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Apoptosis Induced by All-Trans Retinoic Acid in N-Acetylglucosaminyltransferase V Repressed Human Hepatocarcinoma Cells Is Mediated Through Endoplasmic Reticulum Stress

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Abstract We previously demonstrated that endoplasmic reticulum (ER) stress was triggered in human hepatocarcinoma 7721 cells transfected with antisense cDNA of N-acetylglucosaminyltransferase V (GnT-V-AS/7721) which were more susceptible to apoptosis induced by all-trans retinoic acid (ATRA). In the present study, we report that ATRA-induced apoptosis in GnT-V-AS/7721 cells is mediated through ER stress. We show here that ER stress is enhanced in GnT-V-AS/7721 cells with 80 μM ATRA treatment for 24 h, which is evidenced by the increase of GRP78/Bip, C/EBP-homologous protein-10 (CHOP, also known as GADD153) and spliced XBP1. Additionally, activation of caspase-12, caspase-9, and -3 was detected, and apoptosis morphology was observed in GnT-V-AS/7721 cells with ATRA treatment. These results suggest that ATRA enhances the ER stress triggered in GnT-V-AS/7721 cells, which represents a novel mechanism of ATRA to induce apoptosis. We further observed that GnT-V was significantly repressed and the structure of N-glycans was changed in GnT-V-AS/7721 cells with 80 μM ATRA treatment for 24 h, suggesting that repression of GnT-V by ATRA causes the enhanced ER stress and ER stress-mediated apoptosis in GnT-V-AS/7721 cells. J. Cell. Biochem. 100: 773–782, 2007.

Key words: apoptosis; all-trans retinoic acid; N-acetylglucosaminyltransferase V; endoplasmic reticulum stress

Endoplasmic reticulum (ER) is an important eukaryotic organelle. Quality control of proteins in cells is important for normal cellular function, and ER is considered as a site where this event occurs. Occasionally produced misfolded proteins will be retrotranslocated across ER membrane into the cytosol, where ubiquitin-conjugating enzymes target them for proteasomal degradation. This process is known as ER associated protein degradation (ERAD), and guarantees ER against accumulation of unfolded and misfolded proteins in the lumen. When ER functions are perturbed by stimuli, such as calcium homeostasis, or accumulation of mutant proteins, the ER stress is induced to counter the abnormal conditions [Kaufman, 1999; Friedlander et al., 2000; Patil and Walter, 2001]. The unfolded protein response (UPR) is an important adaptive response to ER stress, which includes transcriptional induction of UPR genes, translational attenuation of global protein synthesis, and ER-associated protein degradation. In mammals, UPR is triggered by the sensor proteins, such as ATF6 and IRE1. ATF6 translocates to nucleus as a transcription factor and activates transcription of UPR-target genes including GRP78/Bip and XBP1. XBP1 mRNA is induced by ATF6 and spliced by IRE1 [Yoshida et al., 2001], resulting in production of a highly active transcription factor that can activate the mammalian UPR. If these adaptive

Abbreviations used: ATRA, all-trans-retinoic acid; CHOP, C/EBP homologus protein; ConA, concannavalin A; DSA, Datura stramonium agglutinin; GAPDH, Dehydrogease; GnT-V, N-acetylglucosaminyltransferase V; HRP, horseradish peroxidase; HRP-ConA, HRP-conjugated ConA; HRP-DSA, HRP-conjugated DSA.

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responses are not sufficient to relieve the ER stress, the cell dies through apoptosis.

Apoptosis is primarily mediated by a family of caspases. It has been reported that activation of caspase-3, -9, and -12 is required for ER stress-induced cell death [Nakagawa et al., 2000; Song et al., 2002; Reimertz et al., 2003]. Several ER stress-induced apoptotic pathways have been characterized, including caspase-12-dependent apoptotic pathway [Nakagawa et al., 2000], ASK/JNK pathway [Urano et al., 2000] which induces cytochrome-*c* release from mitochondria as well as activation of caspase-9 and caspase-8 pathway [Jimbo et al., 2003] which also induces cytochrome-*c* release from mitochondria and activation of caspase-9. The predominant apoptotic pathway may differ among cell types. Moreover, C/EBP-homologous protein-10 (CHOP, also known as GADD153) [Wang et al., 1996], a unique transcription factor induced by ER stress, plays a critical role in facilitating ER stress-induced apoptosis.

We previously reported that ER stress was induced in human hepatocarcinoma 7721 cells transfected with antisense cDNA of N-acetylglucosaminyltransferase V (GnT-V-AS/7721) [Fang et al., 2006] and the GnT-V-AS/7721 cells were more susceptible to apoptosis induced by all-trans-retinoic acid (ATRA) [Guo et al., 1999]. In this study, we demonstrate that 80 μM ATRA enhances the ER stress triggered in GnT-V-AS/7721 cells and subsequently induces apoptosis, suggesting that ATRA-induced apoptosis in GnT-V-AS/7721 cells is mediated through ER stress. N-acetylglucosaminyltransferase V (GnT-V) is a key enzyme in the processing of asparagine-linked glycans (N-glycans) during the synthesis of glycoproteins. Downregulation of GnT-V may change the processing mode of N-linked oligosaccharides and lead to accumulation of unfolded and misfolded proteins in ER. We further observed the repression of GnT-V induced by ATRA, which may cause the enhanced ER stress and ER stress-mediated apoptosis in GnT-V-AS/7721 cells. These findings reveal a previously unrecognized mechanism of ATRA-induced apoptosis in human hepatocarcinoma cells.

