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Interferon-γ affects leukemia cell apoptosis through regulating Fas/FasL signaling pathway

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Abstract. – OBJECTIVE: Imbalance of hematopoietic cell proliferation and apoptosis is one of the major causes of leukemia. Enhanced cell proliferation and reduced apoptosis lead to hemocytes accumulation. Fas/FasL signaling pathway promotes cell apoptosis. This study investigated the impact of interferon γ (IFN-γ) on chronic myelogenous leukemia cell proliferation and apoptosis to elucidate its interaction with Fas/FasL signaling pathway.

PATIENTS AND METHODS: Leukemia cells were routinely cultivated and treated with 10 U/ml, 100 U/ml, and 1000 U/ml interferon for 12 h, 24 h, and 48 h, respectively. MTT assay was applied to test cell proliferation. TUNEL assay was adopted to determine cell apoptosis. Western blot was selected to detect Fas/FasL expression.

RESULTS: Different concentrations of IFN-γ inhibited cell proliferation at various time points. IFN-γ at 1000 U/ml treatment for 48 h had the strongest suppressive effect on cell proliferation ($p < 0.05$). IFN-γ intervention enhanced K562 cell apoptosis with concentration and time dependence ($p < 0.05$). Fas and FasL proteins expressions up-regulated after treated by IFN-γ following dose elevation and time extension ($p < 0.05$).

CONCLUSIONS: IFN-γ inhibits leukemia K562 cell proliferation and promotes cell apoptosis via down-regulating Fas and FasL proteins expression.

Word
γ, Leu FasL.

Introduction

The pathogenesis of leukemia is mainly caused by leukemia cells uncontrollable malignant proliferation from the bone marrow and other tissues. Leukemia may appear when they enter the peripheral blood[1]. Following the increase of inflammatory cells, leukemia keeps on progression and infiltration, suggesting that leukemia cells escape the immune surveillance[2]. It is found that Fas/FasL signaling pathway plays an important role in cell apoptosis and immune surveillance[3]. Fas/FasL signaling pathways participate in inducing cell apoptosis, which is an important component of the metabolic process that can effectively remove aged cells. Moreover, it can timely remove the abnormal cells and trigger series of immune reactions[4,5]. Interferon-γ (IFN-γ) involves in cell apoptosis process and can regulate the expression of apoptosis-related genes[6]. In chronic myelogenous leukemia, IFN-α upregulates dendritic cell costimulatory molecules and MHC antigen molecules expressions, thus has a certain stimulatory effect on T cell immune response[7]. However, the mechanism of IFN-γ on chronic myelogenous leukemia is still unclear. This study selected leukemia K562 cells and applied IFN-α for intervention, aiming to investigate the IFN-γ on chronic myelogenous leukemia cell proliferation and apoptosis, and elucidate its interaction with Fas/FasL signaling pathway. MTT assay was applied to test cell proliferation. TUNEL assay was adopted to determine cell apoptosis. Western blot was selected to detect Fas/FasL expression.

Patients and Methods

Experimental Cells

Leukemia K562 cells were provided by the Shanghai Institute of Hematology, China.

Reagents

IFN-γ was purchased from Sinopharm Chemical Reagent Co., Ltd (Lot No. 20050913) (Bei-

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jing, China). TUNEL assay kit, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), rabbit anti-mouse Fas and FasL polyclonal antibodies, and goat anti-rabbit secondary antibody were provided by Santa Cruz Biotechnology (Santa Cruz, CA, USA). Dulbecco's Modified Eagle Medium (DMEM) medium, penicillin-streptomycin, and fetal bovine serum were got from Gibco (Thermo Fisher Scientific, Waltham, MA, USA).

Routine Cell Culture

K562 cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium at 37°C and 5% CO_2.

IFN-γ Intervention

Cells in logarithmic phase were incubated in 2% FBS (fetal bovine serum) for 24 h and further cultured in DMEM containing 10% FBS. IFN-γ was added to treat the cells, while untreated K562 cells were selected as control.

