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

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ABSTRACT

Background: Multiple myeloma (MM), a bone marrow-resident hematological malignancy of plasma cells, has remained largely incurable despite the recent advancement in novel therapies. The heterogeneity of myeloma cells makes risk stratification of MM important for therapeutic regimen planning.

Research design and methods: No immunohistochemical (IHC) predictive and prognostic marker of MM has been constructed yet. Herein, the prognostic value of chemokine (C-X-C motif) 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.

Results: CXCR4-positive patients significantly outperformed CXCR4-negative patients in both 3-year estimated overall survival (93.8% vs 45.8%, P = 0.0392) and progression-free survival (57.1% vs 40.9%, P = 0.0436).

Conclusions: 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.

1. Introduction

Multiple myeloma (MM), a 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 MM was poor around ten years ago, but was improved with a better understanding of MM alongside new treatments [1]. However, the course of MM is heterogeneous due to huge heterogeneity in the response rate and survival outcomes, so the survival time widely varies from a few months to more than 10 years [3]. High-risk MM is associated with elevated serum lactate dehydrogenase, high-risk cytogenetics and extramedullary disease [4]. Nevertheless, no immunohistochemical (IHC) predictive and prognostic marker of MM has been constructed yet.

Two of the major challenges brought by MM are acquired drug resistance and relapse, which make MM incurable yet. Chemokine (C-X-C motif) receptor 4 (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]. When Bortezomib-resistant MM cells express less CXCR4, plasma cells escape from bone marrow extramedullary metastasis in both 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 diseases in younger selected patients [11,12]. Unfortunately, the age at diagnosis of MM is 69 years at the median level, but is above 55 years in three-quarters of patients [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. The possible mechanisms of extramedullary spread include adhesion molecule down-expression and chemokine receptor downregulation. This study, presents for the first time that reduced CXCR4 expression is associated with the extramedullary subentity and predicts poor survival of newly-diagnosed MM patients.

2. Materials and methods

2.1. Patients and clinical features

We analyzed the CXCR4 expressions in IHC bone marrow 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]. The patients were divided by the CXCR4 expression 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.

### 2.2. IHC

An indirect immunoperoxidase method was used. Formalin-fixed and paraffin-embedded sections were utilized for IHC with antibody [15]. During heated antigen retrieval the slides were immersed in an ethylene diamine tetraacetic acid (EDTA) buffer (potential of hydrogen, pH 8.0) and heated for 2 min in a steamer. The sections were incubated overnight at 4°C with primary antibodies specific for anti-CXCR4 antibody (1:100; human, ab124824, Abcam, Shanghai, China). The secondary antibody was goat anti-rabbit IgG H&L (HRP) (1:500; cat, ab96899, Abcam, Cambridge, United Kingdom). The sections were finally counterstained with hematoxylin, then washed and mounted in an aqueous mounting medium. Negative controls were processed in the same manner except that the primary antibody was replaced with phosphate buffer saline (PBS). The sections were observed using an BX53 fluorescence microscope(Olympus, Tokyo, Japan) and the images were analyzed (blue: nucleus, brown: target protein). IHC was evaluated by 2 experienced hematopathologists using a multihed microscope. Then the staining intensity of CXCR4 in the slides was detected. Without prior knowledge about the patients’ outcomes, the two pathologists independently graded the immunostaining intensity as follows: no or low in < 30% of myeloma cells (CXCR4-negative), and strong in ≥ 30% myeloma cells (CXCR4-positive) (Figure 1).

### Table 1. The relationship between CXCR4 and clinical parameters of NDMM.

| Characteristic | CXCR4(+)(n = 21) | CXCR4(-)(n = 27) | P |
|---------------|-----------------|-----------------|---|
| Median age, y(range) | 64(50–76) | 64(37–77) | 0.9636 |
| Female/male | 12/9 | 13/14 | 0.5360 |
| DSS(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 15q | 2 | 1 | 0.4086 |
| IgA/IgG light chain | 6/10 | 5/17 | 0.7100 |
| CD56 expression(-) | 8 | 2 | 0.0094 |
| Extramedullary lesions | 2 | 15 | 0.0009 |
| Bone destruction | 16 | 14 | 0.0840 |
| Bortezomib treatment | 18 | 23 | 0.9589 |
| ASCT | 3 | 4 | 1.0000 |
| CR+/VGPR | 18 | 15 | 0.0110 |
| MRD(-) | 10 | 12 | 0.7700 |
| Chromosomal abnormalities | 1 | 3 | 0.6310 |

CXCR4: chemokine (C-X-C motif) receptor 4; DS: Durie-Salmon; ISS:International Staging System; RISS:Revised International Staging System; NDMM: newly diagnosed multiple myeloma; IGH translocation: t(4,14);t(14,16);t(14,20); FISH: fluorescence in situ hybridization (1q21 gain, 17p-, t (4,14);t(14,16);t (14,20)); ASCT: autologous stem cell transplantation; CR:complete response; VGPR: very good partial response. MRD:Minimal Residual Disease.