MATERIALS AND METHODS

Reagents and Antibodies

ATRA, horseradish peroxidase (HRP), Datura stramonium agglutinin (DSA), and concannavalin A (ConA) were purchased from Sigma Co. (St. Louis, MO). Polyclonal anti-caspase-3 antibody (rabbit anti human), anti-caspase-9 antibody (rabbit anti human), and anti-caspase-12 antibody (rabbit anti human) were purchased from Cell Signaling Technology, Inc. (Beverly, MA). Polyclonal anti-CHOP antibody (rabbit anti human), anti-GRP78 antibody (goat anti human), anti-XBP1 antibody (rabbit anti human), and anti-GnT-V antibody (goat anti human) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

Cell Culture and Transfection

Human hepatocarcinoma 7721 cells (H7721) were obtained from the Institute of Cell and Biochemistry Research of Chinese Academy of Science. GnT-V-AS/7721 cell line was constructed by transfecting the expression plasmid of antisense GnT-V (pcDNA3/GnT-V-AS) into H7721 cells as described earlier [Guo et al., 1999]. The cells transfected with pcDNA3 plasmid as a control was named mock cells. GnT-V-AS/7721 and mock cells were cultured in RPMI-1640 medium (Life Technologies, Inc., GIBCO BRL, Gaithersburg, MA) supplemented with 10% new born bovine serum (Life Technologies, Inc.) and 1% penicillin and streptomycin at 37°C, in a humidified atmosphere of 5% CO_2.

ATRA Treatment

GnT-V-AS/7721 and mock cells were treated with ATRA. The concentration of ATRA in the culture medium was 80 μM, and the cells were harvested after 24 h [Guo et al., 1999].

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Total RNAs (5 μg) extracted from mock and GnT-V-AS/7721 cells with Trizol reagent (Life Technologies, Inc.) according to the manufacture's instructions were used as templates for cDNA synthesis. Reverse transcription was carried out by M-MuLV (Promega Co., Madison, WI) and then cDNAs were subjected to PCR. Primers used for PCR were as follows: GRP78/Bip forward primer 5′-CTGGGTACATTTGATCTGACTGG-3′, and reverse primer 5′-GCATCCTGGTGGCTTTCCAGCCATTC-3′ [Siman et al., 2001]; XBP-1 forward primer 5′-CCTTGTGTAGTTGAGAACCAGG-3′, and reverse primer 5′-GGGGCTTGGTATATAT-

GTGG-3′ [Yoshida et al., 2001]; β-actin forward primer 5′-TGGGCATGGGTCAGAAGGAT-3′, and reverse primer 5′-AAGCATTTGCGGTG-GACGAT -3′. The PCR products were electrophoresed on agarose gel, visualized by ethidium bromide (EB) staining on an image system.

Western Blotting Analysis

Cells were grown to confluence on 80%, rinsed twice in ice-cold Phosphate-Buffer saline (PBS, containing 8 g of NaCl, 0.2 g of KCl, 1.15 g of $Na_2HPO_4 \cdot 12\ H_2O$ and 0.2 g of KH_2PO_4 in 1,000 ml of H_2O) followed by lysis in $1 \times$ SDS buffer with 1% pheylmethylsulfonyl fluoride (PMSF). Proteins were applied to SDS–PAGE. After electrophoresis, proteins were blotted to poly (vinylidene fluoride) (PVDF) membranes and then blocked with 5% skim milk powder with 0.1% Tween-20. Subsequently, membranes were incubated with polyclonal anti-GnT-V antibody (1:500), caspase-9 (1:1,000), caspase-12 (1:1,000), caspase-3 (1:1,000), CHOP (1:500), and GAPDH (1:1,500) respectively. Incubated at 4°C overnight, rinsed by PBST (PBS containing 0.1% Tween-20) three times, the PVDF membranes were incubated with HRP-conjugated bovine anti-goat IgG or bovine anti-rabbit IgG (1:1,000) respectively at room temperature for 2 h. The signal was revealed using the enhanced chemiluminescence (ECL) regents.

Analysis of Nuclear Morphology by Fluorescence Staining

After treatment of 80 μM ATRA at 37°C for 24 h, the cells grown on the cover glass were fixed with methanol/glacial acetic acid (3:1) at 4°C for 5 min. After washing with triplicate distilled water, the cells were stained with Hoechest 33258 at room temperature for 10 min. Then the cover glasses were observed under Nikon phase microscope with fluorescence attachment after fixed with citrate buffer (20 mM citrate, 50 mM monosodium phosphate, 50% glycerol, pH 5.5). Apoptotic cells were counted among 1,000 cells randomly. The apoptotic index was the mean of three independent experiments.

