Reduced CXCR4 expression in associated with extramedullary and predicts poor survival in newly diagnosed multiple myeloma

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Research Article

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Abstract

Multiple myeloma (MM), a bone marrow-resident hematological malignancy of plasma cells, has remained largely incurable despite the advancement of novel therapies in recent years. Because of the heterogeneity of myeloma cells, risk stratification of MM is important for making therapeutic regimens. Nevertheless, no immunohistochemical (IHC) predictive and prognostic marker of MM has been constructed yet. Herein, the prognostic value of C-X-C motif chemokine receptor 4 (CXCR4) expression in 48 newly diagnosed MM patients was explored using IHC. Correlations between CXCR4 expression and clinical features of MM were analyzed. CXCR4-positive patients significantly outperformed CXCR4-negative patients in both 3-year estimated overall survival (93.8% vs 45.8%) and progression-free survival (57.1% vs 40.9%). The incidence of extramedullary lesions in CXCR4-negative patients increased significantly compared with CXCR4-positive patients. Plasma cells that reduce CXCR4 expression have poor prognosis and increase the incidence of extramedullary lesions.

Introduction

Multiple myeloma (MM), a bone marrow-resident hematological malignancy of plasma cells, has remained incurable. It accounts for 1.8% of all malignancies and is the second most common hematologic malignancy.[1–2] The prognosis of this disease was poor around ten years ago, but a better understanding of MM, alongside new treatments, helps to improve the situation.[1] However, due to huge heterogeneity in the response rate and survival outcomes, the course of MM is heterogeneous, so the survival time widely ranges from a few months to more than 10 years.[3] High-risk MM is associated with elevated serum lactate dehydrogenase, high-risk cytogentics and extramedullary disease.[4] Nevertheless, no immunohistochemical (IHC) predictive and prognostic marker of MM has been constructed yet.

Two of the biggest challenges associated with MM are acquired drug resistance and relapse, which make MM incurable yet. CXCR4 an alpha-chemokine receptor specific for stromal-derived-factor-1 (SDF1), reportedly is most widely expressed in tumors.[5] A meta-analysis shows that high CXCR4 expressions in esophagus, gastric and colorectal cancers all predict a worse prognosis.[6] Reportedly, when Bortezomib-resistant MM cells express less CXCR4, plasma cells excape from bone marrow extramedullary metastasis in MM mice and MM patients.[7] The CXCR4/SDF1 axis plays a pivotal role in the proliferation, invasion, dissemination and drug resistance of MM cells.[8,9] Despite wide reporting, the prognostic value of CXCR4 expression in MM remains controversial.

Extramedullary multiple myeloma (EMM) can thrive and grow independent of the bone marrow microenvironment, resulting in a high-risk state associated with increased proliferation, evasion of apoptosis and treatment resistance.[10] High-dose therapy with autologous stem-cell transplantation (ASCT) can overcome the negative prognostic impact of extramedullary disease in younger selected patients.[11,12] Unfortunately, the median age at diagnosis of MM is 69 years, but three-quarters of patients

are diagnosed above the age of 55 years.[13] Even worse, most patients have no access to high-dose chemotherapy.

Despite the improved survival in most MM patients over recent decades, outcomes are generally poor when EMM develops. Possible mechanisms of extramedullary spread include decreased adhesion molecule expression and downregulation of chemokine receptors. This study, presents for the first time that reduced CXCR4 expression is associated with the extramedullary subentity and predicted poor survival of newly-diagnosed MM.

Materials And Methods

Patients and Clinical Features

This study analyzed the CXCR4 expression in bone marrow immunohistochemical (IHC) samples collected from 48 newly-diagnosed MM patients who were treated at Anqing Municipal Hospital between February 2016 and July 2020. All patients were diagnosed as MM according to International Myeloma Working Group.[14] According to the CXCR4 expression, the patients were divided into 2 groups (CXCR4-positive and CXCR4-negative). The clinical features were procured from medical records, including age, sex, stage, fluorescence in situ hybridization (FISH), CD56, extramedullary bone destruction and bortezomib-based treatment (Table 1). This study was approved by the institutional review board, and all patients gave written informed consent.

