

# Unfolded Protein Response Suppresses Cisplatin-Induced Apoptosis via Autophagy Regulation in Human Hepatocellular Carcinoma Cells

(unfolded protein response / Hsp27 / autophagy / cisplatin / HCC cells)

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**Abstract.** It has been shown that drug resistance is extremely common in hepatocellular carcinoma (HCC) and is one of the major problems in HCC chemotherapy. However, the detailed mechanisms remain largely unknown. We have previously shown that endoplasmic reticulum (ER) stress is involved in the tumorigenesis of HCC. Here, we demonstrated that the unfolded protein response (UPR) inhibits cisplatin-induced HCC cell apoptosis. In HCC cells, cisplatin treatment triggers the UPR, which subsequently inhibits cisplatin-induced apoptosis. Importantly, mild ER stress precondition suppresses the sensitivity of HCC cells to cisplatin-induced apoptosis through autophagy regulation. Furthermore, heat-shock protein 27 (Hsp27) is involved in the cytoprotective role of the UPR in cisplatin-induced apoptosis. We also demonstrated that Hsp27 inhibits cisplatin-induced HCC cell death through autophagy activation. Taken together, our results indicate that the UPR inhibits cisplatin-induced apoptosis in HCC cells, at least in part, by Hsp27-mediated autophagy activation.

## Introduction

Hepatocellular carcinoma (HCC) is one of the most common solid malignancies characterized by a high degree of drug resistance (Wakamatsu et al., 2007). Most patients with HCC present in an advanced stage are not amenable to potentially curative treatments (Spangenberg et al., 2009). The development of multidrug resistance (MDR) to chemotherapeutic agents in HCC cells is the major cause for failure of chemotherapy for HCC (Pérez-Tomás, 2006). Cisplatin is a widely used anticancer agent, which is effective against a broad range of tumours, such as HCC, ovarian and testicular cancer (Boulikas and Vougiouka, 2004; Wang and Lippard, 2005; Yoshikawa et al., 2008). However, the clinical application of cisplatin in cancer chemotherapy is limited by acquired or intrinsic resistance of cells to this drug (Gosepath et al., 2005). The molecular mechanisms that underlie cisplatin resistance are poorly understood. Therefore, there is an urgent need to unravel the underlying mechanisms of cisplatin resistance in cancer cells.

The endoplasmic reticulum (ER) is an essential intracellular organelle for the synthesis and maturation of secreted, membrane-bound, and some organelle-targeted proteins. Disruption of ER physiological functions leads to the accumulation of unfolded or misfolded proteins, and triggers an evolutionarily conserved response, termed the unfolded protein response (UPR) (Mori, 2000; Harding et al., 2002; Rutkowski and Kaufman, 2004). During this process, three ER transmembrane signalling molecules PRK (RNA-dependent protein kinase)-like ER kinase (PERK), inositol-requiring gene 1 (IRE1) and activating transcription factor-6 (ATF6) are activated. The activation of UPR is believed to alleviate ER stress and promote cell survival (Gething and Sambrook, 1992; Harding et al., 1999, 2002; Mori, 2000; Rutkowski and Kaufman, 2004). Glucose-regulated protein 78 (GRP78) is one of the best-characterized ER chaperone proteins. In non-stressed cells, the luminal domains of ER stress sensors, PERK, IRE1 and

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Received November 20, 2010. Accepted January 5, 2011.

Research was supported by the projects from National Natural Science Foundation of China (No. 81000886) and supported by the grants from Sichuan (Nos. 2009SZ0117 and 09ZA050).

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Abbreviations: ATF6 – activating transcription factor 6, eIF2 $\alpha$  – eukaryotic translation initiation factor 2 $\alpha$ , ER – endoplasmic reticulum, GADD153 – DNA damage-inducible protein 153, GRP78 – glucose-regulated protein 78, HCC – hepatocellular carcinoma, Hsp27 – heat-shock protein 27, IRE1 – inositol-requiring gene 1, MDR – multidrug resistance, PARP – poly(ADP-ribose) polymerase, PERK – PRK (RNA-dependent protein kinase)-like ER kinase, UPR – unfolded protein response.

ATF6 are occupied by GRP78, which represses the UPR signalling pathways. Upon ER stress and malformed protein accumulation in the ER, sequestration of GRP78 by unfolded proteins activates these sensors (Hendershot, 2004; Schröder and Kaufman, 2005). It is known that GRP78 inhibits apoptotic signalling and protects the cell from apoptosis induced by ER or non-ER stress. Recently, it was discovered that heat-shock protein 27 (Hsp27) plays an important role in inhibiting ER stress-mediated apoptosis (Gupta et al., 2010). However, the mechanisms by which Hsp27 protects eukaryotic cells against cell death under ER stress conditions remain to be explored.

Autophagy is an evolutionarily conserved catabolic process for the degradation and recycling of cellular components in response to nutrient starvation or metabolic stress (Levine and Klionsky, 2004; Lum et al., 2005). When autophagy is induced, a double-membrane structure called autophagosome is formed *de novo* or from the existing membrane to enclose the subcellular components. The functional relationship between apoptosis and autophagy is complicated in that autophagy promotes survival under conditions of stress and starvation, whereas in other cellular situations autophagy constitutes an alternative pathway to cellular demise (Baehrecke, 2005). The importance of autophagy in modulating cancer development and in determining the response of tumour cells to anticancer therapy has been confirmed. Autophagy is one of the most important mechanisms enabling cancer cell survival and potential recurrence after long-term chemotherapy.