Glycoproteins Staining With HRP-Lectin Complexes

Cells were collected and lysed in HEPES containing 2% Triton X-100, 10 μg/ml Leupeptin, 10 μg/ml Pepstatin, 1 mM PMSF. Proteins (80 μg) from each sample were added 5 μU neuraminidase (Sigma Chemical Co.) and incubated at 37°C for 1 h before electrophoresed to remove terminal sialic acid residues. Lysising in $2 \times$ SDS buffer, the samples were electrophoresed by 8% SDS–PAGE and then transferred to PVDF membrane. After blocking with PBS containing 5% BSA overnight, the membrane was incubated with HRP-conjugated ConA (HRP-ConA) or HRP-conjugated DSA (HRP-DSA) for 2 h at room temperature. Following complete washes protein bands were detected with the ECL reagents.

RESULTS

ER Stress Was Enhanced in GnT-V-AS/7721 Cells With ATRA Treatment

GRP78/Bip is the key chaperone for folding and maturation of proteins in ER and its upregulation is the usual marker of ER stress. As shown in Figure 1A,B, GRP78/Bip was overexpressed at both transcriptional and translational levels in GnT-V-AS/7721 cells compared with the mock cells. After 80 μM ATRA treatment for 24 h, GRP78/Bip mRNA and protein in GnT-V-AS/7721 cells were increased markedly.

XBP1 is an important transcription factor during ER stress. Activated IRE1 mediates frame-switching splicing of the XBP1, which results in the formation of a potent tans-activator that upregulates its own expression and that of molecular chaperons [Yoshida et al., 2001]. To evaluate the possible role of ATRA in ER stress, the XBP1 was examined by RT-PCR with primers that permit the detection of both spliced and unspliced (intron-containing) mRNAs. These two bands with 26 bp difference as expected were 442 bp and 416 bp respectively, representing the unspliced and spliced mRNA of XBP1 ($XBP1^U$ and $XBP1^S$). They were observed both in GnT-V-AS/7721 cells treated with ATRA and those untreated (Fig. 1C). However, more spliced XBP1 mRNA was observed in GnT-V-AS/7721 cells treated with ATRA than that in untreated cells (Fig. 1C). XBP1 mRNA contains open reading frame 1 (ORF1) encoding 261 aa. In addition, the mRNA contain another ORF (ORF2), partially overlapping but not in frame with ORF1, encoding proteins of 222 aa. Splicing of XBP1 mRNA is