### 2.3 Statistical analysis

Various statistical analyses were utilized to evaluate the roles of CXCR4 expression in clinicopathological features and prognosis in the newly diagnosed multiple myeloma (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 95% 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 Student’s t-test for either paired or unpaired groups as required. Dichotomic variables were reported as percentage and compared with χ² test or Fisher test as required. The effect of CXCR4 expression on outcomes was analyzed using univariate and multivariate Cox regression.

Figure 1. The expression of the CXCR4 protein was determined by immunohistochemistry in multiple myeloma (blue is the nucleus, brown is the target protein). a: CXCR4 expression was negative; b: CXCR4 expression was positive. (Scan10 × 40). CXCR4:chemokine (C-X-C motif) receptor 4.
models [16]. Our analyses were conducted on GraphPad Prism 5 and *p < 0.05 was considered statistically significant.

3. Results

3.1. Patients

The 48 NDMM 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. None of median age, Durie-Salmon (DS) staging, International Staging System (ISS) staging, Revised ISS (RISS) staging, deletion P53, 1q21 gain, immunoglobulin heavy chain (IGH) rearrangement, ImmunoGlobulins A (IgA)/IgG/light chain, bone destruction,ASCT, minimal residual disease (MRD), chromosomal abnormalities, or bortezomib-based treatment was significantly different between groups (P > 0.05). However, the incidence of extramedullary lesions in the CXCR4-negative group increased significantly (P = 0.0009). CD56-negativity was significantly more frequent in the CXCR4-positive group (P = 0.0094). After 4 cycles of chemotherapy, therapeutic response (CR +VGPR) in the CXCR4-positive group increased significantly (P = 0.011). MRD was negative in the CXCR4-negative group (n = 10) vs. the CXCR4-positive group (n = 12) (P = 0.770).

3.2. Survival analysis

The CXCR4-positive group were significantly better than the CXCR4-negative group in terms of both estimated 3-year OS (93.8% vs. 45.8%, p = 0.0392) and PFS (57.1% vs. 40.9%, p = 0.0436) (Figure 2). The data of the 48 patients was examined by Cox multivariate analysis, which proved CXCR4 as 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) (Figure 3).

4. Discussion

MM remains incurable despite novel treatment and ASCT. Existing prognostic indicators cannot completely predict the prognosis of MM due to its heterogeneity. 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 frequently found at diagnosis [17–19]. EMM patients underwent DNA sequencing for a targeted panel of 50 tumor suppressors and oncogenes, which revealed a high frequency of activating RAS mutations [20]. CXCR4 expression is associated with oral squamous cell carcinoma, esophageal, gastric, colon, liver, pancreas, thyroid, ovary, prostate, lung, kidney, breast, brain, melanoma and leukemia [8]. SDF1 recruited CXCR4-positive inflammatory, vascular and stromal cells to the tumor microenvironment [21–23]. SDF1 is expressed in various human tissues, including liver, lungs, bone marrow, lymphnodes, and stromal and endothelial cells
CXCR4 overexpression in EMM cells is associated with poor prognosis [24–26]. Bortezomib-resistant MM cells express less CXCR4, and plasma cells can escape from bone marrow extramedullary metastasis in MM mice and MM patients [7]. In our study, reduced CXCR4 expression in bone marrow is associated with the extramedullary subtype of NDMM. 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 of NDMM. Twenty-four 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 evidently worse prognosis on contrast to high CXCR4 expression 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.

5. Conclusions
Despite the greatly improved prognosis for MM in general, our current standard therapies still cannot handle in NDMM and do not sufficiently improved the 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. However, the current real-world data are almost entirely limited to retrospective observational studies with small patient populations, indicating large prospective and retrospective studies are needed to establish a better picture. Such developments are expected to facilitate and complement ongoing research that is paving the way toward improved prognostic markers and novel therapy development.
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