Apoptosis (2007) 12:743–751
DOI 10.1007/s10495-006-0018-8

Caspase-3 antisense oligodeoxynucleotides inhibit apoptosis in γ-irradiated human leukemia HL-60 cells

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Published online: 12 January 2007

Abstract To study the inhibitory effects of caspase-3 mRNA antisense oligodeoxynucleotides (ASODNs) on apoptosis, we designed four ASODNs targeting different regions of caspase-3 mRNA and transfected them into human leukemia HL-60 cells. The transfected cells were given 10 Gy γ-irradiation followed by incubation for 18 h and measurement of apoptosis and caspase-3 expression. Our results showed that ASODN-2 targeting the 5′ non-coding region of sites –62 to –46, and ASODN-3 targeting the 5′ coding region of sites –1 to 16, both reduced apoptosis measured by gel electrophoresis and flow cytometry. Hoechst 33258 staining and TUNEL assay revealed that apoptotic indexes in the ASODN-2 and ASODN-3 groups were significantly lower than those in the untransfected and mismatched oligodeoxynucleotide (MODN) groups. Immunocytochemistry, Western blotting and RT-PCR showed that expression levels of caspase-3 protein and mRNA in both ASODN-2 and ASODN-3 groups were decreased compared with those in the untransfected and MODN groups. In conclusion, caspase-3 mRNA ASODNs can inhibit γ-radiation-induced apoptosis of HL-60 cells and reduce expression of caspase-3 protein and mRNA. The results suggest that antisense approach may be useful for therapeutic treatment of certain neurodegenerative diseases in which apoptosis is involved.

Keywords Caspase-3 · Antisense oligonucleotide · γ-Irradiation · Apoptosis · HL-60 cells

The work was supported by a grant from the National Natural Science Foundation of China (No. 39880008).

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Introduction

Apoptosis is known to be involved in various types of pathological phenomena [1]. A large variety of stimuli, including ionizing radiation have been shown to induce apoptosis [2]. Studies concerning the mechanisms of radiation-induced apoptosis have revealed that apoptotic signaling can be initiated in different cellular compartments [3–5]. DNA damage is the critical lesion caused by ionizing radiation and has been widely studied. Radiation induces DNA single-strand breaks, base damages, cross-links and double-strand breaks. Most of these lesions can be repaired, but double-strand breaks are not so easy to cope with and if not repaired, they can induce apoptosis [6].

It has been found that the p53 gene product plays a pivotal role in radiation-induced apoptosis [7, 8]. When cells are irradiated, p53 is activated and causes a delay in cell progression from G1 to S phase, allowing the damaged DNA to be repaired before replication and mitosis occur. If repair fails, however, p53 may trigger cell death through apoptosis by regulating expression of many apoptosis-related molecules, such as Bcl-2 family members (e.g. Bax and Bcl-2) [3], Fas [9, 10] and caspases [3]. On the other hand, p53-deficient cell lines, such as HL-60 cells, retain the ability to undergo radiation-induced apoptosis, suggesting that there are p53-independent pathways of apoptosis [8, 11, 12]. Hara et al. [13] have shown that p53-independent induction of

apoptosis following ionizing radiation exposure is mediated by sphingomyelinase/ceramide via the stress-activated protein kinase or *c*-Jun N-terminal kinase (SAPK/JNK) signaling pathway and involves caspase activation.

Although the upstream factors and regulators are versatile and not completely known, the different members of caspase family have been found to be involved in radiation-induced apoptosis of human promyelocytic leukemia HL-60 cells. We have previously cloned a caspase-3 gene fragment in γ-radiation-induced apoptotic HL-60 cells [11]. Zhuang et al. [14] found that caspase-8 and -3 were activated in ultraviolet light-induced apoptosis of HL-60 cells. Hosokawa et al. [15] showed that in radiation-induced HL-60 cells activity of caspase-9 and -3, but not caspase-8, was increased. Among these caspases, caspase-3, as a downstream "apoptosis executor", plays an important role in apoptosis, and thus was chosen as the target in the present study.

Aberrant apoptosis underlies some human disorders such as neurodegenerative diseases [16, 17]. Recent studies have shown that neuronal loss in the brain of Alzheimer disease (AD) [18] and Huntington disease (HD) patients [19] is related to apoptosis mediated by a caspase cascade, in which caspase-3 is abnormally activated. In some animal models of human diseases, peptidyl or nonpeptidyl small molecule inhibitors of caspases have been reported to protect neurons from apoptotic death [20, 21], suggesting that the inhibition of caspases is a therapeutic strategy for neurodegenerative diseases.

