Cancer Chemother Pharmacol (2012) 69:1205–1212
DOI 10.1007/s00280-012-1823-0

ORIGINAL ARTICLE

Correlation between low-level expression of the tumor suppressor gene TAp73 and the chemoresistance of human glioma stem cells

Xiaogang Hu · Nan Wu · Peiyuan Xia · Sicang Yu · Fengjun Sun · Jianhong Chen

Received: 16 September 2011 / Accepted: 4 January 2012 / Published online: 19 January 2012

Abstract
Objective Glioma stem cells (GSCs) are regarded as the root of glioma growth and recurrence. Chemoresistance is one of the characteristics of GSCs that increases the difficulties in eradicating the cells by anticancer drugs.
Purpose The objective of this study is to investigate the correlation between expression of the tumor suppressor gene TAp73 and the chemoresistance of human GSCs.
Methods MTT and tumor sphere formation assays were used to analyze the chemoresistance phenotype of GSCs derived from primary human glioma specimens under cisplatin exposure. Reverse transcription real-time PCR was applied for assaying mRNA levels of TAp73. Protein levels of TAp73, p21, Bax, and cleared caspase 3 were assayed by western blot. Cell apoptosis was analyzed by flow cytometry after the annexin V fluorescence staining.
Results GSCs exhibited increased chemoresistance compared to differentiated glioma cells (DGCs) derived from the same tumor specimen. The expression of TAp73 was lower in GSCs and was not sensitive to cisplatin treatment as compared to DGCs. Overexpression of TAp73 by transfection increased the apoptosis of GSCs in the presence of cisplatin and reduced the chemoresistance of GSC. TAp73 knockdown by siRNA in DGCs reduced cisplatin-induced apoptosis and increased the resistance to cisplatin.
Conclusion These findings indicate that TAp73 silencing is hallmark of GSC to maintain their chemoresistance phenotype. Thus, targeting TAp73 may provide a novel strategy to eradicating GSCs.

Keywords TAp73 · Glioma stem cells · Chemoresistance · Apoptosis

X. Hu · P. Xia · F. Sun · J. Chen (✉)
Department of Pharmacy, Southwest Hospital, Third Military Medical University, Chongqing 400038, China
e-mail: chenjh-110@263.net

N. Wu
Department of Neurosurgery, Southwest Hospital, Third Military Medical University, Chongqing 400038, China

S. Yu
Institute of Pathology, Southwest Hospital, Third Military Medical University, Chongqing 400038, China

S. Yu
Southwest Cancer Center, Southwest Hospital, Third Military Medical University, Chongqing 400038, China

Introduction

Gliomas are the most common tumors in human brain arising from glial cells. The five-year survival for patients with glioma is only 12–15% despite combined therapies of surgery, radiation and chemotherapy. Glioma stem cells (GSCs) are a minor population in the tumor but are regarded as the root of tumor origin and relapse. One of the characteristic phenotypes of GSCs is chemoresistance [16], which is implicated in the failure of anticancer drugs to decrease the rate of glioma recurrence in patients.

Many anticancer drugs induce DNA damage in tumor cells, which is mediated by p53 family proteins acting as "guardian of the genome." These proteins maintain the integrity of the genome and eliminate damaged cells, in association with changes in the expression of a number of genes involved in cell cycle arrest and apoptosis [33]. p73 is a novel p53 family member, which resembles p53 not only in structure but also in biological activity [14, 15]. The amino acid sequences of p53 family members are highly

homologous, in particular in the domains of DNA binding, transactivation and oligomerization. p73 isoforms originate from alternative splicing of the p73 gene and are divided into two groups: TAp73 with N-terminal transactivation (TA) domain and DNp73 without TA domain. The TA domain and C-terminus determine the p73 activity in cells. Due to its high structural and functional homology to p53, p73 is recognized as an important target for anticancer therapies aiming at selective activation of pro-apoptotic function of TAp73 in malignant cells.

In the present study, we report lower levels of TAp73 expression in GSCs derived from human primary glioblastomas as compared to differentiated glioma cells (DGCs) from the same tumor specimens, and this confers a cisplatin-resistant phenotype to GSCs.

Materials and methods

Glioma specimens

Glioma specimens were obtained from patients in the Department of Neurosurgery, Southwest Hospital, Third Military Medical University, Chongqing, China, with written consent. The tumors were classified by at least two pathologists according to the 2007 WHO classification of central nervous system tumors [17]. All experiments were approved by the institutional ethics committee. None of the patients received chemotherapy or radiotherapy before surgery.

Isolation and identification of GSCs

Tumor tissues from human primary glioblastoma were isolated and identified as previously described [29]. Briefly, primary tumor cells were detached with trypsin (Gibco, USA) and suspended in Dulbecco's modified Eagle's medium/F12 (DMEM/F12, Hyclone, USA) supplemented with penicillin/streptomycin (Sigma, USA), B-27 (1×, Gibco), 20 ng/mL basic fibroblast growth factor (bFGF, Peprotech, USA) and 20 ng/mL epidermal growth factor (EGF, Peprotech, USA) (stem cell medium). After 24 h, most tumor cells became adherent, with about 10% to 20% cells floating and forming spheres. The adherent cells were continuously cultured in DMEM containing 20% fetal calf serum (FCS) and penicillin/streptomycin and were named as differentiated glioma cells (DGCs). After an additional 7 days, spheres expanded in size, with 200 to 300 cells in each sphere. The spheres then were dissociated and plated at a density of 10 cells/well in 0.2 ml stem cell medium to acquire secondary neurospheres. The spheres were enriched in GSCs as measured by a CD133 immunofluorescence staining and flow cytometry. The spheres were dispersed and suspended in CD133 MicroBeads for purification of GSCs by a CD133 cell isolation kit (Miltenyi Biotec, 130-050-801) and MACS MS columns (Miltenyi Biotec, 130-042-201). GSCs were cultured in stem cell medium.

