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The iron chelator deferoxamine decreases myeloma cell survival

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Abstract

Objective: This study evaluated serum ferritin (SF) levels and investigated their relationships with various clinical markers in patients with multiple myeloma (MM). Furthermore, the effects and molecular mechanism of deferoxamine (DFO) in myeloma cells were studied.

Methods: Clinical data from 84 patients with MM were collected to evaluate SF content and its relationship with several important clinical parameters. MM1S and MM1R myeloma cells were chosen to investigate the effects of iron and DFO on cell survival and apoptosis.

Results: Increased SF levels were detected in newly diagnosed patients, especially those with stage III disease or the κ isotype. SF content was positively correlated with β2-microglobulin, interleukin-6, and lactate dehydrogenase expression. Furthermore, patients with progressive or relapsed disease had higher SF levels. Importantly, iron chelation with DFO efficiently inhibited myeloma cell survival and accelerated apoptosis by regulating apoptosis-related genes.

Conclusions: The importance of SF for MM was highlighted. Additionally, it is suggested that DFO may be a good therapeutic option for MM.

Keywords

Serum ferritin, deferoxamine, iron chelation, multiple myeloma, survival, apoptosis

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Introduction

Iron is an indispensable element that is involved in various biologic processes.[1] Dysregulation of iron homeostasis can lead to changes in cell fate and disease.[2,3]

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Both intrinsic and extrinsic factors play key roles in the transformation and progression of malignancies.[4–6] Mounting evidence indicates that deregulated iron metabolism underlies the pathogenesis of cancer.[7,8] A variety of cancers exhibit alterations in iron metabolism, resulting in high intracellular iron levels that both facilitate the growth of cancer cells during early stages and enhance the metastatic cascade during late stages.[9,10] Under specific conditions, iron also contributes to cancer defense by regulating iron metabolism-associated proteins and inducing the production of toxic reactive oxygen species that initiate ferroptosis.[3,11] Nevertheless, iron is a target for cancer therapy, and regulating cellular iron content is considered a promising anti-cancer strategy.

Because of their high affinity for iron, iron chelators, such as deferoxamine (DFO), deferiprone, and deferasirox, have been used clinically to treat iron overload disorders.[12–14] Notably, iron chelation efficiently inhibits cancer cell growth. Several key molecules and pathways that are important for the cytotoxic effects of iron chelation therapy have been elucidated in some solid tumors, such as neuroblastoma, liver cancer, breast cancer, and ovarian cancer.[1,15] Additionally, the remarkable diversity in the mechanisms of iron chelation among various solid tumors may depend on several factors, such as tumor type, disease stage and iron chelators.[16]

Compared with solid tumors, malignancies in the hematopoietic system have different clinical features. DFO, the most widely used iron chelator, caused leukemic cytoreduction in patients with acute leukemia and suppressed the growth of leukemic cells in previous research.[17,18] Multiple myeloma (MM), characterized by the monoclonal proliferation of long-lived plasma cells within the bone marrow, remains incurable despite recent treatment advances.[19] Recently, serum ferritin (SF) has been regarded as a negative prognostic indicator, whereas low ferroportin expression is associated with significantly reduced overall survival in MM.[20,21] However, the further value of SF content in the progression of MM and the underlying mechanism of the effects of DFO on myeloma cells have not yet been fully explored.

In this study, we evaluated SF content and analyzed its relationship with clinical indicators in patients with MM. Furthermore, we studied the effects and molecular mechanism of DFO in myeloma cells. Increased SF levels were detected in patients with newly diagnosed MM, especially those with stage III disease or the κ isotype. SF levels were positively correlated with β2-microglobulin (β2-MG), interleukin (IL)-6, and lactate dehydrogenase (LDH) expression. Moreover, patients with progressive or relapsed disease had higher SF levels. Importantly, DFO efficiently inhibited myeloma cell survival while accelerating apoptosis by regulating apoptosis-related genes. Overall, these findings highlight the importance of SF for MM and suggest that DFO may be a good therapeutic option for this malignancy.

Materials and methods

Patient data analysis

All patients participated voluntarily in the study and provided informed consent. The study was approved by the Ethics Committee of Nanjing First Hospital (Nanjing, China). SF content and its relationships with various clinical parameters, including β2-MG, IL-6, LDH, hemoglobin (Hb), albumin (Alb), and serum creatinine (Scr), were analyzed.

Cell culture

MM1S and MM1R cell lines were obtained from American Type Culture Collection (Manassas, VA, USA). All cells were

cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS, Gibco, Thermo Fisher Scientific, Waltham, MA, USA) and 1% penicillin–streptomycin solution (Sigma-Aldrich, St. Louis, MO, USA) in a humidified atmosphere of 5% CO_2 at 37°C.

Flow cytometric analysis

Cells were harvested as single-cell suspensions in PBS and then resuspended in PBS containing 1% FBS, stained with a combination of antibodies, washed, and then subjected to fluorescence-activated cell sorting (FACS). Standard protocols were followed for all experiments. We used Canto II (BD Biosciences, San Diego, CA, USA) for FACS analysis.

Cellular total iron detection

MM1S and MM1R cells were separately seeded in 96-well plates at a density of 5×10^6 cells/well and treated with various concentrations of DFO (10, 20, and 50 μM, Novartis, Basel, Switzerland), either with or without 10 μM $FeCl_3$ (Sigma-Aldrich), for 12 hours. Cells treated with PBS served as the control group. Following treatment, the concentrations of cellular total iron were quantified using a spectrophotometric iron assay kit (BioVision, Mountain View, CA, USA) according to the manufacturer's protocols.

