Golgi-SNARE GS28 potentiates cisplatin-induced apoptosis by forming GS28–MDM2–p53 complexes and by preventing the ubiquitination and degradation of p53

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INTRODUCTION

Cisplatin is an important anticancer agent used to cross-link DNA and inhibit genome replication in cancer cells. Genotoxic agents such as cisplatin act mainly by inducing apoptosis in treated cells [1,2]. Nonetheless, the development of cisplatin resistance during cancer treatment remains a major clinical problem. Therefore the mechanisms underlying cisplatin resistance and the strategies that could be used to prevent this process have been studied extensively in the past [3–5]. The mechanisms underlying cisplatin resistance are complex and depend on several factors, including an inadequate drug exposure and accumulation of alterations in cancer cells [6]. The alterations observed in chemoresistant cancer cells include increased drug efflux, decreased drug influx, activation of detoxification systems, alterations of the drug’s target(s), increased DNA repair, resistance to apoptosis and unusual oncogene expression [7–11]. Although the cellular targets of cisplatin have been identified, less is known about the processes responsible for the precise molecular components involved in resistance to this drug. Using genome-wide screening, we recently identified several genes, including NAPA (N-ethylmaleimide-sensitive factor attachment protein α) and CITED2 (CBP [CREB (cAMP-response-element-binding protein)]-binding protein/p300-interacting transactivator, with glutamate/aspartate-rich C-terminal domain 2), as being consistently overexpressed in cisplatin-resistant cells [12–14]. Notably, the products of these two ‘chemoresistance’ genes were shown to induce resistance to cisplatin in a p53-dependent manner.

The relevance of the tumour suppressor p53 in cancer is illustrated by the observation that more than 50% of human cancers harbour mutations in the p53 gene (TP53), and a large proportion of the remaining wild-type p53 cancer cells show altered p53 pathways. Mouse studies and clinical evidence suggest that p53 is required for efficient execution of the apoptosis in tumour cells, and that p53 mutations are associated with pleiotropic resistance to chemotherapy, therefore indicating that p53 represents a critical target of chemotherapeutic drugs [15,16]. On the other hand, oncogenes such as the adenovirus E1A gene can sensitize mouse fibroblasts to apoptosis induced by DNA damagers such as ionizing radiation, 5-fluorouracil, etoposide and adriamycin [17]. Complex regulatory circuits repress the activity of p53 in non-stressed cells, and these circuits allow a rapid activation of p53 upon exposure to stress stimuli. In general, low levels of stress induce p53 and activate genes involved in cell-cycle...
arrest, DNA repair and senescence. Thus p53 essentially promotes cellular survival under low levels of stress. In contrast, high levels of stress enhance the accumulation of p53 and activation of pro-apoptotic genes such as PUMA (p53 up-regulated modulator of apoptosis), NOXA (PMA-induced protein 1) and BAX (Bcl-2-associated X protein). These pro-apoptotic proteins then activate caspases, thereby leading to the death and removal of the damaged cells from the host [18–22]. These results suggest that the cytotoxic action of many anticancer agents may depend on the nature of the tumour suppressor and oncogenic signals existing within the treated cancer cells.

Several enzymes catalyse post-translational modifications of the p53 protein [23,24]. Previous studies, such as those performed by the Centre for Protein Engineering (CPE) (reviewed in [25]), have provided critical insights to understand the protein–protein interactions involving p53. However, a complete description of the regulation of p53 remains a daunting task. For instance, several ubiquitin ligases [e.g. COP1 (constitutive photomorphogenic 1), PIRH2 (p53-induced RING-H2), TRIM24 (tripartite-motif-containing 24), β-TrCP (β-transducin repeat-containing protein) and HDM2 (human double minute 2)/MDM2 (murine double containing 24)], may cause ER stress and be associated with diseases such as non-toxic concentrations, suggesting that molecules that stabilize p53 may be used for cancer treatment [27]. Furthermore, many tumours overproduce MDM2 which impairs p53 function, indicating that MDM2 plays a critical role in reducing p53 activity in cancer cells.

GS28, which is also termed GOS28 or GOSR1, is a 28-kDa membrane protein that apparently plays an essential role in mammalian ER (endoplasmic reticulum)–Golgi or intra-Golgi membrane vesicle transport [28,29]. Furthermore, a protein complex consisting of the cis-Golgi vesicle receptor syntaxin 5 in complex with GS28, rab1, rab3a, sec22b and membrin proteins has been isolated by Scheller and colleagues [30]. Interactions of these proteins strongly suggest an intermediate in ER–Golgi transfer reactions in mammalian cells. Abnormal ER–Golgi transfer because of defects in the interaction of these membrane proteins may cause ER stress and be associated with diseases such as neurodegeneration, cancer and immune defects. The release of membrane vesicles, in particular exosomes, has become the subject of increasing interest in intercellular communication [31]. The ERAD (ER-associated degradation) complex may be activated by ER stress to process misfolded proteins. The ERAD process, which includes the p53 ubiquitin ligase synoviolin, a protein involved in the degradation of p53, has also been described [32]. We recently observed that knockdown of SNAP (soluble N-ethylmaleimide-sensitive fusion protein)-α is sufficient to induce ER stress equivalent to that induced by either A23187 or cisplatin, and that this process caused p53 accumulation in an ERAD-dependent manner [33]. Given that GS28 physically associates with SNAP-α during normal protein folding and ER–Golgi transport [28,29], further studies should be designed to determine the potential significance of the link between membrane vesicular proteins and p53 in chemosensitivity of cancer.

To identify additional regulators of p53 involved in the cellular response to cisplatin, we isolated the proteins that interact with p53 using immunoprecipitation and combined protein annotation assays. In the present study, we observed that the Golgi-SNARE (SNAP receptor) GS28 forms a protein complex with both p53 and MDM2. We also show that overexpression of GS28 sensitizes cells to cisplatin in a p53-dependent manner. Conversely, knockdown of GS28 using shRNA (short hairpin RNA) has the opposite effect and can prevent cells from undergoing apoptosis in response to cisplatin.

