Glycoconj J (2007) 24:207–220
DOI 10.1007/s10719-007-9028-1

α1,3 Fucosyltransferase-VII modifies the susceptibility of apoptosis induced by ultraviolet and retinoic acid in human hepatocarcinoma cells

Hao Wang · Qiu-Yan Wang · Ying Zhang · Zong-Hou Shen · Hui-Li Chen

Received: 4 August 2006 / Revised: 27 November 2006 / Accepted: 1 February 2007 / Published online: 15 April 2007

Abstract The role of α1,3fucosyltransferase-VII (α1,3 FucT-VII) in cell apoptosis was studied in human hepatocellular carcinoma H7721 cells. After the cells were transfected with α1,3 FucT-VII cDNA, the expression of apoptotic protease, procaspase-3, was decreased, while the anti-apoptotic proteins, phospho-PKB and phospho-Bad were increased as compared with mock (vector) transfected cells, indicating that α1,3FucT-VII is a potential anti-apoptotic factor in H7721 cells. After "α1,3FucT-VII" cells were irradiated by UV to induce apoptosis, the anti-apoptotic potential of α1,3FucT-VII became more apparent, as evidenced by the less apoptotic cell % and active cleaved caspase-3, more phospho-p38 MAPK and JNK (two anti-apoptotic signaling molecules in H7721 cells responsible to UV stress) when compared with the "Mock" cells. In contrast, "α1,3FucT-VII" cells facilitated the apoptosis induced by *all-trans* retinoic acid (ATRA), which was verified by the greater sub-G1 (apoptotic cells) peak in flow cytometry analysis, more expressions of active caspase-3 and pro-apoptotic protein Bax, as well as less expressions of anti-apoptotic proteins, Bcl-2 and Bcl-X_L. The up regulation of α1,3FucT-VII mRNA and cell surface SLe^x (α1,3FucT-VII product) by UV and down regulation of them by ATRA was speculated to be one of the mechanisms that α1,3FucT-VII decreased and increased the susceptibility of apoptosis induced by UV and ATRA respectively.

Keywords Human hepatocarcinoma cell line · α1 · 3 Fucosyltransferase-VII · Apoptosis · Mitochondria pathway · p38 MAPK/JNK pathways

Abbreviations

ASK	apoptosis signal-regulating kinase
MAPK	Mitogen-activated protein kinase
MEK or MKK	MAPK kinase
ERK	Extracellularly-responsive kinase
JNK	c-Jun N-terminal kinase
PKB	protein kinase B (Akt)
Fuc	Fucose
Gal	Galactose
GlcNAc	*N*-acetylglucosamine
SA	Sialic acid
FucT	Fucosyltransferase
UV	Ultraviolet
ATRA	*all-trans* retinoic acid
DMSO	Dimethyl sulfoxide
EDTA	Ethylenediamine tetraacate
ECL	Enhanced chemiluminescence
RT-PCR	reverse transcriptase-polymerase chain reaction
FACS	Fluorescence activated cell spectra
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide electrophoresis
PBS	Phosphate buffered saline
BSA	Bovine serum albumin

Introduction

Apoptosis or programmed cell death is strictly controlled by a series of molecular mechanisms including death

Hao Wang and Qiu-Yan Wang contributed to this article equally.

H. Wang · Q.-Y. Wang · Y. Zhang · Z.-H. Shen · H.-L. Chen (✉)
Key Laboratory of Glycoconjugate Research, Ministry of Health, Department of Biochemistry, Shanghai Medical College, Fudan University, Shanghai 200032, People's Republic of China
e-mail: hlchen@shmu.edu.cn

receptors on cell surface, signaling pathways related to survival or apoptosis, and apoptosis-related molecules in cell mitochondria [1–5]. Fas (its ligand is FasL) and tumor necrosis factor receptor (TNF-R, its ligand is TNF) are the two representatives of cell surface death receptors [1]. Apoptosis signal-regulating kinase (ASK)−MAPK kinase (MEK or MKK) 3/6–p38 MAPK, and ASK−MKK 4/7−JNK (also referred as stress activated protein kinase, SAPK or p54 MAPK) as well as ERK5-big mitogen activated protein kinase-1(BMK-1) pathways are the three main signing pathways associated with stress signals, including apoptosis-inducing signal [2–4]. It has been well documented that Bcl-2 protein family acts as the gatekeeper of apoptosis in mitochondria [5]. Bcl-2 family includes pro-apoptotic proteins, such as Bax, Bad and Bid, as well as anti-apoptotic (pro-survival) proteins represented by Bcl-2 and Bcl-X_L [6, 7]. When a death signal induces the translocation of pro-apoptotic members from cytosol to mitochondria, the pro-apoptotic proteins stimulate the release of cytochrome c form mitochondria. Oppositely, the anti-apoptotic proteins inhibit the release of cytochrome c [8]. The released cytochrome c activates caspase-9 and the latter turns to activate caspase-3 for the initiation of cell apoptosis [5–8]. Most of Bcl-2 family members can form a homo-dimer or a hetero-dimer. If the hetero-dimer is formed by pro-apoptotic and anti-apoptotic proteins, they can inhibit each other. For example, in the excess of Bcl-2 or Bcl-X_L, the Bcl-2 (Bcl-X_L)/Bax hetero-dimer exhibits an anti-apoptotic effect, whereas, in the excess of Bax, the Bax homo-dimer displays a pro-apoptotic function. Therefore, the activity ratio of Bcl-2 (Bcl-X_L) to Bax is a critical factor to decide the cell survival or death [8, 9]. Caspase, a family of cysteine-dependent aspartate-directed proteases, is the executioner of apoptosis, which is activated by a cascade mechanism [10, 11]. Among the members of the caspase family, caspase-3 is most popular and the final protease in the caspase cascade. Once activated, caspase-3 cleaves a variety of intracellular proteins, including the major structural elements of cytoplasm and nucleus, and the components of DNA repair-related proteins [10].

In addition, Ras−MEK−ERK and PI-3K (phosphatidylinositide-3-kinase)−PDK-1 (phosphotidyl-inositide-dependent kinase 1)−PKB (Protein kinase B, Akt) pathways involved in the signal transduction of growth factors and insulin are also important in the regulation of cell apoptosis and survival [12, 13]. The ERK1/2 in MAPK family activates the phosphorylation of ribosomal S6 kinases (RSK), which in turn catalyses the phosphorylation of the pro-apoptotic protein Bad at Ser-112. Moreover, RSK phosphorylates the transcription factor, cAMP response element-binding protein (CREB), at serine-133 and activate CREB for stimulating the transcription of cell survival genes [12]. PKB itself is activated via phosphorylation at Thr-308 and Ser-473 sites by PDK-1 and PDK-2, the latter was reported to be an integrin-linked kinase (ILK) [14] and mammalian target of rapamycin (mTOR, a protein kinase) [15]. The activated PKB may phosphorylate the pro-apoptotic Bad at Ser-136 to prevent it from binding and inhibiting the anti-apoptotic proteins Bcl-2 and Bcl-X_L. PKB also phosphorylates caspase-9 and blocks its activation by cytochrome c released from mitochondria [13, 16]. Furthermore, PKB has been shown to phosphorylate Fockhead family members, and to block apoptosis through regulation of death receptors [13].

The human fucosyltransferase (FucT) family is composed of three subfamilies, α1,2FucT, α1,3FucT and α1,6 FucT [17]. α1,2FucT (FucT-I, -II) participates in the synthesis of ABO blood group antigens and Lewis antigen Y and B, both Lewis Y and B contain two fucosyl residues with α1-2 and α1-3 (or α1-4) linkages. α1-6 FucT (FucT-VIII) is responsible for the synthesis of core α1-6 fucose in Asn-linked sugar chains (N-glycans). The α1,3FucT subfamily is the main glycosyltransferase catalyzing the final fucosylation step in the synthesis of Lewis antigens. Up to date, six α1,3-FucTs (-III to -VII and -IX) have been identified [18]. Each enzyme has a unique acceptor substrate binding pattern, and each generates a unique range of fucosylated products. Among them, α1,3-FucT-VII fucusylates sialylated substrates and SLe^x [SAα2,3Galβ1,4 (Fuc α1,3) GlcNAc-] is its only product [19]. SLe^x and some other sialylated Lewis antigens can serve as ligands for E- or P-selectin expressed on the surface of vascular endothelial cells, and mediate the adhesion of malignant cells to vascular endothelium [20]. α1,3-FucT-VII is increased in some malignant cells, especially certain cases of leukemia, and is responsible for the increased SLe^x level on leukemia cell surface. However, α1,3-FucT-VII was reported to be also involved in the increased expression of SLe^x in cancer cells of epithelial origin [21]. Our lab has reported that cell surface SLe^x is correlated to the metastatic potential of H7721 human hepatocarcinoma cells after transfection of metastasis-stimulating gene (*c-erb*B2/*neu*) [22], as well as after treatment of proliferation-inducers (EGF, phobol ester) or differentiation-inducers (ATRA, 8-bromo-cAMP) [23]. On the other hand, transfection of α1,3-FucT-VII resulted in the stimulation of cell growth [24] and the up regulation of some growth factor signaling molecules (FEBS J, in press). Therefore, α1,3-FucT-VII can be considered as a cell survival-related enzyme.

In our laboratory, it was discovered that after *N*-acetylglucosaminyltransferase V (GnT-V), an enzyme involved in the synthesis of GlcNAcβ1-6 branch in N-glycans, was down regulated by the transfection of its antisense cDNA (GnTV-AS), the apoptosis of H7721 cells induced by ATRA was facilitated, while ATRA itself could not induce apparent apoptosis in mock cells transfected with the vector [25]. In the study of the molecular mechanism, it was found that GnTV-AS increased the expression of some pro-

apoptotic proteins and reduced some anti-apoptotic proteins. After the GnTV-AS transfected cells were treated with ATRA, some pro-apoptotic proteins were further increased, while some anti-apoptotic proteins were further decreased [26]. GnT-V is a tumorigenesis- and metastasis-associated enzyme [27], it is reasonable that GnT-V is also related to cell survival and anti-apoptosis. Hence, it is interesting to study whether α1,3-FucT-VII, another survival-related enzyme, also exhibits an anti-apoptotic effect.

