View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

FEBS 29267 FEBS Letters 579 (2005) 1279–1284

Overexpression of small glutamine-rich TPR-containing protein promotes apoptosis in 7721 cells

Hanzhou Wang, Hailian Shen, Yanlin Wang, Zejuan Li, Hongyan Yin, Hongliang Zong, Jianhai Jiang, Jianxin Gu*

State Key Laboratory of Genetic Engineering & Gene Research Center, Shanghai Medical College of Fudan University, Shanghai 200032, People's Republic of China

Received 8 October 2004; revised 7 December 2004; accepted 20 December 2004

Available online 26 January 2005

Edited by Michael R. Bubb

Abstract **It is known that small glutamine-rich TPR-containing protein (SGT) is the member of TPR motif family. However, the biological functions of SGT remain unclear. In this paper, we report that SGT plays a role in apoptotic signaling. Ectopic expression of SGT enhances DNA fragment and nucleus breakage after the induction of apoptosis. Increasing mRNA level of SGT is also observed in 7721 cells undergoing apoptosis, knockdown the expression of endogenous SGT contributes to the decrease of apoptosis of 7721 cells. Deletion analysis reveals that TPR domain is critical to pro-apoptotic function of SGT. Furthermore, we demonstrated that the PARP cleavage and cytochrome *c* release are enhanced when SGT is overexpressed in 7721 cells during apoptosis. Collectively, our results indicate that SGT is a new pro-apoptotic factor.**
© 2005 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

Keywords: Small glutamine-rich TPR-containing protein; Heat shock protein 70; Heat shock cognate protein 70; Apoptosis; TPR

1. Introduction

The small glutamine-rich TPR-containing protein (SGT), which was also termed as UBP, was first identified as a binding partner of the non-structural protein of autonomous parvovirus H-1 [1]. Human SGT consists of three known TPR motifs which were thought to mediate in protein–protein interaction [2]. TPR motifs, which are found in a variety of proteins, regulate the cell cycle, protein folding, transcription, protein transport, ubiquitin-protesomes, hormone receptor signaling, and several other pathways [3]. However, the significance of TPR domain for the biological roles of SGT is unknown, and the specific biologic functions of SGT remain to be elucidated.

Recently, some interacting partner of SGT, such as NS1, human immune-deficiency virus-encoded Vpu protein [4,5], the growth hormone receptor [6] and myostatin [7], were characterized one after another, but the biological significance of these interactions is not clear. However, more and more evidences suggest that SGT may be a cochaperone. It was demonstrated that SGT can bind both heat shock cognate protein (Hsc70) and heat shock protein (Hsp70), and regulate their chaperone activity [8,9]. In a *Caenorhabditis elegans* model for Alzheimer disease, SGT was found to be present in protein complexes including β-amyloid peptide, several chaperones and chaperone-like protein. Furthermore, when SGT was knockdown by dsRNA, the toxic effect of exogenous expression of β-amyloid peptide was significantly reduced, implying that SGT may be involved in β-amyloid peptide-dependent pathogenicity in this model system [10]. On the other hand, rat SGT promoted the chaperone activity of Hsc70 in a tripartite complex containing the cysteine string protein (Csp) and Hsc70, which is thought to participate in vesicle-mediated neuronal signal transduction [11]. Most recently, a cellular function of SGT was elucidated. It was showed that SGT is present throughout the cell cycle and accumulated in midzone and the midbody. Depletion of SGT contributes to the increase of the mitotic index [12].

The fact that SGT-like protein is present in almost all eukaryotes such as yeast, worm, fly, and mammals [2] and a great diversity of cellular functions of all known TPR motifs raise the possibility that SGT has an additional cellular function. In this report, we substantiate this idea and present evidences to confirm that SGT is involved in apoptosis.

2. Materials and methods

2.1. Materials

The 7721 hepatocarcinoma cell line and COS-1 cell line was obtained from the Institute of Cell Biology, Academic Sinica. RPMI 1640 medium and DMEM was from Sigma. LipofectAMINETM Reagent was purchased from Invitrogen. Leupeptin, aprotinin, and phenylmethylsulfonyl fluoride were purchased from Sigma. [α-^{32}P]ATP, Hybond polyvinylidene difluoride membrane, goat anti-mouse horseradish, and goat anti-rabbit horseradish peroxidase secondary antibody were from Santa Cruz Biotechnology, the monoclonal Hsp60 antibody was from Stressgen Biotechnologies, the polyclonal cytochrome *c* antibody was from Cell Signaling, the monoclonal PARP antibody was from Calbiochem, the monoclonal GFP antibody was from Roche, and the enhanced chemiluminescence assay kit were purchased from Amersham Biosciences. Annexin-V-FITC recombinant protein was purchased from Bender MedSystems. Cycloheximide (CHX) was from Sigma, Staurosporine (STS) was from Cell Signaling. Other reagents were commercially available in China.