MTT Assay

Different concentrations of IFN-γ (10 U/ml, 100 U/ml, and 1000 U/ml) were used to treat K562 cells for 12 h, 24 h, and 48 h. MTT at 5 mg/ml was adopted to incubate the cells for 4 h. The reaction was stopped by 150 µl DMSO (Dimethyl sulfoxide) and the plate was tested at 570 nm.

TUNEL Assay

The cells were treated with dimethylbenzene, gradient ethanol, and proteinase K sequentially. Next, the cells were incubated with TUNEL mixture, converter-POD, and DAB. At last, the cells were counted under microscope.

Western Blot

The cells in logarithmic phase were treated by IFN-γ. Total 50 µg protein was separated by electrophoresis and incubated in primary antibody (β-actin 1:1000) for 30 min. Next, the membrane was incubated in secondary antibody (1:2000) for 1 h and developed. The membrane was scanned and analyzed by Quantity One software.

Statistical Analysis

SPSS 17.0 software (SPSS Inc., Chicago, IL, USA) was selected for data analysis. Enumeration data was tested by chi-square test, measurement data was depicted as mean ± standard deviation and compared by ANOVA followed by Turkey's multiple comparison test. $p < 0.05$ was considered statistically significant.

Results

MTT Assay Detection of IFN-γ Impact on K562 Cell Proliferation

Different concentrations of IFN-γ were used to treat K562 cells for 12 h, 24 h, and 48 h. Different concentrations of IFN-γ inhibited cell proliferation at various time points. IFN-γ at 1000 U/ml at 48 h exhibited the strongest suppressive effect on cell proliferation ($p < 0.05$) (Table I).

TUNEL Assay Detection of IFN-γ Intervention on 562 Cell Apoptosis

TUNEL assay was used to detect K562 cell apoptosis treated by IFN-γ. IFN-γ intervention enhanced K562 cell apoptosis with concentration and time dependence ($p < 0.05$) (Table II, Figure 1).

Fas and FasL Proteins Expressions in K562 Cells Treated by IFN-γ

Western blot was selected to detect Fas and FasL proteins expressions in K562 cells treated by IFN-γ for 12 h, 24 h, and 48 h. Fas and FasL proteins expressions upregulated after treated by IFN-γ following dose elevation and time extension ($p < 0.05$) (Table III, Figure 2).

Table I. IFN-γ affects K562 cell proliferation ($x \pm s$, %).

Time	Experimental group			Control
	10 U/mL	100 U/mL	1000 U/mL	
12h	0.985 ± 0.072[1]	0.723 ± 0.046[1,2]	0.232 ± 0.024[1,2,3]	0.091 ± 0.023
24h	0.811 ± 0.045[1,4]	0.512 ± 0.034[1,2,4]	0.176 ± 0.016[1,2,3,4]	0.072 ± 0.012
48h	0.321 ± 0.031[1,4,5]	0.256 ± 0.026[1,2,4,5]	0.121 ± 0.011[1,2,3,4,5]	0.034 ± 0.016

[1]$p < 0.05$, compared with control. [2]$p < 0.05$, compared with 10 U/ml. [3]$p < 0.05$, compared with 100 U/ml. [4]$p < 0.05$, compared with 12 h. [5]$p < 0.05$, compared with 24 h.

Table II. IFN-γ affected K562 cell apoptosis ($x \pm s$, %).

Time	Experimental group			Co
	10 U/mL	100 U/mL	1000 U/mL	
12h	11.18 ± 1.02[1]	17.27 ± 1.44[1,2]	21.24 ± 2.45[1,2,3]	± 1.26
24h	15.21 ± 1.33[1,4]	18.48 ± 1.67[1,2,4]	24.37 ± 2.63[1,2,3,4]	.21 ± 1.43
48h	18.26 ± 1.54[1,4,5]	20.85 ± 2.12[1,2,4,5]	28.92 ± 2.77[1,2,3,4,5]	32 ± 1.39

[1]$p < 0.05$, compared with control. [2]$p < 0.05$, compared with 10 U/ml. [3]$p < 0.05$, compared with 100 U/ml. [4]$p <$ mpared with 12 h. [5]$p < 0.05$, compared with 24 h.