In the present investigation, the effect of α1,3-FucT-VII on the cell apoptosis was studied in H7721 cells transfected with the cDNA of α1,3-FucT-VII. Ultraviolet and ATRA were selected as two apoptosis-inducing agents. The former is a physical agent, and the latter is a chemical one. It was found that α1,3-FucT-VII modified the sensitivity of H7721 cells to UV and ATRA, which was mediated by the alteration of the expression of some apoptosis-related molecules in mitochondria and cell signaling pathway.

Materials and methods

Materials

H7721 cell line was obtained from the Institute of Cell Biology, Academic Sinica. RPMI 1640 and DMEM mediums were purchased from GIBCO/BRL. Monoclonal antibodies of human Bcl-2, Bcl-X_L were from Neomarkers Company. Monoclonal antibodies to human β-actin, goat polyclonal antibodies to human S136-phospho-Bad and rabbit polyclonal antibody to human Bax, p53 (wild type), Fas, FasL, JNK and phospho-JNK (p-JNK) were from Santa Cruz Company. Rabbit polyclonal antibodies to human caspase-3, protein kinase B (PKB, Akt), T308-phospho- PKB, p38 and phospho-p38 (p-p38) were the products of Cell Signaling Technology. Horseradish peroxidase (HRP)-labeled second antibodies (goat anti-rabbit IgG and anti-mouse IgG, monkey anti-goat IgG) were purchased from Dako Company. ATRA, fluorescein isothiocyanate (FITC) labeled goat anti-mouse IgM second antibody, 4,6-diamidino-2-phenylindole (DAPI) and SB203580 were from Sigma. ECL assay kit was from Pierce Company. Trizol and DNase I were from Invitrogen Company. Real time-PCR reagent kit ExScript™ was purchased from TaKaRa Company. Monoclonal antibody (IgM) to human SLe^x, KM93, was the product of Chemicon. Other reagents were commercially available in China.

H7721 cell lines transfected with α1,3FucT-VII cDNA (inserted in vector pcDNA3.1) were established in our lab as previously reported [22, 24]. FucT-VII-A and FucT-VII-B were chosen in the present investigation. The expression of α1,3FucT-VII mRNA for FucT-VII-A was higher than that for FucT-VII-B.

Cell culture and treatment

Cells were cultured at 37°C, 5% CO_2 in RPMI-1640 medium containing 10% fetal calf serum, penicillin and streptomycin as previously described [24, 26]. In the case of ultraviolet (UV) light irradiation, 1×10^6 cells were subjected to instantaneous exposure of 10 mJ/cm^2 using CL-1000 Ultraviolet Crosslinker and determined cell apoptosis and caspase-3 after 12 h or measured p38 MAPK and JNK after 1 h. When inhibitor of p38 MAPK [28] was used, 10 μM SB203580 in DMSO was added to the medium 1 h before UV light irradiation. If ATRA was used as inducer of apoptosis, ATRA (dissolved in DMSO) was added to the culture medium at a concentration of 40 μM and the cells were incubated for 24 h. In the control of ATRA and SB203580 treated cells, the same final concentration of DMSO was added in the cell medium.

Observation of cell apoptosis with DAPI staining

Cells (1×10^6) after UV light irradiation were cultured in 96-wells plate for 12 h and fixed with 4% paraformaldehyde in PBS for 10 min. After treated with 0.1% Triton X-100 in PBS, the cells were stained with DAPI for 30 min. and observed under fluorescence microscope. The population of apoptotic cells was counted in six random fields (100×).

Cell apoptosis analyzed with flow cytometry

Briefly, the cells were incubated in 2% fetal calf serum with or without 40 μM ATRA for 24 h. The collected and washed cells were treated with 2 mM EDTA and 75% ethanol in −20°C for 2 h. After the cells were washed with PBS and suspended in PBS (containing 20 μg/ml RNase A, 0.2% Triton-X100, 0.2 mM EDTA and 20 μg/ml propidium iodide) at 37°C for 30 min, and were subjected to FACScan (Becton Dickson) for determination of DNA content. FACS was drawn automatically The sub-G1 peak was defined as the apoptotic cell peak to calculate the percentage of the apoptotic cells.

Analysis of the expression of apoptosis-related molecules using Western immuno-blot

In short, the cells were homogenized in lysed buffer, [pH 6.5 0.1M 2-(N-morpholino)-ethanesulfonic acid (MES) buffer/150 mM NaCl/2% TritonX-100/25% glycerol/ 0.1 mg % leupeptin and pepstatin], and then centrifuged with 1000×g for 15 min. Aliquots of 50 μg of protein samples in the supernatants were subjected to SDS-PAGE

and Western blot. The nitrocellulose membranes were treated with TTBS (Tris-buffered saline containing 0.1% Tween-20) and 5% fat-free dry milk for 1 h, followed by the addition of 1: 500~1,000 (according to previous experiments) diluted antibodies against one of the target proteins (caspase-3, PKB /Akt, T308-phospho-PKB, S136-phospho-Bad, Bax, Bcl-2, Bcl-X_L, p53, FasL, Fas, p38 MAPK, phospho-p38 MAPK, JNK or phospho-JNK) in TTBS with 5% fat-free dry milk and incubated for 2 h. After washing with TTBS, the cells were incubated with 1: 500 diluted HRP labeled corresponding second antibody for 1 h. Beta-actin was used as loading control and stained with 1: 500 diluted monoclonal antibody and HRP-labeled second antibody. Finally, the color was developed with ECL reagents, and followed by densitometric scanning. The intensities of the target protein bands were normalized by the intensity of β-actin.

The protocol for determination of caspase 3 was the same as above, except that the lysed buffer used was 20 mM Tris-HCl pH 8.0/137 nm NaCl/0.1 mM $CaCl_2$/ 1 mM $MgCl_2$/1% NP-40/10% glycerol/10 mg% phenyl-methylsulfonyl fluoride/0.1 mg% aprotinin.

Determination of α1,3 FucT-VII mRNAs with RT-PCR

Total cell RNA was extracted with Trizol and the cDNA was synthesized with oligo(dT)-18 primer and AMV reverse transcriptase from 3 μg total RNA. The RT-PCR was performed in 50 μl of reaction mixture containing 5 μl cDNA, 0.2 μM of the primer pair of α1,3 FucT-VII or β-actin (internal standard), 0.2M d-NTP and 1unit Taq as described previously [29]. The primer of α1,3 FucT-VII was F: 5'-CACCTCCGAGGCATCTTCAACTG-3', R: 5'-CGTTGGTATCGGCTC TCATTCATG-3'. The primer of β-actin was F: 5'-GATATCGCCGCGCTCGTCGTCGAC-3', R: 5'-CAGGAAGGAAGGCTG GAAGAGTGC-3' [29]. The cDNA was subjected to denaturation at 94°C for 5 min, followed by 32 (in the experiment of UV) or 40 (in the experiment of ATRA) cycles (94, 61.5 and 72°C, 1 min for each) of PCR, and incubated at 72°C for 10 min and 4° C for 5 min. Then 10 μl products were applied to 2% agarose gel containing ethedium bromide for electrophoresis. The amplified DNA bands were scanned by Pharmacia Biotech and analyzed with NIH Image software.

Quantitative real-time RT-PCR

Total RNA was extracted using the Trizol reagent. Residual genomic DNA in RNA preparations was removed by DNase I digestion. The DNase I-digested RNA samples were used for reverse transcription with ExScript™ RT Reagent Kit. Quantitative real-time PCR was performed in an ABI PRISM 7300 Sequencing Detector using SYBR-green PCR master mix (Applied Biosystem). Amplification parameters were 2 min at 50°C, 10 min at 95°C, and 40 cycles (each cycle includes 95°C for 15 s and 60°C for 1 min). The data was expressed as relative abundance of the FucT-VII mRNA (the "Mock" cell value was set at 100%). Primers for FucT-VII were F: 5'-AGAGCTGGCGGCTTTCCT-3', R: 5'-CTTCTTTGCCTGGCGTGAC-3', which were designed using the Primer Express 2.0 software. Each sample was analyzed in triplicate,

Cell surface SLe^x detection with flow cytometry

Cells were detached with 2 mM EDTA, washed and blocked with 1% BSA at 4°C for 10 min, then 1×10^6 cells were incubated with 1:50 SLe^x monoclonal antibody (KM93) for 45 min at 4°C. A (−) control sample was set up as a background without the addition of KM93. After washing, the cells were re-incubated for 30 min at 4°C with 1:128 FITC-conjugated second antibody. Then the washed cells were suspended in 0.5 ml PBS and subjected to flow cytometry. Fluorescence activated cell spectra (FACS) were drawn automatically, and the left- or right-shift of the curve indicated the decrease or increase of the mean fluorescence intensity (MFI) respectively as shown by the "M1" bar in the figures. Quantitative data (relative expression of SLe^x) were obtained from relative MFI and set the "untreated Mock" sample as 100%.

Results

Effects of α1,3FucT-VII on the cell apoptosis induced by UV and ATRA

One α1,3FucT-VII transfected H7721 cell line, FucT-VII-A, was chosen in the experiments of UV irradiation. By using DAPI staining and fluorescence microscopic observation, almost no apoptotic cells were found in cell samples before UV irradiation. However, a plenty of apoptotic cells were found in cells mock-transfected with empty vector pcDNA3.1 after UV irradiation, while only a few apoptotic cells were appeared in UV irradiated cells transfected with α1,3FucT-VII cDNA (Fig. 1a). This result suggests that α1,3FucT-VII suppresses the susceptibility of UV induced apoptosis.