2.2. Plasmid construction

Full-length cDNA of SGT was generated through PCR with 0.1 μg of human fetal liver cDNA library [13] as the template and the primers used were primer 1 (sense) (5′-GATGAATTCATGGACAACAAGAAGCGCC-3′) and primer 2 (antisense) (5′-GATCTCGAGTCACTCCTGCTGGTCGTC-3′), the PCR product was inserted in-frame into

*Corresponding author. Fax: +86 21 64164489.
E-mail address: jxgu@shmu.edu.cn (J. Gu).

0014-5793/$30.00 © 2005 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.febslet.2004.12.092

pcDNA3.0 vector via *Eco*RI and *Xho*I restriction sites. The cDNA of SGT without the stop code was cloned to pcDNA3.1-myc-His-A$^-$ vector via *Eco*RI and *Bam*HI restriction sites. Various deletion mutants were generated through PCR using cDNA of SGT as the template and subcloned into pcDNA3.0 vector via the same sites. The sense oligonucleotide (5′-GATCC ACGTGGAGGCCGTGGCTTA TTCAAGAGA TAAGCCACGGCCTCCACGTGC TTTTTTGGAAA-3′) and antisense oligonucleotide (5′-AGCTTTTCCAAAAAA GCACGTGGAGGCCGTGGCTTA TCTCTTGAA TAAGCCACGGCCTCCACGT G-3′) were annealed and then cloned to piSilencer 2.1 vector. Clones were sequenced to identified the siRNA template insert.

2.3. Cell culture, transfection, FCM analysis and cell count

The 7721 Cells were cultured in RPMI-1640 medium supplemented with 10% (v/v) fetal calf serum, 100 units/ml of penicillin and 50 μg/ml of streptomycin at 37 °C under 5% CO_2 in humidified air. African green monkey kidney COS-1 cells (Institute of Cell Biology, Academic Sinica) were cultured in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal calf serum, 100 units/ml penicillin, and 50 μg/ml streptomycin. Cells were transfected with different plasmids using lipofectAMINE according to the manufacturer's recommendation. Clone selection was conducted by culturing with G418 (400 μg/ml). Stable transfectants with high expression of the target genes were identified by Northern-blot analysis.

For growth curves, 3×10^3 cells were plated. Subsequently cells were counted at 72 h intervals in triplicate using a hemocytometer [14].

After fixation in cold ethanol for 12 h, cells were washed again with phosphate-buffered saline (PBS) and then stained with a solution containing 50 μg/ml propidium iodide (PI), 250 μg/ml RNase, and 0.1% Triton X-100 and analyzed by quantitative flow cell cytometry with standard optics of FACScan flow cytometer (BD PharMingen FACStar) employing the Cell Quest program.

2.4. Induction and measurement of apoptosis

Hoechst 33258 staining of nuclei. Morphological changes in the nuclear chromatin in cells undergoing apoptosis were detected by staining with the DNA binding fluorochrome Hoechst 33258. Cells were grown on glass coverslips and treated with either STS or CHX. Cells were washed twice with PBS and fixed by incubation in 4% paraformaldehyde for 30 min. Following washes with PBS, cells were incubated in 25 μg/ml Hoechst 33258 solutions for 30 min in the dark chamber. Coverslips were then washed with PBS and analyzed by fluorescence microscopy.

DNA fragmentation assay. After treating with CHX for the indicated time, cells were harvested and washed with PBS followed by centrifugation at $12000 \times g$ in a microcentrifuge for 30 s at 4 °C. Cells were lysed in solution (0.5% SDS, 10 mM EDTA, 0.5 mg/ml proteinase K, and 50 mM Tris–HCl, pH 8.0). DNA was extracted with a standard phenol–chloroform method and precipitated with cold ethanol. The pellets were dried and resuspended in 100 ml of 20 mM Tris–HCl, pH 8.0. After digesting RNA with RNase (0.1 mg/ml) at 37 °C for 1 h, samples were electrophoresed through a 1.2% agarose gel DNA was visualized with UV light.

Apoptosis analysis by annexin-V PI staining. This analysis was performed as described before [15]. To use annexin-V to detect cell surface PS, the cells were washed twice in PBS and then resuspended in binding buffer (10 mM HEPES/NaOH, pH 7.4, 140 mM NaCl, and 2.5 mM $CaCl_2$). 195 μl of cell suspension containing 10^5 cells was taken, and 5 μl of annexin-V-FITC was added, mixed, and incubated for 10 min in the dark. After that, the cells were washed twice in PBS and resuspended in 190 μl of binding buffer. 10 μl of 20 μg/ml PI was added and then analyzed by fluorescence-activated cell sorting. Apoptotic cells could be stained with annexin-V but not PI.

2.5. Northern blot, Western blot and isolation of mitochondria and cytosolic fraction

Total RNA was isolated using Trizol reagent according to the manufacturer's instructions. RNA concentrations were calculated by measuring UV light absorbance at 260 nm. Each RNA sample (40 μg) was denatured and subjected to electrophoresis in 1% agarose gel containing 2.2 M formaldehyde. RNA was transferred to Hybond-N$^+$ nylon membrane (Amersham, UK) and cross-linked by UV irradiation using a GS Gene Linker UV chamber (Bio-Rad, CA, USA). Northern-blot analysis was performed as described before [16], using the [α-^{32}P]dATP-labeled SGT fragments as probes. The widely used cDNA of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was adopted as an intrinsic standard during Northern-blot analysis.