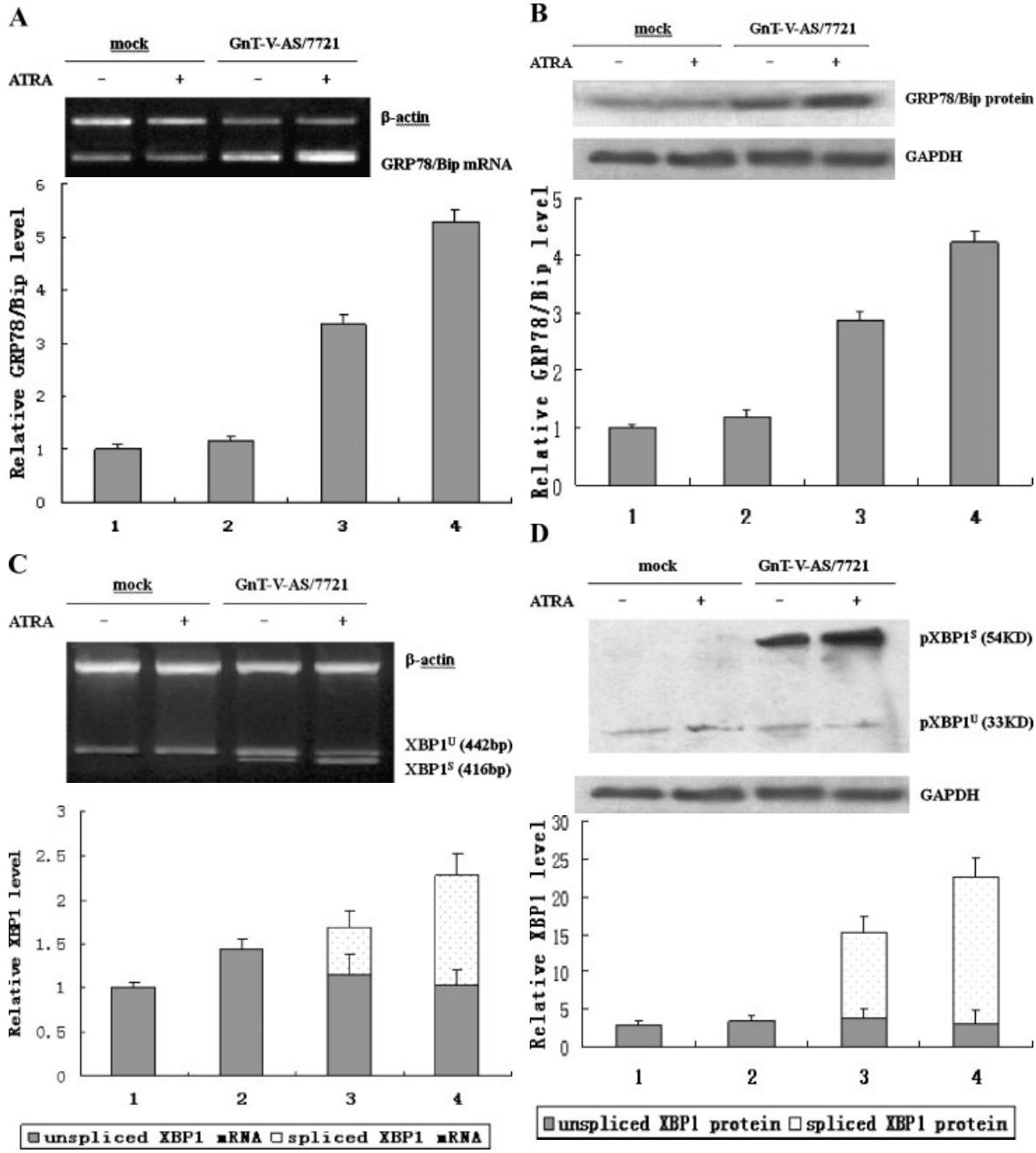

Fig. 1. ATRA enhanced the ER stress triggered in GnT-V-AS/7721 cells. GnT-V-AS/7721 and mock cells were maintained under basal conditions or were treated with ATRA (80 μM) for 24 h. Total RNAs from these cells were analyzed by RT-PCR as described in the Materials and Methods for GRP78/Bip and XBP1. β-actin was used as loading control. The relative levels of mRNAs were determined by quantification of the RT-PCR bands using densitometry and were normalized against the level of β-actin. The standard deviations are shown. Total proteins were analyzed by Western blot as described in the Materials and Methods for GRP78/Bip, XBP1, and CHOP. GAPDH was used as loading control. The relative levels of proteins were determined by quantification of the Western blot bands using densitometry and were normalized against the level of GAPDH. The standard deviations are shown. 1: mock, 2: mock treated with 80 μM ATRA for 24 h, 3: GnT-V-AS/7721, 4: GnT-V-AS/7721 treated with 80 μM ATRA for 24 h. **A**: ATRA upregulated GRP78/Bip mRNA in GnT-V-AS/7721 cells. **B**: ATRA upregulated GRP78/Bip protein in GnT-V-AS/7721 cells. **C**: ATRA induced the splicing of XBP1 mRNA in GnT-V-AS/7721 cells. Amplified products from RT-PCR were subjected to 2% agarose gel electrophoresis. $XBP1^U$: unspliced XBP1 mRNA; $XBP1^S$: spliced XBP1 mRNA. **D**: Spliced XBP1 protein was induced by ATRA. $pXBP1^U$: unspliced XBP1 protein; $pXBP1^S$: spliced XBP1 protein. **E**: ATRA upregulated CHOP in GnT-V-AS/7721 cells.

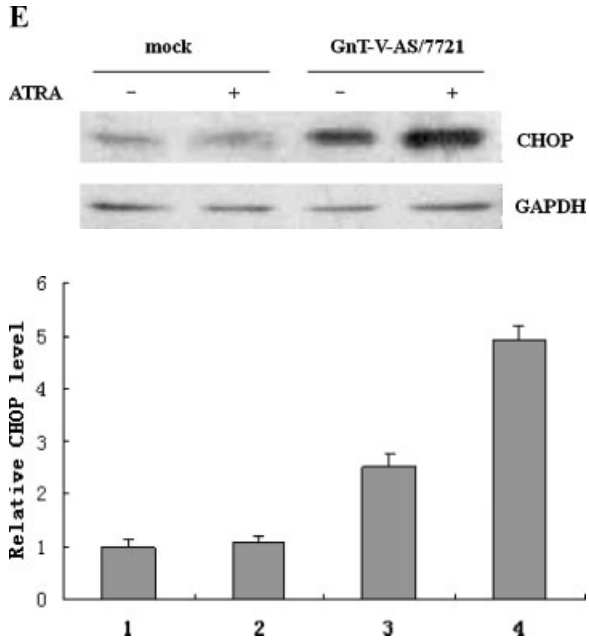

Fig. 1. (*Continued*)

predicted to cause removal of the C-terminal 97 aa from ORF1 and addition of the 212 aa of ORF2 to the N-terminal 164 aa of ORF1, as splicing out of the 26 nt results in a frame shift at aa165. Thus, unspliced and spliced XBP1 mRNA should encode proteins of 261 and 376 aa, respectively. As shown in Figure 1D, the proteins, translated from the $XBP1^S$ and $XBP1^U$, were visualized as the bands of 54 kDa $pXBP1^S$ and 33 kDa $pXBP1^U$ respectively. In accordance to the results of RT-PCR, the Western blot results indicated that more 54 kDa $pXBP1^S$ was expressed in GnT-V-AS/7721 cells treated with ATRA than that in untreated cells (Fig. 1D).