In the experiment of ATRA, two α1,3FucT-VII transfected cell lines (FucT-VII-A and FucT-VII-B) were used. With a more accurate flow-cytometric method, it was found that in the absence of ATRA, both mock- and α1,3FucT-VII-transfected cells did not show any sub-G1 (apoptotic cell) peak and only a very small sub-G1 peak was observed in "Mock" cells after ATRA treatment (Fig. 1b). However, a larger and higher sub-G1 peak was appeared in α1,3FucT-

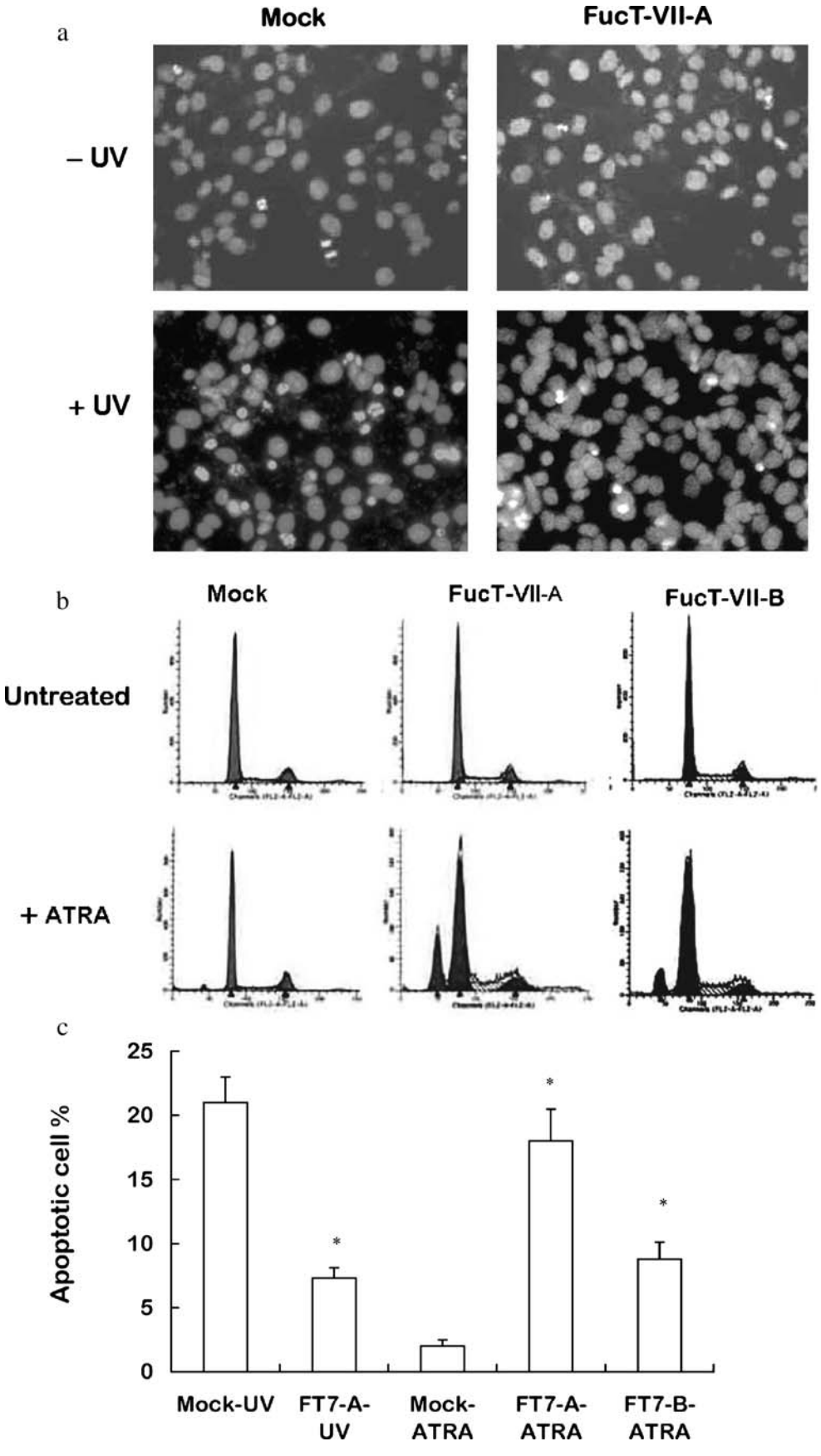


Fig. 1 Effects of α1,3FucT-VII on the cell apoptosis induced by UV and ATRA. **a** Microscopic observation on UV induced cell apoptosis. **b** Flow cytometric analysis on ATRA induced apoptosis (FACS). **c** The percentages of apoptotic cells in "Mock" and "α1,3FucT-VII" cells after UV or ATRA induced apoptosis (n=3). Mock: cell transfected with empty vector pcDNA3.1, FucT-VII-A or FT7-A, FucT-VII-B or FT7-B: two cell lines transfected with the cDNA of α1,3FucT-VII. UV: ultraviolet light; ATRA: *all-trans* retinoic acid. *: $p<0.01$ compared to corresponding "Mock" cells. "A" and "B" are the representatives of three reproducible experiments. Cell apoptosis was determined at 12 h after UV irradiation or 24 h after 40 μM ATRA treatment. The experimental procedures for DAPI staining, cell counting and flow cytometric analysis were described in the "Materials and methods"

VII transfected cells after ATRA treatment, especially in FucT-VII-A. These findings revealed that the susceptibility of apoptosis was facilitated in α1,3FucT-VII transfected cells when ATRA was used as the inducer of apoptosis.

The quantitative data of the apoptotic cell percentage in UV irradiated and ATRA treated cells were shown in Fig. 1c. The p values between "Mock" and α1,3FucT-VII transfected cells were <0.01. FucT-VII-A showed a higher percentage of apoptotic cells than FucT-VII-B.

Effects of α1,3FucT-VII on the caspase-3 during apoptosis induced by UV and ATRA

In the study of molecular mechanism that α1,3FucT-VII modifies the susceptibility of apoptosis, the expression of caspase-3, the final executioner of the apoptosis cascade, was measured without and with UV irradiation. As shown in Fig. 2a, in mock- and α1,3FucT-VII transfected cells without UV irradiation, caspase-3 was only present as inactive procaspase-3 (32 kDa), the level of which was approximately one time higher in "Mock" cells than that in α1,3FucT-VII transfected cells ($p<0.01$). After UV irradiation, the active cleaved caspase-3 (17 kDa) appeared, and the level of procaspase-3 was correspondingly decreased. The expression of active caspase-3 was significantly higher (about 3.4 times) in "Mock" cells than that in "α1,3FucT-VII" cells ($p<0.01$).

If ATRA treatment was used instead of UV irradiation, it was found that the procaspase-3 was slightly more expressed in "FucT-VII" than in "Mock" cells before ATRA treatment and the active cleaved caspase-3 was also not appeared in ATRA untreated cells. After ATRA treatment, however, it appeared as two bands (17 and 19 kDa) in stead of one band in UV irradiated cells (Fig. 2b). The reason is unknown; it is possible that the effects of UV and ATRA on the cleavage of procaspase-3 are different. The level of active caspase-3 in α1,3FucT-VII transfected cells was much higher (approximately more than five times) than that in "Mock" cells ($p<0.01$). Moreover, the level in FucT-VII-A was higher than that in FucT-VII-B, which was correlated with the expression of α1,3FucT-VII in the cell and the apoptotic cell percentage shown in Fig. 1c.

Effects of α1,3FucT-VII on the other apoptosis-related proteins during ATRA induced apoptosis

PKB, Thr-308 phosphorylated PKB, Ser-136 phosphorylated Bad, Bax, Bcl-2 and Bcl-X_L were selected as apoptosis-related proteins to study the effect of ATRA on their expressions in Mock and two α1,3FucT-VII transfected cell lines (FucT-VII-A and B). Fig. 3a indicated that in the absence of ATRA, the expressions of PKB, Bax, Bcl-2, Bcl-X_L were unchanged in α1,3FucT-VII transfected cells as compared with the "Mock" cells. On the contrast, T308-phospho-PKB was elevated in FucT-VII-A and FucT-VII-B, up to 220 and 149% of the "Mock" value respectively (both $p<0.01$). As a result of the increased activity of PKB, S136-phospho-Bad also increased to 315 and 160% respectively (both $p<0.01$) in "A" and "B" cells (Bad itself is a pro-apoptotic protein, but it becomes anti-apoptotic after phosphorylation by PKB [13, 16]). Therefore, the intensity order of both T308-phospho-PKB and S136-phospho-Bad was "FucT-VII-A" > "FucT-VII-B" > "Mock," this order was in accordance with the order of α1,3FucT-VII" expression and the apoptosis sensitivity. After ATRA treatment, both phospho-PKB and phospho-Bad were down regulated and the difference between "α1,3FucT-VII" and "Mock" cells was abolished. On the other hand, in ATRA