Table 1
the relationship between CXCR4 and clinical parameters of NDMM

Characteristic	CXCR4		*P*
	CXCR4(+)(n = 21)	CXCR4(-)(n = 27)	
Median age, y(range)	64(50–76)	64(37–77)	0.9636
Female/male	12/9	13/14	0.5360
DS(I-II/III,stage)	5/16	6/21	0.8967
ISS(I-II/III,stage)	8/13	10/17	0.9401
RISS(I-II/III,stage)	10/11	14/13	0.7711
Deletion P53	2	1	0.4086
1q21 gain	6	10	0.5371
IGH rearrangement	4	7	0.5738
No FISH	11	14	0.9710
IgA/IgG/light chain	6/10/5	5/15/7	0.7100
CD56 expression(-)	8	2	0.0094
Extramedullary lesions	2	15	0.0009
Bone destruction	16	14	0.0840
Bortezomib	18	23	0.9589
IGH translocation: t (4,14);t(14,16);t(14,20); FISH (1q21 gain, 17p-, t (4,14);t(14,16);t(14,20)).			

IHC

Formalin-fixed and paraffin-embedded sections were utilized for IHC with antibody.[15] Heated antigen retrieval was performed by immersing the slides in an EDTA buffer (pH 8.0) and heating them for 2 min in a steamer. Reorganization anti-CXCR4 body (Abcam, Shanghai Laizi Biotechnology Co., Ltd. dilution 1:100) was used in addition to an autostainer following the standard polymer method (Dako Autostainer Plus). IHC was evaluated by 2 experienced hematopathologists using a multihead microscope. Then the staining intensity of CXCR4 in the slides was detected. Without prior knowledge about patients' outcomes, the two pathologists independently graded the immunostaining intensity as follows: no or low staning intensity in < 30% of tumor cells (CXCR4-negative); strong staining intensity in ≥ 30% tumor cells with strong staining intensity (CXCR4-positive) (Fig. 1).

Statistical analysis

Various statistical analyses were utilized to evaluate the roles of CXCR4 expression in clinicopathological features and prognosis in NDMM patients. Overall survival (OS) was computed from the date of diagnosis to the date of either death or the last documented follow-up. Progression-free survival (PFS) was calculated from the date of diagnosis to the date of either progression or death from any cause. PFS and OS rates were estimated using the Kaplan-Meier method and analyzed with Log-Rank test and multiple stepwise Cox analysis; hazard ratio (HR) with 96% confidence intervals (CI) were calculated. Associations between CXCR4 expression and the clinical characteristics of the patients were described by means ± standard deviation and compared with compared with Student's t-test for either paired or unpaired groups as required, dichotomic variables as percentage and compared with $\chi 2$ test of Fisher test as required. The effect of CXCAR4 expression on outcomes was analyzed using univariate and multivariate Cox regression models.[16] For our analyses, GraphPad Prism 5 was employed and $*p < 0.05$ was considered statistically significant.

Results

Patients

The 48 newly-diagnosed MM patients were divided into a CXCR4-positive group (n = 21) and a CXCR4-negative group (n = 27). The main characteristics of the patients at diagnosis are listed in Table 1. The median age, Durie-Salmon (DS) staging, International Staging System (ISS) staging, Revised ISS (RISS) staging, deletion P53, 1q21 gain, IGH rearrangement, IgA/IgG/light chain, bone destruction and bortezomib-based treatment were all not significantly different between groups (P > 0.05). However, the incidence of extramedullary lesions in the CXCR4-negative group increased significantly (P < 0.01). CD56-negative was more likely to appear in the CXCR4-positive group (P < 0.01).

Survival Analysis

The CXCR4-positive group showed significantly better survival compared with the CXCR4-negative group in terms of both 3-year estimated OS (93.8% vs. 45.8%, p = 0.0392) and PFS (57.1% vs. 40.9%, p = 0.0436) (Fig. 2). The data of the 48 patients was examined by Cox multivariate analysis, and CXCR4 was proved to be an independent prognostic factor of survival (Table 2). For patients with 4 risk factors, the Cox multivariate analysis showed that CXCR4 was an independent prognostic factor of PFS (HR 0.1538–0.9536; P = 0.0392) and OS (HR 1.040–14.70; P = 0.0436). Moreover, a subgroup analysis of the CXCR4-negative group was performed. For FISH abnormal and bone destruction risk factor, no significant difference was found between OS and PFS. Moreover, the EMM patients had worse OS (P = 0.0032) and PFS (P = 0.0001) (Fig. 3).