To further verify that ATRA contributes to ER stress in GnT-V-AS/7721 cells, we analyzed the translational level of CHOP, which has been implicated in mediating apoptosis during ER stress and is known to be regulated by PERK/eIF2α. The expression level of CHOP is low under non-stressed conditions, but is markedly increased in response to ER stress. As shown in Figure 1E, the translational level of CHOP in GnT-V-AS/7721 cells treated with ATRA was significantly higher than that in the GnT-V-AS/7721 cells untreated.

Furthermore, there was no difference of GRP78/Bip, XBP1, and CHOP between mock cells treated with ATRA and those untreated (Fig. 1A–E). This indicates that ER stress was not triggered in mock cells with ATRA treatment.

Activation of Caspase-12, -9, and -3 in GnT-V-AS/7721 Cells Treated With ATRA

As the ATRA enhanced ER stress in GnT-V-AS/7721 cells, we examined the activation of caspases in GnT-V-AS/7721 cells with or without ATRA treatment. Focusing on upstream caspases, we detected that caspase-12, residing normally within the ER, became cleaved after ATRA treatment for 24 h in GnT-V-AS/7721 cells. As seen in Figure 2, activation of caspase-12 was shown by the appearance of the cleaved fragment, and this was accordingly accompanied by a reduction in procaspase-12 level. These data indicate that the processing of caspase-12 appeared after ATRA treatment in GnT-V-AS/7721 cells. In addition to caspase-12 activation, ER stress stimulated caspase-9 and caspase-3 activation in GnT-V-AS/7721 cells as well. As shown in Figure 2, the proforms of caspase-9 was slightly decreased and the processed fragment appeared. Similarly, as an effective caspase in the cell apoptosis downstream pathway, caspase-3 was also activated and the processed fragment was detected. However, caspase-12, -9, and -3 were not activated in mock cells with ATRA treatment and GnT-V-AS/7721 cells untreated.

Apoptotic Morphology Was Observed in ATRA-Treated Cells

The nuclear morphology of mock and GnT-V-AS/7721 cells after 24 h induction of ATRA was observed after fluorescent staining with Hoechest 33258. The bright blue fluorescent condensed nuclei and the nuclear fragments were very apparent in the GnT-V-AS/7721 cells with ATRA treatment, while there were very few in other cells (Fig. 3).

GnT-V Was Repressed by ATRA

To observe the effect of ATRA on the expression of GnT-V, Western blot was used. The expression of GnT-V at translational level, slightly decreased in mock cells with ATRA treatment, was markedly downregulated in GnT-V-AS/7721 cells with ATRA treatment (Fig. 4).

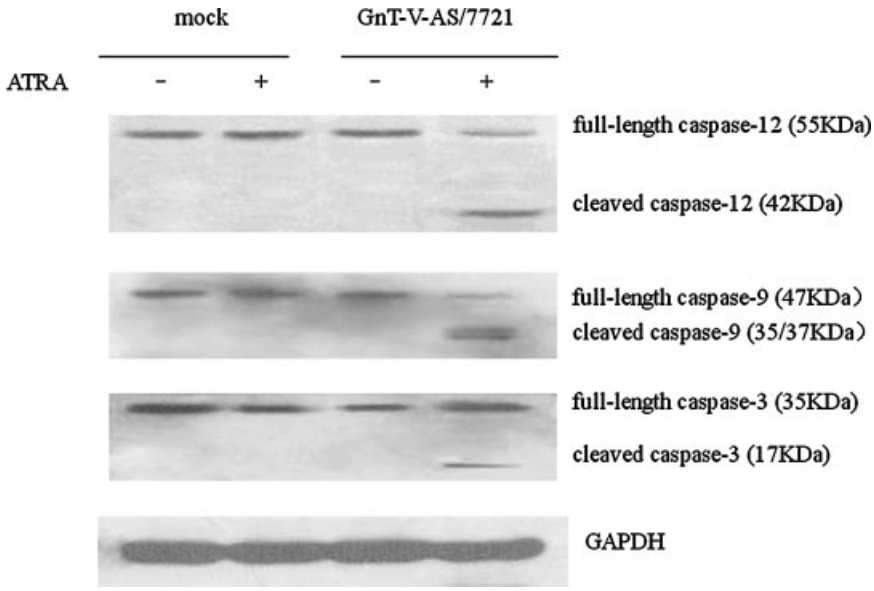

Fig. 2. Activation of caspase-12, -9, and -3 in GnT-V-AS/7721 cells. **A**: GnT-V-AS/7721 and mock cells were maintained under basal conditions or were treated with ATRA (80 μM) for 24 h and then total proteins from these cells were analyzed by Western blot for caspase-12, -9, and -3. GAPDH was used as loading control.

