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Interferon-α promotes heat-induced apoptosis in human myeloid leukemia cells

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Abstract The effects of interferon-α (IFNα) treatment on following heat-induced apoptosis were evaluated in human myeloid leukemia cell line, U937. Incubation of U937 cells with 1000 U/ml of IFNα promoted heat-induced apoptosis in a dose- and time-dependent manner. Another human leukemia cell line, HL60, was also sensitized to heat-induced apoptosis by pretreatment with IFNα. To investigate the mechanism of sensitization, effects of IFNα on the expression of HSP70 and BCL-2, two major anti-apoptotic proteins, were evaluated. Both proteins were upregulated by heat treatment, but irrespective of the presence or absence of IFNα. These results suggest that IFNα promotes heat-induced apoptosis in human myeloid leukemia cells through the mechanism whereby HSP70 or BCL-2 is not involved.

Introduction

Apoptosis is a morphologically unique process of cell death, which is distinct from necrosis. Apoptosis is characterized morphologically by reduced cell volume, condensed chromatin in the nucleus, formation of internucleosomal DNA fragmentation[1]. Heat shock, one of the apoptotic stimuli, is now used for the treatment of solid tumors, designated hyperthermia[2]. Interferons (IFNs) are a family of natural glycoproteins that share antiviral, immunomodulatory, and anti-proliferative effects[3]. Although the combination of anticancer drug or hyperthermia with IFNs enhances antitumor effect[4], the precise mechanism of its synergistic effects has been unknown.

Heat shock protein (HSP) is one of the stress proteins induced by several cellular stresses including heat[5]. It has been reported that quercetin, a plant flavonoid, induces apoptosis by inhibiting the expression of HSP70, suggesting the anti-apoptotic effect of HSP70[6].

In this report, we investigated the sensitization of heat-induced apoptosis by IFNα treatment in human myeloid leukemia cell lines and evaluated possible roles of HSP70 and BCL-2, potent anti-apoptotic proteins, in the mechanism of sensitization.

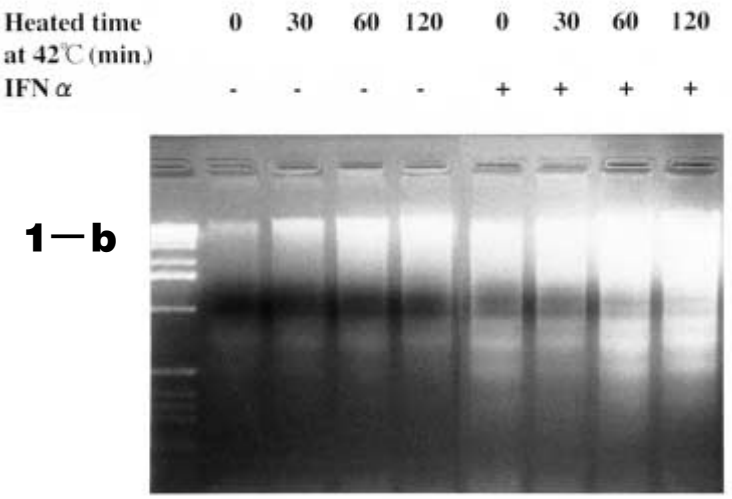
Materials and Method

Cells: Three human myeloid leukemia cell lines (U937, K562, and HL60), obtained from the Japanese Cancer Resource Bank (Tokyo Japan), were cultured in RPMI1640 medium supplemented with 10%(v/v)

heat-inactivated fetal bovine serum containing 50 U/ml of penicillin G and 50 μg/ml of streptomycin. Cells were maintained at 37℃ in a 5% CO_2 humidified atmosphere. In all experiments, cells were washed twice with phosphate-buffered saline (PBS) and resuspended in fresh culture medium 12 hours before use.

Antibodies and reagents: Anti-HSP70 (clone BRM-22) was obtained from Sigma. Anti-BCL-2 (Ab-1) was obtained from Oncogen Research Products. IFN α was kindly provided by Sumitomo Pharmaceutical Co. Japan.

Cell Treatments: Cells preincubated with various concentrations of IFN α for 48 hours were washed and resuspended (1 X 10^6 cells /ml) in the fresh culture medium in the 15 ml conical tube, followed by incubation at 42℃ for indicated periods. Cells were then returned to the 37℃ CO_2 incubator and maintained for 12 hours (recovery period) before evaluation of apoptosis.



