Serum miR-148a And miR-152 Expression As Novel Noninvasive Biomarkers For Diagnosis And Prognostic Prediction of Multiple Myeloma

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Abstract

Objective: Multiple myeloma (MM) as a hematological malignancy remains mostly incurable at present. The aim of the current study was to investigate the potential of serum microRNA (miRNA) as a novel biomarker for MM.

Methods: This study recruited 90 MM patients and 30 healthy controls. Bone marrow samples were obtained from 12 MM patients and 6 healthy controls. The expression level of miR-148a/152 was determined by qRT-PCR. The diagnostic performance and prognostic prediction of miR-148a/152 expression were analyzed by ROC curve and Kaplan-Meier method respectively. Serum IgA, IgG, κ light chain, λ light chain and β_2 microglobulin levels were detected by immunoturbidimetry. Serum LDH level was detected by lactic acid substrate method.

Results: The expression levels of miR-148a and miR-152 were elevated in serum and CD138+ plasma cells of MM patients as compared with controls ($P < 0.05$). There was a statistically significant correlation between serum miR-148a/152 expression and the clinicopathological parameters of MM patients.

Conclusion: The results of the present study suggest that circulating miR-148a and miR-152 may prove to be a marker for diagnosis and prognostic prediction of MM.

Introduction

Multiple myeloma (MM) is the second most common hematologic cancer with a global incidence of 6–7 cases per 100000 persons per year. It is characterized by abnormal proliferation of bone marrow (BM) plasma cells resulting in various clinical symptoms including anemia, hypercalcemia, renal insufficiency and osteolytic lesions [1]. Various laboratory tests including bone-marrow biopsy, determination of serum and urine free light chain, and low-dose whole-body CT, supportive MRI are useful for screening of MM [2]. But clinically, there is still a lack of novel noninvasive, specific and sensitive serum or plasma biomarkers for early diagnosis and follow up survey of MM.

MicroRNAs (miRNAs) as small non-coding RNA molecules that function in RNA silencing and post-transcriptional regulation of gene expression have gained popularity as potential biomarkers [3–5]. Aberrant expression of cellular miRNAs can lead to a variety of cancers; dysregulation of these miRNAs can have significant ramifications on tumor initiation, progression, and metastasis, and are often associated with diagnosis, prognosis and response to therapy. Circulating miRNAs are reported to be associated with tumor initiation, metastasis and chemotherapy resistance by regulating cancer stem cells. miRNA-21 is one of the important miRNAs implicated in the genesis and progression of human cancer. Up-regulation of cellular miRNA-21 can cause tumor development and progression, and circulating miRNA-21 has been described as a biomarker for different tumor entities. Plasma miRNA-21 can be used to differentiate early stage lung cancer patients from healthy non-smoking individuals [6]. Thus, given the characteristics of miRNAs in cell free components of the serum and a variety of other bodily fluids, circulating miRNA profiling can be more promising as biomarkers than earlier reported ones [7–9]. The circulating miRNA profile was reported to have a valuable role in early diagnosis, prognostic prediction, therapeutic decision-making and recurrence monitoring of MM [10–12]. A recent study demonstrated that miRNA-130a could discriminate between myeloma patients with extramedullary disease and healthy donors, offering a sensitivity of 77.1% and a specificity of 90.0% [13]. A combination of miR-720 and miR-1308 provides a powerful diagnostic tool for distinguishing normal healthy controls and patients with unrelated illnesses from patients with monoclonal gammopathy of undetermined significance (MGUS) and myeloma [14]. Previous studies reported the aberrant miRNA expression profile in CD138 + plasma cells from MM patients or MGUS, MM cell lines, the BM extracellular microenvironment, and serum or plasma of myeloma patients [10, 15–17].

miR-148a plays a role in several biological processes, including cellular differentiation and development. Differential expression of miR-148a can be observed in different cancers including gastric, colorectal, pancreatic, liver, esophageal, breast, non-small cell lung and urogenital system cancers. The expression level of miR-148a has been linked to the clinical classification, efficacy and prognosis of the tumor. miR-148a can act on different target genes via different pathways such as SMAD2 (TGF- β /SMAD2 pathway), HPIIP (AKT/ERK/FOXO4/ATFS pathway, mTOR pathway) and MET (HGF/Met/Snail pathway) to affect their functions in various tumors [18]. miR-152 is a member of the miR-148/152 family, which includes miR-148a, miR-148b and miR-152. miR-152 has been implicated in a variety of cancers such as prostate, pancreatic and gastric cancer and glioma. miR-152 was found to be inhibited in gynecological tumors such as ovary, endometrial and breast cancers, while its overexpression was found to be associated with

resistance to cisplatin in ovarian cancer by inhibiting cell proliferation and promoting cell apoptosis via direct suppression of DNMT1 [19]. Thus, the miR-148/152 family plays a predominant role in the progression and chemosensitivity of various cancers. In spite of myriad research on these miRNAs, few studies have investigated their significance in MM. The aim of the present study was to assess the miR-148/miR-152 as potential biomarkers in serum and CD138 + plasma cells of MM patients.

