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Correlation of A-Kinase Interacting Protein 1 With Clinical Features, Treatment Response, and Survival Profiles in Patients With Multiple Myeloma

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Abstract

Objective: The present study aimed to detect A-kinase interacting protein 1 expression and further explore the association of A-kinase interacting protein 1 with clinical features and prognosis in patients with multiple myeloma. **Methods:** Totally, 152 *de novo* symptomatic patients with multiple myeloma and 30 healthy donors were enrolled. Bone marrow mononuclear cells derived plasma cells were collected from patients with multiple myeloma before initial treatment and from healthy donors on the enrollment, respectively, and then A-kinase interacting protein 1 protein/messenger RNA expressions were detected by Western blot and reverse transcription quantitative polymerase chain reaction. Treatment response (complete response and overall response rate) was assessed, and survival profiles (progression-free survival and overall survival) were calculated in patients with multiple myeloma. **Results:** A-kinase interacting protein 1 protein/messenger RNA expressions were elevated in patients with multiple myeloma compared to healthy donors, and A-kinase interacting protein 1 (area under the curve: 0.809, 95% confidence interval: 0.726-0.891)/messenger RNA (area under the curve: 0.839, 95% confidence interval: 0.764-0.914) presented good value in differentiating patients with multiple myeloma from healthy donors. In patients with multiple myeloma, A-kinase interacting protein 1 /messenger RNA expressions negatively correlated with albumin while positively correlated with Beta-2-microglobulin, lactate dehydrogenase, International Staging System stage, and t (4;14). Meanwhile, there were 39 (25.7%) complete response patients, 113 (74.3%) noncomplete response patients, 112 (73.7%) overall response rate patients, and 40 (26.3%) nonoverall response rate patients. Complete response and overall response rates were decreased in patients with high A-kinase interacting protein 1 compared to patients with low A-kinase interacting protein 1. Additionally, progression-free survival and overall survival were reduced in patients with high A-kinase interacting protein 1 compared to patients with low A-kinase interacting protein 1. **Conclusion:** A-kinase interacting protein 1 exhibits the potency as a biomarker for multiple myeloma progression and prognosis, which implies the clinical application of A-kinase interacting protein 1 in multiple myeloma management.

Keywords

A-kinase interacting protein 1, clinical features, multiple myeloma, survival, treatment response

Abbreviations

β2-MG, Beta-2-microglobulin; AKIP1, A-kinase interacting protein 1; ALB, albumin; AML, acute myeloid leukemia; AUC, area under curve; BM, bone marrow; BMMC, BM mononuclear cells; CR, complete response; Hb, hemoglobin; IgA, immunoglobulin A; IgG, immunoglobulin G; ISS, International Staging System; LDH, lactate dehydrogenase; MM, multiple myeloma; mRNA,

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messenger RNA; NF-κB, nuclear factor-kappa B; NSCLC, non-small cell lung cancer non-small cell lung cancer; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; PKAc, catalytic subunit of PKA; PR, partial response; ROC, receiver–operating characteristic; RT-qPCR, reverse transcription quantitative polymerase chain reaction; Scr, serum creatinine; VGPR, very good partial response.

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Introduction

Multiple myeloma (MM), one of the most common hematologic malignancies, is characterized by the abnormal proliferation of malignant plasma cells in bone marrow (BM) and excessive secretion of immunoglobulins, which accounts for approximately 10% of cases of hematological malignancies.[1,2] The clinical outcomes have been improved greatly due to the incorporation of novel therapeutic agents and immunomodulatory drugs into MM treatment approaches.[3,4] Previous studies report that the development and progression of MM is often accompanied with cytogenetic abnormalities, chromosomal translocation, and various oncogenes activations; thus, identification of these genetic abnormalities is imperative in predicting treatment response and survival profiles.[5] However, many patients still fail to achieve favorable treatment response and long-term prognosis.[6] Thus, it is essential to explore novel prognosis indicators to assist with MM management in the clinical setting.

A-kinase interacting protein 1 (AKIP1) is a binding partner of p65 subunit in the nuclear factor-kappa B (NF-κB) signaling pathway, which interacts with catalytic subunit of PKA (PKAc) to enhance the transcriptional activity of NF-kB via phosphorylation.[7,8] As a key regulator of NF-κB signaling, AKIP1 is implicated in various aspects of physiological and biological activities (such as immunoinflammatory responses, cell metabolism), which serve as an oncogenic factor in pathogenesis of leukemia and solid cancers.[9-13] Functionally, AKIP1 promotes tumor angiogenesis and lymphangiogenesis via activating downstream oncogenes expression of NF-κB signaling in several solid tumors.[11,14,15] Regarding the role of AKIP1 in hematological malignancies, clinically, AKIP1 is associated with poor risk stratification and worse prognosis in patients with acute myeloid leukemia (AML).[12] In addition, several studies disclose that activation of NF-κB signaling, which is regulated by AKIP1, is a key factor for the growth and survival of MM cells.[16,17] Furthermore, we performed a preliminary research with a small sample size population and observed that AKIP1 was upregulated in patients with MM compared to healthy donors. According to the aforementioned evidence, we hypothesized that AKIP1 might be involved in the development and progression of MM, while no related research has been published yet. Therefore, the present study was to detect the AKIP1 expression in the plasma cells of patients with MM and healthy donors and further explore the association of AKIP1 expression with clinical features and prognosis in patients with MM.