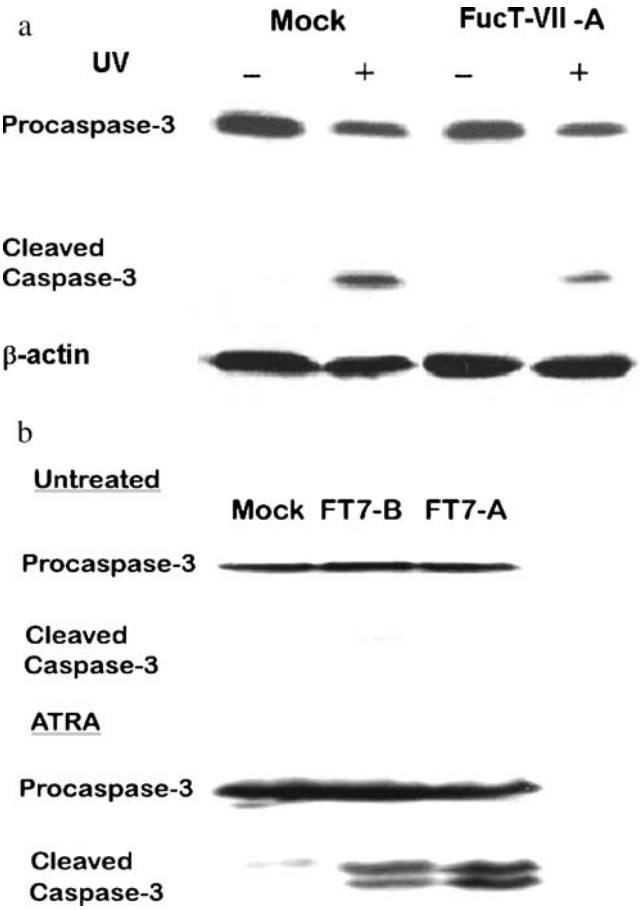


Fig. 2 Caspase-3 expression in "Mock" and "α1,3FucT-VII" cells during UV and ATRA induced apoptosis. **a** Caspase-3 expression in "Mock" and "α1,3FucT-VII" cells with and without UV irradiation. **b** Caspase-3 expression in "Mock" and "α1,3FucT-VII" cells with and without ATRA treatment. Mock, FucT-VII-A, FT7-A, FT7-B, UV, ATRA: same as indicated under the legends of Fig. 1. Caspase-3 was determined at 12 h after UV irradiation or 24 h after 40 μM ATRA treatment. "A" and "B" are the representatives of three reproducible experiments. The experimental procedure for Western immuno-blot of caspase-3 was described in the "Materials and methods"

treated "α1,3FucT-VII" cells, Bax was increased to 230 and 167% of the "Mock" level in cell "A" and "B" respectively (both $p<0.01$), while Bcl-2 was decreased to 73 and 82 %, and Bcl-X_L was reduced to 35 and 61% of the "Mock" value in cell "A" and "B" respectively (all the p values were <0.01). This suggeststhat α1,3FucT-VII shows an anti-apoptotic effect on H7721 cells in the absence of ATRA, but displays a facilitation of apoptosis in the presence of ATRA.

Several anti-oncogenes also participate in the control of apoptosis, such as p53, which has been reported as a substrate of p38 MAPK [30] and a direct activator of Bax in the promotion of mitochondrial permeabilization and release of cytochrome c [31]. The function of p53 is similar to the pro-apoptotic Bid protein. Fig. 3a also showed that p53 expression was not altered in "α1,3FucT-VII" cells with and without ATRA treatment.

Unlike PKB and the Bcl-2 family in mitochondria, the expressions of cell surface death receptor and its ligand, Fas and FasL, were unchanged in "α1,3FucT-VII" cells despite of the presence or absence of ATRA (Fig. 3b).

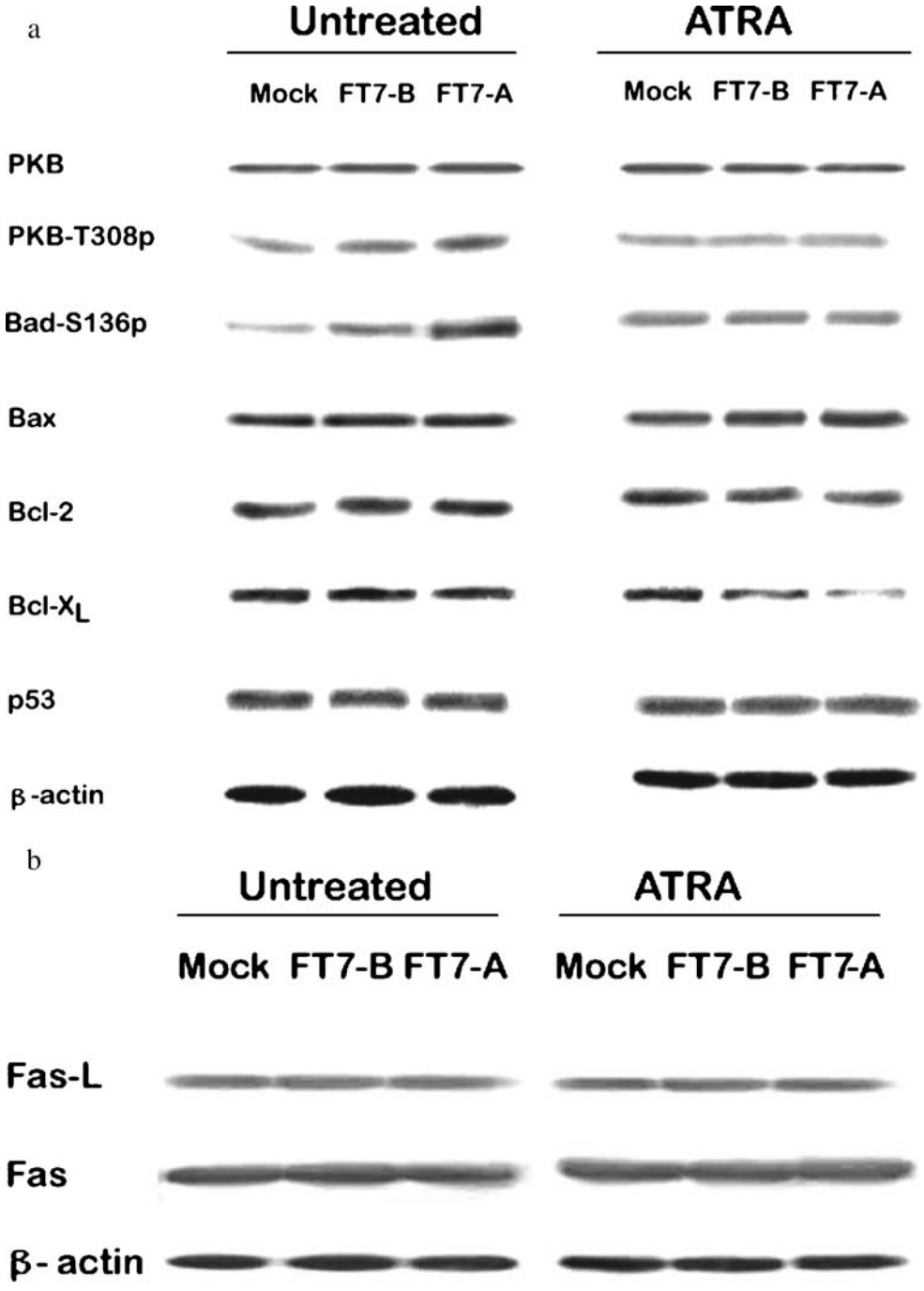


Fig. 3 Expression of other apoptosis-related proteins in "Mock" and "α1,3FucT-VII" cells during ATRA induced apoptosis. **a** Western immuno-blot profiles of PKB, Bcl-2 family proteins and p53 in "Mock" and "α1,3FucT-VII" cells with and without ATRA treatment. **b** Western immuno-blot of FasL and Fas in "Mock" and "α1,3FucT-VII" cells with and without ATRA treatment. Mock, FT7-A, FT7-B, ATRA: same as indicated under the legends of Fig. 1. PKB-T308p: Thr-308 phosphorylated PKB, Bad-S136p: Ser-136 phosphorylated Bad. The duration of 40 μM ATRA treatment was 24 h. "A" and "B" are the representatives of three reproducible experiments. The experimental procedure for Western immuno-blot of apoptosis-related proteins was described in the "Materials and methods"

P38 MAPK signaling pathway is anti-apoptotic for H7721 cells

P38 MAPK and JNK signaling pathways are the two main pathways responsible for stress including UV irradiation, and have been widely investigated [2–4, 32]. Hence, in the case of UV induced apoptosis, our studies were focused on these two pathways to search the relationship between them and α1,3FucT-VII. However, it has been generally accepted that the role of p38 MAPK in apoptosis remains controversial. First, in many systems, such as in T cells and fibroblasts, activation of p38 MAPK activity results in apoptosis, and inhibition of p38 reduces apoptosis, while in other systems, like HeLa cells subjected to photodynamic therapy, p38 MAPK activity inhibits apoptosis [33]. Second, the duration of p38 activation is another critical factor. In mouse erythroleukemia SKT6 cells exposed to osmotic shock, where early activation of p38 MAPK inhibits apoptosis and prolonged activation leads to apoptosis [34]. The pro-apoptotic action of phosphorylated p38 MAPK resulted from the cleavage and activation of Bid and caspase-3 in HL 60 leukemia cells during singlet oxygen induced apoptosis [35], while the anti-apoptotic effect of p38 MAPK was mediated by the elevated expression of anti-apoptotic protein Bcl-X_L [36]. On the other hand, JNK was also suggested to be pro-apoptotic, anti-apoptotic, or had no role in apoptosis depending on the cell type and stimulus used [37, 38]. One of its anti-apoptotic mechanisms is mediated by the phosphorylation of Bad at T201 [37].