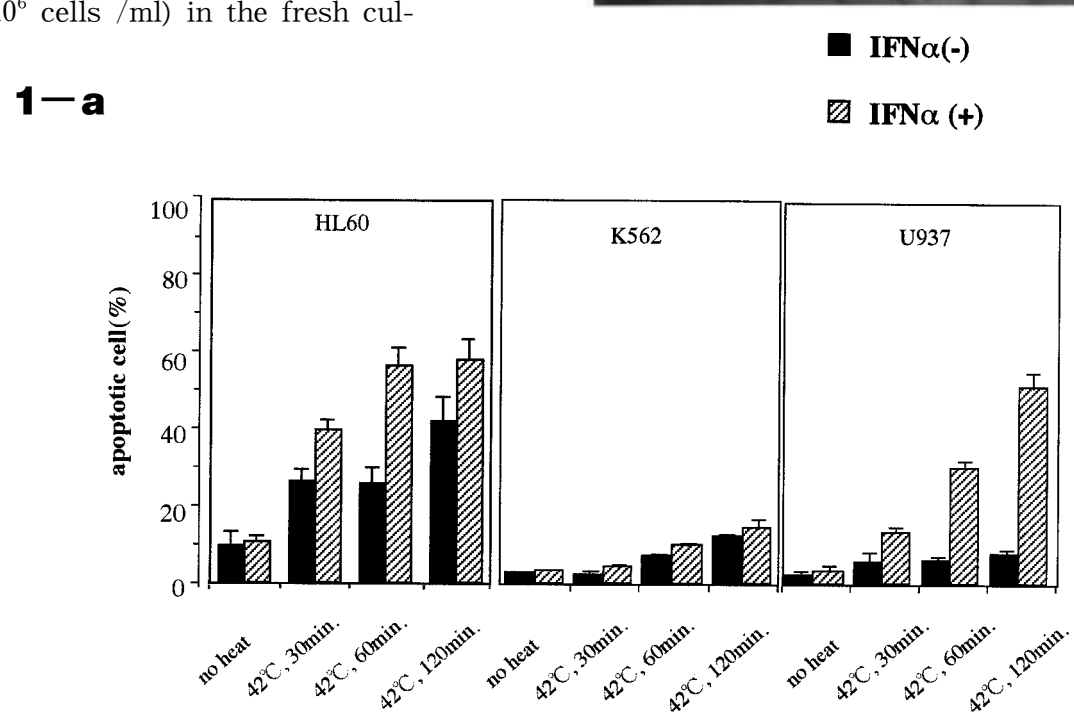


Figure 1

IFN α sensitizes human myeloid leukemia cells to heat-induced apoptosis.

a. Cells were preincubated for 24 hours with or without 1000 U/ml of IFN-α. The cells were washed and exposed to 42℃ for the indicated periods and then incubated at 37℃ for 12 hours (the recovery period). The percentage of apoptotic cells was assessed by staining with Höechst-33258. The results of three independent experiments are expressed as the mean±SEM.

b. DNA fragmentation induced by heat in U937 cells. Cells were treated as described in Figure 1-a. Agarose gel electrophoresis was performed as described in "Materials and Methods".

Cytochemical staining of apoptotic cells: Morphological changes in the nuclear chromatin of apoptotic cells were detected by staining with bisbenzimide trihydrochloride (Höechst-33258). Cells were collected, washed with PBS, and fixed for 30 minutes with 1% glutaraldehyde in PBS. Subsequently, fixed cells were washed and resuspended in PBS before staining with 0.17 μM of Höechst-33258. Apoptotic cells were identified by the presence of chromatin condensation and segmentation under fluorescence light microscopy. Five hundred cells were counted and % apoptosis (apoptotic cell count / total cell count x 100) was calculated. Apoptosis was also evaluated by agarose gel electrophoresis of fragmented DNA as previously reported[7].