Materials and Methods

Participants

From January 2016 to June 2019, 152 *de novo* patients with MM admitted in our hospital were consecutively enrolled in this study. The inclusion criteria were (1) newly confirmed as *de novo* MM according to 2014 International Myeloma Working Group updated criteria for the diagnosis of MM[18]; (2) age between 18 and 80 years old; and (3) no history of chemotherapy radiotherapy or stem cell transplantation. The exclusion criteria were (1) smoldering (asymptomatic) MM, relapsed MM, secondary MM or mixed MM; (2) complicated with other hematological malignancies or solid tumors; (3) serious infection; and (4) pregnant or lactating women. Meanwhile, 30 healthy BM donors were recruited during the same period, and their health conditions were confirmed during the BM transplantation examination. The present study was approved by the Ethics Committee of Huashan Hospital Fudan University with the approval number "2015-341." The written informed consents were provided by the patients and donors before enrollment.

Data and Sample Collection

The clinical features of patients with MM were collected from electronic medical records, which included demographics, immunoglobulin subtype, biochemical indexes level, bone lesion status, renal impairment status, and chromosomal abnormalities. The Durie-Salmon stage of patients with MM was assessed according to the criteria of Durie-Salmon stage system for MM.[19] The International Staging System (ISS) stage of patients with MM was evaluated based on the criteria of ISS for MM.[20] The BM samples of patients with MM were collected before initial treatment, and the BM samples of healthy donors were collected on the enrollment. Bone marrow samples were then processed with gradient density centrifugation to isolate BM mononuclear cells (BMMC). The separated BMMCs were further purified using CD138-coated magnetic beads (Miltenyi Biotec) to obtain plasma cells. Finally, the plasma cells were stored in liquid nitrogen for any further detection.

Western Blot

The AKIP1 protein expression in plasma cells was measured by Western blot. The procedures were carried out as described previously,[13] and the following antibodies were used: Rabbit Anti-AKIP1 antibody (1:500; Abcam), Rabbit Anti-GAPDH

antibody-Loading Control (1:2500; Abcam), and Horseradish peroxidase-conjugated Goat Anti-Rabbit IgG H&L (1:5000; Abcam).

Reverse Transcription Quantitative Polymerase Chain Reaction

The expression of AKIP1 messenger RNA (mRNA) was detected using reverse transcription quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from plasma cells using PureZOL RNA isolation reagent (Bio-Rad) and then reversely transcribed using iScript cDNA Synthesis Kit (Bio-Rad). Following that, qPCR was performed using KOD SYBR qPCR Mix (Toyobo) to quantify AKIP1 expressions. In addition, the expression of AKIP1 was calculated using $2^{-\triangle\triangle Ct}$ method with GAPDH as an internal reference. The primers of AKIP1 and GAPDH used in this study were referenced from the study previously published[21]: Forward primer: CATGGACAACTGTTTGGCGG; reverse primer: CTGTTTCTCTAGGTGGGGCG; GAPDH; forward primer: TGACCACAGTCCATGCCATCAC, reverse primer: GCCTGCTTCACCACCTTCTTGA.

Treatment and Follow-Up

According to the clinical status and willingness, all patients received appropriate treatments. After 2 cycles of treatments, the clinical response including complete response (CR), very good partial response (VGPR), and partial response (PR) were assessed reference to NCCN clinical practice guidelines in Oncology: Multiple Myeloma (2015.V4). Besides, overall response rate (ORR) was defined as the total percentage of patients with CR, VGPR, and PR. Intensive follow-up was conducted for all patients by telephone or clinical visit. The last follow-up date was June 30, 2019, and the median follow-up duration was 22.0 months ranging from 1.0 to 42.0 months. Progression-free survival (PFS) and overall survival (OS) were calculated in this study. The definition of PFS was the duration from initial treatment to disease progression or death, and the definition of OS was the duration from initial treatment to death. The patients who lost follow-up or not known whether the disease had progressed or whether they had died at the last follow-up date were censored on the date of last visit or the date last known to be alive, respectively.