MATERIALS AND METHODS

Cell lines and chemicals

The cell lines used in the present study included human primary lung cells (WI-38), immortalized HEK (human embryonic kidney)-293 cells and lung cancer cells (H1299). These cell lines were obtained from the A.T.C.C. (Manassas, VA, U.S.A.), and were maintained in DMEM (Dulbecco’s modified Eagle’s medium) (Gibco) supplemented with 10% (v/v) FBS (fetal bovine serum), 100 units/ml penicillin and 100 μg/ml streptomycin (Gibco) at 37°C in a humidified atmosphere of 5% CO2 and 95% air. The chemotherapeutic agents cisplatin, vincristine and taxol (also known as paclitaxel) were purchased from Bristol-Myers Squibb. PFT-α (pifithrin-α) was obtained from Biomol Research Laboratories. Other chemicals were purchased from Sigma–Aldrich. All reagents were prepared according to the instructions provided by the supplier.

Protein identification by MALDI–TOF (matrix-assisted laser-desorption ionization–time-of-flight)-MS

For immunoprecipitation, antibodies were cross-linked to Protein A–Sepharose beads with dimethyl pimelidate as described previously [34]. Cell extracts prepared in extraction buffer (20 mM Hepes, pH 7.3, 100 mM KCl, 2 mM EDTA, 1 mM dithiothreitol and 1% Triton X-100) were incubated with Protein A–Sepharose beads coated with an anti-p53 antibody (p53 DO-1, Santa Cruz Biotechnology) for 2 h at 4°C. Beads were centrifuged at 1000 g for 2 min, washed three times in the extraction buffer and twice in the same buffer without Triton X-100, boiled in SDS/PAGE sample buffer, and resolved by SDS/PAGE (10% gels). The proteins obtained this way were identified by MALDI–TOF-MS as described in [35]. Briefly, protein bands were removed and washed twice with Milli-Q water (Millipore). For destaining, a 1:1 (v/v) solution of 30 mM potassium ferricyanide and 100 mM sodium thiosulfate was added before incubation for 10 min. Gel pieces were then washed twice with 50 mM ammonium bicarbonate/acetonitrile (1:1, v/v), dehydrated with acetonitrile, dried by speed vacuum, rehydrated in 25 mM ammonium bicarbonate containing 20 ng/μl trypsin and incubated at 37°C for 16 h. A small volume of acetonitrile containing 1% trifluoroacetic acid was combined with the samples and incubated for 1 h. A 0.5 μl volume of trypsin was added to the sample plate containing 600 μg AnchorChipTM, Bruker Daltonics) and combined with 0.2 μl of matrix solution (2 mg/ml α-cyano-4-hydroxy-cinnamic acid in 50% acetonitrile containing 1% trifluoroacetic acid) containing 1 fmol each of angiotensin II and ACTH-(18–39) (corticotropin) for co-crystallization with the sample. Mass spectra of the tryptic digest were obtained by using an Ultraflex MALDI-TOF/TOF mass spectrometer (Bruker Daltonics) calibrated with the standards and the trypsin autolytic fragments. The peptide mass profiles were evaluated using the Biotools software (Bruker Daltonics). Protein identification of peptide mass fingerprinting was performed with the Mascot search engine (http://www.matrixscience.com; accessed 13 January 2011) based on the complete NCBI or MSDB protein database, with carbamidomethylation on cysteine residues as fixed modifications and oxidation on methionine residues as
variable modifications. Up to one missed trypsin cleavage was allowed. Mass tolerance of 100 p.p.m. was used as an error range for matching peptide mass values.

### Plasmids, transfection and immunoblotting

GS28 (GenBank® accession number NM_004871) cDNA sequences were isolated by PCR using the following primers: GS28, forward primer, 5′-GGGGCTGACGTGGACGACAAAG-3′, and reverse primer, 5′-AGAGTCCCTGAAGATGCTCCCA-3′. The amplified products were cloned into the pGEM-T easy vector and confirmed by automatic sequencing. The FLAG–GS28 expression plasmid was constructed by removing ORFs (open reading frames) from the pGEM-T easy vector and by inserting it into the pcDNA3 vector using the restriction enzymes EcoRI and XhoI, which resulted in the formation of pcDNA3-FLAG-GS28. The p53 expression plasmid (pcep4-p53) was provided by Professor Y.-S. Lin (Academia Sinica, Taipei, Taiwan). To express GS28 or p53, HEK-293 cells were transfected with plasmid cDNA using Lipofectamine® (Invitrogen) [36]. Alternatively, stable cancer cell clones were established as described previously [37].

Total protein extracts for immunoblotting experiments were prepared as described in [38]. Briefly, 50 μg protein extracts were separated by SDS/PAGE (12% gels), followed by transfer on to PVDF membranes and incubation with primary antibodies raised against the following proteins: FLAG (Sigma–Aldrich), phospho-p53 (Ser46) and cleaved caspase 3 (Cell Signaling Technology). GS28, GAPDH (glyceraldehyde-3-phosphate dehydrogenase) (FL-335), Bax (N-20), PARP [poly(ADP-ribose) polymerase] (H-250), MDM2, GFP (green fluorescent protein) and ubiquitin (Santa Cruz Biotechnology). Subsequently, the membranes were incubated with the following secondary antibodies: goat anti-mouse or horseradish-peroxidase-conjugated goat anti-rabbit (Santa Cruz Biotechnology). Subsequently, the membranes were incubated with the following secondary antibodies: goat anti-mouse or horseradish-peroxidase-conjugated goat anti-rabbit (GE Healthcare), and the signal was visualized by enhanced chemiluminescence according to the specifications from the supplier (Pierce). The intensity of the protein bands was determined using a scanning densitometer (Hoefer GS 300).

### Knockdown expression of the GS28 gene using shRNA

pLKO.1 plasmids expressing shRNA for GS28 knockdown were purchased from the National RNAi Core Facility (Academia Sinica, Taipei, Taiwan). A recombinant plasmid expressing luciferase shRNA (shLuc; TRCN0000072244) was used as a negative control. Five recombinant plasmid clones expressing shGS28 were tested for gene-knockdown efficiency in HEK-293 cells, which can be transfected efficiently. One of the plasmids encoding shGS28 (TRCN0000060386) was identified as the most effective plasmid to inhibit GS28 expression, and was therefore used in the present study. Transient transfection of shRNA plasmids was performed by adding 2 μg/well of the plasmids with 3 μl/well Lipofectamine® into cells cultured in six-well plates (1.5 × 10⁴ cells/well) according to the instructions from the manufacturer. The stable cell clones that were efficient for plasmid transfection were infected with recombinant lentivirus expressing shGS28. The recombinant lentivirus also carried a puromycin-resistance gene for selection which was performed according to the methods described by the supplier (National RNAi Core Facility).