Table 2
Survival analysis in 48 patients with multiple myeloma

	parameter	Univariate analysis		Multivariate analysis	
		HR(95%CI)	*P*	HR(95%CI)	*P*
	CD56(-)	0.2034–1.667	0.3136	0.773–0.126	0.781
	Extramedullary	1.506–11.26	0.0058	0.691–13.878	0.138
PFS	Bone destruction	0.2824–2.033	0.5817	0.676–13.286	0.138
	CXCR4(-)	0.1538–0.9536	0.0392	0.058–1.662	0.166
	CD56(-)	0.1995–4.494	0.9454	0.016–2.507	0.195
	Extramedullary	0.09154–1.478	0.1586	0.254–9.320	0.636
OS	Bone destruction	0.5060–10.15	0.2849	0.174–5.106	0.946
	CXCR4(-)	1.040–14.70	0.0436	0.004–1.273	0.033

Discussion

MM remains incurable despite novel treatment and ASCT. Existing prognostic indicators cannot completely predict prognosis due to heterogeneity of MM. Thus, evaluating new clinical markers of MM is crucial for predicting prognosis and making personalized treatment regimens.

In a series of 19 MM patients who progressed to EMM, cytogenetic abnormality was most frequent at diagnosis.[17–20] EMM patients underwent DNA sequencing for a targeted panel of 50 tumor suppressors and oncogenes, which revealed a high frequency of activating RAS mutations.[21] CXCR4 expression was associated with oral aquamous cell carcinoma, oesophageal, gastric, colom, liver, pancreas, thyriod, ovary, prostate, lung, kidney, breast, brain, melanoma and leukemia.[8] SDF1 recruited CXCR4-positive inflammatory, vascular and stromal cells to the tumor microenvironment.[22–24] SDF1 is widely expressed in various human tissues, including liver, lungs, bone marrow, lymphnodes, stromal and endothelial cells.[23,24] CXCR4 overexpression in EMM cells is associated with poor prognosis.[25–27] As for low CXCR4 expression when Bortezomib-resistant MM cells expressed less CXCR4, plasma cells escaped from bone marrow extramedullary metastasis in an MM mouse model and MM patients.[7] In our study, reduced CXCR4 expression in bone marrow is associated with the extramedullary subtype of newly diagnosed MM. The possible reason is that the extramedullary region expressing CXCR4 tissue chemotaxis attracts CXCR4-positive myeloma cells out of the bone marrow, leading to the occurrence of extramedullary lesions.

We find that CXCR4-negativity in bone marrow predicts poor survival in newly-diagnosed MM. A total of 24 studies involving 3637 cases suggest that CXCR4 over-expression is significantly associated with

worse prognosis of gastrointestinal cancer patients.[6] High CXCR4 expression in the cytoplasm indicates poor prognosis on contrast to a better evident prognosis when CXCR4 is highly expressed in the nucleus.[22] The expansion and colonization of aggressive MM cells to secondary metastatic sites is associated by higher SDF1 gradient that promotes the migration and homing of CXCR4-positive MM cells from primary tumor sites.[8] Thus, CXCR4 expressions in different tumors and different parts can affect the prognosis of patients.

Conclusions

Despite the greatly improved prognosis for multiple myeloma in general, our current standard therapies still cannot handle in newly- diagnosed MM and have not sufficiently improved outcomes of EMM patients. Hence, new prognostic factors need to be explored. Our investigation verifies that CXCR4 can be an efficient predictor of clinical outcome, and reduced CXCR4 expression in bone marrow myeloma cells can increase the incidence of extramedullary lesions.

Declarations

Ethics Approval and Permit.

The study was approved by the Ethics Committee of Anqing Municipal Hospital. All patients provided written informed consent to publish the study and accompanying images. All methods were performed in accordance with relevant guidelines and regulations.

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Disclosure

The authors have no conflicts of interest to declare.

