Leukemia Research 33 (2009) 1530–1538

Contents lists available at ScienceDirect

Leukemia Research

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

Role of microRNA-143 in Fas-mediated apoptosis in human T-cell leukemia Jurkat cells

Yukihiro Akao[a,c,*], Yoshihito Nakagawa[a], Akio Iio[a], Tomoki Naoe[b]

[a] Department of Medical Oncology, Gifu International Institute of Biotechnology, 1-1 Naka-Fudogaoka, Kakamigahara, Gifu 504-0838, Japan
[b] Department of Hematology and Oncology, Nagoya University, Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan
[c] United Graduate School of Drug Discovery and Medical Information Sciences, Gifu University, Yanagido, Gifu 501-1193, Japan

ARTICLE INFO

Article history:
Received 29 December 2008
Received in revised form 9 April 2009
Accepted 16 April 2009
Available online 21 May 2009

Keywords:
MiRNA-143
Apoptosis
Fas
Jurkat T-cell
ERK5

ABSTRACT

Treatment of Jurkat T cells with Fas-activating antibody (CH-11) facilitated rapid cell death that was shown to be caspase-dependent apoptosis. The expression of miR-143 was up-regulated during the apoptosis with time. The increased expression of miR-143 emerged from 1 to 2 h after the treatment, at which time the caspases-8 and -3 were also activated; and this increase was almost canceled by the pretreatment with an inhibitor of caspase-3 or -8. Furthermore, the transfection of Jurkat cells with mature miR-143 induced a significant growth suppression and enhancement of CH-11-induced apoptosis. On the contrary, an extracellular signal-regulated protein kinase 5 (*ERK5*), which was determined to be a target of miR-143 in colon cancer DLD-1 cells, was time-dependently down-regulated at the translational level after the treatment. During the apoptosis, the expression level of FasL was maintained and the level of nuclear-Foxo3a was increased in the early phase. These data suggest that the up-regulation of miR-143 could be related to the apoptosis in part by targeting *ERK5*, which leads to promotion of Foxo3a/FasL positive feedback loop.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

MicroRNAs (miRNAs) are a recently discovered class of small non-coding transcripts that regulate gene expression by inhibiting the translation or promoting the degradation of transcribed mRNAs [1]. Although bioinformatic approaches suggest that miRNAs may regulate the expression of a large fraction of the genes [2,3], the determination of miRNA gene targets and biological fractions has been comparatively limited. Recently, approximate 500 miRNAs have been identified in the human genome (miR registry, www.sanger.ac.uk/Software/Rfam/mirna/index.shtml). Emerging studies suggest that many miRNAs may participate in human disease, especially in the case of oncogenesis [4,5]. Changes in the levels of miRNAs alter the control of growth or apoptosis in some cancers [6–8]. We previously found that miRNAs(miRs)-143 and -145 were down-regulated in colon cancer [9,10]. Furthermore, all of the kinds of human cancer cell lines tested exhibited a extremely low-expression of miRs-143 and -145, whereas their originating normal tissues showed a good expression of both [9,10]. It is considered that miRs-143 and -145 act as anti-oncomirs on carcinogenesis. MiR-143 and -145 are located approximately 1.7 kb apart from each other at 5q33, which suggests that both are transcribed as the same primary miRNA.

In the present study, we investigated the expression of miR-143 during Fas-mediated apoptosis in Jurkat cells, and found that the expression of miR-143 in Jurkat cells was up-regulated by treatment of the cells with anti-Fas antibody. Fas, a receptor for Fas-ligand (FasL), belongs to the tumor necrosis factor (TNF) receptor family, and transduces the apoptotic signal into cells [11]. The molecular mechanism for Fas-induced apoptosis is caspase-dependent [11]. Here, we show that miR-143, which was previously defined to be anti-oncomir, positively contributes to the Fas-induced activation of caspases in Jurkat cells and that the target gene of miR-143 is extracellular signal-regulated protein kinase 5 (*ERK5*) which would contribute negatively to the apoptosis [12].