At first, whether p38 MAPK is pro-apoptotic or anti-apoptotic for H7721 cells was studied. The autophosphorylated p38 (p-p38) was detected at 1 h after UV irradiation as an index of p38 activation, since the production of p-p38 is a prompt response to UV stress. It was found that p-p38 was only appeared after UV irradiation (Fig. 4a). Moreover, after the addition of SB203580, an inhibitor of p38 MAPK for competitive with ATP at ATP binding site [28], the expression of p-p38 was dramatically reduced in both "Mock" and "FucT-VII-A"' cells ($p<0.01$) (Fig. 4a). Concomitantly, the active cleaved caspase-3 (Fig. 4b) and the percentage of the apoptotic cells (Fig. 4c) were increased in UV irradiated cells by the addition of SB203580 ($p<0.01$). These findings demonstrated that p38 MAPK is an anti-apoptotic pathway for H7721 cells in our experimental condition, since inhibition of p38 MAPK by SB203580 results in the promotion of apoptosis.

α1,3FucT-VII increases phosphorylated p38 MAPK

When the expression of phospho-p38 MAPK (p-p38) in "Mock" and "α1,3FucT-VII" cells was compared (Fig. 4a), it was discovered that p-p38 was significantly higher in UV

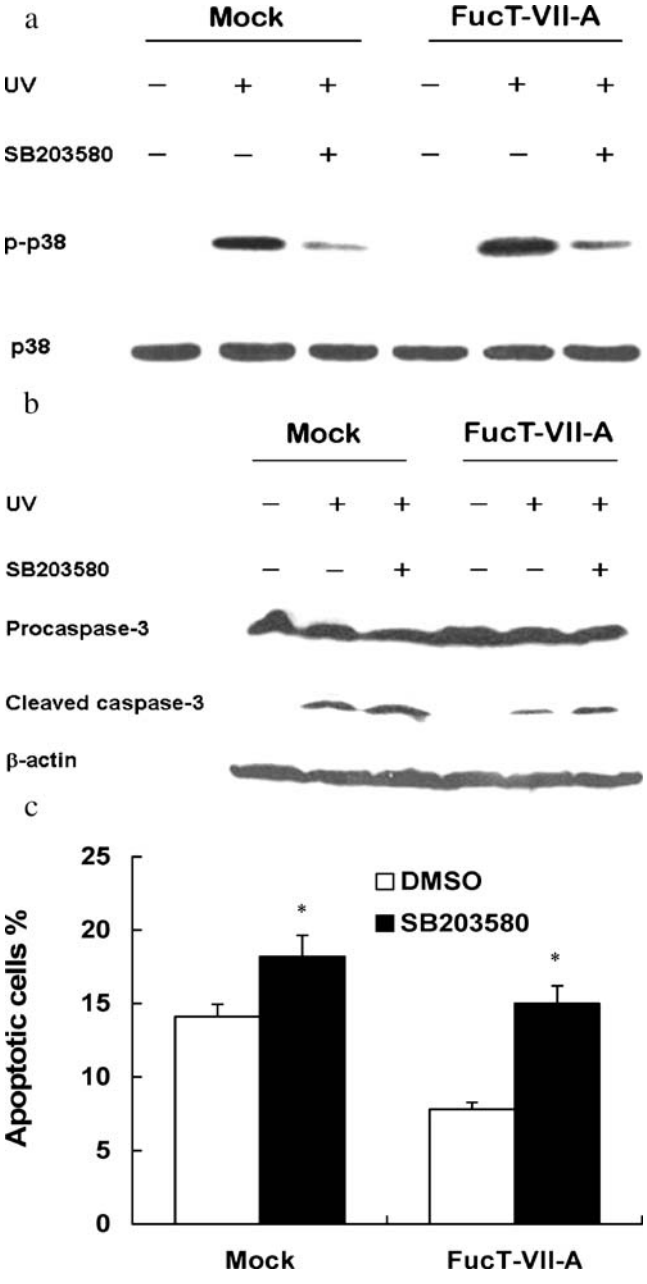


Fig. 4 Effect of SB203580 on the expression of p38 MAPK, caspase-3 and percentage of apoptotic cells in UV irradiated "Mock" and "α1,3FucT-VII" cells. **a** Western immuno-blot profiles of p38 MAPK and p-p38 MAPK in UV irradiated "Mock" and "α1,3FucT-VII" cells in the absence and presence of SB203580. **b** Western immuno-blot profiles of caspase-3 in UV irradiated "Mock" and "α1,3FucT-VII" cells in the absence and presence of SB203580. **c** Effect of SB203580 on the percentage of apoptotic cells in UV irradiated "Mock" and "α1,3FucT-VII" cells ($n=3$). Mock, FucT-VII-A, UV: same as indicated under the legends of Fig. 1 p-38: p-38 MAPK, p-p38: phosphorylated p38 MAPK, DMSO; control cells untreated with SB203580, but only treated with the same concentration of SB203580 solvent, dimethyl sulfoxide. *: $p<0.01$ compared with DMSO control. SB203580 was added at 1 h before and p38 MAPK was determined at 1 h after UV irradiation. Percentage of cell apoptosis was measured at 12 h after irradiation. "A" and "B" are the representatives of three reproducible experiments. The experimental procedures for Western immuno-blot of p38 MAPK and caspase-3 as well as the DAPI staining and cell counting were described in the "Materials and methods"

irradiated "α1,3FucT-VII" cells, being 178 and 213% of the values in "Mock" cells in the absence or presence of SB203580 respectively ($p<0.01$), while unphosphorylated p38 MAPK protein remained unchanged in UV irradiated, SB203580 treated and α1,3FucT-VII transfected cells. Furthermore, the active caspase-3 (Fig. 4b) and apoptotic cell % (Fig. 4c) in UV irradiated cells were lower in "α1,3FucT-VII" cells than those in "Mock" cells in spite of SB203580 was present or not,. The amount of active caspase-3 in "α1,3FucT-VII" cells was only one third of that in "Mock" cells both in SB203580 treated or untreated cells ($p<0.01$). These findings reveal that α1,3FucT-VII shows an up regulation of phosphorylated p38 MAPK in addition to the down regulation of active caspase-3 and apoptotic cells in UV induced apoptosis

α1,3FucT-VII increases phosphorylated JNK

The UV stimulated up regulation of p38 MAPK was compared with that of JNK. Phospho-JNK1/2 (p-JNK) proteins were also not present in UV un-irradiated cells, and unphosphorylated JNK1/2 proteins were also not changed in UV irradiated and α1,3FucT-VII transfected cells (Fig. 5). However, p-JNK proteins were appeared in UV irradiated cells, and the expression of p-JNK 1/2 proteins was also higher in "α1,3FucT-VII" cells than that in "Mock" cells, being 147% of the "Mock" value ($p<0.05$). The higher expression of JNK in α1,3FucT-VII transfected

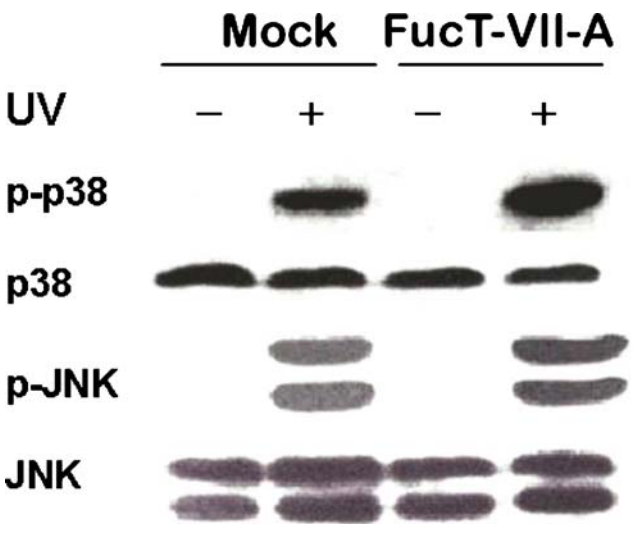


Fig. 5 Comparison of the expression of phosphorylated p38 MAPK and phosphorylated JNK1/2 in UV irradiated "Mock" and "α?α1,3FucT-VII" cells. Mock, FucT-VII-A, UV, p38, p-p38: same as under the legends of Figs. 1 and 4. JNK: c-Jun N-terminal kinase1/2, p-JNK: phosphorylated JNK1/2. p38 MAPK, JNK and their phosphorylated derivatives were determined at 1 h after UV irradiation. The figure is the representatives of three reproducible experiments. The experimental procedure for Western immuno-blot of the above apoptosis-related proteins was described in the "Materials and methods"