Recent data suggest that the UPR can alter the sensitivity of cancer cells to chemotherapeutic agents, making them either more sensitive in some cases or more resistant in others. We have previously shown the potential involvement of the UPR in HCC progression (Dai et al., 2009), but the role of the UPR in HCC remains to be clarified. Recently, it was discovered that autophagy is activated upon ER stress as a defensive mechanism for survival (Ogata et al., 2006; Ding et al., 2007). Therefore, this raised the possibility that the UPR might lead to cisplatin resistance in HCC cells via autophagy regulation. In order to test this hypothesis, dithiothreitol (DTT) and tunicamycin (Tun) were used to induce ER stress response in SMMC-7721, HepG2 and Hep3B cells. We report here that the UPR protects HCC cells against cisplatin-induced apoptosis through autophagy regulation.

## Material and Methods

### Materials

Dithiothreitol (DTT), tunicamycin (Tun) and 3-methyladenine (3-MA) were purchased from Sigma Chemical Company (St. Louis, MO). ATF6, IRE1 $\alpha$ , GRP78 and

Hsp27 siRNA, salubrinal, antibodies against ATF6, IRE1 $\alpha$ , GRP78, GADD153 and Hsp27 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Atg5 siRNA, antibodies against Atg5, Cleaved PARP, LC3A/B, and  $\beta$ -actin were purchased from Cell Signaling Technology (Beverly, MA).

### Cell culture and treatment

SMMC-7721, HepG2 and Hep3B cells were maintained at 37 °C in a humidified incubator containing 5% CO<sub>2</sub>, in Dulbecco's Modified Eagle Medium (DMEM, Sigma) supplemented with 10% foetal bovine serum and 1% penicillin/streptomycin. Dithiothreitol (1.25 mM/ml) and tunicamycin (1  $\mu$ g/ml) were used to induce mild ER stress response. The cells were pre-treated with 10  $\mu$ M/ml 3-MA, a specific inhibitor of endogenous lysosomal protein degradation that targets PI3KC3 but not the other PI3Ks, or 50  $\mu$ M/ml salubrinal for 1 h prior to dithiothreitol, tunicamycin or cisplatin treatment. The protocol used for ATF6, IRE1 $\alpha$ , Atg5, GRP78 and Hsp27 knockdown has been previously described (Li and Lee, 2006; Hara et al., 2008), the control siRNA against GFP was used.

### Western blot analysis

Cells were lysed in Triton lysis buffer (20 mM Tris, pH 7.4, 137 mM NaCl, 10% glycerol, 1% Triton X-100, 2 mM EDTA, 1 mM PMSF, 10 mM NaF, 5 mg/ml aprotinin, 20 mM leupeptin, and 1 mM sodium orthovanadate) and centrifuged at 12,000 g for 15 min. Protein concentrations were measured using the BCA assay (Santa Cruz, CA). Equal proteins were applied to SDS-PAGE. After electrophoresis, proteins were blotted to polyvinylidene fluoride (PVDF) membranes and then blocked with 5% skim milk powder with 0.1% Tween-20. The blots were then probed at 4 °C overnight with the relevant primary antibodies, washed three times with TBST (TBS containing 0.1% Tween-20), and probed with the appropriate horseradish-peroxidase-conjugated secondary antibodies at room temperature for 2 h. Immunoreactive material was detected using the ECL kit (Santa Cruz, CA) according to the manufacturer's instructions.

### Apoptosis analysis

Cells were treated with dithiothreitol and tunicamycin at indicated dose for the indicated time. Apoptosis was detected using Annexin V-FITC Apoptosis Detection Kit (BD PharMingen, Franklin Lakes, NJ) according to the manufacturer's manual. Annexin V staining was analysed by flow cytometry within 1 h. The experiments were repeated three times.