Total protein (40 μg) was extracted and resolved to immunoblot analysis as described before [15]. To isolate the mitochondrial and cytosolic fraction, cells were washed with PBS, resuspended in CFS buffer (10 mM HEPES–NaOH (pH 7.2), 0.22 M mannitol, 68 mM sucrose, 2 mM NaCl, 2.5 mM KH_2PO_4, 0.5 mM EGTA, 2 mM $MgCl_2$, 5 mM sodium pyruvate, 0.1 mM phenylmethylsulfonyl fluoride, and 1 mM dithiothreitol) and homogenized by aspiration through a 22-gauge needle. The homogenate was centrifuged at $700 \times g$ for 5 min, and the resulting supernatant was centrifuged for 10 min at $10000 \times g$ to obtain a pellet enriched in mitochondria and a postmitochondrial supernatant as the cytosolic fraction.

2.6. RT-PCR analysis

Total RNA was extracted from 7721 cells using Trizol reagent. Then, 0.5 μg of RNA from each sample was reverse-transcribed into cDNA according to the manufacturer's manuals. The cDNA was amplified by PCR with the following primers: SGT (950 bp) 5′-GATGAATTCATGGACAACAAGAAGCGCC-3′ (sense primer) and 5′-GATCTCGAGTCACTCCTGCTGGTCGTC-3′ (antisense primer). The expression levels of actin (5′-aaccgtgaaaagatgacccag-3′ sense primer, 5′-ctcctgcttgctgatccacat-3′ antisense primer) analyzed by RT-PCR assays were used as a internal control. The expression level of SGT during apoptosis was quantitated by densitometric analysis.

3. Results

3.1. Ectopic expression of SGT promotes apoptosis in 7721 cell

It is known that three TPR motif proteins (including CHIP, TPR2, and Strap) can modulate cell death in different mechanism [17–19]. Therefore, it raised the possibility that SGT may be also involved in apoptosis. To test this idea, pcDNA3-SGT expression vector and pcDNA3 empty vector were transfected into 7721 cells. After selection of the G418-resisted colonies, the expression of SGT mRNA was highly expressed in pcDNA3-SGT transfected cells (7721/SGT) compared with that in the parent and pcDNA3-transfected cells (Mock, Fig. 1A). Next, we investigated the role of SGT in apoptosis. After exposure to CHX for the indicated time, the genomic DNA were extracted and then resolved in 1.2% agarose gel, Fig. 1B shows that SGT induced fragmentation of chromosomes (lanes 4 and 6), whereas the fragment of chromosomes did not appear in Mock cells (lanes 3 and 5). Furthermore, we quantitated the apoptotic cells by annexin-V and PI staining. After the 7721 cells were incubated with CHX for the indicated time, the percentage of apoptotic cells was not be altered (Fig. 1C, panels a and b). However, overexpression of SGT sensitized 7721 cells to apoptosis, the percentage of apoptotic cells was increased from 4% to 19% (Fig. 1C, panels c and d). Thus, we concluded that SGT is a pro-apoptotic protein, but not an anti-apoptotic factor like other TPR family proteins which are associated with apoptosis.

3.2. Endogenous SGT is associated with apoptosis in 7721 cells

Above results indicated that exogenous SGT displayed the pro-apoptotic activity. Thus, we asked whether endogenous SGT is also involved in apoptosis. We therefore examined whether the mRNA level of SGT was altered during apoptosis. Cells were incubated with either CHX or STS for the indicated time, following a time-course analysis the change of SGT mRNA during apoptosis. The mRNA of SGT was increased when cells treated with STS for 2 h (Fig. 2A, lane 2), while