Materials And Methods

Patients and samples

Based on the guidelines for diagnosis and management of multiple myeloma in China (revised in 2011), a total of 90 newly diagnosed samples from clinically confirmed MM patients from the Hematology Department of the Affiliated Hospital of Nantong University (Nantong, China) between January 2013 and December 2018 were used in this study, with a median follow-up duration of 42 months from diagnosis. Patients with rheumatoid arthritis and other autoimmune diseases, central nervous system diseases, diabetes, various acute and chronic infectious diseases, other tumor diseases, and mental disorders were excluded from the study. The treatment response was evaluated when the newly diagnosed patients received 4 cycles of chemotherapy. Thirty healthy volunteers were recruited as controls.

Venous blood was collected in a serum separator tube containing clot activation additive and a barrier gel (Vacuette, Kresmunster, Austria) before or after bortezomib-based treatment. Samples were centrifuged at 4°C, 1500g for 10 min; the supernatants were further centrifuged at 4°C, 12000g for 10 min and the serum was transferred to a RNase/DNase-free tube and stored at - 80°C until use.

Serum κ and λ light chain as well as serum IgA and IgG were quantitated using commercially available Beckman Coulter kits (IMMAGE Immunochemistry Systems) and the results were analyzed using Image Special Protein Analyzer by immunoturbidimetry. Lactate dehydrogenase (LDH) was measured with Beckman Coulter Automatic Biochemical Analyzer using commercial Beckman Coulter kits by Lactic acid substrate method. Serum β_2 microglobulin was quantified with immunoturbidimetric test in Beckman Coulter Automatic Biochemical Analyzer using commercial Beckman Coulter kits.

BM samples were obtained from 12 MM patients and 6 healthy controls for evaluation of miRNA expression levels in serum-BM paired samples. BM samples were collected by BM aspiration in tubes containing ethylenediaminetetraacetic acid (EDTA) before bortezomib treatment and CD138 + plasma cells were immediately isolated from BM by CD138 + magnetic bead separation technology following manufacturer's instructions (Miltenyi Biotec Corp., Gladbach, Germany). The purities of isolated CD138 + cells from MM patients and healthy donors were around 90% and 78%, respectively. Written informed consent was obtained from all patients, and the study was approved by the Ethics Committee of our Hospital.

RNA isolation and reverse transcription

Total RNA including miRNAs was extracted from 300 μ l serum samples using the mirVana Paris RNA Isolation kit (Ambion, Austin, TX) following the manufacturer's protocol for liquid samples. Total RNA was isolated from CD138 + plasma cells using Trizol (Invitrogen, USA) reagent. The RNA concentration and purity were determined using a spectrophotometer (NanoPhotometerTM, IMPLEN, German). The serum RNA concentration ranged from 10 ng/ μ l to 20ng/ μ l and RNA purity ranged from 1.20 to 1.86 at 260/280 nm, 0.05 to 0.30 at 260/230 nm, respectively. The range of isolated serum RNA amounts in total RNA was 0.30 μ g to 1.20 μ g. 100ng RNA was reverse transcribed into complementary DNA (cDNA) using RevertAid First Strand cDNA Synthesis Kit (ThermoFisher Scientific, Waltham, USA) following the instructions.

Real-time quantitative polymerase chain reaction (qRT-PCR) analysis

qRT-PCR reactions were performed using ABI 7500 Real-Time PCR System (ABI, Abilene, TX, USA) in triplicate. mRNA levels were qualified using Fast SYBR-Green Master Mix (Applied Biosystems Life Technologies) using U6 as the internal control. For detection of miR-148a and miR-152 expression levels, Bulge-loopTM miRNA qRT-PCR Primer Sets (one RT primer and a pair of qPCR primers for each set) specific for miR-148a and miR-152 designed by Ruibo Biotechnologies Co., Ltd (Guangzhou, China) were used, and endogenous U6 snRNA was used as control. All experimental results were calculated using $2^{-\Delta\Delta CT}$ method: $\Delta CT = CT$ (miR-148a and miR-152) - CT (U6), $\Delta\Delta CT = \Delta CT$ (patients) - ΔCT (control). The cutoff value of miR-148a and miR-152 was 1.345 and 0.62, respectively. The PCR products were verified by sequencing.