Antisense oligodeoxynucleotides (ASODNs) specifically inhibit translation of target mRNA [22]. ASODNs not only can be used as an experimental tool to study functions of specific gene products in a loss-of-function manner, but they also might be used as a therapeutic agent to treat disorders caused by overexpression of specific genes. Therefore, in the present study, we synthesized ASODNs targeting different regions of caspase-3 mRNA, transfected them into apoptosis model system of HL-60 cells induced by γ-irradiation, and then measured apoptosis and caspase-3 expression. Our aim is to investigate the inhibitory effect of these ASODNs on radiation-induced apoptosis and caspase-3 expression, and to identify the effective caspase-3 ASODNs for further studies and potential therapeutic use in apoptosis-based human disorders like neurodegenerative diseases.

Materials and methods

Design and synthesis of oligodeoxynucleotides

Four ASODNs targeting 5′ non-coding, initiative interpreting and coding regions of caspase-3 mRNA, and one mismatched oligodeoxynucleotide (MODN), were designed with the software including Oligo 6.4, Omiga 2.0, Vector NTI 5.5 and blast 2.1. All oligodeoxynucleotides were proved to be not homologous with other human genes by computer searches, and synthesized by Beijing Aoke Biology Co. with the 2 bases at both ends of each oligodeoxynucleotide being modified with sulfur phosphate (identified by asterisks). They are termed as ASODN-1, 5′-*C*TCTTCTACAACCGCC*T*-3′ (–137 to –121); ASODN-2, 5′-*G*GATTTCAAGGCGACG*C*-3′ (–62 to –46); ASODN-3, 5′-*T*TTCAGTGTTCTCCAT*G*-3′ (–1 to 16); ASODN-4, 5′-*C*GGCATACTGTTTCAG*C*-3′ (890 to 906); and MODN, 5′-*C*TCGCACTACGTTAGC*A*-3′.

Cell line and treatment

Human promyelocytic leukemia HL-60 cells were provided by the Bio-stomatology Laboratory of the Fourth Military Medical University (FMMU of China) and grown in RPMI1640 (Gibco) containing 10% neonatal bovine serum and maintained at 37°C in 5% CO_2. One ml of 1×10^6 cells were plated in the 24-well culture plate and transfected with ASODNs and MODN at different concentrations for 5 h. The transfection procedure was directed by the instructions provided in the lipofectamine transfection kit (Life Technologies). The cells were then irradiated with 10 Gy of γ-ray at the dose rate of 2 Gy/min. The ^{60}Co radiation source was performed in the Radiation Center of the FMMU. After being cultured for an additional 18 h, the cells were used in the following experiments.

DNA gel electrophoresis

HL-60 cells were transfected with each of four ASODNs at the final concentration of 1, 2, 3, 4 and 5 μM followed by γ-irradiation. Then 0.5 ml hypotonic lysis buffer (100 μg/ml proteinase K in 1 mM EDTA, 10 mM Tris, 100 mM NaCl, and 10% SDS) was added in each well of precipitated cells. After 30 min of reaction at 37°C, the supernatant was extracted with the mixture of hydroxybenzene: chloroform: isoamyl alcohol (25:24:1). DNA was deposited with dehydrated alcohol and sodium acetate, and then dissolved in TE. Electrophoresis was performed in 10% agarose gel (containing 0.5 μg/ml ethidium bromide) for 1 h, and bands were visualized and photographed under transmitted ultraviolet light. Three control groups were set in this experiment: (1) the blank group contained cells untransfected and unirradiated; (2) the untransfected group contained cells not transfected but irradiated; and (3) the MODN group contained cells transfected with MODN and irradiated.

Flow cytometry

HL-60 cells transfected with ASODN-2, ASODN-3 and MODN at final concentration of 3 μM were γ-irradiated with 10 Gy. After centrifugation cells were fixed with 70% ethanol at –20°C for 30 min. RNA was removed by adding RNase (100 μg/ml, Sigma) at 37°C for 2 h. Finally, the cells were stained with propidium iodide (100 μg/ml, Fluka) in phosphate buffered saline (PBS) containing 1%Triton X-100 in the dark for 30 min. DNA content was analyzed by flow cytometry. The percentage of cells with a sub-G1 DNA content was taken as a measure of the degree of apoptosis.

Hoechst 33258 fluorescent staining

HL-60 cells were divided into the following groups. (1) The experimental groups: cells were transfected with ASODN-2 and ASODN-3 at concentrations of 1, 3, 5, and 10 μM, followed by irradiation. Three parallel wells for each concentration were designed. (2) The control groups included the blank, untransfected and MODN groups. After γ-irradiation, cells were harvested and stained with Hoechst 33258 (1 μM in PBS, Sigma) at 37°C for 7 min. Then cells were examined with a fluorescence microscope (Nikon UFX-II) and those with nuclear condensation and fragmentation were taken as apoptotic cells. Apoptotic and total (about 400–500) cells were counted on four slides in each group. For each slide, three 400 $\times$ fields were selected randomly. The apoptotic index (AI) was then calculated according to the following formula: AI = (number of apoptotic cells/number of total cells) $\times$ 100%.