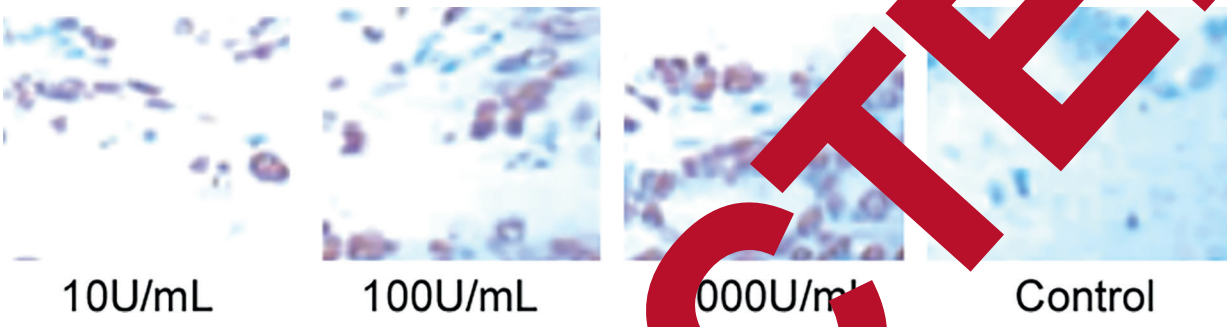

Figure 1. IFN-γ affected K ll apoptosi

Discussion

Apoptosis can remove the redundant, p differentiated, and aged cells that are difficu adapt to the state, thus to ma he norm physiological function[8]. Imb e hema- topoietic cells proliferati d apo sis may cause the occurrence kemi signaling pathway m ate s pla n important role in or oc ce and de- velopment. It w inted out t s did not show significa in most be tumors compared wi norma ue. However, it was reported[10] that Fas downregulated in malignant ss y be related to Fas downregulation or deletion, leading to Fas/FasL signaling pathway dysfunction. Malignant tumor cells may evade immune attack and reduce sensitivity to T lymphocytes by decreasing Fas expression on cell surface[11,12]. IFN-γ can suppress malignant tumor cell proliferation, elevate MHC antigen expression, and inhibit angiogenesis[13]. This study adopted IFN-γ to treat leukemia K562 cells to analyze its function on chronic granulocytic leukemia cell proliferation and apoptosis.

Table s and F L proteins exp sions in in K562 cells treated by IFN-γ ($x \pm s$, μmol/l).

Ite	Experimental group			Control
	/mL	100 U/mL	1000 U/mL	
	0.11 ± 0.21[1]	0.26 ± 0.18[1,2]	0.33 ± 0.11[1,2,3]	0.74 ± 0.37
	0.34 ± 0.15[1,4]	0.44 ± 0.13[1,2,4]	0.41 ± 0.03[1,2,3,4]	0.72 ± 0.38
	0.43 ± 0.13[1,5]	0.57 ± 0.09[1,2,5]	0.64 ± 0.01[1,2,3,4,5]	0.69 ± 0.41
asL				
12h	0.13 ± 0.21[1]	0.27 ± 0.181,2	0.39 ± 0.11[1,2,3]	0.10 ± 0.09
	0.34 ± 0.25[1,4]	0.42 ± 0.21[1,2,4,5]	0.54 ± 0.12[1,2,3,4]	0.10 ± 0.08
n	0.46 ± 0.39[1,4,5]	0.59 ± 0.24[1,2,4,5]	0.73 ± 0.17[1,2,3,4,5]	0.11 ± 0.07

[1]$p < 0.05$, compared with control. [2]$p < 0.05$, compared with 10 U/ml. [3]$p < 0.05$, compared with 100 U/ml. [4]$p < 0.05$, compared with 12 h. [5]$p < 0.05$, compared with 24 h.