Statistical Analysis

Descriptive analyses for variables were expressed as mean $\pm$ standard deviation, median (interquartile range), or count (percentage). Comparison of clinical features between 2 groups was determined by Student t test or χ^2 test. Comparison of AKIP1 protein/ mRNA expressions between 2 groups or among 3 groups was determined by Wilcoxon rank sum test or Kruskal-Wallis H rank sum test. Correlation of AKIP1 protein/mRNA expression with continuous variables was analyzed by Spearman rank correlation test. The performances of AKIP1 protein/mRNA expressions in discriminating patients with MM from healthy donors were assessed by receiver–operating characteristic (ROC) curve and the area under curve (AUC) with 95% CI. Progression-free survival and OS were displayed using Kaplan-Meier curves, and comparisons of PFS and OS between 2 groups were determined by log-rank test. Statistical analyses were performed with the use of SPSS version 24.0 (IBM). Figures were plotted using GraphPad Prism 7.00 (GraphPad Software). P value <.05 was considered as significant.

Results

Clinical Characteristics of Patients With MM and Healthy Donors

The median age of total patients with MM\(N = 152) was 55.0 years (47.3-61.0 years), and there were 61 (40.1%) females/91 (59.9%) males. As for immunoglobulin subtype, 83 (54.6%), 41 (27.0%), and 28 (18.4%) patients presented with immunoglobulin (Ig) G, IgA, and others, respectively. In terms of Durie-salmon stage, 16 (10.5%) and 136 (89.5%) patients were with Durie-salmon stage II and III, respectively. Regarding ISS stage, 35 (23.0%), 43 (28.3%), and 74 (48.7%) patients were of ISS stage I, II, and III, respectively. In healthy donors, the median age of total healthy donors (N = 30) were 52.5 years (45.0-61.5 years), and there were 12 (40.0%) females/18 (60.0%) males. There was no difference of age ($P = .299$) or gender ($P = .989$) between patients with MM and healthy donors. Other detailed clinical characteristics are presented in Table 1.

A-Kinase Interacting Protein 1 in Patients With MM and Healthy Donors

Western Blot representative image presenting AKIP1 protein relative expression in plasma cells of patients with MM and healthy donors is shown (Figure 1A). Further quantitative analysis found that AKIP1 protein intensity was elevated in patients with MM (0.751 [0.460-1.067]) compared to healthy donors (0.309 [0.158-0.554]; $P < .001$; Figure 1B). Expression of AKIP1 mRNA was also increased in patients with MM (2.389 [1.627-3.944]) compared to healthy donors (1.026 [0.386-1.650]; $P < .001$; Figure 1C). In addition, ROC curves analysis presented that AKIP1 protein (AUC: 0.809, 95%CI: 0.726-0.891) and mRNA (AUC: 0.839, 95%CI: 0.764-0.914) expressions were both of good value in discriminating patients with MM from healthy donors (Figure 1D). These data suggested that high expression of AKIP1 correlated with increased MM risk.

Correlation of AKIP1 With Clinical Characteristics in Patients With MM

For continuous variables (Table 2), AKIP1 protein expression was negatively associated with albumin (ALB; $r = -.225$,

Table 1. Clinical Features.[a]

Items	Healthy donors, N = 30	Patients with MM, N = 152	*P* value
Age, years			
Mean ± SD	52.0 ± 10.3	54.8 ± 8.6	.123
Median (IQR)	52.5 (45.0-61.5)	55.0 (47.3-61.0)	.299
Gender, n (%)			.989
Female	12 (40.0)	61 (40.1)	
Male	18 (60.0)	91 (59.9)	
Immunoglobulin subtype, n (%)			–
IgG	–	83 (54.6)	
IgA	–	41 (27.0)	
Others	–	28 (18.4)	
Bone lesion, n (%)	–	111 (73.0)	–
Renal impairment, n (%)	–	61 (40.1)	–
Durie-Salmon stage, n (%)			–
II	–	16 (10.5)	
III	–	136 (89.5)	
ISS stage, n (%)			–
I	–	35 (23.0)	
II	–	43 (28.3)	
III	–	74 (48.7)	
Biochemical indexes, median (IQR)	–		
Hb, g/L	–	99.0 (82.3-113.0)	–
Calcium, mg/dL	–	9.8 (8.5-11.1)	–
Scr, mg/dL	–	1.8 (1.4-2.2)	–
ALB, g/L	–	34.0 (29.0-37.8)	–
β2-MG, mg/L	–	5.4 (2.8-8.7)	–
LDH, U/L	–	213.4 (181.3-250.0)	–
Chromosomal abnormalities, n (%)			–
t(4; 14)	–	17 (11.2)	
t(14; 16)	–	7 (4.6)	
Del(17p)	–	15 (9.9)	

Abbreviations: β2-MG, Beta-2-microglobulin; ALB, albumin; Hb, hemoglobin; IgG, immunoglobulin G; IgA, immunoglobulin A; ISS, International Staging System; IQR, interquartile range; LDH, lactate dehydrogenase; MM, multiple myeloma; Scr, serum creatinine; SD, standard deviation.
[a]Comparison was determined by Student *t* test or χ^2 test.