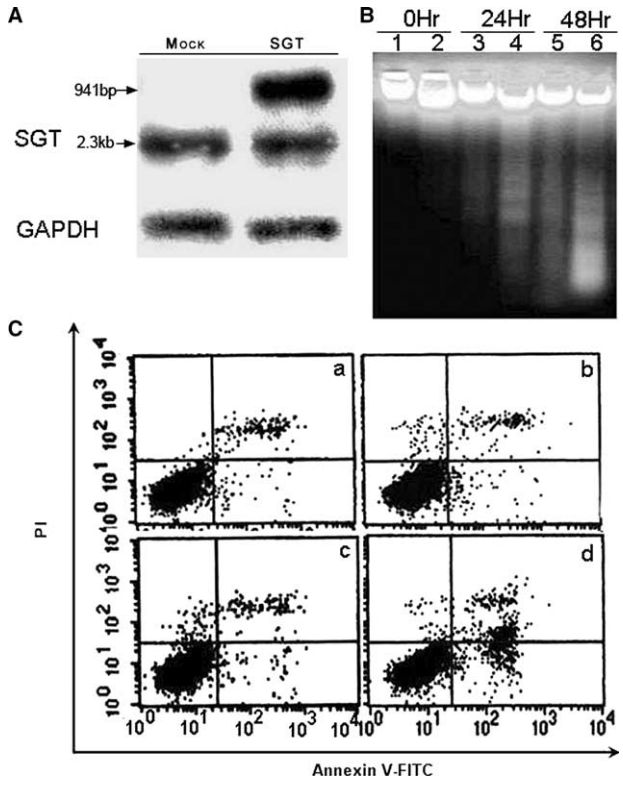

Fig. 1. Ecotopic expression of SGT promotes apoptosis in 7721 cells. (A) Identification of stable 7721 cell lines expressing pcDNA3.0-SGT (7721/SGT). Total RNA (30 μg) from mock cells (lane Mock) and 7721/SGT cells (lane SGT) were extracted and subjected to Northern-blot analysis. (Note: the exogenous SGT mRNA is 941 bp, the endogenous SGT mRNA is 2.4 kb). GAPDH was used as an internal control. (B) Stably transformed 7721 cell lines expressing pcDNA3.0 (Mock, lanes 1, 3, and 5) or 7721/SGT (lanes 2, 4, and 6) were treated with 10 μg/ml CHX for the indicated time. DNA were isolated, resolved on an agarose gel, stained with ethidium bromide, and visualized for DNA fragmentation by UV transillumination. (C) Mock cells (panels a and b) and 7721/SGT cells (panel c and d) were treated with (panels b and d) or without (panels a and c) 10 μg/ml CHX for 24 h, the cells were stained with annexin-V and PI as described in Section 2.

the increase of SGT expression was apparent only after 12 h exposure of cells to CHX (Fig. 2B, lane 2). Furthermore, the upregulation of SGT mRNA is prior to the onset of apoptosis (data not shown). Next, we used RNA interference to suppress the expression of SGT to test our above hypothesis. We first verified the efficiency of the designed siRNA in COS-1 cells (Fig. 2C, left panel), then we used the siRNA to knockdown the endogenous in 7721 cells (Fig. 2C, right panel). It is showed that transfection with the siRNA SGT decreased the apoptosis by about 40%, compared with cells transfected with mock vector. From the above results, we can conclude that the endogenous SGT is also involved in apoptosis in 7721 cells.

3.3. TPR domain is required for the SGT pro-apoptotic activity

Previous studies showed that TPR domain is critical for the anti-apoptotic function of several TPR family proteins [17,18]. To investigate whether the three TPR motifs are required for the pro-apoptotic function of SGT, we constructed a series of deletion mutants of SGT as indicated (Fig. 3B). These mutants were transfected into 7721 cells, respectively, to test its apoptosis-inducing ability. It is to be noted that only the domain containing the three TPR motifs (Fig. 3A, F2, F4 mutant) displayed its capacity of pro-apoptosis. On the contrary, overexpression of the mutant containing the other domain could not induce apoptosis. These results imply that three TPR motifs are crucial for the apoptosis-inducing ability of SGT.

3.4. Ectopic expression of SGT does not affect cellular proliferation or cell cycle progression

We considered the possibility that ectopic expression of SGT might exert its pro-apoptotic activity by affecting the rate of cellular proliferation. We therefore compared the growth kinetics of 7721 cells and 7721/SGT cells. Fig. 4A showed that the growth curves of 7721 cells were similar with those of 7721/SGT cells, which indicated that SGT expression does not affect the cellular proliferation. To gain further insight into whether ectopic expression of SGT has a subtle effect on cell growth, we made use of DNA flow cytometry to compare the cell cycle of 7721/SGT cells with that of mock cells, the data (Fig. 4B) showed that the distribution of 7721/SGT in G1, S, G2/M phase were not different from that of mock cells. Taken together, we concluded that SGT exerts its pro-apoptotic function without affecting cellular proliferation and cell cycle.

3.5. Enhancement of PARP cleavage and release of cytochrome c from mitochondria by the overexpression of SGT

Caspase activity and cytochrome *c* release are known to be the major events in apoptotic tumor cells. Therefore, we asked whether caspase activation and cytochrome *c* release are modulated by the overexpression of SGT. Fig. 5A showed that the number of apoptotic cell was obviously reduced when 7721/SGT cells were incubated with pancaspase inhibitor (V-ZAD-FMK) just one hour before treatment with CHX. The above observation indicated that the activation of caspases may be involved in apoptosis induced by SGT expression. For it has been well documented that the activation of Caspase 3 is responsible for the cleavage of PARP which is required for cell death in various cell lines [20], we therefore examined the cleavage of PARP during apoptosis to assess the activation of Caspase 3. As shown in Fig. 5B, the 89 kDa fragment of PARP in 7721/SGT cells which was cleaved by Caspase 3 was more apparent than that of mock cells. Furthermore, after exposure to STS for 3 h, cytochrome *c* was released from mitochondria to cytosol in 7721/SGT cells but not in mock cells (Fig. 5C). Therefore, we proposed that the activation of caspases and cytochrome *c* release occur in 7721/SGT cells during apoptosis.