Statistical analysis

Statistical analysis was performed using SPSS Statistics 17.0 software (SPSS Inc., Chicago, IL, USA) and related graphs were drawn by GraphPad Prism v 5.0 software (GraphPad Software Inc, La Jolla, CA, USA). Kolmogorov-Smirnov test was used to determine the normality of data distribution in each group. Normally distributed variables are expressed as the mean $\pm$ standard deviation (SD), and non-normally distributed variables are expressed as medians (25th, 75th percentiles). The relative expression of miR-148a and miR-152 in MM and healthy control group was verified by M-W test. Correlation analysis was performed using Spearman tests. Associations of serum miR-148 or miR-152 with the clinicopathological parameters were examined by χ^2 test. Kaplan–Meier survival curves were constructed and Cox regression model was applied to evaluate the prognostic value of each variable to overall survival (OS) of MM patients. Receiver-operating characteristic curves (ROC) and area under the ROC curve (AUC) were used to assess the diagnostic performance of miR-148a and miR-152 for MM. $P < 0.05$ was considered statistically significant.

Results

Baseline participant characteristics

The clinicopathologic characteristics of the 90 participants are listed in Table 1. No significant variation in age and sex distribution was observed between MM patients and healthy controls ($P > 0.05$). The median age of the 90 MM patients and 30 healthy volunteers was 63 (40–84) and 62 years (43–79) years respectively.

Table 1
Characteristics of the study subjects

Characteristics	MM	Control	P
No. of patients	90	30	
Age			0.68
Mean (range)	63 (40–84)	62 (43–79)	
Gender			0.92
Male	56	19	
Female	34	11	
IgG	10.15 (5.79, 25.55)	13.05(10.55, 17.18)	0.39
IgA	0.92 (0.36, 15.23)	2.00 (1.37, 3.91)	0.071
λ light chain	354 (197.30, 1065)	463.50 (408.50, 581)	0.081
κ light chain	1320 (481, 2505)	901.50(782.30, 1065)	0.23
LDH	248 (176.80, 285)	176.50(150.30, 200.30)	< 0.0001
β_2 microglobulin	2.67 (1.61, 4.72)	1.90(1.70, 2.13)	0.001
hemoglobin	89.50 $\pm$ 26.80	138.30 $\pm$ 13.40	< 0.0001
erythrocyte sedimentation rate	72.00 $\pm$ 38.50	6.40 $\pm$ 3.20	< 0.0001
creatinine	100.50(58.80,151.30)	70.00(62.00, 84.75)	0.0075
albumin	31.20 $\pm$ 5.50	43.30 $\pm$ 2.50	< 0.0001
uric acid	443.10 $\pm$ 177.10	318.60 $\pm$ 55.50	0.0001
urea	9.60 $\pm$ 4.00	5.60 $\pm$ 1.10	0.92

Validation of miR-148a and miR-152 in CD138 + plasma cells

It was found in our previous study that miR-148a and miR-152 were upregulated in CD138 + plasma cells of myeloma patients [20], which was further verified by RT-qPCR in this study ($P = 0.02$ and $P = 0.03$ respectively) (Fig. 1a, b). In addition, a significant positive correlation was observed between the relative expression of miR-148a ($r = 0.68$, $P = 0.02$) and miR-152 ($r = 0.60$, $P = 0.04$) in CD138⁺ plasma cells and serum (Fig. 1c, d).

Validation of serum miR-148a and miR-152 expression levels

The relative expression level of miR-148a and miR-152 in serum of MM patients was significantly higher than that in healthy controls (both $P < 0.05$) (Fig. 2a, b). In addition, the relative expression level of miR-148a and miR-152 showed a strong correlation with each other, with a correlation coefficient of 0.3346 ($P < 0.05$) (Fig. 2c). The association of the serum miR-148a and miR-152 expression pattern with the treatment status was further analyzed. As shown in Fig. 3a, b, there was a significant difference in the expression level of miR-152 before and after treatment, whereas the level of miR-148a showed no such a significant difference ($P = 0.0059$ vs. $P = 0.27$). Analysis of serial changes of miR-148a and miR-152 expression in two MM patients who underwent various routine examinations during treatment and follow-up demonstrated that the expression of miR-148a and miR-152 changed dynamically and was associated with the personalized disease status (Fig. 3c, d). The PCR amplification products of miR-148a and miR-152 were further validated by sequencing. These objective gene sequences were in accordance with those provided by the National Center of Bioinformatics Institute (NCBI).