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Figures

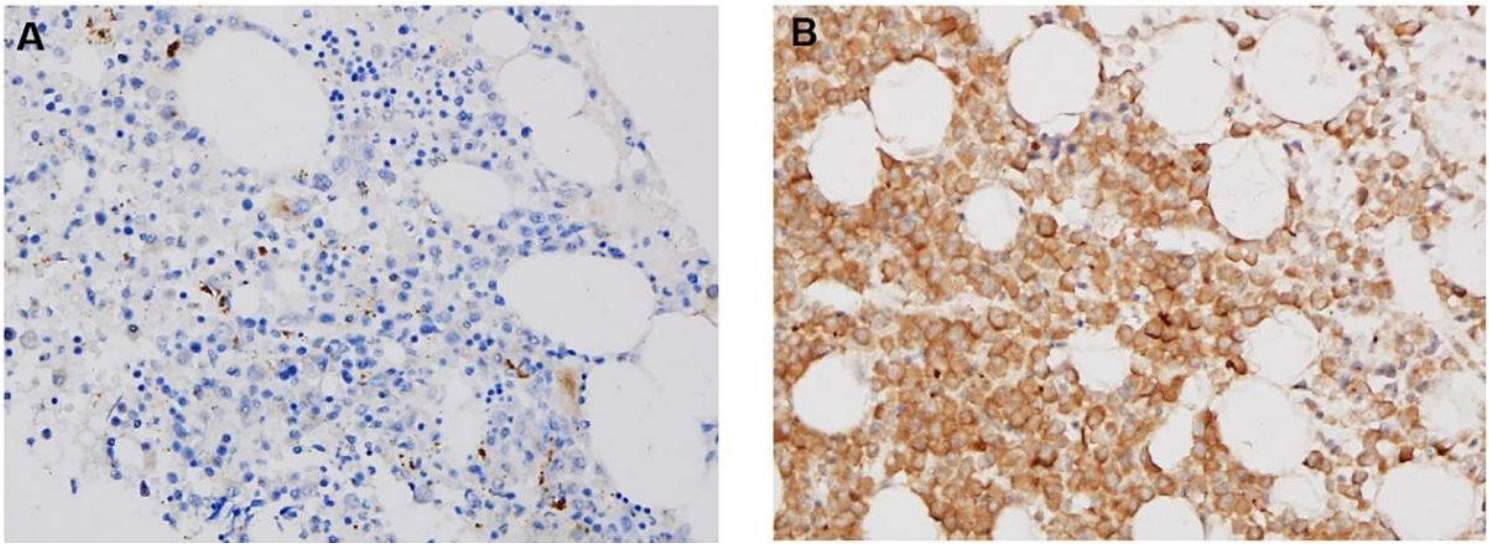

Figure 1

The expression of the CXCR4 protein was deteremined by immunohistochemistry in multiple myeloma. A: CXCR4 expression was negative; B: CXCR4 expression was positive (Scan10×40).

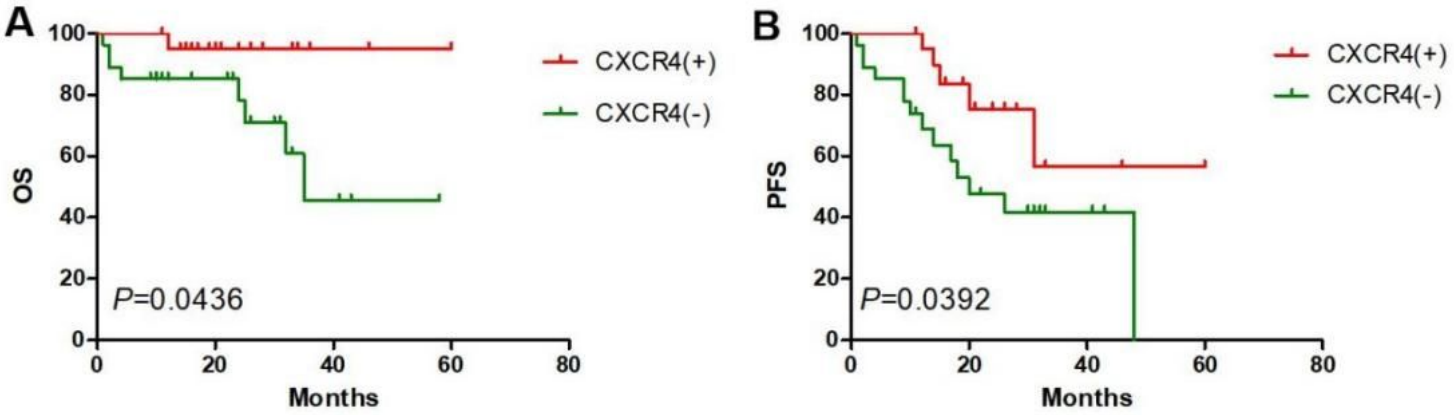

Figure 2

The expression of the CXCR4 protein was deteremined by immunohistochemistry in multiple myeloma. A: CXCR4 expression was negative; B: CXCR4 expression was positive (Scan10×40).

