Bone 110 (2018) 21–28

Contents lists available at ScienceDirect

Bone

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

Full Length Article

Young female patients with multiple myeloma have low occurrence of osteolytic lesion

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ARTICLE INFO

Article history:
Received 7 September 2017
Revised 6 January 2018
Accepted 16 January 2018
Available online xxxx

Keywords:
Multiple myeloma
Osteolytic lesion
MAST4

ABSTRACT

Objective: Osteolytic lesion (OL) and bone damage are common complications in multiple myeloma (MM). This study aimed to analyze the occurrence of OL in MM patient groups of different ages and genders.
Patients and methods: We performed a retrospective study of 762 MM patients admitted to West China Hospital from 2009 to 2014 to investigate the association between OL occurrence with patients' ages and genders. The presence or absence of OL was confirmed by X-ray, computed tomography (CT) or magnetic resonance imaging (MRI) examination. We also downloaded MM patients' published gene expression profiles and performed microarray-based analyses to identify differentially regulated genes and signaling pathways. Finally, we examined target gene expressions in MM bone marrow (BM) biopsies through immunohistochemistry (IHC).
Results: We calculated the frequency of OL in female and male MM patients with different age cut-offs. From West China Hospital data, we found that in young female MM patients aged under 55, the frequency of OL was 16.67%, significantly lower than the frequencies in other groups of patients (young males: 34.38%; old males: 31.04%; old females: 29.24%; $p < .05$). The same was true in another independent MM cohort. Microarray-based analyses showed that Microtubule Associated Serine/Threonine Kinase Family Member 4 (*MAST4*), an estrogen-responsive gene, expression was up-regulated in MM patients without OL and in young female MM patients ($p < .05$). The expression of *MAST4* in MM BM was confirmed by IHC. The perspective of cell signaling network suggested that *MAST4* might interact with phosphatase and tensin homolog (PTEN) and control the expression of a panel of osteoclast-regulatory cytokines, such as TNFSF11 and CCL2.
Conclusions: Young female (<55 years) MM patients have significantly lower OL frequency than other groups. *MAST4* gene expression is thought to be associated with this phenomenon.

1. Introduction

Multiple myeloma (MM) is an incurable hematological cancer characterized by clonal plasma cell accumulation in bone marrow (BM) [1]. The symptoms of MM include hypercalcemia, renal failure, anemia and bone lesions, also known as CRAB symptoms [2]. Pathological bone lesions and damage are the most common symptom in MM; nearly 90% of MM patients have bone disease [3]. Chantry et al. find that an MM patient has positive OL when he/she had >1 focal lesion or a diffuse infiltrate with a diameter > 5 mm [4]. It has been well established that MM bone disease is mainly because the expansion of MM cells in BM generates an osteoclast (OC) promotion and osteoblast (OB) suppression microenvironment that in turn results in increased bone destruction [5,6].

Several factors have been identified as responsive to altered OC activation and OB inhibition in an MM setting [6]. One well-studied factor in MM-associated OL is RANK ligand (RANKL). Dougall et al. found that MM-derived RANKL bound to RANK receptors on OC precursors and induced OC formation [7]. Osteoprotegrin (OPG) inhibited MM OL by decoying RANK receptors [8]. In addition, macrophage inflammatory protein 1 (MIP-1) also played a key role in MM-associated OL. MM-expressed MIP-1alpha and MIP-1beta enhanced osteoclastic bone resorption [9]. Other soluble factors derived from MM cells and/or BM stromal cells might be involved in regulating MM-associated OL, such as IL-6 and IL-3 [10]. The mechanism of MM bone disease is complicated and involves multiple factors. To our knowledge, very little work has been done with regard to the risk-stratification of bone disease

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https://doi.org/10.1016/j.bone.2018.01.021
8756-3282/

occurrence in MM patients. According to Masih-Khan's and Terpos' studies, MM patients with t(4;14) translocation might have increased OL [11,12]. Furthermore, Zhan et al. classified MM using a gene expression profile (GEP) and showed that a group of patients with down-regulated FRZB, DKK1 expression and other signature gene expressions had less OL. Zhan named this group the low bone disease group (LB) [13]. In this study, we perform a retrospective analysis of OL in MM groups of different ages and genders. We also discuss the significance of our discovery.