Western blot analysis of BCL-2 and HSP70 protein : For western blot analysis, 1 X 10^6 cells were lysed in lysis solution (10 mM Tris and 1% SDS) and sonicated. Equivalent amounts of cell lysates (20 μg) were separated by SDS-PAGE on 7.5% (for HSP70) or 15% (for BCL-2) acrylamide gel and electrically transferred onto a nitrocel lulose membrane using a semi-dry transfer system. Heat treatment in this study caused no apparent changes in the amounts of protein in the cell lysates (data not shown). The membranes were preblocked in blocking solution (PBS containing 5% w/v low-fat milk and 0.2% Tween-20) for 1 hour, and then probed with either a 1 : 5000 dilution of the monoclonal anti-HSP 70 or a 1 : 40 dilution of anti-BCL-2, Human (Ab-1). Membranes were washed three times with PBS containing 0.2% Tween-20 (PBS-T) and incubated with PBS-T containing 3% BSA and a 1 : 1000 dilution of sheep anti-mouse IgG peroxidase conjugate (Amersham). After the membranes had been washed three times with PBS-T, proteins were detected using an ECL-Western blot detection kit (Amersham).

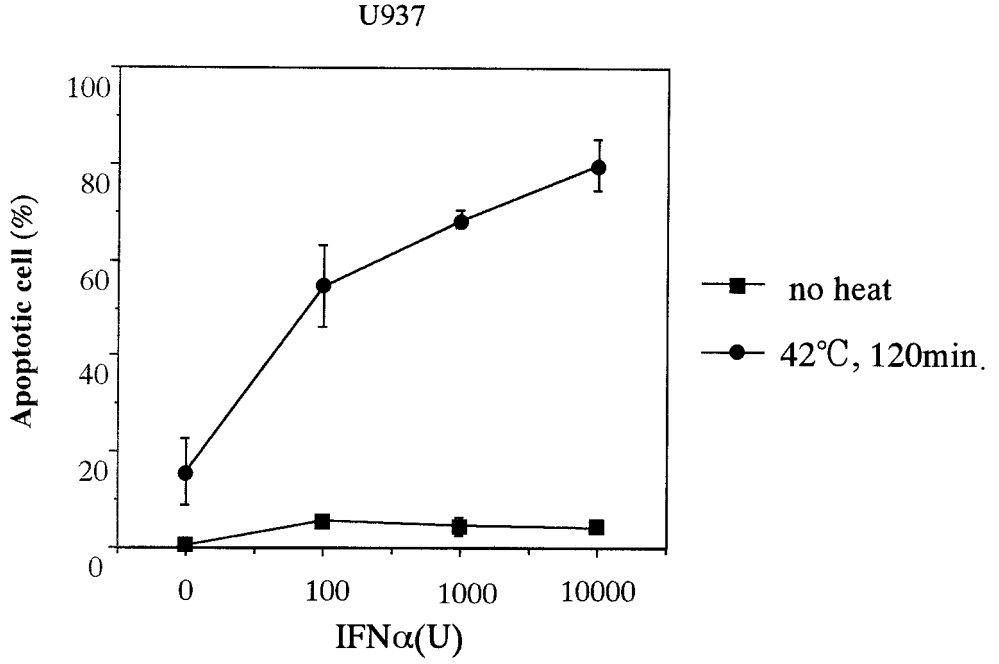


Figure 2
IFN α sensitizes U937 cells to heat-induced apoptosis in a dose-dependent manner.
Cells were preincubated for 24 hours in the absence or presence of various concentrations of IFN-α. The cells were washed and exposed to 42℃ for 120 minutes and then incubated at 37℃ for 12 hours (the recovery period). The percentage of apoptotic cells was assessed by staining with Höechst-33258. The results of three independent experiments are expressed as the mean±SEM.