4. Discussion

The tetratricopeptide, which was originally identified in 1990, comprises a degenerate 34-amino acid repeated motif that is widespread in a variety of different organisms ranging from bacteria to humans [3,21,22]. To date, the known three-dimensional structure data have shown that a TPR motif contains two anti-parallel α-helices, therefore tandem arrays of TPR motifs generate a right-handed helical structure with an amphipathic channel that might accommodate the complementary region of the target protein [3,22]. Owing to the specific structure, TPR mediates protein–protein interactions and the assembly of macromolecular protein complexes.

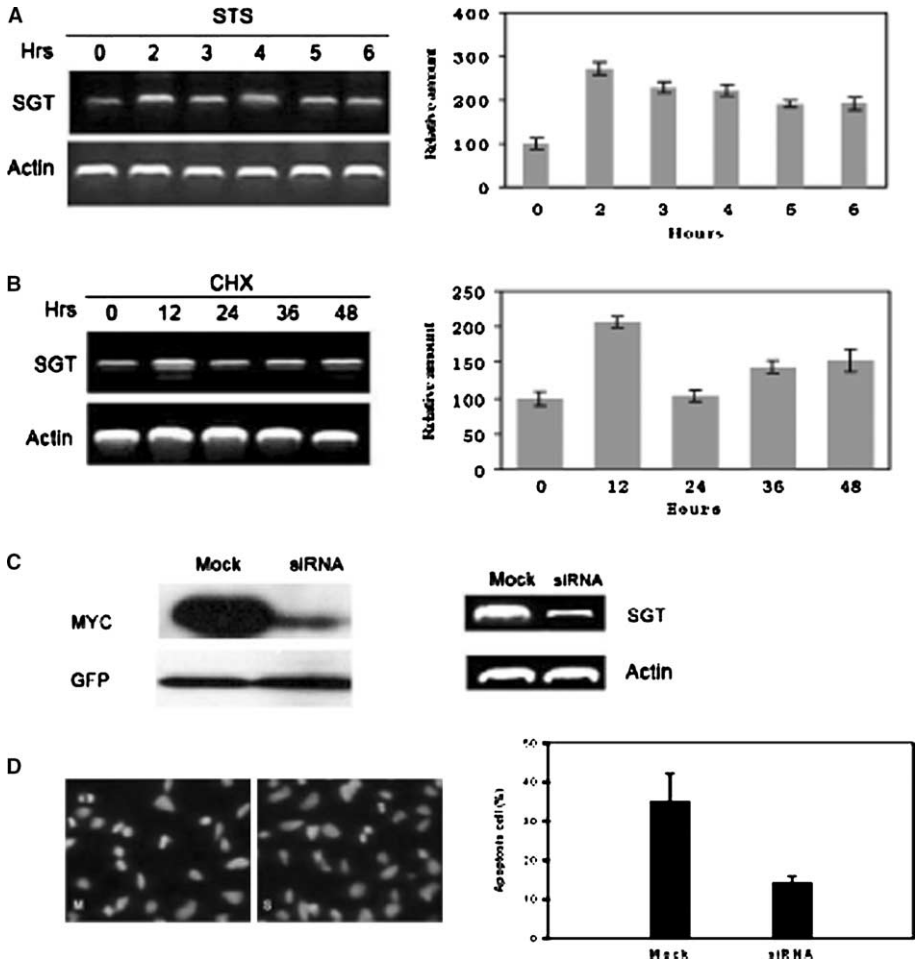

Fig. 2. The endogenous SGT is associated with apoptosis. After exposure of 7721 cells to STS (panel A) or CHX (panel B) for the indicated time, total RNA was isolated from 7721 cells, 0.5 μg RNA was used to perform RT-PCR analysis to assess the level of SGT mRNA, actin was used as a internal control. Results quantitated by densitometric analysis are representative of three repeated experiments. (C) Knockdown of SGT by RNA interference. Left panel: the pcDNA3.1/myc-His(−) A-SGT and pEGFP were cotransfected to COS-1 cells with mock pSilencer vector (lane Mock) or siRNA SGT (lane siRNA), then the expression of SGT was detected by Western blot with anti-myc antibody. Western blot for GFP was used as a control for equal protein and transfection efficiency. Right panel: the mock pSilencer vector (Mock) or siRNA SGT (siRNA) were, respectively, transfected to 7721 cells, the level of SGT mRNA was analyzed by RT-PCR. (D) Suppression of endogenous SGT decreased apoptosis of 7721 cells. The mock pSilencer vector (left panel: M; right panel: Mock) or siRNA SGT (left panel: S; right panel: siRNA) were, respectively, transfected to 7721 cells, 48 h after transfection, cells were treated with STS for 6 h, then cells were fixed and stained with Hoechst 33258. The apoptotic cells were counted by fluorescence microscopy. Representative results (means ± S.D.) from one out of three independent experiments are presented (right panel).

Moreover, extensive evidences indicate that TPR participates in a variety of biological processes, such as cell cycle control, transcriptional repression, protein folding, protein transport and neurogenesis [3,21,22]. For instance, anaphase-promoting complex (APC), which contains TPR motif protein including Cdc16, Cdc23, and Cdc27, functions as a mitotic E3 ligase that target proteins for degradation at the onset of anaphase [23,24]. Ssn6, which contains 10 copies of TPR motifs, forms a complex with other proteins, and then the complex is recruited by different transcription factors to repress gene transcription [25].