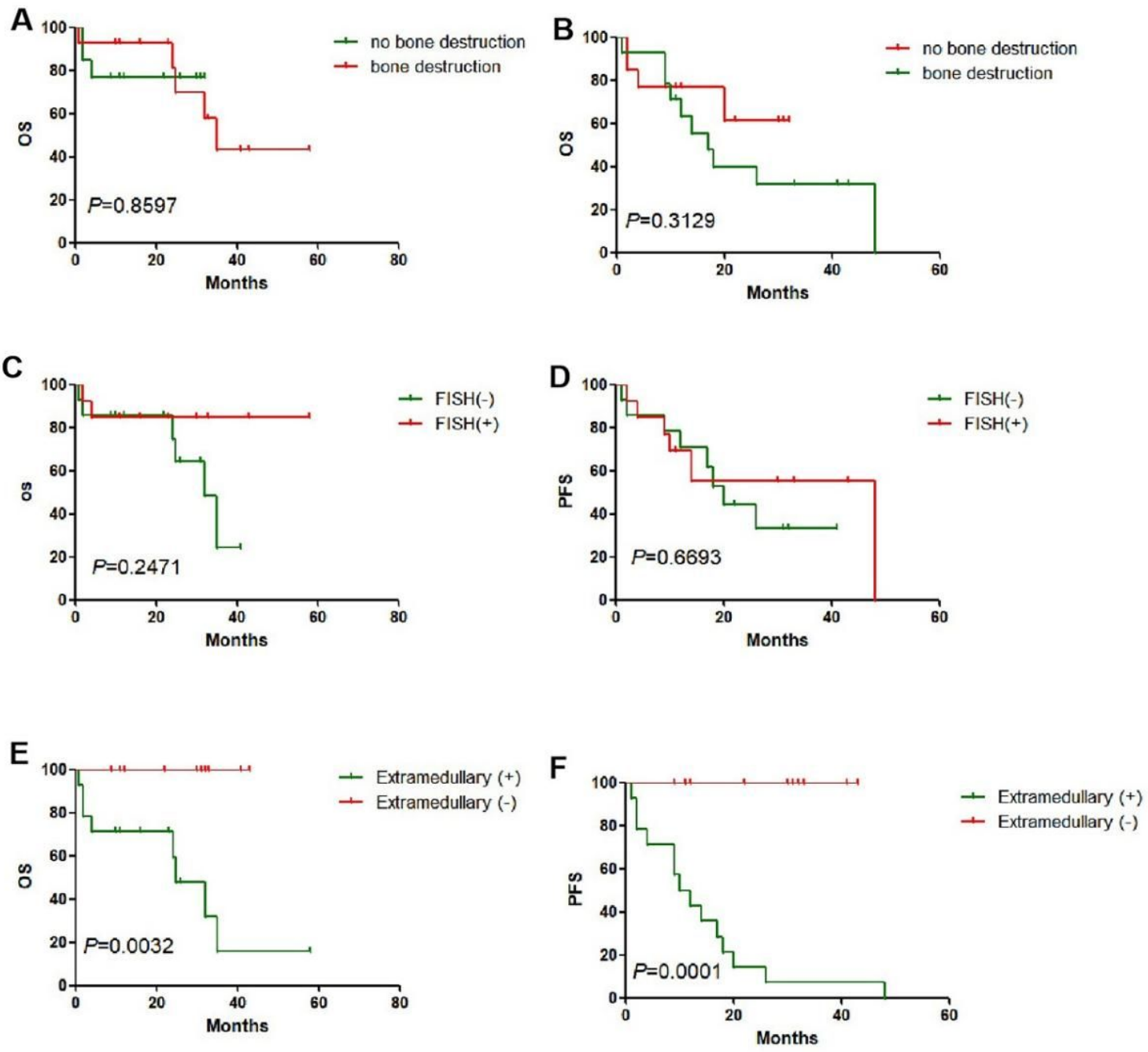

Figure 3

Survival according to CXCR4-negative in 27 multiple myeloma patients with FISH, extramedullary and bone destruction risk factors. For bone destruction: A. Overall survival (P=0.8597), B. Progression-free survival (P=0.3129); For FISH: C. Overall survival (P=0.2471), C. Progression-free survival (P=0.6693); For extramedullary: E. Overall survival (P=0.0032), F. Progression-free survival (P=0.0001).