### Statistical analysis

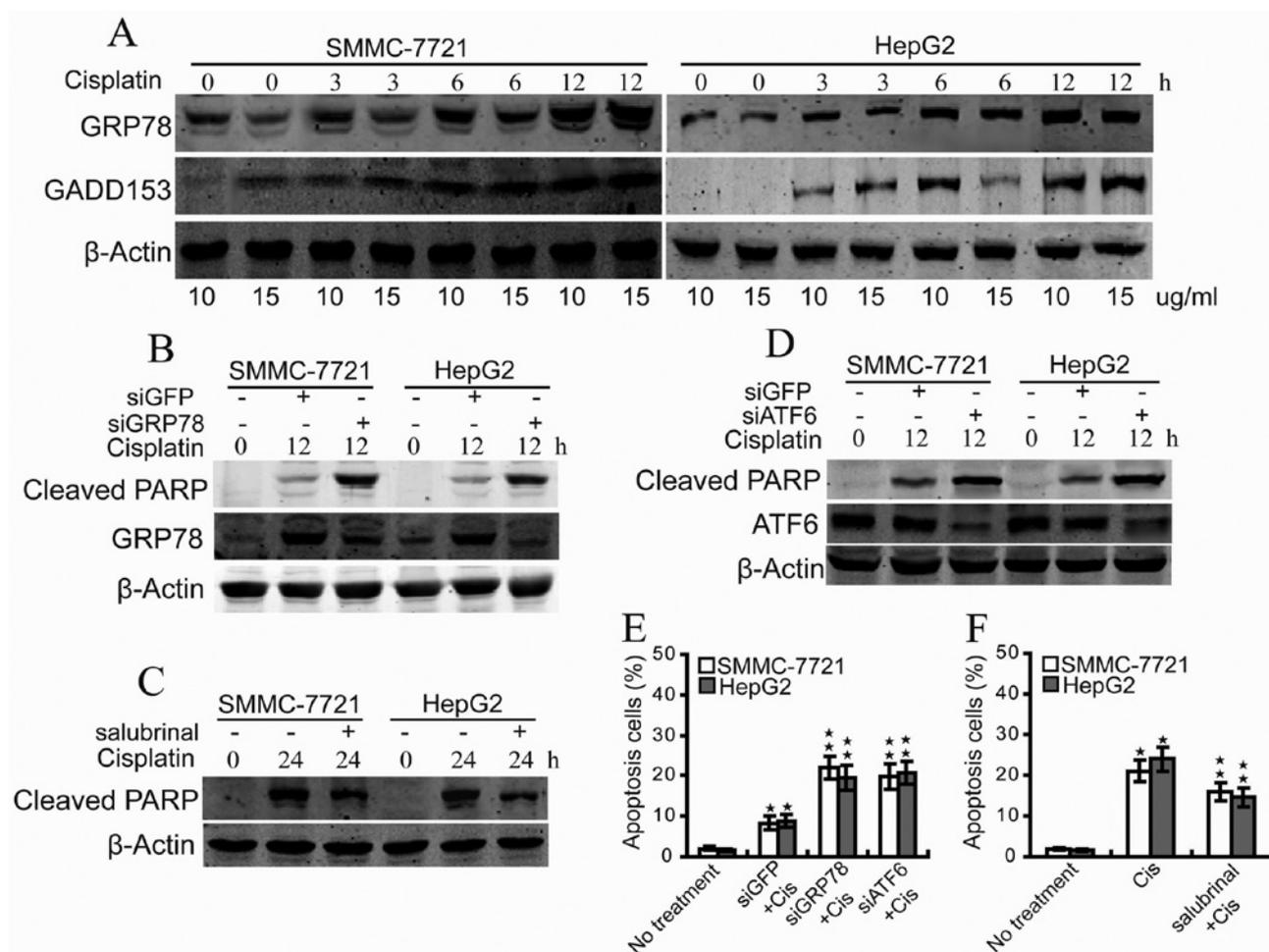
Results are expressed as mean  $\pm$  standard deviation. Statistical analysis was performed using Student's *t*-test.  $P < 0.05$  was considered statistically significant.

## Results

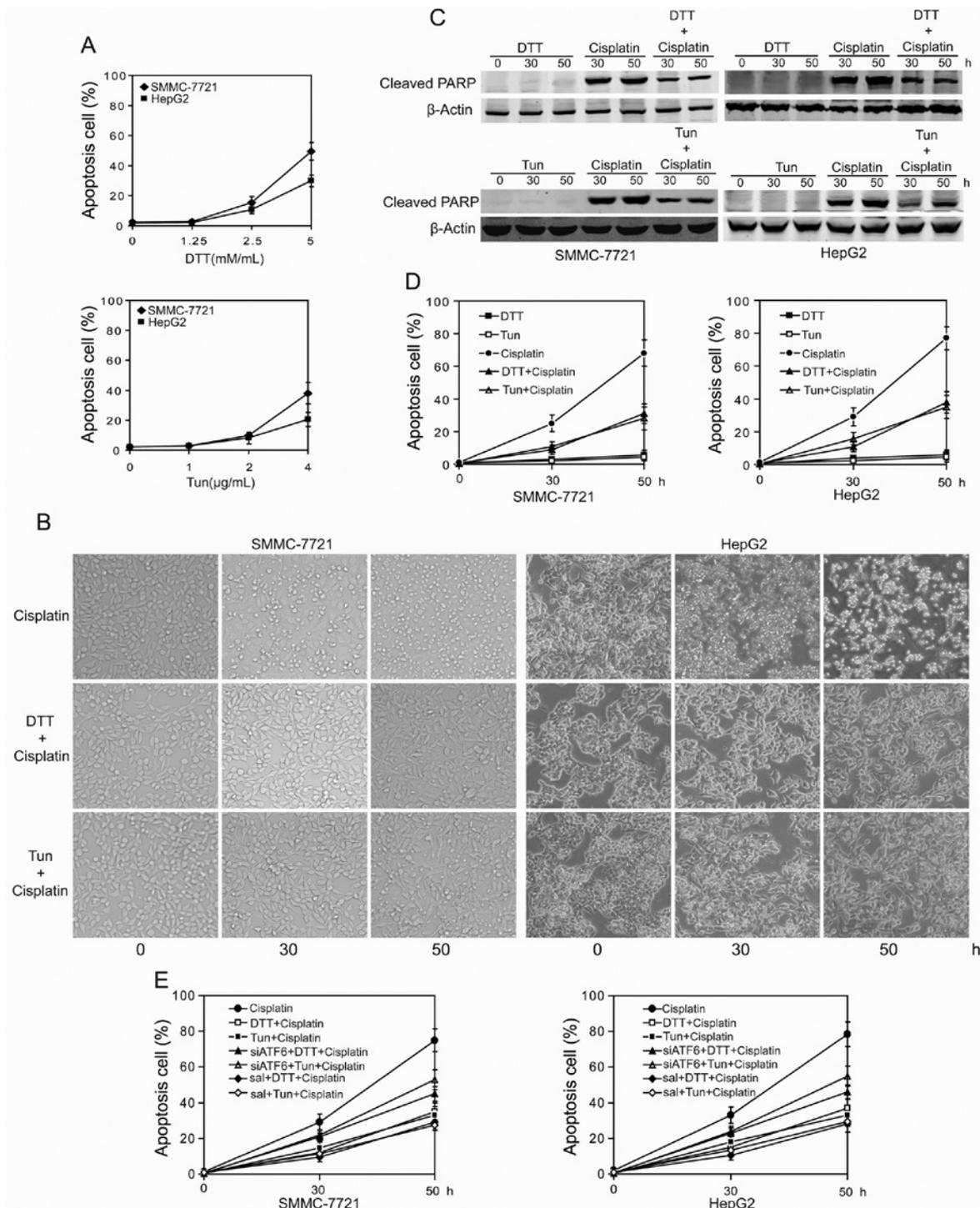
### *ER stress is involved in cisplatin-induced HCC cell apoptosis regulation*