### Real-time qPCR (quantitative PCR)

Real-time qPCR was performed on total RNA extracted with the TRizol® reagent (Invitrogen) as described previously [36]. GenBank® sequences NM_004871 and NM_000996 were used to design primers for GS28 and GAPDH respectively. PCR primer pairs designed using Primer Express 2.0.0 (Applied Biosystems) were used at a concentration of 200 nmol/l. The primers used were as follows: GS28, forward, 5′-GCGATACCGAGATGAAAGA-3′, and reverse, 5′-CCCTGAAAGCCCTTCCAGAAG-3′; and GAPDH, forward, 5′-CTCTGACCACCAACTGCTT-3′, and reverse, 5′-GAGGAGGCCCATCCCATCTTT-3′. All unknown samples as well as the controls were run in triplicate on the same plate. Relative quantification was calculated by the ΔΔCt method and normalized to GAPDH. Namely, the ΔCt for each candidate was calculated with the following equation: 

\[ \Delta C_t = \Delta (C_t - C_t^{\text{GAPDH}}) \]

The relative abundance of the candidate gene X was expressed as

\[ 2^{-\Delta C_t/X - \Delta C_t^{\text{GAPDH}}} \]

### Radiolabelling and immunoprecipitation of p53 protein

The methods used for pulse labelling of cells with [35S]methionine, immunoprecipitation of radiolabelled p53, and analysis of immunoprecipitates by SDS/PAGE were carried out according to established protocols [39].

### Ubiquitination assay

HEK-293 cells were co-transfected with expression plasmids (2 μg each) encoding either shRNA (shGS28) or proteins (pcDNA3 vector control or pcDNA3-FLAG-GS28) along with His-tagged ubiquitin plasmid (provided by Professor R.-H. Chen, Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan) in the presence of MG132 (20 μM). The cells were harvested and lysed 48 h after transfection. Equal amounts (500 μg) of total protein lysates were subjected to immunoprecipitation with anti-p53 (DO-1) antibody. The immunoprecipitation products were analysed by Western blotting using anti-p53 (FL-393) antibody.

### Cell death and FACS analysis

Apoptotic cells were determined by nuclear phenotype as described previously [38]. The percentage of apoptotic cells was calculated as the number of dead cells divided by the total number of cells counted. To analyse drug-induced apoptosis, cell extracts were used for immunoblotting with antibodies directed against cleaved caspase 3 and PARP [36]. Cell clonogenicity and cell growth inhibition were determined by colony formation assay and the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide] colorimetric method respectively [40]. Apoptotic sub-G1-phase cells were measured by flow cytometry [41].

### Statistical analysis

Results were analysed using paired Student’s t tests to determine statistical significance. Results are expressed as means ± S.D.

### RESULTS

GS28 forms a complex with p53

p53 mediates a variety of cellular responses to stress, including chemotherapeutic drugs. To assess the cellular function of p53 in this context, we performed immunoprecipitation experiments to identify the proteins that interact with p53 in HEK-293 cells. A protein with a molecular mass of approximately 28 kDa was found...
cisplatin/shLuc treatments. By using the MTT cell viability assay, we observed that knockdown of GS28 increased the resistance of HEK-293 cells to cisplatin (Figure 2F). To quantify the level of resistance, we used an RF (resistance factor) defined as the IC_{50} for the shGS28 treatment divided by the IC_{50} for shLuc (Figure 2F; RF = 1.5). We obtained similar results by using colony-formation assays to monitor the level of resistance following knockdown of GS28 (Figure 2G; RF = 1.9).

However, the combination of cisplatin and shGS28 resulted in reduced apoptosis (Figure 2D) and a smaller population of apoptotic sub-G1-phase cells (Figure 2E) compared with control (Supplementary Figure S1). Cells that expressed shGS28 and were treated with a high dose of cisplatin also showed lower Bax levels at 24 h, whereas no reduction in Bax levels was detected at 48 h (Supplementary Figures S1A and S1B). Similar to the results obtained using 20 μM cisplatin (Figures 2B–2E), cells treated with a combination of shGS28 and 100 μM cisplatin showed a slight reduction in cleaved caspase 3 and PARP levels (Supplementary Figure S1A). Furthermore, the combination of such high concentrations of cisplatin and shGS28 displayed a reduced apoptosis compared with shLuc control (Supplementary Figure S1C, P < 0.05). These results indicate that GS28 knockdown in HEK-293 cells decreases p53 apoptotic activity and induces resistance to cisplatin.

**Figure 1** GS28 interacts with p53 as revealed by immunoprecipitation and MALDI–TOF-MS analysis

(A) Silver-stained gel of proteins co-immunoprecipitated with p53 antibody. Proteins from HEK-293 cells (50 μg) were immunoprecipitated (IP) with anti-p53 antibody or pre-immune serum, and separated by SDS/PAGE. The protein of interest (indicated by an arrow) was revealed on the gel by silver stain (upper panels). The proteins (50 μg) were examined by immunoblotting (IB) with an anti-p53 antibody (lower panels). (B) GS28 protein sequence. The peptide marked in (A) was identified by MALDI–TOF-MS, and 11 peptides matched GS28, covering 45.6% of the protein sequence. Matched peptides are underlined.

To assess the role of GS28 in modulating chemosensitivity, a lentivirus-based plasmid expressing GS28 shRNA (shGS28) was used to knockdown GS28 expression in HEK-293 cells. The GS28 mRNA level was reduced by 80% compared with control shRNA (shLuc; Figure 2A). The GS28 protein level was also clearly reduced by the shGS28 treatment (Figure 2B). Notably, Bax protein level was reduced in the cells that expressed shGS28 and were treated with cisplatin (Figure 2B). The reduction in Bax protein levels in the cisplatin-treated cells expressing shGS28 was confirmed by measuring the intensity of the protein bands using densitometry (Figure 2C). Although we detected a reduced protein level of p53 following knockdown of GS28 (Figure 2C), the level of pro-apoptotic phosphorylated p53 on Ser46 was not detected (Figure 2B, p-p53-Ser46, lanes 7–9). However, the combination of cisplatin and shGS28 resulted in reduced apoptosis (Figure 2D) and a smaller population of apoptotic sub-G1-phase cells (Figure 2E) compared with cisplatin/shLuc treatments. By using the MTT cell viability assay, we observed that knockdown of GS28 increased the resistance of HEK-293 cells to cisplatin (Figure 2F). To quantify the level of resistance, we used an RF (resistance factor) defined as the IC_{50} for the shGS28 treatment divided by the IC_{50} for shLuc (Figure 2F; RF = 1.5). We obtained similar results by using colony-formation assays to monitor the level of resistance following knockdown of GS28 (Figure 2G; RF = 1.9).