Immunofluorescence staining

After GSCs formed colonies containing 10–30 cells on coverslips, the colonies were fixed in 4% paraformaldehyde for 10 min at room temperature and incubated with mouse antibody against human CD133 (1:200; Abcam, Cambridge, UK). Secondary antibodies PE-conjugated goat anti-mouse IgG (1:500 dilution, Beyotime institute of Biotech, China) were added to the culture for an additional 1 h at room temperature. Cell nuclei were then labeled with DAPI. The coverslips containing differentiating cells were examined under a laser confocal scanning microscope (Leica, Germany).

MTT assay

In vitro chemoresistance of glioma cells to cisplatin (Sigma, St. Louis, MO, USA) dissolved in 100% (v/v) ethanol was measured by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay. Briefly, 100-μL aliquots of cell suspension with a density of 1.6×10^6 viable cells/mL were incubated with 2 μM cisplatin. After 48 h at 37°C, the number of viable cells was measured in a spectrophotometer at 562 nm after the addition of 10 μL MTT (5 mg/ml; Sigma) followed by 6 h of incubation at 37°C and the subsequent addition of 100 μL acidified isopropanol (0.04 M HCl–isopropanol). The percentage of viable cells cultured in the absence of cisplatin was set as 100%.

Reverse transcription and real-time quantitative polymerase chain reaction

RNeasy MicroKit (Qiagen, Valencia, CA, USA) was used to isolate total RNA from tumor cells. cDNA was generated using the Qiagen Sensiscript RT kit. TAp73 mRNA levels were measured by real-time polymerase chain reaction (PCR) using SYBR Green PCR Master Mix reagent (Takara, Dalian, China) and normalized to β-actin. The primers 5′-TGC TGT CCC TGT ATG CCT CTG -3′ and 5′-TGA TGT CAC GCA CGA TTT CC-3′ were used for β-actin and the 5′-GGC TGC GAC GGC TGC AGA GC-3′ and 5′-GCT CAG CAG ATT GAA CTG GGC CAT G-3′ for TAp73 whose PCR products contained exon 2, 3 and part of exon 4 [24]. The expression of cisplatin-induced TAp73 was calculated as the fold of TAp73 mRNA level in cisplatin-treated cells over untreated cells.

Knockdown of TAp73 by TAp73 siRNA

DGCs were transfected by retrovirus containing blank or TAp73 siRNA expression cassettes. The siRNA sequence targeting TAp73 is 5′-GGA TTC CAG CAT GGA CGT CTT-3′ [13, 18, 22], which is also found within p73 exon 3. Therefore, this siRNA does not target deltaNp73. Retroviral vector stocks were produced by transient transfection of Phoenix-Ampho cells with Superfect Transfection Reagent (QIAGEN, Valencia, CA, USA) and 5 μg siRNA expression plasmid (pRNATin-H1.4/Retro, Genscript, Piscataway, NJ, USA). Virus was collected from the culture supernatants on day 2 after transfection, and the cells were transfected with the vectors in the presence of polybrene at 5 μg/mL. DGCs transfected with TAp73 siRNA (siTAp73) were selected and maintained by incubation with 2 μg/mL puromycin and 200 ng/mL hygromycin (BD Biosciences).

Overexpression of TAp73 in GSCs

TAp73 full cDNA (p73alpha) was cloned and amplified with Pfu DNA polymerase (Takara) using reported primers with introduction of 5′XhoI and 3′BglII sites. Purified PCR product was ligated into retrovirus vector pMSCVneo (clontech). The expression plasmids were used to transfect 293T cells with helper plasmids to produce retrovirus particles pMSCV-TAp73 (pTAp73). Virus was collected from the culture supernatant on day 2 after transfection, and the cells infected with retrovirus particles were cultured with G418 to select cells expressing TAp73.

Immunoblot

Tumor cells were lysed in SDS sample buffer, sonicated and boiled. The cell lysate was then centrifuged and electrophoresed on Tris–glycine gels. Proteins were transferred onto Immunoblotin P membranes (Millipore, Billerica, MA, USA), which were then blocked by incubation in 3% nonfat dry milk for 1 h at room temperature. Membranes were then incubated with primary antibodies against TAp73 (Imgenex, IMG-246), β-actin, Bax or p21 (Santa Cruz Biotechnologies) overnight at 4°C. After incubation with a horseradish peroxidase-conjugated secondary antibody, the protein bands were detected with Super Signal Chemoluminescent Substrate Stable Peroxide Solution (Pierce, Rockford, IL, USA).

Tumor microsphere formation

Growth of tumor cells in a semisolid agar was examined. Bacto-Agar was dissolved in a 0.6% (w/v) solution in warmed DMEM containing 10% FCS. The solution was poured into culture plates and allowed to solidify for 15 min at room temperature. And 0.3% agar solution containing 1,200 tumor cells per 100 μL was then layered over the solidified agar. The plates were incubated in 5% CO_2 at 37°C in the presence of 10% FCS/DMEM for 3 weeks. The spheres were numerated based on different sizes under microscope.