Cell viability assay

MM1S and MM1R cells were seeded in 96-well plates at a density of 5×10^4 cells/well and cultured for 24 hours. Subsequently, the cells were treated with various concentrations of DFO (10, 20, and 50 μM), either with or without 10 μM $FeCl_3$, for 12 hours. Cells treated with PBS served as the control group. Finally, cell viability was detected using a CCK-8 kit (Dojindo Laboratories, Kumamoto, Japan) following the manufacturer's protocol.

Cell apoptosis assay

Following specific treatment, single-cell suspensions were prepared. Cells were first incubated with annexin V-FITC antibody (BD Biosciences, San Diego, CA, USA) in binding buffer for 15 minutes. Before flow cytometry, PI in binding buffer was added following the manufacturer's protocols. Cells were then collected for analysis *via* flow cytometry.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated using RNeasy Mini Kit (Qiagen, Hilden, Germany), and reverse transcription was performed using Super Script III (Invitrogen, Thermo Fisher Scientific). qRT-PCR was conducted using the StepOne real-time PCR system (Applied Biosystems, Thermo Fisher Scientific) with SYBR Green PCR Master Mix (Applied Biosystems, Thermo Fisher Scientific). The expression level of target genes was analyzed as the relative quantity calculated using the $^{\Delta\Delta}$Ct method [$^{\Delta\Delta}Ct = (Ct_{TARGET} - Ct_{GAPDH})_{sample} - (Ct_{TARGET} - Ct_{GAPDH})_{calibrator}$]. The sequences for all primers are presented in Table 1.

Statistical analysis

SPSS17.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 6.0 (GraphPad, San Diego, CA, USA) were used for the statistical analyses. Comparisons between two groups were performed using an unpaired Student's *t*-test, whereas multiple-group comparisons were performed using one-way analysis of variance. The correlations of different variables were calculated using Pearson's correlation analysis. All results are expressed as the mean ± SEM.

Table 1. Primers used in real-time polymerase chain reaction.

Gene	Forward (5′-3′)	Reverse (5′-3′)
GAPDH	GGAGTCCACTGGCGTCTTCA	ATTGCTGATGATCTTGAGGCTGTTG
CDK2	CCAGAAACAAGTTGACGGGAGAG	AGTGAGAGCAGAGGCATCCAT
CDK4	GAGCATGTAGACCAGGACCTAAGG	CCACCACTTGTCACCAGAATGTTC
CDK6	GTGACCAGCAGCGGACAAAT	GCAGCCAACACTCCAGAGATC
Cyclin D1	CCAGAGGCGGAGGAGAACAA	GAGGCGGTAGTAGGACAGGAAG
Cyclin D3	GCTTACTGGATGCTGGAGGTATGT	AGCGTGGTCGGTGTAGATGC
Cyclin E1	TGACTATTGTGTCCTGGCTGAATGT	TTCTCTATGTCGCACCACTGATACC
p53	AGTGTGGTGGTGCCCTATGA	GTTGGACAGTGCTCGCTTAGTG
Bcl-2	GTGTGTGGAGAGCGTCAACC	CAGAGACAGCCAGGAGAAATCAAAC
Bcl-xl	GCCACTTACCTGAATGACCACCTA	TGAAGAGTGAGCCCAGCAGAA
Bax	TTCTGACGGCAACTTCAACTGG	GGTGAGGAGGCTTGAGGAGTC
Fas	TGAAGGACATGGCTTAGAAGTGGAA	CTTGGTGTTGCTGGTGAGTGT

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; CDK, cyclin-dependent kinase.

Table 2. Clinical characteristics of all patients with multiple myeloma.

Characteristics	All patients (N = 84)
Median age, years (range)	63 (37–89)
Sex, no. (%)	
Male	48 (57)
Female	36 (43)
Ig isotype, no. (%)	
IgA	24 (29)
IgG	39 (46)
κ	14 (17)
λ	7 (8)
DS stage, no. (%)	
Stage I	10 (12)
Stage II	22 (26)
Stage III	52 (62)

DS, Durie–Salmon.

$P < 0.05$ was considered statistically significant.

Results

Increased SF content was detected in patients during MM progression

This retrospective study included 88 patients with MM. The clinical characteristics of the patients are presented in Tables 2 and 3. The mean levels of SF in all patients, including those who were newly diagnosed (389.1 ± 36.67 ng/mL) and those with progressive (801.9 ± 83.25 ng/mL), stable (347.5 ± 37.62 ng/mL), and relapsed disease (931.6 ± 97.62 ng/mL), were higher than those of healthy controls (88.34 ± 6.91 ng/mL, all $P < 0.001$). SF levels were significantly higher in patients with progressive disease than in those with newly diagnosed MM ($P < 0.001$). Although SF levels were lower in patients with stable disease than in those with progressive disease ($P < 0.001$), they were still equivalent to those of newly diagnosed patients. Importantly, SF levels were highest in patients with relapsed disease ($P < 0.001$, Figure 1a).