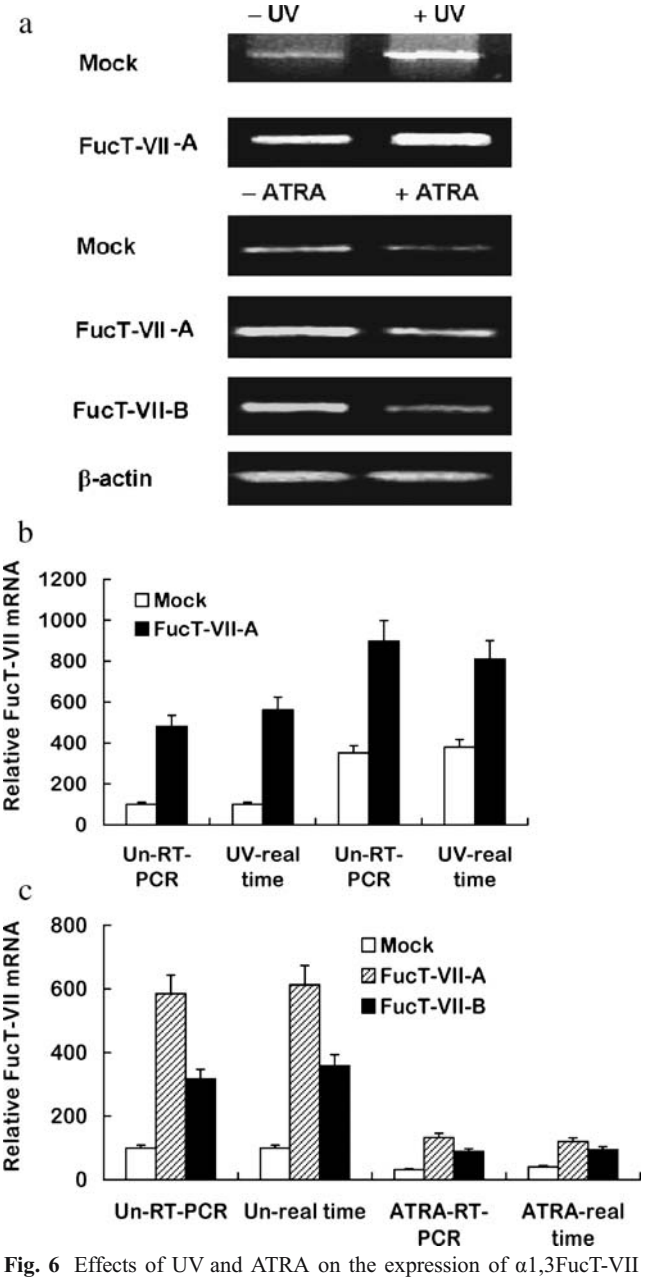
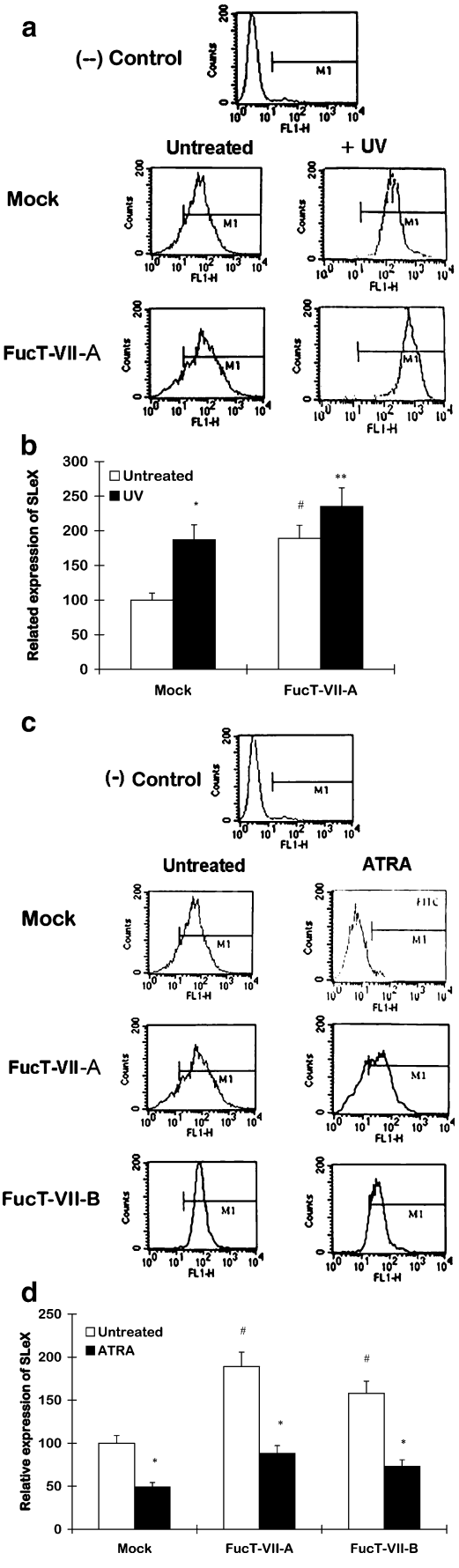


Fig. 6 Effects of UV and ATRA on the expression of α1,3FucT-VII mRNA. **a** RT-PCR of α1,3FucT-VII mRNA in mock-and α1,3FucT-VII transfected H7721 cells before and after UV or ATRA treatment. The PCR was 32 and 40 cycles for the experiment of UV and ATRA respectively. **b** Comparison of RT-PCR and real time-PCR method for determination of α1,3FucT-VII mRNA in UV un- irradiated and irradiated cells. **c** Comparison of RT-PCR and real time-PCR method for determination of α1,3FucT-VII mRNA in ATRA untreated and treated cells. Mock, FucT-VII-A, FucT-VII-B, UV, ATRA: same as under the legends of Fig. 1. Un: Untreated with UV or ATRA. RT-PCR or real time PCR was analyzed 12 h after UV irradiation or 24 h after 40 μM ATRA treatment. "A" is the representative of three reproducible experiments. The experimental procedures for RT-PCR and real time-PCR were described in the "Materials and methods"



cells suggesting that JNK pathway is also anti-apoptotic for H7721 cells.

Effects of UV light and ATRA on the expression of α1,3FucT-VII and SLe^x

The above results indicate that the transfection of α1,3FucT-VII suppresses the sensitivity of apoptosis induced by UV, but increases the susceptibility of ATRA induced apoptosis. Hence, it is important to study the effects of UV and ATRA on the expression α1,3FucT-VII transfected cells. As shown in Fig. 6a, a week expression of α1,3FucT-VII mRNA was found in mock-transfected H7721 cells after 32 cycle of RT-PCR. It was very interesting to discover that UV irradiation up regulated the mRNA of α1,3FucT-VII not only in "Mock" cells, but also in FucT-VII-A cells. Oppositely, after 40 cycles of RT-PCR, it was observed that ATRA down regulated α1,3FucT-VII mRNA in both mock- and two α1,3FucT-VII-transfected cell lines, being 32% for "Mock", 24.4% for FucT-VII-A and 28.1% for FucT-VII-B when compared to the ATRA untreated controls. Using real time PCR, the result was similar to that from RT-PCR. After UV irradiation, the relative concentration of α1,3FucT-VII mRNA was increased to 3.51 and 1.86 times in "Mock" and FucT-VII-A cells respectively when RT-PCR was used, and the increase was 3.79 and 1.44 times in "Mock" and FucT-VII-A respectively with real time-PCR method (Fig. 6b). On the other hand, in ATRA treated cells, the decrease-percentage α1,3FucT-VII mRNA in FucT-VII-A was 77.3% with RT-PCR and 80.5 % with real time PCR, and that in FucT-VII-B was 71.9 and 73.5% with RT-PCR and real time PCR, respectively (Fig. 6c).

In. order to confirm the above results, the expression of SLe^x (the product of α1,3FucT-VII) was determined using flow cytometry combined with SLe^x antibody on mock- and α1,3FucT-VII-transfected cell surface before and after UV or ATRA treatment. It was shown in Fig. 7a and b that

Fig. 7 Effects of UV and ATRA on the expression of SLe^x on cell surface. **a** Immuno-analysis of SLe^x with flow cytometry (FACS) on mock-and α1,3FucT-VII-transfected H7721 cell surface before and after UV irradiation. **b** Quantification of surface SLe^x in A (n=3). **c** Immuno-analysis of SLe^x with flow cytometry (FACS) on mock- and α1,3FucT-VII-transfected H7721 cell surface before and after ATRA treatment. **d** Quantification of surface SLe^x in C (n=3). Mock, FucT-VII-A, FucT-VII-B, UV, ATRA: same as indicated under the legends of Fig. 1. (−) Control: cell sample without the addition of SLe^x monoclonal antibody (KM93). *: $p<0.01$ compared to "Untreated" control; **: $p<0.05$ compared to "Untreated" control; #: $p<0.01$ compared to "Untreated Mock" cells. Cell surface SLe^x was analyzed 12 h after UV irradiation or 24 h after 40 μM ATRA treatment. "A" and "C" are the representatives of three reproducfsible experiments. The experimental procedure for flow cytometric analysis was described in the "Materials and methods"

surface SLe^x expression was elevated by UV irradiation and α1,3FucT-VII transfection, up to 189 and 187 % of the un-irradiated and "Mock" cells, respectively. After α1,3FucT-VII transfected cells were irradiated by UV, SLe^x expression was further increased to 235% of the un-irradiated "Mock" cell value. On the other hand, surface SLe^x was significantly reduced on both mock- and α1,3FucT-VII-transfected cells by the addition of ATRA, down to 49, 46.6 and 46.2 % in "Mock" "FucT-VII-A" and "FucT-VII-B" cells respectively (Fig. 7c and d). These findings were compatible with the alteration of α1,3FucT-VII mRNA after UV irradiation and ATRA treatment as shown in Fig. 6.

Discussion

The purpose of this study was mainly to elucidate the role of α1,3FucT-VII in cell apoptosis, not to compare the molecular mechanisms of apoptosis induced by UV and ATRA. All the results in the present studies from mock- and α1,3FucT-VII-transfected cell, and from various experiments using different methods and different indexes of apoptosis provide strong evidences that α1,3FucT-VII acts as a modifier of apoptosis in H7721 cell, and its effect is mediated by the modified expression of some apoptosis-related molecules.

In the present investigation, we discovered that α1,3FucT-VII showed an anti-apoptotic action on H7721 cells. This conclusion was evidenced by the following observations. (1) After UV irradiation, the apoptotic cells % in "FucT-VII" cells was less than that in "Mock" cells (Fig. 1a and c). (2) The expression of procaspase-3 before UV irradiation and the expression of cleaved active caspase-3 after UV irradiation were also lower in "FucT-VII" cells than in "Mock" cells, despite the addition of SB203580 (Fig. 2a, Fig. 4b) (3) The anti-apoptotic proteins, phospho-PKB and phospho-Bad in "FucT-VII" were higher than those in "Mock" cells before ATRA treatment (Fig. 4a). (4) The anti-apoptotic signaling molecule for H7721 cells, p-p38 MAPK (both in the absence or presence of SB203580) and JNK were also higher in "FucT-VII" than in "Mock" cells after UV irradiation (Fig. 4a, Fig. 5).