Annexin V analysis

To assess the extent of apoptosis, siTAp73, pTAp73, mock-transfected and wild-type (WT) tumor cells were exposed to 2 μM cisplatin for 48 h and then were resuspended in buffer (10 mM Hepes, pH 7.4/140 mM NaCl, 2.5 mM $CaCl_2$) for staining with 5 μL annexin V fluorescein isothiocyanate (BD Pharmingen) and 2.5 μg/mL propidium iodide (PI). The cell suspension was incubated for 15 min at room temperature and analyzed by flow cytometry.

Statistical analyses

All experiments were performed at least three times. A computer-aided *t* test program SPSS (version 11.0) (GraphPad Software, Inc. San Diego, CA, USA) was used to determine the statistical significance of the differences between DGCs and GSCs, and between transfected and mock cells. *P* values equal to or less than 0.05 were considered as statistically significant.

Results

Isolation of GSCs from human glioblastoma specimens

In order to compare the chemoresistance of GSCs and DGCs, we isolated glioma cells from 11 human specimens. Primary glioma cells contained 1.42% $CD133^+$ GSCs (Fig. 1a). In serum-free stem cell culture medium, only GSCs and progenitor cells survived, proliferated and formed neurospheres (Fig. 1b) in which $CD133^+$ cells increased to 7.38% after the culture (Fig. 1c). GSCs were further sorted by CD133 FACS and confirmed by CD133-positive immunofluorescence staining (Fig. 1d–f). $CD133^+$ GSCs in serum-containing medium differentiated into GFAP-, MAP2- and O4-positive cells (data not shown), suggesting the capacity for multi-lineage differentiation.

Correlation between chemoresistance and TAp73 silencing in GSCs

As compared to DGCs, GSCs showed increased survival under exposure to DNA-damaging drug cisplatin (Fig. 2a). The levels of TAp73 mRNA were markedly lower in GSCs

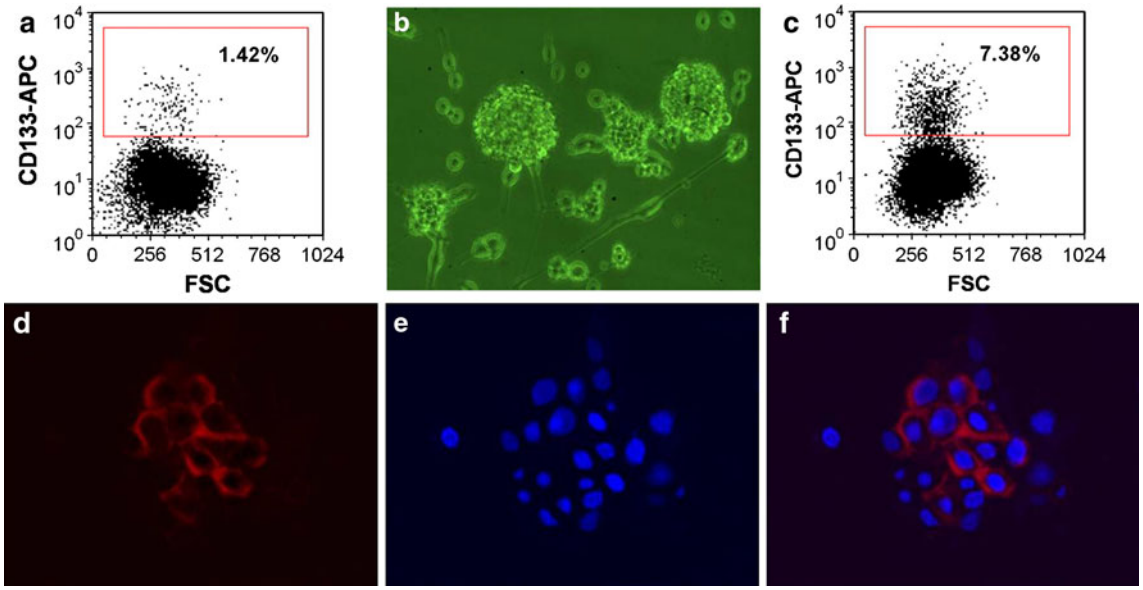

Fig. 1 Isolation and identification of CD133$^+$ cells from human gliomas. Primary glioma cells (**a**) from glioblastoma specimens were cultured and the CD133$^+$ cells were detected by FACS. CD133$^+$ cells were enriched by serum-free culture to form neurospheres (**b**) and were further confirmed by FACS (**c**) and immunofluorescence staining of CD133 (**d–f**, *red color*; counterstained with DAPI showing nuclei in *blue*)