Mean SF levels were further analyzed in all patients with MM according to the Durie–Salmon stage and Ig isotype. Significantly higher SF levels (both $P < 0.01$) were detected in stage III (569.7 ± 46.31 ng/mL) than in stage I (307.5 ± 23.18 ng/mL) and stage II (352.1 ± 29.72 ng/mL, Figure 1b). Different SF levels were observed among the various Ig isotypes, including IgA (382.7 ± 55.44 ng/mL), IgG (528.9 ± 46.84 ng/mL), κ (510.5 ± 45.24 ng/mL), and

Table 3 Clinical characteristics of patients with newly diagnosed MM.

Characteristics	All patients (N = 26)
Median age, years (range)	63 (37–89)
Sex, no. (%)	
Male	15 (58)
Female	11 (42)
Ig isotype, no. (%)	
IgA	8 (31)
IgG	6 (23)
κ	8 (31)
λ	4 (15)
DS stage, no. (%)	
Stage I	4 (15)
Stage II	8 (31)
Stage III	14 (54)

DS, Durie–Salmon.

λ (275.1 ± 21.81 ng/mL). SF levels were significantly higher in patients with the IgG and κ isotypes than in those with the λ isotype (both $P < 0.05$, Figure 1c). This indicated that SF levels increased with disease progression, suggesting that its content is an indicator of tumor burden.

SF levels were positively correlated with β2-MG, IL-6, and LDH expression in patients with newly diagnosed MM

We next focused on SF levels in patients with newly diagnosed MM. SF levels were higher in stage II (361.4 ± 47.99 ng/mL) than in stage I (242 ± 29.71 ng/mL, $P < 0.05$), whereas SF levels were highest in stage III (458 ± 54.73 ng/mL, $P < 0.05$, Figure 2a). Additionally, patients with the κ isotype (557 ± 75.85 ng/mL) had higher SF levels than those with other Ig isotypes, including IgA (312 ± 52.69 ng/mL, $P < 0.05$), IgG (293.8 ± 64.75 ng/mL, $P < 0.05$), and λ (271.2 ± 30.31 ng/mL, $P < 0.01$, Figure 2b).

β2-MG, IL-6, LDH, Hb, Alb, and Scr are important diagnostic markers assessed in the clinic.[22–24] We evaluated the correlations of SF levels with these factors in patients with newly diagnosed MM. Notably, SF levels exhibited positive correlations with β2-MG ($r = 0.510$, $P = 0.011$, Figure 2c), IL-6 ($r = 0.651$, $P = 0.010$, Figure 2d), and LDH expression ($r = 0.524$, $P = 0.009$, Figure 2e). However, SF content was not correlated with Hb ($r = -0.021$, $P = 0.921$, Figure 2f), Alb ($r = 0.072$, $P = 0.738$, Figure 2g), and Scr levels ($r = 0.317$, $P = 0.132$, Figure 2h).

DFO decreased the survival of myeloma cells

DFO is the most widely used iron chelator for treating iron overload disease. Therefore, we studied its effects on myeloma cells. Treatment with 50 μM DFO significantly decreased the iron content in both MM1S and MM1R cells (both $P < 0.05$, Figure 3a). Ferric chloride was added to further examine the effect of DFO on myeloma cells. Ferric chloride increased intracellular iron content in both MM1S and MM1R cells (both $P < 0.01$), while DFO treatment significantly decreased the iron content in both myeloma cells in a concentration-dependent manner ($P < 0.05$, Figure 3b). Then, then CCK-8 assay was used to detect myeloma cell survival. Viability was decreased in both MM1S and MM1R cells following treatment with 20 or 50 μM DFO treatment ($P < 0.05$, Figure 4a). Additionally, compared with the findings in the control group, the survival of both MM1S and MM1R cells was not changed by exposure to ferric chloride alone. In the presence of ferric chloride, the viability of MM1S cells was significantly inhibited by 20 or 50 μM DFO (both $P < 0.05$), whereas treatment with 50 μM DFO also decreased the viability of MM1R cells ($P < 0.05$, Figure 4b).