We also analysed the effects of shGS28 on HEK-293 cells treated with a higher dose (100 μM) of cisplatin (Supplementary Figure S1 at http://www.BiochemJ.org/bj/444/bj4440303add.htm). Cells that expressed shGS28 and were treated with a high dose of cisplatin also showed lower Bax levels at 24 h, whereas no reduction in Bax levels was detected at 48 h (Supplementary Figures S1A and S1B). Similar to the results obtained using 20 μM cisplatin (Figures 2B–2E), cells treated with a combination of shGS28 and 100 μM cisplatin showed a slight reduction in cleaved caspase 3 and PARP levels (Supplementary Figure S1A). Furthermore, the combination of such high concentrations of cisplatin and shGS28 displayed a reduced apoptosis compared with shLuc control (Supplementary Figure S1C, P < 0.05). These results indicate that GS28 knockdown in HEK-293 cells decreases p53 apoptotic activity and induces resistance to cisplatin.

**Overexpression of GS28 sensitizes HEK-293 cells to cisplatin by enhancing accumulation and apoptotic activity of p53**

To confirm the effect of GS28 on chemoresistance, we overexpressed GS28 in HEK-293 cells by transfection of a FLAG-GS28 cDNA and overexpressed GFP as a negative control. A toxic cisplatin concentration (20 μM) was used to test the kinetic activation of apoptotic proteins in these cells. We observed that cleavage of caspase 3 and PARP was higher in cells overexpressing GS28 than in cells expressing GFP (Figure 3A, 48 h). p53 protein levels were also profoundly increased in GS28-overexpressing cells (Figure 3A, lane 4 compared with lane 1). Whereas p53 protein level was increased in the control GFP cells following cisplatin treatment, p53 remained at a high level and was not increased further in GS28-overexpressing cells (Figure 3A). In addition, the increase of phospho-p53 (Ser46) and Bax was more profound in GS28-overexpressing cells compared with the control (Figure 3A). These results indicate that GS28 enhances the accumulation and apoptotic activity of p53. To confirm these results, the protein levels of p53 and Bax were quantified by densitometry (Figures 3B), and this analysis confirmed that both proteins were significantly increased in GS28-overexpressing cells compared with control GFP-expressing cells (i.e. cisplatin treatment at 0 h). As seen for GS28 knockdown, the cells that overexpressed GS28 did not show increased p53 expression following cisplatin treatment for either 24 or 48 h of incubation (Figure 3B). On the other hand, Bax protein levels were increased in GS28-overexpressing cells (Figure 3B), consistent with the increase in phospho-p53 (Ser46) levels observed in GS28-overexpressing cells treated with cisplatin (Figure 3A). To assess whether p53 accumulation was due to increased protein stability, we treated the cells with the inhibitor of protein biosynthesis CHX (cycloheximide) (100 μg/ml) for various lengths of time, and monitored the level of p53 by immunoblots (Figure 3C). The regression slope corresponding to the decrease in p53 protein level in GS28-overexpressing cells was higher than that in GFP-expressing cells (Figure 3C, μ = 0.501 compared with μ = 0.818), suggesting that GS28 overexpression in HEK-293 cells may stabilize p53. This observation may not be due to an increase in protein translation induced by GS28 since we observed no difference in the level of total protein synthesis in cells...
overexpressing either GS28 or GFP (Supplementary Figure S2 at http://www.BiochemJ.org/bj/444/bj4440303add.htm).

Following cisplatin treatment, GS28 overexpression in HEK-293 cells increased apoptosis (Figure 3D) and produced a larger sub-G1-phase cell population (Figure 3E) compared with GFP expression. Using the MTT assay, we found that overexpression of GS28 sensitized HEK-293 cells to cisplatin (Figure 3F). In this case, an SF (sensitization factor), defined as the IC50 for GFP expression divided by the IC50 for GS28 overexpression, was equal to 1.44 (Figure 3F). Similarly, an SF of 1.5 was obtained for the cells overexpressing GS28 as assessed using a colony-formation assay (Figure 3G). These results indicate that GS28 increases p53 apoptotic activity and sensitizes HEK-293 cells to cisplatin.

**Inhibition of p53 impairs GS28-mediated sensitization of HEK-293 cells to cisplatin**

To confirm that GS28 sensitizes cells to cisplatin via p53, we tested the effect of the reversible p53 inhibitor PFT-α [42] on this process (Figure 4A). PFT-α slightly inhibited cisplatin-induced p53 accumulation in cells overexpressing either GS28 or GFP.
Figure 3  Accumulation of p53 protein and apoptotic activity, and sensitization of HEK-293 cells to cisplatin by GS28 overexpression

(A) Increase in p53 protein levels and its apoptotic activity, and caspase 3 activity by FLAG–GS28 expression. HEK-293 cells that overexpressed either FLAG–GS28 or control GFP were treated with 20 μM cisplatin for the indicated lengths of time. Total protein extracts were treated for immunoblotting as in Figure 2. Increased levels of p53, phospho-p53 (Ser46) (p-p53-Ser46), Bax and caspase 3 activity were detected in cells expressing FLAG–GS28 alone and/or combined with cisplatin treatment. Molecular masses are indicated in kDa. (B) Band density and statistical analyses of p53 and Bax in cells expressing shGS28 (as shown in A). (C) Enhancement of p53 stability by GS28 overexpression. Plasmids coding for GFP or FLAG–GS28 were transfected in HEK-293 cells for 24 h, followed by treatment with CHX for the times indicated. The degradation of p53 protein was examined by Western blotting (top panels). The amounts of p53 protein shown in the top panels were quantified using densitometry and the results were plotted below (bottom panel). (D) Increase in cisplatin-induced apoptosis following GS28 overexpression. (E) Increase in cisplatin-induced sub-G1-phase cells by GS28 overexpression. (F) Sensitization of cells to cisplatin after GS28 overexpression as shown by the MTT assay. (D) Sensitization of cells to cisplatin following GS28 overexpression as revealed by colony formation assays. Results are means ± S.D. for experiments performed in triplicate. *P < 0.05; **P < 0.01.