TUNEL assay

HL-60 cells were fixed in 4% paraformaldehyde for 15 min at 4°C. The terminal deoxynucleotide transferase (TdT)-mediated dUTP nick end labeling (TUNEL) assay was carried out according to the protocol of the TUNEL Kit (Boehringer Mannheim). Briefly, cells were incubated in the label buffer containing TdT and digoxigenin-labeled dUTP, followed by biotinylated antibody to digoxigenin and avidin-biotin-peroxidase complex (ABC, Vector). Apoptotic cells were visualized in a chromogen solution containing 3,3-diaminobenzidine (Sigma) and H_2O_2. Substitution of the label buffer for digoxigenin-dUTP was taken as the negative control. Cell counting and AI determination were the same as that mentioned above.

Immunocytochemistry

Immunocytochemical staining for caspase-3 was performed by using the ABC method. The smears of HL-60 cells were fixed with acetone and pretreated with 0.3% H_2O_2 and 3% normal goat serum, respectively. The cells were then incubated for 1 h at 37°C with rabbit anti-human caspase-3 antibody (1:1000, Santa Cruz), followed by incubation in biotinylated goat anti-rabbit IgG (1:100) and ABC (1:200) both for 20 min. 3,3-Diaminobenzidine-H_2O_2 was used in color development. Some slides were counterstained with hematoxylin. Substitution of normal rabbit serum for the caspase-3 antibody in the negative control completely eliminated the immunocytochemical staining. The cells stained brown were considered as caspase-3 positive cells. Caspase-3 positive and total (about 400–500) cells in each group were counted as in the section of Hoechst 33258 staining and the percentage of positive cells was calculated.

Western blotting

Cells were lysed in the cell lysis buffer (Beyotime) containing 1 mM phenylmethylsulfonyl fluoride. Then samples (150 μg protein/lane) were electrophoresed in SDS-PAGE, and transferred onto nitrocellulose membrane. The membrane was blocked with 5% bovine serum albumin and subsequently incubated overnight at 4°C with rabbit primary antibody (1:1000, Santa Cruz) recognizing human procaspase-3. Then the membrane was incubated with a horseradish peroxidase conjugated goat anti-rabbit IgG (1:2000, Santa Cruz) for 2 h. To verify equal loading of samples, the same membrane was incubated with monoclonal β-actin antibody, followed by a horseradish peroxidase conjugated goat anti-mouse IgG (Santa Cruz). Protein bands were visualized in a solution of 3,3-diaminobenzidine and H_2O_2. The optical density (OD) of each band was determined by using Image-Pro Plus 5.0 software, and the procaspase-3 expression was expressed as the ratio of OD of procaspase-3 and OD of β-actin.

One-step RT-PCR

Total RNA of HL-60 cells in each group was extracted by using the trizol reagent (Gibco). One-step RT-PCR was then performed according to the protocol of the kit (Takara). The program was setup as follows: reverse transcription at 50°C for 40 min, extinguishment of the reverse transcriptase at 94°C for 2 min, and followed by 30 (caspase-3) or 24 (β-actin) cycles of 94°C for 1 min, 57°C for 1 min, and 68°C for 1 min, and then extension at 72°C for 10 min. Five μl of the resulting product of each group was added to 1 μl of 6 $\times$ loading dye solution, and agarose gel electrophoresis was conducted under 75 V for 30 min. Quantity 4.4.0 software was used to analyze the OD of electrophoresis bands. To exclude variations due to RNA quantity and quality the data for caspase-3 was adjusted to β-actin expression (OD of caspase-3 mRNA *versus* OD of β-actin mRNA).

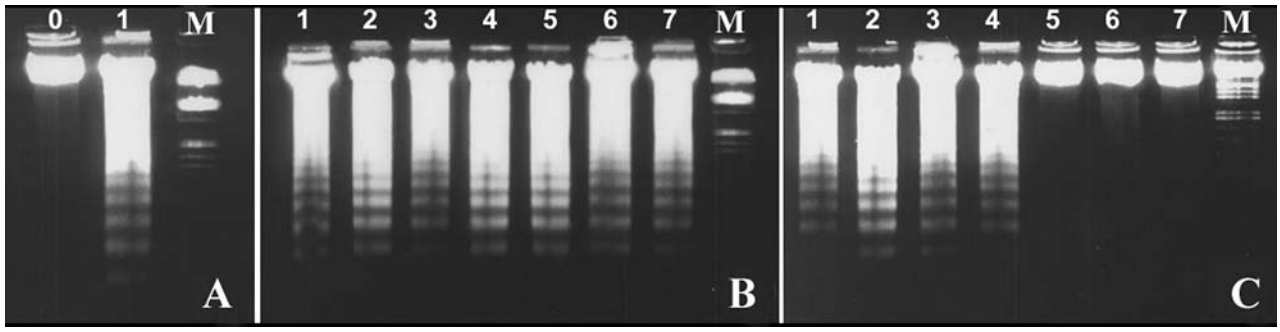

Fig. 1 Agarose gel electrophoresis showing DNA ladder formation. 0: Blank HL-60 cells, no DNA ladder; 1: γ-irradiated, untransfected HL-60 cells showing DNA ladder; M: DNA marker (A). 2: γ-irradiated HL-60 cells transfected with MODN showing DNA ladder; 3, 4, 5, 6 and 7: γ-irradiated HL-60 cells transfected with caspase-3 mRNA ASODN-1 (B) and ASODN-2 (C) at final concentrations of 1, 2, 3, 4 and 5 μM, respectively. No DNA ladder appeared in γ-irradiated cells transfected with ASODN-2 at 3, 4, and 5 μM

Statistical analysis

Data were expressed as mean $\pm$ SD and analyzed by analysis of variance (ANOVA) and Student's t test by using the statistical software SPSS 12.0. Difference was considered significant when $P < 0.05$.