2. Materials and methods

2.1. Cell culture and cell viability

Human T-cell leukemia Jurkat cells were grown in RPMI-1640 medium supplemented with 10% (v/v) heat-inactivated FBS (Sigma, St. Louis, MO, USA) and 2 mM L-glutamine under an atmosphere of 95% air and 5% CO_2 at 37 °C. The number of viable cells was determined by the trypan-blue dye exclusion test. For morphological examination of apoptotic changes, cells were stained with Hoechst33342 (5 μg/ml) at 37 °C for 30 min, washed twice with phosphate-buffered saline (PBS), pipetted dropwise onto a glass slide, and examined by fluorescence microscopy using an Olympus microscope (Tokyo, Japan) equipped with an epi-illuminator and

* Corresponding author at: Gifu International Institute of Biotechnology, 1-1 Naka-Fudogaoka, Kakamigahara, Gifu 504-0838, Japan. Tel.: +81 583 71 4646; fax: +81 583 71 4412.
E-mail address: yakao@giib.or.jp (Y. Akao).

0145-2126/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.leukres.2009.04.019

appropriate filters. Anti-Fas antibody (CH-11, MBL, Nagoya, Japan) or TRAIL (MBL) was used for induction of apoptosis in Jurkat cells. For induction of apoptosis, CH-11 (0.8–0.12 μg/ml) and TRAIL (10 ng/ml) was added into the medium at the concentration of 3–4 × 10^5/ml cells.

2.2. Assessment of apoptosis

Cellular DNA was extracted from whole cells by the procedure described previously [13]. RNase was added to the DNA solution at the final concentration of 20 μg/ml, and the mixture was incubated at 37 °C for 30 min. After electrophoresis on a 2% agarose gel, DNA was visualized by ethidium bromide staining. For assessment of the morphological characteristics of apoptosis, the cells were stained with Hoechst33342 (5 μg/ml) at 37 °C for 30 min, washed once with PBS, resuspended, pipetted dropwise onto a glass slide, and examined by fluorescence microscopy using an Olympus microscope (Tokyo, Japan) equipped with an epi-illuminator and appropriate filters. The cells with condensed and fragmented nuclei stained with Hoechst33342 were assessed to be apoptotic. Approximately 200 cells were counted in 4 different fields and 2 independent experiments were performed.

2.3. Quantitative RT-PCR

In order to examine the expression level of mature miR-143 or -145 in detail we performed TaqMan® MicroRNA Assays (Applied Biosystems, Foster City, CA) using real-time PCR [14]. The threshold cycle (CT) is defined as the fractional cycle number at which the fluorescence passes the fixed threshold. The expression level of miR-143 in each sample was measured and was normalized to *U6* expression which was used as an internal control. In addition, to determine the expression levels of these miRNAs, we also measured their levels by using a mirVana™ qRT-PCR miRNA Detection Kit (Applied Biosystems) and mirVana™ qRT-PCR Primer set (Applied Biosystems) [9,14]. The PCR primer pairs for miR-143 were obtained commercially from Applied Biosystems. The PCR products obtained by using such primer pairs were confirmed to be from the loci of miR-143 by DNA sequencing [9].

2.4. Western blotting

The cells were homogenized in chilled lysis buffer comprising 10 mM Tris–HCl (pH 7.4), 1% NP-40, 0.1% deoxycholic acid, 0.1% SDS, 150 mM NaCl, 1 mM EDTA, and 1% Protease Inhibitor Cocktail (Sigma) and stood for 20 min on ice. After