(Figure 4A). Further studies are required to unravel whether p53 conformation is altered by binding to PFT-α. Notably, the cisplatin-induced accumulation of phospho-p53 (Ser46) and Bax were inhibited by PFT-α in GS28-overexpressing cells (Figure 4A). On the other hand, only cisplatin-induced Bax accumulation was inhibited by PFT-α in GFP-expressing cells (Figure 4A). Densitometric analysis of the protein bands also confirmed that PFT-α inhibited p53 and Bax to a larger extent in cells overexpressing GS28 compared with cells expressing GFP (Figure 4B). To confirm that the sensitizing effect of GS28 to cisplatin is mediated by p53, we treated HEK-293 cells overexpressing either GS28 or GFP with PFT-α before cisplatin treatment (Figure 4C). As expected, PFT-α reduced the level of apoptosis in cells overexpressing either GS28 or GFP and
GS28 sensitizes cells to cisplatin

**Figure 4** Impairment of GS28-induced p53 accumulation and sensitization to cisplatin in HEK-293 cells by the p53 inhibitor PFT-α

(A) Reduction in GS28- and cisplatin-induced p53 and Bax protein levels by PFT-α. Molecular masses are indicated in kDa. (B) Band density and statistical analyses of the decrease in GS28-induced p53 and Bax proteins by PFT-α (as shown in A). (C) Reduction in GS28- and cisplatin-induced apoptotic cells by PFT-α. (D) Reversal of GS28- and cisplatin-induced cell growth inhibition by PFT-α (MTT assay). Results are means ± S.D. for experiments performed in triplicate. *P < 0.05; **P < 0.01.

that were treated with cisplatin (Figure 4C). Using the MTT assay, we observed that blocking p53 using PFT-α was sufficient to increase the resistance of control GFP-expressing cells to cisplatin (Figure 4D; RF = 1.36). Moreover, we confirmed that the sensitizing effect of GS28 overexpression to cisplatin could be inhibited by PFT-α (Figure 4D; RF = 1.93). However, there was no difference in the effects between GS28 plus PFT-α and GFP plus PFT-α. These results confirm that the sensitizing effect of GS28 overexpression to cisplatin is dependent on p53. HEK-293 cells were initially generated through immortalization by human Ad5 (adenovirus 5), an oncogenic virus that expresses proteins (e.g. E1A and E1B) that perturb p53 signalling mechanisms. To evaluate the possibility that GS28 acts by modulating Ad5-associated functions, we conducted the same experiments and acquired similar results with non-virally immortalized H1299 and primary WI-38 cell lines (Figure 5, and Supplementary Figures S3 and S6 at http://www.BiochemJ.org/bj/444/bj4440303add.htm). It is unlikely that the association observed is mediated through Ad5 virus protein (e.g. E1B).

**Desensitization to cisplatin by GS28 knockdown is absent from p53-null H1299 cells, but can be restored by ectopic p53 expression**

To confirm further that the protection effect of GS28 knockdown against cisplatin is p53-dependent, we examined apoptosis and chemoresistance in the p53-null cell line H1299. H1299 cells that were transfected with a p53 expression plasmid or the empty vector were co-transfected with plasmids encoding either shLuc or shGS28. Two independent cell clones expressing shGS28 (shGS28-1 and shGS28-2) were established and both cell lines showed dramatic knockdown of GS28 (Figure 5A). Following ectopic expression of p53, the cells expressing shGS28 (shGS28-1 as an example) displayed reduced p53 accumulation following treatment with a cytotoxic concentration of cisplatin compared with cells expressing shLuc (Figure 5B; 20 μM cisplatin). The associated p53 apoptotic activation, which includes phospho-p53 (Ser46), Bax, cleaved caspase 3 and PARP, was also reduced in cells expressing shGS28 (Figure 5B). The reduction in p53 and Bax protein levels following knockdown of GS28 was confirmed by densitometric analysis (Figure 5C, 24 and 48 h). Following cisplatin treatment, GS28 knockdown in shGS28-1- and shGS28-2-expressing cells displayed reduced apoptotic cells compared with shLuc (Figure 5D). Using the MTT assay, we observed that GS28 knockdown desensitized H1299 cells to cisplatin when the cells overexpressed exogenous p53 (Figure 5E), producing RFs of 1.48 for shGS28-1 and 1.7 for shGS28-2. In contrast, H1299 cells expressing shGS28 did not display desensitization effects to cisplatin in the absence of exogenous p53 expression (Figure 5F). Immunoblotting experiments revealed that H1299 cells expressing FLAG–GS28 showed an increased level of p53 compared with cells expressing GFP (Supplementary Figures S3A and S3B). The cells expressing FLAG–GS28 and which were treated with cisplatin showed significant increases in Bax and cleaved caspase 3 compared with cells expressing GFP (Supplementary Figure S3A). Furthermore, H1299 cells that overexpressed both GS28 and p53 displayed enhanced sensitivity to cisplatin compared with cells expressing GFP and p53 (Figures S3C and S3D). These results indicate that the modulatory effects of GS28 on cisplatin sensitivity can be observed in H1299 cells when exogenous p53 is expressed.

**GS28 interacts with both p53 and MDM2, and reduces ubiquitination of p53**

The results described above indicate that GS28 increases the stability of the p53 protein. To confirm this possibility, we first co-transfected H1299 cells with both p53- and FLAG–GS28-expression plasmids. Cell extracts were then immunoprecipitated with antibodies directed against either p53...
or GS28, followed by immunoblotting experiments to detect p53, FLAG–GS28 or MDM2. Our results showed that GS28 was co-immunoprecipitated with both p53 and MDM2 (Figure 6A, lanes 3 and 4). In contrast, cell extracts immunoprecipitated with unrelated IgG did not retrieve any of these proteins (Figure 6A, lane 2), which were nevertheless detected in 10% input cell extracts (Figure 6A, lane 1). To confirm the interaction between p53 and GS28, increasing amounts of either FLAG–GS28 or p53 were co-immunoprecipitated with the other partner. When p53 was increased with equal FLAG–GS28 levels, the level of FLAG–GS28 that was co-immunoprecipitated in this case did not change (Figure 6B, lanes 3–5). On the other hand, when FLAG–GS28 was increased and p53 remained constant, the level of p53 co-immunoprecipitated was slightly elevated at the highest level of FLAG–GS28 expression (Figure 6B, lanes 6–8). In both panels of co-immunoprecipitation experiments, p53 appeared to associate better with MDM2 than with GS28 (Figure 6B). We noted that FLAG–GS28 clearly co-immunoprecipitated with MDM2 in H1299 cells which do not express p53 (Figure 6B, lane 3), suggesting that the association between p53 and GS28 in our initial experiment (Figure 1A) may be dependent on MDM2. As a control, cell extracts immunoprecipitated with IgG showed only a non-specific protein band when immunoblotted with the anti-p53 antibody (Figure 6B, lane 2).