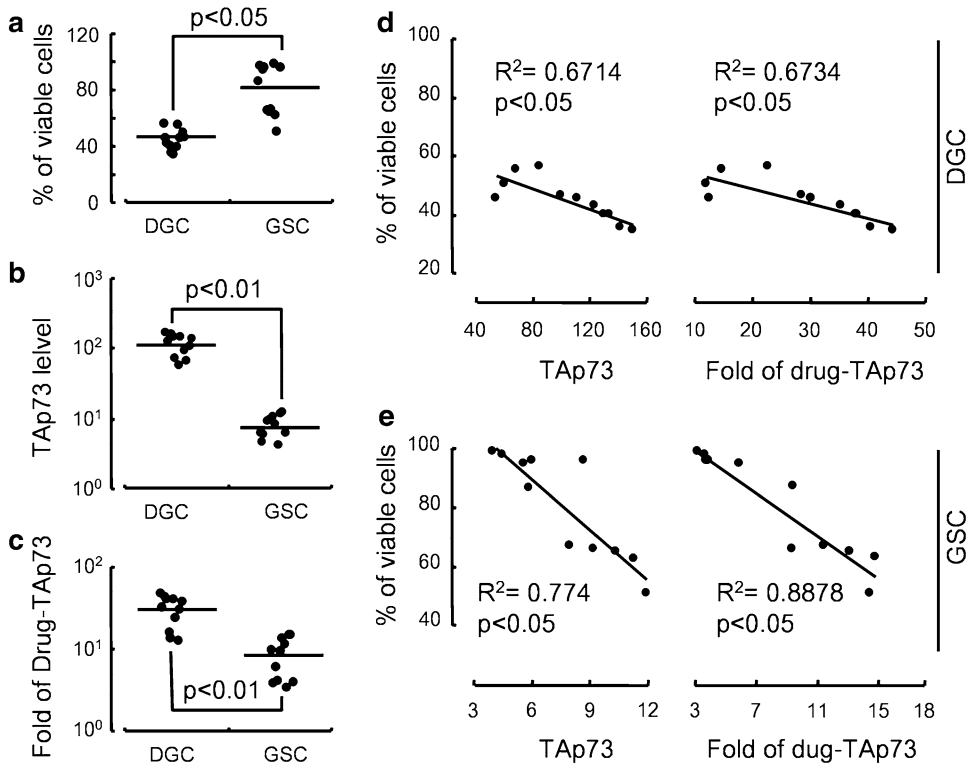

Fig. 2 Correlation between TAp73 expression and chemoresistance. Sorted GSCs and DGCs were treated with cisplatin (2 μM, 48 h). Cell viability was estimated using MTT (**a**). Cell viability was calculated by setting the survival of vehicle-treated cells as 100%. Statistical significance was defined if $P < 0.05$. The level of TAp73 mRNA in DGCs and DGCs was measured by RT^2-PCR (**b**). Cisplatin-induced TAp73 expression was expressed as the fold of the level of TAp73 mRNA in cisplatin-treated DGCs and GSCs relative to vehicle-treated cells (**c**). The correlation between chemoresistance, TAp73 expression and cisplatin-induced TAp73 expression was analyzed by dot-plot of the percentage of viable cells under cisplatin, TAp73 mRNA level and cisplatin-induced fold changes of TAp73 mRNA in DGCs (**d**) and GSCs (**e**)

than in DGCs (Fig. 2b). We then examined the changes in TAp73 transcription in response to cisplatin-induced DNA damage. Cisplatin augmented TAp73 mRNA in GSCs, but the fold increase was lower than in DGCs (Fig. 2c). The number of viable cells under cisplatin exposure was negatively correlated with TAp73 mRNA level and the changes in response to cisplatin treatment in both cells (Fig. 2d, e). GSCs exhibited a more significant negative correlation between the original TAp73 mRNA level and its induction by cisplatin (Fig. 2d, e).

