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MiR-124 contributes to glucocorticoid resistance in acute lymphoblastic leukemia by promoting proliferation, inhibiting apoptosis and targeting the glucocorticoid receptor

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ABSTRACT

Acute lymphoblastic leukemia (ALL) is characterized by the accumulation of abnormal lymphoblasts in the bone marrow and blood. Though great progress has been made for improvement in clinical treatment during the past decades, some children with ALL still relapsed. Glucocorticoid (GC) resistance is an important clinical problem for ALL treatment failure. Therefore, further understanding of the mechanism of GC resistance and exploring novel therapeutic strategies are crucial for improving treatment outcome. The reported involvement of microRNAs (miRNAs) in drug resistance implied that deregulated miRNA expression might contribute to GC treatment response of ALL. However, individual miRNAs and their functional mechanisms potentially involved in the GC response are still largely unknown. In the present study, we found that miR-124 was up-regulated in prednisone insensitive human ALL cell line and prednisone-poor response ALL patients. Furthermore, it was found that miR-124 might contribute to GC resistance by promoting proliferation and inhibiting apoptosis of ALL cells. Importantly, we validated that miR-124, targeted and decreased the expression of glucocorticoid receptor (NR3C1), prevented the inhibitory effect of GC in ALL. These findings strongly suggest that miR-124 is critical in poor GC response and may serve as a potential therapeutic target in ALL with poor GC resistance.

1. Introduction

Acute lymphoblastic leukemia (ALL) arises from the differentiation arrest of lymphoid precursor and malignant proliferation in bone marrow (BM) and blood. The prognosis of ALL has been of great concern because ALL is the most common type of childhood cancer [1]. Glucocorticoids (GC) were one of the key drugs used for the treatment of ALL, and GC (prednisone or dexamethasone) treatment response was a strong independent factor of prognosis [2]. Ten-year event-free survival (EFS) was about 80% for pediatric patients with prednisone-good response (PGR) ALL, however, there were still 57% of prednisone-poor response (PPR) ALL patients had to receive more intensive treatment [3,4]. Therefore, investigating the mechanism of GC resistance and exploring novel therapeutic strategies to reverse the GC resistance are important for treatment failure of ALL patients.

MicroRNAs (miRNAs) have been proven to be an abundant class of small non-coding RNAs which were directly involved in various physiological processes, such as cell proliferation, differentiation and apoptosis, and promote tumorigenesis [5–9]. Some miRNAs have been reported to be associated with drug resistance in ALL. Our previous study found that miR-99a/100 has an indirect influence on treatment response to GC by targeting FKBP51 [10]. It has also been showed that miR-335 promotes GC sensitive by suppressing MAPK1 gene and miR-142-3p plays an oncogenic role in ALL and directly targets GC receptor-α [11]. Recently, a report demonstrated that GC induced the expression of miR-124 which targeted NR3C1 to limit the anti-inflammatory effect of GC and contributed to GC resistance in sepsis [12]. However, whether miR-124 affected on the treatment response to GC in ALL through targeting NR3C1, it should be further researched.

In this study, we demonstrated that miR-124, abnormally expressed in many cancers [13–15], was overexpressed in pediatric PPR ALL. MiR-124 had been shown to play an important role in acute myeloid

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Table 1
Characteristics of patients.

	PGR ALL	PPR ALL
Patients number	31	9
Median age (range), yr	5.5	10
Sex, No.		
Male	21	5
Female	10	4
WBC, $\times 10^9$/L (median)	17.2	64.3
Immunophenotype, No.		
B	29	5
T	2	4
Outcome, No.		
PR[a]	1	2
CR[a]	30	4
Relapsed	0	3

[a] PR: partial remission; CR: complete remission.