MDM2 has been shown to represent a major E3 ubiquitin ligase that mediates the degradation of p53 by the proteasome. To explore the possibility that GS28 affects the ubiquitination of p53, we co-transfected HEK-293 cells with expression plasmids (2 μg each) encoding His6–ubiquitin and pcDNA control, FLAG–GS28 or shGS28, and examined the level of p53 ubiquitination in the presence of the proteasome inhibitor MG132 (20 μM) (Figure 6C). We performed immunoprecipitation of the cell extracts with anti-p53 antibody, followed by immunoblotting for p53 (Figure 6C, top panel). Notably, the overexpression of...
GS28 was originally identified as a SNARE located in the cis-Golgi apparatus, and as a protein that participates in either intra-Golgi or ER–Golgi protein transport [28,29]. In the present study, GS28 was identified as forming a complex with p53 and its ubiquitin ligase MDM2. Knockdown of GS28 protected cells against undergoing apoptosis in response to cisplatin by preventing apoptosis. Knockdown of GS28 also prevented cells from undergoing apoptosis in response to other therapeutic agents such as Adriamycin and etoposide, which also require p53 activity to induce cytotoxic effects (results not shown). Besides, knockdown of GS28 did not influence the viability of HEK-293 cells in response to the mitotic damaging agents vincristine and taxol as determined by cell viability assays and activation of caspase 3 (Supplementary Figure S5 at http://www.BiochemJ.org/bj/444/bj4440303add.htm). Our findings provide the first evidence that a cellular SNARE protein is involved in chemosensitivity in vitro. In contrast, overexpression of GS28 caused an increase in p53 protein levels as well as an increase in cisplatin-induced p53 activity as shown by the accumulation of pro-apoptotic Bax. The possibility that the sensitization effect of GS28 to cisplatin is dependent on p53 was confirmed by showing that the p53 inhibitor PFT-α could prevent this cellular response. Similarly, the sensitization effect of GS28 to cisplatin was not detected in p53-null H1299 cells, unless exogenous p53 expression was introduced. These findings indicate that the combination of GS28 overexpression and cisplatin inhibits cancer cell growth in a p53-dependent manner. Furthermore, overexpression of GS28 also significantly increased p53 in non-virally immortalized H1299 cells and normal primary WI-38 cells (Supplementary Figures S3 and S6 respectively at http://www.BiochemJ.org/bj/444/bj4440303add.htm). These results suggest that the interaction between GS28 and p53 is not confined to HEK-293 cells, but instead it appears to be a more general phenomenon seen in various cell lines.

Interestingly, the level of p53 was not induced by cisplatin treatment in control experiments, but it was significantly lower following silencing of GS28. Whereas p53 may be increased to high levels because of the Ad5 proteins in HEK-293 cells in the absence of cisplatin stress, no induction of p53 was detected following cisplatin treatment (Figure 2B). Given that much lower p53 levels were detected in human HeLa cells which are immortalized with HPV (human papillomavirus), however, the levels of p53 were markedly increased by cisplatin treatment in HeLa cells in which the p53 levels are also significantly lower following silencing of GS28 (results not shown). It is thus unlikely that the reduction in the p53 levels following silencing of GS28 represents an artefact. This conclusion was supported further by the similar findings obtained in non-virally transformed p53-null H1299 cells in which ectopic expression of p53 was introduced (Figure 5). Together, these results suggest that GS28 may affect p53 directly rather than by influencing some aspects of Ad5 viral induction.

The phenomena involved in the protecting effect of GS28 knockdown against cisplatin in HEK-293 cells include a reduction in pro-apoptotic p53 levels. This observation is consistent with the finding made in prostate cancer cells that acetylation of p53 at Lys373, which stabilizes the p53 protein, is required for the p53-mediated induction of cell-cycle arrest and apoptosis [43]. This finding may be explained by the observation that acetylation of p53 is involved in the controlling of p53 activity by GS28. GS28 and p53 form a complex, and GS28 overexpression of GS28 decreased p53 ubiquitination (Figure 6C, lane 1 compared with lane 2). In contrast, the knockdown expression of GS28 by shGS28 dramatically increased p53 ubiquitination (Figure 6C, lane 1 compared with lane 3). Besides, the FLAG–GS28 overexpression induced a higher level of p53 protein (Figure 6C, input, lane 1 compared with lane 2), and shGS28 reduced the level of p53 protein (Figure 6C, input, lane 1 compared with lane 3). These results indicate that GS28 decreases the ubiquitination of p53, thereby increasing the stability of the p53 protein.

The co-immunoprecipitation of endogenous proteins using HEK-293 and lymphoblastoma HOB1 cell lines also showed a physical interaction between p53 and GS28 (Supplementary Figures S4A and S4B respectively at http://www.BiochemJ.org/bj/444/bj4440303add.htm).
of Lys$^{73}$ may also lead to a stronger interaction of p53 with low-affinity DNA-binding sites, which are found in pro-apoptotic target genes [44]. Surprisingly, we observed the formation of GS28–p53–MDM2 complexes that are associated with preventing the ubiquitination and degradation of p53 in cells overexpressing GS28. Thus the protective role of GS28 knockdown may proceed by affecting the expression of pro-apoptotic genes or by promoting expression of anti-apoptotic genes. For example, knockdown of GS28 may block the expression or activity of proteins such as MDM2 that degrade p53. Therefore the accumulation of p53 following GS28 overexpression may be due to a reduced ubiquitination of p53 by MDM2. Furthermore, the slight increase as MDM2 that degrade p53. Therefore the accumulation of GS28 may block the expression or activity of proteins such as MDM2 that degrade p53. Therefore the accumulation of GS28 may be involved in mediating ER-stress-induced regulation of p53 function during apoptosis has not been demonstrated. Further studies are required to clarify this issue. Nevertheless, a reduction in p53 levels in A23187 stressed cells was rescued by FLAG–GS28 (Supplementary Figure S7, lanes 13–16, at http://www.BiochemJ.org/bj/444/bj4440303add.htm), compared with the controls (Supplementary Figure S7, control, lanes 1–4, and shLuc and GFP, lanes 5–8), in HEK-293 cells, suggesting that GS28 may be involved in the ER-stress-induced reduction in p53, at least in the cells tested in the present study. Furthermore, GS28 is not detected in the nucleus following overexpression of GS28 fusion protein in HEK-293 cells (results not shown). It is thus unlikely that the GS28-mediated regulation of p53 occurs through its entry into the nucleus and its alteration of nuclear p53.