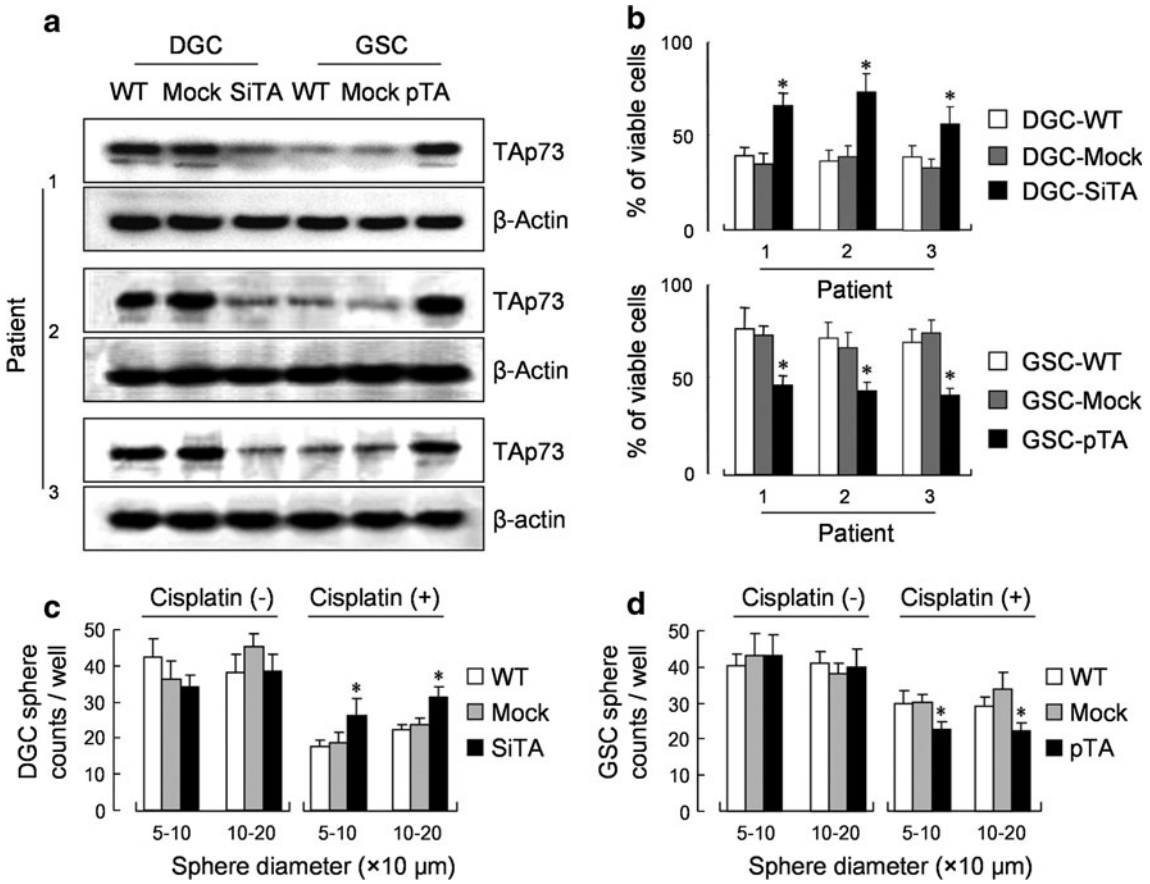

Fig. 3 Effects of TAp73 expression on chemoresistance of glioma cells. The plasmids containing TAp73 siRNA (siTAp73, siTA) or TAp73 expression cassette (pTAp73, pTA) were transfected into DGCs or GSCs. Immunoblot of TAp73 and *β*-actin confirmed the changes in TAp73 in the transfected cells, mock-transfected cells (Mock) and wild-type cells (WT) (**a**). Chemoresistance of the transfected cells was assayed by MTT and expressed as the percentage of viable cells with cisplatin treatment (**b**). Each vertical bar represents the mean ± SD of triplicates. *Asterisk* indicates significantly increased or reduced percentage of viable cells as compared to mock cells ($P < 0.05$) (**b**). Tumor sphere formation of the cells was measured on soft agar. DGCs **(c)** or GSCs **(d)** were treated with 2 μM cisplatin for 48 h and then suspended in 0.3% agar that was layered on solidified bottom agar. The cells were grown in 5% CO_2 at 37°C with medium containing 10% FCS for 3 weeks, and then the tumor spheres were photographed under microscope. The results are expressed as the mean (±SE, $n = 6$) number of spheres. *Asterisk* indicates significantly increased or reduced sphere numbers as compared to mock cells ($P < 0.05$)

Effect of overexpression of TAp73 in GSCs on the chemoresistance to cisplatin

In order to more precisely study the contribution of TAp73 to chemoresistance of GSCs, we transfected GSCs or DGCs with TAp73 gene (PTAp73) or shRNA targeting TAp73 (siTAp73). Western blot confirmed that TAp73 was down-regulated in siTAp73 DGCs and overexpressed in pTAp73 GSCs (Fig. 3a).

Subsequently, we tested the chemoresistance of both cell types. As shown in Fig. 3b, 56.9–73.7% DGCs transfected with TAp73 siRNA survived the cisplatin exposure as compared to 33.5–39.1% of mock-transfected cells. In addition, the survival rate of TAp73-transfected GSCs was reduced to 42.7–47.9% as compared to 68.0–76.3% shown by mock-transfected GSCs and 71.1–77.8% of wild-type cells ($P < 0.01$). In a tumor sphere formation model, we found that TAp73 siRNA attenuated significantly ($P < 0.01$) cisplatin-induced inhibition on formation of DGC tumor spheres as measured by the sphere numbers and size (Fig. 3c). Overexpression of TAp73 further diminished the capacity of GSCs to form larger (50–200 μm) spheres under cisplatin exposure (Fig. 3d). These results confirm that TAp73 silencing enabled GSCs to be more resistant to cisplatin.

TAp73 silencing in GSCs reduced cisplatin-induced apoptosis

Since the level of TAp73 expression was correlated with the survival rate of GSCs and DGCs after cisplatin treatment, we investigated whether TAp73 might promote apoptosis of tumor cells induced by DNA-damaging drugs. We found that after exposure to cisplatin, 8.3% GSCs

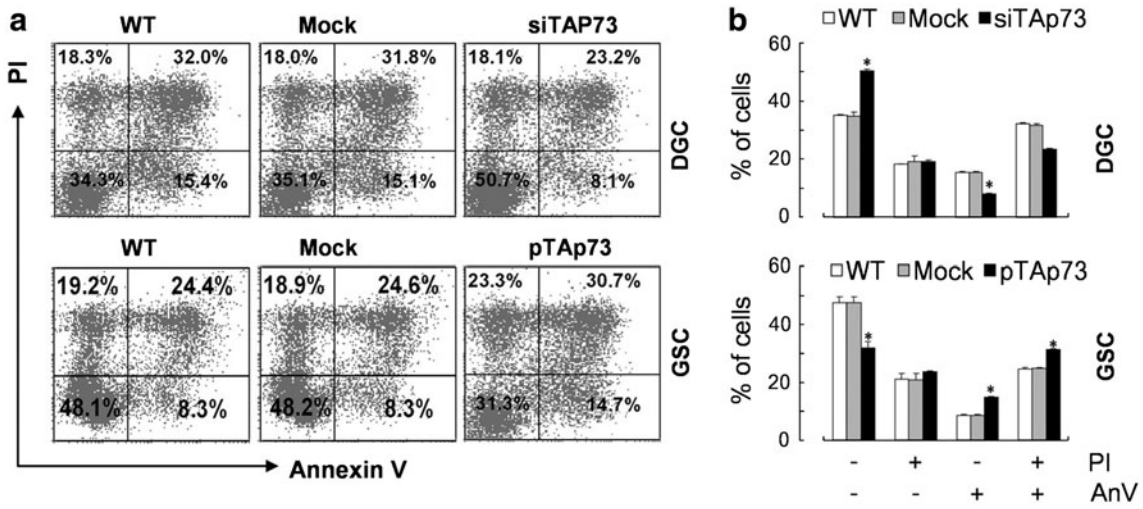

Fig. 4 Effect of TAp73 on cisplatin-induced apoptosis. Apoptotic cells were detected by annexin V surface staining and PI. Shown are dot-plots (**a**) of the fluorescein isothiocyanate-conjugated annexin V versus PI staining for DGCs and GSCs after cisplatin (2 μM, 48 h) treatment. Each bar (**b**) represents the mean ± SD of 6 measures of the percentages of cell number in various quadrants of the dot-plot of annexin V versus PI. *Asterisk* indicates significant changes compared to mock-transfected cells ($P < 0.05$)