Results

Detection of DNA ladder

The results of the DNA electrophoresis experiments are shown in Fig. 1. The DNA ladder was not detected in the blank group, whereas the typical DNA ladder was found in the untransfected group (Fig. 1(A)) and in the MODN, ASODN-1 and ASODN-4 groups at each transfection concentration and followed by 10 Gy γ-irradiation and further incubation for 18 h (Fig. 1(B)). The ASODN-2 and ASODN-3 groups, although irradiated, at the concentrations of ≥ 3 μM showed no DNA ladder (Fig. 1(C)).

Flow cytometry for apoptotic analysis

The results of flow cytometry are shown in Fig. 2. In the MODN group, 25.9% of the irradiated HL-60 cells were detected in the sub-G1 region. In the ASODN-2 ($\geq$ 3μM) and ASODN-3(≥ 3 μM) groups, however, only 4.3% and 7.2% of irradiated cells were in the sub-G1 region, respectively. These data indicate that caspase-3 ASODNs can inhibit γ-radiation-induced apoptosis of HL-60 cells.

Fluorescent staining of apoptotic cells: morphological assay

After Hoechst 33258 staining, the nuclei of viable HL-60 cells showed homogenous pale or bright blue fluores-

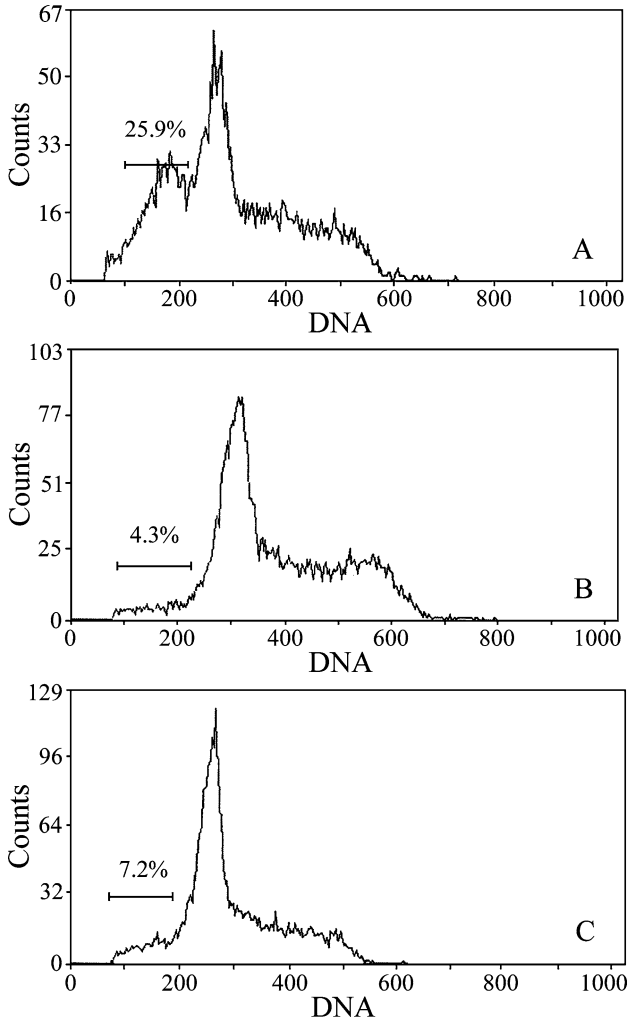

Fig. 2 Inhibitory effect of caspase-3 mRNA ASODNs on γ-irradiation-induced apoptosis of HL-60 cells analyzed with flow cytometry. Cell percentages in the sub-G1 region are indicated. (A) Cells transfected with MODN (3 μM), (B) cells transfected with ASODN-2 (3 μM), (C) cells transfected with ASODN-3 (3 μM)