N-Glycans Structure of Glycoproteins Was Changed by ATRA Treatment

The GnT-V catalyzes the addition of β-1,6-GlcNAc to N-glycan intermediates found on newly synthesized glycoproteins transiting the medial Golgi. Glycosylation of glycoproteins with N-acetylglucosamine (GlcNAc) is a dynamic protein modification of glycoproteins. We proposed that reduced expression of GnT-V might alter the structures of N-glycans. To verify that proposition, glycoproteins from different cells were stained using DSA and ConA labeled by HRP. It is well known that DSA binds to tri- and tetra-antenary glycans, especially those containing β1, 6 GlcNAc branches, while ConA binds to biantannary glycans without β1, 6 GlcNAc and bisecting branches. Our results showed the intensity of HRP-DSA staining was decreased, while the intensity of HRP-ConA staining was increased in GnT-V repressed cells (Fig. 5). This suggested that the β-1,6-GlcNAc branches on glycoproteins were decreased and the biantennary N-glycans without the structures of β-1,6-GlcNAc and bisecting GlcNAc were increased when GnT-V was repressed. The significant changes in GnT-V-AS/7721 cells with ATRA treatment indicate ATRA alters the structure of N-glycans on glycoproteins in GnT-V-AS/7721 cells.

DISCUSSION

In comparison of our previous study [Fang et al., 2006], we demonstrated that ER stress was triggered in GnT-V-AS/7721 cells, which was evidenced by the activation and upregulation of GRP78/Bip, CHOP, and XBP1. However, caspase-12, -9, and -3 were not activated, indicating that the ER stress in GnT-V-AS/7721 cells was chronic and there were no signals of apoptosis. After 80 μM ATRA treatment for 24 h, GRP78/Bip, spliced XBP1, and CHOP were increased in GnT-V-AS/7721 cells, suggesting that ATRA enhances the ER stress triggered in GnT-V-AS/7721 cells. In addition to the enhanced ER stress, we found that GnT-V-AS/7721 cells treated with ATRA presented apoptosis phenomenon, which was verified in previous study [Guo et al., 1999]. Here our findings suggest that ATRA-induced apoptosis in GnT-V-AS/7721 cells is mediated through ER stress.

Caspases are important enzymes for apoptosis. In this study, we detected caspase-12, as the upstream caspase member considered to be concerned with apoptosis mediated by ER stress, was cleaved in GnT-V-AS/7721 cells with ATRA treatment. Caspase-9 and caspase-3, downstream caspase members, were also activated. Caspase-9 and -3 are considered as key caspases during mitochodria-dependent apop-

