Leukemia Research 40 (2016) 44–53

Contents lists available at ScienceDirect

Leukemia Research

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

Small interfering RNA silencing of interleukin-6 in mesenchymal stromal cells inhibits multiple myeloma cell growth

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ARTICLE INFO

Article history:
Received 15 April 2015
Received in revised form 9 October 2015
Accepted 11 October 2015
Available online 17 October 2015

Keywords:
Mesenchymal stromal cells
Interleukin-6
siRNA
RNA interference
Multiple myeloma

ABSTRACT

Studies demonstrated that mesenchymal stromal cells (MSC) from bone marrow stroma produced high concentration of interleukin-6 (IL-6) that promoted multiple myeloma cell growth. In view of the failure of IL-6 monoclonal antibody therapy to demonstrate substantial clinical responses in early clinical trials, more effective methods are needed in order to disrupt the favourable microenvironment provided by the bone marrow stroma. In this study, we evaluated the short interfering RNA (siRNA)-mediated silencing of IL-6 in MSC and the efficacy of these genetically modified MSC, with IL-6 suppression, on inhibition of U266 multiple myeloma cell growth. IL-6 mRNA and protein were significantly suppressed by 72 h post IL-6 siRNA transfection without affecting the biological properties of MSC. Here we show significant inhibition of cell growth and IL-6 production in U266 cells co-cultured with MSC transfected with IL-6 siRNA when compared to U266 cells co-cultured with control MSC. We also show that the tumour volume and mitotic index of tumours in nude mice co-injected with U266 and MSC transfected with IL-6 siRNA were significantly reduced compared to tumours of mice co-injected with control MSC. Our results suggest potential use of RNA interference mediated therapy for multiple myeloma.

1. Introduction

Multiple myeloma is a neoplastic disorder of plasma cells, which accounts for 10% of all haematological cancers [1] and remains an incurable disease with an estimated 5-year survival rate of 46.3% [2]. The development of multiple myeloma is a multistep process involving the accumulation of mutations leading to the deregulation of genes controlling cell cycle, apoptosis or the tumour microenvironment interactions.

Mesenchymal stromal cells (MSC) in the bone marrow microenvironment play a crucial role in the pathogenesis of multiple myeloma [3,4]. The adhesion of multiple myeloma cells to MSC induced the transcription and secretion of cytokines that mediated multiple myeloma cells proliferation and migration [5]. The cytokines secreted include interleukin-6 (IL-6) which is a major growth factor and survival factor for multiple myeloma cells by activating specific signal transduction pathways, most notably the JAK/STAT pathways, to induce proliferation and inhibit apoptosis [6–10].

MSC promote the growth of myeloma cells mainly by paracrine IL-6 production [11,12] with several studies reporting of a positive feedback loop between MSC and myeloma cells leading to enhanced stromal production of IL-6 [13,14]. The extensive role of IL-6 in multiple myeloma pathogenesis makes it an important target in anticancer therapy. Targeted therapy against IL-6 using BE-8 murine derived IL-6 monoclonal antibody showed lowered IL-6 level and disease stabilisation but no clinically significant improvements or patient remission [15–17]. Clinical trial for CNTO 328, a new humanised IL-6 monoclonal antibody, on multiple myeloma

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http://dx.doi.org/10.1016/j.leukres.2015.10.004
0145-2126/