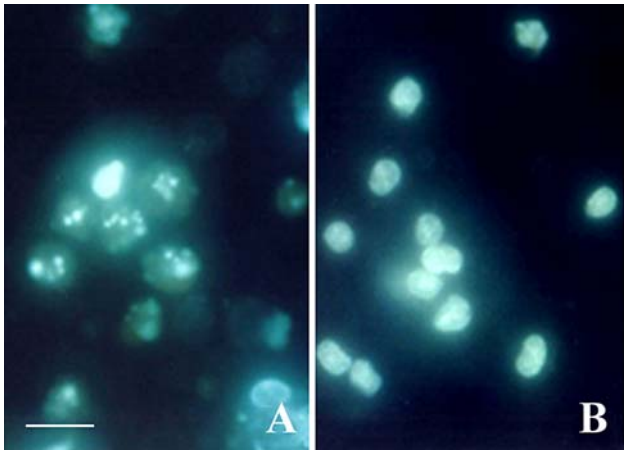

Fig. 3 Morphological changes of γ-irradiated HL-60 cells visualized by Hoechst 33258 staining. (A) Some of HL-60 cells transfected with MODN (5 μM) show intense blue fluorescence, and chromatin condensation and fragmentation of nuclei. (B) Most HL-60 cells transfected with caspase-3 mRNA ASODN-2 (5 μM) show nuclei with homogenous blue fluorescence (bar = 25 μm)

cence, whereas apoptotic cell nuclei exhibited enhanced fluorescence and chromatin condensation and fragmentation (Fig. 3(A)). Fluorescent staining analysis showed that there was no significant difference in the AI between the untransfected group ($17.01 \pm 13.72\%$ –$17.35 \pm 12.07\%$) and MODN group ($14.99 \pm 8.93\%$ –$17.35 \pm 12.15\%$) at each transfection concentration used. The AI in the ASODN-2 ($2.91 \pm 1.93\%$ –$5.38 \pm 2.12\%$) and ASODN-3 ($4.97 \pm 1.92\%$ –$6.94 \pm 2.45\%$) groups, when the transfection concentrations were ≥ 3 μM, was significantly reduced ($P < 0.01$) (Fig. 3(B)) compared with the untransfected and MODN groups.

TUNEL assay for *in situ* detection of apoptosis

The nuclei of HL-60 cells that underwent apoptosis were stained brown and appeared condensed or collapsed, whereas there was no nuclear staining in the negative control (Fig. 4). In the blank group, there were a few positive cells, with the AI being $1.13 \pm 1.02\%$. The results of the TUNEL experiments with radiation-exposed cells are presented in Table 1. The AI in untransfected and MODN groups were not significantly different ($P = 0.458$). Radiation alone increased the AI of HL-60 cells from $\sim 1.13\%$ to $\sim 18.5\%$. In the ASODN-2 and ASODN-3 groups, the AI showed a clear decrease with dose increases. When the final concentration of both ASODNs was ≥ 3 μM, the AI decrease was significantly different compared with the untransfected and MODN groups ($P < 0.01$). The results also demonstrated that ASODN-2 had a stronger effect than ASODN-3 in inhibiting the HL-60 cell apoptosis ($P < 0.05$) (Table 1).

Immunostaining for caspase-3 expression

Most HL-60 cells in the blank group were negatively stained for caspase-3. There were many caspase-3 positive cells with brown-stained cytoplasm in the untransfected and MODN groups. Statistical analysis revealed no significant difference in the percentage of positive cells between the two groups. However, the percentage was significantly decreased ($P < 0.01$) in both ASODN-2 and ASODN-3 groups compared with the untransfected and MODN groups (Figs. 5 and 6). When comparing the immunocytochemical and TUNEL results we found that the percentage of the caspase-3 positive cells was higher than the AI in all the untransfected, MODN, ASODN-2 and ASODN-3 groups ($P < 0.01$) (Fig. 6).

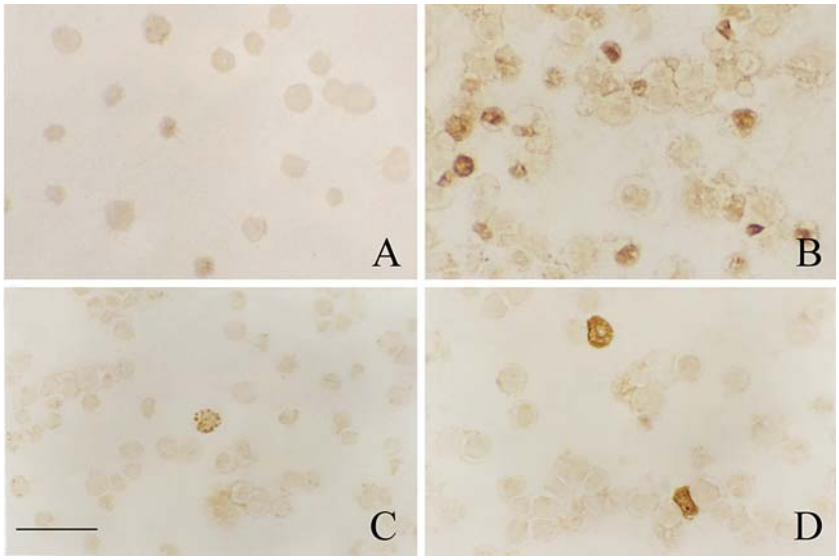

Fig. 4 TUNEL detection of apoptosis in γ-irradiated HL-60 cell cultures. In the negative control without use of digoxigenin-dUTP, there is no nuclear staining in the HL-60 cells (A). Many apoptotic cells with brown, condensed or collapsed nuclei are present in cultures transfected with the MODN (5 μM) (B). In cultures transfected with the caspase-3 mRNA ASODN-2 (5 μM) (C) and ASODN-3 (5 μM) (D), the apoptotic cells are obviously decreased in number (bar = 50 μm)