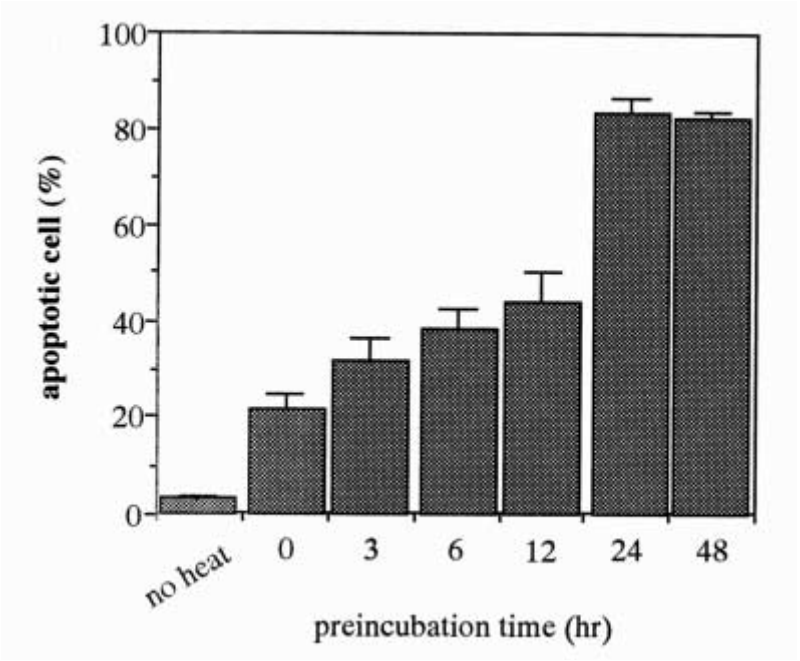


Figure 3
IFN α sensitizes U937 cells to heat-induced apoptosis in the time-dependent manner.
Cells were preincubated for the indicated time with or without 1000 U/ml of IFN-α. The cells were washed and exposed to 42℃ for 120 min. and then incubated at 37℃ for 12 hours (the recovery period). The percentage of apoptotic cells was assessed by staining with Höechst-33258. The results of three independent experiments are expressed as the mean±SEM.

Results

IFN α promotes heat-induced apoptosis in myeloid leukemia cells

Heat-induced apoptosis was evaluated in three myeloid leukemia cell lines (U937, HL 60 and K562) by morphological determination of nuclear fragmentation (Fig.1a). Nuclear fragmentation was also confirmed by electrophoretic determination of fragmented DNA (Fig.1b). Incubation at 42 ℃ for 30 to 120 min, followed by 60 min.recovery at 37 ℃, induced apoptosis in HL60 cells in a time-dependent manner. Heat treatment for 120 min. induced apoptosis in approximately 40% HL60 cells, whereas only 5 to 10% of K562 cells and U937 cells showed apoptosis in the same condition, indicating that latter two cell lines are resistant to heat-induced apoptosis. U937 cells and HL60 cells were sensitized to heat-induced apoptosis when cells were preincubated with 1000 U/ml of IFN α before heat treatment. IFN α alone without heat treatment had little effect on cell apoptosis of these three cell lines. Because U937 cells preincubated with IFN α were most sensitized to heat-induced apoptosis, further evaluation was performed with U937 cells.

When U937 cells were incubated at various concentrations of IFN α followed by heat treatment at 42 ℃ for 120 min. apoptotic cells increased dose-dependently with the concentration of IFN α (Fig. 2). IFN α alone without heat treatment had little effect on apoptosis even at the highest concentration of IFN α (10000 U/ml). In addition, sensitization of U937 cells to heat-induced apoptosis was dependent on the preincubation period with 1000 U/ml of IFN α (Fig. 3).

Expression of HSP70 and BCL-2 protein after treatment with IFN α

From these results, it may be possible that IFN α promotes heat-induced apoptosis by modulating the expression of apoptosis-regulating proteins. We previously reported that non-lethal heat treatment upregulated the expression of HSP70 and BCL-2. Increased expression of these

proteins may be resposible for the resistance to apoptosis induced by otherwise lethal heat and anti-cancer drugs[7]. We therefore examined the possibility that IFN α might promote heat-induced apoptosis by downregulating the expression of HSP70 and/or BCL-2. Figure 4-a shows the expression of HSP70 in the cells incubated with or without 1000 U/ml of IFN α followed by exposure to 42 ℃ for 60 minutes. Regardless of the presence or absence of IFN α, intracellular HSP70 increased gradually after heat treatment, and there was no difference in the expression between two groups. The expression levels of BCL-2 also showed no difference between two groups (Fig.4-b).

Discussion

HSP 70 is known to act as a molecular chaperon, which protects cellular protein from several apoptotic stimuli[5]. Several lines of evidence have suggested that HSP 70 is a potent anti-apoptotic protein. Park et al. reported that Hsp70-transfected melanoma cells shows significantly elevated levels of HSP70 and are highly resistant to ultraviolet B radiation[8]. Rong et al. reported that quercetin, a widely distributed plant flavonoid, induces apoptosis of U937 cells in a dose- and time-dependent manner and suppresses the expression of HSP 70[9]. Moreover, Nylandsted et al. reported that the effective and specific depletion of HSP70 by antisense HSP70 results in massive cell death of all tumorigenic cell lines tested, although this antisense HSP70 has no effect on the survival or growth of fetal fibroblasts or non-tumorigenic epithelial cells of breast[10]. These results suggest that the high expression of HSP70 is a prerequisite for the survival of human cancer cells. We also reported recently that HL60 cells treated with mild hyperthermia become resistant to otherwise lethal

