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Research paper

miRNA-149* promotes cell proliferation and suppresses apoptosis by mediating *JunB* in T-cell acute lymphoblastic leukemia

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ABSTRACT

MicroRNA-149* (miRNA-149*) functions as an oncogenic regulator in human melanoma. However, the effect of miRNA-149* on T-cell acute lymphoblastic leukemia (T-ALL) is unclear. Here we aimed to analyze the effects of miRNA-149* on in vitro T-ALL cells and to uncover the target for miRNA-149* in these cells. The miRNA-149* level was determined in multiple cell lines and bone marrow cells derived from patients with T-ALL, B acute lymphoblastic leukemia (B-ALL), acute myelocytic leukemia (AML), and healthy donors. We found that miRNA-149* was highly expressed in T-ALL cell lines and T-ALL patients' bone marrow samples. *JunB* was identified as a direct target of miR-149*. miRNA-149* mimics downregulated *JunB* levels in Molt-4 and Jurkat cells, while miRNA-149* inhibitors dramatically upregulated *JunB* expression in these cells. miRNA-149* mimics promoted proliferation, decreased the proportion of cells in G1 phase, and reduced cell apoptosis in T-ALL cells, while miRNA-149* inhibitors prevented these effects. miRNA-149* mimics downregulated p21 and upregulated cyclinD1, 4EBP1, and p70s6k in Molt-4 and Jurkat cells. Again, inhibitors prevented these effects. Our findings demonstrate that miRNA-149* may serve as an oncogenic regulator in T-ALL by negatively regulating *JunB*.

1. Introduction

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive type of leukemia that is one of the most common malignancies diagnosed in children and adults [1,2]. Various intracellular mechanisms have been indicated to contribute to disease pathogenesis and progression, including the NOTCH1, PI3K-Akt, and cyclin D3:CDK4/6 (cyclin-dependent kinase 4/6) pathways [3]. Nevertheless, the pathogenesis of T-ALL has not yet been fully elucidated and the therapeutic outcomes of patients with resistance or relapsed disease are still poor [4].

microRNAs (miRNAs) are a family of noncoding RNAs (ncRNAs) that regulate gene expression. Aberrant miRNA regulation has thus been shown to play multiple roles in the pathogenesis of many diseases, including cancer [5–7]. miRNAs are 19–24 nt in length and are processed from large precursor hairpins approximately 70 nt long by the RNase III enzyme Dicer into mature miRNA and miRNA* duplexes (the complementary strands of miRNA that from miRNA/miRNA* duplexes) [8–10].

Microarray analyzes show that treatment with rapamycin greatly reduces the expression of miR-149* in the human T-ALL cell line Molt-4, suggesting that miR-149* may be involved in T-ALL pathogenesis. It has been shown that miR-149* serves as an oncogenic regulator in human melanoma [11]. However, the precise role of miR-149* in regulating gene expression and in mediating the pathogenesis of T-ALL remains unclear.

In the present study, we investigated the expression of miR-149* in cultured leukemia cell lines as well as in bone marrow cells derived from leukemia patients. In addition, the target gene for miR-149* was identified and the effects of miR-149* on biological properties of T-ALL cells were investigated. Our findings provide a potential approach for future miR-149*-targeting therapies for human T-ALL.

2. Materials and methods

2.1. Cell culture

Cell lines, including T-ALL cell line Molt-4, human Burkitt's lymphoma cell line Raja, human pre-B lymphoma cell line Nalm6, human chronic myeloid leukemia cell line K562, and human acute monocytic leukemia cell line THP-1 (all purchased from Amer-