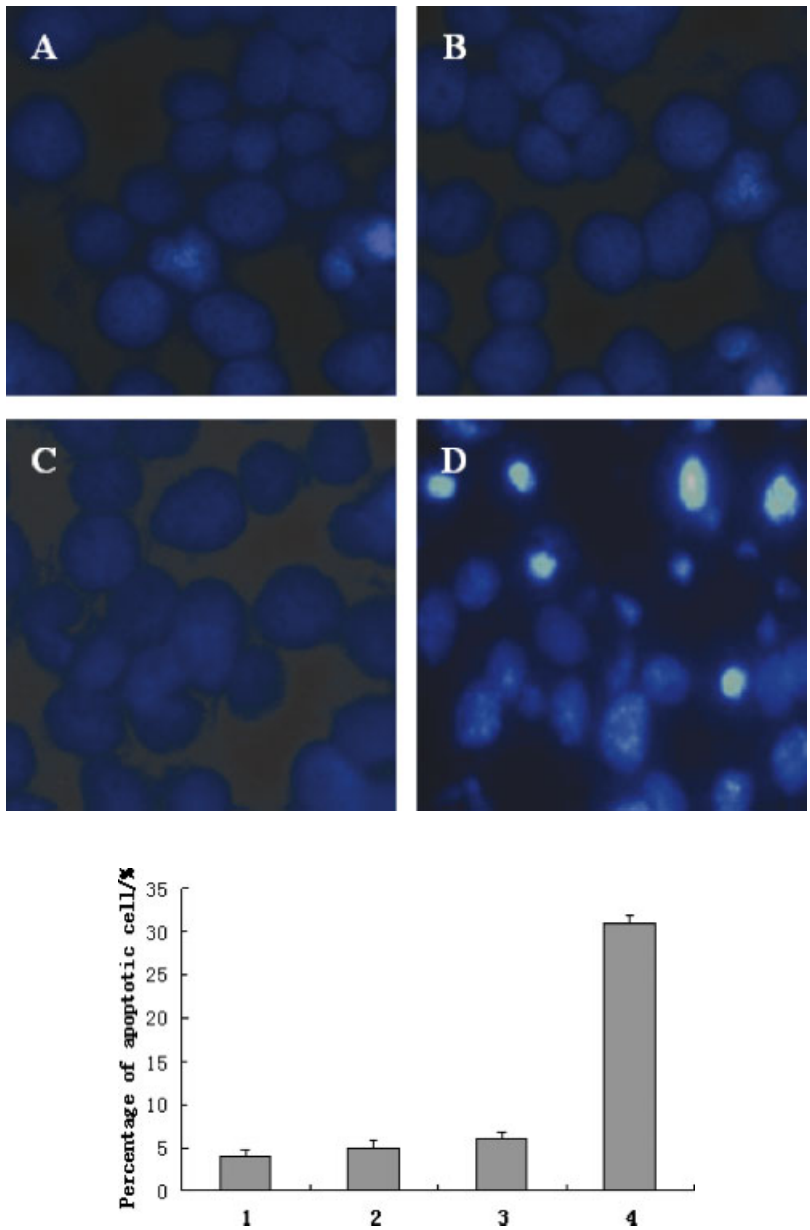

Fig. 3. Fluorescent microscope observation of ATRA-induced apoptosis of GnT-V-AS/7721 cells. Cells were cultured under basal conditions or treated with 80 μM ATRA for 24 h. After the incubation period, cells were washed and stained with Hoechst 33258, and examined under a fluorescence microscope. Apoptotic cells were counted among 1,000 cells randomly. The apoptotic index was the mean of three independent experiments. The standard deviations are shown. **A**: mock, (**B**) mock treated with 80 μM ATRA for 24 h, (**C**) GnT-V-AS/7721, (**D**) GnT-V-AS/7721 treated with 80 μM ATRA for 24 h. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

tosis. But Jayanthi et al. [2004] reported possible cross-talks between endoplasmic reticulum and mitochodria-dependent death cascades in neuronal apoptosis, suggesting that effector caspase-3 might be cleaved by caspase-12. It has also been reported that activation of caspase-3, -9, and -12 plays a central role in ER stress-induced cell death [Nakagawa et al., 2000; Song et al., 2002; Reimertz et al., 2003]. In this study, we further observed CHOP, a transcriptional factor involved in ER stress-induced apoptosis, was increased markedly in GnT-V-AS/7721 cells after ATRA treatment. CHOP was first reported as a molecule involved

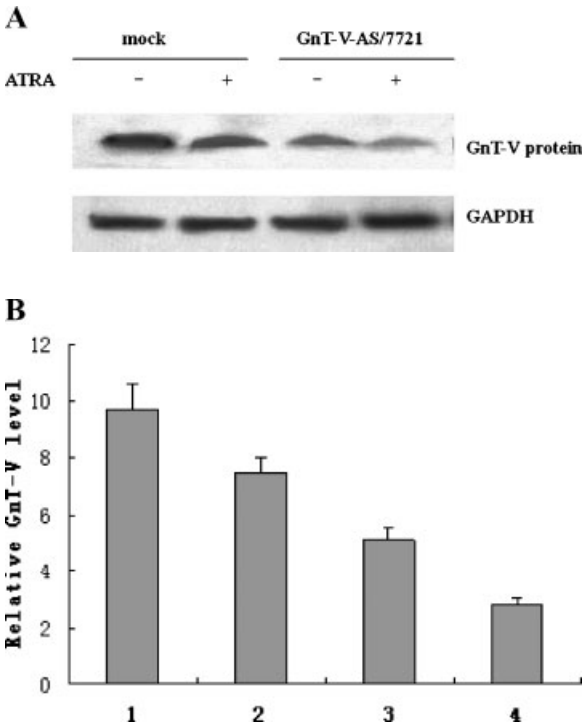

Fig. 4. GnT-V was repressed by ATRA. **A**: GnT-V-AS/7721 and mock cells were maintained under basal conditions or were treated with ATRA (80 μM) for 24 h and then total proteins from these cells were analyzed by Western blot for GnT-V and GAPDH. GAPDH was used as loading control. **B**: Quantification of relative GnT-V protein expression in different cells. The relative amount of GnT-V protein level was normalized against GAPDH. The standard deviations are shown.1: mock, 2: mock treated with 80 μM ATRA for 24 h, 3: GnT-V-AS/7721, 4: GnT-V-AS/7721 treated with 80 μM ATRA for 24 h.

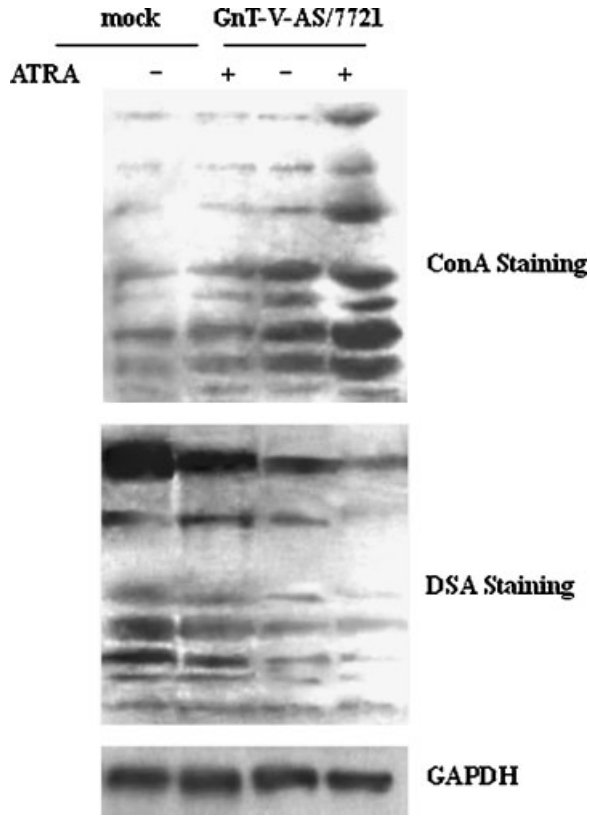

Fig. 5. N-glycans structure of glycoproteins was changed using the staining of HRP-labeled DSA and ConA. GnT-V-AS/7721 and mock cells were maintained under basal conditions or were treated with ATRA (80 μM) for 24 h. Total proteins from cells were stained with HRP-ConA and HRP-DSA respectively. GAPDH was used as loading control.