$P = .005$), while it was positively associated with Beta-2-microglobulin (β2-MG; $r = .273$, $P = .001$) and lactate dehydrogenase (LDH; $r = .248$, $P = .002$); Similarly, AKIP1 mRNA expression was negatively correlated with ALB ($r = -.272$, $P = .001$) but positively associated with β2-MG ($r = .260$, $P = .001$) and LDH ($r = .345$, $P < .001$) as well. Regarding categorical variables (Table 3), AKIP1 protein expression was positively associated with ISS stage ($P = .001$) and t(4;14) ($P = .040$), whereas there was no correlation of AKIP1 protein intensity with t(14; 16) ($P = .391$) or del (17p) ($P = .445$) in patients with MM. Similarly, AKIP1 mRNA expression was positively associated with ISS stage ($P = .001$) and t(4;14) ($P = .004$) as well, while there was no correlation of AKIP1 mRNA expression with t(14; 16) ($P = .702$) or del(17p) ($P = .230$). These suggested that high expression of AKIP1 was associated with poor general disease condition in patients with MM.

Correlation of AKIP1 With Treatment Response in Patients With MM

Among total patients with MM, there were 39 (25.7%) CR patients, 113 (74.3%) non-CR patients, 112 (73.7%) ORR patients, and 40 (26.3%) non-ORR patients (Figure 2A). Relative expression of AKIP1 protein was increased in non-CR patients compared to CR patients ($P = .014$), and it was also elevated in non-ORR patients compared to ORR patients ($P = .022$; Figure 2B and C). Furthermore, AKIP1 mRNA expression was elevated in non-CR patients compared to CR patients ($P = .012$), and it was also increased in non-ORR patients compared with ORR patients ($P = .009$; Figure 2D). These data indicated that AKIP1 predicted unfavorable treatment response in patients with MM.

Correlation of AKIP1 With PFS in Patients With MM

Progression-free survival was decreased in patients with high AKIP1 protein compared to patients with low AKIP1 protein ($P = .001$; Figure 3A). Progression-free survival was also reduced in patients with high AKIP1 mRNA compared to patients with low AKIP1 mRNA ($P = .009$; Figure 3B). These data suggested that AKIP1 expression was negatively associated with PFS in patients with MM.

Correlation of AKIP1 With OS in Patients With MM

Overall survival was decreased in patients with high AKIP1 protein compared to patients with low AKIP1 protein ($P = .007$; Figure 4A). Overall survival was also reduced in patients with high AKIP1 mRNA compared to patients with low AKIP1 mRNA ($P = .028$; Figure 4B). These data suggested that AKIP1 expression was negatively associated with OS in patients with MM.

Correlation of Clinical Response With Difference Treatment Regimens in Patients With MM

There was no difference of CR ($P = .826$) or ORR ($P = .355$) among patients receiving bortezomib/dexamethasone, patients receiving bortezomib/doxorubicin/dexamethasone, patients receiving bortezomib/cyclophosphamide/dexamethasone, and patients receiving bortezomib/thalidomide/dexamethasone. More detailed information of clinical response in patients with MM with different treatment regimens was shown in Supplementary Table 1.

Discussion

In the present study, we found that (1) AKIP1 expression was increased in patients with MM compared to healthy