Nevertheless, the specific functions of the individual TPR protein are still to be elucidated. As a member of TPR family proteins, SGT has been found to interact with several proteins including Myostatin, NS1, Vpu, etc. However, little is know about the biological significance of these interactions. In the present study, we showed that SGT had a role in apoptosis. The apoptotic activity of SGT was confirmed through DNA fragment, Hoechst staining and annexin-V PI staining. In fact, several proteins have been reported to be involved in apoptosis. Moreover, our results showed that the endogenous SGT is also associated with apoptosis (Fig. 3). The first TPR motif protein that was reported to be associated with apoptosis is Strap, which is present in the p300/CBP transcription factor complex. Strap enhances the stability of p53 and in turn regulates apoptosis by modulating MDM2 activity [18]. Next, TPR2, which carries both a J domain and TPR motif, interacts with Rad9 that controls apoptosis by interacting with Bcl-2 protein, therefore it is proposed to be involved in apoptosis [19]. Finally, CHIP, which was originally identified as a protein that interacted with Hsc70 and carries both a TPR motif and U-box, cooperates with Hsp70 and Parkin and inhibits cell death induced by unfolded Pael-R in some types of Parkinsons's disease through the ubiquitin proteasome protein degra-

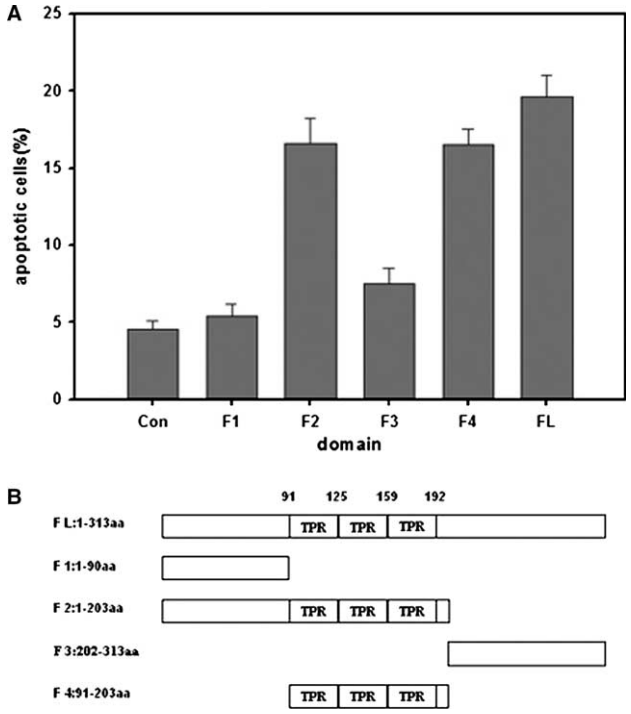

Fig. 3. TPR domain is important for the apoptotic activity of SGT. (A) 7721 cells were transfected with a series of deletion mutants shown in (B), 24 h after transfection, cells were fixed and stained with Hoechst 33258. Then, the apoptotic cells were counted by fluorescence microscopy. Representative results (means ± S.D.) from one out of four independent experiments are presented. (B) Schematic diagram of SGT and its mutants, SGT contains three TPR motifs as indicated.

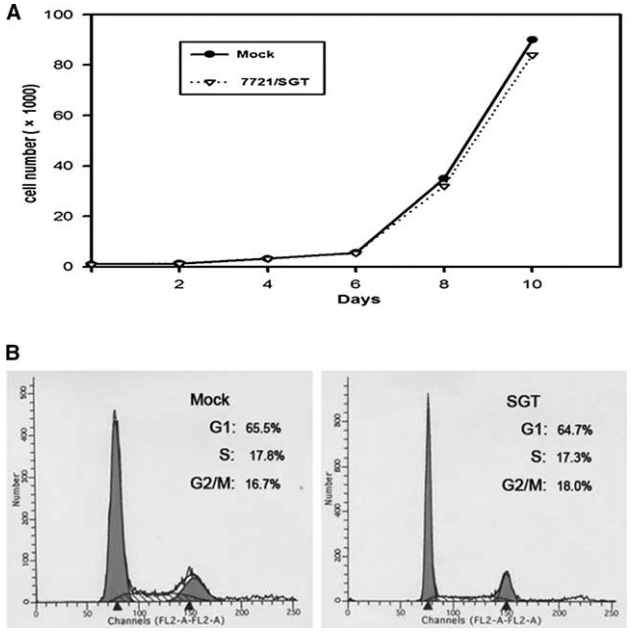

Fig. 4. SGT does not affect cellular proliferation and cell cycle. (A) Cellular proliferation analysis. 3×10^3 cells of 7721 cell (circle) or 7721/SGT (triangle) were seeded and cell number were counted by && at the indicated time. (B) Cell cycle analysis. The distribution of each phase of asynchronous cells including 7721 and 7721/SGT cells were quantitated by flow cytometry. The percentage of G1, S, G2/M are listed as indicated.