Taken together, our results suggest a simple hypothesis to explain the positive regulation of p53 stability by GS28 and the subsequent enhancement of cisplatin-induced apoptosis (Figure 7). In non-stressed cells, p53 is rapidly degraded by the proteasome through MDM2-mediated ubiquitination, and this ubiquitination process can be blocked by overexpression of GS28 (Figure 7, right-hand side). Inhibition of p53 ubiquitination may occur through GS28 binding to MDM2 which interferes with p53 ubiquitination. However, the mechanism underlying the action of GS28 requires further study. A reduced level of p53 ubiquitination due to GS28 overexpression may provide an opportunity for additional modifications of p53. In response to cisplatin stress, p53 is modified to escape from degradation (such as with acetylation by HAT). If the cells are exposed to cytotoxic concentrations of cisplatin (severe DNA damage), p53 is additionally phosphorylated at Ser$^{46}$ by DYRK2, for example, which strongly transactivates Bax and induces apoptotic cell death (left-hand side). The involvement of HAT and DYRK2 is included on the basis of previous studies as mentioned in the Discussion.

Figure 7 Working model of cisplatin-induced stress and related signal pathways implicated in the positive regulation of cisplatin-induced apoptosis by GS28

In unstressed conditions, p53, which is usually rapidly degraded by MDM2-mediated ubiquitination in the proteasome, is blocked by GS28 (right-hand side of the model). In response to cisplatin stress, p53 is modified to escape from degradation (such as with acetylation by HAT). If the cells are exposed to cytotoxic concentrations of cisplatin (severe DNA damage), p53 is additionally phosphorylated at Ser$^{46}$ by DYRK2, for example, which strongly transactivates Bax and induces apoptotic cell death (left-hand side). The involvement of HAT and DYRK2 is included on the basis of previous studies as mentioned in the Discussion.
carry unique domains and possess various functions. These p53 isoforms are capable of affecting the functions of normal p53 in terms of their effects on transcriptional activity, cell growth and apoptosis responses, depending on which p53 target genes are regulated. These observations may help us to better understand how upstream and downstream p53 pathways have evolved relative to specific p53 domains (reviewed in [48]). To confirm the role of GS28 in regulating p53 level and chemosensitivity, we also found that overexpression of GS28 sensitized primary WI-38 cells to cisplatin by enhancing p53 accumulation and apoptotic activity (Supplementary Figures S5 and S5). Two p53 family proteins, p63 and p73, may also transactivate p53-responsive genes and cause cell-cycle arrest and apoptosis (reviewed in [49]). In addition, the anti-p53 antibody DO-1 used in the present study does not detect p53 without TAD1 (transactivation domain 1) [50]. If these TAD1-lacking p53 members are regulated by GS28, this may complicate the cellular response observed. Because of this complexity, we simply focused on the changes in p53 protein level following expression or suppression of GS28, along with the importance of this protein in the regulation of cisplatin chemotherapy. Given that several proteins are now known to interact with p53 physiologically, it is not clear why GS28 seems to stand out alone as a p53-interacting protein in the experiments performed in the present study. Further studies are needed to clarify how both proteins interact. The explanation provided in the present study about the role of GS28 in the regulation of cisplatin-sensitivity awaits validation in vivo. On the basis of the results of the present study, a combination of cisplatin and GS28 overexpression could be used to reverse cisplatin resistance in cancer cells.

AUTHOR CONTRIBUTION

Chuck Chao and Nian-Kang Sun designed the research, Nian-Kang Sun, Shang-Lang Huang and Kun-Yi Chien performed the research, Chuck Chao and and Kun-Yi Chien analysed the data, and Chuck Chao wrote the paper.

ACKNOWLEDGEMENTS

Protein identification by MALDI–TOF-MS was performed at the Proteomics Core Laboratory, Chang Gung University. We also acknowledge the help of Jan Martel during the preparation of the paper.

FUNDING

This study was supported by the National Science Council, Taiwan [contract numbers NSC96-2320-B-182-034 and NSC97-2320-B-182-024-MY3] and Chang Gung University and Hospitals [contract numbers CMRPD150293 (to C.-C.K.C.) and CMRP190041 (to N.-K.S.)].

REFERENCES


36 Sun, C. L. and Chao, C. C. (2005) Cross-resistance to death ligand-induced apoptosis in 
cisplatin-selected HeLa cells associated with overexpression of DDB2 and subsequent 
induction of cFLIP. Mol. Pharmacol. 67, 1307–1314
in UV-resistant HeLa cells by antisense-mediated depletion of damaged DNA-binding 
protein 2 (DDB2). FEBS Lett. 512, 168–172
partially overcomes apoptotic resistance in a cisplatin-selected HeLa cell line. FEBS Lett. 
505, 206–212
39 Chao, C. C. (1992) A single amino acid deletion at the amino terminus of influenza virus 
hemagglutinin causes malfolding and blocks exocytosis of the molecule in mammalian 
cells. J. Biol. Chem. 267, 2142–2148
reactivation of damaged plasmid DNA in HeLa cells resistant to 
cis-diamminedichloroplatinum(II). Cancer Res. 51, 601–605
41 Tsai, S. Y., Sun, N. K., Lu, H. P., Cheng, M. L. and Chao, C. C. (2007) Involvement of 
reactive oxygen species in multidrug resistance of a vincristine-selected lymphoblastoma. 
Cancer Sci. 98, 1106–1114
42 Komarov, P. G., Komarova, E. A., Kondratov, R. V., Chritso-Tselkov, K., Coon, J. S., 
from the side effects of cancer therapy. Science 285, 1733–1737
inhibitors differentially stabilize acetylated p53 and induce cell cycle arrest or apoptosis in 
prostate cancer cells. Cell Death Differ. 12, 482–491
44 Knights, C. D., Catania, J., Di Giovanni, S., Muratoglu, S., Perez, R. and Swartzbeck, A. 
(NSF) and α-soluble NSF attachment proteins (SNAP) mediate dissociation of 
GS28–syntaxin 5 Golgi SNAP receptors (SNARE) complex. J. Biol. Chem. 272, 
25441–25444
47 Bourdon, J., Fernandez, K., Murray-Zmijewski, F., Liu, G., Diot, A., Xirodimas, D. P., 
Saville, M. K. and Lane, D. P. (2005) p53 isoforms can regulate p53 transcriptional 
activity. Genes Dev. 19, 2122–2137
5113–5119
orchestra of isoforms to harmonise cell differentiation and response to stress. Cell Death 
Differ. 13, 962–972
Harbor Perspect. Biol. 2, a000927
SUPPLEMENTARY ONLINE DATA