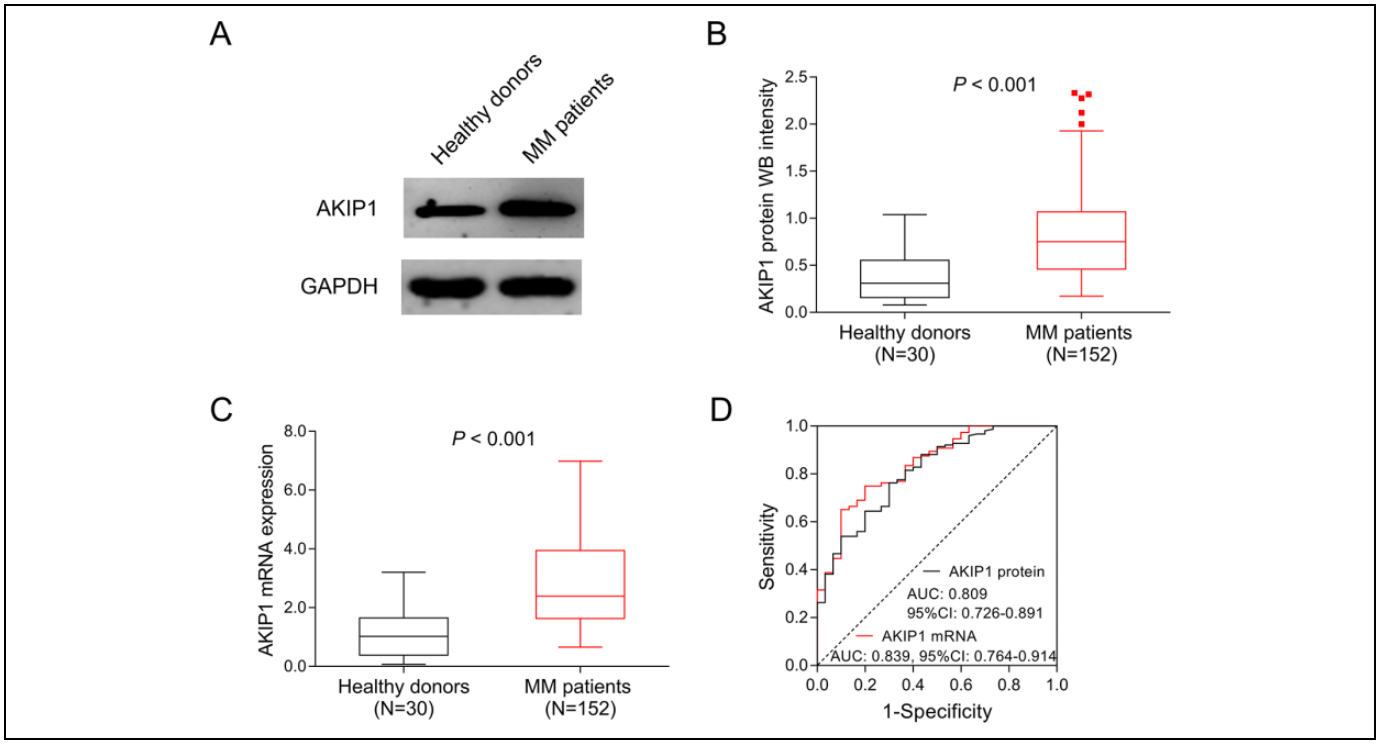

Figure 1. A-kinase interacting protein 1 in patients with MM and healthy donors. Western blot representative image exhibited AKIP1 protein expression in patients with MM and healthy donors (A). Comparison of AKIP1 protein intensity between patients with MM and healthy donors (B). Comparison of AKIP1 mRNA expression between patients with MM and healthy donors (C). The performance of AKIP1 protein/mRNA expression in distinguishing patients with MM from healthy donors (D). AKIP1 indicates A-kinase interacting protein 1; AUC, area under curve; MM, multiple myeloma; mRNA, messenger RNA.

Table 2. Correlation of AKIP1 Protein/mRNA Expressions With Clinical Features (Continuous Variables) in Patients With MM.[a]

	AKIP1 protein expression		AKIP1 mRNA expression	
Items	*P* value	Correlation coefficient (*r*)	*P* value	Correlation coefficient (*r*)
Age	.359	−.075	.642	−.038
Hb	.180	.109	.540	.050
Calcium	.936	.007	.988	−.001
Scr	.326	.080	.121	.126
ALB	.005	−.225	.001	−.272
β2-MG	.001	.273	.001	.260
LDH	.002	.248	<.001	.345

Abbreviations: β2-MG, Beta-2-microglobulin; AKIP1, A-kinase interacting protein 1; ALB, albumin; Hb, hemoglobin; LDH, lactate dehydrogenase; MM, multiple myeloma; mRNA, messenger RNA; Scr, serum creatinine.
[a]Correlation was determined by Spearman rank correlation test.

donors, and it was positively associated with MM risk. (2) Expression of AKIP1 positively correlated with β2-MG, LDH, ISS stage, and t(4;14) but negatively correlated with ALB in patients with MM. (3) Expression of AKIP1 correlated with poor treatment response and unfavorable survival in patients with MM.

A-kinase interacting protein 1 binds to the amino terminal tail of PKAc and facilitates translocation of PKAc into the nucleus, which functions as an important regulator for PKAc-dependent NF-κB activation.[8] Recent researches demonstrate that AKIP1 is implicated in the immunoinflammatory responses, cell proliferation, and survival of several tumors. For example, AKIP1 overexpression promotes cell proliferation, clonogenicity capacity, migration, and invasion via regulating NF-κB signaling in hepatocellular carcinoma and non-small cell lung cancer (NSCLC).[15,22] Clinically, AKIP1 expression is increased in NSCLC tissues compared to adjacent tissues, and it is positively associated with tumor size, lymph node metastasis, and TNM stage in patients with NSCLC.[15] In addition, accumulating researches reveal that NF-κB signaling regulates growth and survival of MM cells, which is vital in the development and progression of MM.[16] Therefore, it was assumed that AKIP1 might serve as an important oncogenic mediator for the pathological progression of MM, and the present study was performed to understand the clinical significance of AKIP1 in MM. Bone marrow samples of patients with MM and healthy donors were collected to detect AKIP1 expression by RT-qPCR and Western blot. It was observed that AKIP1 protein and mRNA expressions were upregulated in patients with MM compared to healthy donors, which was consistent with the previous findings that AKIP1 was upregulated in patients with AML compared to healthy controls.[12] The ROC curve analysis further presented good value of AKIP1 in differentiating patients with MM from healthy donors. The possible