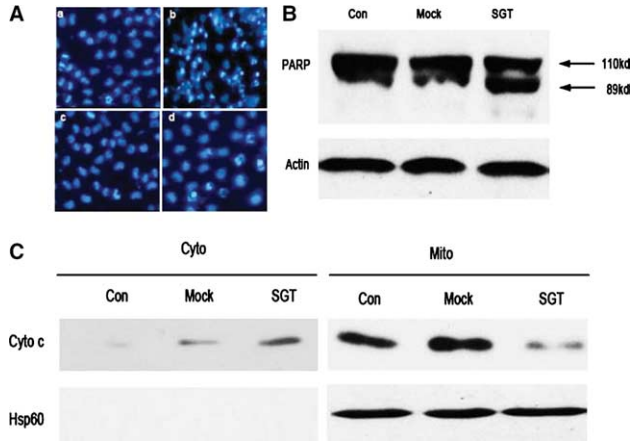

Fig. 5. Caspase 3 activity and cytochrome *c* release are induced by SGT overexpression. (A) Mock cells (panels a and c) and 7721/SGT cells (panels b and d) were incubated with (panels b and d) or without (panels a and c) the pancaspase inhibitor Z-VAD-FMK (50 μM) for one hour followed by treating with 10 μg/ml CHX for 24 h. After staining by Hoechst 33258, the apoptotic cells were counted by fluorescence microscopy. (B) Control 7721 cells (con), 7721 stably expressing pcDNA3.0 (Mock) and 7721 stably expressing pcDNA3.0-SGT (7721/SGT) were incubated with 0.2 μM STS for 3 h, 40 μg total protein were extracted and subjected to immunoblot analysis for PARP. (C) Three cell lines described above were, respectively, incubated with 0.2 μM STS for 3 h, cytosolic and mitochondrial fractions were isolated and subjected to immunoblot analysis for cytochrome *c* and Hsp60.

dation pathway [17]. Recently, CHIP was reported to induce trimerization and transcriptional activation of HSF1 and confers protection against apoptosis [26]. Above all, the TPR domain of all protein described above are important for their anti-apoptotic activity. As expected, we found that the TPR domain of SGT is also responsible for its function in apoptosis. However, the TPR domain of SGT is not anti-apoptotic like other TPR family protein but is pro-apoptotic.

Despite that our observations indicated that SGT exerts a role in apoptosis, it is unlikely that SGT is a direct effector in apoptosis according to our known knowledge that the known TPR motif proteins are not the effector [17–19,26]. On the contrary, the regulator role of SGT in apoptosis depends on its ability to interact with other proteins. Therefore the identification of the apoptosis-associated interacting partner for SGT should help define its molecular mechanism in apoptosis. At the molecular level, Hsp70 and Hsc70 may be the best candidate binding partners which are involved in apoptosis. Hsp70 or Hsc70 protects cells against multiple apoptotic stimuli including DNA damage, UV irradiation, serum withdrawal and a variety of chemotherapeutic agents [27]. Much of existing evidence seem to indicate that Hsp70 protects apoptosis within the mitochondria-mediated pathway and specifically (but not necessarily) downstream of mitochondria at the level of the apoptosome. For example, Saleh et al. [28] showed that the interaction between Hsp70 and Apaf-1 inhibited the oligomerization of Apaf-1 and subsequent recruitment and activation of procaspase-9. But another report showed that Hsp70 maintain the oligomer in a conformation incompatible with procaspase-9 recruitment by preventing exposure of the Apaf-1 CARD domain [29]. On the other hand, it is showed that Hsp70 prevents cleavage and activation of Bid, independent of its chaperone activity [30]. Moreover, recent

data also indicated that Hsp70 cooperates with Hsp40 to prevent the translocation of BAX from cyotosol to mitochondria and subsequent inhibit nitric-oxide-induced apoptosis [31]. Based on the above observations, it is reasonable to speculate that SGT can negatively modulate the chaperone activity of Hsc70 or Hsp70 [8,9] and in turn make 7721 cells more susceptible to apoptosis because the chaperone activity of either Hsc70 or Hsp70 is necessary to its capacity of anti-apoptosis [32,33], but it is to be noted that the chaperone activity of Hsc70 can also be positively regulated by SGT [11]. The contrary results imply that SGT may also protect apoptosis, which is consistent with the observation reported by Winnefeld et al. [12] in NBE cells. A rational explanation for above controversial observations is that SGT exerts its anti-apoptotic function or pro-apoptotic function through two different pathways, respectively, in different cell lines. That is to say, it is possible that SGT has dual functions in apoptosis. However, in 7721 cells it displays the pro-apoptotic activity.

In brief, our study demonstrated that SGT is involved in apoptosis in 7721 cells, and like other TPR motif protein, the TPR domain is important for its function in apoptosis, the caspase activation and cytochrome *c* release, the pivotal events in apoptosis, are also observed in the SGT overexpressing cells. However, further work need to be performed to elucidate how SGT sensitizes cells to apoptosis.

Acknowledgments: This work was supported by National Natural Scientific Foundation of China (30330320) and Mizutani Foundation for Glycoscience of Japan (040025).