Table 1 Repressing effects of ASODNs on γ-radiation-induced HL-60 cell apoptosis detected by TUNEL

Group	Apoptotic index (%) (mean ± SD)				
	0 μM	1 μM	3 μM	5 μM	10 μM
MODN	18.74 ± 1.51	18.96 ± 7.67	18.49 ± 3.46	18.60 ± 2.88	18.34 ± 1.56
ASODN-2	18.50 ± 4.26	16.36 ± 2.10	8.66 ± 1.98*†	3.18 ± 1.74*†	1.86 ± 0.75*
ASODN-3	18.73 ± 2.73	16.47 ± 3.70	11.65 ± 2.84*	5.50 ± 1.53*	2.11 ± 1.03*

*$P < 0.01$, compared with the untransfected (0 μM) and MODN groups; †$P < 0.05$, compared with the caspase-3 mRNA ASODN-3 group.

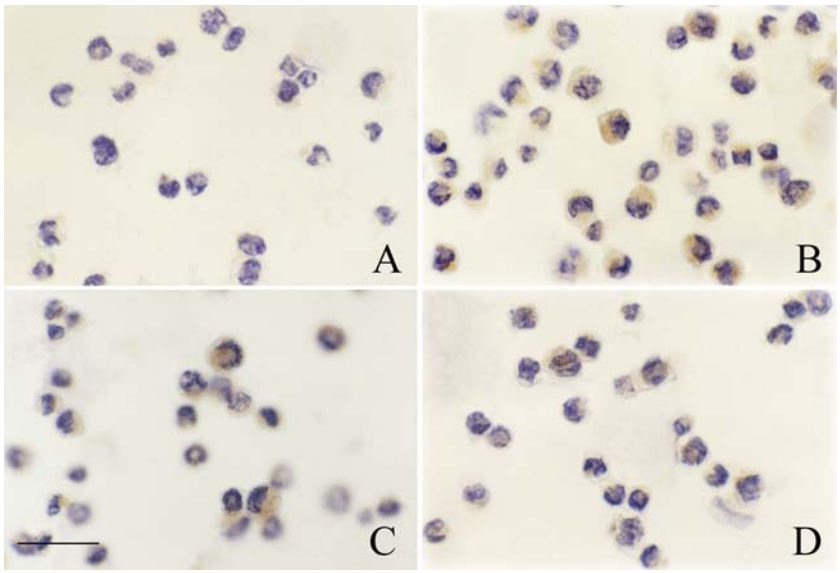

Fig. 5 Immunocytochemical staining of caspase-3 in γ-irradiated HL-60 cells. In the blank group (A) there is almost no expression of caspase-3. Many caspase-3 positive cells in the MODN (5 μM) group (B) show brown cytoplasm with a condensed, crescent or marginally located nucleus. In the caspase-3 mRNA ASODN-2 (5 μM) (C) and ASODN-3 (5 μM) (D) groups, the number of caspase-3 positive cells are obviously decreased (ABC method, counterstained with hematoxylin, bar = 50 μm)

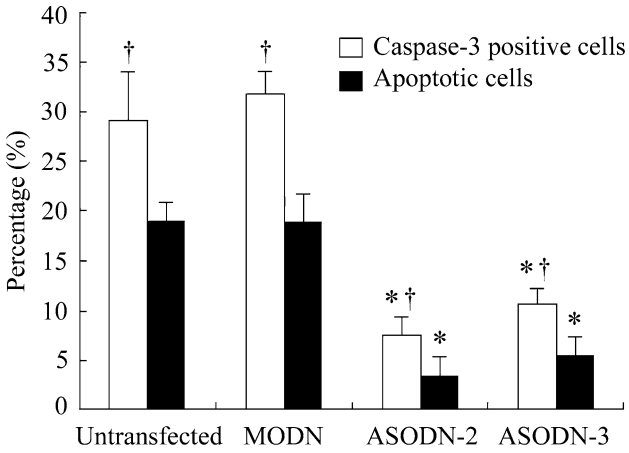

Fig. 6 Comparison of percentages of caspase-3 positive cells and apoptotic cells detected by immunocytochemistry and TUNEL, respectively, in γ-irradiated HL-60 cell cultures. Cells were untransfected or transfected with caspase-3 mRNA ASODN-2, ASODN-3 or MODN at the concentration of 5 μM. *$P < 0.01$, compared with the untransfected group and MODN group; †$P < 0.01$, compared with the percentages of apoptotic cells in the same groups