Table 3. Comparison of AKIP1 Protein/mRNA Expressions With Clinical Features (Categorical Variables) in Patients With MM.[a]

Items	AKIP1 protein expression	*P* value	AKIP1 mRNA expression	*P* value
Gender, median (IQR)		.406		.507
Female	0.741 (0.439-0.961)		3.544 (2.401-4.812)	
Male	0.759 (0.470-1.145)		4.286 (2.378-5.542)	
Immunoglobulin subtype, median (IQR)		.352		.319
IgG	0.641 (0.459-0.924)		2.311 (1.429-3.182)	
IgA	0.840 (0.466-1.194)		2.791 (1.772-4.729)	
Others	0.832 (0.438-1.182)		2.531 (2.039-4.423)	
Bone lesion, median (IQR)		.108		.105
No	0.602 (0.427-0.961)		2.240 (1.347-3.081)	
Yes	0.779 (0.467-1.193)		2.462 (1.743-4.069)	
Renal impairment, median (IQR)		.435		.339
No	0.749 (0.430-1.054)		2.342 (1.490-3.549)	
Yes	0.753 (0.475-1.313)		2.456 (1.730-4.181)	
Durie-Salmon stage, median (IQR)		.893		.633
II	0.706 (0.425-1.047)		2.170 (1.584-4.033)	
III	0.751 (0.461-1.067)		2.402 (1.627-3.944)	
ISS stage, median (IQR)		.001		.001
I	0.470 (0.322-0.824)		2.015 (1.070-2.401)	
II	0.612 (0.471-1.054)		2.382 (1.733-3.182)	
III	0.854 (0.543-1.366)		2.904 (2.099-4.722)	
t(4; 14), median (IQR)		.040		.004
No	0.710 (0.488-1.018)		2.374 (1.465-3.566)	
Yes	0.928 (0.652-1.469)		3.539 (2.279-5.674)	
t(14; 16), median (IQR)		.391		.702
No	0.758 (0.460-1.089)		2.382 (1.568-3.942)	
Yes	0.572 (0.448-0.862)		2.509 (1.796-4.695)	
Del(17p), median (IQR)		.445		.230
No	0.726 (0.450-1.071)		2.374 (1.586-3.718)	
Yes	0.853 (0.572-0.995)		3.454 (1.755-4.597)	

Abbreviations: AKIP1, A-kinase interacting protein 1; IgG, immunoglobulin G; IgA, immunoglobulin A; IQR, interquartile range; ISS, International Staging System; MM, multiple myeloma; mRNA, messenger RNA.
[a]Comparison was determined by Wilcoxon rank sum test or Kruskal-Wallis H rank sum test.

reasons might include that according to the previous study, activation of NF-κB signaling pathway is associated with several diverse genetic abnormalities, including cytogenetic abnormalities and chromosomal translocation, which lead to the initiation and progression of malignant MM events.[23] Overexpression of AKIP1 might activate the interaction between PKAc and NF-κB, which enhances the activation of NF-κB signaling; thus, high expression of AKIP1 is associated with increased MM risk.

Existing studies illustrate the positive association between AKIP1 with clinical tumor features in some tumors.[14,15] For example, AKIP1 expression is increased in colorectal cancer tissue compared to noncancerous colorectal mucosa, and it positively correlates with tumor diameter, TNM stage, and lymph node metastasis in patients with colorectal cancer.[14] Another study indicates that high AKIP1 expression correlates with advanced tumor stage, tumor size, and lymph node metastasis in patients with breast cancer.[24] As for the correlation of AKIP1 with clinical features in MM, the present study disclosed that AKIP1 protein and mRNA expression were positively associated with β2-MG, LDH, ISS stage, and t(4;14) but were negatively associated with ALB, which suggested that AKIP1 correlated with poor general disease condition in patients with MM. The possible reasons might include that (1) high expression of AKIP1 might activate NF-κB signaling, which not only promoted MM cell proliferation and migration but also induced cytogenetic abnormalities and chromosomal translocation, thus contributing to the progression of MM. (2) high expression of AKIP1 might stimulate NF-κB-mediated anti-apoptotic signaling and promote the expression of oncogenic gene (such as cyclin D1), which resulted in the poor general disease condition in patients with MM.