Association of serum miR-148a and miR-152 expression with the clinicopathologic parameters

Spearman bivariate analysis showed that the relative expression of miR-148a/miR-152 was significantly correlated with IgA + IgG ($r = 0.28$, $P = 0.01$ / $r = 0.26$, $P = 0.01$), $\kappa + \lambda$ light chain ($r = 0.25$, $P = 0.02$ / $r = 0.24$, $P = 0.02$), β_2 microglobulin ($r = 0.26$, $P = 0.01$ / $r = 0.25$, $P = 0.02$) and LDH ($r = 0.23$, $P = 0.03$ / $r = 0.22$, $P = 0.03$) (Fig. 4a-d / Fig. 4e-h). MM patients were stratified by age, gender, international staging system, plasma cell percentage in BM, anemia, renal insufficiency, bone disease and treatment response. Based on the median level of miR-148a or miR-152, MM patients were categorized into a low-expression group and a high-expression group. The results showed that high serum expression of miR-152 and miR-148a was significantly associated with the international staging system ($P = 0.02$, 0.0063), plasma cell percentage in BM ($P = 0.034$, 0.01) and treatment response ($P = 0.0011$, 0.0002), but not with the other clinicopathologic parameters (all $P > 0.05$, Table 2).

Table 2
Correlations of serum miR-148a and miR-152 expressions with the clinicopathologic characteristics of MM patients

Characteristics	n	miR-152 expression			miR-148a expression		
		High	Low	P	High	Low	P
Age (years)							
< 60	31	15	16	1.0	13	18	0.38
≥ 60	59	30	29		32	27	
Gender							
Male	56	30	26	0.51	29	27	0.83
Female	34	15	19		16	18	
International staging system							
Stage 1, n (%)	29	9	20	0.023	8	21	0.0063
Stage 2 + 3, n (%)	61	36	25		37	24	
Percentage of plasma cell in bone marrow (%)							
> 10%	41	26	15	0.034	27	14	0.011
< 10%	49	19	30		18	31	
Anemia, n (%)							
Negative	34	16	18	0.83	13	21	0.13
Positive	56	29	27		32	24	
Renal insufficiency, n (%)							
Negative	34	14	20	0.28	21	13	0.13
Positive	56	31	25		24	32	
Bone disease, n (%)							
Negative	41	19	22	0.67	18	23	0.40
Positive	49	26	23		27	22	
Treatment response							
CR + VGPR + PR, n (%)	63	24	39	0.0011	23	40	0.0002
SD + PD, n (%)	27	21	6		22	5	

Diagnostic performance of serum miR-148a and miR-152

ROC analysis was used to investigate the diagnostic value of both miRNAs. AUC of miR-148a was 0.75 (95% CI: 0.66–0.84, $p < 0.0001$) with 58.89% sensitivity and 90% specificity. Serum miR-152 was slightly better in differentiating between MM patients and healthy controls with an AUC of 0.84 (95% CI: 0.77–0.92, $p < 0.0001$), sensitivity of 72.22% and specificity of 86.67%. Notably, multivariate logistical regression analysis suggested that the combination of miR-152 and miR-148a could improve the predictive value, offering an AUC value of 0.86 (95% CI: 0.79 to 0.93, $P < 0.0001$), a sensitivity of 77.78% and a specificity 93.33% for MM, all of which were significantly higher than serum miR-148a or miR-152 alone (both $P < 0.05$) (Fig. 5a). Further ROC analysis showed that the combination of miR-148a, miR-152 and β_2 microglobulin had an increased AUC value to 0.94 (95 % CI: 0.89 to 0.98) with a sensitivity of 90% and a specificity of 90% (Table 3).