It has been reported that cisplatin can induce apoptosis through the UPR induction in human melanoma cells (Mandic et al., 2003). We firstly investigated the effect of cisplatin on the UPR induction in HCC cells. Incubation of SMMC-7721 and HepG2 cells with cisplatin for the indicated time markedly elevated GRP78 and growth arrest and DNA damage-inducible protein 153

(GADD153) levels (Fig. 1A), indicating the UPR activation (Yoshida et al., 2001; Hu et al., 2004). As the UPR is an important cytoprotective mechanism under stress conditions, we examined whether the UPR can protect HCC cells against cisplatin-induced death. Fig. 1B and E showed that GRP78 knockdown significantly promoted cisplatin-induced apoptosis in SMMC-7721 and HepG2 cells. To directly prove the role of the main components (PERK/eIF2 $\alpha$ , ATF6 and IRE1 $\alpha$ ) of UPR in protection against cisplatin-induced death, SMMC-7721 and HepG2 cells were treated with cisplatin in the presence or absence of salubrinal (50  $\mu$ M/l), which selec-



**Fig. 1.** ER stress is involved in cisplatin-induced HCC cell apoptosis regulation. (A) Cisplatin induces the UPR in SMMC-7721 and HepG2 cells. After SMMC-7721 and HepG2 cells were treated with cisplatin (10  $\mu$ g/ml) for indicated times, GRP78 and GADD153 protein levels were detected by Western blot. (B) GRP78 knockdown promotes cisplatin-induced HCC cell apoptosis. SMMC-7721 and HepG2 cells were treated with cisplatin (10  $\mu$ g/ml) for 12 h with or without GRP78 siRNA transient transfection. Cleaved PARP was detected by Western blot. (C) Salubrinal inhibits cisplatin-induced HCC cell apoptosis. Western blot analysis for cleaved PARP in cisplatin-treated SMMC-7721 and HepG2 cells for 24 h with or without salubrinal (50  $\mu$ M/l) pre-incubation for 1 h. (D) ATF6 knockdown promotes cisplatin-induced HCC cell apoptosis. SMMC-7721 and HepG2 cells were treated with cisplatin (10  $\mu$ g/ml) for 12 h with or without ATF6 siRNA transient transfection. Cleaved PARP was detected by Western blot. (E) SMMC-7721 and HepG2 cells were treated with cisplatin (10  $\mu$ g/ml) for 12 h with or without GRP78 or ATF6 siRNA transient transfection. Apoptosis was measured using flow cytometry after staining with FITC-conjugated Annexin V and propidium iodide. The data are expressed as the mean  $\pm$  SD for the three determinations in triplicate; bars, SE. (F) SMMC-7721 and HepG2 cells were treated with cisplatin (10  $\mu$ g/ml) for 24 h with or without salubrinal (50  $\mu$ M/l) pre-incubation. Apoptosis was measured using flow cytometry after staining with FITC-conjugated Annexin V and propidium iodide. The data are expressed as the mean  $\pm$  SD for the three determinations in triplicate; bars, SE.



**Fig. 2.** ER stress precondition inhibits cisplatin-mediated HCC cell apoptosis. **(A)** DTT and Tun induce HCC cell apoptosis in a concentration-dependent manner. SMMC-7721 and HepG2 cells with DTT and Tun treatment for 48 h at indicated dose were subjected to measurement of apoptosis using flow cytometry after staining with FITC-conjugated Annexin V and propidium iodide. Points, mean of three individual experiments; bars, SE. **(B, C and D)** Mild ER stress precondition inhibits cisplatin-induced HCC cell apoptosis. After DTT (1.25 mM/l) and Tun (1 μg/l) pre-treatment for 6 h, SMMC-7721 and HepG2 cells were treated with cisplatin (10 μg/ml) for indicated times. Apoptosis was detected by morphological examination (B) and Western blot analysis (C). After DTT (1.25 mM/l) and Tun (1 μg/l) pre-treatment for 6 h, SMMC-7721 and HepG2 cells were treated with cisplatin (10 μg/ml) for indicated times (D). Apoptosis was measured using flow cytometry after staining with FITC-conjugated Annexin V and propidium iodide. Points, mean of three individual experiments; bars, SE. **(E)** After DTT (1.25 mM/l) and Tun (1 μg/l) pre-treatment for 6 h with or without ATF6 suppression and salubrinal (50 μM/l) treatment, SMMC-7721 and HepG2 cells were treated with cisplatin (10 μg/ml) for indicated times. Apoptosis was measured using flow cytometry after staining with FITC-conjugated Annexin V and propidium iodide. Points, mean of three individual experiments; bars, SE.