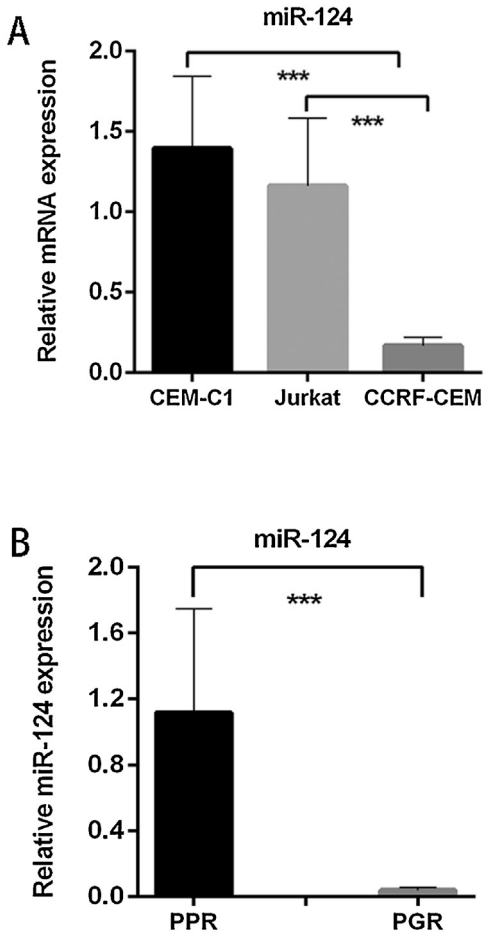

Fig. 1. The expression of miR-124 by qRT-PCR in CEM-C1, Jurakt and CCRF-CEM cell lines (A), PPR and PGR patients (B). $^{***}P < 0.001$.

leukemia (AML) [16,17] and was involved in B-cell lymphomas through the NF-κB pathway [18]. Remarkably, our study found miR-124 induced resistance to GC treatment by targeting GC receptor (NR3C1) in ALL. These findings indicated that the overexpression of miR-124 was a critical prognosis factor of pediatric ALL, which could open a new venue to develop effective strategies against pediatric ALL by targeting miR-124 to reverse GC resistance.

2. Materials and methods

2.1. Patients

A total of 31 patients with PGR ALL and 9 with PPR ALL from the First Affiliated Hospital of Sun Yat-sen University were enrolled. The clinical information about the patients was presented in Table 1. Informed consent was obtained from all patients, and the study was approved by the Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University. All analyzed samples were collected at the time of diagnosis before treatment.

2.2. Cell culture and RNA/protein isolation

Human CCRF-CEM, Jurkat and CEM/C1 cells were cultured in RPMI 1640 medium (Invitrogen). Both cultures were supplemented with 10% fetal bovine serum (fetal bovine serum, Australia) and sodium pyruvate, and cultured at 37 °C in a humidified atmosphere consisting of 5% CO_2. Total RNA and proteins were isolated from clinical samples with Trizol (Invitrogen, Carlsbad, CA) according to the instructions of the manufacturer.

2.3. Quantitative real-time PCR analysis for miR-124 expression

Quantitative real-time reverse transcriptase PCR (qRT-PCR) was performed to detect miR-124 expression. Briefly, 0.2 ug of small RNA extracted from cell samples was reverse-transcribed to cDNA using M-MLV reverse transcriptase (Promega) and amplified with specific designed miRNA RT-primers and PCR amplification primers (Taqman probe, Invitrogen). The expression level of each miRNA was measured using the 2-DeltaDeltaCt method.

2.4. MTT assay

CCRF-CEM and CEM/C1 cells were respectively plated at 1×10^4 per well. The cells were transfected with 100 nM miR-124 mimics/NC (miR-124/scrambled oligonucleotides) or inhibitor-miR-124/NC using Lipofectamine 2000 (Invitrogen) following manufacturer's recommendation and were then incubated with dexamethasone in different concentration, such as 0, 3.7 nM, 11 nM, 33 nM and 99 nM, for 24 h, 48 h and 72 h, respectively. Next, the cells were incubated with Dye Solution (15 uL) for another 4 h until purple precipitate was visible. Lastly, after 100uL Stop Mix was added, the cells were left at room temperature in the dark for 2 h and the absorbance was recorded.

2.5. Apoptosis assay

CCRF-CEM and CEM/C1 cells were transfected with miR-124 mimics/NC or inhibitor-miR-124/NC (100 nM) using Lipofectamine 2000 (Invitrogen) following manufacturer's recommendation. Then the cells were incubated with dexamethasone in different concentration as mentioned above. The cells were collected at 96 h post transfection respectively, and centrifuged and resuspended in 500 ul of staining solution (containing annexin V fluorescein and propidium iodide in HEPES buffer) (annexin V: FITC apoptosis detection kit; BD Pharmingen, San Diego, CA). After incubation at room temperature for 15 min, cells were analyzed by flow cytometry.

2.6. Transient transfection

The cells were transfected using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. The following were purchased from GenePharma (Shanghai, China): miR-124 mimics, miR-NC (a negative control), miR-124 inhibitor with a sequence complementary to mature miR-124 and miR-inhibitor NC (a negative control). The transfection efficiencies were shown in Supplementary Fig. 1.