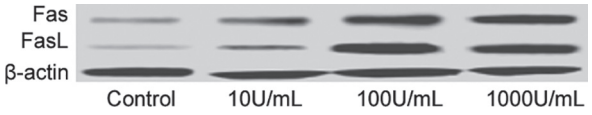

Figure 2. Fas and FasL proteins expressions in K562 cells treated by IFN-γ.

In this investigation, we used different concentrations of IFN-γ to treat K562 cells for 12 h, 24 h, and 48 h. It was found that different concentrations of IFN-γ inhibited cell proliferation at various time points. IFN-γ at 1000 U/ml treatment for 48 h exhibited the strongest suppressive effect on cell proliferation, indicating that IFN-γ can suppress K562 cell proliferation with dose-time dependence. It was reported[14] that IFN-γ increased CML-DCs costimulatory molecules and MHC antigen molecules expressions, and promoted T cell proliferation. IFN-γ can elevate the number of DC with normal function and has a certain corrective effect on DCs with function defect[15].

This study detected K562 cell apoptosis affected by IFN-γ. It was showed that IFN-γ intervention enhanced K562 cell apoptosis with [illegible] centration and time dependence, revealing IFN-γ can promote leukemia K562 cell apop[illegible]s. It was confirmed that Fas-mediated cell apop[illegible] was inhibited in malignant cells. Elevating [illegible] expression to enhance cell sens[illegible] Fas-mediated cell apoptosis is an [illegible]por[illegible] mechanism of IFN-γ to trigger [illegible]tosis. R[illegible]arches[16] demonstrated that IFN-γ [illegible]trigge[illegible] sion and increase cell [illegible]opt[illegible]

This study furth[illegible]opted [illegible]n blot to test Fas and FasL exp[illegible]ns in K562 [illegible]treated by IFN-γ. Fas and [illegible]teins expres[illegible]s upregulated after tr[illegible]ed by [illegible] following dose elevation an[illegible]e extensio[illegible]icating that IFN-γ can up[illegible]ate Fas and FasL [illegible]s in K562 cells. A p[illegible]us st[illegible][17] suggested that IFN-γ can en[illegible] Fas [illegible]ression in cholangiocarcinoma and g[illegible]ncer w[illegible]dose-time dependence. [illegible]erth[illegible] IFN-[illegible]n elevate FasL expression [illegible]ious [illegible]t tumors. Fas is a type of rec[illegible]r, whil[illegible]sL is a legend. Their binding m[illegible]e immune system attack, leading to [illegible]ap[illegible]. Under the function of IFN-γ, Fas/[illegible]sL signaling pathway can induce malignant [illegible]r cell apoptosis. FasL can bind with Fas to[illegible]se malignant tumor cell apoptosis[19,20]. It was proposed that IFN-γ induction may enhance the apoptosis of leukemia cells treated with cytarabine chemotherapy. For the chemotherapy drug targeting Fas/FasL, application of IFN-γ can produce a synergistic effect to enhance the therapeutic effect.

Conclusions

IFN-γ can suppress K562 cell pr[illegible]tion and induce cell apoptosis by ele[illegible]ng Fa[illegible]FasL proteins expressions. Fas[illegible]L signalin[illegible] way mediated cell ap[illegible]sis inv[illegible]es mu[illegible] cytokines with comp[illegible]ed me[illegible]ism. Further in-depth investigati[illegible] [illegible]ed in th[illegible]thogenesis, chemot[illegible]py re[illegible]ce, an[illegible]mune surveillance o[illegible]kemia. A [illegible]mor may be classifie[illegible]s/FasL leve[illegible]e future to further a[illegible]ze t[illegible]gnosis. Fas/FasL signaling pathway provide[illegible] thinking and strategy for t[illegible]ment of leu[illegible], which is of great si[illegible]cance for the early [illegible]agnosis and clinical [illegible]ment of leuk[illegible]ia.

Co[illegible]st

The Au[illegible]e that they have no conflict of interests.

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