Table 3
Diagnosis performance of circulating miR-148a and miR-152 for MM

	Accuracy	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Positive likelihood ratio	Negative likelihood ratio	Youden index	Cutoff point
miR-148a	66.67%	58.89%	90%	94.64%	42.19%	5.89%	45.68%	48.89%	1.35
miR-152	75.83%	72.22%	86.67%	94.20%	50.98%	5.42%	32.05%	58.89%	0.62
miR-148a + miR-152	81.67%	77.78%	93.33%	97.22%	58.33%	11.66%	23.81%	71.11%	0.75
β_2 microglobulin	65%	54.44%	96.67%	98%	41.43%	16.33%	47.13%	51.11%	2.52
miR-148a + miR-152 + β_2 microglobulin	90%	90%	90%	96.43%	75%	9%	11.11%	80%	0.59

Prognostic prediction of serum miR-148a and miR-152 in MM patients

The median follow-up duration was 42 (34–60) months. Kaplan-Meier survival analysis revealed that OS in MM patients with high miR-148a and miR-152 expression levels was significantly reduced as compared with that in those with low miR-148a ($P = 0.024$, Fig. 5b) and miR-152 expression levels ($P = 0.027$, Fig. 5c). As shown in Fig. 5d, OS was low in MM patients with both miR-148a and miR-152 high expressions as compared with that in patients with other combinations of high or low levels of these miRNAs. Parameters significantly related to OS in the univariate analysis were subjected to multivariate analysis to identify independent factors for prognosis and the results showed that miR-148a level ($P = 0.034$) and miR-152 level ($P = 0.039$) were independent prognostic factors for OS in MM with their combined effect being the strongest predictor ($P = 0.008$) (Table 4).

Table 4
Univariate and multivariate Cox proportional hazards regression model analysis of overall survival in MM patients

Parameters	Categories	Univariate analysis		Multivariate analysis	
		HR (95% CI)	P	HR (95% CI)	P
Age	< 60 vs. ≥ 60	2.38 (1.011–5.60)	0.047		0.053
Sex	Male vs. female	2.16 (0.85–5.48)	0.11		
miR-148a	Low vs. high	2.69 (1.14–6.35)	0.024	2.96 (1.08–8.06)	0.034
miR-152	Low vs. high	2.70 (1.12–6.50)	0.027	3.18 (1.06–9.55)	0.039
miR-148a + miR-152	Low vs. high	3.56 (1.51–8.38)	0.0036	3.92 (1.43–10.75)	0.0080
International staging system	Stage 1 vs. Stage 2 + 3	0.35 (0.14–0.83)	0.017	3.82 (1.13–12.88)	0.031
Treatment response	CR + VGPR + PR vs. SD + PD	0.069(0.029–0.16)	< 0.0001	114.70(3.50–3790.10)	0.0080

Discussion

The current study on the miR-148/152 family in MM revealed that the expression of miR-148a and miR-152 was significantly higher in the serum & CD138 + plasma cells of MM patients as compared with healthy controls. There was significant difference in miR-152 expression before and after treatment. The expression of miR-148a and miR-152 was positively correlated with the clinicopathological factors, as well as with the international staging system, plasma cell percentage in BM and treatment response.

Some studies reported that miR-148a was downregulated in gastric, non-small cell lung cancer and cervical cells [21–23], while others reported that it was upregulated [24–26]. Our results demonstrated that miR-148a was significantly upregulated in both serum and plasma cells of MM patients, which is consistent with the report that serum miR-148a-3p level was increased in prostate cancer specimens as compared with normal controls [27]. In addition, miR-148a was also shown to be up-regulated in serum samples from

Type 1 diabetes patients vs. non-diabetic controls [28]. In contrast, Peng et al [29] observed downregulation of miR-148a in the serum of patients with colorectal cancer when compared with healthy controls. It was previously reported that miR-152 acted as a tumor suppressor and it was lowly expressed in cervical cancer tissues and cell lines [30–32]. Contrary to other studies [33–35], we found that miR-152 was upregulated in both the serum and plasma cells of MM patients. Consistent with our research, Jiang et al [36] also observed an upregulation of miR-152 in the serum of patients with bladder cancer, and that miR-152 was an independent prognosis factor for tumor recurrence in patients with non-muscle invasive bladder cancer (NMIBC). Similarly, miR-152 was reported to be up-regulated in BM and cells of patients with chronic myeloid leukemia compared with healthy controls. Further studies showed that miR-152 promoted the proliferation of K562 cells and inhibited cell apoptosis [37]. Another study revealed that miR-152 plasma levels were increased in diabetic nephropathy cases compared with normal controls [38]. However, a downregulation of serum miR-152 was detected in patients with uterine sarcoma [39]. We think that this discrepancy is likely due to the fact that tumor micro-environment varies in different types of cancers and therefore the roles of miRNAs, their target genes and relative mechanisms may be different depending on the type and location of cancer. However, the mechanistic insights of miR-148/miR-152 regulation in carcinogenesis and their significant contributions to tumor progression need to be further investigated in different types of cancer. Notably, we found that the expression of miR-148a and miR-152 changed dynamically and was associated with the personalized disease status, which may aid in improving treatment outcomes by monitoring the patients for these parameters.

