Leukemia Research 49 (2016) 73–79

Contents lists available at ScienceDirect

Leukemia Research

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

Research paper

MiR-15a/16 regulates the growth of myeloma cells, angiogenesis and antitumor immunity by inhibiting Bcl-2, VEGF-A and IL-17 expression in multiple myeloma

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ARTICLE INFO

Article history:
Received 6 July 2016
Received in revised form 21 August 2016
Accepted 26 August 2016
Available online 28 August 2016

Keywords:
miR-15a/16
Bcl-2
VEGF-A
IL-17
Multiple myeloma

ABSTRACT

miRNAs have been reported to be involved in the pathogenesis of many cancers. In this article, we investigated the role and the mechanisms of miR-15a/16 in the pathogenesis of multiple myeloma (MM). We found that miR-15a/16 was down-regulated in bone marrow-derived mononuclear cells (BM-MNCs) of newly diagnosed patients with MM and the downregulation of miR-15a/16 was correlated with International Staging System (ISS) stage. We then demonstrated miR-15a/16 inhibited myeloma cells proliferation, and increased apoptosis rate of U266 cells by suppressing the expression of anti-apoptosis protein Bcl-2. We also found miR-15a/16 could decrease VEGF-A and IL-17 levels in the supernatant of myeloma cells. These results indicate that miR-15a/16 may function as a tumor suppressor in MM through multiple regulatory mechanisms and they may be potential targets for the therapy of MM.

1. Introduction

Multiple myeloma (MM) is a malignant B cell clonal disease whose pathogenesis is not clear. Many studies have reported that the chromosomal abnormalities and the abnormal angiogenesis occupied important places in the complicated pathogenesis [1,2]. Chromosome 13q14 deletion is one of the most common cytogenetic abnormalities which was accounted for about 54% of MM patients. miR-15a and miR-16 which were previously indicated to be dysregulated in MM are located at the chromosome 13q14 [3–5]. Previous studies have found that miR-15a/16 took an important part in the pathogenesis of MM, and it has been reported that inhibition of miR-15a/16 can promote cell proliferation and angiogenesis in MM [6,7].

Angiogenesis and the production of angiogenic factors are fundamental for tumor progression in the form of growth, invasion and metastasis [8]. VEGF-A, one of the important members of the family of vascular endothelial growth factor (VEGF), can induce angiogenesis, promote myeloma cell proliferation and migration, increase osteoclast activity and regulate the immune cell activity in the pathogenesis of MM [9–11]. Some studies demonstrated that the expression of VEGF is correlated with tumor progression and poor prognosis in patients with MM [12,13]. These indicated that VEGF plays a vital role in the development of MM. We found miR-15a/16 could bind to the 3'-UTR of VEGF-A from the online tools Target Scan. In this study, we will determine whether miR-15a/16 can influence angiogenesis by regulating VEGF-A expression in MM.

More recently, several studies have shown that MM is an immunologically relevant disease which subverts and suppresses immunity, owing to the perturbation of immuno-regulatory responses [14]. Abnormal secretion of related cytokines played an important role in the initiation and progression of MM. IL-17 is one of cytokines that is predominantly expressed by Th17 cells which can promote myeloma cell growth but inhibit immune functions in MM [15]. And some studies have reported that miRNAs have effect on the expression of IL-17 [16,17]. However, there were very few studies have addressed the regulation of IL-17 by miR-15a/16 in MM.

Moreover, the miR-15a/16 cluster has been shown to play very important roles in regulating cell proliferation and apoptosis by targeting the antiapoptotic Bcl-2 gene in several cancers [18]. Based

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http://dx.doi.org/10.1016/j.leukres.2016.08.013
0145-2126/

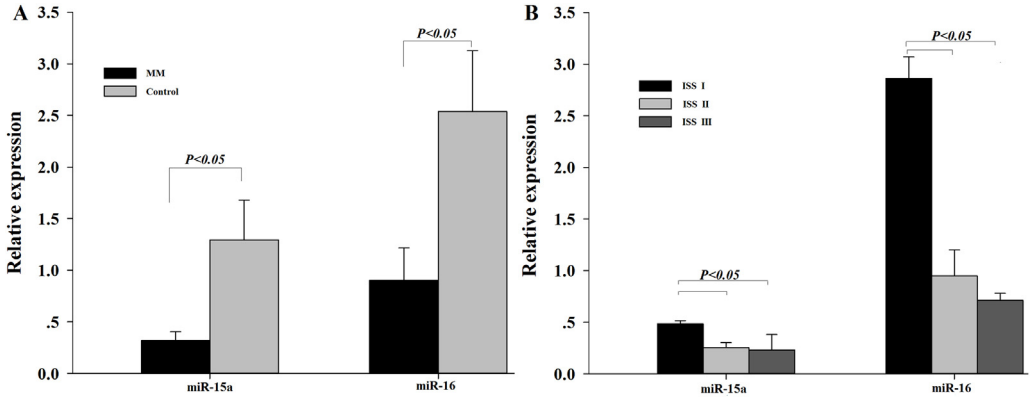

Fig. 1. qRT-PCR analysis of miR-15a and miR-16 expression in BM-MNCs of newly diagnosed patients with MM. The relative expression of miR-15a and miR-16 was detected by qRT-PCR in MM patients ($n = 62$) and healthy donors ($n = 20$) (A), in MM patients of different ISS stages (stage I $n = 11$, stage II $n = 19$ and stage III $n = 32$) (B).