Oppositely, transfection of α1,3FucT-VII led to the increased susceptibility of ATRA induced apoptosis. It was verified by the following findings. (1) The apoptotic cells % was higher in "FucT-VII" cells than in "Mock" cells after ATRA treatment (Fig. 1b and c). (2) In "FucT-VII", the expression of active caspase-3 was also higher than that in "Mock" cells (Fig. 2b). (3) After ATRA treatment, the pro-apoptotic Bax was increased more in "FucT-VII" than in "Mock," while the anti-apoptotic proteins, Bcl-2 and Bcl-X_L, were decreased more in "FucT-VII" than in "Mock." (4) The above alterations in apoptotic cells and related proteins were proportional with the amount of α1,3FucT-VII mRNA in "FucT-VII -A" and "FucT-VII -B" different cell lines.

In the study of the effect of UV and ATRA on the expression of α1,3FucT-VII mRNA, it was found that UV up regulated, but ATRA down regulated the mRNA of α1,3FucT-VII. In our previous work using Northern blot, it has been demonstrated that α1,3FucT-VII mRNA was decreased by ATRA in a dose dependent manner [23]. The SLe^x (product of α1,3FucT-VII) content on cell surface was correspondingly changed in proportion with the α1,3FucT-VII mRNA (Figs. 6, and 7). Therefore, the increase of α1,3FucT-VII/surface SLe^x in UV irradiated and the decrease of α1,3FucT-VII/surface SLe^x in ATRA treated cells may be one of the mechanisms to explain the opposite effect of α1,3FucT-VII on UV and ATRA induced apoptosis. It was generally considered that the effect of UV irradiation should be mediated by cell membrane, while that of ATRA is mediated by its intracellular and intra-nuclear receptor. This difference may be another reason that α1,3FucT-VII displays opposite effect on the apoptosis induced by UV and ATRA.

Whether the alteration of apoptosis and its related proteins is caused by the change of α1,3FucT-VII itself or its product SLe^x is an interesting problem. It has been reported by our lab that the change of SLe^x on cell surface can modify the signaling of some surface receptors, resulting in promotion or suppression of the expression of some proteins, including the transcription factors, since the alterations of the protein expressions can be significantly abolished by the monoclonal antibody of SLe^x [24, FEBS J, in press]. This result suggests that the changed expression of apoptosis-related proteins may be mainly mediated by the altered amount of surface SLe^x. However, the possibility that the altered expression of apoptosis-related proteins is unrelated to SLe^x or even unrelated to the glycosylation effect of α1,3FucT-VII cannot be ruled out. Saito at al [39] reported that tumor angiogenesis induced by a secreted type of GnT-V was not mediated by glycosylation, since the highly basic domain of GnT-V induced the release of fibroblast growth factor-2 (FGH-2) from surface glycans and/or extracellular matrix, leading to the FGH induced angiogenesis. Recently, it has been reported by Okajima *et al*. that in *Drosophila*, another kind of fucosyltransferase named O-fucosyltransferase-1 (O-FUT1), which transfers Fuc to Ser/Thr residues in epidermal growth factor-like domains of Notch (a surface receptor) is a molecular chaperone. The ability of O-FUT1 to facilitate the folding of Notch does not require its fucosylation activity [40]. In our laboratory, a plasmid containing a mutant at the catalytic domain of α1,3FucT-VII with deletion of enzyme activity is under construction, which will be used to elucidate whether the modification of

apoptosis by α1,3FucT-VII is related to the glycosylation effects of α1,3FucT-VII.

The effect of UV on apoptosis is very likely mediated by the phosphorylation of p38 MAPK and JNK, because the activation of p38 MAPK and JNK is far earlier than the increase of α1,3FucT-VII mRNA. We have found that transfection of α1,3FucT-VII resulted in the promotion of Ras-MEK-MAPK and PI-3K-PKB signaling pathways (FEBS J, in press), and these pathways also show anti-apoptotic effect [4, 13]. Hence, the effect of UV irradiation and α1,3FucT-VII overexpression can be added together. This may explain why UV can further up regulate α1,3FucT-VII mRNA in α1,3FucT-VII transfected cells. However, it is still unknown how the p38 MAPK/JNK and Ras-MEK/PI-3K-PKB pathways affect the expression of α1,3FucT-VII mRNA.

Azuma *et al*. reported [41] that the expression of α1,3FucT-IV and its products, Lewis X (Le^x) and Lewis Y (Le^y), was elevated after apoptosis of Jurket cells (a human T cell line) induced by Fas antibody. The elevation of Le^x and Le^y could be blocked by the inhibitor of Caspase-3 and -8, suggesting that the enhancement of α1,3FucT-IV and its products was mediated by a molecule down stream of caspases. Kioke *et al*. also reported that the transcription of α1,3FucT-VII was induced by hypoxia stress via the stimulation of hypoxia inducing factor [42].We considered that the mechanism of the up regulation of α1,3FucT-VII mRNA by UV irradiation in our study might be similar to the elevation of α1,3FucT-IV in Fas antibody induced apoptosis, and the increase of mRNA was probably at the transcription level. In addition, it was previously reported that at the late stage of apoptosis in thymocytes and P185 cells induced by dexamethasone, gliotoxin or thapsigargin, the exposure of fucose residues on cell surface was increased [43]. Therefore, the up regulation of fucosylated sugar chains on the cell surface may be a common phenomenon and consequence of apoptosis induced by a number of chemical or physical agents, but ATRA is an exception, which reduces the fucose-containing SLe^x on cell surface.

It is very interesting to find that apoptosis induced by UV elevates the expression of α1,3FucT-VII and surface fucose-containing SLe^x, and the increased α1,3FucT-VII and surface SLe^x reduces the susceptibility of UV induced apoptosis. This may be seemed as a feedback mechanism for the protection of cells from apoptosis.

However, the biological effect of UV and ATRA are very wide and multiple. Many other factors should involve in the apoptosis induced by UV and ATRA. ATRA was characterized as a differentiation agent for H7721 hepatocarcinoma cells in our lab [44]. It suppresses proliferation-associated genes, including GnT-V [45] and stimulates differentiation-associated genes. ATRA also up regulates the metastasis suppressive gene *nm23*-H1 [46]. Moreover, *nm23*-H1 can down regulate a number of glycosyltransferases participating in Lewis antigen synthesis, such as α1,3FucT-III, -IV, -VI and -VII and sialyltransferase ST3Gal-I and ST3Gal-III [47]. In this study, it was also observed that ATRA inhibited the expression of phospho-PKB as shown in Fig. 3a. On the other hand, the consequence of α1,3FucT-VII transfection is also complicated. Our lab found that numerous signaling proteins were up regulated by α1,3FucT-VII [24], including the mRNA of α5 integrin (to be published). It is possible that in the presence of ATRA, α1,3FucT-VII may also affect the expression of some unknown apoptosis-related molecules other than caspase-3, Bcl-2 family, p-38 MAPK and JNK reported in this study, leading to a enhanced sensitivity to apoptosis induced by ATRA, but not UV, and the total effects of these apoptosis-promoting molecules may exceed the anti-apoptotic effect of α1,3FucT-VII. Consequently, the susceptibility to ATRA induced apoptosis is facilitated, and the facilitation is positively related to the amount of α1,3FucT-VII in the cells.

In summary, α1,3FucT-VII is not only a growth-stimulating glycosyltransferase as previously reported by our lab [24], but also an apoptosis-regulating enzyme, which can enhance or suppress the sensitivity or resistance to apoptosis induced by different physical or chemical agents.

References

1. Ashkenazi, A., Dixit, V.M.: Death receptor signaling and modulation. Science **281**, 1305–1308 (1998)
2. Kyriakis, J.M., Avruch, J.: Mammalian mitogen-activated protein kinase signaling pathways activated by stress and inflammation. Physiol. Rev. **81**, 807–869 (2001)
3. Kyriakis, J.M.: Signaling by germinal center kinase family of protein kinases. J. Biol. Chem. **274**, 5258–5262 (1999)
4. Johnson, G.L., Lapadat, R.: Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. Science **298**, 1911–1912 (2002)
5. Kroemer, G.: Mitochondrial control of apoptosis: an introduction. Biochem. Biophys. Res. Commun. **304**, 433–435 (2003)
6. Adams, J.M., Cory, S.: Life-or-death decisions by the Bcl-2 protein family. Trends Biochem. Sci. **26**, 61–66 (2001)
7. Tsujimoto, Y., Shimizu, S.: Bcl-2 family: life-or-death switch. FEBS Lett. **466**, 6–10 (2000)
8. Scorrano, L., Korsmeyer, S.J.: Mechanisms of cytochrome c release by proapoptotic BCL-2 family members. Biochem. Biophys. Res. Commun. **304**, 437–444 (2003)
9. Antonsson, B., Martinou, J.C.: The Bcl-2 protein family. Exp. Cell Res. **256**, 50–57 (2000)