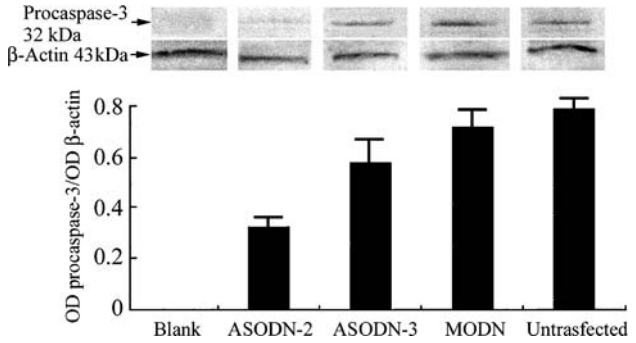

Fig. 7 Procaspase-3 expression in γ-irradiated HL-60 cells of different experimental groups. The upper part shows procaspase-3 and β-actin protein bands detected by Western blot. The lower part is the bar graph showing the relative expression levels of procaspase-3 protein, and each bar represents the average of three separate assays. The procaspase-3 expression is apparently decreased in cells transfected with the caspase-3 mRNA ASODN-2 and ASODN-3 compared with cells transfected with MODN and untransfected cells

Western blotting analysis for procaspase-3 expression

Procaspase-3 expression could not be found in the blank group. In other groups in which HL-60 cells were γ-irradiated, procaspase-3 was differently expressed (Fig. 7). Image analysis showed that the procaspase-3 expression levels (OD of procaspase-3 *vs*. OD of β-actin) in the untransfected and MODN groups were similar (0.7868 and 0.6578, respectively). The procaspase-3 expression was reduced by 58.85% in the ASODN-2 (0.3238) and by 26.64% in the

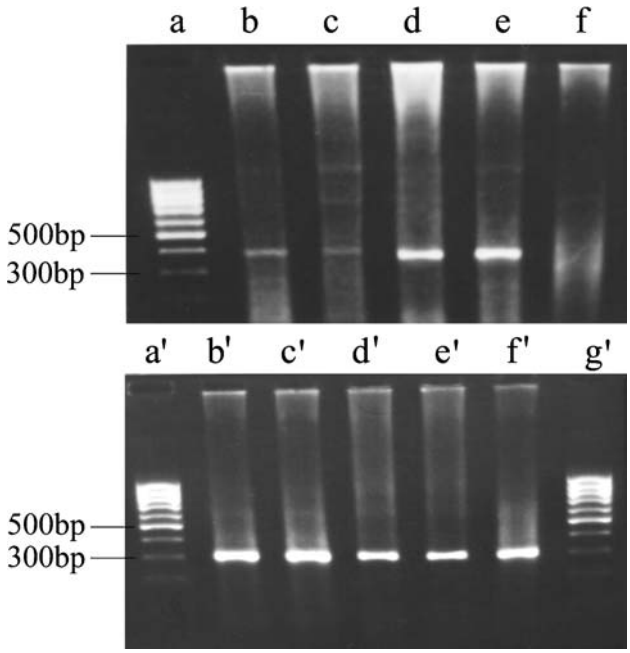

Fig. 8 RT-PCR amplification showing caspase-3 mRNA expression in γ-irradiated HL-60 cells of different experimental groups. Upper: caspase-3 mRNA; lower: β-actin mRNA. a, a′ and g′: DNA marker; b and b′: cells transfected with caspase-3 mRNA ASODN-3; c and c′: cells transfected with ASODN-2; d and d′: cells transfected with MODN; e and e′: untransfected cells; f and f ′: blank cells

ASODN-3 (0.5772) groups, respectively, compared with the untransfected group (Fig. 7).

RT-PCR for caspase-3 mRNA

RT-PCR showed no expression of caspase-3 mRNA in the blank group. In the untransfected and MODN groups, caspase-3 mRNA was strongly expressed with a 374 bp product. The expression of caspase-3 mRNA was apparently reduced after ASODNs transfection (Fig. 8). The adjusted expression of caspase-3 mRNA (OD of caspase-3 mRNA *vs*. OD of β-actin mRNA) was decreased by 73.16% in the ASODN-2 group (0.2294) and by 45.64% in the ASODN-3 group (0.4647), respectively, compared with the untransfected group (0.8548).

Discussion

In this study we used the antisense technique to examine the expression status of caspase-3 and its correlation to apoptosis in γ-irradiated human leukemia HL-60 cells. According to our results, the typical apoptosis and apparent caspase-3 expression were found in γ-irradiated HL-60 cells. The reason for these changes may lie in the re-synthesis (*de novo* synthesis) of caspase-3 mRNA after exposure to γ-radiation [23]. However, when cells were respectively transfected with caspase-3 mRNA ASODNs targeting the 5′ non-coding region of –62 to –46 and initial translation region of –1 to 16 at concentrations of ≥ 3 μM before γ-irradiation, both apoptosis and caspase-3 expression were significantly suppressed, the effect being ASODN sequence-specific and concentration-dependent. Our results also indicated that the caspase-3 mRNA ASODN targeting the 5′ non-coding region was more effective than that targeting the initial translation region. The reason for this difference in effectiveness may be that the ASODN targeting the 5′ non-coding region can inhibit information flow of translation, which starts from the 5′ non-coding region, and finally block the expression of caspase-3 protein [22].