tively inhibits eIF2 $\alpha$  dephosphorylation. The results showed that salubrinal inhibited SMMC-7721 and HepG2 cells to cisplatin-induced apoptosis (Fig. 1C and F). Furthermore, ATF6-specific siRNA transient transfection significantly promoted cisplatin-induced apoptosis, indicating that ATF6 signal was involved in the protective role of UPR (Fig. 1D and E). However, IRE1 $\alpha$  suppression had no apparent effects on the protective role of UPR against cisplatin-induced HCC cell death (data not shown). These results reveal that PERK/eIF2 $\alpha$  and ATF6 pathways are involved in protecting HCC cells against cisplatin-mediated apoptosis.

#### *ER stress precondition inhibits cisplatin-mediated HCC cell apoptosis*

During tumour development, several ER stress activators, such as hypoxia and low glucose, are known to induce resistance to chemotherapy through UPR-dependent mechanisms (Tsuruo et al., 2003). Since the UPR is obviously activated in HCC (Dai et al., 2009), dithiothreitol and tunicamycin were used to establish ER stress microenvironment in SMMC-7721 and HepG2 cells. Based on this mimicked ER stress microenvironment, we investigated whether the ER stress precondition plays some role in regulating cisplatin-mediated HCC cell death.

As the UPR cellular signal will result in stressed cell death under aggravated ER stress condition, SMMC-7721 and HepG2 cells were treated with different doses of dithiothreitol and tunicamycin to optimize the concentration which initiates mild ER stress without undergoing apoptosis. As shown in Fig. 2A, SMMC-7721 and HepG2 cells were relatively resistant to ER stress-induced apoptosis triggered by dithiothreitol and tunicamycin treatment at relatively high doses (DTT 2.5 mM/ml and Tun 2  $\mu$ g/ml) and for a relatively long time (< 20 % apoptotic cells at 48 h); 1.25 mM/ml dithiothreitol and 1  $\mu$ g/ml tunicamycin resulted in little cell death. Accordingly, the dosage of 1.25 mM/ml dithiothreitol and 1  $\mu$ g/ml tunicamycin were used to induce optimum ER stress before cisplatin treatment in SMMC-7721 and HepG2 cells. The results showed that dithiothreitol and tunicamycin pre-incubation obviously decreased cisplatin cytotoxicity in SMMC-7721 and HepG2 cells (Fig. 2B). In order to confirm the role of the UPR in inhibiting cisplatin-induced HCC cell apoptosis, cleavage of poly(ADP-ribose) polymerase (PARP) was detected by Western blot analysis. The results indicated that dithiothreitol and tunicamycin pre-incubation inhibited cisplatin-induced SMMC-7721 and HepG2 cell apoptosis (Fig. 2C). Furthermore, Annexin V-FITC staining (Fig. 2D) confirmed that dithiothreitol and tunicamycin pre-incubation inhibited cisplatin-induced HCC cell apoptosis. However, severe ER stress precondition rendered SMMC-7721 and HepG2 cells more sensitive to cisplatin-induced apoptosis (data not shown).

As PERK/eIF2 $\alpha$  and ATF6 pathways are involved in protecting HCC cells against cisplatin-mediated apoptosis, the effects of PERK/eIF2 $\alpha$  and ATF6 signals in the

protective role of UPR pre-activation were investigated. As shown in Fig. 2E, ATF6 suppression inhibited the protective effect of ER stress precondition in cisplatin-treated SMMC-7721 and HepG2 cells. Furthermore, salubrinal pre-treatment enhanced the protective function of ER stress precondition (Fig. 2E). These data imply that optimum UPR protects HCC cells against cisplatin-induced apoptosis.