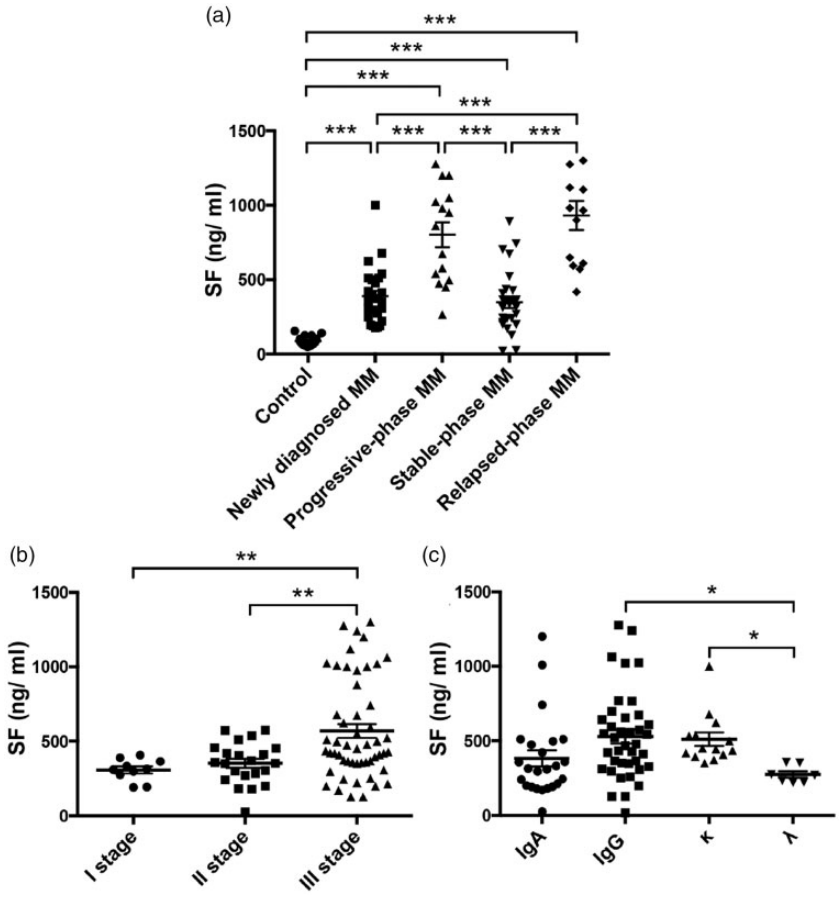

Figure 1. SF levels in patients with MM. (a) SF levels in healthy controls and different subsets of patients with MM stratified by disease status. SF levels in patients with MM stratified by the Durie–Salmon stage (b) and Ig isotype (c). One-way analysis of variance was performed (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).
SF, serum ferritin; MM, multiple myeloma.

DFO decreased the expression of some genes positively correlated with the proliferation of myeloma cells

To investigate the underlying molecular mechanism of DFO, we focused on the expression of several cell proliferation-related genes, including cyclin-dependent kinase 2 (CDK2), CDK4, CDK6, cyclin D1, cyclin D3, and cyclin E1. CDK2 and cyclin E1 expression was decreased in DFO-treated MM1S and MM1R cells (all $P < 0.05$), whereas the expression of CDK4, CDK6, and cyclin D3 was decreased in DFO-treated MM1R cells (all $P < 0.05$). Ferric chloride supplementation significantly increased the expression of CDK4, CDK6, cyclin D3, and cyclin E1 in MM1S cells (all $P < 0.05$) and promoted the expression of CDK4, cyclin D1, and cyclin D3 in MM1R cells (all $P < 0.05$). Furthermore, in the presence of ferric chloride, DFO decreased the expression of CDK2, CDK4, CDK6, cyclin D3, and

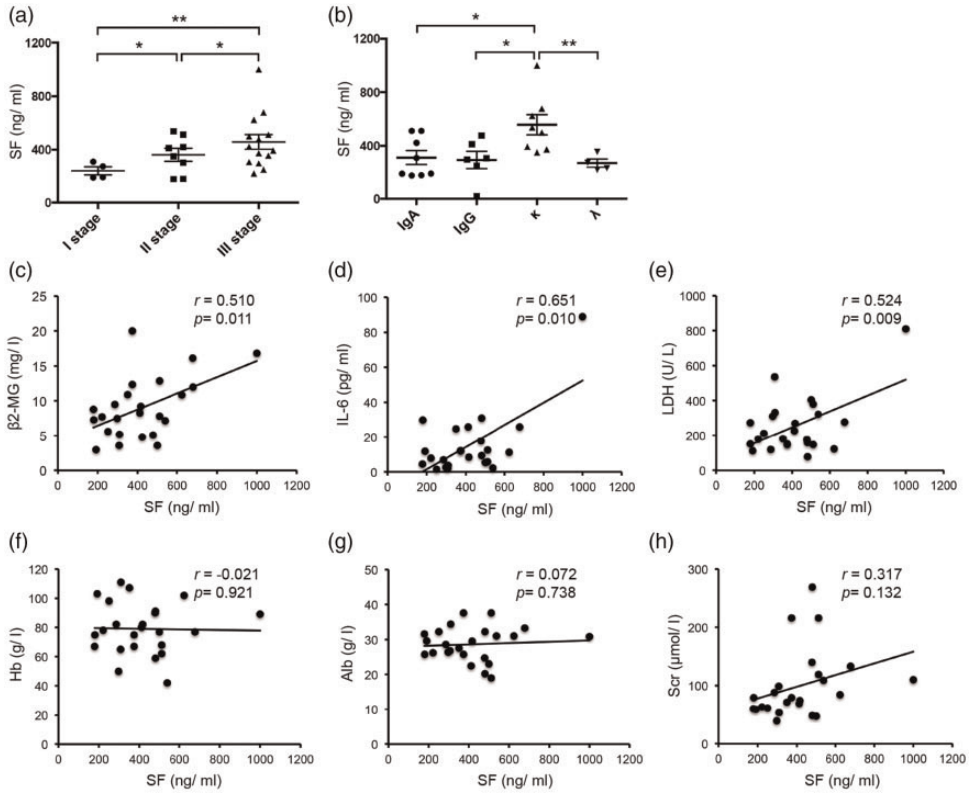

Figure 2. SF levels in patients with newly diagnosed MM and their correlations with diagnostic variables. SF levels in patients with newly diagnosed MM stratified by the Durie–Salmon stage (a) and Ig isotype (b). The correlations of SF levels with β2-MG (c), IL-6 (d), LDH (e), Hb (f), Alb (g), and Scr levels (h) were calculated. Pearson's correlation analysis and one-way analysis of variance were performed (*$P < 0.05$; **$P < 0.01$)
SF, serum ferritin; MM, multiple myeloma, β2-MG, β2-microglobulin; IL-6, interleukin-6; LDH, lactate dehydrogenase; Hb, hemoglobin; Alb, albumin; Scr, serum creatinine.

cyclin E1 in MM1S cells and that of CDK4 and cyclin D3 in MM1R cells compared with the findings in cells treated with ferric chloride alone (all $P < 0.05$, Figure 5a, 5b). These results demonstrated that DFO downregulated some genes associated with the proliferation of myeloma cells.