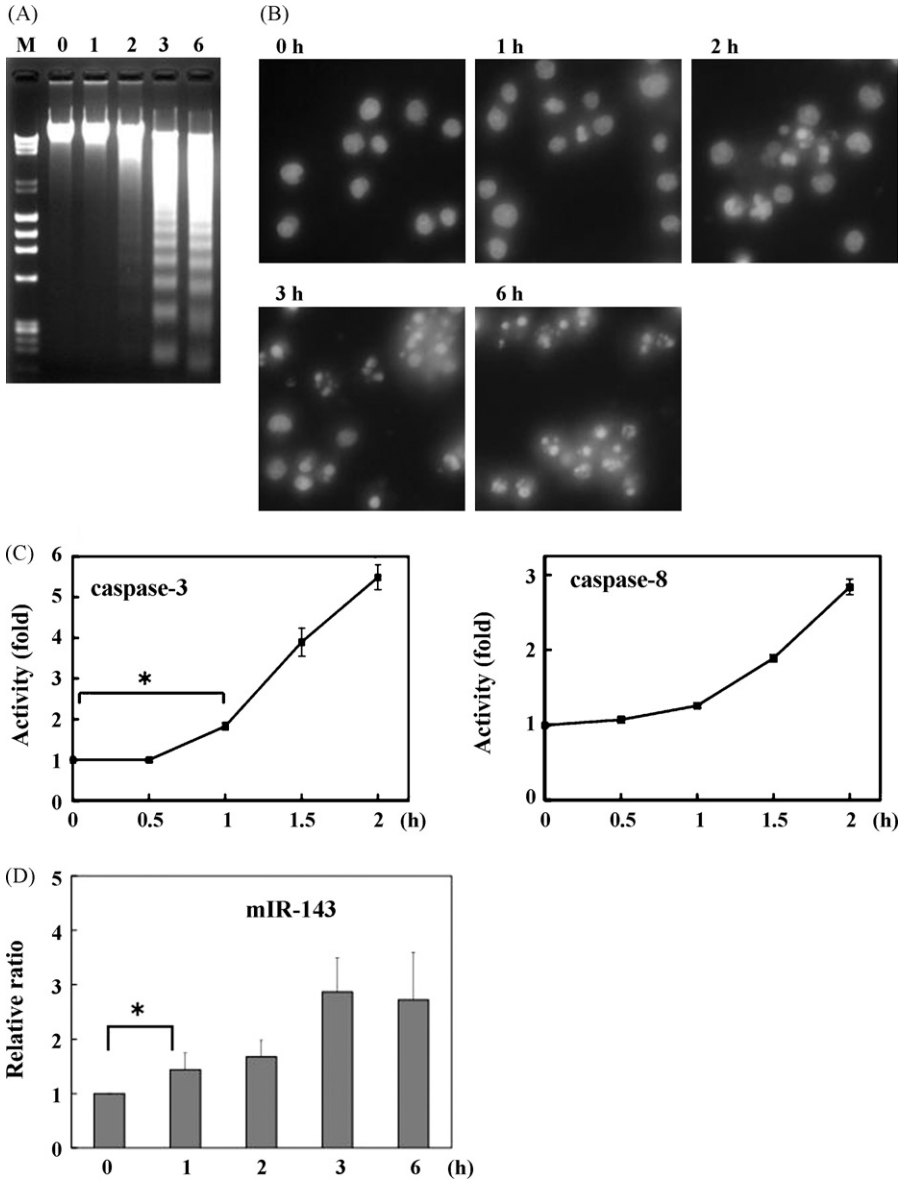

Fig. 1. Fas-mediated apoptosis induced by anti-Fas antibody (CH-11) in Jurkat cells. (A) Nucleosomal DNA fragmentation of the cells after treatment with CH-11. Three micrograms of DNA was loaded onto each lane. Lane M contained DNA size markers. (B) Morphological aspects of the cells after the treatment. The cells were stained with Hoechst33342 (5 μg/ml) for 30 min and then observed by fluorescence microscopy. (C) Changes in the activity of caspase-3 and caspase-8 after the treatment with CH-11. (left panel, caspase-3; right panel, caspase-8), *$p < 0.05$. (D) Expression levels of miR-143 by TaqMan assay after the treatment with CH-11, *$p < 0.05$.

Download English Version:

https://daneshyari.com/en/article/10909347

Download Persian Version:

https://daneshyari.com/article/10909347

Daneshyari.com