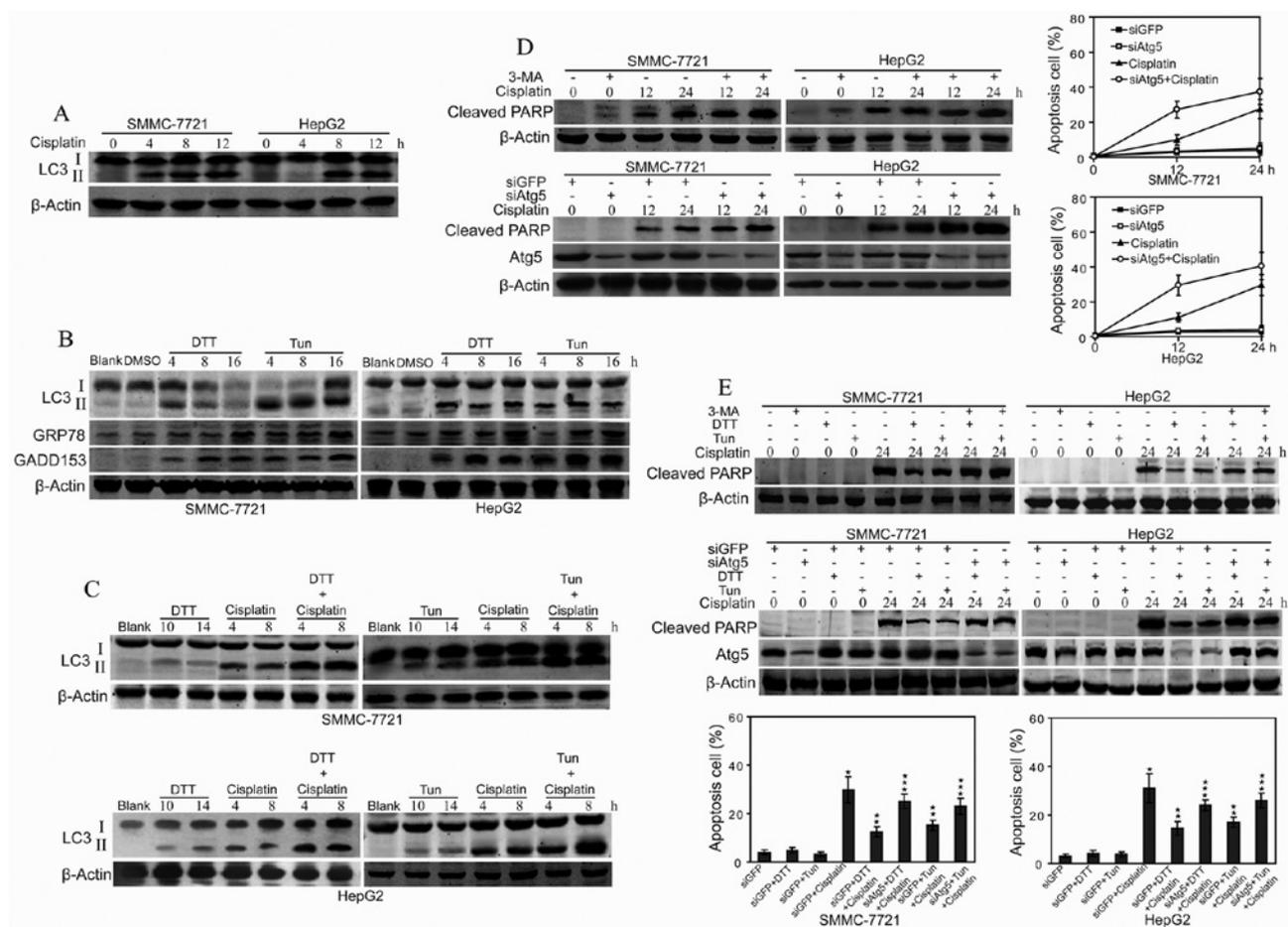
#### *Autophagy contributes to the cytoprotective function of ER stress in cisplatin-induced HCC cell apoptosis*

Considering that autophagy is activated upon ER stress as a defensive mechanism for survival, it seems that ER stress may suppress cisplatin-mediated HCC cell apoptosis through autophagy regulation. To validate this hypothesis, the effect of the UPR on cisplatin-mediated autophagy in HCC cells was investigated. Figure 3A shows that cisplatin treatment induced endogenous LC3 conversion in SMMC-7721 and HepG2 cells at indicated time points. Moreover, autophagy was activated in dithiothreitol- and tunicamycin-treated SMMC-7721 and HepG2 cells (Fig. 3B). In order to make sure whether the UPR may promote autophagy in cisplatin-treated HCC cells, SMMC-7721 and HepG2 cells were treated with dithiothreitol and tunicamycin for 6 h before cisplatin administration. As shown in Fig. 3C, dithiothreitol and tunicamycin pre-treatment promoted endogenous LC3 conversion in cisplatin-treated SMMC-7721 and HepG2 cells. Taken together, these data indicate that the UPR promotes cisplatin-induced autophagy in HCC cells.

To confirm the protective role of autophagy in preventing HCC cells against cisplatin-induced apoptosis, autophagy was inhibited by Atg5 knockdown or 3-methyladenine (3-MA) treatment in SMMC-7721 and HepG2 cells. Figure 3D shows that inhibition of autophagy by 3-MA treatment or Atg5 suppression substantially increased the sensitivity of HCC cells to cisplatin-induced apoptosis. More importantly, 3-MA pre-incubation and Atg suppression prevented the cytoprotective function of dithiothreitol and tunicamycin in cisplatin-treated SMMC-7721 and HepG2 cells (Fig. 3E). These results suggest that the cytoprotective function of the UPR in cisplatin-treated HCC cells is dependent, at least in part, on autophagy activation.

#### *Hsp27 is implicated in the cytoprotective function of ER stress in cisplatin-induced HCC cell apoptosis*

Hsp27 has been shown to prevent cell death by a wide variety of cytotoxic stimuli (Arrigo, 2007; Lanneau et al., 2007). Since Hsp27 is involved in inhibiting cisplatin-induced apoptosis (Garrido et al., 1997; Wachsberger et al., 1997), we also studied whether Hsp27 protects HCC cells against cisplatin-induced death. Figure 4A shows that cisplatin treatment obviously resulted in