Induction of myeloma cell apoptosis by iron chelator DFO

Apoptosis in myeloma cells was also studied to investigate the mechanism by which DFO decreases myeloma cell viability. The proportions of both Annexin V^+ propidium iodide $(PI)^-$ and Annexin V^+ PI^+ apoptotic MM1R cells were increased by DFO treatment (both $P < 0.05$). Ferric chloride supplementation decreased the proportion of Annexin V^+ PI^+ apoptotic MM1S cells ($P < 0.05$) and those of both Annexin V^+ PI^- and Annexin V^+ PI^+ apoptotic MM1R cells (both $P < 0.05$). Additionally, with ferric chloride supplementation, the numbers of Annexin V^+ PI^- MM1S and MM1R cells were increased by DFO treatment compared with the findings for DFO treatment alone (both $P < 0.05$, Figure 6a, 6b).

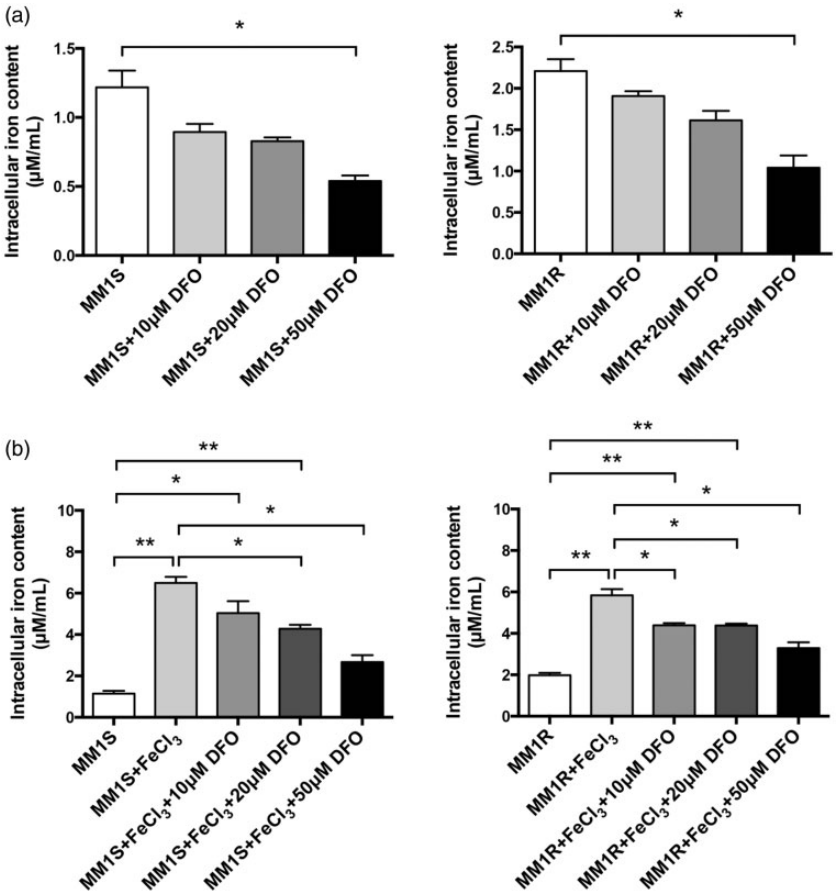

Figure 3. Iron content of myeloma cells after treatment with DFO. (a) Iron content in MM1S (left) and MM1R cells (right) was assessed after treatment with different concentrations of DFO (0, 10, 20, 50 μM) for 12 hours. (b) Iron content in MM1S (left) and MM1R cells (right) was assessed after treatment with 10 μM $FeCl_3$ and/or different concentrations of DFO (0, 10, 20, 50 μM) for 12 hours. Data are representative of three independent experiments. One-way analysis of variance was performed (*$P < 0.05$; **$P < 0.01$). DFO, deferoxamine.

We then assessed the expression of several apoptosis-related genes, including p53, Bcl-2, Bcl-xl, Bax, and Fas. DFO increased the expression of Bcl-2, Bcl-xl and Bax in MM1S cells and that of p53 in MM1R cells (all $P < 0.05$). Conversely, p53, Bcl-xl, and Bax were downregulated in ferric chloride-treated MM1S and MM1R cells (all $P < 0.05$). Bcl-2 and Fas expression was decreased by ferric chloride supplementation in MM1R cells (both $P < 0.05$). Furthermore, in the presence of ferric chloride, Bcl-xl expression was decreased by DFO treatment in both MM1S and MM1R cells (both $P < 0.05$). Additionally, p53 expression was decreased by combined treatment with ferric chloride and DFO in MM1R cells ($P < 0.05$, Figure 6c, 6d). These results suggested that DFO promoted cell apoptosis by regulating apoptosis-related genes.