10. Earnshaw, W.C., Martins, L.M., Kaufmann, S. H.: Mammalian caspases: structure, activation, substrates, and functions during apoptosis. Ann. Rev. Biochem. **68**, 383–424 (1999)
11. Budihardjio, I., Oliver, H., Lutter, M., Luo, X., Wang, X.: Biochemical pathways of caspase activation during apoptosis. Annu. Rev. Cell Dev. Biol. **15**, 269–290 (1999)
12. Bonni, A., Brunet, A, West, A.E., Datta, S.R., Takasu, M.A., Greenberg, M. E.: Cell survival promoted by the RAS-MAPK signaling pathway by transcription-dependent and -independent mechanisms. Science **286**, 1358–1362 (1999)
13. Datta, S.R., Brunet, A., Greenberg, M.E.: Cellular survival: a play in three Akts. Genes Dev. **13**, 2905–2927 (1999)
14. Shoukat, D., Benjamin, W., Gregory H.: Integrin-linked kinase (ILK): a regulator of integrin and growth factor signaling. Trends Cell Biol. **9**, 319–323 (1999)
15. Sarbassov, D.D., Guertin, D.A., Ali, S.M., Sabatin, D.M.: Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science **307**, 1098–1101 (2005)
16. Kennedy, S.G., Kandel, E.S., Cross, T.K., Hay, N.: Akt/protein kinase B inhibits cell death by preventing the release of cytochrome c from mitochondria. Mol. Cell. Biol. **19**, 5800–5810 (1999)
17. de Vries, T., Knegtel, R.M.A., Holmes, E.H., Macher, B.A.: Fucosyltransferases: structure/function studies. Glycobiology **11**, 119–128 (2001)
18. Narimatsu, H.: Human fucosyltransferases, Tissue distribution of blood group antigens, cancer associated antigens and fucosyltransferase. Protein, Nucleic Acid and Enzyme (in Japanese) **48**, 2394–2403 (1998)
19. Sasaki, K., Kurata, K., Funayama, K., Nagata, M., Watanabe, E., Ohta, S., Hanat, N., Nishi, T.: Expression cloning of a novel α1,3-fucosyltransferase that is involved in biosynthesis of the sialyl Lewis X carbohydrate determinants in leukocytes. J. Biol. Chem. **269**, 14730–14737 (1994)
20. Kannagi, R., Izawa, M., Koike, T., Miyazaki, K., Kimura, N.: Carbohydrate-mediated cell adhesion in cancer metastasis and angiogenesis. Cancer Sci. **95**, 377–384 (2004)
21. Kannagi, R.: Molecular mechanism for cancer-associated induction of sialyl Lewis X and sialyl Lewis A expression—the Warburg effect revisited. Glycoconj. J. **20**, 353–364 (2004)
22. Liu, F., Qi, H.L., Zhang, Y., Zhang, X.Y., Chen, H.L.: Transfection of *c-erb*B2/*neu* gene up regulates the expression of sialyl Lewis X, α1,3 fucosyltransferase VII and metastatic potential in human hepatocarcinoma cell line. Eur. J. Biochem. **268**, 3501–3512 (2001)
23. Liu, F., Qi, H.L., Chen, H.L.: Regulation of differentiation- and proliferation-inducers on Lewis antigens, α-fucosyltransferase and metastatic potential in hepatocarcinoma cells. Br. J. Cancer **268**, 3501–3512 (2001)
24. Wang, Q.Y., Guo, P., Duan, L.L., Shen, Z.H., Chen, H.L.: α-1,3-Fucosyltransferase-VII stimulates the growth of hepatocarcinoma cells via cyclin dependent kinase inhibitor $p27^{Kip1}$. Cell. Mol. Life Sci. **62**, 171–178 (2005)
25. Guo, H.B., Liu, F., Chen, H.L.: Increased susceptibility to apoptosis of human hepatocarcinoma cells transfected with antisense *N*-acetylglucosaminyltransferase V cDNA. Biochem. Biophys. Res. Commun. **264**, 509–517 (1999)
26. Guo, P., Chen, H.J., Wang, Q.Y., Chen, H.L.: Down regulation of *N*-acetylglucosaminyl-transferase V facilitates *all-trans* retinoic acid to induce apoptosis of human hepatocarcinoma cells. Mol. Cell. Biochem. **284**, 103–110 (2005)
27. Taniguchi, N., Miyoshi, E., Ko, J.H., Ikeda, Y., Ihara, Y.: Implication of *N*-acetyl-glucosaminyltransferases III and V in cancer: gene regulation and signaling mechanism. Biochim. Biophys. Acta **1455**, 287–300 (1999)
28. Young, P.R., McLaughlin, M.M., Kumar, S., Kassis, S., Doyle, M. L., McNulty, D., Gallagher, T.F., Fisher, S., McDonnel, P.C., Carr, S.A., Huddleston, M.J., Siebel, G., Porter, T.G., Livi, G.P., Adams, J.L., Lee, J.C.: Pyridinyl imidazole inhibitors of p38 mitogen activated protein kinase bind in the ATP site. J. Biol. Chem. **272**, 12116–12121 (1997)
29. Guo, P., Zhang, Y., Shen, Z.H., Zhang, X.Y., Chen, H.L.: Effect of *N*-acetylglucosaminyl-transferase V on the expressions of other glycosyltransferases. FEBS Lett. **562**, 93–96 (2004)
30. Eom, Y.W., Too, M.H., Woo, C.H., Hwang, K.C., Song, W.K., Yoo, Y.J., Chun, J.S., Kim, J.H.: Implication of small GTPase Rac1 in the apoptosis induced by UV in Rat-2 fibroblasts. Biochem. Biophys. Res. Commun. **285**, 825–829 (2001)
31. Chipuk, J.E., Kuwana, T., Bouchier-Hayes, L., Droin, N.M., Newmeyer, D.D., Schuler, M., Green, D.R.: Direct activation of Bax by p53 mediates mitochondrial membrane permeabilization and apoptosis. Science **303**, 1010–1014 (2004)
32. Obata, T., Glenn, E., Yaffe, M.B.: MAP kinase pathways activated by stress: The p38 MAPK pathway. Crit. Care Med. **28**, 67–77 (2000)
33. Assefa, Z., Vantieghem, A., Declercq, W., Vandenabeeles, P., Vandenheede, J.R., Merlevede, W., de Witts, P., Agostinis, P.: The activation of the c-JunN-terminal kinase and p38 mitogen-activated protein kinase signaling pathways protect HeLa cells from apoptosis following photodynamic therapy with hypericin. J. Biol. Chem. **274**, 8788–8796 (1999)
34. Nagata, Y., Todokaro, K.: Requirement of activation of JNK and p38 for environmental stress-induced erythoid differentiation and apoptosis and of inhibition of ERK for apoptosis. Blood **94**, 853–863 (1999)
35. Zhuang, S.G., Demirs, J.T., Kochevar I.E.: p38 mitogen-activated protein kinase mediates Bid cleavage, mitochondrial dysfunction, and caspase-3 activation during apoptosis induced by singlet oxygen but not by hydrogen peroxide. J. Biol. Chem. **275**, 25939–25948 (2000)
36. Bachelor, M.A., Bowden, G.T.: Ultraviolet A-induced modulation of Bcl-XL by p38 MAPK in human keratinocytes. Post transcriptional regulation through the 3′-untranslated region. J. Biol. Chem. **279**, 42658–42668 (2004)
37. Yu, C.F., Minemoto, Y., Zhang, Y.M., Liu, J., Tang, F.M., Bui, T. N., Xiang, J.L., Lin, A.N.: JNK suppresses apoptosis via phosphorylation of proapoptotic Bcl-2 Family protein BAD. Mol. Cell **13**, 329–340 (2004)
38. Curtin, J.F., Cotter, T.G.: JNK regulates HIPK3 expression and promotes resistance to Fas-mediated apoptosis in Du 145 prostate carcinoma cells. J. Biol. Chem. **279**, 17090–17100 (2004)
39. Saito, T., Miyoshi, E., Sasaki, K., Nakano, N., Eguchi, H., Honke, K., Taniguchi, N.: A secreted type of β1,6-*N*-acetylglucosaminyltransferase-V (GnT-V) induces tumor angiogenesis without mediation of glycosylation. J. Biol. Chem. **277**, 17002–17008 (2002)
40. Okajima,T., Xu, A.G., Lei, L., Irvin K.D.: Chaperone activity of protein O-fucosyltransferase 1 promotes Notch receptor folding. Science **307**, 1559–1603 (2005)
41. Azuma, Y., Ito, M., Taniguchi, A., Matsumoto, K.: Expression of cell surface Lewis X and Y antigens and FUT4 mRNA is increased in Jurket cells undergoing apoptosis. Biochim. Biophys. Acta **1672**, 157–163 (2004)
42. Koike, T., Kimura, N., Miyazaki, K., Yabuta, T., Kumamoto, K., Takenoshita, S., Chen, J., Kobayashi, M., Hosokawa, M., Taniguchi, A., Kojima, T., Ishida, N., Kawakita, M., Yamamoto,

H., Takematsu, H., Suzuki, A., Kozutsumi, Y., Kannagi, R.: Hypoxia induces adhesion molecules on cancer cells: A missing link between Warburg effect and induction of selectin-ligand carbohydrates. Proc. Nat. Acad. Sci. U. S. A. **101**, 8132–8137 (2004)

43. Russell, L., Waring, P., Beaver, J.P.: Increased cell surface exposure of fucose residues is a late event in apoptosis. Biochem. Biophys. Res. Commun. **250**, 449–452 (1998)
44. Ai, Z.W., Zha, X.L., Liu, Y., Chen, H.L.: Effects of retinoic acid on hepatocarcinoma cell line. J. Tumor Mark. Oncol. **5**, 59–70 (1990)
45. Chen, H.L., Dong, S.C., Ju, T.Z., Yang, X.P.: Effect of retinoic acid on the structure of N-glycans on the surface of human hepatocarcinoma cells and its enzymatic mechanism. J. Cancer Res. Clin. Oncol. **121**, 397–401 (1995)
46. Liu, F., Qi, H.L., Chen, H.L.: Effects of *all-trans* retinoic acid and epidermal growth factor on the expression of *nm23*-H1 in human hepaocarcinoma cells. J. Cancer Res. Clin. Oncol. **126**, 85–90 (2000)
47. Duan, L.L., Guo, P., Zhang, Y., Chen, H.L.: Regulation of metastasis-suppressive gene *nm23*-H1 on glycosyltransferases involved in the synthesis of sialyl Lewis antigens. J. Cell. Biochem. **94**, 1248–1257 (2005)