Golgi-SNARE GS28 potentiates cisplatin-induced apoptosis by forming GS28–MDM2–p53 complexes and by preventing the ubiquitination and degradation of p53


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Figure S1  Knockdown of GS28 reduces p53 apoptotic activity in HEK-293 cells in response to a high concentration of cisplatin

(A) Decrease in apoptotic Bax following knockdown of GS28. HEK-293 cells which express either shGS28 or shLuc, or the mock control, were treated with 100 μM cisplatin for the indicated length of time as in Figure 2(B) of the main text. Molecular masses are indicated in kDa. p-, phospho-. (B) Band density and statistical analyses of the decrease in Bax in cells expressing shGS28 (as shown in A). (C) Reduced apoptotic response to cisplatin in cells following knockdown of GS28 compared with control shLuc. Results are means ± S.D. for experiments performed in triplicate. *P < 0.05.

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Figure S2 Accumulation of total p53 protein, but not nascent p53 protein, in HEK-293 cells overexpressing GS28

(A) Accumulation of p53 protein by GS28 overexpression. (B) Lack of increase in nascent p53 protein levels by GS28 overexpression. HEK-293 cells were transfected for the indicated lengths of time with GFP, FLAG–GS28, shLuc or shGS28 plasmids and were labelled with $^{35}$S-methionine for 20 min. The cells were then washed three times and then treated for 15 min at 37°C with DMEM containing 5 pg/ml trypsin followed by preparation of cell extracts for SDS/PAGE and fluorography. (C) Quantification of nascent p53 protein of the experiments shown in (B) and performed in triplicate. Protein intensity on X-ray film by $^{35}$S-radioactivity or immunofluorescence antibodies was detected by fluorography. The proportion of nascent p53 protein was calculated as the fluorescence of $^{35}$S-labelled p53 divided by the fluorescence of total p53. (D) Lack of decrease in nascent p53 protein levels after GS28 knockdown. (E) Quantification of nascent p53 protein levels of the experiments shown in (D). IP, immunoprecipitation. Results are means ± S.D. for experiments performed in triplicate.
Figure S3  Overexpression of GS28 increases p53 protein and cisplatin-induced apoptotic activity in H1299 cells that ectopically express p53

(A) Effect of GS28 overexpression on gene expression of H1299 cells that ectopically express p53. H1299 cells which express either shLuc (lanes 1–3) or shGS28-1 (lanes 4–6) through lentivirus vectors, were transfected with p53 expression plasmid for 48 h, before treatment with 20 μM of cisplatin for the indicated lengths of time. Total protein extracts (50 μg) were used for immunoblotting experiments to detect cleaved caspase 3 and PARP, as well as the protein levels of p53, phospho-p53 (Ser46), Bax and GAPDH. Molecular masses are indicated in kDa. (B) Band density and statistical analyses of the decrease in p53 and Bax levels in H1299 cells overexpressing GS28 (as shown in A). (C) Enhanced apoptotic response to cisplatin in cells that overexpress both GS28 and p53 compared with control GFP. (D) Reduced cell viability of cisplatin-treated cells that overexpress both GS28 and p53. Results are means ± S.D. for experiments performed in triplicate. *P < 0.05; **P < 0.01.

Figure S4  Co-immunoprecipitation of GS28 and p53 in HEK-293 and lymphoblastoma HOB1 cells

(A) Immunoblot of 10% input proteins. (B) Co-immunoprecipitation of GS28 and p53. Endogenous cell proteins were prepared from HEK-293 and lymphoblastoma HOB1 cell lines, and used sequentially for immunoprecipitation (IP) and immunoblotting as described in Figure 6 of the main text for H1299 cells. Cell proteins from H1299 cells expressing p53 plasmids were also used as control. Molecular masses are indicated in kDa. n.s., non-specific binding.

Figure S5  Lack of sensitization effect to taxol (A) and vincristine (B) in HEK-293 cells that overexpress GS28

Results are means ± S.D. for experiments performed in triplicate. *P < 0.05; **P < 0.01.
Sensitization of WI-38 cells to cisplatin following overexpression of GS28 occurs via enhanced p53 accumulation and apoptotic activity

(A) Increase in p53 protein levels and its apoptotic activity, and caspase 3 activity following FLAG–GS28 expression. WI-38 cells that overexpressed either FLAG–GS28 or control GFP were treated with 2 μM cisplatin for the indicated lengths of time. Total protein extracts (50 μg) were treated for immunoblotting with antibodies directed against the indicated proteins. Molecular masses are indicated in kDa. (B) Band density and statistical analyses of p53 and Bax in cells overexpressing GS28 (as shown in A). (C) Increase in cisplatin-induced apoptotic cells following GS28 overexpression. (D) Sensitization of cells to cisplatin by GS28 overexpression (MTT assay). Results are means ± S.D. for experiments performed in triplicate. *P < 0.05; **P < 0.01.

Reduction in p53 levels in A23187-stressed cells was partially rescued by FLAG–GS28

(A) HEK-293 cells treated with A23187 (7 μM) displayed a severe ER stress response as revealed by dramatic accumulation of BiP (immunoglobulin heavy-chain-binding protein) and reduced levels of p53 protein in control shLuc/GFP- and shGS28-treated cells. Reduction in p53 levels in A23187-stressed cells was partially rescued by ectopic expression of FLAG–GS28. Molecular masses are indicated in kDa. (B) Quantification of p53 protein of the experiments shown in (A). Compared with control (shLuc/GFP), FLAG–GS28-treated cells displayed increased p53 levels.

Received 23 December 2011/1 March 2012; accepted 8 March 2012
Published as BJ Immediate Publication 8 March 2012, doi:10.1042/BJ20112223

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