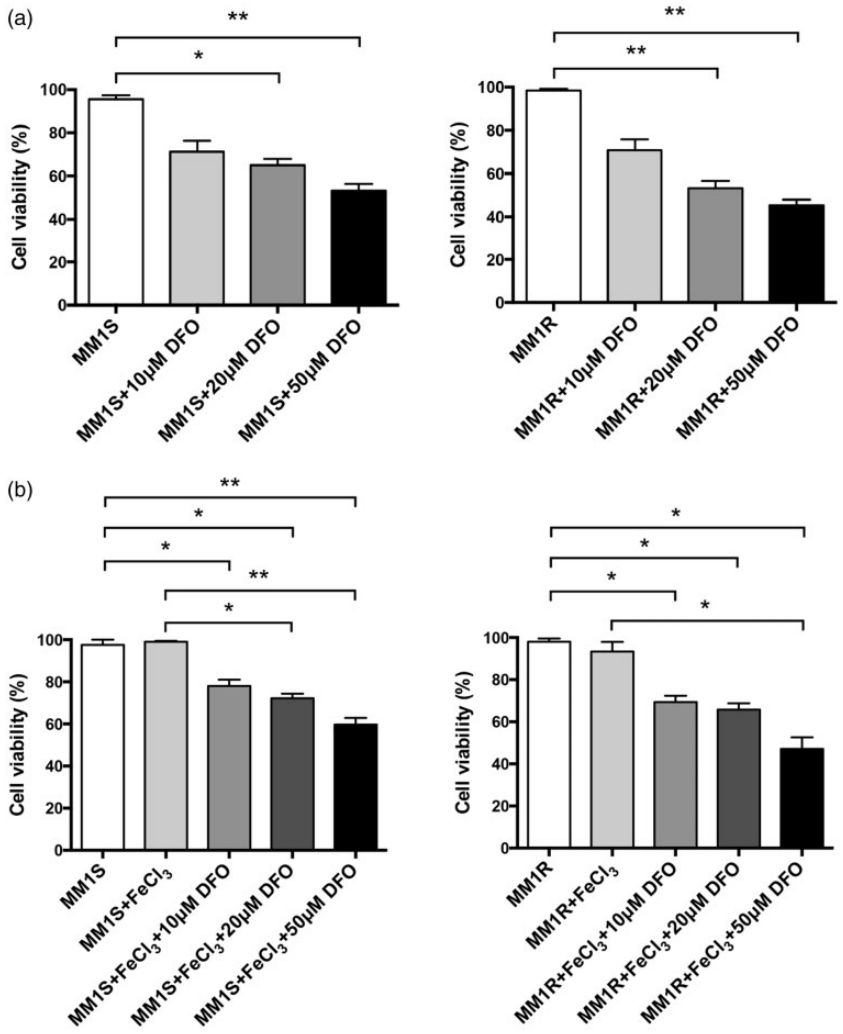

Figure 4. Effects of DFO on the survival of myeloma cells. (a) The viability of MM1S (left) and MM1R cells (right) was assessed using the CCK-8 assay after treatment with different concentrations of DFO (0, 10, 20, 50 μM) for 12 hours. (b) The viability of MM1S (left) and MM1R cells (right) was assessed using the CCK-8 assay after treatment with 10 μM $FeCl_3$ and/or different concentrations of DFO (0, 10, 20, 50 μM) for 12 hours. Data are representative of three independent experiments. One-way analysis of variance was performed (*$P < 0.05$; **$P < 0.01$).
DFO, deferoxamine.

Discussion

Iron is attracting increasing attention from researchers investigating various malignancies because of its vital role and therapeutic potential.[25] Dysregulation of iron homeostasis has been identified in both malignant and microenvironmental cells.[26] However, the significance of iron remains unclear because opposing effects have also been observed as a result of various factors.[16] Excess iron promoted both tumor initiation and tumor growth in hepatocellular, lung,