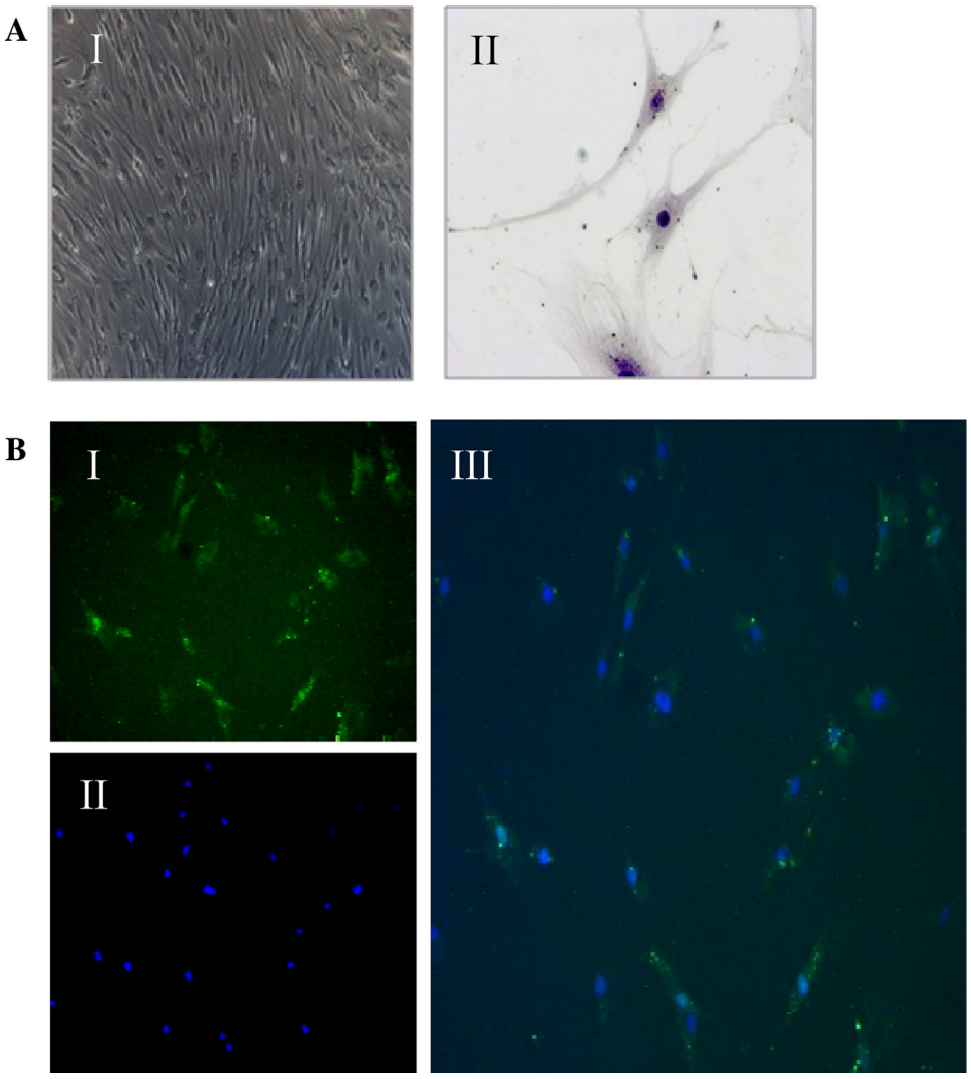

Fig. 1. Transfection efficiency of siRNA in MSC. (A) MSC at Passage 2, prior to transfection, displayed (I) adherent (magnification ×50) and (II) long and spindle-shaped morphologies (magnification ×200). (B) Representative images of BLOCK-iT fluorescent oligo (green) (I) localising in MSC 24 h post transfection (III). MSC nuclei counterstained with DAPI (blue) (II). Transfection efficiency was calculated based on the average of three fields per transfection for three independent experiments. Original magnification was ×100. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

patients resistant to second-line chemotherapy reported sustained suppression of C-reactive protein indicating inhibition of IL-6 activity but again no clinically significant responses [18]. However, in phase I trial on relapsed or refractory multiple myeloma patients in Japan, 66% of patients showed complete or partial response without dose-limiting toxicities observed [19].

Targeted IL-6 monoclonal antibody-based therapy is hampered by the lack of clinically significant improvements or complete remission for patients inflicted with multiple myeloma. New approaches are needed to target IL-6 production by MSC to disrupt the favourable microenvironment provided by the bone marrow for multiple myeloma cell growth. RNA interference (RNAi) provides a new targeted approach to silence overexpression of IL-6 in the development of new multiple myeloma therapy. In this study, we transfected human bone marrow-derived MSC with IL-6 siRNA using lipid-based transfection reagent and evaluated the effect of the transfected MSC on the *in vitro* growth of U266 human multiple myeloma cell line. We also evaluated the antitumour efficacy of MSC transfected with IL-6 siRNA in a murine subcutaneous model of human multiple myeloma.

2. Methods

2.1. Isolation of BM-MSC

Human MSC were isolated from the bone marrow of patients admitted to Universiti Kebangsaan Malaysia Medical Centre for bone marrow aspiration after informed consent according to UKM Research Committee and Ethics Committee guidelines (UKM 1.5.3.5/244/FF-030-2010). Samples from patients with malignancy or history of malignancy within 5 years, and/or positive results for HIV, hepatitis B and hepatitis C infections and who did not consent to participate in the study were excluded. Four mililitres of bone marrow aspirate were layered on top of 3 ml of Ficoll-Paque PLUS (GE Healthcare Biosciences) and centrifuged at 400 × *g* for 20 min. Mononuclear cell layer was extracted and washed twice with PBS. The cells were centrifuged at 100 × *g* for 10 min. The pelleted cells were resuspended in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco) containing 10% Fetal Bovine Serum (FBS) (Gibco), 1% L-Glutamine and 1% penicillin-streptomycin (Gibco). The cells were cultured in 37 °C, 5% CO_2 incubator. Upon confluency, cells were detached using 1 ml of 0.25% trypsin-EDTA (Gibco).