Pathology – Research and Practice 210 (2014) 586–590

Contents lists available at ScienceDirect

Pathology – Research and Practice

journal homepage: www.elsevier.com/locate/prp

Original Article

Interleukin-17 receptor expression on vascular endothelial cells of masses of skeletal extramedullary disease in myeloma patients

Jian-Zhu Yang[a,1], Yan Li[b,1], Li-Xia Sun[b,*], Jie Fang[b], Ling-Juan Kong[b], Jin-Qiao Zhang[b]

[a] Department of Pathology, Third Affiliated Hospital of Hebei Medical University, Shijiazhuang, China
[b] Department of Hematology, Third Affiliated Hospital of Hebei Medical University, Shijiazhuang, China

ARTICLE INFO

Article history:
Received 12 January 2014
Received in revised form 13 April 2014
Accepted 30 April 2014

Keywords:
Multiple myeloma
IL-17 receptor
Myeloma bone diseases
Skeletal extramedullary disease
Microvessel density

ABSTRACT

The goal of the study was to investigate the expression of interleukin-17 (IL-17) and IL-17 receptor (IL-17R) in patients with myeloma bone diseases (MBD) and skeletal extramedullary disease (skeletal EMD). The levels of IL-17 were determined using ELISA. The expression of IL-17R on vascular endothelial cells of bone marrow (BM) and masses of skeletal EMD was detected using immunohistochemistry. The results showed an elevated IL-17 level in BM of BMD and skeletal EMD patients. The microvessel density (MVD) was significantly increased in the masses of skeletal EMD. IL-17R was almost exclusively expressed by endothelial cells, not by myeloma cells in the masses of skeletal EMD patients. We concluded that EMD masses showed increased angiogenesis mediated by IL-17 pathway and in part this may help in myeloma cell-growth under these conditions.

Introduction

Myeloma bone diseases (MBD) were found in about 80% of patients with newly diagnosed multiple myeloma (MM) [1]. Bone destruction can result in bone pain, pathological fractures requiring surgery and radiation, and spinal cord compression [2]. Some patients are characterized by soft-tissue masses arising from bone lesion, as classified as skeletal extramedullary disease (skeletal EMD) [3,4]. At diagnosis, 68% to 85% of soft-tissue extramedullary plasmacytomas arising from bone lesions could be observed [5,6]. The skeletal EMD is common in MM and the most frequent mechanism of myeloma spread [4,6], which is characterized by less undifferentiated and usually shows plasmacytic morphology [7–9] and associated with a poor prognosis [10,11]. However, the mechanisms of skeletal EMD remain incompletely understood.

Available data suggested that interleukin-17 (IL-17) plays an important role in MM [12]. Noonan et al. [13] showed that IL-17 level in BM directly correlated with the extent of MBD. IL-17 binds to its receptor, IL-17 receptor (IL-17R) [14]. The IL-17R is expressed on the surface of various cells, and can be considered a ubiquitous receptor [15]. Prabhala et al. [16] demonstrated that myeloma cells from 7 of 10 patients expressed IL-17R. Several studies suggested that IL-17 promoted angiogenesis [17] and was positively associated with vascular endothelial growth factors (VEGF) [18]. Increased bone marrow angiogenesis has been found in MM [19–21]. Currently, no data are available on the expression of IL-17R in patients with MBD and those with skeletal EMD. Therefore, we investigated the expression of IL-17R in BM biopsy sections and masses from MBD and skeletal EMD patients. In addition, we determined the IL-17 level of BM in these patients in order to find new insights for myeloma.

Materials and methods

Patients

From January 2010 to May 2013, thirty newly diagnosed MBD patients (the mean age was 62.27 ± 4.27 years. 23 cases were male, 7 female) and twenty-eight patients with skeletal EMD (mean age was 59.54 ± 6.21 years, 19 cases were male, 9 female) were included in this study. There was no significant difference about age between the two groups ($P = 0.055$). Of the newly diagnosed MBD, 21 patients were IgG type, 5 IgA type, 1 IgM type, and 3 light chain type. Of the skeletal EMD, 20 patients were IgG type, 3 IgA type, 1 IgM type, and 4 light chain type. Twenty age and sex-matched healthy volunteers were used as controls. Approval for this study was obtained from the Ethical Committee of the Third Affiliated Hospital of Hebei

* Corresponding author at: Department of Hematology, Third Affiliated Hospital of Hebei Medical University, Shijiazhuang 050051, China. Tel.: +86 0311 88602036; fax: +86 311 87023626.
E-mail address: happys.ok@163.com (L.-X. Sun).
[1] The two authors contributed equally to the work.

http://dx.doi.org/10.1016/j.prp.2014.04.018
0344-0338/

Medical University. Informed consent for the study was obtained from all subjects.

Detection of IL-17 using enzyme-linked immunosorbent assay method

BM were collected in sterile tubes containing ethylenediamine tetra acetic acid (EDTA) and centrifuged at 2500 × *g* cycle. The acquisition of the BM aspirates was performed by an experience physician, and a total of 0.5–1 ml BM was harvested in order to avoid contamination by peripheral blood. The plasma was divided into aliquots and stored at −70 °C until the assay was performed. IL-17 levels in the plasma of BM were studied via enzyme-linked immunosorbent assay method (ELISA), according to the instruction of the producer of the kits (EIAAB Science Co., Ltd. Wuhan, China).