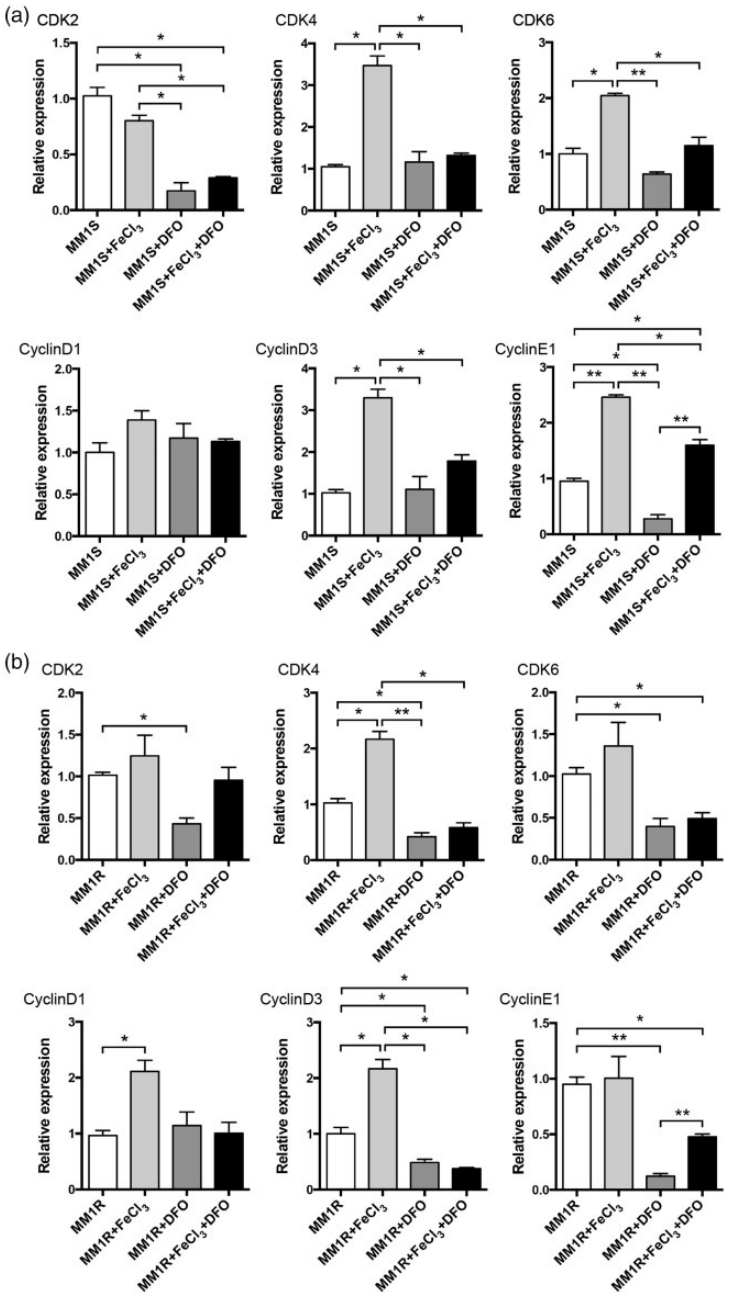

Figure 5. Effects of DFO on the expression of cell proliferation-related genes in myeloma cells. MM1S and MM1R cells were treated with 10 μM $FeCl_3$ and/or 50 μM DFO for 12 hours. MM1S (a) and MM1R cells (b) were collected, and the expression of genes, including CDK2, CDK4, CDK6, cyclin D1, cyclin D3, and cyclin E1, was detected using real-time polymerase chain reaction. For each gene, the relative expression of cells treated with PBS was designated as 1.000. Data are representative of at least three independent experiments. One-way analysis of variance was performed (*$P < 0.05$; **$P < 0.01$).
DFO, deferoxamine, CDK, cyclin-dependent kinase.

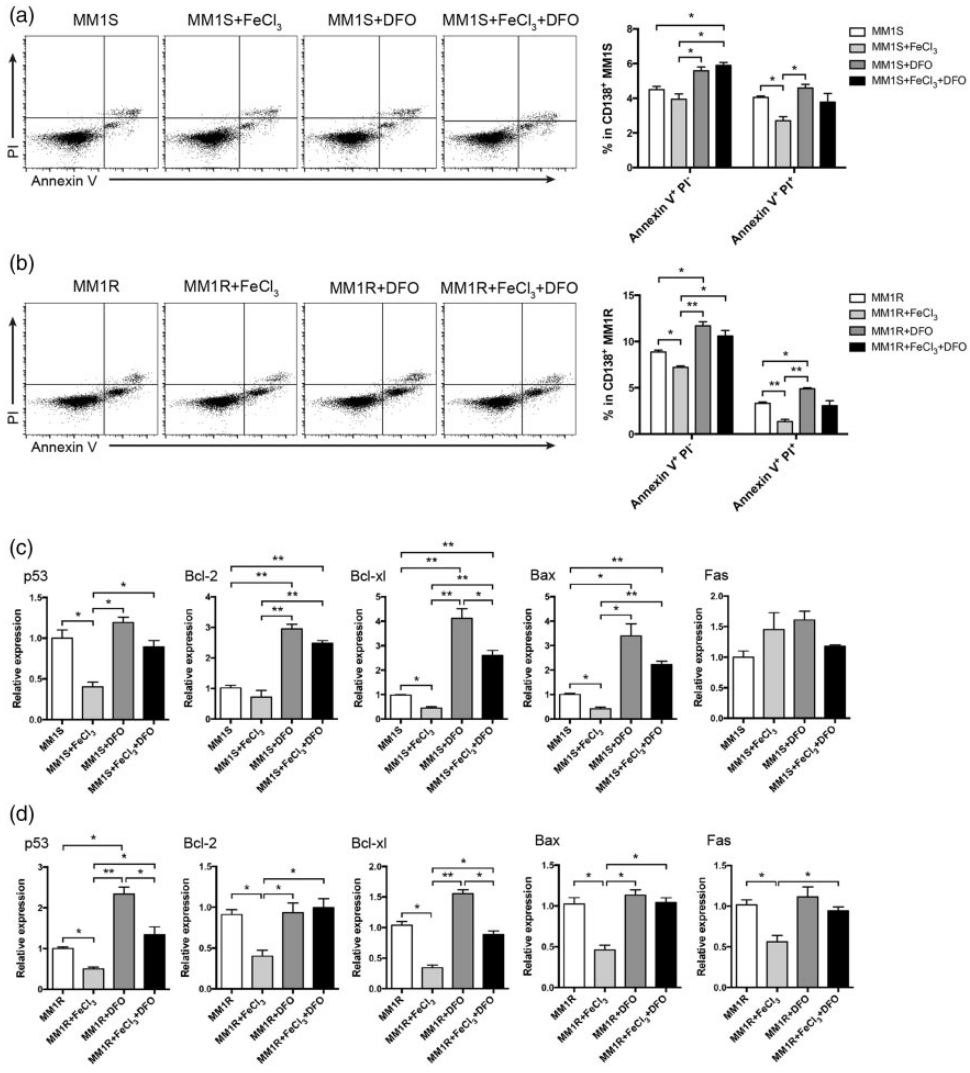

Figure 6. Induction of myeloma cell apoptosis by DFO. MM1S and MM1R cells were treated with 10 μM $FeCl_3$ and/or 50 μM DFO for 12 hours. The apoptosis of MM1S (a) and MM1R cells (b) was assessed using annexin V staining. MM1S (c) and MM1R cells (d) were collected, and the expression of genes, including p53, Bcl-2, Bcl-xl, Bax, and Fas, was detected using real-time polymerase chain reaction. For each gene, the relative expression of cells treated with PBS was designated as 1.000. Data are representative of at least three independent experiments. One-way analysis of variance was performed (*$P < 0.05$; **$P < 0.01$). DFO, deferoxamine.