in ER stress-induced apoptosis [Wang et al., 1996]. It was reported that overexpression of CHOP promoted apoptosis [Friedman, 1996], and deficiency of CHOP could protect cells from ER stress-induced apoptosis [Zinszner et al., 1998; Oyadomari et al., 2002]. Caspase-12 and CHOP are ER-specific signals during apoptotic pathways, while caspase-9 and -3 involve in ER stress-mediated apoptosis. The activation of caspase-12, -9, -3, and CHOP agrees with the hypothesis that the apoptosis in GnT-V-AS/7721 cells induced by ATRA is mediated through ER stress.

It was reported that retinoic acid changed the structure of N-glycans on the cell surface and the enzymatic mechanisms involved the decrease of GnT-V [Chen et al., 1995]. In previous report, ATRA was used as an inhibitor of GnT-V to alter the N-glycans of cell surface [Zhang et al., 2004]. In this study, downregulation of GnT-V was observed in both mock and GnT-V-AS/7721 cells with ATRA treatment, indicating that ATRA at a concentration of 80 μM may exert a permissive function for downregulation of GnT-V. GnT-V, an important cancer-associated enzyme, is located in trans-Golgi apparatus and like other GlcNAc transferases, it regulates the branch formation in complex-type N-linked oligosaccharides. We previously reported that blocking of GnT-V in GnT-V-AS/7721 cells altered the processing mode of N-linked oligosaccharides and produced deficient glycoproteins, which caused the ER stress in GnT-V-AS/7721 cells [Fang et al., 2006]. We presently demonstrate that chronic ER stress but not apoptosis is induced in GnT-V-AS/7721 cells, whereas ATRA enhances the ER stress and induces the apoptosis of those cells. In our study, markedly decrease of GnT-V was observed in GnT-V-AS/7721 cells with ATRA treatment. Furthermore, the results from staining with HRP-lectin indicate that

ATRA has a significant effect on N-galycans structure of GnT-V/7721 cells. Thus, it is reasonable that ATRA-induced downregulation of GnT-V causes more deficient glycoproteins in GnT-V-AS/7721 cells, which enhances the ER stress and subsequently induces apoptosis. However, ER stress had not be observed in mock cells treated with 80 μM ATRA for 24 h, suggesting ATRA can not be a radical stimulus inducing ER stress. As shown in Figures 4 and 5, GnT-V was slightly repressed in H7721 cells with ATRA treatment, which could hardly change the structure of the N-glycans and might not induce ER stress.

ATRA was reported to induce apoptosis at different concentrations in varied cells. Our department found that 80 μM ATRA could induce the apoptosis in H7721 cells after 36–60 h treatment in a time-dependent manner, but proportion of the apoptotic cells were not significantly increased after 24 h treatment [Guo et al., 1999]. However, 80 μM ATRA induces the apoptosis of GnT-V-AS/7721 cells after 24-h treatment. This suggests downregulation of GnT-V facilitates ATRA to induce apoptosis of human hepatocarcinoma cells. GnT-V overexpression and its upregulation during the process of cancerous transformation were observed in many cancers, such as human mammary, colon, and hepatic tumor [Dennis and Laferte, 1987, 1989; Granovsky et al., 2000]. For liver, in normal hepatic tissue, the GnT-V activity is low, but increases in human hepatocellular carcinoma tissue, which is correlated with its progression [Dennis et al., 1999; Guo et al., 2002]. The effect of high expression and activity can be blocked by GnT-V suppression, which initiates ER stress and confronts hepatocarcinoma cells with high susceptibility to ATRA-induced apoptosis. Therefore our results suggest that co-operation of GnT-V repression and ATRA treatment may be a potential way in the treatment of hepatocellular carcinoma.

ATRA is a promising anticancer agent, which has been found regulating a wide of biological process, including cell proliferation, differentiation, and morphogenesis. We here establish an unmentioned role of ATRA to induce apoptosis via ER stress. Except that, ATRA may induce apoptosis through other pathways. In previous work, we found that protein kinase B (PKB) was downregulated by ATRA in GnT-V-AS/7721cells [Chen et al., 2003]. Recently Guo et al. [2006] reported that phosphorylated PKB was further decreased in GnT-V-AS/7721 cells treated with ATRA. Based on our results above and published, a novel mechanism of ATRA-induced apoptosis in GnT-V-AS/7721 cells involves PKB and ER stress, as shown by Hyoda et al., [2006] that PI3K-Akt inactivation induced CHOP expression in ER-stressed cells.

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