Immunohistochemistry and measurement of MVD

BM trephine biopsies were performed from the posterior iliac crest. Specimens of masses from skeletal EMD patients were obtained from surgical operations. Biopsies were fixed in 10% neutral formalin. Paraffin-embedded samples were cut into 4-μm section and processed for immunohistochemistry. Following deparaffinization and rehydration of the tissues sections, antigen retrieval was performed in the pressure cooker in 10 mM citrate buffer, pH 6.0, for 2 min. Endogenous peroxidase was blocked with 3% peroxide for 10 min. Primary IL-17R antibody (Goat polyclonal, Catalog Number: AF2275, R&D Systems, Minneapolis, MN, USA) was applied at 10 μg/ml. Primary CD34 antibody (Mouse monoclonal, clone QBEnd/10, Maixin-Bio, Fuzhou, Fujian, China), Primary CD138 antibody (Mouse monoclonal, Clone MI15, Maixin-Bio, Fuzhou, Fujian, China) were applied at 1:100 dilution. As a negative control, samples were incubated using 10 mM TBS (pH 7.4) instead of a primary antibody. The IL-17R antibody was validated using human kidney. Antibody staining was performed using a streptavidin–horseradish peroxidase system (catalog nos. KIT-9709 and KIT-9710, Maixin-Bio, Fuzhou, Fujian, China). Staining was performed with DAB and counterstaining with Meyer's hematoxylin. Immunostaining was evaluated by two different pathologists. For evaluation of IL-17R expression on myeloma cells a score corresponding to the sum of both (a) and (b) was used. (a) Staining intensity (0 = negative; 1 = weak; 2 = intermediate; 3 = strong). (b) Percentage of positive cells (0 = 0% positive cells; 1 = <25% positive cells; 2 = 26–50% positive cells; 3 = >50% positive cells). The sum of (a) + (b) reached a maximum score of 6. A score greater than 2 was the value of a positive immunohistochemical assay [22]. The agreement between the 2 scoring authors was 90%. Any discrepancies were resolved by joint review over a double-headed microscope.

Microvessel density (MVD) was highlighted by immunostaining endothelial cells with a monoclonal antibody to CD34 and assessed according to Weidner et al. [23]. Analysis was performed by two independent observers who were blinded to the clinical outcome. The final value for each field and hot spot was the mean of the two independent counts. Hot spots were detected at low power (40), verified at 100× magnification and the individual microvessels were counted at 400× magnification. After microvessels counting, the mean MVD (per field of 0.24 mm^2) of the three hot spots was calculated.

Statistical analysis

The normal distribution data were summarized as the mean ± standard deviation (SD). Comparisons were performed using the student *t*-test. Differences were evaluated by means of the Mann–Whitney *U*-test. The X^2 test was used to compare differences in the groups. A value of $P < 0.05$ was considered statistically significant for 2-sided tests. All analyses were performed with SPSS software (version 11.5).

Table 1
The plasma levels of IL-17 in BM of newly diagnosed MBD ($n = 30$), skeletal EMD ($n = 28$) and normal controls ($n = 20$). The data were presented as mean ± SD.

Groups	IL-17 (pg/ml)
MBD	44.85 ± 17.44*
Skeletal EMD	38.93 ± 9.46*
Control	6.04 ± 2.26

IL-17: interleukin-17. BM: bone marrow. MBD: myeloma bone diseases. Skeletal EMD: skeletal extramedullary disease.
* $P < 0.001$ compared with control.

Results

The changes of IL-17 levels in newly diagnosed MBD and skeletal EMD patients

The IL-17 levels in the BM from MBD patients and healthy controls were measured by ELISA. Table 1 showed that the newly diagnosed MBD patients had a higher level of IL-17 in the BM compared with normal controls ($P < 0.001$). The BM levels of IL-17 in skeletal EMD patients were significantly increased compared with normal controls ($P < 0.001$). But the BM levels of IL-17 did not differ significantly between the patients with MBD and skeletal EMD ($P = 0.112$).

The expression of IL-17R in MBD and skeletal EMD patients

We observed the expression of CD138 and IL-17R by evaluating the paraffin-embedded BM biopsy sections and masses biopsy sections with immunohistochemistry. Plasma cells characterized by strong membranous immunopositivity for CD138 in BM biopsy sections and masses from skeletal EMD patients were depicted in Fig. 1. Table 2 demonstrated that the expressions of IL-17R on myeloma cells were positive in 12 of 30 MBD patients (40.0%). There was no significant difference in the proportion of IL-17R expression on BM slides between patients with MBD and skeletal EMD ($P = 0.593$). However, the expression rate of IL-17R on myeloma cells in the masses of skeletal EMD was reduced to 7.1% compared to the BM sections of skeletal EMD ($P = 0.040$). As shown in Fig. 2, a majority of cells of BM biopsy sections expressed IL-17R in controls and the expression of IL-17R was mainly localized in the cytomembrane. Few myeloma cells were positively stained with IL-17R in the BM sections of MBD patients. Although IL-17R was slightly expressed on MM cells, it was almost all positive on the vascular endothelial cells not only in the BM sections but also the masses of skeletal EMD patients.

Table 2
The expression rate of IL-17R on myeloma cells in groups.

Group	Cases	Myeloma cells	
		Positive	Negative
BM of MBD	30	12 (40.0%)	18 (60.0%)
BM of skeletal EMD	28	9 (32.1%)*	19 (67.9%)
Masses of skeletal EMD	28	2 (7.1%)	26 (92.9%)

IL-17R: interleukin-17 receptor. BM: bone marrow. MBD: myeloma bone diseases. Skeletal EMD: skeletal extramedullary disease.
* $P < 0.05$ compared with masses of skeletal EMD.