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0145-2126/

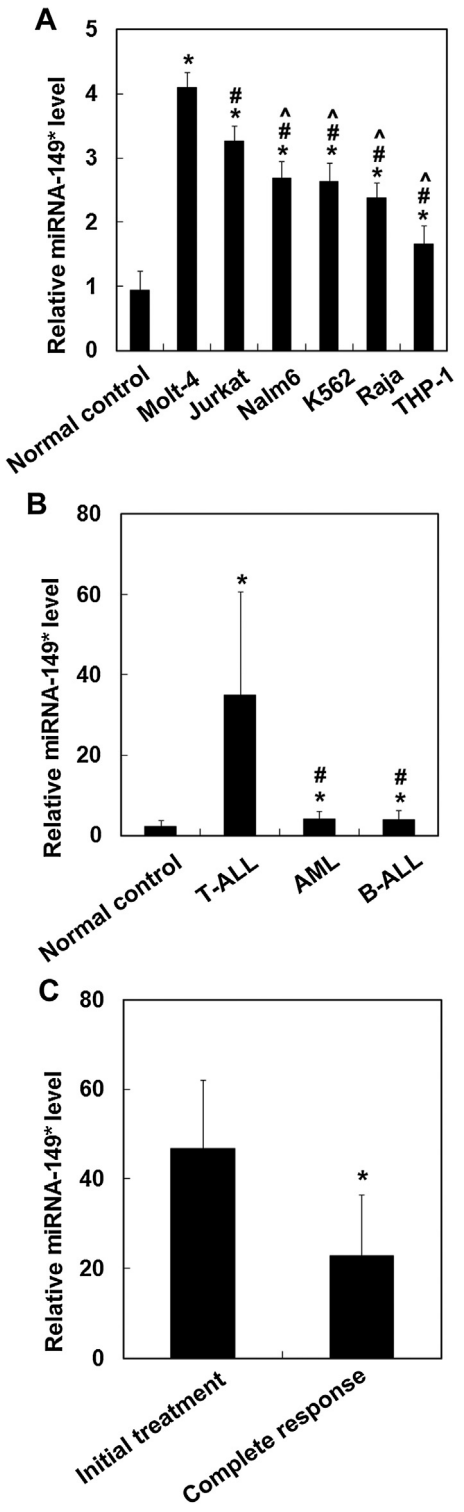

Fig. 1. miRNA-149* is highly expressed in T-ALL cell lines and T-ALL patients' bone marrow samples.
(A) miRNA-149* levels in Molt-4, Jurkat, Raja, Nalm6, K562, THP-1, and normal control cells were examined by qRT-PCR. Data were calculated from three independent experiments. $^{*}P < 0.05$ compared with normal control; $\#P < 0.05$ compared with Molt-4; $\hat{}P < 0.05$ compared with Jurkat. (B) miRNA-149* levels in AML ($n = 20$), B-ALL ($n = 20$), T-ALL ($n = 15$) and healthy donors ($n = 15$) were examined by qRT-PCR. $^{*}P < 0.05$ compared to normal control; $\#P < 0.05$ compared to T-ALL. (C) miRNA-149* levels in T-ALL patients at diagnosis ($n = 8$) and with complete response ($n = 8$) were examined by qRT-PCR. $^{*}P < 0.05$.

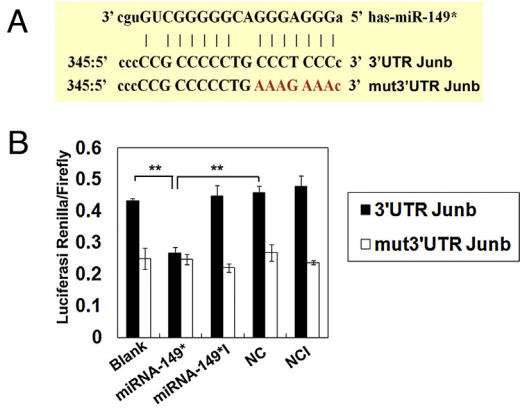

Fig. 2. *JunB* is a potential target for miRNA-149*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.) (A) The predicted binding sites for miRNA-149* and *JunB*. The sequence of the mutant JunB 3′UTR is also presented, with the mutated sites highlighted in red. (B) Luciferase assays showed that the relative luciferase activity was decreased in HEK293 cells co-transfected with miRNA-149* and *JunB*, but was increased in cells co-transfected with miRNA-149* inhibitor and *JunB*. A mutated 3′UTR *JunB* plasmid was used as a control. Control cells were co-transfected with negative control (NC) miRNA or NC inhibitor (NCI) together with *JunB* plasmids. $^{**}P < 0.01$.

ican Type Culture Collection, Manassas, VA, USA), were used in this study. The human T-cell lymphoblast-like cell line Jurkat was kindly provided by the Shanghai Cell Bank, Chinese Academy of Sciences, China. All of the normal control cells were peripheral blood mononuclear cells derived from the healthy donors. Cells were maintained in 1640 culture medium (Hyclone, Logan, Utah, USA) containing 10% fetal bovine serum (FBS, Hyclone, Logan, UT, USA), 100 U/mL penicillin, and 100 U/ml streptomycin, and were incubated in a 5% CO_2-humidified incubator at 37 °C.

2.2. Isolation and primary culture of human blood mononuclear cells

To determine the miRNA-149* levels among different diseases, bone marrow cells were derived from T-ALL ($n = 15$), B-ALL ($n = 20$), AML ($n = 20$) patients, and healthy donors ($n = 15$) at the First Affiliated Hospital of Harbin Medical University, Heilongjiang, China. All participants signed an informed consent. The average age of T-ALL, B-ALL, and AML cohorts was 30.27 ± 16.50 years, 38.50 ± 14.28 years, and 47.45 ± 17.89 years, respectively. According to the French–American–British (FAB) classification, the AML group consisted of one AML-M1 case, 13 AML-M2 cases, three AML-M4 cases, and three AML-M5 cases. The percentages of leukemic cells in mononuclear cells derived from bone marrow cells of T-ALL, B-ALL, and AML cohorts were over 80%. To compare the miRNA-149* levels between T-ALL patients with complete response and at diagnosis, a total of eight male T-ALL patients were included in the study. These patients ranged from 16 to 50 years old. The mRNA expression of miRNA-149* in bone marrow cells was determined at diagnosis and at the time of complete response. Bone marrow samples were obtained by bone marrow puncture upon diagnosis. Ethical approval for this study was granted by the First Affiliated Hospital of Harbin Medical University, Heilongjiang, China. Bone marrow mononuclear cell suspension was prepared using the Ficoll cell separation method. Cells were cultured in the same medium and same conditions as cell lines.

2.3. Real-time quantitative reverse transcription–polymerase chain reaction (qRT-PCR)

Total RNA was extracted using TRIzol (Invitrogen, Carlsbad, CA, USA), total RNA (1 μg) was reverse-transcribed, and qRT-PCR was