As for the prognostic value of AKIP1 in hematologic malignancies, only 1 previous study indicates that AKIP1 overexpression is negatively associated with accumulating event-free survival and accumulating OS in patients with AML.[12] In the present study, we found that high expression of AKIP1 was associated with unfavorable CR, ORR, PFS, and OS in patients with MM. Interestingly, we also revealed that no difference in treatment response was observed among patients receiving different chemotherapy strategy. The possible reasons might involve that (1) in the present study, it was disclosed that high expression of AKIP1 was positively associated with β2-MG, LDH, ISS stage, and t(4;14), which contributed to poor prognosis in MM. (2) Overexpression of AKIP1 might lead to increased drug resistance and self-renew ability of MM cell via

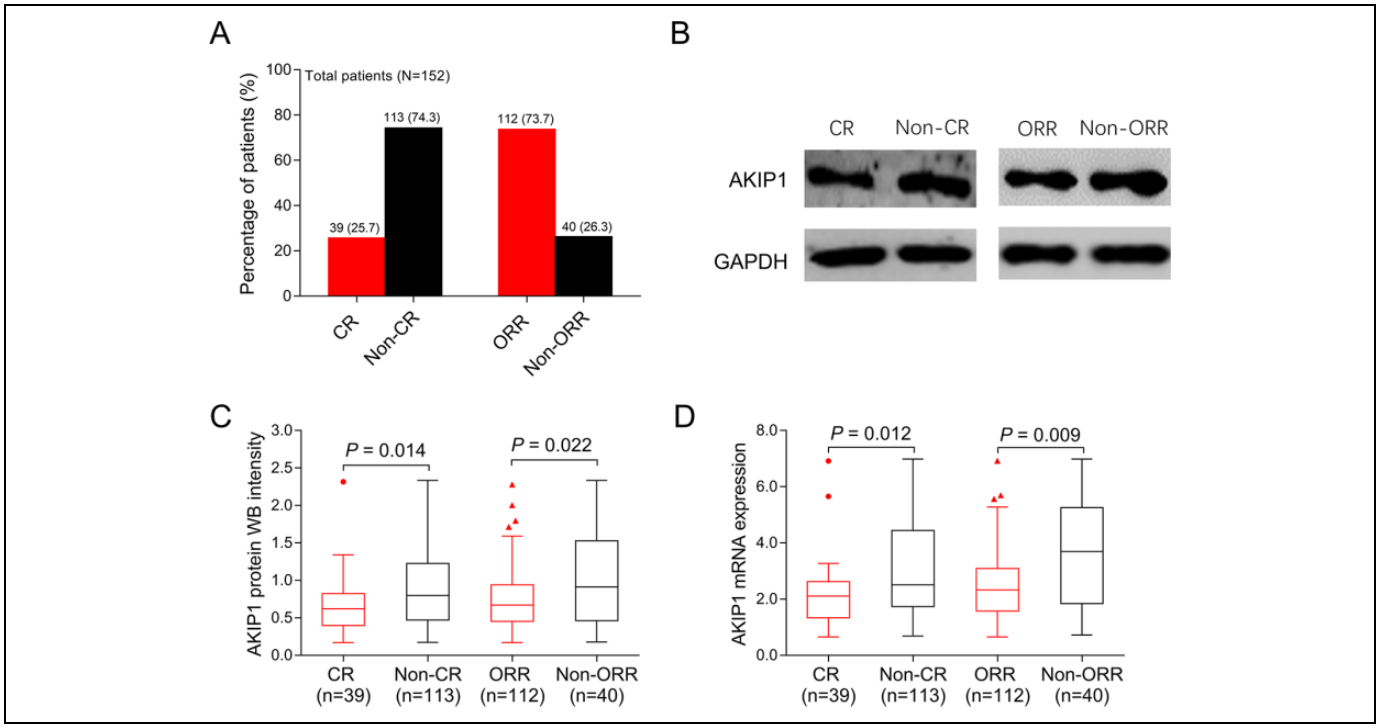

Figure 2. A-kinase interacting protein 1 in CR, non-CR, ORR, and non-ORR patients. Percentage of patients with CR, non-CR, ORR, and non-CRR (A). Western Blot representative image showing AKIP1 protein expression between CR and non-CR patients, between ORR and non-ORR patients (B). Comparison of AKIP1 protein intensity (C)/messenger RNA expression (D) between CR and non-CR patients, between ORR and non-ORR patients. AKIP1 indicates A-kinase interacting protein 1; CR, complete response; MM, multiple myeloma; ORR, overall response rate.

