and SKA3 in renal cell carcinoma (31).

Besides, there existed several limitations in this study: (I) the statistical power might be slightly diminished due to the relatively small sample of our study; (II) the precise molecular functions of the 5 candidate miRNAs including miR-103a-3p, miR-449c-3p, let-7a-5p, miR-877-5p and miR445-3p in the development or progression of RI in MM were not evaluated in this study; (III) the prognostic roles of the 5 candidate miRNAs for RI-MM patients were not analyzed in our study.

In conclusion, miRNA expression profile is closely implicated in the RI development, and miR-103a-3p, miR-449c-5p, miR-877-5p, miR-455-3p as well as let-7a-5p may serve as novel biomarkers for RI risk in MM patients.

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### Footnote

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/tcr.2020.01.41>). The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The present study was approved by

the Institutional Review Board of our hospital with ethical approval number 2015-018, and all patients provided the written informed consents.

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