underwent early apoptosis (Fig. 4a, b) and 24.4% GSCs underwent late apoptosis. The rate of both early and late apoptosis in GSCs was significantly lower than the rate of DGCs (15.4 and 32.0%, respectively) (Fig. 4a, b). These results suggest that GSCs exhibited better anti-apoptosis capacity than DGCs. TAp73 siRNA transfection in DGCs markedly reduced the rate of early and late apoptosis under cisplatin exposure as compared to mock-transfected cells ($P < 0.01$, Fig. 4a, b). In contrast, overexpression of TAp73 in GSCs significantly enhanced their early and late apoptosis as compared to mock-transfected cells ($P < 0.01$, Fig. 4a, b).

TAp73 silencing in GSCs reduced the expression of apoptosis-related genes induced by cisplatin

Western blot showed that cisplatin increased the levels of TAp73 in DGCs (Fig. 5a), but not in GSCs (Fig. 5b). The results confirmed TAp73 silencing in GSCs. To evaluate the mechanisms by which TAp73 promotes apoptosis in DGCs and GSCs, we examined the expression of apoptosis-related genes. p21 is a cyclin-dependent kinase inhibitor and regulates cellular cycle progression and apoptosis. Cisplatin-induced expression of p21, as shown by the relative density to untreated cells (without cisplatin) in Fig. 5a, b, was significantly attenuated after TAp73 was depleted in DGCs by siRNA (Fig. 5a), but was not increased after TAp73 overexpression in GSCs (Fig. 5b). Nevertheless, the absolute p21 level of pTAp73 cells was higher than mock cells in the presence or absence of cisplatin. The phenomenon might be due to TAp73-mediated regulation of p21 expression. Next, we examined Bax expression, a

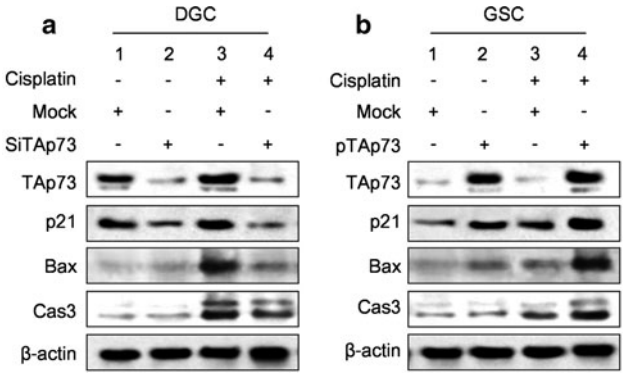

Fig. 5 Expression of apoptosis-related proteins in DGC and GSC with or without cisplatin treatment. After treatment of cisplatin, the lysates of DGCs infected with retrovirus containing siRNA (siTAp73) targeting TAp73 (**a**) or GSCs with expression plasmid of TAp73 (pTAp73) (**b**) and mock-transfected cells (Mock) were subjected to western blot to detect TAp73, $p21^{WAF}$ (p21), Bax, caspase 3 (Cas3), and β-actin

pro-apoptotic gene. We found that cisplatin induced Bax expression in mock DGCs or pTA-GSCs, which contained high levels of TAp73, but not or slightly in mock GSCs or TAp73 siRNA DGCs, which contained low levels of TAp73 (Fig. 5a, b). The level of cleaved caspase 3 induced by cisplatin treatment was increased by eightfold in untreated DGCs (Fig. 5a), but only by twofold in cisplatin-treated GSCs (Fig. 5b). Furthermore, the expression of cleaved caspase 3 was reduced by TAp73 depletion in DGCs (Fig. 5a), but was increased by the overexpression of TAp3 in GSCs (Fig. 5b). These results suggest that TAp73 participates in DNA damage–induced expression of apoptosis-related genes.

Discussion

In this study, we demonstrated that the expression of TAp73 gene is at lower levels and is not easily induced by DNA-damaging chemotherapeutic drug in GSCs than in DGCs. This "TAp73 silencing" may enable GSCs to show higher degree of chemoresistance than DGCs.

In addition to cisplatin, many genotoxic agents such as adriamycin, taxol and etoposide have been shown to induce accumulation of TAp73 protein in tumor cells [3, 13]. p73 including p73 isoforms, p53 and p63 form a complex network to regulate the balance between pro- and anti-apoptosis [9]. Since the discovery of Np73, studies have distinguished pro-apoptotic and anti-apoptotic isoforms of p73 and found accumulation of anti-apoptotic p73 in human tumors [8, 21, 25, 32]. Resistance to chemotherapeutic agents involves inactivation of the pro-apoptotic function of p73 or activation of its anti-apoptosis activity [3, 13]. Chemotherapeutic drug–induced accumulation of pro-apoptotic protein TAp73 may break the balance.

Abrogation of TAp73 expression and function by RNA interference reduced chemotherapeutic agent–induced apoptotic tumor cells and led to chemoresistance [12, 13]. Inactivation of TAp73 by a dominant negative mutant also resulted in resistance of tumor cells to apoptosis induced by genotoxic agents [13]. $p73^{-/-}$ fibroblasts are more resistant to genotoxic agent–induced apoptosis than cells with wild-type p73 [13]. Therefore, TAp73 is an important component in cell response to cytotoxic agents.