2. Materials and methods

2.1. Study population

In this retrospective study, we enrolled 762 MM patients admitted to West China Hospital from 2009 to 2014. The exclusion criterion was the diagnosis of an additional malignancy other than MM. Eleven patients were excluded for lymphoma (2), bladder cancer (2), esophageal carcinoma (3) and nasopharyngeal carcinoma (4). The presence or absence of OL was confirmed by X-ray, computed tomography (CT) or magnetic resonance imaging (MRI) examination. MRI is recommended by the International Myeloma Working Group at 2009 to detect OL in MM patients [14]. This study was approved by the Ethical Committee of Sichuan University, West China Hospital. Written informed consent was obtained from all patients or their legal guardians.

2.2. Bioinformatics

MM GEP datasets GSE26760 [15], GSE13591 [16], GSE2658 [13], GSE4452 [17],GSE19784 [18] and GSE5900 [19] were downloaded from the NCBI Gene Expression Omnibus database. The clinical patient information was downloaded from Oncomine (www.oncomine.org). We also downloaded MM GEP datasets and patient information from the Multiple Myeloma Research Consortium (MMRC) database (www.themmrc.org). The estrogen-responsive gene list was acquired from the Molecular Signatures Database v6.0 (MSigDB) [20,21] HALLMARK_ESTROGE_RESPONSE gene set (http://software.broadinstitute.org/gsea/msigdb/cards/HALLMARK_ESTROGEN_RESPONSE_EARLY.html). According to the MSigDB description, the estrogen-responsive gene set was generated by data mining differentially regulated genes in breast cancer cell lines under estradiol treatment.

To identify a molecular basis for the lower occurrence of OL, we hypothesized that the patient group with a differential occurrence of OL had a specific GEP. Different GEPs were analyzed to address the molecular basis of differential OL occurrence.

To identify target gene cell signaling transduction to OC regulatory factors, we used the pathway exploration tool STRING Interaction Network (http://version10.string-db.org) following the user instructions. In addition, we used MSigDB to identify canonical signaling pathways that the target gene set involved. (http://software.broadinstitute.org/gsea/index.jsp). Specifically, the target gene sets were used to conduct an enrichment analysis using an H collection of gene sets in the MSigDB, which summarized well-defined biological states or processes and displayed coherent expressions.

2.3. Statistics

The statistical significance of OL occurrence among the different groups was determined by the chi-square test. The relation of patient characteristics and the presence of OL was determined by the one-way analysis of variance (ANOVA) and the chi-square test. Comparisons between the two groups were analyzed by the student *t*-test. A $p < .05$ was considered statistically significant.

Table 1
The frequency of OL in young female myeloma patients and the other myeloma patients with different age cut-offs in West China Hospital.

Cut-off age	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65
OL in young female	20.00% (8/40)	17.78% (8/45)	16.33% (8/49)	17.31% (9/52)	16.95% (10/59)	16.67% (11/66)	18.31% (13/71)	20.51% (16/78)	21.35% (19/89)	22.00% (22/100)	23.01% (26/113)	24.22% (31/128)	23.70% (32/135)	23.18% (35/151)	24.20% (38/157)	24.12% (41/170)
OL in others	30.19% (218/722)	30.40% (218/717)	30.58% (218/713)	30.56% (217/710)	30.73% (216/703)	30.89% (215/696)	30.82% (213/691)	30.70% (210/684)	30.76% (207/673)	31.12% (206/662)	30.82% (200/649)	30.76% (195/634)	30.84% (194/627)	31.26% (191/611)	31.07% (188/605)	31.25% (185/592)
P value	0.213	0.091	0.036	0.058	0.026	0.016	0.029	0.067	0.083	0.078	0.096	0.168	0.098	0.059	0.096	0.086