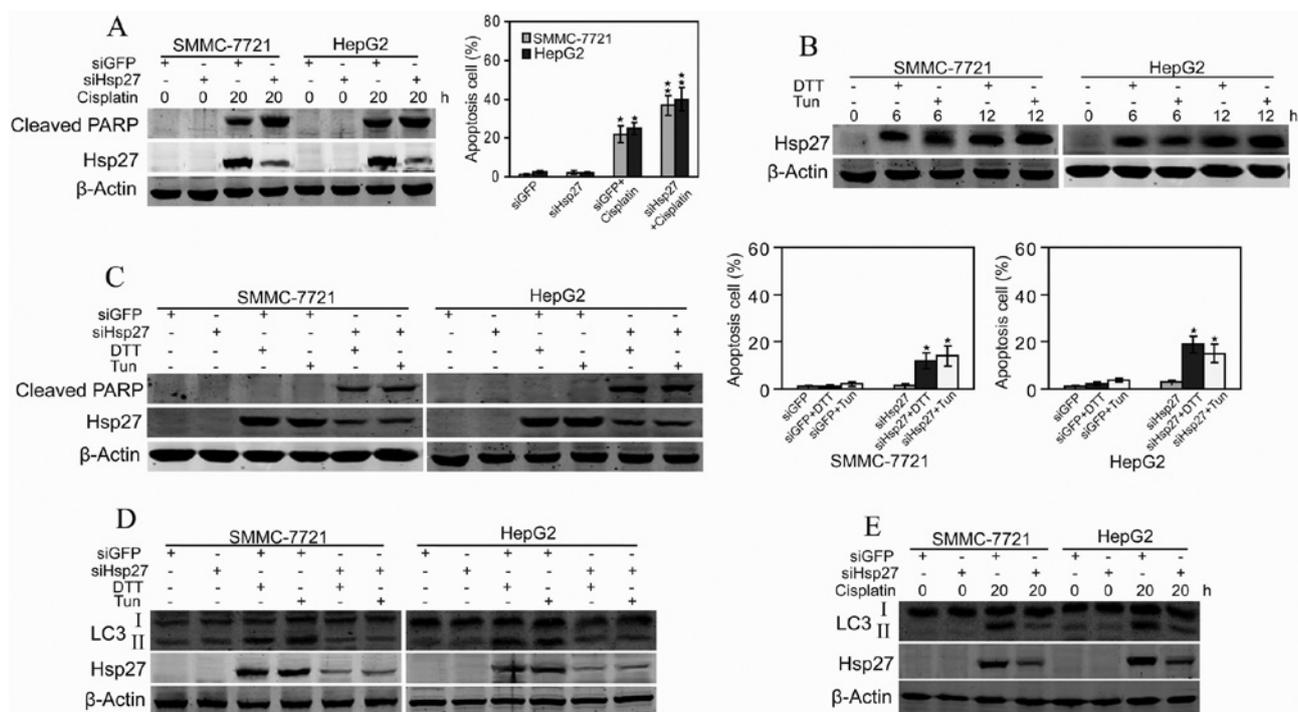
**Fig. 3.** Autophagy contributes to the cytoprotective function of ER stress in cisplatin-treated HCC cells. (A) Cisplatin triggers autophagy activation. Cisplatin (10  $\mu$ M)-treated SMMC-7721 and HepG2 cells were subjected to Western blot analysis for LC3 conversion. (B) ER stress triggers autophagy activation. DTT (1.25 mM/l)- and Tun (1  $\mu$ g/l)-treated SMMC-7721 and HepG2 cells were subjected to Western blot analysis for LC3 conversion. (C) ER stress promotes cisplatin-triggered autophagy activation. After SMMC-7721 and HepG2 were treated with cisplatin (10  $\mu$ M) at indicated times with or without DTT (1.25 mM/l) or Tun (1  $\mu$ g/l) pre-incubation for 6 h, LC3 conversion was detected by Western blot. (D and E) Autophagy inhibitor 3-MA pre-incubation for 1 h and siAtg5 transient transfection not only promotes cisplatin (10  $\mu$ M)-mediated SMMC-7721 and HepG2 cell apoptosis but also inhibits the protective function of DTT (1.25 mM/l) or Tun (1  $\mu$ g/l) in cisplatin (10  $\mu$ M)-treated SMMC-7721 and HepG2 cells. Apoptosis was evaluated by cleaved PARP and reconfirmed by Annexin V-FITC flow cytometry. Data are presented as mean values  $\pm$  SD of three measurements; bars, SE.

Hsp27 accumulation in SMMC-7721 and HepG2 cells. Furthermore, Hsp27 suppression by specific siRNA substantially increased the sensitivity of SMMC-7721 and HepG2 cells to cisplatin-mediated apoptosis (Fig. 4A). Recently, it was discovered that exogenous Hsp27 expression inhibits ER stress-induced apoptosis (Gupta et al., 2010). We tested the role of Hsp27 in ER stress-mediated HCC cell death. Our data showed that dithiothreitol and tunicamycin treatment triggered Hsp27 induction (Fig. 4B), and Hsp27 knockdown rendered SMMC-7721 and HepG2 cells more sensitive to dithiothreitol- and tunicamycin-induced apoptosis (Fig. 4C). More importantly, Hsp27 knockdown inhibited both UPR- and cisplatin-induced autophagy activation in SMMC-7721 and HepG2 cells (Fig. 4D and E). Taken together, these data indicate that Hsp27 plays a

pivotal role in preventing cisplatin-induced HCC cell apoptosis, at least in part, through autophagy activation.

## Discussion

Hypoxia and anoxia are pathophysiologic characteristics of most solid tumours. Evidence is emerging that hypoxia and anoxia play an important role in drug resistance of solid tumours. Both hypoxia and anoxia can result in ER stress, initiating the UPR. We have shown that the UPR is activated in HCC, but how this contributes to chemoresistance in HCC cells remains largely unknown. In this study, we demonstrated that the UPR protects HCC cells against cisplatin-induced apoptosis through autophagy regulation.