and colorectal cancers via different mechanisms.[27–29] Typically, the deregulation of iron-regulated genes activates downstream signaling pathways, which also facilitate the proliferation and metastasis of cancer cells.[30] In hematopoietic malignancies, abnormal iron function has been detected. SF is regarded as a negative prognostic indicator of MM.[20] Decreased ferroportin levels can accelerate myeloma cell

growth.[21] However, both increased ferritin and SF levels have been found in some cancers, and the decreased expression of the main iron efflux pump ferroportin was also detected.[31,32] Because of the complexity of iron metabolism, including the processes of uptake–export, storage, and regulation of numerous genes involved in these processes, changes in both intracellular and extracellular iron content depend on a variety of genes. Conversely, excess iron levels can lead to lipid oxidation, which restricts malignant plasma cell expansion and inhibits proteasome function, thereby potentiating the effects of bortezomib.[33] Thus, the role of iron in MM appears ambiguous, and the importance of iron in MM progression has not yet been fully elucidated. We found that SF levels were elevated in newly diagnosed patients. Patients with stage III disease or the κ isotype had significantly higher SF levels, suggesting an increased tumor burden. Furthermore, SF levels were positively correlated with those of diagnostic markers, including β2-MG, IL-6, and LDH,[24,34,35] in patients with newly diagnosed MM. Importantly, patients with relapsed MM displayed increased SF levels, suggesting the potential role of SF for monitoring disease recurrence. Because of the heterogeneity of the clinical symptoms and cytogenetic performance, a single prognostic factor is insufficient for determining prognosis. Therefore, it is essential to explore new prognostic factors and combine them with various factors for diagnosing and treating MM. Given the limits of the clinical data, we could only provide the statistical relationships of SF with clinical values in this study, which suggested the importance of SF for MM. We plan to evaluate the utility of SF for improving the staging and prognosis of MM in further studies.

Iron chelators have been used to diminish cellular iron, and they also have antitumor roles in malignancies.[36] Because of the safety profiles of iron chelators, early studies of their anti-tumor activity used DFO, which inhibited the growth of cancer cells in many solid tumors.[37–39] In hematopoietic malignancies, DFO dramatically induced apoptosis in both acute myeloid leukemia cells and T-cell acute lymphoblastic leukemia cells.[17] Additionally, DFO displayed synergistic activity with three acute lymphocytic leukemia-specific drugs, namely dexamethasone, doxorubicin, and L-asparaginase.[40] However, the specific effect and underlying mechanism of DFO on MM remain unclear. We demonstrated that DFO efficiently suppressed MM cell survival after decreasing cellular iron content. On the one hand, DFO suppressed the expression of proliferation-related genes in MM cells. The expression of CDK2 and cyclin E1 was decreased in both MM1S and MM1R cells following DFO treatment. Additionally, CDK4, CDK6, and cyclin D3 expression was decreased by DFO treatment in MM1R cells. With iron supplementation, DFO decreased the levels of CDK2, CDK4, CDK6, cyclin D3, and cyclin E1 in MM1S cells and those of CDK4 and cyclin D3 in MM1R cells. On the other hand, DFO accelerated myeloma cell apoptosis by promoting the expression of apoptosis-related genes in MM cells. DFO promoted the expression of Bcl-2, Bcl-xl, and Bax in MM1S cells and that of p53 in MM1R cells. Iron supplementation maintained the survival of both cells lines, although no significant promotion effect has been found at present, possibly because MM cells were already fully saturated with intracellular iron, in line with previous findings.[21] Furthermore, following iron supplementation, the effects of DFO on cell apoptosis-related gene expression were decreased in both MM1S and MM1R cells. p53, Bcl-xl, and Bax expression was detected in MM1S and MM1R cells treated with both ferric chloride and DFO.

Additionally, Fas expression was decreased in MM1R cells treated with ferric chloride and DFO. Therefore, strategies based on diminishing intracellular iron content using iron chelators should offer promising treatment options for MM therapy. In addition to iron chelation, other methods, including impairing the redox status by elevating intracellular iron levels and targeting iron-associated proteins for drug delivery, have been confirmed to be effective for cancer treatment.

Taken together, we evaluated SF levels and then further analyzed their relationships with various clinical markers in patients with MM. We also studied the effects and molecular mechanism of DFO in myeloma cells. Increased SF content was detected in patients with newly diagnosed MM, especially those with stage III disease or the κ isotype. SF content was positively correlated with β2-MG, IL-6, and LDH expression. Moreover, patients with progressive or relapsed disease had higher SF levels. Importantly, iron chelation using DFO efficiently inhibited myeloma cell survival and accelerated apoptosis by regulating apoptosis-related genes. Overall, these findings demonstrate the value of SF for MM and suggest that DFO may be a good therapeutic option for MM. The mechanisms of iron homeostasis maintenance and epigenetics, as well as the microenvironment of iron in MM initiation and development, require further exploration.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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