Quantitative determination of immunoglobulins, LDH, β_2 microglobulin, determination of free light chains (including free light chain ratio) are considered a standard diagnostic approach for MM [2]. In our study, AUC was 0.75 and 0.84 for serum miR-148a and miR-152 respectively, while AUC was slightly increased to 0.86 when the two miRNAs were combined. We also observed a statistically significant correlation of serum miR-148a or miR-152 expression with LDH, IgA + IgG, β_2 microglobulin and $\kappa + \lambda$ light chain. In addition, higher miR-148a and miR-152 expression levels respectively correlated with shorter OS of MM patients. Interestingly, miR-148a level, miR-152 level, international staging system and treatment response were independent prognostic factors for OS of MM patients. Higher levels of these two miRNAs were also correlated with shorter OS of MM patients. These findings suggest that a combination of these two miRNAs might be better for identifying the pathological stage and predicting the prognosis of MM patients more accurately than detection of a single miRNA.

Several limitations are recognized in our study. Firstly, it is unknown whether miR-148a and miR-152 are capable of distinguishing newly diagnosed MM from others such as MGUS, smoldering MM, relapsed and refractory MM. Secondly, the sample size is relatively small, so further studies and validation with larger cohorts including long-term clinical data with well-defined clinical staging and outcomes are needed to confirm our conclusion. Thirdly, the biological functions of the miR-148/152 family were not investigated, and hence their exact roles in the development and progression of MM remain to be further clarified in future.

In summary, this is the first report describing the aberrant expression of the miR-148/152 family in both CD138 + plasma cells and serum of MM patients. It was found that the expression of miR-148a and miR-152 in the serum of MM patients was upregulated with high sensitivity and specificity. Notably, the combination of miR-148a and miR-152 could improve the diagnostic and predictive value. A high level of serum miR-148a and miR-152 was associated with tumor progression and poor clinical outcomes of MM. These findings may lay a foundation for the development of novel noninvasive tests to diagnose and predict the prognosis of MM and pave the way for further study of innovative therapeutic strategies in clinical practice for MM.

Declarations

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Declaration of Conflicting Interests

The authors declare that there is no conflict of interest.

Statement of Ethics

Written informed consent was obtained from all patients, and the study was approved by the Ethics Committee of the Affiliated Hospital of Nantong University (2012-005).

Author Contributions

Lingling Xie contributed to designing and drafting the manuscript. Guangfei Xie, Xiuying Shi and Hongming Huang were responsible for analysis. Shaoqing Ju and Xudong Wang critically revised the manuscript. All the authors gave final approval of this article.

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Figures

Figure 1

RT-qPCR analysis of the relative expression of miR-148a(a) and miR-152(b) in CD138+ plasma cells of 12 MM patients and 6 healthy controls. The correlations analysis with regard to the expression of miR-148a(c) and miR-152(d) between plasma cells and serum samples were analyzed in 12 paired samples of MM patients. n=12.

Figure 2

qRT-PCR analysis of the relative expression level of miR-148a(a) and miR-152(b) in the serum of MM patients. Correlation of the expression level of circulating miR-148a vs. miR-152(c) in MM patients was analyzed. n=90.

Figure 3

Line charts representing serum miR-148a(a) and miR-152(b) in MM patients before and after treatment. Expression of serum miR-148a(c) and miR-152(d) during treatment and follow-up in two representative patients. n=50.

Figure 4

Correlations of serum miR-148a level with IgA + IgG(a), κ light chain + λ light chain (b), β_2 microglobulin(c), LDH(d). Correlations of serum miR-152 level with IgA + IgG(e), κ light chain + λ light chain(f), β_2 microglobulin(g), LDH (h). n=90.

Figure 5

The diagnostic value of circulating miR-148a and miR-152 for MM(a). Kaplan–Meier curves for overall survival of MM patients were drawn according to the levels of serum miR-148a (b), miR-152(c) and combined miRNAs(d). n=90.