**Fig. 4.** Hsp27 is implicated in the cytoprotective function of ER stress in cisplatin-treated HCC cells. **(A)** Cisplatin induces Hsp27 accumulation and Hsp27 knockdown promotes cisplatin-mediated HCC cell apoptosis. After transient transfection with Hsp27 siRNA for 24 h, SMMC-7721 and HepG2 cells were treated with or without cisplatin (10  $\mu$ g/ml) for another 20 h. Hsp27 and cleaved PARP were detected by Western blot. **(B)** ER stress induces Hsp27 accumulation in HCC cells. DTT (1.25 mM/l)- and Tun (1  $\mu$ g/l)-treated SMMC-7721 and HepG2 cells were subjected to Western blot analysis for Hsp27. **(C)** Hsp27 knockdown promotes ER stress-mediated HCC cell apoptosis. After transient transfection with Hsp27 siRNA for 24 h, SMMC-7721 and HepG2 cells were treated with or without DTT (1.25 mM/l) and Tun (1  $\mu$ g/l) for another 36 h. Apoptosis was evaluated by cleaved PARP and reconfirmed by Annexin V-FITC flow cytometry. Data are presented as mean values  $\pm$  SD of three measurements; bars, SE. **(D)** Hsp27 knockdown inhibits ER stress-induced autophagy activation. After transient transfection with Hsp27 siRNA for 24 h, SMMC-7721 and HepG2 cells were treated with DTT (1.25 mM/l) and Tun (1  $\mu$ g/l) for another 6 h. LC3 conversion was detected by Western blot. **(E)** Hsp27 knockdown inhibits cisplatin-induced autophagy activation. After transient transfection with Hsp27 siRNA for 24 h, SMMC-7721 and HepG2 cells were treated with cisplatin (10  $\mu$ g/ml) for another 20 h. LC3 conversion was detected by Western blot.

Cisplatin had been reported to initiate ER stress, and ER might be a cytoplasmic target of cisplatin (Mandic et al., 2003; Peyrou et al., 2007). Here, we found that cisplatin treatment resulted in GRP78 and GADD153 induction, indicators of UPR activation, in SMMC-7721 and HepG2 cells. Furthermore, the findings that knockdown of GRP78 or ATF6 by siRNA enhanced apoptosis induced by cisplatin strongly suggest that the UPR protects HCC cells against cisplatin-induced apoptosis. To confirm the cytoprotective role of the UPR in cisplatin-mediated HCC cell death, mild ER stress was triggered before cisplatin treatment. Our data showed that optimum concentration of dithiothreitol and tunicamycin pre-incubation significantly reduced cisplatin-induced apoptosis in HCC cells. However, severe ER stress precondition rendered HCC cells more sensitive to cisplatin-induced apoptosis (data not shown). We therefore conclude that mild UPR promotes HCC cell survival under cisplatin stress.

A recent report has revealed that tunicamycin represses cisplatin-induced apoptosis via p53 protein nuclear ex-

port in HepG2 cells (Zhang et al., 2009). We found that mild ER stress precondition also protected Hep3B (p53-null HCC cells) against cisplatin-mediated death (data not shown). It seems that there are other mechanisms involved in the cytoprotective role of the UPR. There is an accumulation of evidence that highlights the important function of autophagy in drug resistance (Carew et al., 2007; Apel et al., 2008; Chen and Karantza-Wadsworth, 2009). Considering that both cisplatin and ER stress inducers dithiothreitol and tunicamycin activate autophagy in HCC cells, it is reasonable that the UPR might promote cisplatin-treated HCC cell survival through autophagy. This hypothesis is supported by our data, which demonstrated that cisplatin-induced apoptosis in HCC cells can be enhanced by autophagy inhibitor 3-MA or Atg5 knockdown. Furthermore, 3-MA treatment or Atg5 knockdown suppressed the cytoprotective role of mild ER stress precondition in cisplatin-treated HCC cells. Mechanistically, these results suggest that the UPR can inhibit cisplatin-induced HCC cell apoptosis through autophagy regulation.

Recently, it has been reported that Hsp27 is implicated in ER stress-mediated cell death regulation (Gupta et al., 2010). To examine whether Hsp27 is responsible for autophagy activation induced by ER stress and cisplatin in HCC cells, we employed siRNA to knock down Hsp27 expression. We found that Hsp27 knockdown suppressed both ER stress- and cisplatin-induced autophagy activation and apoptosis in HCC cells. This provides evidence that Hsp27 is involved in the cytoprotective function of the UPR in inhibiting cisplatin-mediated HCC cell apoptosis, at least in part, by autophagy activation.

In brief, we reported that mild ER stress protects HCC cells from cisplatin-induced apoptosis. The cytoprotective role of the UPR under cisplatin treatment is mediated, at least in part, by autophagy activation. We also showed that Hsp27 is involved in cisplatin- and ER stress-triggered autophagy activation in HCC cells. Further studies on the detailed mechanisms of Hsp27-mediated autophagy regulation in HCC cells will contribute to the development of new therapeutic strategies against HCC.

### Acknowledgments

R. Chen, R. Y. Dai and C. Y. Duan contributed equally to this work. There is no conflict of interest.

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