The main biological effect of irradiation is the induction of DNA damage, and p53 protein has been regarded as the regulating center in the process of radiation-induced cell apoptosis [24–26]. Some reports concerning radiation-induced apoptosis, however, have revealed a p53-independent pathway, in which caspase-8 and -3 were activated in HL-60 cells [14] and caspase-8 in glioma cells [12]. Hara et al. [13] showed that in glioma U87-W E6 cells, which lost functional p53, radiation-induced apoptosis were associated with formation of ceramide by acid sphingomyelinase and activation of caspase-3. Hosokawa et al. [15] reported that radiation-induced HL-60 cell apoptosis is independent of caspase-8, but dependent on release of cytochrome *c* from mitochondria and activation of caspase-9 and -3. In our previous [11] and present studies we cloned the caspase-3 gene in γ-radiation-induced apoptotic HL-60 cells and found that caspase-3 mRNA ASODNs can inhibit the apoptosis. These results indicate that γ-radiation-induced apoptosis of HL-60 cells is a p53-independent and caspase-related process, where caspase-3 plays an important role as a final death executor.

Apoptosis dysregulation also contributes to many medical disorders for which adequate prevention or therapy is currently lacking [20]. Recent studies provide evidence that chronic neurodegenerative diseases are related to upregulation of apoptosis mediated by the caspase cascade [27, 28]. Matsui et al. reported that the caspase pathway from caspase-8 to caspase-3 and/or -7 may contribute to neuronal loss in the AD brain. Moreover, the amyloid precursor protein (APP) has been found to be a substrate for caspase-3 [29]. Microinjection of the amyloid beta peptide (Aβ), a fragment generated by proteolytic processing of APP, into the hippocampal region of mice resulted in significant neuronal loss in wild-type mice but not in caspase-3-deficient mice [30]. Disruption of the HD gene in mice causes increased neuronal apoptosis and behavioral and motor disorders reminiscent of human HD [31]. The Huntingtin protein also is a substrate of caspase-3, and neuronal death in HD is associated with the accumulation of Huntingtin fragments in the HD brain [32]. Cid et al. demonstrated that caspase-3 was activated

in neuronal apoptosis induced by cerebrospinal fluid from multiple sclerosis patients, and Ac-DEVD-cmk, an inhibitor of caspase-3 protected the neurons from apoptotic cell death and largely attenuated DNA fragmentation [33]. Because of the caspase-3 activation in these neurodegenerative diseases, caspase-3 inhibition by its ASODNs may be an alternative approach to the treatment of the disorders.

The principle of the antisense technique is that the sequence-specific binding of an ASODN to the target mRNA prevents the mRNA from being converted into a protein, and thereby blocks the action of the gene [34–36]. In studying the transfection function of a novel cationic liposome, Noguchi et al. [37] transfected the bcl-2 mRNA ASODN into Hela and NIH3T3 cells, and found that the bcl-2 ASODN was mainly present in the nuclei of two cell lines. The nuclear distribution may indicate that ASODNs not only inhibit the translation process, but also function as a DNA transcription repressor.

There is a potential role for antisense strategies in the treatment of human diseases. Masui et al. have used the Bcl-XL ASODN to enhance radiation-induced apoptosis of *in vitro* and *in vivo* pancreatic cancer, and found that the ASODN against Bcl-XL could be a good therapeutic tool for radiosensitization of pancreatic cancer [38]. In our study, we found that caspase-3 ASODNs could effectively inhibit radiation-induced caspase-3 expression and apoptosis of HL-60 cells. Since activation of caspase-3, a main final common executor of apoptosis has been found in most, if not all, chronic neurodegenerative diseases, our results may have the potentially important therapeutic use in the treatment of these diseases. However, the signaling pathways of apoptosis employed by different cell types responding to different stimuli may vary considerably. Therefore, our result of apoptosis inhibition via caspase-3 ASODNs in the radiation-induced HL-60 cell model needs further studies in neuronal cells both *in vitro* and *in vivo*.

In conclusion, our results provide the first evidence that caspase-3 mRNA ASODNs can decrease caspase-3 expression and inhibit apoptosis in γ-irradiated HL-60 cells. These findings may provide the experimental foundation for caspase-3 ASODNs being used as a therapeutic agent for aberrant apoptosis-related disorders, such as neurodegenerative diseases caused by upregulation of caspase-3.

Acknowledgments The work was supported by a grant from the National Natural Science Foundation of China (No. 39880008). We are most grateful to Prof. Dwight C. German (Department of psychiatry, University of Texas Southwestern Medical Center) for his suggestions and careful review of the manuscript.

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