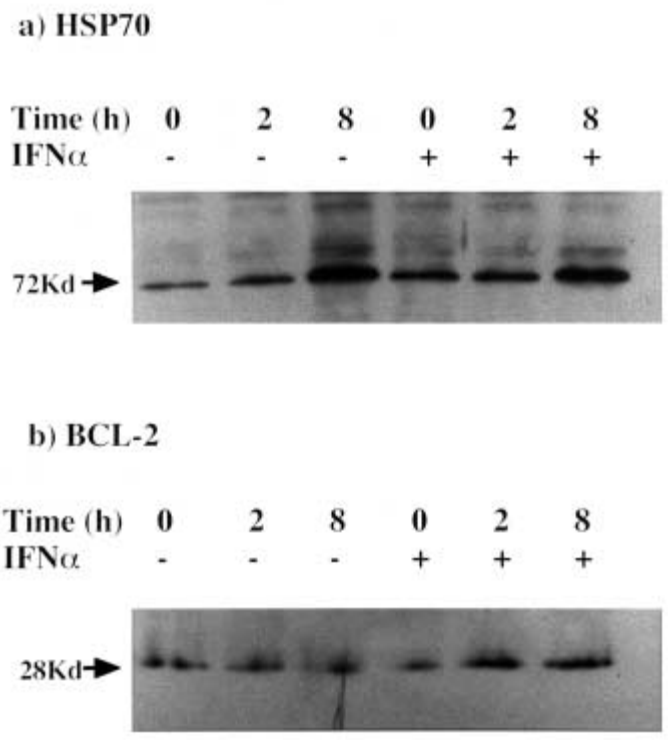


Figure 4
Effect of IFN α on the expression of HSP70 and BCL-2.
Cells were preincubated for 48 hours with or without 1000 U/ml of IFN-α. The cells were washed and exposed to 42℃ for 60 min. and then allowed to recover for the indicated periods at 37℃. Western blot analysis of HSP70 and BCL-2 was performed as described in "Materials and Methods". Results are representative of at least three independent experiments.

hyperthermia and anti-cancer agents, and that induction of HSP70 and BCL-2 play a major role for this resistance[7]. Our results are supported by a recent report that inhibition of the expression of HSP70 by blocking the heat shock transcription factor 1 function with its dominant-negative mutant sensitizes the thermotoletant breast cancer cell line to heat-induced apoptosis [11] .From these results, we postulated that IFN α might promote heat-induced apoptosis of myeloid leukemia cells by suppressing cellular expression of HSP70. The present study, however, showed that there was no difference in HSP70 expression between cells treated with or without IFN α, indicating no major role of HSP70 in the sensitization to heat-induced apoptosis by IFN α.

Varela et al. recently reported that IFN γ sensitizes U937 cells to death receptor-mediated apoptosis induced by tumor necrosis factor or agonistic CD95 antibody and that treatment of U937 cells with IFN γ up-regulates the expression of caspase-8 and down-regulates the expression of BCL-2. These results suggest that IFN γ sensitizes human leukemia cells to a death receptor-induced, mitochondria-mediated pathway of apoptosis[12]. In contrast, in our experiments, treatment of U937 cells with IFN α does not alter the expression of BCL-2.

Kim et al. reported that the expression of Fas and Fas ligand increases the apoptotic sensitivity against Mistletoe lectin-II, a potent apoptosis inducer in IFN γ-treated U937 cells[13]. We also observed the increased expression of Fas in IFN α-treated U937 cells by flow cytometry (data not shown), although it is not clear whether Fas/Fas ligand system is involved in sensitization of heat-induced apoptosis by IFN α.

Hyperthemia is a practical therapeutic modality for solid tumors[14], and is also a promising procedure for eliminating tumor cells which are present in the bone marrow harvested for autologous bone marrow transplantation[15]. Our present results suggest that appropriate combination of IFN α and heat might be an effective treatment for solid tumors as well as hematological malignancies.

In summary, the present results suggest that IFN α promotes heat-induced apoptosis in human myeloid leukemia cells through the mechanism whereby HSP70 or BCL-2 is not involved.

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