TAp73 induced by DNA-damaging drugs cooperates with wild-type p53 to promote the expression of genes essential for apoptosis, cell cycle arrest and DNA repair [9, 13, 23]. Induction of apoptosis by p53 requires the presence of TAp73 [10], which in turn induces apoptosis in tumor cells that lack functional p53 [13]. TAp73 and p53 form a large transcriptional complex composed of p73 and/or p63 and p53 to sequester three proteins close to the promoter to regulate the expression of apoptosis-related genes and their products [27].

Drug-induced TAp73 accumulation has been shown to be due to increased transcription and protein stabilization [1, 5, 6, 30]. Interaction between p73 isoforms and p53 and/or p63 leads to the inactivation of transactivational function of p73 [2, 7, 19, 20, 26] by dominant negative regulation. Following cisplatin-induced DNA damage, Chk1/Chk2 (checkpoint kinases) activate and stabilize the transcription factor E2F1, which binds to the promoter of p73 and enhances p73 transcriptional expression of the p73 gene [33]. The activity of ZEB repressor (zinc finger E-box-binding homeobox) is also involved in the regulation of p73 at transcriptional level. ZEB can bind to regulatory region of p73 gene and attenuate the activity of the p73 promoter in the presence of E2F1. In addition to such p73 silencing, epigenetical alterations could also be involved in the p73 silencing. Although epigenetic alterations in p73 are rare in human tumors, it is not known whether they exist in cancer stem cells. Methylation in the promoter region of the p73 gene was detected in some malignant glioma cells, but not in benign glioma cells, and p73 gene methylation was associated with loss of its expression [28]. In fact, our preliminary data suggest that p73 gene was hypermethylated in GSCs as compared to DGCs, which may explain its resistance to regulation by genotoxic agents. It has been reported that GSCs exhibited hypermethylation in caspase 8 promoter so that the cells expressed low levels of caspase 8 mRNA and protein [4]. However, the mechanisms by which TAp73 in GSCs failed to respond to genotoxic drugs require further study. DNA damage also induces several posttranslational modifications of p73. After cisplatin activates the cellular Abelson leukemia (c-Abl) tyrosine kinase, the SH3 domain of c-Abl kinase interacts with the p73 proline-rich region. The interaction can lead to the phosphorylation of p73 at Tyr-99 and thereby its stabilization [1, 11, 31]. Therefore, c-Abl is involved in the regulation of p73 accumulation after cisplatin treatment. It is not clear whether c-Abl contributes to TAp73 silence in glioma stem cells. Regardless, increasing the expression and function of pro-apoptotic TAp73 isoforms may prove useful in overcoming chemoresistance in human GSCs.

Acknowledgments The authors thank Dr. Ji Ming Wang (Laboratory of Molecular Immunoregulation, Cancer and Inflammation Program, Center for Cancer Research, National Cancer Institute at Frederick, Frederick, MD 21702, USA) for reviewing the manuscript. This project was supported by the grants from the National Natural Science Foundation of China (NSFC, No. 30873034 and 30973493).

References

1. Agami R, Blandino G, Oren M, Shaul Y (1999) Interaction of c-Abl and p73alpha and their collaboration to induce apoptosis. Nature 399:809–813
2. Bensaad K, Le Bras M, Unsal K, Strano S, Blandino G, Tominaga O, Rouillard D, Soussi T (2003) Change of conformation of the DNA-binding domain of p53 is the only key element for binding of and interference with p73. J Biol Chem 278:10546–10555
3. Bergamaschi D, Gasco M, Hiller L, Sullivan A, Syed N, Trigiante G, Yulug I, Merlano M, Numico G, Comino A, Attard M, Reelfs O, Gusterson B, Bell AK, Heath V, Tavassoli M, Farrell PJ, Smith P, Lu X, Crook T (2003) p53 polymorphism influences response in cancer chemotherapy via modulation of p73-dependent apoptosis. Cancer Cell 3:387–402
4. Capper D, Gaiser T, Hartmann C, Habel A, Mueller W, Herold-Mende C, von Deimling A, Siegelin MD (2009) Stem-cell-like glioma cells are resistant to TRAIL/Apo2L and exhibit down-regulation of caspase-8 by promoter methylation. Acta Neuropathol 117:445–456
5. Chen X, Zheng Y, Zhu J, Jiang J, Wang J (2001) p73 is transcriptionally regulated by DNA damage, p53, and p73. Oncogene 20:769–774