References

[1] Cziepluch, C., Kordes, E., Poirey, R., Grewenig, A., Rommelaere, J. and Jauniaux, J.-C. (1998) J. Virol. 72, 4149–4156.
[2] Kordes, E., Savelyeva, L., Schwab, M., Rommelaere, J., Jauniaux, J.C. and Cziepluch, C. (1998) Genomics 52, 90–94.
[3] D'Andrea, L.D. and Regan, L. (2003) Trends Biochem. Sci. 28, 655–662.
[4] Callahan, M.A., Handley, M.A., Lee, Y.H., Talbot, K.J., Harper, J.W. and Panganiban, A.T. (1998) J. Virol. 72, 8461.
[5] Callahan, M.A., Handley, M.A., Lee, Y.H., Talbot, K.J., Harper, J.W. and Panganiban, A.T. (1998) J. Virol. 72, 5189–5197.
[6] Schantl, J.A., Roza, M., De Jong, A.P. and Strous, G.J. (2003) Biochem. J. 373, 855–863.
[7] Wang, H., Zhang, Q. and Zhu, D. (2003) Biochem. Biophys. Res. Commun. 311, 877–883.
[8] Angeletti, P.C., Walker, D. and Panganiban, A.T. (2002) Cell Stress Chaperones 7, 258–268.
[9] Wu, S.J., Liu, F.H., Hu, S.M. and Wang, C. (2001) Biochem. J. 359, 419–426.
[10] Fonte, V., Kapulkin, V., Taft, A., Fluet, A., Friedman, D. and Link, C.D. (2002) Proc. Natl. Acad. Sci. USA 99, 9439–9444.
[11] Tobaben, S., Thakur, P., Fernandez-Chacon, R., Sudhof, T.C., Rettig, J. and Stahl, B. (2001) Neuron 31, 987–999.
[12] Winnefeld, M., Rommelaere, J. and Cziepluch, C. (2004) Exp. Cell. Res. 293, 43–57.
[13] Zhang, S., Cai, M., Zhang, S., Xu, S., Chen, S., Chen, X., Chen, C. and Gu, J. (2002) J. Biol. Chem. 277, 35314–35322.
[14] Liu, W., et al. (2004) J. Biol. Chem. 279, 10167–10175.
[15] Chen, S., et al. (2003) J. Biol. Chem. 278, 20029–20036.
[16] Zhu, D., Shen, A., Wang, Y., Gu, X. and Gu, J. (2003) FEBS Lett. 538, 163–167.
[17] Imai, Y., Soda, M., Hatakeyama, S., Akagi, T., Hashikawa, T., Nakayama, K.I. and Takahashi, R. (2002) Mol. Cell 10, 55–67.
[18] Demonacos, C., Krstic-Demonacos, M. and La Thangue, N.B. (2001) Mol. Cell 8, 71–84.
[19] Xiang, S.L., Kumano, T., Iwasaki, S.I., Sun, X., Yoshioka, K. and Yamamoto, K.C. (2001) Biochem. Biophys. Res. Commun. 287, 932–940.
[20] Boulares, A.H., Yakovlev, A.G., Ivanova, V., Stoica, B.A., Wang, G., Iyer, S. and Smulson, M. (1999) J. Biol. Chem. 274, 22932–22940.
[21] Lamb, J.R., Tugendreich, S. and Hieter, P. (1995) Trends Biochem. Sci. 20, 257–259.
[22] Blatch, G.L. and Lassle, M. (1999) BioEssays 21, 932–939.
[23] King, R.W., Peters, J.M., Tugendreich, S., Rolfe, M., Hieter, P. and Kirschner, M.W. (1995) Cell 81, 279–288.
[24] Lamb, J.R., Michaud, W.A., Sikorski, R.S. and Hieter, P.A. (1994) EMBO J. 13, 4321–4328.
[25] Tzamarias, D. and Struhl, K. (1995) Genes Dev. 9, 821–831.
[26] Dai, Q., et al. (2003) EMBO J. 22, 5446–5458.
[27] Samali, A. and Orrenius, S. (1998) Cell Stress Chaperones 3, 228–236.
[28] Saleh, A., Srinivasula, S.M., Balkir, L., Robbins, P.D. and Alnemri, E.S. (2000) Nat. Cell Biol. 2, 476–483.
[29] Beere, H.M., et al. (2000) Nat. Cell Biol. 2, 469–475.
[30] Gabai, V.L., Mabuchi, K., Mosser, D.D. and Sherman, M.Y. (2002) Mol. Cell. Biol. 22, 3415–3424.
[31] Gotoh, T., Terada, K., Oyadomari, S. and Mori, M. (2004) Cell Death Differ. 11, 390–402.
[32] Mosser, D.D., Caron, A.W., Bourget, L., Denis-Larose, C. and Massie, B. (1997) Mol. Cell. Biol. 17, 5317–5327.
[33] Mosser, D.D., Caron, A.W., Bourget, L., Meriin, A.B., Sherman, M.Y., Morimoto, R.I. and Massie, B. (2000) Mol. Cell. Biol. 20, 7146–7159.