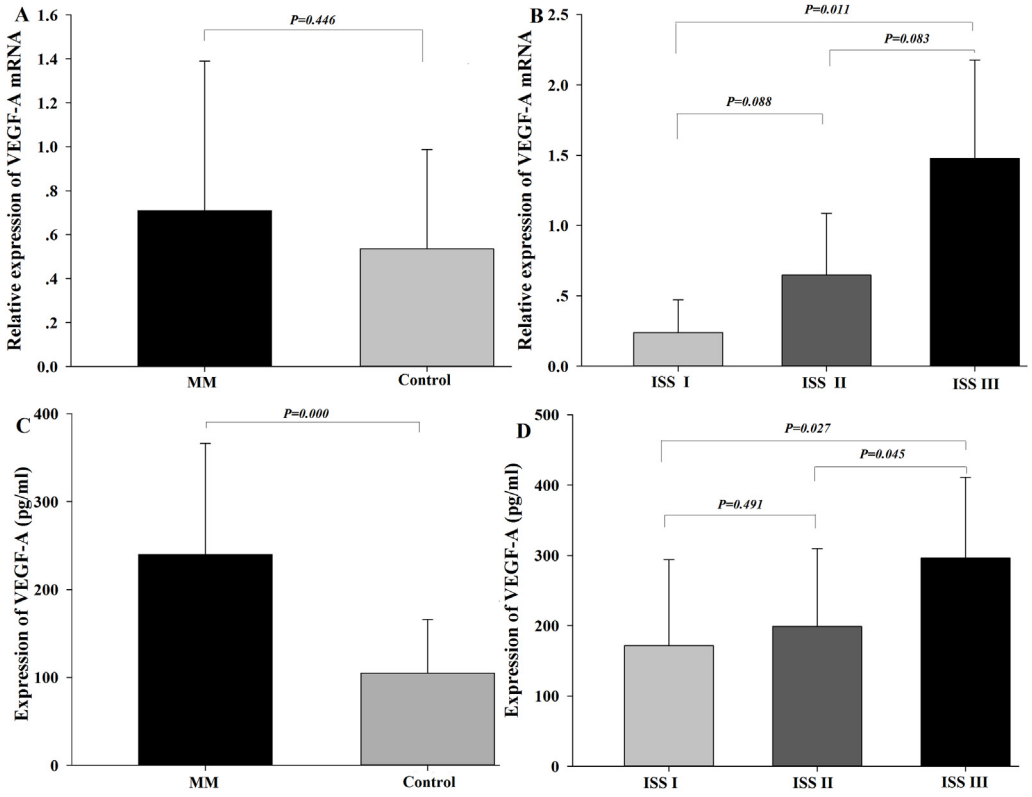

Fig. 2. qRT-PCR and ELISA analysis of VEGF-A in newly diagnosed patients with MM. The relative expression of VEGF-A in BM-MNCs was assessed by qRT-PCR in MM patients ($n = 62$) and healthy donors ($n = 20$) (A), in MM patients of different ISS stages (stage I $n = 11$, stage II $n = 19$ and stage III $n = 32$) (B). The levels of VEGF-A in bone marrow supernatant were analyzed by ELISA in MM patients ($n = 62$) and healthy donors ($n = 20$) (C), in MM patients of different ISS stages (stage I $n = 11$, stage II $n = 19$ and stage III $n = 32$) (D).

on these, we will evaluate the expression of miR-15a/16 in MM patients. Furthermore, we will determine whether miR-15a/16 can affect proliferation and apoptosis of myeloma cells, angiogenesis and anti-MM immune response in MM by regulating the expression of Bcl-2, VEGF-A and IL-17.

2. Materials and methods

2.1. Patients

A cohort of 62 subjects from the Department of Hematology of the Affiliated Hospital of Xuzhou Medical University during the period of October 2013 through May 2015 was enrolled in this study. The newly diagnosed MM patients were 34–82 years old (median 60) and were composed of 34 men and 28 women. All patients were diagnosed based on 2001 World Health Organization diagnostic criteria of multiple myeloma [19]. Diagnoses were confirmed when there were more than 15% clonal plasma cells in bone marrow (BM) samples of the iliac crest. According to the International Staging System (ISS) [20], the MM patients were further divided into 3 subgroups including 11 cases at stage I, 19 at stage II and 32 at stage III. Twenty healthy donors served as a control group, consisting of 12 men and 8 women. No significant differences were observed between MM patients and healthy control regarding age