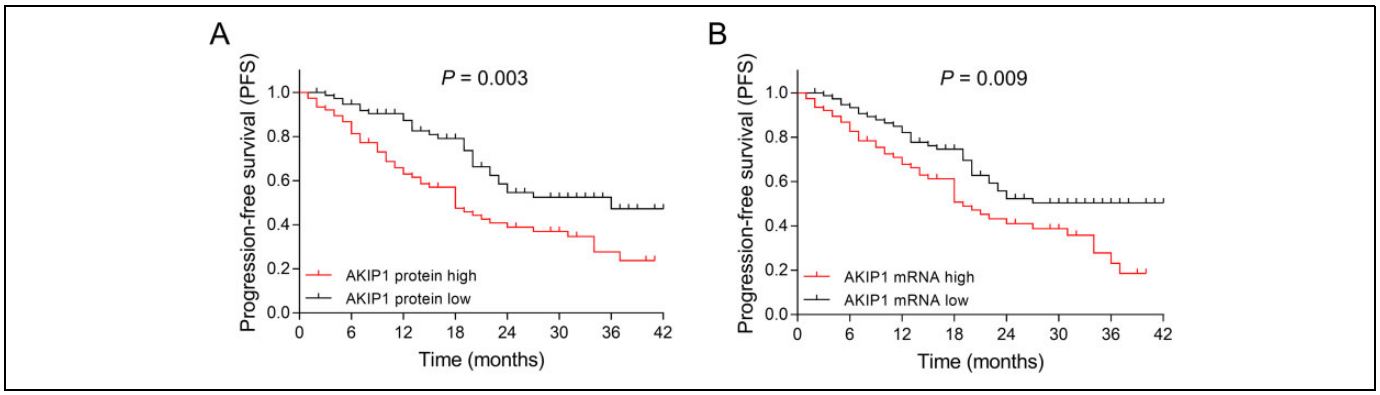

Figure 3. Progression-free survival in AKIP1 high patients with MM and AKIP1 low patients with MM. Comparison of PFS between patients with MM with high AKIP1 protein and patients with MM with low AKIP1 protein (A). Comparison of PFS between patients with MM having high AKIP1 mRNA and patients with MM having low AKIP1 mRNA (B). AKIP1 indicates A-kinase interacting protein 1; MM, multiple myeloma; mRNA, messenger RNA; PFS, progression-free survival.

activating NF-κB signaling; therefore, patients with MM might have unfavorable treatment response and poor long-term prognosis.[16]

There still existed some limitations in the present study. (1) Given that the median follow-up duration was 22.0 months in the present study, further study with a longer follow-up period was needed for validation. (2) The molecular mechanism of AKIP1 in MM was not included in our clinical study, therefore further cellular experiments were essential. (3) Since we excluded the patients with smoldering (asymptomatic) MM, relapsed MM, secondary MM, or mixed MM, further studies were needed to explore the clinical significance of AKIP1 in these patients. In conclusion, AKIP1 is associated with increased MM risk and correlates with poor general disease condition, worse treatment response, and unfavorable survival profiles in patients with MM.

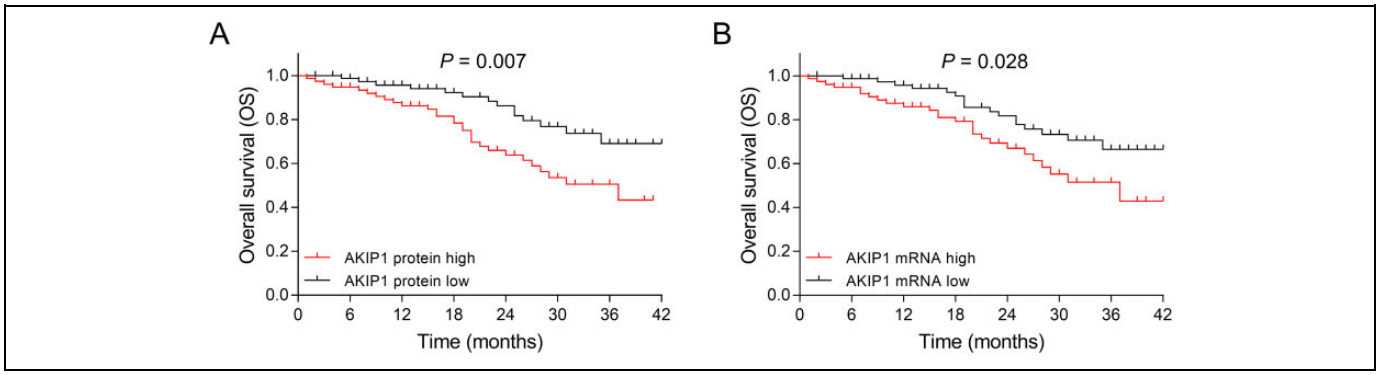

Figure 4. Overall survival in AKIP1 high patients with MM and AKIP1 low patients with MM. Comparison of OS between patients with MM having high AKIP1 protein and patients with MM having low AKIP1 protein (A). Comparison of OS between patients with MM having high AKIP1 mRNA and patients with MM having low AKIP1 mRNA (B). AKIP1 indicates A-kinase interacting protein 1; MM, multiple myeloma; mRNA, messenger RNA;OS, overall survival.

Author' Note

Wei Wang and Yinghua Xie contributed equally to this work. The Ethics Committee of Huashan Hospital, Fudan University approved this study, and the written informed consents were provided by the patients and donors before enrollment.

Declaration of Conflicting Interests

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Supplemental Material

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