6. Costanzo A, Merlo P, Pediconi N, Fulco M, Sartorelli V, Cole PA, Fontemaggi G, Fanciulli M, Schiltz L, Blandino G, Balsano C, Levrero M (2002) DNA damage-dependent acetylation of p73 dictates the selective activation of apoptotic target genes. Mol Cell 9:175–186
7. Di Como CJ, Gaiddon C, Prives C (1999) p73 function is inhibited by tumor-derived p53 mutants in mammalian cells. Mol Cell Biol 19:1438–1449
8. Douc-Rasy S, Barrois M, Echeynne M, Kaghad M, Blanc E, Raguenez G, Goldschneider D, Terrier-Lacombe MJ, Hartmann O, Moll U, Caput D, Benard J (2002) DeltaN-p73alpha accumulates in human neuroblastic tumors. Am J Pathol 160:631–639
9. Flores ER, Tsai KY, Crowley D, Sengupta S, Yang A, McKeon F, Jacks T (2002) p63 and p73 are required for p53-dependent apoptosis in response to DNA damage. Nature 416:560–564
10. Foster BA, Coffey HA, Morin MJ, Rastinejad F (1999) Pharmacological rescue of mutant p53 conformation and function. Science 286:2507–2510
11. Gong JG, Costanzo A, Yang HQ, Melino G, Kaelin WG Jr, Levrero M, Wang JY (1999) The tyrosine kinase c-Abl regulates p73 in apoptotic response to cisplatin-induced DNA damage. Nature 399:806–809
12. Irwin MS (2004) Family feud in chemosensitvity: p73 and mutant p53. Cell Cycle 3:319–323
13. Irwin MS, Kondo K, Marin MC, Cheng LS, Hahn WC, Kaelin WG Jr (2003) Chemosensitivity linked to p73 function. Cancer Cell 3:403–410
14. Jost CA, Marin MC, Kaelin WG Jr (1997) p73 is a simian [correction of human] p53-related protein that can induce apoptosis. Nature 389:191–194
15. Kaghad M, Bonnet H, Yang A, Creancier L, Biscan JC, Valent A, Minty A, Chalon P, Lelias JM, Dumont X, Ferrara P, McKeon F, Caput D (1997) Monoallelically expressed gene related to p53 at 1p36, a region frequently deleted in neuroblastoma and other human cancers. Cell 90:809–819
16. Kang MK, Kang SK (2007) Tumorigenesis of chemotherapeutic drug-resistant cancer stem-like cells in brain glioma. Stem Cells Dev 16:837–847
17. Kleihues P, Louis DN, OD W (2007) WHO grading of tumours of the central nervous system. In: Louis DN, Ohgaki HWO, Cavenea WK (eds). WHO classification of tumours of the central nervous system 4th edn. IARC, Lyon, pp 10–49
18. Leong CO, Vidnovic N, DeYoung MP, Sgroi D, Ellisen LW (2007) The p63/p73 network mediates chemosensitivity to cisplatin in a biologically defined subset of primary breast cancers. J Clin Invest 117:1370–1380
19. Martinez LA, Naguibneva I, Lehrmann H, Vervisch A, Tchenio T, Lozano G, Harel-Bellan A (2002) Synthetic small inhibiting RNAs: efficient tools to inactivate oncogenic mutations and restore p53 pathways. Proc Natl Acad Sci USA 99:14849–14854
20. Melino G, De Laurenzi V, Vousden KH (2002) p73: friend or foe in tumorigenesis. Nat Rev Cancer 2:605–615
21. O'Nions J, Brooks LA, Sullivan A, Bell A, Dunne B, Rozycka M, Reddy A, Tidy JA, Evans D, Farrell PJ, Evans A, Gasco M, Gusterson B, Crook T (2001) p73 is over-expressed in vulval cancer principally as the Delta 2 isoform. Br J Cancer 85:1551–1556
22. Rocco JW, Leong CO, Kuperwasser N, DeYoung MP, Ellisen LW (2006) p63 mediates survival in squamous cell carcinoma by suppression of p73-dependent apoptosis. Cancer Cell 9:45–56
23. Senoo M, Manis JP, Alt FW, McKeon F (2004) p63 and p73 are not required for the development and p53-dependent apoptosis of T cells. Cancer Cell 6:85–89
24. Stiewe T, Tuve S, Peter M, Tannapfel A, Elmaagacli AH, Putzer BM (2004) Quantitative TP73 transcript analysis in hepatocellular carcinomas. Clin Cancer Res 10:626–633
25. Stiewe T, Zimmermann S, Frilling A, Esche H, Putzer BM (2002) Transactivation-deficient DeltaTA-p73 acts as an oncogene. Cancer Res 62:3598–3602
26. Strano S, Fontemaggi G, Costanzo A, Rizzo MG, Monti O, Baccarini A, Del Sal G, Levrero M, Sacchi A, Oren M, Blandino G (2002) Physical interaction with human tumor-derived p53 mutants inhibits p63 activities. J Biol Chem 277:18817–18826
27. Urist M, Prives C (2002) p53 leans on its siblings. Cancer Cell 1:311–313
28. Watanabe T, Huang H, Nakamura M, Wischhusen J, Weller M, Kleihues P, Ohgaki H (2002) Methylation of the p73 gene in gliomas. Acta Neuropathol 104:357–362
29. Yi L, Zhou ZH, Ping YF, Chen JH, Yao XH, Feng H, Lu JY, Wang JM, Bian XW (2007) Isolation and characterization of stem cell-like precursor cells from primary human anaplastic oligoastrocytoma. Mod Pathol 20:1061–1068
30. Yuan ZM, Shioya H, Ishiko T, Sun X, Gu J, Huang YY, Lu H, Kharbanda S, Weichselbaum R, Kufe D (1999) p73 is regulated by tyrosine kinase c-Abl in the apoptotic response to DNA damage. Nature 399:814–817
31. Yuan ZM, Utsugisawa T, Huang Y, Ishiko T, Nakada S, Kharbanda S, Weichselbaum R, Kufe D (1997) Inhibition of phosphatidylinositol 3-kinase by c-Abl in the genotoxic stress response. J Biol Chem 272:23485–23488
32. Zaika AI, Slade N, Erster SH, Sansome C, Joseph TW, Pearl M, Chalas E, Moll UM (2002) DeltaNp73, a dominant-negative inhibitor of wild-type p53 and TAp73, is up-regulated in human tumors. J Exp Med 196:765–780
33. Zawacka-Pankau J, Kostecka A, Sznarkowska A, Hedstrom E, Kawiak A (2010) p73 tumor suppressor protein: a close relative of p53 not only in structure but also in anti-cancer approach? Cell Cycle 9:720–728